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E-mail: paolo.romagnoli@unifi.it - ijae@unifi.it

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ABSTRACTS

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Paolo Mascagni and Alessandro Moreschi: the intellectual property right on the discovery of the vascular structure of urethral tissue

Emanuele Armocida[1] and Gianfranco Natale[2]

[1] Società Italiana di Storia della Medicina, Dipartimento di Medicina e Chirurgia, Università degli Studi di Parma, Parma, Italia
[2] Società Italiana di Anatomia e Istologia, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italia

In the beginning of the XIX century, when both vascular and cellular texture theories concerning the penis structure were still coexisting, three figures were involved in the controversy about the priority of the discovery of the vascular nature of human erectile tissues: Paolo Mascagni (1755-1815), represented by his pupil Tommaso Farnese (1780-1829), and Alessandro Moreschi (1771-1826). In the Elogio del celebre anatomico Paolo Mascagni (1816), Farnese attributed to his mentor the demonstration in 1809 of the continuity between arteries and veins and the description of venous plexuses, this term replacing the previous and misleading name of spongy body attributed to the inner part of penis. But in 1817 Moreschi inflamed the dispute, claiming for the priority of that discovery, with the publication of his anatomical work and a polemical essay against Farnese [1]. Farnese promptly replied with Note addizionali del Dottore Tommaso Farnese al suo elogio di Paolo Mascagni (1818) [2], where he reported a meeting with Moreschi in Bologna in 1810. In that occasion, Farnese explained a Mascagni's technique to perfuse urethral blood vessels that Moreschi would have plagiarized. Furthermore, Farnese also included eight testimonies claiming to have seen Mascagni performing such injections before 1810. The Prodromo della grande anatomia, a posthumous work of Mascagni edited in1819, includes a plate dedicated to the structure of the urethra and a comprehensive view of this scientific story. In short, Mascagni developed a technique to inject urethral blood vessels, but Moreschi was the first to publish an accurate work on this subject. For this reason, many Italian and international authors have attributed to the latter the discovery of the venous circulation of the urethra.

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Key words

Paolo Mascagni, Alessandro Moreschi, Tommaso Farnese, urethra, erectile tissue.

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An immunohistochemical study of TLR-4 and -7 expression during murine embryonic development: respiratory apparatus and peripheral nervous system

Francesca Arnaboldi[1], Emanuela Opizzi [1], Marco Rasile[2], Elena Menegola[3], Francesca Di Renzo[3], Isabella Barajon[4]

[1] Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute, Milano, Italia
[2] Istituto di Ricovero e Cura a Carattere Scientifico Humanitas, Istituto di Ricovero e Cura a Carattere Scientifico Humanitas, Rozzano (Milano), Italia
[3] Università degli Studi di Milano, Department of Environmental Science and Policy, Milano, Italia
[4] Hunimed University, Department of Biomedical Sciences, Rozzano (Milano), Italia

Toll-Like receptors (TLRs) are the mammalian orthologue of the type-I transmembrane receptor Toll originally identified in Drosophila for its function in embryonic developmental patterning [1]. In mammals, TLRs are known to function in innate immunity by recognizing molecular motifs unique to pathogens or injured tissue. However, literature data are emerging about a morphogenetic role of TLRs during development also in mammals, in particular of the nervous system [2]. We assessed TLRs expression in murine peripheral nervous system during the embryonic development, focusing on the innervation of the respiratory apparatus. Mouse embryos from stages E12 to E18 were excised, fixed in paraformaldehyde and paraffin embedded. Immunohistochemical stainings were performed to study the expression of TLR4 and TLR7, and to visualize the developing peripheral nervous system by the neural marker beta-3 tubulin. TLR7 immunoreactivity was already present at E12 in the dorsal root ganglia (DRG) and in the nodose ganglion and, by E14, in the sympathetic ganglia (PVG), vagus nerve, and also in nervous fibers and ganglia in the respiratory apparatus. Instead, TLR4 started to be weakly detected at E14 in DRG, PVG, and vagus nerve and, by E17, also in the smooth muscle, nervous fibers, and little ganglia of the respiratory apparatus. In conclusion the earlier expression of TLR7 could suggest for this receptor a role in the developmental processes while, the late detection of TLR4 might indicate it is most probably related to the maturation of immunity mechanisms in preparation for birth.

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Key words

Toll-Like Receptors, embryonic development, respiratory apparatus, peripheral nervous system.

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FVIII induced expression in adipose-derived Mesenchymal Stem Cells for Haemophilia A cell therapy

Silvia Barbon[1], Elena Stocco[1], Senthilkumar Rajendran[2], Paolo Radossi[3], Giuseppe Tagariello[3], Andrea Porzionato[1], Veronica Macchi[1], Raffaele De Caro[1], Pier Paolo Parnigotto[4] and Claudio Grandi[5]

[1] Sezione di Anatomia Umana, Dipartimento di Neuroscienze, Università degli Studi di Padova, Padova, Italia
[2] Dipartimento di Scienze Chirurgiche, Oncologiche e Gastroenterologiche, Università degli Studi di Padova, Padova, Italia
[3] Centro di Emofilia ed Ematologia, Ospedale San Giacomo, Castelfranco Veneto, Treviso, Italia
[4] Fondazione per la Biologia e la Medicina della Rigenerazione, Tissue Engineering and Signaling, T.E.S., Onlus, Padova, Italia
[5] Dipartimento di Scienze del Farmaco, Università degli Studi di Padova, Padova, Italia

In the last decades, the use of multipotent adult stem cells has paved way for the identification of new therapeutic approaches for the treatment of acquired or degenerative pathologies. Monogenic diseases such as Haemophilia A are also ideal candidates for the treatment by these emerging therapies, which may success in correcting alterated protein expression resulting from genetic mutation. Recently, adipose tissue has proven to serve as an accessible and rich source of adult stem cells with multidifferentiative properties, suitable for regenerative medical applications. Although they have already been studied for these purposes, adipose-derived stem cells are still poorly considered for Haemophilia A cell therapy and few research work have focused on their capacity to produce coagulation factor VIII when properly stimulated. This work has studied the in vitro differentiation of an adipose-derived stem cell line towards the endothelial lineage, considered to be responsible for coagulation factor production. At this end, adipose-derived stem cells were cultured into differentiation medium enriched with endothelial growth factors up to 21 days. After the induction treatment, changes in cell morphology, migratory capacity and specific gene/protein expression were evaluated by optical microscopy, transwell migration assay and qPCR/Dot Blot analysis, respectively. Our data highlighted that, already after 7 days of induction treatment, cell cultures showed to change their fibroblastoid morphology, starting to form capillary-like structures. They significantly responded to the chemotactic stimuli of endothelial growth factors, and up-regulated the expression of specific endothelial markers (CD34, PDGFRα, VE-cadherin, CD31, vWF) as the time of induction increased. Most important, we gave the first evidence that adipose stem cells are capable of secreting factor VIII after specific endothelial stimulation and without any need for gene transfection. On the base of that, stem cells from adipose tissue seem to be promising candidate for Haemophila A cell therapy.

Key words

Adipose-derived stem cells, Haemophilia A, coagulation factor VIII, stem cell therapy.

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Endurance training induces apoptosis in the tumor mass in the C26-bearing mouse model

Rosario Barone[1], Filippo Macaluso[2], Daniela D'Amico[3], Caterina Gargano[4], Medhi Hassani[5], Zhigang Xue[5], Francesco Cappello[6], Giovanni Zummo[6], Sergio Adamo[7] , Felicia Farina[6], Dario Coletti[5] and Valentina Di Felice[6]

[1] Università degli Studi di Palermo, Dipartimento di Biomedicina e Neuroscienze, Palermo, Italia
[2] Università eCampus, Università eCampus, Novedrate, Italia
[3] Univerità di Palermo, Dipartimento di Biomedicina Sperimentale e Neuroscienze, Palermo, Italia
[4] Università degli Studi di Palermo, Dipartimento BIONEC, Palermo, Italia
[5] Sorbonne Universitè, Institute of Biology, Paris, Francia
[6] Università degli Studi di Palermo, Dipartimento BioNeC, Palermo, Italia
[7] Università La Sapienza di Roma, Dipartimento di Scienze Anatomiche, Istologiche, Medico-legali e dell'Apparato Locomotore (SAIMLAL), Roma, Italia

Cachexia, sarcopenia and anorexia are characterised by muscle wasting. This condition is a weakening, shrinking, and loss of muscle caused by a disease or lack of use. The loss of muscle causes a decrease in strength and inability to move compromising the quality of life. Recently we demonstrated that the skeletal muscle of endurance trained Balb/c mice release IL-6 and Hsp60 (inside exosomes) in the blood stream.

We studied the expression of Hsp60 in the muscles of trained and untrained C26-bearing mice, to understand if Hsp60 was over-expression may improve muscle performance and reduce cachexia. Four different interleukins have been also studied in cachectic mice, to understand which was their effect on Hsp60 expression both in the tumor mass and the trained muscle.

In the present study we demonstrated that: 1) IL-6 is released by the trained muscle; 2) IL-6 is release also by the tumor mass, 3) in animals inoculated with the C26 tumor and trained after inoculation, IL-6 is synthesized mainly by the skeletal muscle and the tumor mass undergo apoptosis.

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Key words

Colon carcinoma, metastasis, cachexia, Hsp60, interleukin-6.

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Nandrolone decanoate interferes on testosterone biosynthesis and alters blood-testis barrier

Rosario Barone[1], Alessandro Pitruzzella[2], Antonella Marino Gammazza[1], Francesca Rappa[1], Valentina Di Felice[1], Giuseppe Bonaventura[1], Angelo Leone[1], Cristoforo Pomara[3], Francesco Cappello[1]

[1] Department of Experimental Biomedicine and Clinical Neurosciences (BioNeC), University of Palermo, Palermo, Italia
[2] Department of stress biology, human longevity, sport and health, Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italia
[3] Department of Clinical and Experimental Medicine, Forensic Pathology, University of Foggia, Foggia, Italia

Nandrolone decanoate (ND) is a synthetic testosterone analogue considered one of the most commonly abused anabolic androgenic steroids by adolescents and athletes. ND is alleged to promote an increase in muscle mass and improves both physical appearance and sporting performance, but ND abuse is often associated with serious adverse effects, interfering with the endocrine system and the reproductive system. In a previous study, we demonstrated that ND treatment of Leydig cells interferes with the biosynthesis of testosterone in a dose increase-dependent fashion [1]. As a consequence of the results obtained in vitro, in this study an animal model was utilized to better understand the side effects of ND administration in sedentary and trained mice. A group of mice underwent endurance training while another set led a sedentary lifestyle. All experimental groups were treated with either ND or peanut oil at different doses for 6 weeks. Testosterone serum levels were measured via liquid chromatography–mass spectrometry. Western blot analysis and quantitative real-time PCR were utilized to determine gene and protein expression levels of the primary enzymes implicated in testosterone biosynthesis and gene expression levels of the blood–testis barrier (BTB) components. Immunohistochemistry and immunofluorescence were conducted for testicular morphological evaluation. The study demonstrated that moderate to high doses of ND induced a diminished serum testosterone level and altered the expression level of the key steroidogenic enzymes involved in testosterone biosynthesis. At the morphological level, ND induced degradation of the BTB by targeting the tight junction protein-1 (TJP1). ND stimulation deregulated metalloproteinase-9, metalloproteinase-2 (MMP-2) and the tissue inhibitor of MMP-2. Moreover, ND administration resulted in a mislocalization of mucin-1. In conclusion, ND abuse induces a decline in testosterone production that is unable to regulate the internalization and redistribution of TJP1 and may induce the deregulation of other BTB constituents via the inhibition of MMP-2. ND may well be considered as both a potential inducer of male infertility and a potential risk factor to a low endogenous bioavailable testosterone.

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Key words

Nandrolone decanoate, testosterone, blood–testis barrier.

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PGC1α isoforms expression in skeletal muscle of trained and/or CLA supplemented mice

Rosario Barone, Claudia Sangiorgi, Antonella Marino Gammazza, Daniela D'Amico, Francesco Cappello, Giovanni Zummo, Felicia Farina, Valentina Di Felice and Filippo Macaluso

Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, Palermo, Italia

It has been reported that Conjugated Linoleic Acid (CLA) improves muscle hypertrophy [1], steroidogenesis [2], physical activity, and endurance capacity in mice [3]. Recently, it has been reported that endurance exercise increased the expression of PGC1 isoforms in murine skeletal muscle [4]. The aim of the present study was to quantify the expression of any of the peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) isoforms in gastrocnemius and plantaris muscles of trained and/or CLA supplemented mice. Mice were randomly divided in four groups: placebo sedentary, CLA sedentary, placebo trained, or CLA trained. The CLA groups were gavaged with 35 µl per day of Tonalin® FFA 80 food supplement containing CLA throughout the 6-week experimental period, whereas the placebo groups were gavaged with 35 µl sunflower oil each day. Each administered dose of CLA corresponded to approximately 0.7 g/kg or 0.5%, of the dietary daily intake. Trained groups ran 5 days per week on a Rota-Rod for 6 weeks at increasing speeds and durations. Mice were sacrificed by cervical dislocation and hind limb posterior muscle groups were dissected and used for histological and molecular analyses. Endurance training increased the expression of PGC1α isoforms (tot, α1, α2, and α3), but CLA supplementation did not increased PGC1α isoforms expression in trained and/or sedentary mice. In the plantaris muscle, CLA supplementation induced a fibre-type-specific hypertrophy of type IIx muscle fibres.

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Key words

PGC1α, endurance exercise, skeletal muscle, muscle fibres.

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Connectivity based segmentation of the human red nucleus

Gianpaolo Antonio Basile[1], Demetrio Milardi[2], Debora Di Mauro[1], Giuseppina Rizzo[1], Giuseppina Cutroneo[3]

[1] University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia - University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia -
[2] IRCCS Neurolesi Bonino Pulejo, via Casazza 98100, Messina, Italy -
[3] University of Messina, Department of Clinical and Experimental Medicine, Messina, Italia

The Red Nucleus (RN) is a large, iron-loaded nucleus located in the ventral midbrain. In monkeys, itis subdivided into a small caudoventral magnocellular part (mRN) and a large rostrodorsal parvicellular part (pRN). The mRN is more connected with motor and premotor regions of the cortex, interposed nuclei and spinal motor neurons via the rubrospinal tract [1]. By contrast, the pRN is more connected with cortex and is part of the dento-rubro-olivary pathway involved in movement preparation and motor learning. In humans, functional neuroimaging studies suggest RN involvement in complex motor, but also in sensory and cognitive functions. However, none of these studies was able to distinguish between topographically organized subregions of the RN and just a few studies focused on its structural and functional connections. In this regard, we have previously employed Constrained Spherical Deconvolution (CSD) tractography in order to characterise the structural connectome of the RN in the human brain and in vivo, showing robust connectivity profiles with the cerebellum, thalamus, paracentral lobule, postcentral gyrus, precentral gyrus and superior frontal gyrus [2]. Herein, we use high-quality 3T structural and diffusion MRI data from the Human Connectome Project (HCP) and CSD tractography with a connectivity-based segmentation approach, in order to identify topographically distinguished subregions of the RN according to their different cortical and subcortical connectivity profiles. We tracked connections of RN both with inferior olivary nuclei (IONs), interposed nuclei (INs) and dentate nuclei (DNs), as well as with frontal motor and associative cortices. We found that each RN can be subdivided according to its connectivity into two clusters: a large dorsolateral one, more connected with DNs and IONs, and a smaller ventromedial one, more connected with IN. Topographical connections between cortical areas and these two clusters was also evaluated. Our results are in line with previous literature and confirm CSD-based tractography and connectivity-based segmentation as valuable tools for the evaluation of human neuroanatomy.

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Key words

Red nucleus, cerebellum, magnocellular part, parvicellular part, connectivity, tractography, segmentation

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The bone diagenesis: post-mortem alterations in human bone

Petra Rita Basso[1], Alberto Colombo[2], Mario Raspanti[1]

[1] Dipartimento di Medicina e Chirurgia, Università dell'Insubria, Varese
[2] ASST Settelaghi, Ospedale di Circolo, Varese

Post mortem interval (PMI) estimation is a crucial issue in forensic science. Post-mortem microscopic changes of bone structure were first reported in the second half of the XIX century, yet there is still no general consent about the onset or the speed of these degenerative changes. An "exogenous model" of bioerosion, which hypothesizes that the alterations are brought about by environment-associated microorganisms acting once the hard tissues are skeletonized [1], is opposed to an "endogenous model" where the alterations are caused by bacteria already present in the human body [2]. In the present research 74 human bone samples were collected from the cranial vault of thirty-seven donors during neurosurgical procedures (craniectomy); each sample consisted of a small, irregularly shaped full-thickness bone fragment, with a major axis of approximately 10-12 mm. The specimens were tested for sterility and then exposed in vitro for as long as 48 weeks to a mixture of six strains of harmless microorganisms harvested from the oral cavity of 12 healthy patients. In time the bone specimens showed a progressive demineralization and, starting from the fifth week of incubation, revealed an unexpected complete equivalence of the diagenetic phenomena with the ones we ourselves observed in forensic bone samples and in archaeological bone. These observations indicate that post-mortem morphological changes in bone (a) can result from the interactions between various harmless bacterial strains; (b) are consistent with the endogenous model, being caused by bacterial strains already present in the oral cavity; (c) can be visible as soon as five weeks after exposure, well before the skeletonization as is usually hypothesized.

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Key words

Bone, diagenesis, forensics.

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Ultrastructural analysis of articular structures of normal and pathological human knee

Michela Battistelli[1], Marta Favero[2], Debora Burini[3], Giovanni Trisolino[4], Dante Dallari[5], Lucia De Franceschi[5], Giuseppe Filardo[6] Brunella Grigolo[7] and Eleonora Olivotto[8]

[1] Urbino University Carlo Bo, Università degli Studi di Urbino, Urbino, Italia
[2] University Hospital of Padova, Rheumatology Unit, Dep. of Medicine (DIMED), Padova, Italia
[3] Urbino University Carlo Bo, DiSB, Dep. of Biomolecular Sciences, Urbino, Italia
[4] IRCCS Istituto Ortopedico Rizzoli, Bologna, Pediatric Orthopedic and Traumatology, Bologna, Italia
[5] IRCCS Istituto Ortopedico Rizzoli, Bologna, Reconstructive Orthopedic Surgery Innovative Techniques - Musculoskeletal Tissue Bank, Bologna, Italia
[6] IRCCS Istituto Ortopedico Rizzoli, Bologna, NABI Laboratory, Bologna, Italia
[7] IRCCS Istituto Ortopedico Rizzoli, Bologna, RAMSES Laboratory, RIT Department, Bologna, Italia
[8] IRCCS Istituto Ortopedico Rizzoli, Bologna, RAMSES Laboratory, RIT Department, Laboratory of Immunorheumatology and Tissue Regeneration, Bologna, Italia

Human meniscus plays a crucial role for knee load transmission and distribution, shock absorption, joint stability, lubrication and congruity [1], while cartilage is a smooth tissue that promotes the sliding of the articular strands. The aim of this work is to compare healthy (control) and pathological articular structures. We have analyzed samples of meniscus and articular cartilage obtained from 3 multiorgan donors (median age, 66 years), 5 patients with traumatic meniscal tear (median age, 41years) and 3 patient undergoing total knee replacement for osteoarthritis (OA) (median age, 72 years). In the different conditions we evaluated the ECM component (collagen fiber organization and proteoglycan presence), the appearance and distribution of calcification areas, and the modifications of the cellular structure. Ultrastructural analysis of control menisci and cartilage show rare condensed chromatin masses in diffuse chromatin and well preserved organelles. Both in trauma and in OA, increasing chromatin condensation, organelle degeneration and cytoplasmic vacuolization appear [2]. In pathological conditions, particularly in OA, autophagic vacuoles, which probably represent a cellular self-protection mechanism, also appear. The most evident ultrastructural changes have been observed when surgery takes place long time after trauma. In this case a high chromatin condensation, a large cytoplasmic vacuolization with degeneration of organelles and several necrotic cells can be observed. Calcification areas occur both in traumatic and osteoarthritic menisci and cartilage. In particular, specimens from traumatic menisci have a structure similar to osteoarthritic ones, especially if trauma occurs in a more adult subject. In both disorganization of collagen fibers, replaced with proteoglycans, appears. A reduction of collagen fibers sizes can be also observed, if compared to control condition. We can conclude that trauma might induces an increasing meniscal and cartilage degeneration, comparable to physiological aging. In all experimental conditions, in particular in traumatic meniscal tear, we observed apoptotic-like features. Traumatic and degenerative meniscal lesions have peculiar anatomic features and different proposed etiologies, yet both are associated with development or OA progression.

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Key words

Meniscal tear, osteoarthritis, collagen fiber, ultrastructural analysis.

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A new model of liposome-human cell interaction for iron supplying

Michela Battistelli[1], Sara Salucci[1], Thomas De Paoli[2], Bruno Riccardi[2], Elisabetta Falcieri[1]

[1] Urbino University Carlo Bo, 61029 Urbino (PU), Italy, Department of Biomolecular Sciences, Urbino, Italia
[2] Lipotech S.A., Tres Arroyos 329 -Buenos Aires- Argentina, Bueno-Aires, Argentina

Approximately one third of the entire population is affected by iron deficiency, with heavy consequences on health. Its request may not be completely satisfied with a regular diet, due to a low iron bioavailability and to nutritional factors that limit its absorption.

Iron supplementation is considered a global economic and effective strategy to prevent and control anemia, in particular, during pregnancy [1]. Therefore, to develop new experimental protocols to improve its bioavailability appears very important.

Liposomes [2] are vesicular structures with double lipid layer membrane, which are proved to be particularly interesting in the biomedical field as a delivery system of pharmaceutically active substances [3].

In this study, ultrastructural analyses have been carried out to verify if liposome dehydration process induces structural alterations of lipid membranes, which could compromise carrier viability and function. Morphological integrity of Biofer and Lifervit (two new commercial iron carriers), both in liquid form and in dried powder, has been investigated by means of transmission electron microscopy on negatively stained samples [4]. Moreover, liposome interaction with U937 cell line, a well-known human model, characterized by a great phagocytic ability, has been evaluated on thin sections.

Both compounds revealed a good stability and are easily internalized into cells, interacting with cytoplasmic organelles without inducing, at least apparently, any ultrastructural damage. Therefore, Biofer and Lifervit do not cause cell toxicity, and for that they can be considered, in agreement with the current literature, potential candidates in iron vehiculation.

Further studies are in progress to evaluate their interaction in human intestinal cell models.

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Key words
Iron deficiency, anemia, U937 human cells

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Curcumin affects Hsp60 expression and function in a human neuronal cells

Celeste Caruso Bavisotto[1], Felicia Farina[1], Antonella Marino Gammazza[1], Everly Conway de Macario[2], Alberto J.L. Macario[3], Antonio Palumbo Piccionello[4], Claudia Campanella[1]

[1] University of Palermo, Department of BioNeC, Section of Human Anatomy, Palermo, Italia
[2] University of Maryland, Department Of Microbiology and Immnuology, School of Medicine, Baltimore, Italia
[3] University of Maryland, Department Of Microbiology and Immnuology, School of Medicine, Baltimore, Stati Uniti D' America
[4] University of Palermo, Department of Stebicef, Palermo, Italia

Heat-shock protein (Hsp)60 is a mitochondrial protein involved in assisting the correct folding of other mitochondrial client proteins [1]. Recently, this chaperonine has been considered as an emerging target for Alzheimer's Disease (AD) because seems to be able to mediate the translocation of Amyloid Precursor Protein (APP) and Amyloid Beta peptide (Aβ) to the mitochondria [2]. The fundamental challenge on fighting the Alzheimer's Disease (AD) is the development of neuro-protective agents, able to interfere with biochemical pathways responsible for the protein aggregation process whose clinical signature is represented by the plaques deposition. In this study we investigated the effect of curcumin, an emerging lead-compound for the development of neuro-protective drugs, on Hsp60 gene, protein expression and folding activity using a neuroblastoma cell line (LAN5). We demonstrated that the treatment of LAN5 cells with curcumin caused a down-regulation of mitochondrial Hsp60 protein and gene expression. On the other hand, curcumin enhanced the folding activity of the chaperonine. The ability of curcumin to affect Hsp60 expression as well as its ability to interact with the Hsp60/Hsp10 folding machine, open new frontiers in the use of putative therapeutic properties of curcumin as a switch from cancer therapy to AD treatment.

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Key words

Protein-misfolding diseases, Alzheimer's disease, β-amyloid, tau, molecular chaperones, chaperonotherapy.

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Exosomal Hsp60 levels and related miRNA in brain tumor cells

Celeste Caruso Bavisotto[1], Francesca Graziano[2], Felicia Farina[1], Francesca Rappa[3], Antonella Marino Gammazza[1], Sabrina David[1], Giusi Alberti[1], Everly Conway de Macario[4], Alberto J.L. Macario[5], Francesco Cappello[1], Domenico Gerardo Iacopino[2], Claudia Campanella[1]

[1] University of Palermo, Department of BioNeC, Section of Human Anatomy, Palermo, Italia
[2] University of Palermo, Department of BioNeC, Section of Neurosurgery, Palermo, Italia
[3] University of Palermo, Department of BioNeC, Section of Humana Anatomy, Palermo, Italia
[4] University of Maryland, Department of Microbiology and Immunology, School of Medicine, Baltimore, Stati Uniti D' America
[5] University of Maryland, Department of Microbiology and Immunology, School of Medicine, Palermo, Italia

One of the many pathologic conditions still without a satisfactory solution is that of brain tumors. The prognosis is poor even after surgical resection followed by post-operatory chemo- and radio-therapie [1]. It is, therefore, cogent to find innovative treatment tools. Three recent developments may provide elements to discover novel treatment strategies and means. These developments are: the discovery that molecular chaperones can be determinant factors in the process of tumorigenesis [2]; the elucidation of the role of miRNAs in gene regulation and determination of protein functions, including molecular chaperones; in the various cell compartments [3]; the increasing understanding and characterization of exosomes (exo), particularly in what refers to their release by tumor cells, contents including chaperones and miRNA, and ability to travel and interact with target cells near their origin or far [4]. The aim of the current study is to research a particular molecular chaperone, the HSP60 presence, levels, expression and distribution in tumor and peritumoral cells of primary brain tumors in vivo. The presence and level of HSP60 and some miRNAs involved in his regulation in exo isolated by blood samples obtained from patients with cancer before and after ablative surgery were also investigated. A total of 45 brain surgeries were performed. Blood and pathological tissue sample were taken from patient on the day of the surgery. For each patient, blood samples were collected at one week, one month and three months after surgery. Blood samples were collected from each patients and processed for plasma isolation, from which exo were isolated. The tumor and normal tissue section were used to perform the immunomorphological analyses and was assessed the valuation of HSP60 and microRNAs HSP60-related in exo obtained from blood of patients. Our work provided evidences about presence and levels of the main miRNA involved in HSP60 regulation in tumor brain, which would be useful in detecting the disease and monitoring its progression.

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Key words

HSP[60], exosomes, brain tumor, new therapeutic tools.

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Stress proteins and circulating miRNAs as biomarkers of hippocampal remodelling in drug-resistant temporal lobe epilepsy (DR-TLE)

Celeste Caruso Bavisotto[1], Leila Zummo[1], Rosario Barone[1], Everly Conway de Macario[2], Alberto A.J. Macario[2], Felicia Farina[1], Francesco Cappello[1], Antonella Marino Gammazza[1]

[1] Università degli Studi di Palermo, Dipartimento di Biomedicina Sperimentale e Neuroscienze Cliniche, Palermo, Italia
[2] University of Maryland, Department of Microbiology and Immunology, School of Medicine, Baltimore, Stati Uniti D' America

Among the mediators of stress response, Heat Shock Proteins (HSPs) play essential roles in cell survival, protein folding, trafficking and degradation [1]. In particular, HSPs alterations were associated with temporal lobe epilepsy (TLE) [2] and recently, specific microRNAs (miRNA) have been proposed as regulators of HSPs expression [3].

The significance of HSP60 in hippocampus, derived from patients affected by drug resistant TLE with hippocampal sclerosis and associated controls, was investigated by immunohistochemistry while circulating levels of this protein were detected by ELISA test. qRT-PCR was used to evaluate the expression levels of HSP60 and associated miRNA such as miR1 and miR206 in hippocampus. Moreover, miR-8071, miR-663, miR-146a and miR-124 expression levels associated with clinical features of TLE were also investigated. Our findings show that HSP60 is localized inside neurons somata and neuropil. Hsp60 expression levels were correlated to those of miR1 and miR206. Moreover, plasma Hsp60 levels in patients were higher than those of controls. Finally, circulating levels of miR-8071, miR-663, miR-146a and miR-124 decreased in TLE patients and were correlated to neuroinflammation and seizure recurrences.

Our work suggests that Hsp60 and associated miRNA levels are altered in relation to epileptogenesis and disease progression and may serve as a target for new therapeutic approaches in the management of TLE patients.

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Key words

Epilepsy, microRNA, molecular chaperones.

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The use of cephalometric analysis on fetal MRI to investigate the development of head and neck anatomical structures

Giacomo Begnoni, Gaia Pellegrini, Marilisa Toma, Federica Musto, Daniela Carmagnola, Riccardo Rosati, Natalia Khomchyna, Claudia Dellavia

Dipartimento di scienze biomediche chirurgiche e odontoiatriche, Università degli Studi di Milano, Milano, Italia

Magnetic resonance imaging (MRI) is a second-level screening exam prescribed during pregnancy for the assessment of fetal craniofacial malformations. Aim of the present study was to assess if MRI scans performed for gynecologic reasons are useful in morphologic field to investigate the development of head and neck anatomical structures.

Twenty-eight fetal MRI of patients from 20th to 32nd weeks of gestation taken to dispel doubts about the presence of skeletal malformations were analyzed and divided into 3 groups according to different gestational weeks: group A from 20th to 22nd, group B from 23rd to 25th, group C from 27th to 32nd.

In each image, cephalometric landmarks were set on cranial base, maxilla, mandible and hyoid bone and used to calculate linear and angular measurements. The vertical and sagittal dimensions were computed to investigate the skeletal growth of the different anatomical structures and the position of maxilla and mandible in relation to the anterior cranial base. Oropharyngeal and nasopharyngeal areas were defined to study the development of functional spaces. Descriptive and inferential statistics were applied to each parameter. Also, correlations between linear, angular data and areas were performed.

At the analysis, the growth of the analyzed anatomical structures was linear in both vertical and sagittal directions during the first interval (from group A to B) and peaked during the second interval (from group B to group C) ($p< 0.05$, Wilcoxon-Mann-Whitney test). On sagittal plane, a significant retrusion of maxilla in relation to anterior cranial base was observed at the second interval ($p< 0.05$, Wilcoxon-Mann-Whitney test). Both oropharyngeal and nasopharyngeal spaces increased significantly during the first interval with a peak of growth during the second interval ($p< 0.05$ Wilcoxon-Mann-Whitney test), however growth of nasopharyngeal area was significantly lower than oropharyngeal area ($p<0.05$, Wilcoxon signed-rank test).

The present study found a harmonic growth of skeletal structures and functional spaces of head and neck anatomical region. These findings are in continuity with the existing literature that assessed craniofacial growth of fetus by cephalometric analysis on radiographies [1]. Cephalometric analysis on MRI images of fetus seems to be a useful and non-invasive method to investigate craniofacial development.

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Key words

Growth, Craniofacial, Development, Oropharyngeal, Nasopharyngeal.

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Effects of Sicilian Opuntia ficus-indica juice on heart rate variability after a maximal exercise in young physically active women

Marianna Bellafiore, Angelo Cataldo, Dario Cerasola, Ewan Thomas, Giuseppe Battaglia, Antonino Bianco, Marcello Traina, Antonio Palma

Università degli Studi di Palermo, Dip. di Scienze Psicologiche, Pedagogiche e della Formazione, Palermo, Italia

The consumption of Opuntia ficus-indica (OFI) has been shown to increase the heart rate variability (HRV), a non-invasive marker of cardiac autonomic control [1], in high-level athletes [2]. The aim of this study was to investigate the effects of Sicilian OFI juice supplementation on post-exercise recovery using HRV analysis in young physically active women . This study was a randomized, double blind, placebo controlled and crossover design. Eight women (23.25±2.95 years old, weight of 54.13±9.05 kg, height of 157.75±0.66 cm and BMI of 21.69±0.66 kg/m2) were randomly divided into 2 groups and each group was supplied with either 50 ml OFI, diluted to 170 ml with water, or 170 ml Placebo (PL) containing the same concentration of fruit juice ingredients except for Vitamin C and indicaxanthin. They consumed OFI or PL every day for 3 days before of maximal effort test on cycle ergometer and continued to take it for 2 consecutive days after testing. HRV variables (LF, HF, LF/HF and rMSSD) were recorded pre- and post-test, 24 h and 48 h post-test in both groups using a portable heart rate monitor and analysed with Kubios HRV 2.2 software. The differences were calculated with ANOVA analysis and considered significant with $P<0.05$.

Sympathetic activity (LF) was significantly lower in OFI than PL group 24h post-test. In OFI group, LF was significantly lower 24h post-test than post-test value.

In conclusion, OFI supplementation might reduce the metabolic stress induced by intense exercise and improve recovery status in physically active women.

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Key words

Antioxidant supplementation, heart rate variability, autonomic nervous system, metabolic stress.

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Ultrastructural analysis of mouse blastocysts cultured in vitro under different oxygen concentrations

Manuel Belli[1], Maria Grazia Palmerini[1], Ling Zhang[2], Xiaowei Liu[2], Annemarie Donjacour[2], Elena Ruggeri[2], Stefania Annarita Nottola[3], Paolo Rinaudo[2] and Guido Macchiarelli[4]

[1] University of L'Aquila, Dept. of Life, Health and Environmental Sciences, L'Aquila, Italia
[2] University of California, San Francisco, Dept. of Obstetrics, Gynecology and Reproductive Science, San Francisco, Stati Uniti D' America
[3] Sapienza University, Dept. of Anatomy, Histology, Forensic Medicine and Orthopaedics, Rome, Italia
[4] University of L'Aquila, Dept. of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italia

During the last years, embryo development in vitro was studied in different culture conditions and oxygen (O2) concentrations. Higher developmental blastocyst (Bl) rates were obtained with embryos cultured under a physiological O2 tension (5%), respect to those cultured under atmospheric O2 conditions (20%) [1], but the mechanisms responsible for this, during the preimplantation embryogenesis remain unclear. This study aimed to evaluate the effect of physiologic or atmospheric O2 tension on the ultrastructure of mouse Bl. In vivo, Bl were flushed out of the uterus after natural fertilization (controls). In vitro fertilization (IVF) was performed using KSOM medium and Bl were then cultured under an O2 tension of 5% and 20% for 5 days [2]. After collection, Bl were washed in PBS, fixed in 2.5% glutaraldehyde/PBS and subjected to standard preparative for transmission electron microscopy (TEM) [3]. Morphometric analysis was done on ultrathin sections. The cells of the trophoblast (TE) formed a single, continuous layer of flattened cuboidal cells. In all the group, both inner cell mass (ICM) and TE showed the presence of extensive regions of less dense, granular cytoplasm. Microvilli were distributed on the apical surface, projecting toward the zona pellucida. Nuclei were delimited by integral nuclear membranes and contained dispersed euchromatin with patches of heterochromatin. Cells in mitotic division, with well-defined chromosomes, were occasionally identified. Isolated mitochondria and vacuoles were numerous. Mitochondria, in both ICM and TE, had an elongated and tubular shape, delimited by a double electron-dense membrane. The numerical density of mitochondria was lower in vitro than in vivo, especially under 20% O2. Interestingly, this alteration in density in vitro was associated to an increased vacuolization, both at 5% and 20% O2. These results indicated that alterations in the Bl ultrastructure, especially at 20% O2, can be connected to the O2 concentration and can motivate the higher developmental rates obtained at lower O2 concentration.

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Key words

Blastocyst, IVF, oxygen concentration, TEM, ultrastructure.

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Systematic review and meta-analysis of frequency and diameters of midline lingual foramina

Sara Bernardi, Maria Adelaide Continenza, Serena Bianchi, Sevastiani Antonouli, Guido Macchiarelli

Dipartimento di Medicina Clinica, Sanità Pubblica, Scienze della Vita e dell'Ambiente, Università degli Studi dell'Aquila, L'Aquila, Italia

Lingual mandibular foramina represent an anatomical feature found on the oral bone surface of the mandible. These structures host vascular and nervous anastomosis derived from the branches of the submental artery, lingual artery and the mylohyoid nerve. Studies on cadavers and on radiological images in-vivo reported the content, the frequency and the dimension of these foramina [1]. In surgical practice, this area is considered to be in low-risk from potential accidents, but reports in english-language literature showed intra-operative massive bleeding when implant interventions occurred in this area [2]. This systematic review and meta-analysis aims to summarize qualitatively and quantitatively the features of the lingual foramina on the midline of the inferior jaw. Systematic review and meta-analysis has been performed complying the PRISMA statement, and registered on PROSPERO database. The frequency rate of the foramina were resulted to have a point estimate of 0.965. The means of the diameters were shown to have a point estimate of 0.840 with a standard error of 0.06. The results showed a significant high frequency that has been reported in literature of this variation, presenting a consistent diameter dimensions, sign of significant caliber of the related vessels and nerves. Even if this variation is underestimated in current textbooks of oral anatomy, this evidence based anatomy [3] study suggests an accurate pre-surgical planning as well as a proper risk management preparation to minimize intra-operatory-and post-operatory injures.

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Key words

Systematic review, Evidence-based anatomy, Meta-analysis, Midline lingual foramina, Cone beam computed tomography.

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Wisp2 overexpression induced by short Teriparatide treatment affects IDG-SW3 osteogenic differentiation

Jessika Bertacchini[1], Alberto Smargiassi[1], Marta Checchi[1], Maria Sara Magarò[1], Francesco Potì[2], Elena Tenedini[3], Giuliana Montosi[4], Jonathan Vinet[1], Delphine Maurel[5], Carla Palumbo[1]

[1] University of Modena and Reggio Emilia, Department of Biomedical, Metabolic and Neuronal Sciences, Modena
[2] University of Parma, Department of Medicine and Surgery, Parma
[3] University of Modena and Reggio Emilia, Department of Medical and Surgical Sciences, Modena
[4] University of Modena and Reggio Emilia, Department of Internal Medicine II, Modena
[5] University of Bordeaux, BIOTIS, INSERM, Bordeaux

The Osteocyte, recognized as a major orchestrator of osteoblast and osteoclast activity, is the most important key player during bone remodeling processes. Imbalances occurring during bone remodeling, caused by hormone perturbations or by mechanical loading alterations, can induce bone pathologies such as osteoporosis or sarcopenia. The active fraction of parathormone [PTH (1-34)], a drug named Teriparatide, has long been chosen as election treatment for osteoporosis. The effect of such therapy is dependent on the temporal manner of administration, in fact it has been largely demonstrated that a short administration of Teriparatide increases bone formation but a long administration of the same agent leads to an increased bone resorption. The molecular reasons why the type of administration regimen is so critical for the outcame of bone mass recovery are numerous and not yet well known. The short administration of PTH (1-34) was demonstrated to induce osteoblast hyperplasia and to increase osteoblast survival, in parallel augmenting their ability to differentiate and to induce the preosseus matrix mineralization. On the contrary, the long term treatment with PTH (1-34), leads to the increment of osteoclast number and to the increase of their activity during bone resorption. Based on these considerations, our study attempts to analyze diverse signaling pathways directly activated in osteocytes (using the well-known in vitro model, the MLO-Y4 osteocyte) by Teriparatide treatment. In particular, by the use of a gene array platform, we found many molecules upregulated or downregulated in osteocytes, depending on the temporal administration modes, suggesting that the drug affects differently the osteocyte-related signaling pathways. Further, we paid attention to Wisp2, a well-established marker of canonical WNT activation. In particular we found that in MLO-Y4 cell line, a short Teriparatide treatment is able to induce β-Catenin nuclear translocation and a subsequent transcription of its target genes including Wisp2. Moreover, we found that Wisp 2 was secreted in MLO-Y4 medium and is responsible of increased matrix mineralization during osteoblast differentiation process.In conclusion, these data support the importance of osteocytes in controlling the action of the other bone cells and suggest that the perturbation of certain signaling cascades, such as the Wnt pathway, is crucial for the positive regulation of bone formation.

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Key words

PTH(1-34), Bone remodelling, Wisp2, Wnt, osteocyte.

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Vav1 down-modulates activation and/or expression of specific Akt isoforms in invasive breast cancer

Valeria Bertagnolo[1], Silvia Grassilli[1], Federica Brugnoli[1], Rossano Lattanzio[2], Marco Marchisio[3] and Silvano Capitani[1]

[1] Università degli Studi di Ferrara, Dipartimento di Morfologia, Chirurgia e Medicina Sperimentale, Ferrara, Italia
[2] Università degli Studi "G. d'Annunzio", Chieti-Pescara, Dipartimento di Scienza Mediche, Orali e Biotecnologiche, Chieti, Italia
[3] Università degli Studi "G. d'Annunzio", Chieti-Pescara, Dipartimento di Medicina e Scienze dell'Invecchiamento, Chieti, Italia

Targeting different members of the Akt pathways is a promising therapeutic chance in solid tumors including breast cancer. The variable expression levels of Akt isoforms with opposite effects on tumor growth and metastasis, however, makes it difficult to select the inhibitors to be used for specific breast tumor subtypes [1]. By using in vitro and in vivo models, we demonstrated that Vav1, ectopically expressed in cells derived from invasive breast tumors [2], in which it shows a peculiar localization inside the nucleus [3], down-modulates Akt acting at expression and/or activation levels depending on tumor subtype. The decreased p-Akt1 (Ser473) levels, which are a common effect of Vav1 up-modulation, suggest that in breast tumor derived cells and independently of their phenotype, Vav1 interferes with signaling pathways ended to specifically recruit Akt1. We also found that, only in ER negative cell lines, the silencing of Vav1 induced the expression but not the activation of Akt2. Finally, a retrospective analysis of early invasive breast tumors allowed to establish the prognostic significance of the p-Akt/Vav1 relationship. Remerkably, low Vav1 levels negatively influence the follow-up of patients with low p-Akt in their primary tumors and subjected to adjuvant chemotherapy. As the use of specific or pan-Akt inhibitors may not be sufficient or may even be detrimental, to increase the levels of Vav1 could be a new approach to improve breast cancer outcomes. This might be particularly relevant for tumors with a triple negative phenotype, for which effective target-based therapies are not currently available.

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Key words
Breast cancer, Vav1, Akt.

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Pulvinar: structural connectivity and topographic organization

Salvatore Bertino[1], Fabio Trimarchi[1], Ludovico Magaudda[1], Silvia Marino[2], Daniele Bruschetta[1], Giorgio Cacciola[1], Demetrio Milardi[2]

[1] University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia
[2] University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia - IRCCS Neurolesi Bonino Pulejo, via Casazza 98100, Messina, Italy

The pulvinar is the largest thalamic nucleus and it is considered as a association nucleus connecting different cortical areas to each other. Strong connections between pulvinar and brain areas belonging to the dorsal and the ventral visual streams, along with several regions of the prefrontal cortex and subcortical structures have been demonstrated both in animals and humans. This extremely various array of connections led to the idea that pulvinar acts as a "meta-controller" of attention. The pulvinar has been subdivided into an anterior, dorsal and inferior subdivisions by means of neurochemical markers [1] and functional MRI [2]. Herein, we employed probabilistic tractography implemented with constrained spherical deconvolution, spherical deconvolution informed filtering tractograms (SIFT) and anatomically constrained tractography (ACT) on high quality diffusion data of 30 subjects from the human connectome project (HCP) to characterize connectivity profiles of the pulvinar. Streamlines connecting pulvinar to frontal, parietal, temporal and occipital lobes as well as to subcortical structures have been reconstructed. Interestingly, a contingent of fibers from the spinothalamic tract, separated from the ones reaching the ventroposterior lateral nucleus has been observed, in line with the rising hypothesis of a nociceptive role of the pulvinar. Considering its wide number of connections to a wide range of nervous structures, we hypothesize that the pulvinar could be subdivided into structurally segregated sub-regions. Indeed, the connectivity- based segmentation identified segregated topographically organized sub-regions within the pulvinar. To the best of our knowledge, the present work represents the first attempt to characterize the topographical organization of the cortical and subcortical connectivity patterns within the human pulvinar.

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Key words

Pulvinar, Connectivity parcellation, attention, tractography.

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The pharmacological inhibition of JNK-pathway reduces severity of Spinal Muscular Atrophy in mice

Marina Boido[1], Roberta Schellino[1], SIlvia Biggi[2], Tiziana Borsell[3], Alessandro Vercelli[1]

[1] Neuroscience Institute Cavalieri Ottolenghi, Univ. Torino, Dip. Neuroscienze, Orbassano (TO)
[2] IRCCS-Istituto Di Ricerche Farmacologiche "Mario Negri", Dip. Neuroscienze, Milano
[3] Univ. Milano, Dip. Scienze Farmacologiche e Biomolecolari, Milano

Spinal muscular atrophy (SMA) is a recessive autosomal neuromuscular disease, characterized by motor impairment, muscle atrophy and premature death following motor neuron (MN) degeneration, due to the lack of SMN (survival motor neuron) protein. Currently, the cellular and molecular mechanisms underlying MN death are only partly known [1], although recently it has been shown that the JNK-signalling pathway might be involved in the SMA pathogenesis. After confirming the activation of JNK in our SMA mouse model (SMN2+/+; SMNΔ7+/+; Smn-/-), we tested on these mice a synthetic JNK-inhibitor peptide (D-JNKI), by chronic administration from postnatal day 1 (P1) to P10; then, at age P12, we analyzed their spinal cords and quadriceps muscles. We observed that D-JNKI administration delayed MN death and decreased neuroinflammation in the spinal cord. Moreover, by inhibiting JNK pathway, the muscular fibers and the neuromuscular junctions appeared respectively more trophic and mature. The histological/molecular results positively correlated with improved motor performances and hindlimb muscular tone. Finally, the treatment slightly, but significantly increased lifespan in SMA mice. Overall, our results identify JNK as a promising target to reduce MN cell death and progressive skeletal muscle atrophy, providing insight into the role of JNK-pathway for developing alternative pharmacological strategies for the treatment of SMA.This work was supported by grants from CRT Foundation, Girotondo/ONLUS and SMArathon-ONLUS foundations.

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Key words

Motor neuron disease, apoptosis, innervation, neurodegeneration, therapy.

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Mitochondrial involvement in fibromyalgia and melatonin protective effect

Francesca Bonomini, Gaia Favero, Claudio Lonati, Lorena Giugno, Lorenzo Franceschetti, Rita Rezzani

Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italia

Fibromyalgia (FM) is considered as one of the most common musculoskeletal disorder associated with a chronic pain condition. The principal characteristic of this condition is a widespread pain often associated with sleep disorders, fatigue and consequent anxiety and/or depression. Due to the prevalence of this pathology, recent studies aimed at increasing understanding of both its pathogenesis and treatment have been conducted, but the knowledge is far behind other chronic illnesses in both mechanism understanding as well as appropriate therapeutic approaches [1]. Recent studies reported that in FM patients the metabolism of a powerful antioxidant that is Coenzyme Q10 (CoQ10), called also ubiquinone, is altered showing a new potential marker for this disorder. For study FM several potential animal models have been described, among that reserpine-induced myalgia (RIM) rats are considered a putative model of this pathology showing musculoskeletal alterations and also depressive-like behaviours. Recent evidences suggest that melatonin, an indoleamine with multitasking properties, among which anti-inflammatory and anti-oxidant effects, may be suitable in FM treatment [2].

In this study, we hypothesized that dietary melatonin administration in RIM animal model would support anti-inflammatory and anti-oxidant response in skeletal muscles reducing so the FM symptoms. In particular we focalized our attention to mitochondrial involvement investigating the roles of CoQ10, mitofusin 2 (MFN2) and peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC1α) expression.

Our results showed that melatonin modulates mitochondria homeostasis and oxidative stress in RIM rats.

In summary this study showed that melatonin could be a potential tool in the prevention and treatment of FM symptoms.

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Key words

Fibromyalgia, Mitochondria, Melatonin, Oxidative stress.

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Red LED light in skin regeneration: an in vitro study on human dermal fibroblasts

Elisa Borsani[1], Giorgio Sigorini[1], Mariangela Rucco[2], Stefania Castrezzati[1], Fabio Rinaldi[2] and Elisabetta Sorbellini[2]

[1] University of Brescia, Division of Anatomy and Physiopathology, Department of Clinical and Experimental Sciences, Brescia, Italia
[2] International Hair Research Foundation (IHRF), International Hair Research Foundation (IHRF), Milano, Italia

The use of noncoherent light (light-emitting diodes, LEDs), in particular the Red LED light, represents an innovative approach to tissue regeneration. In dermatological field, experimental studies on its regenerative effect started about ten years ago (1) but the exact mechanism of its action should be better elucidated. Today, a new treatment, named "Dermodinamicaâ" (Elisor, Milan), uses this approach with a wavelength of 630nm to promote skin regeneration. Dermal fibroblast is a primary cell type responsible for synthesis and remodeling of extracellular matrix in human skin. Several studies have reported cytokine-dependent changes in extracellular matrix composition and in particular transforming growth factor (TGF)-b1 is a fibroblast stimulating cytokine effective on both type I collagen and hyaluronan production (2). Moreover, ROS-detoxifying enzymes, such as superoxide dismutases (SOD) and heme-oxygenase 1 (HO-1), have an important role in cutaneous wound repair. The aim of the present in vitro preliminary study was to evaluate the effect of Red LED light (Dermodinamicaâ, Elisor, Milan) on normal human dermal fibroblast (NHDF) at 24h from exposition monitoring: cell viability using MTT assay; TGF-b1 production using ELISA kit; expression of SOD-1 and HO-1 using immunohistochemical technique. Moreover, short (15 min exposition) and prolonged (30 min exposition) treatments were investigated. The results showed a progressive increase in TGF-b1 release respectively in the short treatment (15 min exposition) and in the prolonged treatment (30 min exposition). Moreover, the ROS-detoxifying enzymes were modulated by this treatment and the cell viability was maintained. These data support the hypothesis of the positive influence of Red LED light in the biological processes involved in skin regeneration.

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Key words

Red LED light, skin, regeneration.

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Anatomical variation or pathological feature? Conditioning of the clinical diagnosis in the ex-ante and ex-post forensic evaluation setting. A prospective cross-over pilot study

Rafael Boscolo-Berto[1], Veronica Macchi[1], Massimo Montisci[2], Santo Davide Ferrara[2], Andrea Porzionato[1], Raffaele De Caro[1]

[1] Institute of Human Anatomy, Department of Neurosciences, University of Padova, Padova, Italia
[2] Section of Legal Medicine, Department of Legal and Occupational Medicine, University-Hospital of Padova, Padova, Italia

Medical malpractice often implies the interpretation of anatomo-radiological findings of uncertain clinical value [1]. Crucial importance is played by the perspective adopted in the evaluation of documentary data, which must take an ex-ante approach, consisting in ideally placing himself in the same circumstances of place and time of the facts analysed, reproducing that informative scenario [2]. On the other hand, the daily-observed ex-post perspective included information collected after the time of the events being analysed [3].

We aimed to identify any predictive factor of interpretative errors committed by analysing ex-post an anatomo-clinical imaging of alleged medical professional responsibility.

We submitted selected radiograms being doubt for anatomical variation versus disease (5) or negative (5) to 15 Urologists, who received (or not) collateral information related to the clinical epilogue.

The interpretative errors made by analyzing the images lackingof any additional information proved to be significantly lower than what was observed in the presence of collateral information about the epilogue of the clinical event (13.3% vs. 28.3%, $p < 0.05$). The type of information provided represents the only independent predictive variable ($p < 0.05$) for the occurrence of interpretative error with respect to the real significance of a given radiogram. The ex-ante interpretation showed a sensitivity of 86.2%, specificity of 90.3%, positive predictive value 89.3% and negative predictive value 87.5%. The ex-post interpretation showed a sensitivity of 76.7%, specificity of 73.3%, positive predictive value of 74.2% and negative predictive value 75.9%. Collateral information about the epilogue of the clinical event increased the relative risk of making an interpretative error by 4.4 times, and the total removal of the risk factor itself would reduce the interpreting errors by 18.4%.

Compared to the ex-ante approach, by ex-post analysis we introduce an interpretative distortion shifting the evaluation towards the epilogue communicated, perceiving an anatomical variation as a pathologic feature and vice-versa.

This study confirms the emerging role of forensic clinical anatomy in defining the critical issues related to the evaluation of alleged medical professional responsibility and suggesting the compensatory methodological adjustments to prevent any possible distortion in the assessment.

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Key words

Anatomical variation, medical malpractice, forensic clinical anatomy, radiology.

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Lymphatic drainage of the prostate: an anatomical insight to solve a clinical dilemma

Rafael Boscolo-Berto[1], Veronica Macchi[1], Andrea Porzionato[1], Gloria Sarasin[1], Salvatore Siracusano[2], Raffaele De Caro[1]

[1] Department of Neurosciences, Institute of Human Anatomy, University of Padova, Padova, Italia
[2] Urologic Clinic, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italia

The evidence of pelvic lymph node metastases after radical prostatectomy (RP) with pelvic lymph node dissection (PLND) is one of the strongest prognostic factors for poor oncologic outcome [1]. In this clinical scenario, the extent of PLND, although representing a crucial step in RP, is still controversial [2]. Currently, there is a critical drawback in clinical practice due to the lack of congruence between the known lymphatic drainage and cancer dissemination despite its management by a surgical approach [3].

We hypothesized that some landmarks of the lymphatic drainage of the prostate were not actually considered in clinical daily practice. We carried out a systematic review of the anatomic description of nodal drainage of prostate reported by the original texts since the 18th century. Moreover, we performed an anatomical dissection of a human body made available by the Body Donation Program at the University of Padova.

The cadaver was prepared by a novel anatomical dissection technique developed to solve this particular anatomical-clinical question, by highlighting the efferent lymphatic pathways of the prostate with special reference to the posterior one drainage, in order to verify what is reported in the historical literature. The overall evidence resulting from the historical anatomical treatises and cadaver dissection confirmed that three groups of lymphatics carry out prostatic nodal drainage, as follows. A) ascending ducts from the cranial gland leading to the external iliac nodes; B) lateral ducts leading to the hypogastric nodes; C) posterior ducts from the caudal prostate leading to the lateral and promontory sacral nodes.

The overall evidence resulting from the historical anatomical treatises and cadaver dissection confirmed that posterior ducts from the caudal prostate leading to the lateral and promontory sacral nodes.

These anatomical evidences demonstrate that lymphatic drainage of the prostate extends beyond standard nodal templates actually considered in clinical daily practice. Based on our observations, clinicians have to implement a critical revision of their conception of the prostatic drainage.

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Key words

Prostate, lymphatic drainage, radical prostatectomy, cancer.

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Ultrasound-induced bbb opening: a morphological study in an in vitro cellular model

Jacopo Junio Valerio Branca[1], Matteo Boninsegna[2], Gabriele Morucci[1], Donatello Carrino[1], Massimo Gulisano[1], Leonardo Bocchi[3], Alessandra Pacini[1]

[1] University of Florence, Department of Experimental and Clinical Medicine, Florence
[2] University of Florence, Department of Information Engineering, Florence
[3] University of Firenze, Department of Information Engineering, Florence

Degenerative diseases of the central nervous system are significant causes of mortality among aging population in industrialized countries, as well as expensive for the national health systems and caregivers [1]. Nowadays, pharmacological therapies are still not decisive because they are hindered by the blood brain barrier (BBB) [2]. Although in the last decades many researchers have conducted in vivo experiments to better identify a therapeutic approach of ultrasound (US) in neurodegenerative diseases, little is known about their role in increasing the BBB permeability [3,4]. The present study aims to identify, the effect of focused US on a rat brain endothelial cell line (RBE4). After ultrasound stimulation (10-20-30 min.), MTT and western blotting assay were conducted to demonstrate the non-toxic effects of US. Furthermore, immunostaining of stimulated cells was performed to detect changes in cytoskeletal F-actin fibers and Zonula occludens-1 (ZO1) tight juntion. BBB opening was evaluated by measuring the extent of the intercellular space, in Papanicolaou stained cells. The results evidenced an ultrasonic-dependent mechanical action on F-actin fibers that altered their distribution within the cells showing the formation of numerous stress fibers. F-actin alterations were accompanied by an alteration of the ZO-1 distribution, exhibiting a "zipper-like" staining pattern and holes that became visible between cells. Papanicolaou staining confirm the opening of the BBB evidencing many wider areas of intercellular space. All these structural changes on RBE4 cell line occurred without significant alterations in metabolic activity as well as in absence of apoptotic or endoplasmic reticulum stress markers. In conclusion, these results confirm and highlight the potential role of ultrasound in the permeabilization of the BBB, thus suggesting new ways for drugs administration.

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Key words

BBB in vitro model, RBE4 cell line, permeabilization, ultrasound.

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Anomalous branching pattern of the aortic arch associated with retroesophageal right subclavian artery

Barbara Buffoli[1], Elisa Borsani[1], Alessio Sollima[1], Gianluca Guida[1], Greta Gandolfi[1], Mattia Arturi[1], Lena Hirtler[2]

[1] Sezione di Anatomia e Fisiopatologia, Dipartimento di Scienze Cliniche e Sperimentali, Università di Brescia, Brescia, Italia
[2] Department of Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria

During dissection practice for the students at the University of Brescia, we found an anomalous branching patter of the aortic arch in a female cadaver. Aberrant right subclavian artery originating from the distal part of the aortic arch and following retroesophageal course was recognize; next to it, the left subclavian artery and, proceeding from the left to the right, the left common carotid artery and the right common carotid artery branches, respectively. Anomalous origin of the vertebral arteries was also noted; the left vertebral artery originated directly from the aortic arch, closed to the origin of the left common carotid artery, whereas the right vertebral artery originated from the right common carotid artery.

Even if, in the literature aberrant right subclavian artery is reported as a relatively rare aberration in the general population with a female predominance [1-2], the concomitant anomalous branching pattern of the aortic arch and, in particular, the origin of the vertebral arteries, represents a rare case that appears interesting to describe. Therefore, this case report alerts anatomists and clinicians to the possibility of these simultaneous variants.

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Key words

Retroesophageal sublclavian artery, aortic arch, vertebral arteries, dissection.

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Unusual branch of the lingual artery supplies the infrahyoid muscles: a dissection study

Barbara Buffoli, Vincenzo Verzeletti, Mauro Labanca, Stefania Castrezzati, Manfred Tschabitscher, Luigi F. Rodella

Università di Brescia, Sezione di Anatomia e Fisiopatologia, Dipartimento di Scienze Cliniche e Sperimentali, Brescia, Italia

The lingual artery arises from the external carotid artery and supplies the oral floor and the tongue. During its course gives four branches: deep lingual artery, sublingual artery, the dorsal lingual artery and the suprahyoid or hyoid artery. This last runs along the upper border of the hyoid bone supplying the muscles attached to it.

In this work, we reported a unilateral anatomical variation of the branching pattern of the lingual artery, which was observed during dissection studies at the University of Brescia. We found an accessory branch originating from the starting part of the lingual artery. This branch run down and medially and supplied the infrahyoid strap muscles, which normally are supplied by arteries originating from the superior thyroid artery, the inferior thyroid artery, and the internal mammary artery.

So, considering that similar case has been not yet reported in literature and considering the current use of the infrahyoid strap muscles as pedicled myocutaneous flaps for reconstructing surgical defects in the head and neck [1-2], this vascular variation appears interesting to be reported for appropriate clinical and surgical technical consideration.

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Key words

Lingual artery, infrahyoid muscles, accessory branch, dissection.

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Calcium pyrophosphate dihydrate crystal deposition disease in shoulder soft tissues: a morphological investigation

Debora Burini[1], Sara Salucci[1], Sabrina Burattini[1], Francesco Fardetti[2], Vittorio Calvisi[2], Alessandro Beccarini[3], Pietro Gobbi[1], Elisabetta Falcieri[1] and Davide Curzi[1]

[1] University of Urbino Carlo Bo, Department of Biomolecular Sciences, Urbino, Italia
[2] University of L'Aquila, Department of Life, Health and Environmental Sciences, L'Aquila, Italia
[3] Porta Sole, Hospital, Perugia, Italia

Calcium pyrophosphate dihydrate crystal deposition disease (CPPD) is a rheumatological disorder featured by the presence of calcifications in the soft tissues (1). CPPD onset is strictly correlated to aging, the most relevant risk factor together with previous joint trauma. Knee and articular cartilage are respectively the most affected joint and tissue, even if this kind of disorder may also affect other anatomical areas (2, 3). The aim of the study is to investigate calcium crystal distribution and their interaction with cell behavior in glenohumeral joint. Specimens were withdrawn from patients with CPPD during shoulder arthroplasty, and then processed for morphological analysis. Humeral articular cartilage, joint capsule and long head of biceps brachii tendon sheath seem to reveal a relationship between crystal sediment position and cellular impairment. In particular, close to crystal deposits, chondrocytes and fibroblasts show necrotic features, such as chromatin changes, numerous vacuoles, swollen organelles, plasmatic and nuclear membrane rupture. On the other hand, cells far from crystals display a good vitality, as shown by well preserved cytoplasm and nucleus. These findings reveal how crystal deposits appear to affect cell behavior, suggesting a possible relationship between calcium crystals accumulation and cell death.

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Key words

CPPD, calcium crystals, shoulder.

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Identifying latent brain networks geometry markers: clinical applications in disorders of consciousness

Alberto Cacciola[1], Giuseppe Anastasi[2], Alessandro Muscoloni[3], Antonino Naro[4], Rocco Salvatore Calabrò[4], Carlo Vittorio Cannistraci[3,5]

[1] Department of Biomedical, Dental Sciences and Morphological and Functional Images, University of Messina, Italy - IRCCS Centro Neurolesi 'Bonino Pulejo', Messina, Italy
[2] University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia
[3] Biomedical Cybernetics Group, Biotechnology Center (BIOTEC); Center for Molecular and Cellular Bioengineering (CMCB), Technische Universität Dresden, Dresden, Germany
[4] IRCCS, Centro Neurolesi "Bonino Pulejo", Messina, Italia
[5] Brain Bio-Inspired Computing (BBC) Lab, IRCCS Centro Neurolesi "Bonino Pulejo", Messina, Italy

The complexity of the human connectome arises from several, integrated and segregated distributed networks around critical and participating cortical epicenters embedded in their physical space. The network topology is often intricately related to the physical distances between the nodes of the network: brain regions that are spatially close have a relatively high probability of being interconnected, while longer white matter projections are more expensive in terms of their material and energy costs. The observed topological properties arise from a hidden geometric space, where the nodes represent points and the closer they are the higher the likelihood to be connected. In recent years it has been demonstrated that the hyperbolic space might be a good geometric space of representation for complex networks, despite mapping a given network in the hyperbolic space remained a challenging issue. More recently, coalescent embedding, a class of topological-based unsupervised nonlinear dimension reduction machine learning has been developed in order to perform efficient mapping of complex networks in the 2D and 3D hyperbolic space, and potentially also in higher-dimensions [1]. Applying this new class of algorithms, we previously unsupervisedly discloses the hidden geometry of structural brain networks, demonstrating that it strongly relates to the known neuroanatomy [2]. Herein, we explore a more complex topic such as the human consciousness and its related disorders (DOC). We will demonstrate how simple geometric measures allow to identify latent network geometry changes and to distinguish between patients in minimally conscious state (MCS) and unresponsive wakefulness syndrome (UWS), starting from the mere functional connectivity estimated by resting state EEG recording. By contrast, the original distribution of the connectome weights cannot uncover significant differences between the functional connectomes of UWS and MCS patients ($p>0.05$). Therefore, coalescent embedding in the hyperbolic space enhanced our understanding of the whole-brain network geometry changes of brain connectomes, pioneering the new framework of network latent geometry markers characterization of brain diseases with application in DOC.We hope that this scenario has the potential to improve diagnosis, prognosis and therapeutic treatment evaluation of DOC patients.

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Key words

Connectome, consciousness, hyperbolic space, markers, network geometry, machine learning.

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Aberrant functional brain network organization in disorders of consciousness

Alberto Cacciola[1], Antonino Naro[2], Rocco Salvatore Calabrò[2], Alessia Bramanti[2], Salvatore Bertino[3], Gianpaolo Antonio Basile[3], Giuseppe Anastasi[3]

[1] Department of Biomedical, Dental Sciences and Morphological and Functional Images, University of Messina, Italy - IRCCS Centro Neurolesi 'Bonino Pulejo', Messina, Italy
[2] IRCCS, Centro Neurolesi "Bonino Pulejo", Messina, Italia
[3] University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia

The human connectome is a comprehensive description of neural elements and connections reflecting the complex organization of the brain. Modern network neuroscience has led to a paradigmatic improvement in understanding the brain-network organization and has challenged the traditional framework that many neurological disorders involves exclusively focal alterations. Consciousness is the product of multiple brain structures and depends on the brain's ability to integrate different complex patterns of internal communication. Although several studies demonstrated that the fronto-parietal and default mode networks play a key role in in conscious processes [1,2], it is still not clear whether the brain network organization is altered at the global level in patients with disorders of consciousness (DOC). Herein, we investigated the functional connectivity of DOC patients, diagnosed either as unresponsive wakefulness syndrome (UWS) or minimally conscious state (MCS), from a network perspective. EEG recording was performed in resting-state condition and pairwise brain connectivity between cortical areas was estimated across all time in order to compute a weighted functional network. Network-based statistical analysis revealed a subnetwork of decreased functional connectivity in UWS compared to the MCS patients ($p = 0.004$). Interestingly, apart from a few intrahemispheric pathways linking limbic regions with frontal and parietal areas, these patterns of reduced connectivity mainly involved the interhemispheric fronto-parietal network. Robust correlations between the strength of the connectivity patterns and the CRS-R were found. Global network topological analysis identified increased values of LCP-corr, as well as of high clustering coefficient and modularity in UWS patients compared to MCS patients. At the nodal level, the UWS patients showed increased nodal degree and betweenness centrality in several limbic and temporo-parieto-occipital regions. Taken together, our results highlight i) the involvement of the interhemispheric fronto-parietal network in the pathophysiology of consciousness disorders and ii) an aberrant network organization both at the global and at the nodal level in UWS compared to the MCS patients.

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Key words

Connectome, consciousness, fronto-parietal connectivity, network analysis, topology.

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Validity of L5 Pedicle Lateral Tilt (L5 PLT) Classification: a retrospective analysis on 23 misplaced screw

Giorgio Cacciola[1], Fabio Trimarchi[1], Ludovico Magaudda[1], Daniele Bruschetta[1], Giuseppe Santoro[1], Alessandro Pisani[2], Pietro Cavaliere[2], Federico De Meo[2], Andrea Barbanera[3], Angelo Favaloro[1]

[1] University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia
[2] Istituto Ortopedico del Mezzogiorno d'Italia, "Franco Scalabrino", Messina, Italia
[3] A.O.N. SS Antonio e Biagio e Cesare Arrigo, Neurochirugia, Alessandria, Italia

Posterior fixation of the lumbar spine using screw and bar is a commonly used surgical approach for the treatment of a wide series of spine pathologies. Despite the improvement in surgical technique and in the development of the material, the incidence of misplaced screw at the lumbar spine is about 4.8% (range, 3% to 6%) [1]. The L5 pedicle lateral tilt classification was introduced recently [1]. This classification is based on the morphological changes that occur at the L5 and L4 bone anatomy and on the distance between pedicles and nervous structures. It subdivides the population in three groups based on the lateral tilt of L5 pedicle. The purpose of this study is to evaluate the validity of the L5 PLT Classification. After obtaining internal institutional aboard approval we retrospectively analyzed data from 218 patients that underwent posterior spine stabilization of one or more level. In total 436 screws were positioned at the pedicles of L5. Screw positioning was evaluated using Zdchivasky classification on a CT scan of the lumbar spine. The incidence of screw misplacement was 5.3% (23 screws), a statistically significant difference was found between people that belong in group U (narrowed lateral tilt) and people that belongs to the other two groups W and V (p-value < 0.05). The results obtained in this study was conducted to confirm the theorized hypothesis that the lateral tilt of L5 pedicles must be considered as risk factor for screw misplacement during lumbar spine posterior stabilization. In conclusion the L5 PLT classification is valid.

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Key words

Pedicles, lumbar spine, hip.

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A possible role of mesenchymal stem cells in age-related regression of cervical intraepithelial neoplasia

Miriam Caffarini[1], Monia Orciani[1], Giovanni Delli Carpini[2], Raffaella Lazzarini[1], Andrea Ciavattini[2] and Roberto Di Primio[1]

[1] Dipartimento di Scienze Cliniche e Molecolari, Università politecnica delle Marche, Ancona, Italia (1)
[2] Clinica Ostetrica e Ginecologia, Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche, Università Politecnica delle Marche, Ancona, Italia

High grade cervical intraepithelial neoplasia (CIN2) is a pre-cancerous lesion of uterine cervix that affects between 250,000 and 1 million of American women every year. CIN2 may either resolve spontaneously with a regression or degenerate in CIN3 requiring treatment by loop electrosurgical excision procedure-LEEP (conization). Several studies showed that CIN can occur at any age and the rate of a natural regression is more evident in young woman, around 65%, rather than in old woman, from 15 to 23% [1]. Recent researches are focusing on the involvement of inflammation in CIN progression to evaluate the use of anti-inflammatory drugs in the treatment of CIN [2]. It is known that in addition to the different cells of the immune system, mesenchymal stem cells (MSCs) are able to modulate an inflammatory process. The purpose of our study was to isolate MSCs from cervix of young (yC-MSCs) and old patients (oC-MSCs), in order to evaluate if age can affect their properties and immunobiology; since CIN may progress towards cervical cancer, indirect co-culture with HeLa cells were performed and the effects tested. Our results show that both oC-MSCs and yC-MSCs attain the minimun criteria for MSCs definition [3] even if oC-MSCs display a greater degree of senescence than yC-MSCs. Furthermore, yC-MSCs express higher level of cytokines related to acute inflammation than oC-MSCs. HeLa cells, in co-culture with oC-MSCs, produce an increase in the expression of genes referred to tumor development. In conclusion, the immunobiology of MSCs derived from cervix is affected by the age of donors and this can influence the regression rate of CIN through a paracrine effect. In addition, MSCs from young cervixes drive an anti-tumoral effect by sustaining an acute inflammatory environment.

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Key words

Inflammation, cervical intraepithelial neoplasia, MSCs, HeLa, cervix.

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Underwater investigation of leg veins morphology and heamodynamics

Alberto Caggiati[1], Christopher Lattimer[2], Evi Kalodiki[2], Sara Oberto[3], Giorgio Bergamo[4], Dimitrios Kontothanassis[5]

[1] Dept SAIMLAL, Sapienza University, roma, Italia
[2] Josef Pflug Vascular Laboratory, Ealing Hospital and Imperial College & Wes London Vascular and Interventional Centre, UK
[3] Department of Vascular Surgery, IRCCS multimedica, Sesto San Giovanni, Milan, Italy
[4] Microlab Elettronica, Padua, Italy
[5] Istituto Flebologico Italiano, Ferrara, Italy & Abanomed, Abano Terme, Padua, Italy

No study by duplex ultrasound (DU) has evaluated the effects of Hydrostatic Compression HC on venous morphology and hemodynamics. The aim of this pilot, proof-of-concept study was to assess the technical feasibility of DU in evaluating venous morphology and hemodynamics in subjects standing in a water pool.

Vein morphology and flow were initially evaluated in standing position out of the pool and the sites of venous measurements were marked by a water-proof marker. The measurements were repeated after immersion into the pool, water level 120 cm.

The DU allows an excellent underwater evaluation of both the superficial and deep veins morphology and hemodynamics. Under the water, the subcutaneous tissue appears more echogenic. The HC significantly reduced the diameter of the deep (femoral vein: P = .004; popliteal: P =.008:) and superficial veins (GSV: P =.045 at the thigh but not P =.012 at the ankle). In legs with varicose Valsalva and compression/release maneuvres showed a significant reduction of blood reflux during immersion.

This study has clearly demonstrated the feasibility of underwater DU evaluation of venous morphology and flow. The HC significantly reduces venous diameters in normal and varicose veins and reflux when present. The present findings are the basis for future studies on the effects of HC on venous morphology and blood return, in healthy and pathologic conditions.

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An in vivo Microcomputed Tomographic 3D reconstruction of vasculature and organs of Rat

Gaetano Felice Caldara

Animal welfare body, University of Palermo, Palermo, Italia

The morphological and volumetric investigation of organs and disease progression in experimental animal models involve many invasive procedures such as the handling and restraint of the animal. These acts cause animal pain, suffering or distress and impact on the animal welfare influencing experimental outcome. Microcomputed tomography (micro-CT) is an ideal technique for in vivo quantifying both acute and chronic diseases in diachronic studies and for understanding the morphological development and regeneration of organs without interfere with animal welfare. The micro-CT detection improve the 3Rs principle for performing more humane animal research reducing the number of animals used during longitudinal in vivo tests as recommended by the Directive 2010/63/EU and Italian D.Lgs 26/2014. This technique clarify the inter-hierarchical relationship between microscopic and organ-level tissue deformation dynamics if associated with the In Vivo Imaging System (IVIS) scanner revealing the molecular and cellular activities of drugs. The information provided by this technique is complementary to histological and anatomopathological evaluation and it is used by researchers to investigate preclinical animal models: oncology, angiogenesis, neurodegenerative disorders, inflammation, cardiovascular, infectious diseases, etc. Therefore, non-invasive micro-CT imaging technique provide reliable images of skeleton, organs, tumours, and responses to exogenous substances can be spatially and temporally monitored is associate with IVIS scanner. Furthermore, it allows serial studies on the same animal, reducing inter-animal variation and the number of samples needed to achieve statistical significance. This study provide an anatomical study by using a Micro-CT scan as compendium about the in vivo anatomy of the rat to show details of the internal organs of the whole body and improve the knowledge on the animal model used in many experimental model. The images performed illustrate the high precision of scanned organ anatomy and monitoring differences in shapes during organogenesis and diseases. This high-resolution imaging study is a highly accurate anatomical atlas performed by a nondestructive method and explores the orientation-independent measures of the organ structure in the animal models virtually moving the sample and exploring it from any perspective to gain a greater understanding of the morphologic characteristics.

I would like to express my special thanks to my Professor Enrico Cabassi.

Key words

In Vivo imaging, animal model, vascolature, rat anatomy organs, micro-computed tomography.

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Migrants and inclusive communities: rights, citizenship practices and "risk prevention": biological and medical aspects

Gabriele Candela, Fabio Massimo Perrotta, Ennio Lubrano di Scorpaniello and Germano Guerra

Università degli Studi del Molise, Dipartimento di Medicina e Scienze della Salute "Vincenzo Tiberio", Campobasso, Italia

University of Molise under the PNR - Programma Nazionale per la Ricerca 2015-2020; proposed a project entitled: "Migranti e comunità inclusive: diritti, pratiche di cittadinanza e prevenzione dei rischi". With CIPE resolution no. 78 of 7 August 2017 published in the Gazzetta Ufficiale of 27.11.2017, € 950,000 were allocated to the research project in question by the Interministerial Committee for Economic CIPE programming. The multidisciplinary project was drawn up by multidisciplinary working group, made up of professors belonging to different Departments. Prof. Germano Guerra has been designated as responsible for issues related to the biological-health field. The part of the project that will deal with the biological-health issues will be carried out by the authors of the abstract. The aim of this part of the project including the constitution, with a view to implementing the quality of life of migrants, equipped spaces within reception facilities, dedicated to the performance of motor / sport activities in an optimal manner. The end point of the study could be, beyond the already highlighted benefits, the start up of specific motor / sports activities in order to bring out sports potentials to be cultivated up to the competitive level. A more specific medical aspect will be the evaluation of eating habits in the context of intercultural and interreligious differences in migrants, with particular attention to the identification and stratification of different habits for the purpose of proper nutrition, prevention of diseases with a high social impact (eg vitamin D deficiency and diseases bone) and an improvement in the quality of life.

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Key words

Migration, Health, Physical activity, Sport, Vitamin D deficit.

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Alterations in Endoplasmic Reticulum and Lysosomal–Mitochondrial Axis in Monocytes after treatment with Different Campylobacter jejuni Lysates

Barbara Canonico, Erica Cesarini, Francesca Luchetti, Gianna Di Sario, Loris Zamai, Raffaella Campana, Wally Baffone and Stefano Papa

Università Degli Studi Di Urbino, Carlo Bo, Disb, Urbino, Italia

Campylobacter jejuni, a Gram-negative spiral-shaped bacterium, often explicates its virulence through the cytolethal distending toxin (CDT). Infection by C. jejuni is commonly associated with human gastroenteritis, however it may also generate the development of the Guillain–Barré Syndrome, an acute peripheral neuropathy. An inflammatory response consequence of CDT-induced cell death is a possible cause of the disease. Monocytes are potent producers of both pro- and anti-inflammatory cytokines, playing a major role in innate immunity and in non-specific host responses. For this reason, we tested the effect of C. jejuni lysates obtained from different strains (expressing wild type or mutated-less functional CTD) on donor monocytes. The alteration induced on monocyte mitochondria and lysosomes were specifically evaluated by flow cytometry and confocal microscopy. Lysates from all strains induced endoplasmic reticulum (ER) stress in monocytes, suggesting that ER stress was not associated with CDT but to other C. jejuni virulence factors. The C. jejuni ISS 1 wild-type strain mostly induced lysosomal alterations, whereas the C. jejuni ATCC 33291 strain induced the most relevant mitochondrial alterations consistent with the induction of an intrinsic apoptotic pathway. Differently, the presence of CDT wild-type produced alteration in lysosomal acidic compartments and p53 down-regulation. In conclusion, the inhibition of p53 expression induced by CDT wild-type, would suggest that CDT, beside its direct cell death effects, is able to promote an apoptotic stimuli-resisting pathway.

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A brief anatomo-surgical dissection guide to human mediastinal anatomy: results of the collaboration between the University of Palermo and the University of Malta

Francesco Carini[1], Giovanni Tomasello[1], Margherita Mazzola[2], Serena Nobile[2], Francesca Sole[2], Alfonso Venezia[2], Benedetto Spataro[2], Benedetto Fazio[2], Fabiana Cipolla[2], Valentina Polisano[2], Francesca Rappa[1], Fabio Bucchieri[1], Felicia Farina[1], Christian Zammit[3], Cristoforo Pomara[3], Francesco Cappello[1]

[1] Sezione di Anatomia, Università di Palermo, Palermo, Italia
[2] Sezione di Anatomia, Scuola di Medicina, Palermo, Italia
[3] Anatomy, University of Malta, Msida, Malta

In the summer of 2017, thanks to an agreement between the University of Malta and the University of Palermo, a group of students from the University of Palermo, who had already taken the anatomy exams and had a good knowledge of English, went for a 4 week period to the University of Malta to follow a dissection course . The students dissected skin, the sternum, the vessels, the nerves, analyzed the pericardium, the lungs and all the mediastinal organs. This work proves to be a small dissection guide for young medical students who want to learn the basics of dissection and the relevant topographical anatomy.

The students were selected by the University of Palermo because of the good quality of their university career and their excellent knowledge of the English language.

The aim of this work was to analyze the mediastinum, a space between the right lung, the left lung, the sternum and the vertebral column. The mediastinum is located between a complex of organs: the heart with the pericardium, the large vascular trunks, the intermediate and distal part of the extrapulmonary respiratory tract, the thoracic portion of the esophagus, the lymphatic system with the lymph nodes, and the nervous trunks. The mediastinal space is also filled with connective tissue that fills the empty spaces between the various organs in such a way that they can maintain both an anatomical and functional independence.

Anatomically and surgically the mediastinum is divided, according to a frontal plane, in anterior and posterior mediastinum or, according to a transverse plane, in an upper and lower mediastinum.

This experience has given excellent results and we hope to make further collaborations with the University of Malta in the future.

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A brief anatomo-surgical dissection guide to the human neck: results of a collaboration between the University of Palermo and the University of Malta

Francesco Carini[1], Giovanni Tomasello[1], Serena Nobile[2], Margherita Mazzola[2], Giorgia Cavallo[2], Sofia Musso[2], Stefania Gentile[2], Stefania D'Accardo[2], Alessia La Mantia[2], Francesca Rappa[1], Fabio Bucchieri[1], Felicia Farina[1], Christian Zammit[3], Cristoforo Pomara[3], Francesco Cappello[1]

[1] Sezione Di Anatomia, Università Degli Studi, Palermo, Italia
[2] Sezione Di Anatomia, Scuola Di Medicina, Palermo, Italia
[3] Anatomy, University Of Malta, Msida, Malta

The aim of this work was to offer a guide to young students and medical doctors that want to learn the bases of neck dissection.

In the summer of 2017 a group of students from the University of Palermo that had already passed the Human Anatomy exams took a 4 weeks dissection course at the University of Malta. The students were provided with a dissection kit, video recording equipment and cameras for taking pictures.

Medicine is a science that requires both a practical and theoretical approach, and the last one, unfortunately, often is not valued by our University. Studying Human Anatomy, which is the basis for a doctor's education, exclusively through books and atlases, is indeed partially lacking. For this reason a group of students from the University of Palermo have been selected, based on their academic scores and English proficiency, to take part in an anatomic dissection course at the University of Malta.

The course took place in the university's dissection hall. The students spent their time dissecting, analyzing and separating the various anatomical structures under the supervision of tutors from both universities.

The dissections were preceded by a thorough review of the anatomy of the neck, using books and atlases for a recognition of the correct anatomical planes. The anatomical limits of the neck allow it to be distinguished from the head and thorax. Superiorly, in an anterior-posterior direction, the limits are the mandibular profile, the auricular back line and the superior nucal line. Inferiorly, in an anterior-posterior direction, the limits are the jugular notch, the superior border of the clavicle and the spinous process of C7.

This experience has given excellent results and we hope to make further collaborations with the University of Malta in the future.

Key words

Cadaveric study, surgical dissection course, topographical anatomy.

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Exploring the role of Fragile X Mental Retardation Protein in melanoma progression and invasiveness-related pathways in melanoma cells

Simone Carotti[1], Francesca Zalfa[1], Vincenzo Panasiti[2], Maria Zingariello[1], Vincenzo Roberti[3], Laura Sancillo[4], Giuseppe Perrone[5], Rosa Alba Rana[6], Jean-Christophe Marine[7], Claudia Bagni[8], Sergio Morini[1]

[1] University Campus Bio-Medico of Rome, Department of Medicine, Unit of Microscopic and Ultrastructural Anatomy, Rome, Italia
[2] University Campus Bio-Medico of Rome, Department of Medicine, Plastic and Reconstructive Surgery Unit, Rome, Italia
[3] University of Rome 'La Sapienza', Department of Dermatology, Rome, Italia
[4] University of Chieti 'G d'Annunzio', Department of Medicine and Science of Aging, Chieti, Italia
[5] University Campus Bio-Medico of Rome, Department of Anatomical Pathology, Rome, Italia
[6] University of Chieti 'G d'Annunzio', Department of Medicine and Science of Aging, Rome, Italia
[7] VIB/Center for the Biology of Disease, Laboratory for Molecular Cancer Biology, Leuven, Belgio
[8] University of Lausanne, Department of Fundamental Neuroscience, Lausanne, Svizzera

The Fragile X Mental Retardation Protein (FMRP) is an RNA binding protein, involved in multiple steps of RNA metabolism in neurons [1]. FMRP is lacking or mutated in patients with the Fragile X syndrome (FXS), a form of inherited mental retardation. Recently it has been also demonstrated that FMRP modulates metastasis formation in breast cancer, regulating the metabolism of mRNAs involved in cancer progression [2]. But the role of this protein has never been investigated in other types of cancer. Considering the similarities existing in the embryological origin between neurons and melanocytic cells, the aim of the present study is to investigate the role of FMRP in melanoma progression. FMRP overexpression is found in melanomas characterized by high Breslow's thickness and high Clark level, suggesting an association between FMRP increased expression and metastatic phenotype in melanoma. Furthermore, a reduction of FMRP in metastatic melanoma cell lines affecting their migration, invasion and adhesion properties, is found. Next-generation sequencing in human melanoma cells revealed that FMRP regulates a large number of mRNAs involved in relevant processes of melanoma progression. These data clearly show that FMRP in melanoma, as in breast cancer, is associated with an invasive phenotype and potentially related with cancer progression, suggesting in this way possible future therapeutic targets in melanoma.

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Key words

Fragile X mental retardation protein, melanoma, tumoral invasion.

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Hepatic Stem Cells and Adipocytokines in Nonalcoholic Fatty Liver Disease pediatric patients after Laparoscopic Sleeve Gastrectomy

Guido Carpino[1], Antonio Franchitto[2], Diletta Overi[2], Valerio Nobili[3] and Eugenio Gaudio[2]

[1] Università di Roma "Foro Italico", Dipartimento di Scienze Motorie, Umane e della Salute, Roma, Italia (1)
[2] Sapienza Università di Roma, Dipartimento di Scienze Anatomiche, Istologiche, Medico Legali e dell'Apparato Locomotore, Roma, Italia
[3] Ospedale Pediatrico Bambino Gesù, Hepatometabolic Diseases Unit, Roma, Itali

Hepatic stem/progenitor cells (HpSCs) are facultative bipotential stem cells [1], located in Canals of Hering and surrounded by a specialized niche [2]. We aimed to investigate the modulation of HpSC niche and the modification of adipocytokine expression induced by laparoscopic sleeve gastrectomy (LSG) in adolescents with nonalcoholic fatty liver disease (NAFLD). Twenty obese adolescents who underwent LSG and with biopsy-proven NAFLD were included. At baseline (T0) and 1 year after treatment (T1), patients underwent clinical evaluation, blood tests, and liver biopsy. HpSCs, hepatic stellate cells (HSCs), macrophages, and adipocytokines were evaluated by immunohistochemistry and immunofluorescence. Liver biopsies after LSG demonstrated a significant improvement of NAFLD Activity Score and fibrosis. Immunohistochemistry indicated a significant reduction of hepatocyte cell cycle arrest, HpSC activation, activated HSC, and macrophage number after LSG compared with T0. Hepatocyte expression of adiponectin was significant higher after LSG than at T0. Moreover, LSG caused decreased resistin expression in Sox9+ HpSCs compared to T0. The number of S100A9+ macrophages was also reduced by LSG correlating with resistin expression in HpSC. Finally, serum levels of proinflammatory cytokines significantly correlated with macrophages and activated HSC numbers. The histologic improvement induced by LSG is associated with the reduced activation of local cellular cross-talks, thus, strengthening the role of stem cell niche and hepatic adipocytokine production in the pathogenesis of NAFLD.

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Key words

Liver, Stem Cell, NAFLD, adiponectin.

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An anatomical description of the anterior ethmoidal artery: clinical and surgical considerations

Filippo Cascio[1], Alberto Cacciola[2], Simona Portaro[3], Alexandre Wady Debes Felippu[4], Andrè Wady Debes Felippu[4], Simona Pergolizzi[5], Felice Cascio[1], Demetrio Milardi[2]

[1] Department of Otorhinolaryngology, Papardo Hospital, Messina, Italia
[2] Department of Biomedical, Dental Sciences and Morphological and Functional Images, University of Messina, Messina, Italia
[3] IRCCS, Centro Neurolesi "Bonino-Pulejo", Messina, Italia
[4] Disciplina de Otorrinolaringologia, Instituto Felippu, Sao Paulo, Brasile
[5] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italia

Anterior Ethmoidal Artery (AEA) is a small vessel, branch of the ophthalmic artery: it arises in the orbit, reaches the ethmoidal labyrinth passing through its bony canal and finally reaches the olfactory fossa, through the lateral of the cribriform plate, along the so-called anterior ethmoidal sulcus [1]. Its anatomy and variations are of outstanding clinical relevance in rhinosinusal surgery, considering its role as a surgical landmark [2], its importance in the therapy of epistaxis [3] and the high risks related iatrogenic injuries [4]. In the present work we provide an anatomical description of anterior ethmoidal artery course and relationships, using in vivo CT Direct Volume Rendering in 18 subjects. The topographical location of 36 anterior ethmoidal arteries was assessed as shown: 10 dehiscent (27.8%), 20 intracanal (55.5%), 6 incomplete canal (16.7%). This work demonstrates that CT-DVR is a valid imaging technique for visualizing topographic anatomical details such as the AEA course, its relations with its bony canal and its possible dehiscence. In addition, it allows to achieve important information in vivo thus representing a useful tool for pre-operatory assessments.

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Key words
Anterior ethmoidal artery, CT, volume rendering.

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Morphofunctional study of human temporomandibular joint disc

Piero Cascone[1], Michele Runci Anastasi[2], Antonio Centofanti[2], Debora Di Mauro[2] and Claudia Previti[2]

[1] "Sapienza" University of Rome, Department of Maxillo-Facial Surgery, Roma, Italia
[2] University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia

During temporomandibular joint movement, the articular disc translates antero-posteriorly, supported the anterior attachment to the lateral pterygoid muscle and posterior attachment to the retrodiscal tissue. The articular disc is describe as a biconcave lens with two faces, superior and inferior, two margins, medial and lateral, and two extremities, anterior an posterior. The articular disc can be also divide into three different functional portions: posterior band, intermediate zone and anterior band. The extracellular matrix of the disc is composed mainly of collagen I and elastin and their distribution is different in the three functional zone [1]. The collagen I is the predominant ECM component. This protein forms a network and it's very important for resisting tensile forces. The elastin is also present in entire disc, but its distribution is different depending on the region; this protein is associated with resistance and elasticity and it is responsible to maintain the shape after deformation. Collagen I and elastin lie parallel to each other; some authors have demonstrated that collagen I is more present in the posterior and lateral zones in respect to elastin [2]. On this basis, here we studied the localization of collagen I and elastin in normal human temporomandibular joint disc by confocal laser scanning microscopy and scanning electron microscopy. Our results demonstrated that both proteins are present in entire disc and they run parallel to each other. In particular, collagen I and elastin, in intermediate zone, have an antero-posterior orientation, with longitudinal direction in condylar surface, while oblique orientation in temporal surface. In medial margin, the tested proteins have a similar staining pattern, and they cross each other with an oblique orientation. In lateral zone, the staining pattern of collagen I is more represented than to elastin and they form a thick network. Moreover, these preliminary immunofluorescence results are confirmed by scanning electron microscopic observations. The present results suggest that, during temporomandibular joint movements, the lateral margin of disc is submitted to a major compression forces due to a major presence of collagen I; however, the medial margin, corresponding to attachment of lateral pterygoid muscle, is submitted mainly to elastic forces because we observed similar staining patterns for both tested proteins.

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Key words

Temporomandibular joint disc, collagen I, elastin.

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Alpha-synuclein immunoreactivity in the enteric nervous system of human small intestine

Arianna Casini[1], Piero Chirletti[2], Rosa Vaccaro[1]

[1] Dipartimento di Scienze Anatomiche, Istologiche, Medico Legali e dell'Apparato Locomotore, Sapienza Università di Roma, Roma, Italia
[2] Dipartimento di Scienze Chirurgiche, Sezione di Chirurgia Interdisciplinare, Sapienza Università di Roma, Roma, Italia

Alpha-synuclein (α-syn) is a 140 amino acid protein, belonging to the synuclein family, expressed in mammalian neurons. Structural alterations of α-syn as well as its overexpression have been related to the onset and the progression of several human neurodegenerative diseases, as Parkinson's diseases (PD). Indeed, α-syn aggregates are the main component of the Lewy bodies (Lbs), considered as pathological hallmarks of neurodegenerative diseases [1-2], known as synucleinopathies. PD is a multicentric neurodegenerative process that affects several neuronal structures in the central and peripheral nervous system, among which is the enteric nervous system (ENS). Remarkably, recent reports have shown that the lesions in the ENS occurred at very early stage of the disease, even before the involvement of the central nervous system. So, the ENS could be critical in the pathophysiology of PD [3-4] and the pathological alterations within the ENS could be involved in the gastrointestinal dysfunction frequently encountered by parkinsonian patients. Although at present Lbs, as well as α-syn pathological aggregates, have been evidenced throughout the autonomic nervous system projecting to the gut of patients affected by PD or other neurodegenerative diseases, however data on the distribution of α-syn in human normal ENS are lacking. Our study focused on the immunohistochemical distribution of α-syn in the ENS of proximal tract of human normal small intestine. Surgical specimens of duodenum and proximal jejunum, collected from patients submitted to a pancreaticoduodenectomy, were fixed and paraffin embedded. Intestinal slices underwent immunohistochemical procedure using monoclonal anti α-syn antibody. Alpha-syn immunoreactive (ir) structures were detected along both myenteric and submucosal plexuses as well as in the circular and longitudinal muscular layers. We found perivascular α-syn-ir fibers in the submucosa and a dense ir periglandular network projecting up to the axis of the villi in the mucosa. The immunohistochemical distribution pattern of α-syn has been compared with that of major enteric neurotransmitters. Our preliminary observations confirm a physiological role of α-syn in the ENS, and may contribute to clarify its role in the peripheral nervous system.

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Zfp423/ZNF423 regulates Purkinje cell and cerebellar nuclei development

Filippo Casoni, Laura Croci, Ottavio Cremona, Giangiacomo G. Consalez

Istituto Scientifico San Raffaele, Università Vita e Salute San Raffaele, Milano, Italia

The Zfp423/ZNF423 gene encodes a 30-zinc-finger transcription factor involved in key developmental pathways. Although null Zfp423 mutants develop cerebellar malformations, the underlying mechanism is only partially characterized. In humans, ZNF423 mutations are associated with cerebellar vermis hypoplasia and Joubert Syndrome (JS), a ciliopathy causing congenital ataxia. ZNF423 participates in the DNA-damage response (DDR), suggesting that its mutation may slow down neural progenitor cell cycle progression in cerebellar development. To characterize in vivo the function of ZFP423 in neurogenesis, we analysed allelic murine mutants in which distinct functional domains are deleted. In Purkinje cell (PC) progenitors, located in the cerebellar ventricular zone (VZ), the two mutations produce different alterations in mitotic spindle orientation, maintenance of the progenitor pool and neuronal differentiation. In both mutants, cell cycle progression is remarkably delayed and DDR markers are upregulated in VZ and rhombic lip (RL) progenitors. In the RL, Zfp423 mutants display an increase in cell death at key developmental stages, and clear alterations in cerebellar nuclei (CN) development. Our results reveal protein-domain-specific roles played by ZFP423 in different aspects of PC and CN neurogenesis, and at the same time strengthen the emerging notion that an impaired DDR may be a key factor in the pathogenesis of JS and other ciliopathies.

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Scaffold of decellularized human dermis for cardiac repair and regeneration

Clotilde Castaldo[1], Franca Di Meglio[1], Immacolata Belviso[1], Anna Maria Sacco[1], Antonia Carfora[1], Veronica Romano[1], Diana Massai[2], Daria Nurzynska[1], Fabrizio Schonauer[1] and Stefania Montagnani[1]

[1] University of Naples Federico II, Department of Public Health, Napoli, Italia
[2] Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Torino, Italia

Skin shares properties of elasticity with muscular tissue. Since elasticity is mostly conferred by muscle cells or elastic fibers, after decellularization the removal of muscle cells causes in decellularized muscles loss of such property, while decellularized skin retains elasticity as skin ECM is rich in elastic fibers that are retained after decellularization. Additionally, mechanic properties are fundamental to ensure myocyte differentiation1 and alignment in myocardium. We developed a fast and efficient protocol of decellularization for human skin using skin fragments from patients undergoing plastic surgery. After decellularization, content of elastin was quantified by quantitative dye-binding method. Additionally elastin content and distribution was evaluated on histological sections by Paraldehyde Fuchsin Gomori and Weigert Van Gieson stainings. Decellularized Human Skin (d-HuSk) obtained was then sectioned into 600um thick sections and used as scaffold to prepare three-dimensional culture of cardiac primitive cells (CPCs). We evaluated, then, CPC survival and ability to differentiate, in vitro, towards cardiomyocytes at gene and protein level when cultured on d-HuSk. Decellularization procedure yielded the acellular extracellular matrix (ECM) with preserved tissue architecture, named d-HuSk. Importantly, histological and quantitative analysis clearly showed the retention of elastic fibers by d-HuSk. CPCs seeded on d-Husk engrafted and survived, and their ability to differentiate towards cardiomyocytes was not lost, as shown by preserved expression of markers specific for cardiac muscle cells, both at protein and gene level. Such results suggest that common signals and properties act both in cardiac and skin microenvironment, making skin a potential powerful and off-the-shelf biological scaffold for cardiovascular regenerative medicine. Although emerging from an in vitro study, the evidence that progenitors of cardiac muscle lineage retain the ability to differentiate on biological scaffold obtained from different, more easily accessible, anatomic site, represents an important advance in cardiovascular regenerative medicine. Specifically, d-HuSk is an alternate biological scaffold that overcomes problems related to the preparation of myocardial biological scaffolds.

Key words

Cardiac regeneration, Decellularized-ECM, Biological scaffold.

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Actigraphic analysis of activity levels in obese with binge eating disorder

Lucia Castelli[1], Antonino Mulè[1], Letizia Galasso[1], Eleonora Bruno[2], Patrizia Pasanisi[2], Andrea Caumo[1], Eliana Roveda[1] and Angela Montaruli[1]

[1] Università degli Studi di Milano, Dipartimento delle Scienze Biomediche per la Salute, Milano, Italia
[2] IRCCS Istituto Nazionale Tumori, Dipartimento di Medicina Preventiva e Predittiva, Milano, Italia

Altered Rest-Activity circadian Rhythm (RAR) are associated with a compromised health status. RAR abnormalities have been assessed in pathological conditions, such as neurological, cancer, and cardiovascular diseases [1]. Binge Eating Disorder (BED), characterized by obesity and motor inactivity, could produce RAR disruption with negative consequences on health-related quality of life. The aim of the present study was to evaluate RAR by actigraphy in obese patients with BED compared to a body mass index-matched control group (Ctrl).

Sixteen participants (8 obese women with and 8 obese women without BED diagnosis) were recruited to perform a 5-day actigraphic monitoring (MotionWatch 8®, CamNtech, Cambridge, UK) to estimate RAR.

The population mean cosinor applied to BED and Ctrl showed the presence of a significant circadian rhythm in both groups ($p<0.001$). The MESOR (170.0 vs 301.6 a.c., in BED and Ctrl, respectively; $p<0.01$) and amplitude (157.66 vs 238.19 a.c., in BED and Ctrl, respectively $p<0.05$) resulted significantly different between the two groups. There were no differences between BED and Ctrl referring to Acrophase. The dysfunctional RAR found in BED cannot be related to obesity per se because the participants of the two groups were all obese with similar BMIs (31.3 ± 1.0 vs 31.6 ± 0.7 Kg/m2 in BED and Ctrl, respectively).

These data represented the first actigraphy-based evidence of RAR disruption in women with BED. The circadian approach can represent a novel tool useful in the treatment of patients with eating disorders. The rest-activity circadian parameters should be assessed and managed to enhance interventions able to normalize the spontaneous activity level and improve the quality of life in BED patients.

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Key words

Binge eating disorder, circadian rhythm, rest-activity cycle, actigraphs.

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Academic performance in Italian students of Sport Science: the Circadian Typology is related to theoretical or practice exams?

Lucia Castelli, Antonino Mulè, Letizia Galasso, Gianni Michielon, Raffaele Scurati, Andrea Caumo, Eliana Roveda and Angela Montaruli

Università degli Studi di Milano, Dipartimento delle Scienze Biomediche per la Salute, Milano, Italia

Circadian rhythms play an important role on activation level. The expression of circadian rhythms differs among individuals and these differences define three Circadian Typology (CT): Morning-types [M-types], Evening-types [E-types] and Neither-types [N-types]. In particular, M-types and E-types differ in the sleep-wake timing and mental or physical activation during the 24 hours. The peak of mental and physical performance is reached in the first part of the day by M-types and in the second half by E-types [1]. School schedule in Italy imposes wake up and activation timing not compliant to E-types innate circadian preferences. This condition doesn't respect the so-called synchrony effect, for which people perform better in agreement with activation peak. Because of this, E-types students may be disadvantaged respect to M-types, which are more aligned with the daily social program [2]. In order to evaluate the academic performance referring to the circadian preferences, we recruited 427 subjects (female=133; male=294; mean age 20.8 ±2.2 years). They are all attending the School of Sport Science' University of Milan and recruited at the beginning of the university career for four consecutive academic years, from 2010 to 2014. The students filled in the Morningness-Eveningness Questionnaire (MEQ), for the determination of the chronotype. Exam grades of theoretical (Anatomy, Physiology and Sport Medicine) and of practice subjects (Athletic, Swim, Volleyball and Basket) were collected. The statistical analysis showed that the mean values for all exams considered are higher for M-types respect to N-types and E-types students. As regard E-types, these students appear to be disadvantaged more in practice than in theoretical subjects respect to M-types students.

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Key words

Academic achievement, circadian typology, theoretical subjects, practical subjects.

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From Embriology to Eurgical Anatomy in Colorectal Surgery

Sergio Castorina[1], Carla Loreto[2]

[1] Dipartimento di Scienze Mediche Chirurgiche e Tecnologie Avanzate,Università di Catania, Catania, Italia
[2] Dipartimento BIOMETEC, Università di Catania, Catania, Italia

Nowadays, the gross anatomy is not longer considered a static discipline because it is constantly evolving along with surgical progresses. In particular, laparoscopic surgery, in the last twenty years, has further contributed to the deepening of some aspects of the dissection in peritoneal cavity, especially in colorectal surgery.

In 1982 Heald et al. introduced the concept of total mesorectal excision (TME) as an anatomical-surgical basis for a correct resection of rectal cancer [1]. In 2009 Hohenberger et al. introduced the same principle for complete mesocolic excision (CME) [2]. The knowledge of the different organogenetic phases in the development of the gut and of the mesentery is extremely useful in clinical applications, since it allows, in particular with a laparoscopic approach, to identify the peritoneal attachment layers and the mesenteric limits.

The aim of our study was to deepen the knowledge of the mesenteric embriology with the dissection on cadaver, the laparoscopic dissection, the histology and the virtual reconstruction of anatomical structures using Nuclear Magnetic Resonance imaging as starting point.

Moreover, we propose a video in which we show the mesorectal excision on cadaver and patient with the new transanal approach (Transanal total mesorectal excision,TaTME).

We want to show that in human body only a mesenter is present and it starts from the inferior region of the esophagus and reaches the cloaca, where the rectum has its own meso, called mesorectum, which separates the organ from the posterior part of the pelvis.

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Key words

Mesenter, colorectal surgery.

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The chitinases role in osteoclasts activity

Paola Castrogiovanni, Rosa Imbesi, Marta Anna Szychlinska, Silvia Ravalli, Giuseppe Musumeci and Michelino Di Rosa

Università degli studi di Catania, Scienze Biomediche e Biotecnologiche - Sezione di Anatomia Umana ed Istologia, Catania

The integrity of skeletal mass require continuous bone renewal by a combination of modeling and remodeling, mediated by osteoclasts (OCs) and osteoblasts (OBLs). Osteolysis mediated by OCs is a hallmark of different bone diseases [1]. In multiple myeloma (MM), osteolytic bone disease is a manifestation that leads to progressive skeleton destruction and is the most severe cause of morbidity. Our hypothesis was that a family genes called Chitinases could play a role in bone loss mediated by OCs. These genes exert important biological functions in the monocyte lineage and chronic inflammatory diseases [2]. In this respect, we evaluated the chitinases expression in OCs differentiation and in MM cell lines under Bortezomib (BO) treatment. Our results showed an increasing of CHI3L1 and CHIT1 during the osteoclastogenesis. As well, the confocal immunofluorescence (IF) and immunohistochemistry (IHC) analysis demonstrated the presence of CHI3L1 and CHIT1 uniformly distributed into the OCs. The OCs treatments with chitosan, a natural ligand for chitinases, and the silencing with small interfering RNA (siRNA) of CHI3L1 and CHIT1, resulted in a significant reduction in bone resorption. Based on this results, the OCs treatments with BO during the osteoclastogenesis, had reduced the digestive activity and the chitinases mRNA and protein expression levels. Moreover, the IF evaluation of mature OCs showed that BO was able to induce CHI3L1 translocations into the nucleus, while CHIT1 remained into the cytoplasm. Since MM cell lines showed high levels of CHIT1 activity, we analyzed bone resorption ability of U266. Silencing chitinases proteins in U266 cell line with siRNA, resulted in pits number reduction on dentine disks. Overall, all these results have demonstrate crucial role of chitinases in promoting bone resorption. In the light of all this, the chitinases are new potential candidate markers for therapeutic targeting in bone loss.

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Key words

Osteoclast, chitinase, multiple myeloma.

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Comparison between dual-energy X-ray absorptiometry and anthropometric predictive equations in assessing percentage body fat in soccer players with lower limb amputation

Valentina Cavedon, Carlo Zancanaro and Chiara Milanese

Università di Verona, Dip. di Neuroscienze Biomedicina Movimento, Verona, Italia

Anthropometric equations are an accessible and cost-effective method to assess percentage body fat (%BF) in different athletic populations, but their reliability in athletes with limb amputation is unclear [1]. The aim of this study was to compare, in amputee soccer players, %BF estimated with several commonly used equations and dual-energy X-ray absorptiometry (DXA) taken as the reference method. Body density was assessed in 10 male soccer players aged 33.9±11.9 years with transfemoral (n=7) or transtibial (n=3) lower limb amputation using five currently used anthropometric equations established for able-bodied subjects [Durnin and Womersley (1974), Jackson and Pollock 7-sites (1978), Sloan and Weir (1970), Wilmore and Behnke (1969), and Katch e McArdle (1973)]; body density was converted to %BF according to Siri (1961). %BF measured with DXA (Hologic) was used for assessing the validity of anthropometric equations (paired-sample t-test); the agreement between methods was assessed with the coefficient of determination and the standard error of estimate. Results showed that all the anthropometric equations significantly underestimate %BF (-2.7%÷-6.0 %; p, 0.012÷<0.001), but the Durnin and Womersley equation, which significantly overestimates %BF by +4.0%. The highest adjusted coefficient of determination was found for the Wilmore and Behnke equation (R2=0.805, p=0.001) and the lowest (R2=0.422, p=0.025) was found with the Durnin and Womersley equation. The standard error of estimate ranged from 2.37% (Wilmore and Behnke equation) to 4.08% (Durnin and Womersley equation). Further comparative studies are required to confirm or refine the accuracy of practical, non-invasive methods for monitoring %BF in the amputee athletic population. Impairment-specific equations may be needed in amputee soccer players with lower limb amputation.

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Key words

Anthropometry, soccer, amputee, body composition.

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Application of a thermosealing industrial process of packaging to human anatomical prosections in interactive teaching

Gabriele Ceccarelli, Laura Benedetti, Gabriella Cusella

Anatomy Institute, Department of Public Health, Experimental Medicine and Forensic, University of Pavia, Pavia, Italia

Due to the inability to use cadaver dissection, for both legal and economic reasons [1], in the institute of Human Anatomy of Pavia, we started to use a thermosealing industrial process of packaging (VGP 60 Skin, Orved) to wrap and seal off fixed brains and cranial sections [2]. The purpose of this procedure is to improve students' preparation in relation to skull osteology and topography of the central nervous system. Designed according to the most demanding hygienic requirements, the "Skin" effect is a packaging system that allows a specific film to adhere perfectly to the sample like a "second skin", ensuring airtight packaging with no type of drop and, at the same time, perfect preservation, allowing an excellent visibility too [2]. These preparations, very quick and easy to prepare, are no toxic anymore because lacking of formalin [3] and could be used by medical students to improve their approach to anatomic nervous system structures. First, we have washed brain samples in running water, then frozen to harden and finally sealed. We noted that this strategy largely improved student's level of exam preparation on human prosectioned speciments in full compliance with the current safety regulations.

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Key words

Thermosealing industrial process, fixed brains, human anatomical prosections, interactive teaching.

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The effects of micro-grafts in the treatment of androgenetic alopecia

Gabriele Ceccarelli[1], Laura Benedetti[1], Flavio Lorenzo Ronzoni[1], Maurilio Sampaolesi[1], Letizia Trovato[2], Antonio Graziano[2], Ciro De Sio[3] and Gabriella Cusella[1]

[1] Università degli Studi di Pavia, Dipartimento Sanità Pubblica, Medicina Sperimentale e Forense e Centre for Health Technologies, Pavia, Italia
[2] HBW, Human Brain Wave s.r.l, Torino, Italia
[3] Private Practice, Specialist in Plastic Surgery, Roma, Italia

Androgenetic alopecia (AGA) is a hereditary androgen-dependent, progressive thinning of scalp hair affecting 60–70% of the adult population worldwide [1]. In AGA, hair is lost in a well-defined pattern, beginning above both temples. Over time, hairline recedes to form a characteristic "M" shape. Pharmacological treatment offers moderate results and hair transplantation represents the only permanent treatment option [2]. Here we describe a clinical approach, based on autologous micro-grafts, called Rigenera® that is able to restore hair loss using a promising CE-certified medical device called Rigeneracons. Its efficacy was demonstrated in the wound care including the management of chronic or non-healing wounds and for hard tissues and cartilage regeneration [3]. A preliminary in vivo study on three patients reported that autologous micro-grafts obtained by Rigenera® protocol promote hair growth even two months after the surgical procedure. The aim of this study was to demonstrate long-term efficacy of Rigenera® protocol in the treatment of AGA performing histological evaluations on scalps after 6 and 9 months from micro-grafts application with respect to controls. Morphological evaluations were performed by Haematoxylin/Eosin and Mallory Trichrome staining on 4-mm punch of scalps from volunteers patients. Results showed that, after 6 months of micro-grafts application, the number of hair follicles in the scalp is increased with a beginning of cuticle formation and dermal papilla in proliferation. After 9 months, we reported a well-organized derma, more regular and structured collagen fibres, and hair follicles in Anagen IV/Mesanagen phase. In summary, micro-grafts application improve hair restoration with a positive patient's subjective assessment.

This work was supported by grant from NATO 2016 ("RAWINTS" G-984961): RApid Skin Wound healing by INtegrated Tissue engineering and Sensing).

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Key words

Androgenetic alopecia, autologous micro-grafts, morphological evaluations, hair restoration.

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Categorical evaluation of Umami perception in Europe

Maria Paola Cecchini[1], Antti Knaapila[2], E. Hoffmann[3], Thomas Hummel[3], Emilia Iannilli[3]

[1] University of Verona, Department of Neurosciences, Biomedicine and Movement Sciences, Section of Anatomy and Histology, School of Medicine, Strada le, Verona, Italia
[2] University of Helsinki, Department of Food and Nutrition, Helsinki, Finlandia
[3] University of Dresden, Interdisciplinary Center "Smell & Taste", Department of Otorhinolaryngology, Germany, Dresden, Germania

The umami taste has been known for more than 100 years [1]. Although taste researchers have known about Ikeda's work for decades, it is only recently that umami has gradually gained wider public recognition as the fifth primary taste, distinct from the other four basic tastes. Recent progress in molecular biology identified umami taste receptors in tongue's taste buds. Umami taste is elicited by L-glutamate, typically as its sodium salt, the monosodium glutamate (MSG), some amino acids and purine nucleotides [2]. MSG is found in a wide range of foods (e.g. meat, fish, tomatoes, soy sauce, potatoes, parmigiano cheese, and mushrooms). Despite that, in European countries umami taste is not generally included in taste evaluation methods, because it has been found to be hardly conceptualized by the European population [3]. On the other hand, Japanese subjects are familiar with this taste because they ordinarily eat foods rich in umami substances such as dashi, a broth made of kelp (L-glutamate) or dried bonito flakes (inosinate) [4]. In addition, in Japan methods to assess umami taste sensitivity by means of MSG have been developed and currently clinically used [5]. To the best of our knowledge, a comprehensive survey evaluation of umami perception on European countries is lacking. On the basis of these premises, the goal of this study was a survey on the umami perception among different countries and cultures in Europe. For that reason, we chose three countries representative of northern (Finland,), central (Germany) and southern Europe (Italy). Each group included respectively 300, 271 and 252 samples. In this point of view, we aimed to collect the categorical descriptors naturally expressed from volunteers just after the tasting of an Umami solution alone and also in comparison with a salty and water solution together with the hedonic value perceived. Here we report and discuss the correlations among responses in the three different countries.

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Key words

Taste, umami perception, European countries.

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Adult thyroid stem cells as a novel source of brown adipose tissue: clues to bioengineered rat models of tissue implants for innovative treatment of obesity

Leonardo Cecotto[1], Elia Consolini[1], Fulvio Barbaro[1], Giorgio Gallo[1], Davide Dallatana[1], Nicoletta Zini[2], Elena Bassi[1], Marco Alfieri[3] and Roberto Toni[4]

[1] Laboratory of Regenerative Morphology and Bioartificial structures (Re.Mo.Bio.S.), Unit of Biomedical, Biotechnological and Translational Sciences, Section of Human Anatomy, Department of Medicine and Surgery, University of Parma, Parma
[2] CNR National Research Council of Italy, IGM, Rizzoli Orthopaedic Institute, IRCCS, Biologna
[3] Laboratory of Regenerative Morphology and Bioartificial structures (Re.Mo.Bio.S.), Unit of Biomedical, Biotechnological and Translational Sciences, Section of Human Anatomy, Department of Medicine and Surgery, University of Parma; UCD Conway Institute of Biomelocular and Biomedical Research, Parma, Italy; Dublin, Ireland
[4] Laboratory of Regenerative Morphology and Bioartificial structures (Re.Mo.Bio.S.), Unit of Biomedical, Biotechnological and Translational Sciences, Section of Human Anatomy, Department of Medicine and Surgery, University of Parma; Division of Endocrinology, Diabetes and Metabolism, TUSM-TMC, Parma, Italy; Boston, MA, USA

Brown adipose tissue (BAT) can provide a novel therapeutic to treat obesity. Using adult male rat, thyroid stem cells (TSC) recently isolated by our group we have studied their differentiation potential to BAT as a cell source to engineer metabolically-active biomaterial-based tissue implants. TSC were obtained as colony forming unit-like cultures [1] whereas adipose differentiation was reached based on a protocol with 4 cycles (6 d/cycle) of white adipogenic induction. Presence of lipid droplets was assessed by light microscopy (LM) using oil red O histochemistry, their ultrastructural morphology studied by TEM, and difference in the 3D cellular morphology screened by SEM. Finally, a morphometric analysis was conducted with LM to determine the contribution of different cell phenotypes at control and differentiated levels, and their relevant subcellular features. More than 90% of control, adult TSC displayed a multipolar morphology, and flattening at increasing times as opposed to less than 10% of cells that were fibroblastoid. Similar, adipoblast-like cells exhibited a multipolar and, less frequently fibroblastoid morphotypes characterized by the absence of intracellular triglycerides. In contrast, white preadipocytes were identified as multipolar and ovoid cells containing small lipid droplets fusing into bigger ones. Differently, brown preadipocytes displayed a multipolar filamentous-rich phenotype, with abundant lipid droplets around 50% less in size than those of the white counterpart. Finally, few mature brown adipocytes were observed, depicting a polygonal shape with a central nucleus surrounded by sizeable lipid droplets, and a nucleus/cytoplasm ratio lower than that of all preadipocytes. At 21 days of induction, 70% of adipoblast-like cells were replaced by white preadipocytes; however, brown preadipocytes increased in number throughout the differentiation time, reaching 14% of all cells at 28 days. Our results show that adult male rat TSC have a remarkable potential to differentiate in culture to the brown lineage even in the absence of specific browning stimuli, providing an innovative source to engineer metabolically-active bioimplants for the treatment of obesity.

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Key words

Adult Thyroid Stem Cells, BAT, Bioimplant, Obesity.

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SEM and CLSM study on steam sterilization of equine bone blocks

Antonio Centofanti, Elena Filippini, Emanuele Magaudda, Roberto Lo Giudice, Giuseppe Lo Giudice

University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia

Equine bone blocks are mainly used in bone regeneration technique if the bone volume is not sufficient to provide a long-term stability during prosthetic restorations [1]; this techniques is very useful also in post-traumatic and reconstructive interventions. Moreover, in order to realize a faithful shape of bone block, the shaping is performed by hand or using a Computer Numerical Control (CNC) milling machine [2]. The real problem for this technique is the realization of shape in a sterile ambient in order to avoid potential risk of transmission of bacteria, viruses and prions. On this basis, here we aim to evaluate if the steam sterilization could provide an useful sterilization of bone blocks and to estimate the possible variations in bone structure and in collagen organization after different steam sterilization cycles with traditional autoclave. For this, we obtained 16 samples from 2 blocks of equine bone. 1 sample was used as control, while other 15 samples were infected with a Streptococcus faecalis bacterial culture; these samples were divided in 3 groups (A, B, and C) and treated with different cycles of autoclave sterilization (Gr. A: 121°C, 1,16 bar for 20'; Gr. B: 134°C, 2,16 bar for 4'; Gr. C: 134°C, 2,16 bar for 3.30'). For each group, 2 samples were evaluated for the sterility, 3 samples were evaluated at S.E.M. and at confocal laser scanning microscope in order to evaluate possible morphological and collagen organization variations. Our observations at S.E.M. showed that no morphological variations were present in samples; while our findings obtained by confocal laser scanning microscope showed a more uniform and preserved organization of collagen in samples of group C. These results demonstrated that autoclave steam sterilization represents an indicate technique to obtain sterilization of equine bone blocks.

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Key words

Bone, collagen I, biomaterials.

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Synovial-Derived Stem Cells (SDSCs) and Telocytes: possible involvement in osteoarticular pathologies

Giorgia Cerqueni[1], Manuela Dicarlo[2], Caterina Licini[3], Iolanda Iezzi[1], Huijuan Tang[1] and Monica Mattioli Belmonte[1]

[1] DISCLIMO, Università Politecnica delle Marche, Ancona, Italia
[2] Laboratory of Experimental Immunopathology,, IRRCS "S de Bellis", Castellana Grotte (BA), Italia
[3] DISCLIMO/DISAT, Università Politecnica delle Marche/ Politecnico di Torino, Ancona, Italia

Osteochondral defects may progress in osteoartithis (OA) that is one of the most common causes of articular pain and disability in an ageing population [1]. OA affects cartilage, the subchondral bone and the synovial tissue; therefore, it is currently defined as a disease of the whole joint [2]. Recent researches are focusing on an in-depth characterisation of synovial membrane (SM) and cells isolated from OA to elucidate their role in the pathogenesis and/or regeneration of joint diseases. SM is a specialized mesenchymal tissue that includes two layers, the intima and the subintima, and hosts mesenchymal stromal cells, called Synovial-Derived Stem Cells (SDSCs) [3] that confer an intrinsic ability of regeneration to SM and/or may be involved in early stages of osteoarticular diseases. Recently, the presence of telocytes (TCs), a peculiar type of interstitial cells characterized by extraordinary long cytoplasmic processes (telopodes) has been demonstrated in SM [4]. The aim of our research was to isolate SDSCs from osteoarthritic subjects and evaluate their morphology, phenotype, differentiation potential and capability to activate Peripheral Blood Mononuclear Cells (PBMCs) in comparison with cells isolated form healthy subjects. A peculiar attention to the presence of TCs was paid. SM was obtained during surgery for total knee arthroplasty in OA subjects (mean age 76±3). Control SM was harvested form 2 young subjects, gender matching, undergoing leg amputation. Histological and ultrastructural analyses evidenced the presence of TCs in SM of both normal and OA subjects. No significant differences in SDSCs behaviour were observed between healthy and OA subjects except for the isolation and maintenance of TCs that was possible only from SM of OA patients. Co-culture of SDSCs with PBMCs highlight the generation of active osteoclasts from PBMCs only in the presence of SDSCs derived from OA subjects, whilst control SDSCs could generate multinucleate but not active osteoclasts. Further studies are still necessary to clarify the role of telocytes and its secretome in osteoarthritis to develop new effective therapeutic strategies.

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Key words

Synovial membrane, Stem cells, Telocytes, Osteoartithis.

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Angiogenic and inflammatory potential of Scleral Ossicles, novel natural biomaterials for bone regeneration

Marta Checchi[1], Jessika Bertacchini[1], Maria Sara Magarò[2], Antonella Sola[3], Francesca Bisi[3], Massimo Messori[3], Domenico Ribatti[4], Delphine Maurel[5] and Carla Palumbo[1]

[1] Università di Modena e Reggio Emilia, Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Modena, Italia
[2] Università di Modena e Reggio Emilia, University of Modena and Reggio Emilia, Modena, Italia
[3] Università di Modena e Reggio Emilia, Dipartimento di Ingegneria "Enzo Ferrari", Modena, Italia
[4] Università di Bari, Dipartimento di Scienze mediche di base, neuroscienze e organi di senso, Bari, Italia
[5] Université de Bordeaux, BioTiS - Bioingénierie Tissulaire, Bordeaux, Francia

Recovering and regeneration of significant bone defects is one of the big challenge that researchers would like to win in the field of regenerative medicine. When a fracture happens it is possible to incur in a so called critical-size defects, a sever lesion that affect a skeletal segment by preventing its self-recovery. Current standard treatments include autografting, allografting and other implant techniques which may imply some issues, such as limitations connected to costs and side effects like potential infections and nonunion. The tissue engineering directed its efforts in developing new scaffold to combine with cells and stimuli with the aim to reproduce the interaction between cells and Extracellular Matrix during the osteogenesis process. In particular, many different scaffolds have been developed with different properties, proposing new materials to be used for new 3D printing techniques in order to optimize the cell growth; a variety of different exogenous chemical or physical stimuli were tested, such as soluble growth and differentiation factors as well as mechanical forces; finally many types of cells have been used alone or in co-culture. The most important obstacle emerged so far is the lack of a proper vascularization by which cells inside the scaffold receive a sufficient blood supply. The aim of this work is the analysis of the angiogenic and inflammatory potential of the Scleral Ossicles (SOs), already analysed by the structural viewpoint [1], and the development of a functionalized-SOs-construct. Recently, we have already characterized the SO proposing it as innovative and naturally decellularized material easily available at no cost [2]. Currently, the SOs has been tested for angiogenic potential in ovo utilizing the Chorioallantoic Membrane (CAM) system. The preliminary results have shown the induction by SO of a strong vasculo-proliferative reaction on CAM in which the neo-formed vessels have an extremely tortuous and irregular course. The inflammatory potential will be evaluate in vivo by means of subcutaneous implant of SOs in rat models. Finally, we are developing a 3D-printed scaffold made by Dental SG Resin, which will host the SOs, with the aim to adapt this functionalized-SOs-construct (3D scaffold + SOs) to the dimensions of a critical fracture in diverse species.

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Key words

Scleral Ossicle, CAM, inflammation, angiogenesis, 3D printing.

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Photobiomodulation with 635 nm diode laser stimulates osteoblast differentiation via Akt signaling activation

Flaminia Chellini[1], Alessia Tani[1], Marco Giannelli[2], Daniele Nosi[1], Sandra Zecchi-Orlandini[1] and Chiara Sassoli[1]

[1] Università degli Studi di Firenze, Dipartimento di Medicina Sperimentale e Clinica, Firenze, Italia
[2] Odontostomatologic Laser Therapy Center, Odontostomatologic Laser Therapy Center, Firenze, Italia

Low Level Laser Therapy (LLLT), more recently termed photobiomodulation (PBM), has been used for bone regenerative purposes in different fields of medicine and dentistry [1,2]. However, at present, univocal standardized guidelines for its use PBM are not available. This is mainly due to the variety of wavelenghts, light source types used, disparate energy output modes and setting parameters, which have produced many different treatment protocols with different and sometimes contradictory outcomes hampering meaningful comparison of the results and demanding a skeptical look for the promising and potential beneficial effects of this approach [2,3]. In addition, the molecular mechanisms by which PBM induces different biological responses have not been fully clarified [4]. In this in vitro study we evaluated the PBM potentiality by 635±5 nm diode laser operating in continous wave with a 0.4 J/cm2 energy density to influence osteoblast progenitor cell viability, proliferation, adhesion and osteogenic differentiation. Red light did not alter viability (PI/Syto16 and MTS assays). Confocal immunofluorescence and RT-PCR analyses indicated that photobiomodulation by 635 nm increased vinculin-rich clusters, osteogenic markers expression (Runx-2, alkaline phosphatase, osteopontin) and mineralized bone-like nodule structure deposition. Interestingly, osteoblast responses to 635 nm laser treatment were mediated by Akt signaling activation which seems to positively modulate reactive oxygen species (ROS) levels. Although within the limitations of an in vitro experimentation, this study may suggest PBM by 635 nm laser operating as indicated, as a potential effective option for promoting/improving bone regeneration.

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Key words

Photobiomodulation (PBM), low level laser therapy (LLLT), osteoblasts, bone regeneration, Runx-2, ostepontin, Akt signaling, ROS.

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Effects of a four-month judo training on gait performance in old male and female practitioners

Simone Ciaccioni, Giancarlo Condell, Roberta Forte, Caterina Pesce and Laura Capranica

University of Rome "Foro Italico", Department of Movement, Human and Health Sciences, Roma

The fall-related injuries are a crucial health, social, and economic problem in older populations and there is a need of effective programmes to prevent falling1. To quantify locomotion changes with aging and to monitor the effects of therapeutic interventions, gait variability is a sensitive and clinically relevant parameter2. The aim of this study was to investigate the effects of a 4-month judo training (1-hr training session, twice a week) on gait performance in older individuals (age: 60-76 yrs). The experimental group (JG) included 16 (F=8, M=8; 69.3±3.9 yrs) participants to a 4-month judo programme, whereas the control group (CG) encompassed 14 (F=5, M=9; CG: 70.1±4.5 yrs) moderately active controls (CG). Average values (AVG) and coefficients of variation (CV) of step length during habitual (HWS) and maximal walking speed (MWS) were computed in a flat path (flat), walking on a narrow (20 cm wide) corridor (corridor), and walking on a path with hurdles (hurdles). A 2 (gender) x 2 (group) x 2 (time) ANOVA for repeated measures was applied to ascertain differences between groups in the different conditions ($p<0.05$). A main effect emerged for time ($p=0.042$), whilst significant interactions time x group ($p=0.004$) and time x group x gender ($p=0.019$) were revealed. Post hoc analysis (adjusted p for 12 comparisons = 0.0041) did not confirm the differences for the time effect. Regarding the time x group interaction, significant reductions were confirmed in JG for CV in the HWSflat (pre: 4.79±2.08%, post: 3.74±1.27%, $p=0.03$), MWSflat (pre: 4.71±1.34%, post: 3.89±1.22%, $p=0.013$), and HWShurdles (pre: 11.26±2.58%, post: 9.62±1.62%, $p=0.012$). In CG significant increases were confirmed for CV in the MWSflat (pre: 3.47±0.94%, post: 4.67±2.21%, $p=0.039$) and MWScorridor (pre: 4.85±1.54%, post: 6.24±2.16%, $p=0.007$). Regarding the interaction time x group x gender, significant differences emerged for CV in HWShurdles ($p=0.003$). Post hoc analysis maintained significant differences for the female JG (pre: 11.81±3.42%, post: 8.92±1.47%, $p=0.017$). These findings indicate a positive effect of judo training on gait performance in novice senior judo population, particularly in women.

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Key words

Gait analysis, gait variability, older persons, judoka, physical activity.

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3D virtual morphometry of human myometrium and uterine fibroids performed by Synchrotron Radiation-based Microtomography

Pasquapina Ciarmela[1], Stefania Greco[1], Giuliana Tromba[2], Serena Pacilè[2], Alessandro Zannotti[1], Giovanni Delli Carpini[3], Stefano Raffaele Giannubilo[3], Andrea Ciavattini[3] and Alessandra Giuliani[3]

[1] Università Politecnica delle Marche, Dipartimento Medicina Sperimentale e Clinica, Ancona
[2] Elettra, Sincrotrone Trieste S.C.p.A., Trieste
[3] Università Politecnica delle Marche, Dipartimento di Scienze Cliniche, Ancona

Uterine leiomyomas, or fibroids, are the most common gynecological tumors originating inside the myometrium. Leiomyomas bulk is caused by a disorder of fibrosis, characterized by production of large amount extracellular matrix (ECM) and by its disruption (1-3).

The ECM can be easily detected by histochemical methods. However, conventional microscopy techniques are limited to two-dimensional images hampering the quantitative analysis or requiring digital 3D reconstruction of serial stained sections.

The impact of the microtomography (microCT) technique, performable with third-generation synchrotron light sources, has been revolutionary, enabling the observation of internal sample details with unprecedented definition, high resolution up to 0.2 mm and allowing the calculation of different morphometric parameters.

We performed microCT experiments on paraffin embedded leiomyoma and myometrial biopsy at the Italian Synchrotron Facility, ELETTRA (Basovizza-TS), using phase-contrast settings optimized for non-mineralized biological tissues. For each biopsy, several subvolumes were analyzed: each of them was a 3D portion fully included in the sample bulk and the complete set of them allowed to achieve the whole retrieved sample mapping. The quantitative analysis was based on the structural indices usually measured for bone samples: Collagen-Fibers-specific-volume, Collagen-Fibers-specific-surface, Mean Collagen Fiber thickness, Mean Collagen Fiber number and Mean Collagen Fiber spacing. Furthermore, as Collagen Fibers could vary their orientation depending on the pathology, we also extracted information about the anisotropy of the collagen structure, i.e. the presence of preferential orientation. The anisotropy degree index measures the similarity of a fabric to a uniform distribution and varies between 0 (all observation confined to a single plane or axis) and 1 (perfect isotropy). Finally, the morphometric analysis was also applied in order to derive a descriptor for the interconnectivity between the structures.

This preliminary investigation opened new methodologic possibilities for future studies to evaluate the ECM in soft tissues.

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Key words

Myometrium, leiomyoma, morphometry, microtomography, synchrotron radiation.

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The effect of a physical training with the use of an exoskeleton in the rehabilitation of institutionalized elderly patients at high risk of falls

Antonello Ciccarelli[1], Walter Verrusio[2], Filippo Tajani[3] and Maurizio Ripani[4]

[1] Università degli studi di Roma "Foro Italico", Dipartimento di Scienze Motorie, Umane e della Salute, Roma, Italia
[2] Università degli studi di Roma "Sapienza", Department of Cardiovascular, Respiratory, Nephrological, Anesthesiological and Geriatric Sciences, Roma, Italia
[3] Università degli studi "Foro Italico", Dipartimento di Scienze Motorie, Umane e della Salute, Roma, Italia
[4] Università degli studi di Roma "Foro Italico, Dipartimento di Scienze Motorie, Umane e della Salute, Roma, Italia

Physical training exerts several systemic beneficial effects in elderly, also in reducing the risk of falls and fractures [1]. The aim of this study was to evaluate the effects of a physical training with the exoskeleton (HBP) [2,3] on gait and balance in the institutionalized elderly at severe risk of falls. The study included a series of elderly subjects (n = 16; mean age: 87, ds = ± 6.8 males 5) living in nursing home and at high risk of falls (Tinetti score <19). Baseline evaluation included: i) the Tinetti balance and Gait evaluation scale; ii) the short physical performance battery (SPPB). Participants were randomly assigned to one of the two groups: i) in the HBP group, subjects received physical exercise training using HBP; ii) in the Exercise group subjects received physical exercise training without the use of the HBP. Each patient was engaged in three sessions of physical exercise a week under instruction of a therapist. The HBP is a fully-articulated orthosis, consisting of four basic elements which come into contact with different anatomical zones. As a result, users enjoy great freedom of movement and continuous central reprogramming of the users' postural attitude.

Exercise with the HBP seems to be more efficacious in quickly improving balance and gait in the elderly compared to the effectiveness of exercise without the HBP, with a significant reduction of the risk of falls. In elderly, compensatory response to neuronal deficits or loss of neuronal specialization produce hyperactivation of specific brain areas, particularly in the pre-frontal cortex (PFC) to improve task accuracy, posture and gait. Age-related decline in brain activity can reduce these compensatory response especially in oldest old. A recent study showed that the HBP rehabilitation device may improve motor control by stimulating the PFC [3].

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Key words

Exoskeleton, elderly, rehabilitation.

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Endothelial progenitor cells senescence is accelerate by ROS Urea-induced

Anna Laura Colia[1], Maria D'Apolito[2], Massimo Pettoello-Mantovani[2], Michael Brownlee[3], Ida Giardino[1], Angela Bruna Maffione[1]

[1] Università, Università degli Studi di Foggia/Dipartimento di Medicina Clinica e Sperimentale, Foggia, Italia
[2] Università, Università degli Studi di Foggia/Dipartimento di Scienze Mediche e Chirurgiche, Foggia, Italia
[3] Università, Albert Einstein College of Medicine/Department of Medicine, New York, Stati Uniti D' America

Cardiovascular disease is one of the leading contributors to morbidity and mortality in chronic renal failure (CRF) patients. Endothelial injury caused by various cardiovascular risk factors is a responsable for atherosclerosis [1]. In uremic patients, evidence of endothelial dysfunction has been identified at early stages of the disease [2]. Endothelial progenitor cells (EPCs) play a key role in the maintenance of vascular integrity by promoting endothelial repair mechanisms and new endothelial growth. Uremia and compromised renal function are associated with a greater reduction in EPC availability and function [3]. Here we investigate the hypothesis that increased concentrations of urea associated with CRF increase ROS production directly in EPCs, causing abnormalities associated with coronary artery disease risk. Human EPCs were isolated from peripheral blood mononuclear cells of healthy donors and cultured in the presence or absence of 20 mmol/L urea. Urea, at concentrations seen in CRF, induces reactive oxygen species production in endothelial progenitor cells through the activation of mitochondrial and cytosolic mechanisms. Urea-induced ROS production impairs EC-CFU morphology and number, reduced the uptake and binding of Dil-Ac-LDL and lectin-1, and the ability to differentiate into CD31- and vascular endothelial growth factor receptor 2 positive cells. Moreover, urea-induced ROS generation accelerated the onset of EPC senescence, leading to a senescence-associated secretory phenotype (SASP). Normalization of mitochondrial ROS production prevented each of these effects of urea. These data suggest that urea itself causes both reduced EPC number and increased EPC dysfunction, thereby contributing to the pathogenesis of cardiovascular disease in CRF patients.

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Key words

Urea, EPC, ROS.

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Palmitate induces Ros generation and actives inflammation pathway

Anna Laura Colia[1], Maria D'Apolito[2], Massimo Pettoello-Mantovani[2], Michael Brownlee[3], Ida Giardino[1] and Angela Bruna Maffione[1]

[1] Università, Università degli Studi di Foggia/Dipartimento di Medicina Clinica e Sperimentale, Foggia, Italia
[2] Università, Università degli Studi di Foggia/Dipartimento di Scienze Mediche e Chirurgiche, Foggia, Italia
[3] Università, Albert Einstein College of Medicine/Department of Medicine, New York, Stati Uniti D' America

Obesity is a social and economical problem. The prevalence of obesity has risen to epidemic proportions and continues to be one of the major worldwide health problem. Concurrent with the global obesity epidemic, there is an increasing number of people of all ages developing chronic kidney disease associated with obesity(1). Although obesity is often associated with diabetes and hypertension, which are two of the most common risk factors for the development of end-stage renal disease (ESRD), obesity in itself can be an independent risk factor for both chronic kidney disease (CKD) and ESRD. The signaling pathways leading to renal pathology in obesity are not well understood. Here we investigate the hypothesis that physio-pathological concentration of palmitate induces reactive oxygen species production in conditioned human podocytes cell line through the activation of mitochondrial mechanisms , induces endoplasmic reticulum (ER) stress and increases HMGB1 expression and inflammation. The conditionally immortalized human podocytes cell line were differentiated and then treated with/without palmitate conjugated with BSA in a control and physio-pathological condition(2) and cell morphology, under sperimental conditions, were evaluated. Physio-pathological palmitate concentrations stimulate ROS generation in human podocyte and induces endoplasmic reticulum (ER) stress in podocytes. Moreover palmitate- induced ROS caused the activation of pro-inflammatory pathways (p65/NfkB and MCP-1), up-regulation of TGF-β well identified as a central mediator in renal fibrosis and induces a significantly increase of gene expression of HMGB1. Normalization of mitochondrial ROS production prevented each of these effects of palmitate. These results showed that palmitate at physio-pathological concentrations is able to induce ROS production, ER stress, inflammation, fibrosis and dysregulation of HMGB1 in human podocytes.

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Key words

Obesity, podocytes, ROS, palmitate.

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Identification of CLL stereotyped BCR in splenic B cell subsets using Next Generation Sequencing analysis

Monica Colombo[1], Davide Bagnara[2], Daniele Reverberi[1], Serena Matis[1], Martina Cardillo[1], Fabio Ghiotto[2], Silvia Ravera[3], Silvia Bruno[2], Giovanna Cutrona[1], Manlio Ferrarini[2], Franco Fais[2]

[1] Molecular Pathology Unit, IRCCS Ospedale Policlinico San Martino, Genova, Italia
[2] Department of Experimental Medicine, University of Genoa, Genoa, Italia
[3] Department of Pharmacology, University of Genoa, Genoa, Italia

Chronic Lymphocytic Leukemia (CLL) is characterized by the accumulation in the blood, bone marrow and lymphoid tissues of monoclonal B lymphocytes with a distinctive phenotype characterized by the expression of CD5 and CD23 antigens and a low expression of surface Immunoglobulins (sIG). The course of the disease is heterogeneous with some patients showing an aggressive form and a second group with a more stable disease; these groups are well defined by the absence or presence of somatic mutation on the IGHV gene, respectively.

In CLL clones the rearrangements of the B-cell Receptor (BCR) IGV regions exhibit distinctive features. In spite of the enormous diversity that can be created in the IGHV region up to 30% of CLL clones exhibit very similar (stereotyped) BCR which have been categorized in Subsets. Of these, 19 are most represented therefore are defined as "major Subsets". The identification of stereotyped receptors is codified on the basis of HCDR3 features in the context of the use of certain IGHV genes.

The above observations sustain the notion of an antigen drive role in CLL ontogeny. In addition, stimulation via BCR appears to be critical for leukemic cell survival/proliferation as suggested by the possibility to inhibit CLL growth by modern inhibitors of BCR-associated kinases. Furthermore, has been reported existence of a BCR homotypic interaction between BCR epitopes leading to receptor de-clustering and allowing the activation of autonomous signaling. Thus, this homotypic BCR–BCR interaction provides a further mechanism of CLL cell stimulation.

Whether CLL-like stereotyped BCR are present in the B-cell repertoire of healthy subjects remains largely unexplored. We addressed this issue in splenic B lymphocyte subpopulations and used a high throughput sequencing technique to collect IGV gene rearranged sequences. In particular, we separated and analyzed follicular mantle B cells (sIgDbright IgM+ CD38-CD27-), germinal center (GC) B cells (CD38+, sIgD-, CD24-), marginal zone B cells (sIgDlow, sIgM+, CD38-) and switched memory B cells (sIgD-sIgM-CD38-). In addition, we focus on IGV rearrangements using IGHV1 family genes because these are the most represented among the major subsets identified in CLL. Our study shows that CLL stereotyped receptors can be traced in a sizeable proportion of most of splenic B-cell subpopulations indicating that these receptors are part of the normal B-cell repertoire.

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Immune System/ Bone/ Fat cross-talk: the role of LIGHT/ TNFSF14

Silvia Colucci[1], Giacomina Brunetti[1], Sara Bortolotti[2], Giuseppina Storlino[2], Graziana Colaianni[2], Lorenzo Sanesi[2], Luciana Lippo[2], Carl F. Ware[3] and Maria Grano[2]

[1] Università degli Studi "Aldo Moro", Dipartimento di Scienze mediche di base, neuroscienze e organi di senso, Bari, Italia
[2] Università degli Studi "Aldo Moro", Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy, Italia
[3] Sanford Burnham Prebys Medical Discovery Institute, Infectious and Inflammatory Disease Center, La Jolla, Stati Uniti D' America

LIGHT/TNFS14 is a cytokine produced by immune cells. We demonstrated its role in regulating basal and pathological bone remodeling whereas other authors showed its pro-adipogenic role. Based on the "immune system/bone/fat" cross-talk, in this study we investigated whether LIGHT could be a new linker of this interaction. In bone marrow cell extracts of Tnfsf14-/- mice (KO), in which we proved the reduced trabecular bone, here we firstly detected a reduced expression of PPARγ, the key pro-adipogenic transcription factor. Consistently, we detected a lower weight of visceral and inguinal white adipose (iWAT) tissues respect to the WT mice, suggesting an impairment of adipocyte precursors in LIGHT deficient mice. Moreover, in the iWAT of these mice, we detected a lower number of brown adipocytes and lower mRNA levels of Wnt10b, involved in browning response, respect to WT mice, indicating that LIGHT-deficiency alters the adipose phenotype together with the bone one. These effects are mediated by immune cells, indeed, by using Rag-/Tnfsf14-mice lacking mature B/T-cells and LIGHT expression, the levels of PPARγ in bone marrow extracts and the number of brown adipocytes in iWAT are rescued respect to KO mice. These findings indicate LIGHT as new linker in immune system/bone/fat cross-talk and a potential target in obesity.

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Autoantibodies against brain neurons in Systemic Lupus Erythematosus

Giulia Corda[1], Matteo Piga[2], Alessandro Mathieu[2], Nicoletta Sainas[3], Gian-Luca Ferri[4], Maria Maddalena Angioni[2], Silvia Pinna[2], Cristina Cocco[3]

[1] Università di Cagliari, Scienze biomediche, Monserrato, Italia
[2] Università di Cagliari, Scienze Mediche e sanità pubblica, Cagliari, Italia
[3] Università di Cagliari, Scienze biomediche, Cagliari, Italia

Systemic lupus erythematosus (SLE), is an autoimmune disease characterized by multiple autoantibodies (AutoAbs). Neuropsychiatric syndromes appear when SLE affects brain neurons. However, the physiophatological mechanisms involved in Neuropsychiatric Lupus (NP-SLE) are unknown as well as the specific biomarkers. The project aim was to identify AutoAbs to neuronal epitopes possibly involved in specific NP-SLE clinical features. The search of AutoAbs was done through immunohistochemistry on perfused rat brains (4% paraformaldehyde), using sera from patients affected by: (1) NP-SLE (n=25), (2) SLE without neuropsychiatric features (n=39), (3) Multiple Sclerosis as no-autoimmune disease (MS, n=22) and age-matched controls (n=82). When sera were tested (1:60–600), a high percentage of patients contained AutoAbs labeling neurons of the cortex, hippocampus and cerebellum (72% and 30.7% in NP-SLE and SLE, respectively) while control subjects and MS patients were negative. Patients' sera stained a high number of perikarya within the cortex as well as within hippocampus and cerebellum (ImageJ optical density: p= 0.0003 and 0.00000001 respectively, positive patients vs. controls). To investigate the neuonal types involved in the autoimmune reaction, we carried out double stainings with the relevant neurotransmitters/proteins. No colocalization profiles were found with antibodies to the vesicular acetylcholine transporter, GAD-65 or glutammate but we observed that the staining of patients' sera was largely revealed within the same cells labeled also by the anti NeuN antibody in all the areas studied (cortex, hippocampus and cerebellum). Many patients showed a virtually 90% of colocalization profile, while other ranged 50–75%. The NeuN protein is mainly localized in the nuclei of specific brain neurons while NeuN antibodies have applied in the differential morphological diagnosis of cancer. The role of AutoAbs to NeuN in our cohort of patients remains to be identified. In conclusion, we found high titer neuronal AutoAbs in the majority of the analyzed NP-SLE/SLE patients, probably reacting against the NeuN protein.

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Morphological correlation between Psoriasis Vulgaris and Guttate and a 3D in vistro psoriatic microenvironment

Laura Cornaghi[1], Giulia Lombardo[1], Elena Donetti[1] and Francesca Prignano[2]

[1] Università degli Studi di Milano, Department of Biomedical Sciences for Health, Milan, Italia
[2] Università degli Studi di Firenze, Department of Surgery and Translational Medicine, Florence, Italia

Psoriasis is characterized by a great variety of clinical manifestations and they vary according to different phenotypes. Guttate or eruptive psoriasis (PG) [1] shares genetic similarities with psoriasis vulgaris (PV) [2], the most represented clinical form. Cell types and molecules of both the innate and adaptive immunosystem are involved in the pathogenesis/progression of the disease, but several data concerning the early phase of the disease lack. A three dimensional model of organotypic cultures of normal human skin biopsies represents an useful approach for investigating the cellular mechanism(s) involved in the early epidermal response to proinflammatory psoriatic cytokines [3;4]. The aim of this study was to compare cellular proliferation, the expression of Toll-like receptors (TLR) 7 and 9, and the innate immune response in lesional and perilesional skin of patients affected by PV or PG and in our model of organotypic culture after exposure to a cytokine mix (IL-17, IL-22, IL-23, and TNF-alpha) in a time-course study. Parallel ultrastructural analysis was performed. Keratinocyte proliferation in non lesional skin of both PG and PV was comparable, with PV lesional area as the most proliferative. In PG cell proliferation was exclusively localized in the basal layer. After mix incubation, a progressive decrease of cell proliferation was detected as an early response to proinflammatory stimulus. TLR9 was present in the granular layer of non lesional skin and mix samples and in the suprabasal layers of PV/PG lesional skin. TLR7 distribution was clearly different in each group, highlighting a specific response to the specific microenvironment.

In conclusion, these results prove that a psoriatic microenvironment is able to modify the expression of TLR7 and TLR9 in our model from human skin. These observations provide also new insights regarding the specific localisation of these two receptors and this could be an important detail for the many new small molecules targeted against TLRs for the therapy of chronic inflammatory disease, including psoriasis.

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Key words

Cytokine, keratinocyte proliferation, Toll-like receptors.

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Dental pulp during orthodontic tooth movement: immunohistochemical study

Giuseppina Cutroneo[1], Cecilia Spoto[2], Elena Filippini[2], Angela Militi[2], Marco Matarese[2], Giovanna Vermiglio[2], Michele Runci Anastasi[2], Antonio Centofanti[2]

[1] Department of Clinical and Experimental Medicine,University of Messina, Messina, Italia
[2] Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italia

Dental pulp is formed by connective tissue and it is localized in cavity of tooth. In the dental pulp is verified all defensive processes so as in all connective tissues. This tissue is continuously exposed to mechanical stresses during the phases of orthodontic therapy [1]. The progression of the inflammatory process in human pulp fibroblasts apparently depends on stimulation by neuropeptides and production of inflammatory cytokines. A recent report described apoptosis in dental pulp tissues of rats undergoing orthodontic treatment [2]. The literature shows conflicting results for correlation of pulpal changes incident to orthodontic force. Some reports suggested permanent damage to pulpal tissue from orthodontic force, but others claimed no significant long-lasting effects on the pulp [2]. However tissue reactions incident to orthodontic tooth movement depend mainly on the pattern of stress-strain distribution in the paradental tissue. In recent years, the alterations in pulpal vasculature and blood flow in response to orthodontic force have gained much attention. The clinical impact of these studies was to determine whether any alterations in pulpal tissue could jeopardize the long-term vitality of the teeth. In this study we analyzed in vivo human samples of dental pulp of 18 subjects, scheduled for orthodontic treatment at the Department of Dentistry of Messina University. The premolars were subjected to a buccally directed tipping force (50 g) with Nickel Titanium closed coil spring (American Orthodontics). On dental pulp samples, after extraction of the premolars, were monitored by analysis of the expression of different proteins that compose it and by analysis of Vascular Endothelial Growth Factor (VEGF). We have demonstrated an initial decrease in blood flow at 7 days, followed by an increase in blood flow at 28 days.

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Key words
Dental pulp, VEGF, orthodontic force.

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NAP modulates inflammatory cytokines release and counteracts outer blood retinal barrier breakdown in diabetic rat retina

Agata Grazia D'Amico[1], Grazia Maugeri[2], Daniela Maria Rasà[3], Concetta Federico[3], Salvatore Saccone[3], Claudio Bucolo[4], Velia D'Agata[5]

[1] San Raffaele Open University of Rome, Department of Human Science and Promotion of quality of Life, Roma
[2] Section of Human Anatomy and Histology, University of Catania, Department of Biomedical and Biotechnological Sciences, University of Catania, Italy, Catania
[3] Section of Animal Biology, University of Catania, Department of Biological, Geological and Environmental Sciences, University of Catania, Italy;
[4] Section of Pharmacology, University of Catania, Department of Biomedical and Biotechnological Sciences, University of Catania, Italy,
[5] Section of Human Anatomy and Histology, University of Catania, Department o Biomedical and Biotechnological Sciences, University of Catania, Italy,

Diabetic retinopathy (DR) is a common microvascular complication of diabetes. Prolonged hyperglycaemia triggers inflammatory response mediated by release of some cytokines. Combination of these events leads to thinning retinal thickness and blood retinal barrier (BRB) impairment. Many evidences have shown the protective effect played by a small peptide, known as NAP, in some retinal diseases [1]. To this regard, we have recently demonstrated that it reduces apoptotic cell death and interferes with HIFs/VEGF system during early stage of DR [2, 3]. However, the effect of NAP on inflammatory process affecting hyperglycaemic retina has not been identified, yet.

In the present work, we have studied the effect of this peptide in retina of STZ-injected rats, mimicking an in vivo model of diabetes. Furthermore, we have also characterized its role on outer-BRB impairment following exposure of human retinal epithelial cells (ARPE 19) to hyperglycaemic/inflammatory insult.

Results have demonstrated that a single intraocular injection of NAP modulates inflammatory cytokines expression by downregulating IL-1β and related receptors and upregulating IL-1Ra level in diabetic rat retina. These data have been confirmed by immunofluorescence analysis using confocal microscopy. IL-1β immunosignal increased in all retinal layers of diabetic rats as compared to control. NAP treatment reduced cytokine immunoreactivity in inner plexiform layer (IPL), outer plexiform layer (OPL) and rod and cone layer (RCL). We have also evaluated the effect of NAP on the outer-BRB integrity. ARPE19 monolayer hyperpermeability induced by hyperglycaemic/inflammatory insult was significantly reduced after NAP addition in the culture medium. This effect is also mediated through enhancement of tight-junction related proteins expression levels. In conclusion, characterization of NAP action mechanism may be useful to develop a new strategy to prevent retinal damage during DR.

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Key words

Diabetic retinopathy, NAP, blood retinal barrier, inflammatory cytokines.

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Urea-induced ROS caused endothelial dysfunction in chronic renal failure

Maria D'Apolito[1], Anna Laura Colia[2], Massimo Pettoello-Mantovani[1], Michael Brownlee[3], Ida Giardino[2] and Angela Bruna Maffione[2]

[1] Dipartimento di Scienze Mediche e Chirurgiche, Università degli Studi di Foggia, Foggia, Italia
[2] Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Foggia, Foggia, Italia
[3] Department of Medicine, Albert Einstein College of Medicine, New York, Stati Uniti D' America

The pathogenic events responsible for accelerated atherosclerosis in patients with chronic renal failure (CRF) are poorly understood [2] .Here we investigate the hypothesis that concentrations of urea associated with CRF and increased ROS production in adipocytes3 might also increase ROS production directly in arterial endothelial cells, causing the same pathophysiologic changes seen with hyperglycemia [1]. For these purpose, confluent primary human aortic endothelial cells (HAECs) were incubated with either 20mM urea or with 20mM mannitol used as osmotic control, for 48 hours. Urea induces mitochondrial reactive oxygen species in HAEC and cause pro-inflammatory changes in endothelial cells. Breafly, PGI2 Synthase activity, NFκB p65 and NFκB-specific target genes mRNA expression (MCP-1 and VCAM-1) [3] and their protein levels were evaluated. Moreover, we have shown that urea-induced ROS production increases PKC activity, hexosamine pathway activity and intracellular AGE formation in HAEC. In addition, urea-induced ROS decrease GAPDH activity, increase DNA strand breaks and increase PARP activity in HAEC. In summary, urea increases mitochondrial ROS production in arterial endothelial cells, thereby activating pro-atherosclerotic pathways and directly inactivating PGI2 synthase, a critical endothelial-specific antiatherosclerotic enzyme in vitro. The present findings provide further insight into the underlying mechanisms that contribute to the enhanced cardiovascular risk associated with chronic renal failure.

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Key words

Urea, endothelial cells, ROS.

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The graph theory applied to the study of the human locomotor system: a simulated amputation changes the characteristics of the system

Daniele Della Posta, Massimo Gulisano, Ferdinando Paternostro, Ugo Santosuosso, Cristiana Veltro and Piergiorgio Francia

Università degli Studi Di Firenze, DMSC, Firenze, Italia

The study of the relationships between the different structures of the human locomotor system still to date raises great interest. In fact, the human body networks and in particular the myofascial system "myofascial system network" underlie posture and movement and new knowledge could be useful and applied to many fields such as medicine and prosthetics. The hypothesis of this study was to verify the possibility of creating a structural network representing the human locomotor system as well as to study and describe the relationship between the different structures considered.

The graph theory was applied to a network of 2339 body parts (nodes) and 7310 links, representing the locomotor system. The open source platform software Cytoscape was used for data entry (nodes and links) as well as for debugging. In addition, the "Network Analyzer" plugin was used for the descriptive statistics of the network obtained. In order to achieve a better rendering, the results of the network parameters gained were then imported into Gephi (www.Gephi.org).

At the end of this procedure, we obtained a image of a human being in an orthostatic position with a precise distribution of the nodes and links.

By simulating the common amputations at the level of the foot and leg (as for necrotic complications of diabetes) the balance between the parts and the whole structure of the graph are profoundly modified.

Key words

Locomotor system, Graph theory, Tensegrity, Diabetes.

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Innovative experimental approach for the morphological characterization of cancer stem cells spheroids

Anna Di Vito[1], Chiara Mignogna[2], Flavia Biamonte[3] and Tullio Barni[1]

[1] Magna Græcia University, Department of Experimental and Clinical Medicine, Catanzaro, Italia
[2] Magna Græcia University, Department of Health Science, Catanzaro, Italia
[3] University Magna Graecia, Department of Experimental and Clinical Medicine, Catanzaro, Italia

It's widely accepted the involvement of EMT in the onset of cancer metastasis given its ability to enable both cancer cells dissemination and self-renewal. Many researches have showed that the generation of circulating tumor cells (CTCs) with stem cells-like properties is responsible for metastasis successful; despite the advances in CTCs detection systems, the molecular characterization appears tough since their existence in a very small amount. For this purpose, increasing numbers of studies have developed multiple methodological tools for the culture of cancer cells exhibiting stem cells-like properties, including 3D-spheroids propagation [1]. Our group have previously showed that ferritin heavy chain (FHC) exerts a negative role on both ovarian cancer stem cells expansion and EMT, via the application of the in vitro 3D spheroid assay [2]. Here, we applied an innovative experimental approach for the characterization of cancer stem cell spheroids. FHC-silenced (shFHC) and control shScr SKOV3-cells were cultured in ultra-low attachment plates and maintained with RPMI supplemented with 10% FBS. The first generation of spheroids derived from a 10-day cultures of FHC-silenced and control shScr SKOV3-cells were characterized for their number, size and morphology. The expression of proliferative markers, extracellular matrix components and stem cells markers was evaluated in both adherent and spheroids cells using the cell block technique. First, shFHC cells showed a significant greater number and larger 3D spheroids than control shScr SKOV3-cells in 10-day cultures. Proliferative activity as determined by Ki-67 immunoreactivity showed an even distribution in adherent cells. Otherwise, in FHC-silenced spheroids, proliferation was predominant in the peripheral areas. The spheroid cell cultures also exhibited a distinct network of CD44 and CD56. We suggest our experimental approach as a useful tool for testing the role of FHC in the acquisition of stem cells-like properties.

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Key words

Cancer stem cells spheroids, cell block technique, ferritin.

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Moringin Treatment on Mesenchymal Stem Cells from Periodontal Ligament Induces Neural Differentiation

Francesca Diomede[1], Letizia Romeo[2], Ilaria Merciaro[1], Emanuela Mazzon[2] and Oriana Trubiani[1]

[1] Università "G. d'Annunzio" Chieti e Pescara, Dipartimento di Scienze Mediche, Orali e Biotecnologiche, Chieti
[2] IRCCS Centro Neurolesi "Bonino Pulejo", Laboratorio di Neurologia Sperimentale, Messina

Neurodegenerative disorders are a broad-ranging and highly complex group, with diverse etiologies and frequently overlapping clinical manifestations and marked by the loss of neurons within the brain and/or spinal cord [1]. The therapeutic strategies for neurodegenerative diseases still represent a vast research field because of the lack of targeted, effective and resolutive treatment for neurodegenerative diseases [2]. The use of stem cell-based therapy is an alternative approach that could lead to the replacement of damaged neuronal tissue[3]. For this purpose, adult mesenchymal stem cells (MSC), including periodontal ligament stem cells (hPDLSCs), could be very useful for their differentiation capacity, easy isolation and the ability to perform an autologous implant [4]. The aim of this work was to test whether the Moringin [4- (α-L-rhamnosyloxy) benzyl isothiocyanate; GMG-ITC], an isothiocyanate extracted from Moringa oleifera seeds, was able to produce an effect on hPDLSCs in terms of neural differentiation profile expression. Recently moringin effects have attracted the attention of scientists for its chemopreventive activity. Moringin treatment showed an increased expression of genes involved in neuron cortical development by means next-generation transcriptomics sequencing analysis, in particular the profile is near to neuron belonging to upper and deep cortical layers. Moreover, genes involved in osteogenesis and adipogenesis were modulated with moringin treatment although with a lower fold change compared to upregulated genes involved in neuronal differentiation. Moringin did not induce the expression of oncogenes and it can be considered a safe treatment. bA better understanding of the mechanisms underlying neurodegeneration should lead to more effective, disease-modifying treatments in the future.

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Key words

Oral stem cells, moringin, neurodegenerative disease, neurogenic differentiation.

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Anatomical variation of infraorbital foramen position according to sex, side and cranium size

Claudia Dolci[1], Alessia Borlando[2], Annalisa Cappella[2], Francesca M.E. Rusconi[1], Cristina Cattaneo[2]

[1] Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milano, Italia
[2] LABANOF, Laboratorio di Antropologia e Odontologia Forense, Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milano, Italia

Anaesthetic procedures focusing on the infraorbital nerve play an important role in dentistry and plastic surgery, as well as for treatment of pharmacologically resistant forms of trigeminal neuralgia; therefore, the anatomical localization of the infraorbital foramen (IOF) has been widely analysed by literature in relation with sex and side [1,2]. However, no study has so far considered the possible effect of general cranium size on the sexual dimorphism of measurements used for pinpointing the position of infraorbital foramen. This study aims at providing additional data concerning the position of infraorbital foramen assessing the possible influence of cranium size on sexual dimorphism. Three measurements (distances from anterior nasal spine and inferior orbital rim, and angle at the intersection between the line from anterior nasal spine and the transversal plane parallel to the Frankfurt plane) were assessed on 100 skulls belonging to a contemporary skeletal collection with known sex and age (50 males and 50 females, mean age 68.4±19.1 years). Maximum cranial length, maximum cranial breadth, cranial heigth and bizygomatic breadth were measured as well, together with horizontal cephalic index and Giardina Y-index. Possible differences according to sex and side were assessed through two-way ANOVA test ($p<0.05$). Measurements showing statistically significant differences according to sex were further assessed through one-way ANCOVA test including cranial measurements and indices as covariates ($p<0.05$). Statistically significant differences according to sex and side were found respectively for the distance from the anterior nasal spine and the angle at infraorbital foramen ($p<0.05$). One-way ANCOVA test verified that the sexual dimorphism of the distance from the anterior nasal spine distance was independent from the all the assessed measurements and indices of cranium. The present study proved that sexually dimorphic parameters useful for the localization of IOF do not depend upon the cranium size.

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Key words

Infraorbital foramen, skeletal collection, maxillofacial surgery, maxillary nerve block.

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Association between morphometric facial features and other systemic manifestations in patients with Marfan syndrome

Claudia Dolci[1], Claudia L.P. Ferreira[2], Susan Marelli[3], Giuliana Trifirò[4], Alessandro Pini[3]

[1] Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milano, Italia
[2] Departamento de Fonoaudiologia, Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brasile
[3] Centro Malattie Rare, Marfan Clinic, U.O. Cardiologia, ASST Fatebenefratelli-Sacco, Milano, Italia
[4] U.O. Pediatria, ASST Rhodense, Rho (Milano), Italia

Marfan syndrome (MFS) is a rare hereditable disorder of connective tissue which results from mutations in the gene encoding fibrillin-1 (FBN1), an extracellular matrix component found in non-collagenous microfibrils. To date, MFS is a clinical diagnosis mostly based on family history and alterations in skeletal, ocular and cardiovascular systems. Early recognition of MFS is essential to prevent its most severe complications but it can be difficult, because of the phenotypic variability of the syndrome. In a recent study we analyzed facial dysmorphism in MFS, defining some quantitative morphometric features which could elude a mere visual inspection of the patients [1]; in the current study we investigated their association with other systemic manifestations of the syndrome. Facial linear distances and angles of 41 Italian adult subjects with MFS (14 males, 36±16 years; 27 females, 41±14 years) were computed from the 3D coordinates of soft-tissue landmarks obtained by stereophotogrammetry [2]. Corresponding z score values were calculated comparing patients with 779 healthy reference subjects matched for sex and age. For each facial measurement, patients were assigned into two groups on the basis of z-score values lower or higher than ½1.5½. Patients underwent multidisciplinary clinical examinations in order to evaluate the presence/absence of systemic signs of the syndrome, according to revised Ghent nosology. Patients with ectopia lentis (61%) or pectus deformities (51%) showed a more reduced facial width/height ratio (Student's t test, $p<0.05$), the value being mainly influenced by a longer face in patients with ectopia lentis ($p<0.01$) and a narrower face in patients with pectus deformities ($p<0.01$). A significant association between ectopia lentis and facial width/height ratio was found (Fisher's exact test, $p<0.05$). Results are promising and suggest that quantitative features of the face should be included among information which clinicians should be aware to improve the recognition of MFS; nevertheless they need to be confirmed on more patients.

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Key words

Facial anthropometry, Marfan syndrome (MFS), stereophotogrammetry, clinical correlation.

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Morphological features induced by interleukin 17 in a 3D organotypic cultures of normal human skin are promptly reverted by a specific biological inhibitor

Elena Donetti[1], Laura Cornaghi[1], Giulia Lombardo[1], Francesca Arnaboldi[1], Franz Baruffaldi Preis[2] and Francesca Prignano[3]

[1] Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute, Milano, Italia
[2] Istituto Ortopedico Galeazzi, Istituto Ortopedico Galeazzi, Milano, Italia -
[3] Università degli Studi di Firenze, Department of Surgery and Translational Medicine, Firenze, Italia

Psoriatic plaque is the result of a strict interaction among epidermal cells, immune system, and soluble cytokines. Interleukin 17 (IL-17) is a well-known proinflammatory psoriatic cytokine mainly produced by the T helper subclass Th17. In the last decade we standardized a 3D model organotypic cultures of normal human skin for studying the early, intrinsic and specific effects induced by IL-17. We demonstrated that IL-17 elicited Langerhans cell (LC) activation and migration, keratin 17 expression, Toll like receptor 7 and 9 expressions and profoundly altered filaggrin expression, without affecting the suprabasal distribution of keratin 10 and keratin 14. Moreover, this cytokine early inhibited keratinocyte proliferation, strongly suggesting that this event can be the basis for the response to injury leading to the psoriatic characteristic hyperproliferation observed in lesional plaques. In the present study, we incubated bioptic skin fragments obtained after aesthetic surgery of healthy young women (n=5) with i) IL-17 alone, ii) with a combination of IL-17 and an IL-17 biological inhibitor, iii) with the IL-17 biological inhibitor alone. Control samples were in parallel cultured. Incubation lasted for 24 and 48 hours with skin at the air-liquid interface. Immunofluorescence experiments and transmission electron microscopy (TEM) analysis were carried out. Samples incubated with the IL-17 biological inhibitor were comparable to controls. By immunofluorescence, the combination reverted IL-17-induced effects at all considered time-points. By TEM, LCs appeared less activated as shown by the paucity of Birbeck granules and the highly dispersed nuclear chromatin. The epidermal ultrastructure was comparable in all groups, with well-preserved desmosomes, interspersed keratin filaments and terminally differentiated granular keratinocytes/corneocytes. These results highlight the clinical usefulness of this experimental approach for identifying the early psoriatic processes that can be modulated by last generation biological agents.

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Thrombin in the peripheral nervous system as regulator of Schwann cell neurotrophic potentials

Cinzia Fabrizi[1], Elena Pompili[1], Viviana Ciraci[1], Stefano Leone[2], Roberto Matassa[1], Giuseppe Familiari[1], Lorenzo Fumagalli[1]

[1] Department of Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza University, Roma
[2] Department of Science, University of Roma Tre, Roma

Coagulation and inflammation are tightly and reciprocally regulated. Inflammation initiates clotting, decreases the activity of natural anticoagulant mechanisms and impairs the fibrinolytic system. Thrombin is the main effector protease in hemostasis and it also plays a role in various non-hemostatic biological and pathophysiologic processes, predominantly mediated through activation of protease-activated receptors (PARs).

PAR-1 is the main thrombin receptor in peripheral nerves and it is highly expressed at the level of non-compacted Schwann cell myelin microvilli of the nodes of Ranvier.

After nerve crush thrombin is locally generated at the site of injury [1]. Thrombin generation is generally believed to have long-term beneficial effects for tissue repair. In this connection, our previous data indicate that PAR-1 activation on Schwann cells favors their ability to promote axonal regrowth after lesion [2]. On the other hand, it has also been reported that an excessive generation of thrombin can be detrimental for nerve functions. Our present data obtained by confocal and environmental scanning electron microscopy (ESEM) show that the morphology of the nodes appears to be deeply affected by high concentrations of thrombin (0.1-1 microM). In thrombin-treated nerves we also observe a redistribution of S100b to the paranodes and to the Schmidt-Lanterman incisures.

Controlling thrombin concentration may preserve neuronal health during nerve injury and represent a novel possible target for pharmacologic therapies.

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Key words

Schwann cells, peripheral nervous system, regeneration, thrombin, protesase-activated receptors.

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A New AKT in RNA Editing: AKT Associates with and Phosphorylates the Adenosine Deaminases, ADAR-1 and -2

Irene Faenza[1], William Blalock[2], Alberto Bavelloni[3], Enrico Focaccia[4], Giulia Ramazzotti[1], Giulia Adalgisa Mariani[1], Desiree Martini[1], Lucia Manzoli[1] and Lucio Cocco[1]

[1] Department of Biomedical Sciences, University of Bologna, Bologna, Italia
[2] Institute of Molecular Genetics-National Research Council of Italy (IGM-CNR), UOS Bologna, Bologna, 40126 Italy, Rizzoli Orthopedic Institute (IOR), Bologna, Italia (
[3] Laboratory of Musculoskeletal Cell Biology, Rizzoli Orthopedic Institute (IOR), Bologna, Italia
[4] Institute of Molecular Genetics-National Research Council of Italy (IGM-CNR), Rizzoli Orthopedic Institute (IOR), Bologna, Italia

A central component of many non-infectious diseases is a chronic inflammatory state resulting in selected metabolic alterations affecting DNA repair, apoptosis and autophagy, metabolism and protein synthesis. The proto-oncogene AKT and the dsRNA-dependent kinase PKR are two of the best known kinases demonstrated to significantly influence these mechanisms. In order to identify signaling intermediates common to both kinases, we isolated potential AKT substrates from CCRF-CEM nuclear lysates, using a phospho-AKT substrate antibody and tandem mass spectrometry (MS/MS); then compared these to a list of proteins known to interact with PKR. Among the proteins of interest identified was ADAR1p110, the adenosine deaminase acting on dsRNA. It was determined that the ADAR1 identified corresponded to ADAR1p110, the constitutively expressed and most abundant form of ADAR1 in the nucleus. It was found that not only AKT1, but also AKT2 and AKT3 interact with ADAR1p110 as well as ADAR2 and phosphorylate these RNA editases. The ADARs and AKTs were found to reciprocally influence each other with AKT family members altering ADAR1 and 2 expression; and ADAR1 and ADAR2 suppressing AKT expression. Thus, AKT activation may have a direct and major impact on RNA processing.

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Cancer stem cell miRNAs in early diagnosis and prognosis of colorectal cancer patients

Cristiano Farace[1], Andrea Pisano[1], Fabrizio Scognamillo[2], Andrea Montella[1] and Roberto Madeddu[1]

[1] Dept. of Biomedical Sciences, University of Sassari, Sassari, Italia
[2] Dept. of Surgical, Microsurgical and Medical Specialities, University of Sassari, Sassari, Italia

Colorectal cancer is a leading tumor whose worldwide patients' mortality is still increasing in the last years, calling for more early and robust diagnostic and therapeutic procedures. To this end, a number of molecules have been investigated as diagnostic biomarker and therapeutic targets in biological matrix of patients, but a real translation into the clinical practice is still missing. Cancer stem cells (CSC) denote a small subpopulation of the cancer cell with the unique ability to initiate and propagate the tumor mass. Moreover, CSC have been shown to play a crucial role in epithelial-to-mesenchymal transition, a phenotype switch at the bottom of the metastatic process. Hence, CSC and their regulatory molecules represent optimal cellular and molecular targets in cancer eradication strategies. On the other hand, microRNA (miRNA) represent a class of small RNA with gene expression function and high potential as biomarker. In presence of a specific miRNA, the related mRNA is committed degradation, resulting in the specific downregulation of its expression. Since miRNAs are 19-23bp long, these short molecules display high stability and integrity in body tissues and fluids. Hence, miRNA represent optimal tissue- and blood-based candidate biomarker in diagnosis, prognosis and therapeutic intervention and prediction.

Here, we unraveled the role of colorectal CSC-related miRNA in colorectal cancer patients' clinic. Firstly, we assessed through qRT-PCR a set of 9 miRNA in CSC models derived from 3 different established cell lines (HCT-116, HT-29 and T84). Then, the same miRNA were investigated in tumor and healthy tissues, and blood of 13 colorectal cancer patients, and statistical analysis tasks applied to highlight miRNA contribute in colorectal cancer clinic. The results analysis have shown that 1) CSC display different miRNA pattern in relation to the molecular background of the established cells; 2) some CSC-related miRNA are overexpressed in cancer tissues and blood, corroborating our hypothesis; 3) a pattern of miRNA was correlated with cancer staging, sex, AST, alkaline phosphatase, LDL, triglicerides; 4) some miRNA were positively correlated with metastases (miR16). Hence, we found a pattern of CSC-related candidate miRNA which play important role in colorectal patients' clinic, even at level of cancer staging and metastases, paving the way to new miRNA-based biomarkers useful in colorectal cancer early diagnosis and prognosis.

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Testosterone and physical execise positively modulate synaptic ultrastructure in old mice

Patrizia Fattoretti[1], Manuela Malatesta[2], Carlo Zancanaro[3]

[1] INRCA, Center for Neurobiology of Aging, Ancona, Italia
[2] Università di Verona, Dip. di Neuroscienze biomedicna e Movimento, Verona, Italia
[3] Università di Verona, Dip. di Neuroscienze Biomedicina e Movimento, Verona, Italia

Androgenic steroids affect numerous aspects of central nervous system function inclusive of cognition [1]. The hippocampus is an anatomical model to investigate the neuronal structural dynamics because of its prominent plasticity. Androgens modulate the structure and function of the hippocampus by affecting patterns of dendritic arborisation, dendritic spine and spine synapse density. Synaptic contacts, synaptic strength, and plasticity are reduced in the ageing hippocampus as well as neurogenesis. However, evidence of a structural effect of testosterone on the aged hippocampus of normal rodents is lacking. Physical exercise is beneficial to the ageing hippocampal. Recently, we showed that aerobic physical exercise positively modulates synaptic ultrastructural dynamics in the old mice hippocampus [2]. In this work, old (27mo) mice were randomly assigned to one of four groups including 5 mice each: control (C), testosterone administration (10 mg/kg once a week, TA), treadmill training (30 min a day, five days a week at belt speed 8m/min, TT), and treadmill training plus testosterone administration (TTTA). The experimental period was one month. One-way ANOVA of morphometric ultrastructural results obtained in the inner molecular layer of the hippocampal dentate gyrus showed that the numeric and surface density of synapses were significantly different within the four groups ($p<0.05$) whereas the average size of synaptic contacts was not. Post-hoc analysis (Bonferroni's) showed that both testosterone and physical exercise tended to increase synaptic density, the combination of the two treatments yielding no further increase. It is concluded that testosterone and physical exercise are both able to positively modulate synaptic density in the inner molecular layer of the hippocampal dentate gyrus without affecting synaptic size.

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Key words

Testosterone, physical exercise, synapse, morphometry.

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Extracellular matrix remodeling of subcutaneous small resistance arteries during essential hypertension

Gaia Favero[1], Francesca Bonomini[1], Lorena Giugno[1], Enrico Moretti[1], Caterina Franco[1], Lorenzo Franceschetti[1], Damiano Rizzoni[2] and Rita Rezzani[1]

[1] Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italia
[2] Division of Medicine, Department of Clinical and Experimental Sciences, University of Brescia and Istituto Clinico Città di Brescia, Brescia, Italia

Remodeling in microvascular structure may impair organ flow reserve and may be important in the support and also in the progressive worsening of hypertensive disease [1, 2]. In the development of hypertensive microvascular remodeling, a relevant role may be played by changes in extracellular matrix proteins [3]. Aim of this study was evaluate some extracellular matrix components within the tunica media of subcutaneous small arteries of 9 normotensive subjects and 12 essential hypertensive patients. Subcutaneous small resistance arteries were dissected and mounted on an isometric myograph and the tunica media to internal lumen ratio was measured. In addition, fibronectin, laminin, transforming growth factor-beta1 (TGF-β1) and emilin-1, important extracellular matrix components, were evaluated together with total collagen content and collagen subtypes. Small arteries of normotensive controls presented less total and type III collagen amounts with respect to hypertensive patients. Fibronectin and TGF-β1 contents were significantly greater in essential hypertensive patients, compared with normotensive subjects; while laminin and emilin-1 contents were lesser in essential hypertensive patients with respect to normotensive controls. Furthermore, a significant correlation was observed between fibronectin content and media to lumen ratio. In conclusion, our results indicated that in small resistance arteries of patients with essential hypertension may be detected a relevant fibrosis with increased fibronectin and TGF-β1 tunica media contents and decreased laminin and emilin-1 contents. These extracellular matrix changes might be involved in the remodeling of human small resistance artery and so extracellular matrix proteins may be possible targets for new anti-hypertensive drugs.

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Key words

Extracellular matrix, essential hypertension, microvascular remodeling, small resistence artery.

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Surgical anatomy of the parapharyngeal space: a multiperspective, quantification-based study

Marco Ferrari[1], Alberto Schreiber[1], Davide Mattavelli[1], Davide Lombardi[1], Vittorio Rampinelli[1], Francesco Doglietto[2], Piero Nicolai[1], Luigi Fabrizio Rodella[3]

[1] Unit of Otorhinolaryngology – Head and Neck Surgery, Department of Surgical Specialties, Radiological Sciences, and Public Health,University of Brescia, Brescia, Italia
[2] Unit of Neurosurgery, Department of Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, Brescia, Italia
[3] Section of Anatomy and Physiopathology, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italia

Several surgical approaches to the parapharyngeal space (PPS) have been proposed. An objective description of advantages and limitations of the surgical routes is lacking [1, 2]. Ten cadaver heads were dissected using the transnasal (medial, lateral), sublabial, transoral (transpharyngeal, transvestibular, transmandibular), transcervical (transcervical, transparotid, transmandibular, transmastoid), and type C and D infratemporal approaches. Neurovascular and musculoskeletal structures encountered were analyzed. A navigation-based quantification of working volume and exposure of PPS compartments was accomplished. Transnasal approaches exposed the upper PPS, though with limited working volume. Transoral approaches exposed the middle PPS, minimizing neurovascular structures crossed. Only transcervical and skull base approaches exposed the entire PPS exposing several neurovascular structures. A tentative systematization of the surgical approach(es) to PPS in relation to different targets is provided: unicompartmental resection can be performed with a single, conservative access, whereas multicompartmental dissections frequently require a wider or multiportal approach.

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Key words

Parapharyngeal space, surgery.

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Interaction between mineral and skeletal homeostasis in rats fed different calcium content diets with/without PTH (1-34)

Marzia Ferretti[1], Francesco Cavani[1], Jessika Bertacchini[1], Marta Checchi[1], Maria Sara Magarò[2] and Carla Palumbo[2]

[1] Università di Modena e Reggio Emilia, Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Modena, Italia
[2] Università di Modena e Reggio Emilia, Dipartimento di Scienze Mediche e Chirurgiche Materno-Infantili e dell'Adulto, Modena, Italia

Aim of the study is to analyze how mineral and skeletal homeostases influence both the bone loss due to calcium-diet deprivation and the successive bone mass recovery after calcium-diet restoration, with/without concomitant PTH(1-34) administration. The present investigation, performed on 3-months-old Sprague-Dawley male rats, is the second step (concerning the normal-diet restoration) of a previous one (concerning the calcium-free diet)1, conducted to determine the factors mainly affecting amounts and deposition sites during bone mass recovery. Observations emerged allowed to: 1) define times and modalities of bone mass recovery; 2) identify the most involved bony architecture (trabecular or compact) in bone response to dietary regimen; 3) verify eventual effects of intermittent PTH(1-34) administration in modifying the process of bone recovery. Histomorphometric evaluations of static/dynamic bone parameters and immunohistochemical analysis for Sclerostin expression were conducted on vertebral bodies and femurs. Serum analysis for calcium, phosphorus, osteoprotegerin, bone alkaline phosphatase, CrossLaps and PTH(1-34) was also performed. Results evidenced the greater involvement of trabecular bone with respect to the cortical one, in answering to different calcium diet content, and the effect of PTH mostly in the recovery of trabecular bony architecture. Observations clearly show that the integration between mineral and skeletal homeostases occurs in determining bone response in different sites of the skeleton (axial or appendicular) with different architecture. As expected, Sclerostin expression resulted to be higher in animals fed calcium-deprived-diet with respect to the other animal groups. In conclusion, the main finding of the present study is to strengthen the importance of interplay between mineral and skeletal homeostases in modulating and guiding bone answers to mineral alterations and to underline that the more involved bony architecture is the trabecular one, the most susceptible to the dynamical balance of the two homeostases. Clinical strategies in recovery of any skeletal impairment (of metabolic or traumatic origin) must take into account: i) mostly the type of the bone architecture involved regardless of the amount of bone mass to recover and ii) the evidence that the main target of PTH(1-34) is the trabecular bone. Thus, therapeutic treatments cannot fail to consider these aspects to optimize the drug effect and speed up the recovery.

This work was supported by grants from FAR-dip. 2017 and from Eli-Lilly, USA, which also provided PTH (1-34).

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Key words

Calcium diet content, mineral/skeletal homeostasis, trabecular bone, PTH(1-34), rat.

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How nuclear phospholipase C beta 1 can regulate gene transcription?

Roberta Fiume[1], Cristina Mazzetti[1], Alessandro Poli[2], Ester Orsini[1], Roberto Mambelli[1], Anna Maria Billi[1] and Lucio Cocco[1]

[1] Università di Bologna, DIBINEM, Bologna, Italia
[2] INGM, INGM, Milano, Italia

Since 80s the importance for nuclear phosphoinositides regulating enzymes has emerged, revealing that they are different and independent from the counterpart in the cytosol. While it is still not clear how phosphoinositides are presented in the nucleus nor how they are controlled, scientists have shown that their levels are changed in response to many different types of stimuli and that they are able to interact with and regulate proteins that are involved in nuclear functions such as transcription, mRNA processing and export and DNA conformation. Nuclear phospholipase C beta 1 (PLCβ1) has been shown to directly regulate cell cycle progression, differentiation and gene expression in a variety of different cell types [1-4].

We used human acute myeloid leukaemia THP-1 cells to investigate a possible role of PLCβ1 in epigenetic signalling. Epigenetic signalling is a mechanism by which environmental stimuli can impact on both short and long term gene transcriptional output and thereby control cell fate decisions. Here we shown that PLCβ1 directly regulates histone methyltransferase and demethylase, and as consequence, histone tails profiles that lead to transcription factors accessibility at the promoter of key genes involved in acute myeloid leukaemia.

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The perivascular neuron type and the blood brain barrier

Paolo Flace

Università degli Studi di Bari 'Aldo Moro', Dipartimento di Scienze Mediche di Base, Neuroscienze e Organi di Senso, Bari, Italia

The blood brain barrier (BBB) is involved in the transport mechanisms between the blood and the central nervous system (CNS). The BBB protect the CNS from injurious agents and modulates selectively the passage of pharmacological agents. The BBB is composed by a layer of endothelial cells, incompletely covered by pericytes surrounded by the extracellular matrix sheathed by endfeet astrocyte processes. The endfeet astrocytes are the mainly target of neuronal processes, the exact neuronal control on the BBB and the neuronal role in the neurovascular unit it is not completely know [1]. Studies revealed the presence of perivascular neuronal processes involved in the modulation of the BBB and few studies demonstrate in the CNS the presence of neuronal cell bodies in close relationship with the wall of vessels [2-6]. The goal of this study of chemical neuroanatomy is to investigate on the presence of monoaminergic and peptidergic perivascular neuronal elements in the human cerebellum.

The study was carried out on autoptic fragments of human cerebellum fixed in an aldehyde picric acid solution, embedded in paraffin, cut into 5μm sections and subjected to light microscopic immunohistochemistry with rabbit polyclonal antibodies for serotonin (5-HT), dopamine transporter (DAT), dopamine type 2 receptor (DRD2), neurotensin (NT), neurotensin receptor type 1 (NTR1). The immunoreaction revealed in the molecular layer, in the three zones of the granular layer of the cerebellar cortex, in the dentate nucleus the presence of neuronal cell bodies and processes in close relationship with the wall of microvessels positive for 5-HT, DAT, DRD2, NT and NTR1.

Although, this data provides further insights, we suggest that the perivascular neuron may be considered a new specific neuron type of the neurovascular unit involved in the permeability control mechanisms of the BBB.

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Key words

Cerebellum, perivascular neuron, blood brain barrier, microvessels, immunohistochemistry.

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Multidisciplinary morphological approaches to the intrinsic human cerebellar dopaminergic system, its projection and clinical role

Paolo Flace[1], Demetrio Milardi[2], Alberto Cacciola[3], Gianpaolo Basile[4]

[1] Società Italiana di Anatomia e Istologia, Università degli Studi di Bari 'Aldo Moro' - Dipartimento di Scienze Mediche di Base, Neuroscienze e Organi di Senso, Bari, Italia
[2] Società Italiana di Anatomia e Istologia, Università degli Studi di Messina - Dipartimento di Scienze biomediche, odontoiatriche e delle immagini morfologiche e funzionali, Messina, Italia
[3] Società Italiana di Anatomia e Istologia, IRCCS Centro Neurolesi "Bonino Pulejo" , Messina , Italy., Messina, Italia
[4] Università degli Studi di Bari 'Aldo Moro', Università degli Studi di Bari di Messina - Dipartimento di Scienze biomediche, odontoiatriche e delle immagini morfologiche e funzionali, Messina, Italia

Introduction. Studies suggest that the cerebellum plays a role in dopaminergic disorders, such as Parkinson's disease (PD) and Schizophrenia (SCZ). Data on an intrinsic human cerebellar dopaminergic system and on a connection of the cerebellum with basal ganglia and the dopaminergic midbrain area are lacking. Studies demonstrate mainly in rodents an extrinsic dopaminergic fiber system in the cerebellum, originating from the A10 dopaminergic area, and few dopaminergic neurons composed by Purkinje neurons of the cerebellar cortex and by neurons of the deep cerebellar nuclei. Studies on the connection of the cerebellum with the brain area involved in PD and in SCZ are also lacking.

Aim. The aim of this study is to make an immunohistochemical investigation of the presence of an intrinsic human cerebellar dopaminergic system. Recent developments in Diffusion Magnetic Resonance Tractography (DMRT) may allow in vivo studies of the cerebellar connections with basal ganglia and the midbrain traditional dopaminergic area. Material and Methods. Autoptic fragments of human cerebellum were fixed in an aldehyde picric acid solution, embedded in paraffin, cut into 5μm sections and subjected to light microscopic immunohistochemistry with rabbit polyclonal antibodies for dopamine transporter (DAT) or dopamine type 2 receptor (DRD2).

A 3T Achieva Philips scanner was used; a SENSE 8 channels head coil, acquiring T1 weighted 3D TFE, DTI sequences: data were analyzed using the contrastained spherical deconvolution technique (CDS). Results. DAT and DRD2 positivity was observed in the cerebellar cortex in Purkinje neurons, granules and in some non-traditional neuron types. In the dentate nucleus positivity was observed in large and small neuron types. We demonstrated with CSD cerebellar-subcortical connections. In particular, we found a direct route linking the dentate nucleus to the substantia nigra as well as to the ventro tegmental area.

Conclusions. This study demonstrates for the first time, the existence of an intrinsic human cerebellar dopaminergic system and a cortico-dentate and direct dentate nucleus connection with the substantia nigra and with the ventro tegmental area. Finally, we suggest that the cerebellum may be involved in dopaminergic brain disorders.

Key words

Cerebellum, substantia nigra, ventro tegmental area, dopamine, connectivity, tractography, immunohistochemistry.

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Interactions between nuclear inositide signalling and leukemic bone marrow microenvironment

Matilde Y. Follo[1], Sara Mongiorgi[1], Valentina Salvestrini[2], Alessandra Cappellini[3], Antonio Curti[2] and Carlo Finelli[4]

[1] Università di Bologna, Dipartimento di Scienze Biomediche e Neuromotorie, Bologna, Italia
[2] Università di Bologna, Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale, Bologna, Italia
[3] Università di Cassino, Dipartimento di Scienze Umane, Sociali e della Salute, Cassino, Italia
[4] Policlinico S. Orsola-Malpighi, Istituto di Ematologia "L. e A. Seràgnoli", Bologna, Italia

Although hematopoietic stem cell studies led to the development of new targeted therapies for patients with acute myeloid leukemia (AML), the role of leukemic stem cells has to be clearly disclosed. Recent investigations showed that the perturbation of the bone marrow niche can initiate myeloid neoplasms, including AML and myelodysplastic syndromes (MDS), inducing leukemic stem cell proliferation and preventing drug-induced toxicity [1]. That is why several new therapies now target both leukemic stem cells and the bone marrow niche. Nuclear Phospholipase Cbeta1 (PI-PLCbeta1) is a key enzyme involved in hematopoietic regulation, and is particularly implicated in the progression of MDS to AML [2]. Here we studied the relationship between the bone marrow microenvironment and AML cells using in vitro co-culture experimental models. At first, we used hematopoietic cell lines, such as KG-1 (macrophage-like), THP-1 (monocytes) and HL-60 (promyeloblasts), then we switched to primary cells. We analyzed both the expression and topographic localization of inositide-dependent regulators (i.e. phospholipases and protein kinases) and hematopoietic differentiation markers, by means of Real-Time PCR, immunocytochemistry and flow cytometry. In addition, we are now performing experiments based on nuclear PI-PLCbeta1 overexpression or silencing in co-culture models. Our findings show that the presence of leukemic cells can perturb the bone marrow niche, inducing the gene expression of specific inositide players, such as PI-PLCbeta1, PI-PLCgamma1, PI-PLCgamma2 and protein kinase alpha. On the other hand, also specific hematopoietic proliferation and differentiation markers, like CD34, CD33, CD11b and CD14, are affected. All in all, our results not only show that nuclear inositides interact with the bone marrow niche, but also that their expression is altered during leukemic stem cell proliferation and is affected by nuclear PI-PLCbeta1 modulation, possibly paving the way to the development of innovative targeted therapies.

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Key words

Nucleus, PI-PLCbeta1, Hematopoiesis.

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Epithelial-to-mesenchymal transition markers are differently expressed in 2D and 3D cell cultures of prostate cancer cells

Fabrizio Fontana[1], Michela Raimondi[1], Monica Marzagalli[2], Francesca Bianchi[3], Michele Sommariva[4], Patrizia Limonta[1] and Nicoletta Gagliano[4]

[1] Università degli Studi di Milano, Department of Pharmacological and Biomolecular Sciences, Milano, Italia
[2] City of Hope National Medical Center, Department of Immuno-Oncology, Beckman Research Institute, Duarte, CA, Stati Uniti D' America
[3] Fondazione IRCCS Istituto Nazionale dei Tumori, Molecular Targeting Unit, Milano, Italia
[4] Università degli Studi di Milano, Department of Biomedical Sciences for Health, Milano, Italia

Three-dimensional (3D) cell cultures allow to mimic the functions of living tissues and provide key information encoded in the tissue architecture [1]. Considered the pivotal role of epithelial-to-mesenchymal transition (EMT) in carcinoma progression, including prostate cancer (PCa) [2], we aimed at investigating the effect of the 3D arrangement on the expression of some key markers of EMT in cultured human prostate cancer (PCa) cells to better understand PCa cell behaviour.

PC3 and DU145 PCa cells were cultured in RPMI cell culture medium either in 2D-monolayers or in 3D-spheroids. The main EMT markers E-cadherin, N-cadherin, α-smooth muscle actin (αSMA), vimentin, Snail, Slug, Twist and Zeb1 were evaluated by confocal microscopy, real-time PCR and Western blot.

Confocal microscopy revealed that E-cadherin was similarly expressed at the cell boundaries on the plasma membrane of PCa cells grown in 2D-monolayers as well as in 3D-spheroids, but resulted up-regulated in 3D-spheroids, compared to 2D-monolayers, at the mRNA and protein level. Moreover, markers of mesenchymal phenotype were expressed at very low levels in 3D-spheroids, suggesting important differences in the phenotype of PCa cells grown in 3D-spheroids or in 2D-monolayers.

Considered as a whole, our findings contribute to a clarification of the role of EMT in PCa and confirm that a 3D cell culture model could provide deeper insight into the understanding of the biology of PCa.

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Key words

Epithelial-to-mesenchymal transition, prostate cancer, 3D-spheroids, E-cadherin.

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The consequences of interrupting adapted physical activity program on a population of elderly subjects

Piergiorgio Francia[1], Francesca Barbagallo[1], Michele Sorelli[2], Giulia Iannone[3], Ugo Santosuosso[1] and Massimo Gulisano[1]

[1] School of Human Health Sciences, University of Florence, Florence
[2] Engineering, Dept. of Information Engineering, Florence
[3] ANIMO, Associazione nazionale indirizzo motorio, Florence

Exercise is generally recommended for elderly subjects although the effects of the different programmes used in the adapted physical activity (APA) centers are not always properly verified. The aim of this study was to evaluate the response of elderly subjects attending classes of APA to a moderate aerobic physical activity.

In 15 subjects (2/13 M/F), mean age 67,8±13,8 yrs, body mass index 23,7±3,5 Kg/m2 attending an APA class we evaluated ankle joint mobility (inclinometer), hand strength (Jamar hand grip), walking speed and step length (10 and 20 meters), aerobic capacity and endurance (6 Minute Walk Test-6MWT), lower extremity function (SPPB: short physical performance battery), posture (sagittal images), and peripheral microcirculation (Laser doppler flowmetry). The subjects were evaluated in the following 3 stages: at the end of a 8-months APA period, after 4 months of inactivity and 4 months after recommencing of a new period of the same programme. All the subjects included in the study performed a training programme of APA twice a week for 1 hour each. The training programme consisted in 10 minutes of organic activation, 30 minutes of moderate physical activity at 45-65% of VO2 peak and toning, 20 minutes of exercises on the floor: breathing and stretching. After 4 months of inactivity, the subjects investigated showed a significant and widespread reduction of the gait parameters investigated: 6MWT (446.5 ± 91.4 vs 429 ± 89.4 mt, $p < 0.01$); 10 meters (step length: 0.67 ± 0.09 vs 0.63 ± 0.08 mt – time: 7.6 ± 1.7 vs 8.5 ± 1.7''); 20 meters (step length: 0.71 ± 0.08 vs 0.67 ± 0.06 mt – time: 14.0 ± 2.1 vs 15.4 ± 2.0''). After 4 months of training there was a significant, even if partial recovery of the parameters investigated in comparison to the results achieved after the inactivity period,: 6MWT (438.05 ± 92.3 mt, $p < 0.01$); 10 meters (step length: 0.65 ± 0.07 mt – time: 8.0 ± 1.7'', $p < 0.01$); 20 meters (step length: 0.68 ± 0.07 mt, $p = 0.15$ – time: 14.6 ± 2.1'', $p < 0.01$). The 4 meter walking speed (SPPB) evaluated in the subjects investigated was correlated with 10 mt ($r = 0.69$, $p < 0.01$) and 20 mt ($r = 0.6$, $p < 0, 05$). The length of the step measured in the 10 and 20 meters tests was correlated with the ankle mobility (10 mt: $r = 0.7$, $p < 0.01$; 20 mt: $r = 0.62$, $p < 0.5$). The results of this study show that in a population of elderly subjects a programme of APA, as scheduled and performed in this study, significantly improves the gait parameters. However, a short interruption of the APA training significantly reduced the walking speed and step length. This study, in addition to underlining the importance of the APA programmes, also shows that it is necessary to reduce or avoid interruptions of these physical activities.

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Key words

Adapted physical activity, Sedentary , Gait speed.

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The effects of sport practiced on joint mobility, flexibility and muscle strength of young subjects

Piergiorgio Francia[1], Alessandro Vittori[1], Giulia Iannone[2], Paolo Bosi[1] and Massimo Gulisano[1]

[1] School of Human Health Sciences, University of Florence, Florence
[2] ANIMO, Associazione nazionale indirizzo motorio, Florence

It is known that joint mobility (JM), flexibility, muscle strength and posture can determine the quality of movement and could also adversely affect the body development of young subjects practicing sport. The aim of this study was to investigate how the practice of different sports affects these parameters.

We enrolled 109 young subjects practicing different sports: soccer (M/F:22/10), mean age 12,6±1,4 yrs, BMI:18,3±2,3 kg/m2; volleyball (M/F:13/14), mean age 12,0±1,3 yrs, BMI 17,4±2,2 kg/m2; basketball (M/F:20/2), mean age 11,1±0,8 yrs, BMI 20,9±4,4 kg/m2; gymnastics (M/F:0/11), mean age 14,5±1,1 yrs, BMI 18,9±2,1 kg/m2 and dance (M/F:0/17), mean age 11,7±3,1 yrs, BMI 18,3±2,8 kg/m2. In these subjects we evaluated ankle JM (inclinometer), trunk flexibility (sit & reach test), muscle strength (standing long jump, vertical jump and Jamar hand grip), posture (images on the sagittal plane) and lifestyle (IPAQ-C, IPAQ-A). The individual sporting history was investigated by a specific questionnaire.

The tests performed showed a significantly higher ankle JM in young dancers (155,8±10,3°) compared to all other groups excluding volleyball players ($p<0,001$). In particular, the subjects practicing soccer showed a significant reduction of the ankle JM (125,2±22,3°) compared to all the other groups of subjects investigated ($p<0,01$).

Gymnasts showed a greater flexibility of the trunk than that measured in all other groups (18,3±3,5 cm; $p<0,001$), while basketball players showed lower trunk flexibility (-7.7 ± 7.0 cm). In the muscle-strength tests performed the dancers showed the following results (hand grip: 18.7 ± 6.6 kg, long-jump standing 119.8 ± 29.2 cm) that are significantly reduced compared to the gymnasts (hand grip: 26.0 ± 4 , 2 kg: $p <0.005$, long jump standing 163.8 ± 12.7 cm: $p <0.001$) and to volleyball players (standing long jump 152.5 ± 27.9 cm: $p <0.001$).

The practice of specific sports can significantly modify the ankle JM and the flexibility of the trunk as well as affect muscle strength even in young subjects. It is not entirely clear whether these effects may induce negative consequences on health and development of the anatomical structures involved; therefore, further studies are needed to verify the conclusions and the possible usefulness of APA programmes in the prevention or recovery of such significant effects.

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Key words

Sport, Joint mobility, Muscle strength.

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Store glutaraldehyde fixed samples at -80°C: is it possible?

Alberto Fucarino, Alessandro Pitruzzella, Fulvio Barone, Vanessa Matranga, Francesco Carini, Sabrina David, Felicia Farina, Francesco Cappello and Fabio Bucchieri

Dip. di Biomedicina Sperimentale e Neuroscienze Cliniche, Università degli studi di Palermo, Palermo, Italia

The design and realization of experiments aboard the International Space Station (ISS) often clashes with greater difficulties than at ground level. Dimensions, weight, materials used, transport, extreme conditions are just some of the possible variables. During the planning of a research project, involving the study of microgravity effects on human bronchial mucosa on the ISS, we stumbled upon an "unusual" problem: is it possible, in order to minimize containment levels of the fixatives, to store biological samples fixed in 2.5% glutaraldehyde at -80 ° C without affecting transmission electron microscopy (TEM) analysis? Normal fixation procedures involve storage of fixed samples at 4°C. For reasons related to crew safety in fact, fixed samples should not be handled in the incubator (BIOLAB) and must be stored at a -80°C. Therefore, we had to verify that once brought back to room temperature, samples fixed at -80°C did not show morphological and ultrastructural alterations. Small bronchial tissue biopsies (about 0.2/0.3 mm3) were fixed in a glutaraldehyde solution at 2.5%, for 45'. After this passage the fixative solution was removed and samples were dried under the cabinet or replaced with DMSO 10% in Millonig's buffer. . In both cases samples were stored at -80°C for one week. At the end of the week we proceeded with the inclusion in EPON 812 resin for the subsequent analysis by TEM. Analysing both types of preparations through TEM we didn't observe any alteration or loss of morphology. The cellular structures were practically identical to the control samples fixed with the standard procedure and stored at 4°C. Even structures such as mitochondrial crests have retained their morphological integrity. In conclusion, we demonstrate preservation of samples fixed in glutaraldehyde for TEM at a temperature of -80 ° C is feasible and does not lead to the loss of tissue integrity. This will allow to store the samples of the experiments conducted under microgravity conditions, meeting the engineering requirements and safety for the astronauts on board.

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Key words

Electron Microscopy, Microgravity Environment, Space Medicine.

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Human bronchial mucosa equivalents in extreme space conditions

Alberto Fucarino, Alessandro Pitruzzella, Angelo Giosuè Mezzoiuso, Maria Concetta Zarcone, Francesco Carini, Sabrina David, Felicia Farina, Francesco Cappello and Fabio Bucchieri

Dip. di Biomedicina Sperimentale e Neuroscienze Cliniche, Università degli Studi di Palermo, Palermo, Italia

In view of the growing international interest towards manned long-term space exploration, possible effects of exposure to microgravity conditions affecting the respiratory system are subject of interest by major space agencies (NASA and ESA primarily). It becomes very relevant to understand how space conditions may affect the bronchial mucosa. We have developed an advanced 3d tissue model of the human bronchial mucosa that includes bronchial epithelial cells (ciliated and goblet cells) and fibroblasts where it is possible to study [1]: structure and functionality of the ciliary apparatus, mucus production and the production of antimicrobial peptides [2]. Our in vitro culture model not only presents accurate histological features of the human bronchial mucosa but it also has excellent resistance to different chemo-physical variables (such as temperature, CO2 levels and nutrients) that play a major role before and during transport from earth to the International Space Station (ISS). We have conducted experiments that have validated the ability of the model to resist, with minimal variations, to temperatures lower than growth optimum (up to 4°C for short periods), to reduced concentrations of CO2 (0.02% indefinitely, providing a significant reduction in maintenance and transport cost) and to prolonged starvation (at least up to 96 hours). 3D cultures were analysed at the end of the treatments evaluating their morphology and monitoring their Trans Epithelial Electric Resistance. The results obtained demonstrated how this culture model is able to guarantee a likely test bench to conduct experiments in microgravity conditions on the ISS that can easily overcome the critical phases of the journey (transport) and any unexpected events that may occur. The data that will be obtained from these experiments will derive exclusively from automated cultures without the need to obtain biological samples from astronauts, which until today has been the only source of study regarding the respiratory system subjected to microgravity.

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Neural crest- and primary mesoderm-derived morphogenetic-like fields in the nasal septum of the human embryo: implications for engineering of the articular cartilage

Giorgio Gallo[1], Fulvio Barbaro[1], Elia Consolini[1], Leonardo Cecotto[2], Marco Alfieri[3], Davide Dallatana[2], Barbero Andrea[4], Ivan Martin[4] and Roberto Toni[5]

[1] Laboratory of Regenerative Morphology and Bioartificial Structures (Re.Mo.Bio.S), Unit of Biomedical, Biotechnological, and Translational Sciences, Section of Human Anatomy, Department of Medicine and Surgery, University of Parma/Laboratory of Tissue Engineering, University Hospital Basel, Parma (Italy) / Basel (Switzerland), Italia
[2] Laboratory of Regenerative Morphology and Bioartificial Structures (Re.Mo.Bio.S), Unit of Biomedical, Biotechnological, and Translational Sciences, Section of Human Anatomy, Department of Medicine and Surgery, University of Parma, Parma (Italy), Italia
[3] Laboratory of Regenerative Morphology and Bioartificial Structures (Re.Mo.Bio.S), Unit of Biomedical, Biotechnological, and Translational Sciences, Section of Human Anatomy, Department of Medicine and Surgery, University of Parma/UCD Conway Institute of Biomolecular and Biomedical Research, Parma (Italy) / Dublin (Ireland), Italia
[4] Departments of Surgery and Biomedicine, Laboratory of Tissue Engineering, University Hospital Basel, University of Basel, Basel (Switzerland), Svizzera
[5] Laboratory of Regenerative Morphology and Bioartificial Structures (Re.Mo.Bio.S), Unit of Biomedical, Biotechnological, and Translational Sciences, Section of Human Anatomy, Department of Medicine and Surgery, University of Parma/Division of Endocrinology, Diabetes and Metabolism, TUSM-TMC, Parma (Italy) / Boston - MA (USA), Italia

The cartilage of the adult human nasal septum has recently been found as a source of neuroectodermal-derived chondrocytes exhibiting capacity to regenerate the articular cartilage [1,2]. To shed light on the key cellular players of this differentiation, we studied the contribution of neural crest (NC)- and primary mesoderm (Mes)-derived stem cells to the development of the nasal septum in the human embryo. Ninety-nine sagittal and horizontal, paraffin-embedded and formaldehyde-fixed sections of 8 human embryos (CRL 11, 19, 20, 24, 30) from the collection of the Museum and Historical Library of Biomedicine (BIOMED) of the University of Parma were used. After dewaxing and rehydration, tissue epitope retrieval was achieved soaking sections in boiling citrate buffer pH 6.0 for 20 min, followed by immunocytochemical labelling with primary antibodies to Wnt1, Notch1, Msi1, Nestin, Sox10, and Chromogranin A (Chrom A) as NC markers, and Brachyury (T) as Mes marker. Immunoreactive (IR) material was detected using either the peroxidase or alkaline phosphatase - ABC techniques, and DAB or Vector Red as chromogens, respectively and analyzed with light microscopy. A topographical arrangement was apparent for both Mes- and NC-derived stem cells: vertical stripes of Wnt1-IR cells segmented the lateral aspects of the septal primordium, moving from a posterior to an anterior direction. These cell fields alternated with either Notch1-IR, T-IR, or Nestin-IR cell columns / groups, the latter two diffusing also into the medial aspects of the septum. In contrast, Sox10-IR, T-IR, Chrom A-IR, and Msi1-IR cell groups contributed to the most anterior portion of the lateral aspects of the septal cartilage, giving rise to a caudally- to cranially- oriented pile of individual stem cell fields. These results raise the possibility that development of the human nasal septum is driven by a place-dependent, morphogenetic code provided by both NC- and Mes-derived stem cells. In addition, it suggests that different areas of the adult nasal septum may provide different types of stem cells, whose differentiation potential could be selectively exploited in bioengineering of cartilaginous grafts for the repair of a variety of mesodermal tissues including the articular and intervertebral disk cartilages.

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Key words

Stem cells, neural crest, nasal septum, cartilage.

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School backpack transportation and its effects on posture: a case study

Laura Galuppo[1], Prisco Mirandola[2], Daniela Galli[2], Giulia Pozzi[3], Roberta Zoni[3], Silvia Capici[4], Licia Veronesi[3], Giuliana Gobbi[2], Marco Vitale[5], Cesira Isabella Maria Pasquarella[3]

[1] University of Parma, Department of Medicine and Surgery and Laboratorio di Analisi del Movimento e Postura, AUSL Parma, Parma, Italia
[2] University of Parma, Sport and Exercise Medicine Center SEM and Department of Medicine and Surgery, Parma, Italia
[3] University of Parma, Department of Medicine and Surgery, Parma, Italia
[4] Presidio di Riabilitazione Specialistica Ambulatoriale "terapia fisica e riabilitazione", Roma, Italia
[5] University of Parma, Sport and Exercise Medicine Centre (SEM) and Department of Medicine and Surgery and Laboratorio di Analisi del Movimento e Postura, AUSL Parma, Parma, Italia

Heavy school backpacks, or their incorrect transportation, represent a risk for musculoskeletal disorders. According to the recommendations of the MIUR and the Ministry of Labor, Health and Agriculture, the weight of the backpack should not exceed 10-15% of body weight. Studies in literature have not shown conclusive data about the effect of the weight of backpack and the duration of its transport on the musculoskeletal system [1]. The objective of this case study is to verify the variation of spatiotemporal gait parameters and pelvis angles walking 10 meters, in a 13-year-old girl (body weight:50 kg, height:155 cm), carrying a school backpack. We performed a clinical and instrumental evaluation with 3 different weight percentages relative to body weight (T1:10%; T2:15%; T3:20%) using a wearable inertial sensor (G-sensor BTS Engineer) placed at L5 level. The T0 evaluation without backpack, showed a scapular and shoulder height-right deviation in the frontal plane. Adam test showed dorsal hump 0,4 cm. Different stature triangles (>right); counterclockwise pelvic rotation (10°). Spatiotemporal parameters were symmetrical: right and left propulsion index 8,5; 9; tilt 98; pelvic obliquity 79,2; pelvic rotation 62,8. Data "on both shoulders" modality transport at T1, T2, T3 were respectively: left propulsion index 9,4; 7,5;7,8; right propulsion index 7,6; 8; 9,2; tilt 98,6; 90,4; 93; pelvic obliquity 96,5; 98,6; 86,7; pelvic rotation 79,6; 98,6; 99. Data "on one shoulder" modality transport (T1, T2, T3) were: left propulsion index 9,4; 9,4; 6,1. Right propulsion index:10,1; 8,7; 6,5. Tilt: 68,7; 37,8; 79,2; Pelvic obliquity: 96,7; 97,3; 51,1; Pelvic rotation: 91,4; 59,7; 95,7. Data trolley modality at 20% of body weight showed left and right propulsion index: 12,5; tilt 81,2; pelvic obliquity 98,6; pelvic rotation 99,2. Data of right "one hand" at 10% of body weight were: left propulsion index 9,8; right propulsion index 8,2; tilt 77,9; pelvic obliquity 75,4; pelvic rotation 57,7. Compared to T0, the best modality seems trolley mode. After that "on one shoulder" and "on both shoulders" show a progressive loss of pelvic movement at 15% of body weight. The worst modality is "one hand" in which pelvis seems fixed. In conclusion, a prolonged period of high weight transport and incorrect modality could produce or worsen postural abnormalities.

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Key words

School backpack, musculoskeletal disease, gait, pelvis.

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ERBB2 activation leads to an anti-oncogenic signalling

Marco Gaviraghi[1], Andrea Rabellino[2], Matthias Brand[3], Paola Bagnato[4], Giuseppina De Feudis[1], Andrea Raimondi[1], Alberta Locatelli[5], Daniela Tosoni[5], Davide Mazza[1], Luca Gianii[5], Tiziana Daniele[1] and Carlo Tacchetti[1]

[1] Istituto Scientifico San Raffaele, Centro Imaging Sperimentale, Milano, Italia
[2] Qimr Berghofer Medical Research Institute, Brisbane, Queensland, Australia
[3] Cemm Research Center For Molecular Medicine of The Austrian Academy of Sciences, Vienna, Austria
[4] Dipartimento Medicina Sperimentale, Università di Genova, Genova, Italia
[5] Istituto Scientifico San Raffaele, Dipatimento di Oncologia, Milano, Italia

Approximately 20% of breast cancers display ERBB2 (HER2) gene amplification and/or protein overexpression. In cancer cells, ERBB2 can function both in the homodimeric and heterodimeric form. Moreover, it represents the preferred partner of other members of the ERBB receptor family. Strikingly, ERBB2-containing heterodimers are more oncogenic than other ERBB combinations, mostly by promoting cell proliferation via ERK activation and cell survival via the AKT pathway. AKT signalling plays a fundamental role in driving breast carcinogenesis and is downmodulated in response to the binding of the humanized therapeutic antibody Trastuzumab (TZ) to ERBB2. How ERBB2 differentially modulates AKT in ERBB2-overexpressing BrCa cells upon TZ treatment, remain unclear. Our findings show that TZ treatment of ERBB2-positive breast cancer cell lines triggers the homodimerization and the activation of ERBB2, leading to an previously unidentified signalling cascade causing an ERK-dependent AKT dephosphorylation, via PP2A Ser/Thr phosphatases. The immunophilin Cyclophilin A (CyPA) plays a key role in this pathway, as a negative modulator of AKT de-phosphorylation, by competing with PP2A phosphatases for binding to AKT. Upon TZ treatment ERK promotes CyPA redistribution to ERBB2, allowing the binding of PP2A to phospho-AKT. Finally, we report that CyPA silencing reverts TZ-resistant human ERBB2-positive breast cancer cell lines to a TZ-responsive state. In conclusion, we show that in breast cancer cells TZ promotes ERBB2 activation, working as a "putative" ligand of the receptor, and that the outcome of the ERBB2 activity depends on the dimerization status: pro-oncogenic in the hetero- and anti-proliferative in the homodimeric form.

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Key words

Breast cancer, ERBB2, HER2, AKT, ERK, signalling, dimerization, trastuzumab, resistance, Cyclophilin A, Ser/Thr phosphatase, PP2A.

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Communication between Median and Musculocutaneous nerve at the level of cubital fossa - a case report

Clarissa Ann Elisabeth Gelmi[1], Francesca Alice Pedrini[1], Matteo Fermi[2], Nicolò Rossi[1], Giulia Adalgisa Mariani[1], Marilisa Quaranta[1], Ester Orsini[1], Roberta Fiume[1], Stefano Ratti[1], Anna Maria Billi[1]

[1] Dipartimento di Scienze biomediche e neuromotorie, Università di Bologna, Bologna, Italia
[2] Dipartimento di otorinolaringoiatria - Chirurgia di testa e collo, Università di Modena, Modena, Italia

The anatomical variations of the brachial plexus are important to be reported, in order to avoid their damage during the upper-limb surgical procedures. Despite of the frequent documented abnormalities in the pathway of the musculocutaneous and the median nerves, the anatomical variation described in the present case is unusual to see: it does not perfectly come under any of the classifications proposed in literature.

During the dissection of the right brachial plexus in an old male Caucasian cadaver a communicating branch between the musculocutaneous nerve (C5-7) and the median nerve (C5-T1) was exposed. Proximally It originated by the musculocutaneous nerve, after its perforation of the coracobrachialis muscle; distally it joined the median nerve only at the level of the cubital fossa. The musculocutaneous nerve and the median nerve maintained their normal courses, supplying all the collateral and terminal branches. In the same arm an atrophied short head of biceps brachii muscle was also found, despite this its musculocutaneous nerve's branch was discovered during the dissection.

This anatomical variation assumes a considerable importance for neurophysiological studies and for surgical practice. Appropriate neurophysiological examinations through electroneurography allow us to identify the specific functions of its fibers, to be useful during surgical procedures. In neurosurgery it can be used in those cases of lesions or pathologies of the median nerve which require surgery (for example neurofibromatosis), thanks to its length and its distal position, because it can supply the innervation of those structures which are normally innervated by the median nerve. It can also be involved in the neurotization procedure, when a proximal lesion at the brachial plexus afflicts the muscolocutaneous nerve functionality. Finally the possible presence of this comunicating branch must be also considered by orthopedics, general surgeons and neurosurgeons to avoid damages during surgeries of elbow's region and proximal forearm, which could cause alteration of the motility and the sensibility of forearm and hand.

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The impact of T cell mediated immune surveillance on epithelial cancer cells with mismatch repair alterations

Giovanni Germano[1], Simona Lamba[2], Giuseppe Rospo[2], Alessandro Magrì[2], Silvia Marsoni[3], Maurizio D'Incalci[4], Enrico Giraudo[5], Federica Di Nicolantonio[1] and Alberto Bardelli[1]

[1] Candiolo Cancer Institute, Dipartimento di Oncologia, Università degli Studi di Torino, Candiolo, Italia
[2] Candiolo Cancer Institute, FPO, IRCCS, Candiolo, Italia
[3] IFOM - The FIRC Institute of Molecular Oncology, Precision Oncology, Milan, Italia
[4] IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Head, Department of Oncology, Milan, Italia
[5] Candiolo Cancer Institute – FPO, IRCCS,, Laboratory of Transgenic Mouse Models, Candiolo, Italia

The impact of T cell mediated immune surveillance on epithelial cancer cells with mismatch repair the conflicting models of colorectal Lynch syndrome pathogenesis—MMR deficiency as a late event 3,8,53 versus MMR deficiency as an early event 17,19,20 —can only be reconciled by a unifying model tha t accepts the existence of distinct pathways of colorectal carcinogenesis in Lynch syn- drome (Fig. 5). Indeed, our study provides histological and molecular evidence that Lynch syndrome-associated colorectal cancers do not follow one single pathway, but three path- ways separated from each other by the type and timing of key mutation events: colorectal cancers in Lynch syndrome can in fact grow out from MMR-profici ent adenomas after secondary inactivation of the MMR system the conflicting models of colorectal Lynch syndrome pathogenesis—MMR deficiency as a late event 3,8,53 versus MMR deficiency as an early event 17,19,20 —can only be reconciled by a unifying model tha t accepts the existence of distinct pathways of colorectal carcinogenesis in Lynch syn- drome (Fig. 5). Indeed, our study provides histological and molecular evidence that Lynch syndrome-associated colorectal cancers do not follow one single pathway, but three pathways separated from each other by the type and timing of key mutation events: colorectal cancers in Lynch syndrome can in fact grow out from MMR-profici ent adenomas after secondary inactivation of the MMR system Mismatch repair (MMR) is a DNA repair mechanism that ensures the fidelity of DNA replication. In highly proliferative organs the MMR mechanism is relevant for maintaining the correct genetic information. In the colon, the bottom of each crypt, 4-6 stem cells contribute to the enormous amount of colonocytes and host the potential of accumulating genetic and epigenetic changes. Mismatch Repair alterations are considered early event in the transition from colonocytes to adenoma and some colorectal cancer developed from MMR-deficient precursor lesions. Interestingly, recent evidences demonstrate that a subset of patients with alterations in MMR respond prominently to immune checkpoint blockade leading to the hypothesis that the presence of high number of somatic mutations may be responsible for effective recruitment of immune-cells and consequently of surveillance. To prove the hypothesis, we genetically inactivated a component of MMR machinery, MutL homolog 1 (MLH1), in murine colorectal cancer cells. MMR inactivation increased the mutational burden and led to dynamic mutational profiles, resulting in persistent renewal of neoantigens over time. The histological analyses of tissues didn't reveal differences in size and morphology of CT26 colorectal cancer cell lines. Interestingly, isogenic MMR deficient cancer cells were unable to form tumors when injected subcutaneously or orthotopically in syngeneic mouse models according to cell-passage number. To distil the role of the immune system, we injected MMR deficient cells in the presence of depleting CD8 antibody whereas isotype matched antibodies served as controls. Interestingly, MMR deficient cells readily formed tumors in syngeneic mice only when CD8 T cells were suppressed. To test the effects of DNA repair inactivation also on fully established tumors we implanted a fragment of MLH1 KO tumour in syngeneic mouse models. The immunological repertoire of MLH1 KO tumours revealed an increased level of CD45+ cells and activation of cytotoxic CD8+ T cells by IFNγ suggesting that functionally reactive T cells might be responsible for the impaired tumorigenesis of MMR deficient cells. To test the activation of T cells, IFNγ and CD8+ cells were stained by immunofluorescence in control and Mlh1 KO clones. These results led us hypothesize that enforced increase of the number of mutations in cancer cells could foster T cell infiltration restricting cancer growth and this might be beneficial for therapeutic purposes.

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Functional Properties of Cardiomyocytes Derived from Human Cardiopoietic Amniotic Fluids

Barbara Ghinassi[1], Maria Angela D'Amico[1], Pascal Izzicupo[1], Giulia Gaggi[1], Andrea Di Credico[1], Maria Addolorata Mariggio[2] and Angela Di Baldassarre[1]

[1] Università "G. d'Annunzio" di Chieti-Pescara, DMSI, Chieti, Italia (1) –
[2] Università "G. d'Annunzio" di Chieti-Pescara, Dep of Neuroscience, Imaging e Clinical Scienes, Chieti, Italia

Human amniotic fluid (hAF) cells share characteristics of both embryonic and adult stem cells. They proliferate rapidly and can differentiate into cells of all embryonic germ layers but do not form teratomas. Embryoid-bodies obtained from hAF have cardiac differentiation potential, but terminal differentiation to cardiomyocytes (CMs) has not yet been described. Our purpose was to promote cardiac differentiation in hAFcells. Cells were exposed to inducing factors for up to 15 days. Only the subset of hAF cells expressing the multipotency markers SSEA4, OCT4 and CD90 (CardiopoieticAF cells) responded to the differentiation process by increasing the expression of the cardiac transcription factors Nkx2.5 and GATA4, sarcomeric proteins (cTnT, α-MHC, α-SA), Connexin43 and atrial and ventricular markers. Furthermore, up to 90% of differentiated cells were positive for the calcium pumps CACNA1C and SERCA2a, with approximately 30% of CardiopoieticAF-derived CM-like cells responding to caffeine or adrenergic stimulation. Some spontaneous beating foci were also observed. In conclusion, we demonstrated that CardiopoieticAF cells can differentiate into a population of CM-like cells, characterized by cardiac-specific molecular, structural, and functional properties, that are useful for the development of in vitro models of genetic cardiac disorders, for drug discovery and testing, and for the regenerative medicine.

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Key words

Cardiac Differentiation, Amniotic Fluid, Stem Cells.

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Disclosing the "hidden" anatomical structures of the cranium: morphological and metrical assessment of pterygopalatine fossa and intrapetrous internal carotid artery through 3D segmentation on CT-scan

Daniele Gibelli[1], Michaela Cellina[2], Annalisa Cappella[1], Annalisa Gadler[1], Gianluca M. Tartaglia[1], Antonio Giancarlo Oliva[2]

[1] Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milano, Italia
[2] Fatebenefratelli Sacco, Reparto di Radiologia, Ospedale Fatebenefratelli, Milano, Italia

The pterygopalatine fossa (PPF) and the intrapetrous carotid artery (IPCA) are among the most unexplored anatomical regions of the cranium, as they cannot be directly visualized, with consequent limits in research and didactics. Yet, the precise knowledge of PPF and IPCA is important in different surgical fields [1,2]. 3D segmentation software can overcome this limit, obtaining a 3D model of anatomical structures from CT and MRI scans. The present study aims at applying the 3D segmentation procedures to the identification of PPF and IPCA from 100 CT-scans (50 males. 50 females aged 18-91 years). Both the structures were manually segmented through ITK-SNAP segmentation software and the following measurements were assessed: height and volume from PPF, and angles of the posterior and anterior genu, diameter and length of the horizontal portion and volume from IPCA, respectively. Statistically significant differences according to sex and side for all the measurements were assessed through two-way ANOVA test ($p<0.05$). On average PPF height was 24.1±3.5 mm in males, and 22.8±3.4 mm in females, whereas volume was 0.930±0.181 cm3 in males and 0.817±0.157 cm3 in females, with statistically significant differences according to sex ($p<0.05$), but not to side ($p>0.05$). For what concerns IPCA, on average the posterior genu angle was 120.1±10.4° in males, 119.5±9.2° in females, whereas the anterior genu angle was 118.0±10.0° in males, 117.6±10.3° in females. Average length and diameter of the horizontal part were respectively 25.5±2.9 mm and 5.8±0.8 mm in males, and 24.0±2.3 mm and 5.3±0.8 mm in females. The volume of IPCA was 0.941±0.215 cm3 in males, and 0.752±0.159 cm3 in females. Length and diameter of horizontal portion, and volume of IPCA showed statistically significant differences according to sex ($p<0.05$). This study shows that 3D segmentation may be useful for the morphological and metrical assessment of "hidden" anatomical structures.

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Key words

Pterygopalatine foramen, intrapetrous carotid artery, 3D segmentation, surgery.

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Three-dimensional assessment of patients with facial palsy: thirds-based evaluation of the success of reanimation procedures

Daniele Gibelli[1], Sofia Restelli[2], Efrem Pozzi[2], Filippo Tarabbia[3], Fabiana Allevi[2], Virgilio F. Ferrario[1] and Federico Biglioli[2]

[1] Università degli Studi di Milano, LAFAS, Laboratorio di Anatomia Funzionale dell'Apparato Stomatognatico - Dipartimento di Scienze Biomediche per la Salute, Milano, Italia
[2] Università degli Studi di Milano, Maxillo-Facial Surgical Unit, Dipartimento di Scienze della Salute, Ospedale San Paolo, Milano, Italia
[3] Università di Napoli "Federico II", Division of Maxillofacial Surgery, Department of Neurosciences, Reproductive and Odontostomatological Sciences, Napoli, Italia

The effects of facial palsy can severely compromise aesthetic and functional characteristics of face, that may be ameliorated by facial reanimation procedures providing new neural stimuli and/or muscle grafts (1,2). However, there is no consensus about an objective and quantitative method for assessing the success of surgical procedures in different parts of face. This study aims at testing a method for the 3D assessment of mimicry in different facial thirds of patients treated through facial reanimation. Twelve patients aged between 42 and 77 years affected by recent facial palsy (onset between 6 and 18 months) were treated through triple innervation procedure (masseteric nerve, 30% of the hypoglossal fibers, and contralateral facial nerve through two cross-face sural nerve grafts). Each patient underwent five facial 3D scans: at rest, smiling on the healthy side (facial stimulus), biting (masseteric stimulus), moving the tongue (hypoglossal stimulus), and Mona-Lisa smile. Each scan was registered onto the scan in rest position, and the point-to-point root mean square (RMS) value was automatically calculated on the upper, middle and lower facial thirds defined on the territories of trigeminal branches. An index of asymmetry was computed. Two-way ANOVA test was applied to verify statistically significant differences in RMS and asymmetry index according to the type of stimulus and facial thirds ($p<0.05$). On the rehabilitated hemiface, the widest facial movements were performed by the masseteric and hypoglossal stimuli in the upper third of face, and by the hypoglossal one in the middle and lower thirds ($p<0.05$), whereas no statistically significant differences were observed on the healthy side ($p>0.05$). Facial stimulus evoked the most asymmetrical movement, whereas the hypoglossal one produced the most symmetrical expression, for all the facial thirds. The 3D facial assessment may provide an important contribution for verifying the role of different stimuli in evoking facial movements in patients treated through reanimation procedures.

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3D-3D superimposition, facial palsy, facial reanimation, stereophotogrammetry.

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The non-neuronal cholinergic system in the inflamed adipose tissue of obese mice

Antonio Giordano, Ilenia Severi, Jessica Perugini, Martina Senzacqua, Abbatelli Silvia, Saverio Cinti

Marche Polytechnic University, Section of Neuroscience and Cell Biology, Department of Experimental and Clinical Medicine, Ancona, Italia

A key feature of morbid obesity is white adipose tissue (WAT) inflammation. In obese animals and humans WAT is infiltrated by macrophages that are mainly found at sites where adipocytes die by pyroptosis [1]. Here, macrophages sequester and digest adipocyte debris, forming distinctive crown-like structures [2]. Acetylcholine (ACh) was the first neurotransmitter to be discovered. However, within the past decades increasing experimental evidence has shown that ACh is also produced by non-neuronal cells and tissues, including the immune cells, where it acts as a secreted messenger. Here we evaluated whether the non-neuronal cholinergic system occurs in obese and inflamed fat. By RT-qPCR, we found that all the components of the non-neuronal cholinergic system molecular machinery significantly increased in subcutaneous and visceral WAT from high-fat diet obese mice compared with mice fed a normal diet. By immunohistochemistry and confocal microscopy, we found that about 40-50% of macrophages infiltrating obese WAT expressed choline acetyltransferase (ChAT), choline transporter-1 (ChT-1) and the vesicular ACh transporter (VAChT), whereas the white adipocytes expressed the butyrylcholinesterase (BChE). In vitro studies showed that white adipocytes differentiated from human multipotent adipose-derived stem cells not only produced BChE but also, and to a larger amount, acetylcholinesterase (AChE). Collectively, these data suggest that a consistent proportion of macrophages infiltrating obese WAT produce and secrete ACh that may act on ACh receptor-bearing adipocytes; diffusion of this potent molecule is prevented by ACh re-uptake by macrophages (through VAChT) or by adipocyte degradation of ACh (through BChE and AChE) into acetate and choline, which is quickly taken up by the macrophages (through ChT-1). Promoting the anti-inflammatory effect [3] of the non-neuronal cholinergic system present in obese fat could represent a novel and effective therapeutic approach to obesity and associated diseases.

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Key words

Acetylcholine, inflammation, macrophages, obesity.

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Neuroanatomical features of the Locus Coeruleus in neurodegeneration

Filippo Sean Giorgi[1,2], Marina Flaibani[1], Francesca Biagioni[3], Gloria Lazzeri[1], Gianfranco Natale[1], Riccardo Ruffoli[1], Paola Soldani[1], Francesco Fornai[1], Francesco Fornai[1,3]

[1] University of Pisa, Human Anatomy, Department of Translational Research and New Technologies in Medicine and Surgery, Pisa, Italia
[2] Neurology Unit, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italia
[3] IRCCS INM Neuromed, via Atinense 18, 86077 Pozzilli, Unit of Neurobiology of Movement Disorders, Isernia, Italia

The nucleus Locus Coeruleus (LC) is placed in the upper part of the pons, is mainly formed by medium-sized neurons containing neuromelanin and it is part of the so-called isodendritic core of the brain stem reticular formation. LC is the main source of noradrenaline in the brain. In humans, each one of two symmetrical LC nuclei is formed by up to 60.000 neurons, which send axons profusely branching and innervating the entire cerebral and cerebellar cortices. Noradrenaline is released through "bouton en passage" by LC axons, and thus it modulates the activity of several cortical areas. In particular, LC modulates sleep/wake cycle, different cognitive functions (such as attention, alerting and novelty orienting and memory consolidation) and electroencephalogram activity. It also plays an important role in neural plasticity and neuroprotection.

Several post-mortem studies showed a significant LC cell loss in Parkinson's Disease (PD) and in cases of severe Alzheimer's Disease (AD) dementia, in post-mortem studies.

Recent histological data suggest a very early involvement of LC in the pathogenesis of AD. In particular accumulation of phospho-tau deposits in the axons of LC neurons may precede their occurrence in limbic regions in Mild Cognitive Impairment (MCI, which is the prodromal phase of Dementia) or even in pre-MCI stages. Experimental data show that LC impairment may accelerate beta amyloid plaques deposition and neuroinflammation.

Recently, specific 1,5 and 3,0 Tesla Magnetic Resonance Imaging (MRI) protocols and post-processing analysis have been developed in order to detect neuromelanin-containing LC neurons in vivo in controls and in PD patients, as well as in dementia. In this presentation, we report the state of the art of the neuroanatomical features of LC in models of degenerative diseases, as well as more recent evidence in humans. These data disclose a novel scenario for LC, stemming from in vivo neuroanatomy to neurobiology of neurodegenerative diseases.

Key words

Neurodegenerative diseases, noradrenaline, neuromelanin.

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Mitochondria and Cytoskeleton rearrangement in Drp1 overexpressing skeletal muscle

Matteo Giovarelli[1], Silvia Zecchini[2], Claudia Moscheni[1], Patrizia Procacci[3], Patrizia Sartori[3] and Clara De Palma[2]

[1] Università degli studi di Milano, Department of Biomedical and Clinical Sciences "Luigi Sacco", Milano, Italia
[2] Ospedale Luigi Sacco, Department of Biomedical and Clinical Sciences "Luigi Sacco", Milano, Italia
[3] Università degli studi di Milano, Department of Biomedical Sciences for Health, Milano, Italia

In skeletal muscle mitochondrial fusion and fission define the mitochondrial network morphology regulating myofiber differentiation, muscle contraction and the response to stress conditions 1-3. Mitochondrial fission is mainly mediated by dynamin-related protein 1 (Drp1) which represents a critical player in myogenesis and its inhibition suppresses myotube formation 4-5.Our work is focused on a transgenic mouse overexpressing Drp1 specifically in skeletal muscle (Drp/MC). Drp/MC mice show growth defects starting from P7 mainly due to an impairment of glycolytic muscles development; indeed, in adult phase they display an overall 20% reduction of body weight and a drop of locomotor performance without any increasing in catabolic processes. Drp/MC mice exhibit low mitochondrial DNA levels which trigger mitochondrial stress and upregulate the unfolding proteins response (mtUPR) together with an impairment of Growth Hormone anabolic pathway. Interestingly, we observe a strong remodeling of mitochondria distribution with a depletion of inter-myofibrillar mitochondria and an enrichment of the sub-sarcolemmal pool. In parallel, we observe a perturbation of cytoskeleton framework characterized by the disruption of Desmin network (the main skeletal muscle intermediate filament connecting mitochondria to cytoskeleton) with the presence of Desmin aggregates inside myofibers and its accumulation beneath the sarcolemma. Moreover, in vivo time-lapse imaging of both skeletal muscle fibers and satellite cells-derived myotubes, indicates an increased mitochondrial mobility in Drp/MC mice; therefore, our aim is the evaluation of the role of different motor proteins, such as the kinesin (Kif5b and KLC1) and dynein, in the Drp/MC dysregulated muscular and mitochondrial phenotype.

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Molecular mechanisms underlying oleic acid-induced cell death of hepatocarcinoma cell lines

Federico Giulitti, Simonetta Petrungaro, Luana Tomaipitinca, Sara Mandatori, Eugenio Gaudio, Elio Ziparo and Claudia Giampietri

Sapienza Università di Roma, Dipartimento di Scienze Anatomiche, Istologiche, Medico-Legali e dell' Apparato Locomotore, Roma, Italia

Extra virgin olive oil, containing high levels of monounsaturated fatty acids such as oleic acid, has been shown to exert several protective effects on the liver, reducing hepatic steatosis, fibrogenesis and lipid peroxidation. Remarkably, oleic acid is known for its anti-cancer effects in different tumors through cell death induction [1] and autophagy modulation [2]. The aim of the present study is to investigate the effects of oleic acid on hepatocellular carcinoma. More in detail, we are investigating the effects of oleic acid treatment in two hepatocarcinoma cell lines, namely Huh7.5 and Hep3B, by evaluating lipid accumulation (through oil red staining), cell death index (through annexin and propidium iodide assay), expression of endoplasmic reticulum stress- and lipid synthesis- markers as well as analyzing autophagic flux (by Western blot assays). We found that the storage levels of neutral lipids in Huh7.5 are different than in Hep3B cell lines. This is true both under basal conditions and upon oleic acid treatment. Conversely, cell death index is increased in both cell lines upon high oleic acid concentration (300 mM). Unexpectedly, such increased cell death inversely correlates with the endoplasmic reticulum stress marker BIP, since it displays a mild reduction after high oleic acid treatment in both cell lines (300 mM). Interestingly, oleic acid treatment dose-dependently (50 to 150 mM) leads to a reduction of peroxisome proliferator-activated receptor (PPAR)-g expression in both cell lines, thus suggesting a reduction of de novo lipid synthesis at low-dose oleic acid treatments. Furthermore, reduced autophagic flux upon high oleic acid treatment (300 mM) occurs in both cell lines, as demonstrated by LC3 lipidation in the presence of bafilomycin. We are currently investigating if autophagy modulation after oleic acid treatment in these cell lines depends on phospho-glycogen synthase kinase-3 (P-GSK-3) and phospho AMP-activated protein kinase (P-AMPK). We therefore hypothesize that autophagy may have a crucial role in controlling cell death after high oleic acid treatment in the two investigated hepatocarcinoma cell lines, unrevealing new insights on the mechanisms underlying autophagy in hepatocarcinoma.

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Key words

Liver, autophagy, fatty acids.

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Evaluation of muscle activation in bench press exercises with different types of barbells

Giuliana Gobbi[1], Andrea Melani[2], Daniela Galli[1], Maria Luisa Arcari[3], Cecilia Carubbi[3], Elena Masselli[3], Cristina Micheloni[3], Valentina Presta[4], Mauro Vaccarezza[5], Marco Vitale[1] and Prisco Mirandola[1]

[1] University of Parma, Sport and Exercise Medicine Centre (SEM) and SBiBiT Unit of Department of Medicine and Surgery, Parma, Italia
[2] University of Cassino and Southern Lazio, PhD program in "Systems, technologies and devices for movement and health", Cassino, Italia
[3] University of Parma, SBiBiT Unit of Department of Medicine and Surgery, Parma, Italia
[4] Master Degree Curriculum in Theory and Methods of Preventive and Adapted Physical Activities, University of Parma, Parma, Italia
[5] Curtin University, School of Pharmacy and Biomedical Sciences, Perth, Australia

Bench Press exercise (BP) is generally used to induce adult muscle hypertrophy and it is based on the use of barbells to lift weights. During exercise performance the range of motion, the grip strength and the speed of the exercise represent factors that can be affected during training. Moreover, BP is a multiarticular exercise that involves several muscle groups [1]. For example, involved muscle groups include the large pectoral (GP), the elbow extensors represented by the brachial triceps (TB), the deltoid front bands (DA), the serratus anterior muscle (GD) and the elbow flexors represented by the biceps brachialis (BB) as well as all the peri-articular shoulder muscle. Several studies reported that muscle kinematic and electromyographic parameters have been correlated to specific rockers [2, 3]. Here we studied, by electromyography, the muscle activation during the performance of the bench press exercise with a traditional rocker and a variable inter-handle distance (IHD) rocker. The aim of using the IHD rocker is to increase the muscle activation regardless the external loads and reduce the articular stress.

Surface electromyography data were collected from healthy adult subjects who performed the exercise with both barbell types to reduce inter-individual variability. Moreover, data were normalized to obtain a mean value of contraction for each analyzed muscle. Data distributions were evaluated using the Kolmogorov-Smirnov test. For GP, DA and TB statistical significance was calculated using Wilcoxon test, while for BB Student t test was applied.

Results showed a significant increase in GP (19,5%) and BB (173%) muscle activity using the IHD rocker. For DA and TB differences in electromyographic signals were not significant.

Although several research points still to remain to be analyzed we think that the new concept rocker represents a valid method to make more efficient the training in the bench press exercise.

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Key words

Bench press, inter-handle distance barbell.

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Quercetin and Indole 3-carbinol downregulate extracellular matrix expression in human primary uterine leiomyoma cells

Stefania Greco[1], Soriful Islam[1], Alessandro Zannotti[1], Giovanni Delli Carpini[2], Stefano Raffaele Giannubilo[2], Andrea Ciavattini[2], Mario Castellucci[1], Felice Petraglia[3] and Pasquapina Ciarmela[1]

[1] Università Politecnica delle Marche, Department of Experimental and Clinical Medicine, Faculty of Medicine, Ancona
[2] Università Politecnica delle Marche, Department of Medical Biotechnology and Department of Molecular and Developmental Medicine, Obstetrics, and Gynecology, Ancona
[3] Università degli studi di Firenze, Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Obstetrics and Gynecology, Firenze

Uterine leiomyomas (fibroids or myomas) are the most common benign tumors of female reproductive tract, originating from myometrial smooth muscle cells of the uterus. The affect about 77% of women of reproductive-age and approximately 25% of reproductive-age women bear clinically apparent tumors. The incidence and severity of symptoms typically depend on the size, number, and location of the fibroids. The common symptoms are irregular and excessive menstrual bleeding, pelvic pain, pressure on the bladder, miscarriage, and infertility. They are the leading indication for hysterectomy. Unfortunately, no long-term medical treatments are available. The cause of uterine leiomyomas remains unknown, but the current understanding is that stem cells, genetic and epigenetic factors, sex steroids, growth factors, cytokines, chemokines, and extracellular matrix (ECM) components are known factors involved in the development and growth of leiomyomas. The ECM components, mainly collagen1A1, fibronectin and versican are over- expressed in leiomyoma, and their upregulation is induced by activin A. ECM proteins transmit mechanical signals from the outside of the cell to the cell interior through transmembrane integrin proteins. In modern pharmaceutical industries, dietary phytochemicals are used as source of new potential drugs for many kinds of tumors. Dietary phytochemicals may exert therapeutic effects by interfering with key cellular events of the tumorigenesis. Quercetin (3,3',4',5,7-pentahydroxyflavone) is a plant bioflavonoid, found in most edible fruits and vegetables. Indole-3-carbinol (I3C; 1H-indol-3-ylmethanol) is produced from naturally occurring glucosinolates contained in a wide variety of plants, including members of the family Cruciferae and particularly members of the genus Brassica [2,3].In the present study, we aimed to investigate if quercetin and indole-3-carbinol can regulate ECM in human myometrium and leiomyoma cells.In the previous time we investigated about the purity of cells was assessed by immunocytochemical staining with a specific smooth muscle cell marker. All cells were strongly positive for alfa-sma Leiomyoma and myometrial cells, were treated with Quercetin and 13C (10 μg/ml; 50 μg/ml; 100 μg/ml; 250 μg/ml) for 48 h to measure mRNA and proteins expressions of ECM. Quercetin and I3C significantly decreased collagen1A1 and fibronectin mRNA and protein expression in leiomyoma cells. Immunocytochemical stained showed the decreased expression of fibronectin in primary leiomyoma and myometrial cells after treatments of quercetin and indol-3-carbinol. This study suggests that quercetin and indole-3-carbinol extracts can be developed as therapeutic and/or preventive agent for uterine leiomyomas.

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Key words

Quercetin, indole-3-carbinol, uterine fibroid, antifibrotic, dietary phytochemicals, extracellular matrix.

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Tumor necrosis factor-alpha affects human cholinergic neuron development by epigenetic mechanisms

Giulia Guarnieri[1], Erica Sarchielli[1], Pasquale Gallina[2], Gabriella Barbara Vannelli[1], Annamaria Morelli[1]

[1] University of Florence, Department of Experimental and Clinical Medicine, Florence, Italia
[2] University of Florence, Department of Surgery and Translational Medicine, Florence, Italia

Loss of basal forebrain cholinergic circuits within the nucleus basalis of Meynert (NBM) is responsible for cognitive deficits in neurodegenerative disorders (ND), such as Alzheimer's disease. Despite the pathogenesis underlying the cholinergic damage is still unclear, growing evidence points to neuroinflammation (NI) as a critical trigger for this process [1]. Here we studied the effects of tumor necrosis factor alpha (TNF-α), the major proinflammatory cytokine involved in NI, in a model of cholinergic neurons from the human fetal NBM (hfNBMs) [2]. Phenotypic characterization, performed by quantitative RT-PCR, flow cytometry and immunocytochemistry analyses, demonstrated that hfNBMs express functional TNF-α receptors and exhibit significant changes upon TNF-α exposure, such as the reduction of immature neuronal markers (nestin, beta-tubulin III), the increase of the mature marker microtubule-associated protein 2 and neurite outgrowth, when compared to untreated cells. Interestingly, TNF-α exposure significantly reduced TrkA, the high affinity nerve growth factor (NGF) receptor, essential for cholinergic neuron survival, while increased p75, the low affinity NGF receptor that mediates apoptotic signals. Given the compelling implication of inflammation-related epigenetic mechanisms in ND we performed a genome-wide methylome analysis of hfNBMs under inflammatory insult. TNF-α exposure for 24-48h altered the methylation pattern of target genes involved in neuronal differentiation and migration. In particular, we observed the promoter hypermethylation of genes involved in neuronal commitment, such as chordin like-1 (CHRDL1) and mesoderm specific transcript (MEST) after 48h stimulation with TNF-α. Accordingly, mRNA expression of both genes was significantly reduced by TNFα treatment. Taken together our results suggest that the TNF-α-mediated inflammatory insult may affect hfNBMs development most likely interfering with the DNA methylation status.

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Neuroinflammation, NGF, DNA methylation.

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Glutamate triggers intracellular Ca2+ oscillations and nitric oxide release by inducing NAADP- and InsP3-dependent Ca2+ release in mouse brain endothelial cells

Germano Guerra[1], Estella Zuccolo[2], Dlzar Ali Kheder[3], Dmitry Lim[4], Angelica Perna[5], Francesca Di Nezza[6], Angela Lucariello[5], Domenico Tafuri[7], Teresa Soda[8], Antonio De Luca[9], Luigi Ambrosone[5], Egidio D'Angelo[8], Francesco Moccia[10]

[1] Università degli studi del Molise, Dipartimento di Medicina e Scienze della Salute "Vincenzo Tiberio", Campobasso, Italia
[2] Università degli studi di Pavia, Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", Pavia, Italia
[3] Università di Zakho, Zakho, Kurdistan-Region of Iraq, Dipartimento di Biologia, Zakho, Iraq
[4] Università del Piemonte Orientale "Amedeo Avogadro", Dipartimento di Scienze Farmaceutiche, Novara, Italia
[5] Università degli Studi del Molise, Dipartimento di Medicina e Scienze della Salute "Vincenzo Tiberio", Campobasso, Italia
[6] Università degli Studi del Molise, Dipartimento di Bioscienze e Territorio (DIBIT), Pesche (IS), Italia
[7] Università degli Studi di Napoli "Parthenope", Dipartimento di Scienze Motorie e del Benessere, Napoli, Italia
[8] Università degli Studi di Pavia, Dipartimento di Scienze del Sistema Nervoso e del Comportamento, Pavia, Italia
[9] Università della Campania "Luigi Vanvitelli", Dipartimento di Medicina Fisica e Salute Mentale e Preventiva, Napoli, Italia
[10] Università degli Studi di Pavia, Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", Pavia, Italia

The neurotransmitter glutamate increases cerebral blood flow (CBF) by activating postsynaptic neurons and presynaptic glial cells within the neurovascular (NVU) unit. Glutamate does so by causing an increase in intracellular Ca2+ concentration ([Ca2+]i) in the target cells, which activates the Ca2+/Calmodulin-dependent NO synthase (NOS) to release NO. It is unclear whether brain endothelial cells also sense glutamate through an elevation in [Ca2+]i and NO production. The present study assessed whether and how glutamate drives Ca2+-dependent NO release in bEND5 cells, an established model of brain endothelial cells. We found that glutamate induced a dose dependent oscillatory increase in [Ca2+]i, which was maximally activated at 200 μM and inhibited by MCPG, a selective blocker of Group 1 metabotropic glutamate receptors. Glutamate-induced intracellular Ca2+ oscillations were triggered by rhythmic endogenous Ca2+ mobilization and maintained over time by extracellular Ca2+ entry. Pharmacological manipulation revealed that glutamate-induced endogenous Ca2+ release was mediated by inositol-1,4,5-trisphosphate-sensitive receptors and NAADP-gated two-pore channel 1 (TPC1). Constitutive SOCE mediated Ca2+ entry during ongoing Ca2+ oscillations. Finally, glutamate evoked a robust, although delayed increase in NO levels, which was blocked by pharmacologically inhibition of the accompanying intracellular Ca2+ wave. Of note, glutamate induced Ca2+-dependent NO release also in hCMEC/D3 cells, an established model of human brain microvascular endothelial cells. This investigation demonstrates for the first time that metabotropic glutamate-induced intracellular Ca2+ oscillations and NO release have the potential to impact on neurovascular coupling in the brain.

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Key words

Glutamate, endothelial cells, nitric oxide, neurovascular coupling, Ca^{2}+ oscillations.

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Stim and Orai mediate constitutive Ca2+ entry and control endoplasmic reticulum Ca2+ refilling in primary cultures of colorectal carcinoma cells

Germano Guerra[1], Estella Zuccolo[2], Umberto Laforenza[3], Pawan Farisa[4], Angela Lucariello[1], Angelica Perna[1], Antonio De Luca[5], Domenico Tafuri[6], Marcello Maestri[7], Dlzar Ali Kheder[8], Paolo Pedrazzoli[9], Daniela Montagna[10] and Francesco Moccia[2]

[1] Università degli Studi del Molise, Dipartimento di Medicina e Scienze della Salute "Vincenzo Tiberio", Campobasso, Italia
[2] Università degli Studi di Pavia, Dipartimento di Biologia Biotecnologie "Lazzaro Spallanzani", Pavia, Italia
[3] Università degli studi di Pavia, Dipartimento di Medicina Molecolare, Pavia, Italia
[4] Università di Salahaddin, Erbil, Kurdistan-Region of Iraq, Iraq, Dipartimento di Biologia, College of Science, Erbil, Iraq
[5] Università degli Studi della Campania "Luigi Vanvitelli", Dipartimento di Salute Fisica e Mentale e Medicina Preventiva, Caserta, Italia
[6] Università degli Studi di Napoli "Parthenope", Dipartimento di Scienze Motorie e del Benessere, Napoli, Italia
[7] Fondazione IRCCS Policlinico San Matteo, Unità Operativa Complessa di Chirurgia Generale, Pavia, Italia
[8] Università di Zakho, Zakho, Kurdistan-Region of Iraq, Dipartimento di Biologia, Zakho, Iraq
[9] Università degli Studi di Pavia, Dipartimento of Scienze Clinico Chirurgiche, Diagnostiche e Pediatriche, Pavia, Italia
[10] Fondazione IRCCS Policlinico San Matteo, Laboratorio di Immunologia e Trapiantologia, Pavia, Italia

Store-operated Ca2+ entry (SOCE) provides a major Ca2+ entry route in cancer cells. SOCE is mediated by the assembly of Stim and Orai proteins at endoplasmic reticulum (ER)-plasma membrane junctions upon depletion of the ER Ca2+ store. Additionally, Stim and Orai proteins underpin constitutive Ca2+ entry in a growing number of cancer cell types due to the partial depletion of their ER Ca2+ reservoir. Herein, we investigated for the first time the structure and function of SOCE in primary cultures of colorectal carcinoma (CRC) established from primary tumor (pCRC) and metastatic lesions (mCRC) of human subjects. Stim1-2 and Orai1-3 transcripts were equally expressed in pCRC and mCRC cells, although Stim1 and Orai3 proteins were up-regulated in mCRC cells. The Mn2+-quenching technique revealed that constitutive Ca2+ entry was significantly enhanced in pCRC cells and was inhibited by the pharmacological and genetic blockade of Stim1, Stim2, Orai1 and Orai3. The larger resting Ca2+ influx in pCRC was associated to their lower ER Ca2+ content as compared to mCRC cells. Pharmacological and genetic blockade of Stim1, Stim2, Orai1 and Orai3 prevented ER-dependent Ca2+ release, thereby suggesting that constitutive SOCE maintains ER Ca2+ levels. Nevertheless, pharmacological and genetic blockade of Stim1, Stim2, Orai1 and Orai3 did not affect CRC cell proliferation and migration. These data provide the first evidence that Stim and Orai proteins mediate constitutive Ca2+ entry and replenish ER with Ca2+ in primary cultures of CRC cells. However, SOCE is not a promising target to design for alternative therapies for CRC.

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Key words

Colorectal cancer, store-operated Ca2+ entry, Stim, Orai, proliferation.

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Berberine downturns B-cell chronic lymphocytic leukaemia metabolism and cell cycle progression

Elena Gugiatti[1], Silvia Ravera[2], Ermanno Ciccone[3], Zbigniew Darzynkiewicz[4], Franco Fais[3] and Silvia Bruno[1]

[1] Università di Genova, Dipartimento di Medicina Sperimentale, Genova, Italia
[2] Universita di Genova, Dip. Farmacia, Genova, Italia
[3] Universita di Genova, Dipartimento di Medicina Sperimentale, Genova, Italia
[4] New York Medical College, Brander Cancer Research Institute, Valhalla, Stati Uniti D' America

B-cell chronic lymphocytic leukemia (CLL) was believed to result from clonal accumulation of resting apoptosis-resistant malignant B lymphocytes. However, it became increasingly clear that CLL cells undergo, during their life, iterative cycles of re-activation and subsequent clonal expansion. Drugs interfering both with CLL cell survival and cell cycle entry would be greatly beneficial in the treatment of this still uncurable disease. Berberine (BRB), an isoquinoline quaternary alkaloid isolated from medicinal plants that has a long history of use in old Chinese medicine, is currently used as a dietary nutritional supplement to treat a variety of different conditions, which include metabolic and cardiovascular disorders, type 2 diabetes, atherosclerosis, senile osteoporosis, Alzheimer's disease, hypercholesterolemia, and diabetes-induced renal inflammation. In addition to the reported beneficial effects in different fields of medicine, BRB has recently received attention for its potential antitumor activity. We wondered whether BRB has apoptotic and anti-proliferative activity on leukemic cells derived from CLL patients. BRB was administered in vitro either to quiescent cells or during CLL cell activation stimuli, provided by classical co-culturing with CD40L-expressing fibroblasts. At doses (in the microM range), that were totally ineffective on normal lymphocytes, BRB induces apoptosis of quiescent CLL cells and inhibition of cell cycle entry when CLL are stimulated by CD40-CD40L ligation. This cytostatic effect is accompanied by decreased expression of survival- and proliferation-associated proteins, adhesion- and homing-molecules. Importantly, the activity of signaling pathways specifically involved in CLL disease progression such as STAT3/NF-kB, were remarkably down-regulated. In drug combination experiments, BRB lowered the apoptotic threshold of classical and novel antitumor molecules. Our results indicate that, while CLL cells after stimulation are in the process of building their full survival and cycling armamentarium, the presence of BRB may affect this process.

Key words

Apoptosis, cell cycle, chronic lymphocytic leukemia.

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Imaging, immunohistochemistry and ultrastructure of a primary vaginal leiomyosarcoma

Rosemarie Heyn[1], Vincenzo Petrozza[2], Natale Porta[2], Camilla Certelli[3], Ezio Battaglione[1], Giacomo Corrado[4], Enrico Vizza[3], Giuseppe Familiari[1]

[1] Laboratory of Electron Microscopy Pietro Motta, Department SAIMLAL, Sapienza Università di Roma, Roma
[2] Histology Unit, Department of Medico-Surgical Sciences and Biotechnologies, Sapienza Università di Roma, ICOT Hospital, Latina
[3] Gynecologic Oncologic Unit, Department of Oncologic Surgery, National Cancer Institute Regina Elena-IFO, Roma
[4] Gynecologic Oncology Unit, Department of Obstetrics and Gynecology, Università Cattolica del Sacro Cuore, Roma

Primary vaginal leiomyosarcomas (LMS) are rare, recurrent tumours with an unknown etiology; the prognosis is poor and there is no consensus guideline on their management (1-2). Surgical resection is generally the gold standard. Due to their rarity there are few studies in the literature including immunohistochemistry and/or ultrastructure (2-4). Herein a primary vaginal LMS is analyzed. Magnetic Resonance Imaging identified a nodular mass in the anterior vaginal wall of a 58-year-old previously hysterectomized woman; it infiltrated the urethra but not the rectovaginal septum. Iliac lymph nodes were negative and a total body CT excluded the presence of distant metastasis. An anterior pelvic exenteration was performed with continent urostomy and creation of a neovagina. The biopsy showed a vaginal LMS that was positive for vimentin, α-smooth muscle actin, caldesmon, desmin, p16 and p53. The sample was fixed and prepared for light microscopy, transmission and scanning electron microscopy. The ultrastructural features showed hypercellularity, moderate mitotic index, nuclear pleomorphism, indented nuclear membrane, prominent nucleoli, absence of intercellular junction complexes, and a dense stroma. No dark, intermediate nor light cells could be recognized as reported (3), may be due to a highly undifferentiated and malignant tumor. After 2 years of surveillance follow-up, the patient is fine and without recurrence. According to the literature, there is still no consensus on the fact that this tumor arises de novo or as a malignant change from a leiomyoma (5-6). The patient had uterine leiomyomas and the vaginal hysterectomy might have seeded atypical cells in the vagina. However, routine uterine histology showed no atypical cells. In conclusion, best outcomes occur when the tumour is small, localized, and can be removed surgically with wide, clear margins, as in this case. As there are different kinds of malignant mesenchymal tumors, biopsy followed by immunohistochemistry and electron microscopy still represents a good diagnostic choice. The question regarding the origin of vaginal LMS still remains open.

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Key words

Leiomyosarcoma, vagina, electron microscopy, immunohistochemistry, imaging.

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Dental pulp stem cells: senescence mechanisms and regenerative perspectives

Iolanda Iezzi[1], Giorgia Cerqueni[1], Alessandra Nori[2], Guendalina Lucarini[1], Caterina Licini[3] and Monica Mattioli Belmonte[1]

[1] DISCLIMO, Università Politecnica delle Marche, Ancona, Italia
[2] Odontostomatological and Special Surgery Unit, Ospedali Riuniti Ancona, Ancona, Italia
[3] DISCLIMO/DISAT, Università Politecnica delle Marche/Politecnico di Torino, Ancona, Italia

The aging of population is a worldwide phenomenon that brings a new set of challenges in the field of regenerative medicine. In the development of strategies able to face these needs, it is important to gain information on possible changes that could affect cell behaviour during aging. For dental and maxillofacial reconstruction, human Dental Pulp Stem Cells (hDPSCs) are an attractive option as they have a great self-expansion and differentiation capabilities [1]. Pulp tissue undergoes to age-related modifications [2] such as volume reduction, decrease of vascularization, innervation and cell availability, therefore it could be of interest investigate these changes at the cellular level to offer valid in vitro tools to investigate regenerative strategies. Aim of the present study has been the in vitro investigation of age-related changes in hDPSCs morphology, multipotency and differentiation ability in view of their possible use in regenerative approaches for elderly. Cells were isolated from patients undergoing third molar extraction and divided into three age groups. Cell morphology and senescence features as well as proliferation capability, gene/protein expression profile, odontogenic and neurogenic potential were assessed. hDPSCs isolated from the young donors demonstrated increased proliferation and stemness properties compared with old cells. The latter displayed typical sign of aging, such as the expression of Senescence Associated-β-Galactosidase (SA β-Gal) and p16ink4a. Our observation indicated that hDPSCs of young group were more prone to differentiate into osteogenic, odontogenic and neurogenic lineages in comparison to cells from the aged group. In conclusion our results pointed out age dependent modifications in hDPSCs. Our results could also be considered a valid in vitro tool for the study and/or development of regenerative strategies solving the challenges of an aging population.

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Key words

Dental Pulp Stem Cells, Aging, Regenerative Medicine.

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Histopathological remodelling of small arteries isolated from patients with essential hypertension

Chiara Ippolito[1], Cristina Segnani[1], Sauro Dini[1], Rosa Maria Bruno[2], Emiliano Duranti[2], Giulio Di Candio[3], Massimo Chiarugi[4], Stefano Taddei[2], Agostino Virdis[2], Amelio Dolfi[1] and Nunzia Bernardini[1]

[1] Histology Unit of Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italia
[2] Internal Medicine Unit of Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italia
[3] General Surgery Unit, Department of Surgery, Translational and New Technologies in Medicine, University of Pisa, Pisa, Italia
[4] Department of Surgical Pathology, Medical, Molecular and Critical Area, University of Pisa, Pisa, Italia

Background. Essential hypertension is a chronic multifactorial disease that arises from combined actions among polygenic/environmental/behavioral factors (diet, physical activity, obesity) [1] and aging. It is associated with cardiovascular, cerebrovascular and renal complications [2], which are causes of mortality in hypertensive patients. Surprisingly, to date detailed morphological studies on the histopathological wall rearrangement of the resistance vessels in essential hypertension are lacking and, therefore, are highly expected.

Aim. To analyze the morphological remodeling of the wall of small arteries from young (<30 years) to old (>60 years) normotensive subjects (NT) and hypertensive patients (HT) and to connect the hypertensive effect on age-related vascular changes.

Methods. Formalin fixed and paraffin embedded small arteries (150-300 μm diameter) were isolated from the subcutaneous tissue of the region undergoing abdominal surgery; cross-sectioned samples were examined to evaluate the histopathological vessels architecture and detect collagen by double histochemical staining Sirius Red/Fast Green. Confocal laser scanning microscopy highlighted the production of reactive superoxide anion (ROS) by dihydroethidium fluorescent staining in frozen section. Morphometric parameters were obtained from fresh small arteries mounted in a pressurized myograph.

Results and Conclusions. In small arteries morphological and functional remodeling was found. Deposition of collagen fibers, ROS generation along with values of the media/lumen ratio and media transversal area in the vascular wall were increased with aging and, even more, with HT. Taken together these data suggest that in normotensive subjects the physiological aging of small resistance arteries entails eutrophic and, only late, hypertrofic remodeling. On the contrary, in HT the vascular fibrotic/hypertrofic remodeling occurs precociously, suggesting an early vascular aging in this pathological condition.

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Key words

Hypertension, remodeling, aging, fibrosis, resistance vessels.

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Mitochondrial localization of melatonin in salivary glands: ultrastructural evidences

Michela Isola[1], Raffaella Isola[1], Jörgen Ekström[2], Francesco Loy[1]

[1] Dipartimento di Scienze Biomediche, Università di Cagliari, Monserrato, Italia
[2] Institute of Neuroscience and Physiology, The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Svezia

The storage of melatonin in acinar secretory granules of human salivary glands and the release of the hormone by granule exocytosis following the regulated secretory pathway were recently reported [1-4]. Melatonin was detected principally in secretory granules, but also in small vesicles, rough endoplasmic reticulum and in the nucleus of the cells. Recently, the occurrence of melatonin has been reported in mitochondria [5] as a peculiar site of its function, but the ultrastructural demonstration of melatonin localization in these organelles has not been delved deeper. In this study we showed the fine localization of this hormone in mitochondria by transmission electron microscopy. Bioptic samples of human parotid, submandibular and labial glands were fixed, dehydrated, embedded in Epon Resin and processed to demonstrate melatonin reactivity by the immunogold staining method. In each type of major and minor human salivary glands, the melatonin reactivity was detected in the mitochondrial membranes in secretory cells. Moreover, melatonin was found in mitochondria of ductal cells. Our data provided new morphological evidences of the melatonin localization in mitochondria, fundamental prerequisite for a better understanding the roles of melatonin in human secretory cells.

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Key words

Melatonin, mithocondria, immunogold method, trasmission electron microscopy.

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Interruption of sedentary behavior with intermittent physical activity mitigates hypercoagulation associated with prolonged sitting

Pascal Izzicupo[1], Barbara Ghinassi[1], Maria Angela D'Amico[1], Giulia Gaggi[1], Andrea Di Credico[1], Ana Cordellat[2], Andrea Di Blasio[2] and Angela Di Baldassarre[1]

[1] Università degli Studi "G. D'Annunzio", Dipartimento di Medicina e Scienze dell'Invecchiamento, Chieti, Italia
[2] University of Valencia, Physical Education and Sports Department, Valencia, Spagna

Sedentary behavior (SB) in post-menopause has been associated with an elevated cardiovascular risk, in particular venous thrombosis, regardless of physical activity (PA) levels. Interrupting SB with intermittent light-intensity or moderate to vigorous-intensity physical activity (LPA and MVPA, respectively) can avoid hypercoagulation associated with prolonged sitting. However, no studies examined this effect in free-living context using objectively measured SB and PA. This study was aimed to investigate the association between objectively measured SB patterns (i.e. duration and number of sedentary bouts) and hemostatic parameters. Fifty-two non-smoker post-menopausal women (age: 59.7 ± 6.2 yo; BMI: 27.3 ± 4.4) worn a multisensory device (Sensewear Armband, BodyMedia, Inc., Pittsburgh, PA) for at least three days to measure SB and PA. Blood samples were collected to measure hemostatic parameters. Data were log-transformed (log-) when non-normally distributed. Correlation was analyzed by Pearson's correlation coefficient, controlling for BMI, log-MVPA or total sedentary time (ST), depending on the need. Difference between women with daily SB bouts lasting at least 1 hr and women with shorter bouts was analyzed by Mann-Whitney U-test. Women were really active (102.5 ± 82.6 min/d MVPA) and with a large amount of SB (589.2 ± 132.5 min/d SB). Plasma fibrinogen was directly correlated with both ST and lying time, independently from BMI and log-MVPA (r = .338, P = .016; r = .374, P = .007) and inversely correlated with log-MVPA (r = -.376, P = .006). No correlation was found for sleep time. The number of bouts of SB lower than 5 or 10 min were not associated with fibrinogen, while the number of bouts of SB between 11 and 15, 11 and 20, and 21 and 30 min were directly correlated with plasma fibrinogen, regardless of BMI, ST, and log-MVPA (r = .276, P = .05; r = .315, P = .028; r = .275, P = .05, respectively). Furthermore, women with daily uninterrupted SB longer than 1 hr had higher plasma fibrinogen than other participants (323.9 ± 41.4 mg/dl; 287.7 ± 48.8 mg/dl, respectively; Z = -2.53, P = .011). In conclusion, uninterrupted SB is associated with adverse fibrinogen levels, regardless of ST and PA, while shorter bouts of SB are not directly associated with it. These results suggest that activity breaks mitigate the procoagulant effects of uninterrupted sitting, in post-menopausal women.

Key words

Sedentary Behaviors, sitting interruption, hemostasis.

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Dermal fibroblasts in morphologic monitoring of biodegradable materials: methodological basis of potential application evaluation in dog dentistry

A.V. Krasnikov[1], V.V. Annikov[1], E.S. Krasnikova[1], R.F. Kapustin[2] and N.Y. Starchenko[3]

[1] Saratov State Agrarian University named after N.I. Vavilov, Department of Animal Diseases and Veterinary-sanitary examination, Saratov, Russia
[2] Belgorod State Agrarian University named after V.J. Gorin, Department of Morphology and Physiology, Maiskii, Russia
[3] Belgorod Interregional Veterinary Laboratory, Department of diagnostics, Belgorod, Russia

Objective: To evaluate functional state of dermal fibroblasts cultivated on titanium pieces for implants with different concentrations of a polymer film and propolis put on their surface; to determine polymer and propolis concentrations which may provide biodegradable coating nontoxicity and the required adhesive and proliferative cell culture potential (patent RU 2117997, 2240602, 240603, 22464304, 22464305, 2271139, 2271140, 2303436, 2323694, 2323695).

Materials and method As a material for our research, human dermal fibroblasts extracted from healthy donor skin were used. Culture viability and proliferative activity were evaluated in 1 mm of medium by means of an autocellcounter. Before the experiment was started the study titanium samples had been treated in the dry air sterilizer ("Across", Russia), then placed in 24-well plate ("Costar", USA) with the follow-up cell culture seeding (concentration – 1*105 cells per a sample in 2 ml of the medium). The research was conducted with adhering to all existing ethical practices.

Findings In the course of the experiment the optimal doses of the composite components which did not exert an inhibitory effect on fibroblasts were determined. The research demonstrated good cell adhesion (the first 5 types). Close to the presented samples high proliferative activity was also being observed. Then inhibition of cell growth followed by cell death was being observed, as evidenced by shape change (rounding) and growth cessation (types 6 and 7). The concentration of the polymer was consistently decreasing, the cell culture adhesive and proliferative potential were being improved (types 8, 10), as evidenced by the cells of the distinctive shape close to the sample. The best results were received for type 10. In 24 hours after the control microscopy it was indicated that the fibroblast culture was in good condition, the cells were mainly fusiform, the processes are prominent, the nucleuses are clearly contoured.

Conclusion For creating a biodegradable coating on the implant material surface the following concentrations are to be used: propolis (as a an active agent) – 1.25 mg per 1 ml, polymer - maximum of 0,0001%. Absence of culture cell damage (type 10) with a possibility of the required adhesion and proliferation on the study substances is an evidence of the biodegradable coating nontoxicity.

Key words

Dermal fibroblasts, biodegradable materials.

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Epigenetic control of gene expression in human mesenchymal stem cells during osteogenic differentiation

Marcella La Noce, Luigi Mele, Vincenzo Desiderio, Virginia Tirino, Gianpaolo Papaccio and Francesca Paino

University of Campania L. Vanvitelli, Section of Medical Histology, Department of Experimental Medicine, Napoli

Adult stem cells are widely used in cellular therapy not only because of their intrinsic potential but also because their use does not raise ethical issues. Dental pulp is an interesting source of postnatal progenitor cells/stem cells [1].

Stem cell lineage commitment and differentiation are regulated by epigenetic mechanisms. Epigenetic modification of DNA and DNA-associated histone proteins has been demonstrated to control and regulate the renewal and pluripotency of stem cell populations. The activities of the nuclear enzymes, histone deacetylases, are increasingly being recognized as potential targets for pharmacologically inducing stem cell differentiation[2; 3].

The aim of this study was to evaluate the osteogenic differentiation of dental pulp stem cells (DPSCs) treated with different histone deacetylase inhibitors (HDACi): Valproic acid (VPA), Entinostat (MS275), Trichostatin A (TSA) and Vorinostat (SAHA).

The effects of these inhibitors on cell proliferation, viability, bone-associated gene expression and matrix mineralization were determined.

VPA was found to be the drug that most induced osteogenic differentiation: at low concentration it was sufficient to significantly enhance matrix mineralization by increasing osteopontin and bone sialoprotein expression. In contrast, osteocalcin levels were decreased, an effect induced at the transcriptional level, and were strongly correlated with inhibition of HDAC2. In fact, HDAC2 silencing with shRNA produced a similar effect to that of VPA treatment on the expression of osteoblast-related markers.

Our in vitro data were confirmed by in vivo studies. H&E and Alizarin red stainings have highlighted a strong trend of VPA-treated cells to form a dense connective tissue. Within this tissue is visible a rather large portion that is even more dense and organized, highly colorable (very eosinophilic), similar to a bone ossification center. Immunofluorescence analysis to evaluate the expression of OPN and OC confirmed the results obtained in vitro and in vivo.

By means of RT-PCR, immunofluorescence and Western blotting, a series of biomarkers involved in the osteogenic pathway have been screened, identifying the glucocorticoid receptor (GCr), which is upregulated during the treatment with VPA and shHDAC2 cells, as the main responsible for the downregulation of osteocalcin.

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Key words

Dental pulp stem cells, epigenetic, bone differentiation.

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Bologna – Redemption wax, Redemption flesh Wax modeling for the studying and teaching of Anatomy at the University of Bologna

Luisa Leonardi[1], Giuliano Bettini[2], Lucia Corrain[3], Cristian Mancini[4], Alessandra Ruggeri[4] and Carlo Sarti[5]

[1] Anatomical Wax "Luigi Cattaneo" Collection SMA – DiBiNem, Bologna, Italia
[2] Veterinary Pathology "Alessandrini Ercolani" SMA – DiMeVet , Bologna, Italia
[3] Museum of "Palazzo Poggi" SMA, DARvipem, Bologna, Italia
[4] DiBiNem, Bologna, Italia
[5] Giovanni Cappellini" Geology Collection SMA, Bologna, Italia

The video depict a historical reconstruction of the birth and development of anatomical ceroplastics work in the "felsinea" city. The first anatomical wax modelings were prepared in 1742, in the scientific laboratories by Ercole Lelli (1702-1766), Giovanni Manzolini (1700-1750) and Anna Morandi (1714-1774). By the end of the eighteenth century, the affirmation of the anatomo-pathological paradigm gave to the study of "diseases" a comparative twist: new diagnosis began to relay on experience acquired during investigations of similar cases made in the past. To achieve this goal, they recorded experiences not only through written words, but also through anatomic modelings. The ductility of the waxes was instrumental to reproduce the various aspects of an illness bridged the gap between life and death since the replication of the visible consequences of an illness made when the patient was still alive allowed scientists to observe and study the damage inflicted by the disease also after the patient had been long dead. This transition from medicine to the art applies also to animal disease, which progressively acquires its own autonomy and is given birth to a very large waxes collection made by leading ceroplasts Giuseppe Astorri (1785-1852) and Cesare Bettini (1814-1885) who produced wax reproductions of normal and pathological human anatomy and pathological veterinary anatomy. The large collection of wax models is retained in Museum of "Palazzo Poggi", in Museum of Anatomical Waxes "Luigi Cattaneo" and in the Museum of Veterinary Pathology "Alessandrini Ercolani", all togheter part of the University Museum System (SMA).

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3D Volumetric Tomography as an innovative bridge between medicine, diagnostics, scientific and industrial research

Luisa Leonardi[1], Elios Sequi[1], Cristian Mancini[1], Maria Pia Morigi[2], Fauzia Albertin[3], Matteo Bettuzzi[2] and Rosa Brancaccio[2]

[1] Unibo, Dibinem, Bologna, Italia
[2] Unibo, Difa, Bologna, Italia
[3] Study and Research Center Enrico Fermi, Unibo Difa, Roma, Italia

3D X-ray volumetric tomography can be a bridge between scientific research, industrial research, medicine and diagnostics. Some innovative diagnostic techniques, developed by the researchers of the DIFA, the SMA and the Dibinem of the University of Bologna in collaboration with the Centro Studi e Ricerche Enrico Fermi of Roma and are illustrated in the images. To increase the effect of integrating the acquired scientific information, the 3D tomographic data, transformed and processed from raw images, are shown in 4D visualizations through innovative devices that generate virtual holograms. These devices are able to display, rotate, disintegrate and reassemble the 3D images produced by reproducing them in holographic-virtual films. The feasibility study (extensible to medical tomography images) was made on fine anatomical waxes made from Bolognese ceroplasts from the 18th and 19th centuries.

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Effects of treatment with maraviroc a CCR5 inhibitor on a human hepatic stellate cell line

Angela Lucariello[1], Angelica Perna[1], Marcella Contieri[1], Eleonora Hay[1], Antonio Ascione[2], Germano Guerra[3], Domenico Tafuri[2] and Antonio De Luca[1]

[1] Università degli studi della Campania "Luigi Vanvitelli", Anatomia umana, Napoli, Italia
[2] Università Parthenope, Sport, salute, benessere, Napoli, Italia (2) - Università del Molise, Medicina e salute, Campobasso, Italia

After an acute liver damage, tissue regeneration repairs lesions with degradation of deposed fibrotic material, while mechanisms of tissue restoration are persistently activated following several repeated injuries, inducing deposition of extracellular matrix. (ECM).

Factors responsible for ECM remodeling have been identified in a pathway involving a family of zinc-dependent enzyme matrix metalloproteinases (MMPs), together with tissue inhibitor of metalloproteinases (TIMPs). Recent experimental models suggested a role of CCR5 receptor in the genesis of liver fibrosis. Drawing from these background we decided to evaluate the effects of the treatment with the CCR5 inhibitor Maraviroc on LX-2, a human hepatic stellate cell line (HSC). Treatment with Maraviroc resulted in a block in S phase of LX-2 cells with increased expression levels of cyclin D1 and p21 while the expression of p53 was reduced. Treatment with Maraviroc was also able to block the accumulation of fibrillar collagens and extracellular matrix proteins (ECM), as demonstrated by the decrease of specific markers as Collagen type I, α-SMA and TGF-β1. In addition we observed a down regulation of both metalloproteins (MMP-2, MMP-9), used for the degradation of the extracellular matrix and their inhibitors (TIMP-1, TIMP-2). The identification of a compound that may modulate the dynamic of liver fibrosis could be crucial in all chronic liver diseases. Maraviroc could play an important role because, in addition to its own anti-HIV activity, it could reduce the release of pro-inflammatory citokynes implicated in liver fibrogenesis.

Key words

Maraviroc, human hepatic stellate cell line, liver fibrosis.

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Peroxisome proliferator-activated receptor γ coactivator 1α expression levels in soleus and EDL muscles after exercise

Filippo Macaluso[1], Federica Scalia[2], Daniela D'Amico[2], Francesco Cappello[2], Felicia Farina[2], Valentina Di Felice[2] and Rosario Barone[2]

[1] eCampus University, SMART Engineering Solutions & Technologies (SMARTEST) Research Cente, Palermo, Italia
[2] University of Palermo, Department of Experimental Biomedicine and Clinical Neurosciences (BioNeC), Palermo, Italia

Peroxisome proliferator-activated receptor γ coactivator 1α (PGC1 α) is a transcriptional coactivator that controls the expression of gene involved in the regulation of fatty acid oxidation and glucose metabolism. PGC1 α is considered the "master regulator of mitochondria", as it regulates mitochondrial transcription factors. It has been reported that PGC1 α and its isoforms are involved in mitochondrial biogenesis, fibre type switching, stimulation of fatty acid oxidation, and resistance to muscle atrophy. Recently, we observed that endurance exercise increased the expression of PGC1 α1, α2, and α3 isoforms in murine soleus muscle (1). In the present study we used thirty healthy male and female mice (BALB/c AnNHsd) divided in sedentary (CN) and trained (TR) groups. TR mice ran for 60 min at a speed of 5.5 m/min and were sacrificed after 30 and 240 minutes after the end of acute bout of endurance exercise (TR-30′ – TR-240′ respectively). CN mice did not perform any controlled physical activity. All mice were sacrificed by cervical dislocation and soleus and Extensor Digitorum Longus (EDL) muscles were dissected. Further, PGC1 α isoform expression levels were evaluated by qRT-PCR. The obtained results showed a significant increase in total PGC1 α isoform in response to acute exercise in the soleus and EDL muscles in TR-30′ mice compared to all the other groups ($p<0.05$). Acute exercise induced significant increase of PGC1 α1 isoform gene expression levels in the soleus muscle of TR30′ male and female mice ($p<0.05$) while a significant decrease was observed in EDL muscle ($p<0.05$). Moreover, PGC1 isoform α2 and α3 gene expression increased in male and female TR30′ groups only in EDL muscle ($p<0.05$). We did not observed any change soleus muscle. Furthermore, PGC1 isoform α4 gene expression level was not detected in any muscle samples. These preliminary results, showing the increased expression levels of the isoform α2 and α3 only in EDL muscle immediately after acute exercise, should represent a very interesting and innovative data that might open new ways in the study of the role of these proteins in the skeletal muscle adaptation during exercise.

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Key words

PGC1 α, exercise, EDL.

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Morphological and volumetric analysis of the suprapatellar fat pad compared to infrapatellar fat pad in normal population and in osteoarthritis

Veronica Macchi[1], Chiara Giulia Fontanella[2], Elisa Belluzzi[3], Edgardo Enrico Edoardo Picardi[1], Gloria Sarasin[1], Marco Rossato[4], Roberta Ramonda[5], Marta Favero[5], Arturo Natali[6], Andrea Porzionato[1], Raffaele De Caro[1]

[1] Istituto di Anatomia Umana, Dipartimento di Neuroscienze, Università di Padova, Padova, Italia
[2] Department of Biomedical Sciences, University of Padova, Padova, Italia
[3] Musculoskeletal Pathology and Oncology Laboratory, Department of Orthopedics and Orthopedic Oncology, University of Padova, Padova, Italia
[4] Clinica Medica 3, Department of Medicine – DIMED, University of Padova, Padova, Italia
[5] Rheumatology Unit, Department of Medicine – DIMED, University of Padova, Padova, Italia
[6] Department of Industrial Engineering, University of Padova, Padova, Italia

The suprapatellar fat pad (SFP) is located above the patella and behind the suprapatellar joint recess with the function of increasing the congruency of the extensor mechanism. Osteoarthritis SFP has been demonstrated to produce high amount of inflammatory molecules and to be more fibrous than subcutaneous adipose tissue in osteoarthritis (OA) patients. The aim of this study was to analyze 1. the morphological characteristics of the SFP compared to that of the infrapatellar fat pad (IFP) in normal subjects; 2. the magnetic resonance (MR) volumetric characteristics of the IFP and the SFP in non-OA controls compared to moderate and end-stage OA patients. Five specimens of SFP were sampled from bodies of the donation program of the University of Padova without history of OA. The SFP consisted of white adipose tissue, of lobular type, with lobules delimited by thin connective septa. Forty-four MR images were collected: a) 17 control; b) 15 patients with moderate OA ; and c) 12 patients with end-stage OA. Volume, depth, femoral and tibial arch lengths of IFP were quantified. The SFP volume, oblique, antero-posterior and, cranio-caudal lengths were determined. A decrease of IFP volume, depth, femoral, and tibial arch lengths in moderate and end-stage OA compared to controls were observed. A difference in IFP hypointense signal was found between groups. No differences were found in SFP characteristics between the groups. In controls and moderate OA patients, correlations were found among the different MRI characteristics of both IFP and SFP, while in the end-stage OA group correlations were found only in SFP. Differences of the IFP MRI morphometric characteristics between the groups analyzed supports an important role of IFP in OA pathology and progression. On the contrary, no differences were highlighted in SFP analysis suggesting that this fat pad is not clearly involved in OA, probably due to its peculiar localization and different function.

Key words

Suprapatellar fat pad, magnetic resonance imaging.

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The Computed Tomography of two Mummies from Ancient Egypt

Veronica Macchi[1], Andrea Porzionato[1], Massimo Favat[2], Claudia Gambino[3], Sandro Benetti[2], Cinzia Oliva[4], Matteo Crosera[5], Paola Zanovello[6], Emanuele Ciampini[7], Raffaele De Caro[1]

[1] Istituto di Anatomia Umana, Dipartimento di Neuroscienze, Università di Padova, Padova, Italia
[2] Radiology Department, Azienda Ulss 5, Rovigo, Italia
[3] Progetto Egitto Veneto, Università di Venezia, Venezia, Italia
[4] Venaria Reale, Università di Torino, Torino, Italia
[5] Arcadia Arte srl, Arcadia Arte srl, Venezia, Italia
[6] Dipartimento Dei Beni Culturali: Archeologia, Storia Dell'arte, Del Cinema E Della Musica (Dbc), University of Padova, Padova, Italia
[7] Dipartimento di Studi Umanistici, Università ca Foscari, Venezia, Venezia, Italia

In forensic clinical anatomy an objective non-destructive documentation of the body surface and of the interior of the body is given by computed tomography (CT), which is used in post-mortem radiological investigation. This technique is also applied in mummy studies, with the aim of providing a permanent record of the mummy's features. For the exhibition "L'Egitto Ritrovato", promoted by Fondazione Cassa di Risparmio di Padova e Rovigo, in Rovigo, more than 500 pieces of the entire Valsè Pantellini Collection of the Accademia dei Concordi of Rovigo have been showed. In particular two original mummies, one of a young woman, and the second of a child, underwent a process of a survey and restoration, which was conducted in such a way as to be visible also by visitors. A whole-body CT was performed on the two mummies. The CT examination showed the preservation of skeleton and documented the presence of conservative material inside the bodies, as well as the sites of incision to remove the organs. In fact the artificial mummification had the aim to preserve that person's morphologic features by delaying or arresting the decay of the body. The ancient Egyptians used to eviscerate the bodies, followed by desiccation with natron (a compound of sodium salts) to halt putrefaction and prevent rehydration. CT demonstrated to be a non-destructive method to investigate mummies, in order to acquire data on the individual anatomy and the preservation of the body. It allows for non-invasive insight, revealing detailed information about the mummy's sex, age, constitution, injuries, health, and mummification techniques. Moreover, CT allows not only the acquisition of sectional images but also, thanks to dedicated software, the post-processing and reconstruction of three-dimensional models, that can be used also for public displays.

Key words

Computed tomography, Mummies, Forensic clinical anatomy.

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Muscle-to-bone crosstalk: the Wnt/β-catenin pathway is a candidate mechanism mediating the signalling between C2C12 muscle cells and 2T3 osteoblasts

Maria Sara Magarò[1], Jessika Bertacchini[1], Francesco Potì[2], Marta Checchi[3], Marta Benincasa[1], Paola Sena[1] and Carla Palumbo[1]

[1] University of Modena and Reggio Emilia, Department of Biomedical, Metabolic Science and Neuroscience – Section of Human Morphology, Modena, Italia
[2] Department of Medicine and Surgery, Unit of Neurosciences, University of Parma, Parma, Italia
[3] University of Modena and Reggio Emilia, Department of Biomedical, Metabolic Science and Neuroscience – Section of Human Morphology, 41124, Modena, Italia

Bone and muscle have been recognized as endocrine organs since they produce and secrete "hormone like factors", osteokines and myokines, that can mutually influence each other and other tissues, giving rise to "bone-muscle crosstalk" [1]. Recently, evidences emerged that myokines have profound effects on osteocytes in culture and vice versa [2][3]. Besides, skeletal muscle secreted factors have been found to promote osteocytes and osteoblasts viability through activation of the Wnt/β-catenin pathway [2]. To determine whether myokines can potentially regulate osteogenesis, we conditioned the differentiation of 2T3 osteoblasts with 25% medium from early (day 1), mid (day 7) or late (day 14) myotubes. Osteoblast conditioned differentiation was investigated from different viewpoints: i) analysis of mRNA and protein levels of marker genes of differentiation; ii) characterization of the functional maturation by studying the deposition of mineralized matrix and the activity of alkaline phosphatase enzyme. To date, we have demonstrated that the early conditioned medium (day 1, CM 1) decreased significantly the mineralization degree of osteoblasts compared to control. Besides, CM 1 induced statistically significant increase in the expression of Sclerostin, both SOST mRNA transcription and protein synthesis during osteoblast differentiation. Sclerostin is a secreted glycoprotein mainly expressed in bone and cartilage matrix, considered a negative regulator of bone growth due to its role as an antagonist of the Wnt/β-catenin pathway. It has also been found to inhibit the pre-osteocyte differentiation of osteoblasts in vitro [4]. These data prompted us to analyze the protein content of CM1 and surprisingly we found that myotubes at day 1 and 4 of differentiation secrete Sclerostin. Therefore, we hypothesize that muscle cell-secreted Sclerostin may act as an endocrine negative regulator of 2T3 differentiation through inhibition of Wnt/β-catenin signaling and such effect may be enhanced by the induced osteoblast isoform acting in an autocrine loop.

Further experiments are needed to test the involvement of Sclerostin as mediator of the effects of the early CM. Thus, we are planning the following experiments: i) 2T3 differentiation with recombinant Sclerostin combined with its antibody-mediated neutralization, ii) overexpression of Sclerostin in 2T3 cells, iii) treatment of C2C12 cells with SOST siRNA and 2T3 conditioned differentiation with C2C12 siRNA-treated CM.

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Key words

Bone-muscle interactions, Sclerostin, Wnt/β-catenin, osteoblast differentiation.

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The clinic-pathological meaning of MCT1, MCT4, and GLUT1 in testicular germ cell tumors

Rocco Malivindi, Vittoria Rago, Marta Santoro, Saveria Aquila

Department of Pharmacy and Sciences of Health and Nutrition, University of Calabria, Cosenza, Italia

Testicular germ cell tumors (TGCTs) are the most frequent malignant tumors in male patients aged 15–45 years, their incidence is increasing in recent years. There are two main subclasses of TGCTs: seminomas (SE) and non-seminomatous germ cell tumors (NSGCTs). NSGCTs have varying degrees of differentiation such as embryonal carcinoma (EC) which tends to be metastatic at presentation and a worse prognosis than SE. Although the majority of patients with TGCTs have good responses to treatment, some of them resist to therapy presenting disease progression. Thus, it is important to better understand the biological heterogeneity of TGCTs to optimize treatments by exploring new pathways involved in tumor development and progression. Alterations in cellular metabolism are among the most consistent hallmarks of cancer. Depending on cellular context, cancers manifest an array of metabolic phenotypes. Here, aimed to identify immunohistochemistry (IHC) prognostic markers which could be used to the TGCTs management, we investigated the relevance of some metabolic target in TGCTs and normal testicular tissue (NT). Further, we evaluated the region adjacent to TGCTs (40% of samples) showing the presence of abnormal seminiferous tubules with decreased tubular diameters, identified as intratubular germ cell neoplasia (IGCNU) and representing testicular carcinoma in situ. There is evidence that these tumors are highly glycolytic, although just one study evaluated the monocarboxylate transporters MCT1 and MCT4 expression, while findings on GLUT1 in TGCTs are lacking. The glycolytic phenotype, a metabolic reprogramming in which cancer cells produce energy through glycolysis, leads to a greater glucose consumption and lactate production than normal metabolic profile. To avoid intracellular acidification some proteins are upregulated, including MCT1 and 4 mainly associated with lactate efflux. Glucose uptake is higher in some tumors by an increase of glucose transporters (GLUTs) and GLUT1 usually correlates with cancer malignancy. Interestingly, from our IHC data it emerged that: MCT1 was higher expressed in EC with respect SE and IGCNU, although there were no significant differences with respect NT; MCT4 expression increased in EC with respect SE, IGCNU and NT; GLUT1 levels augmented in EC and in a lesser extent in SE with respect IGCNU and NT where it is absent. The association of MCT1, MCT4 and GLUT1 expression with characteristics of worse prognosis, such as nonseminomatous histology, higher stages, metastasis occurrence, vascular invasion, reflect the function of these proteins in lactate efflux, thus maintaining an extracellular acid pH, and a greater influx of glucose, corroborating the glycolytic phenotype of these tumors.

This study discovered that GLUT1 may be considered of clinic-pathological significance in TGCTs and as an IHC prognostic marker in the differentiation of these tumors.

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The role of the secretin/secretin receptor axis in the modulation of liver fibrosis via changes in TGF-1β-mediated biliary senescence

Romina Mancinelli[1], Antonio Franchitto[2], Gianfranco Alpini[3], Paolo Onori[2], Luigi Pannarale[2] and Eugenio Gaudio[2]

[1] Sapienza University of Rome, Dipartimento scienze anatomiche, istologiche, medico legali e dell'apparato locomotore, Roma, Italia
[2] Sapienza Università di Roma, Dipartimento scienze anatomiche, istologiche, medico legali e dell'apparato locomotore, Roma, Italia
[3] Texas A&M Health Science Center, Department of Medicine and Medical Physiology, Temple, Texas, Italia

The biliary epithelium and its cells, cholangiocytes, are the target in several human cholangiopathies including primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), which are diseases characterized by extensive fibrosis (1). Proliferating cholangiocytes display neuroendocrine characteristics and secrete and respond to several neuropeptides and gastrointestinal hormones that modulate cholangiocyte responses to injury via autocrine/paracrine mechanisms (2). Secretin (Sct) exerts its effects through secretin receptor (SR), which is expressed in the liver by large cholangiocytes. Enhanced biliary proliferation during cholestasis is associated with increased SR expression on cholangiocytes and increased cAMP dependent secretin-stimulated ductal secretion (3). Our aim was to define the role of Sct-regulated cellular senescence and demonstrated that liver fibrosis is significantly reduced in Sct-/-, SR-/- and Sct-/-/SR-/- BDL mice compared to BDL wild-type (WT) mice. The reduction in hepatic fibrosis in Sct-/-, SR-/- and Sct-/-/SR-/- BDL mice was accompanied by reduced TGF-β1 levels in serum and cholangiocyte supernatant as well as decreased expression of markers of cellular senescence in cholangiocytes in contrast to increased expression of cellular senescence in hepatic stellate cells (HSCs) compared to BDL WT mice. Sct directly stimulated the senescence of cholangiocytes and regulated by a paracrine mechanism the senescence of HSCs and liver fibrosis via modulation of TGF-β1 biliary secretion. Targeting senescent cholangiocytes may represent a novel therapeutic approach for ameliorating hepatic fibrosis during cholestatic liver injury.

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Key words

Biliary epithelium, cholangiocytes, fibrosis, secretin.

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Role of autophagy in T regulatory cells (Tregs)-polarization during atherosclerosis disease

S. Mandatori[1], D. Starace[1], S. Piconese[2], I. Pacella[2], M. Caprio[3,4], A. Armani[3], V. Marzolla[3], C. Mammi[3], F. Padula[1], E. Ziparo[1], F. Cecconi[5,6,7] and A. Filippini[1]

[1] Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Sapienza University of Rome
[2] Department of Internal Medicine and Medical Specialties, Sapienza University of Rome
[3] Laboratory of Cardiovascular Endocrinology, IRCCS San Raffaele Pisana, Rome, Italy.
[4] Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Roma Open University, Rome, Italy
[5] Department of Biology, University of Rome Tor Vergata, Rome, Italy.
[6] Department of PaediatricHaematology and Oncology, IRCCS Bambino Gesù Children's Hospital, Rome, Italy.
[7] Unit of Cell Stress and Survival, Danish Cancer Society Research Center, Copenhagen, Denmark.

Atherosclerosis is a chronic inflammatory disorder of the large arteries and represents the primary cause of heart disease and stroke. The exact cause of atherosclerosis is not known. A variety of studies show that autophagy deficiency may be pro-atherogenic and the role of autophagy in smooth muscle cells, macrophages and endothelial cells has been investigated [1]. However, to date no studies addressed the effect of autophagy on leukocyte subsets playing a role in plaque formation and development. The presentproject aims to better clarify the role played by autophagy in lymphocyte homeostasis in human atherosclerotic plaques and in APOE-KO, a mouse model of atherogenesis [2]. In particular, we will investigate cell-autonomous autophagy in mouse Tconv/Tregfunctions, evaluate autophagy-driven T cell polarization in stabilizing atherosclerotic plaques in a mouse model mouse of atherosclerosis. The comprehension of the role of autophagy as a further mechanism underlying Treg induction and stability may open new therapeutic avenues for atherosclerosis.

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Key words
Tregs, autophagy, atherosclerosis, ApoEKO.

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Using an adapted version of "PCEM" to test "Adage"

Elena Mannacio[1], Maurizio Ripani[2], Antonello Ciccarelli[1], Filippo Tajani[1] and Antonio Tessitore[1]

[1] Università degli studi di Roma "Foro Italico", Dipartimento di Scienze Motorie, Umane e della Salute, Roma, Italia
[2] Università degli studi di Roma "Sapienza", Dipartimento di Scienze Motorie, Umane e della Salute, Roma, Italia

Classical ballet is a high-intensity intermittent form of exercise [1], characterized by an aesthetic component, which requires the dancers to show their grace, elegance and beauty. All these features are expressed by the 'adage', which workload, in terms of exposure and duration, can vary considerably in relation to influence of training [2]. The aim of this study was to assess the workload's effects on the aesthetic components of an 'adage' performed before and after a structured training. Nine female classical ballet dancers (age: 19.3±3.3 years; height: 165±4.6 cm; weight: 52.6±2 kg; years of dance training: 10.5±2.3), free from injury, were recruited and submitted the following procedure: 1) 'adage' assessment (PRE); 2) specific training protocol; 3) 'adage' assessment (POST). Both the 'adage' (70-second) and training (60-minute) were expressly choreographed for this study. Moreover, the PRE and POST 'adage' were video recorded and observed by a very experienced ballet teacher through a modified version of the Performance Competence Evaluation Measure (PCEM) questionnaire [3] to assess: 1) Full Body Involvement (FBI); 2) Body Integration and Connectedness (BIC); 3) Articulation of Body Segments (ABS); 4) Movement Skills(MS).

The Wilcoxon Signed Ranks Test showed no significant differences between pre- and post-rehearsal 'adage' performances [FBI, $p=0.527$; BIC, $p=0.317$; ABS, $p=0.083$; MS, $p=0.059$). Despite results were not significantly, FBI showed lower post-training mean values (performance decrement), which can be speculated as a consequence of fatigue. It is also interesting to note that all other parameters showed higher post-training mean values, which can be explained by the positive effects of classical ballet exercise on fluency, rhythm and quality of movements. In conclusion, a ballet routine lesson (training) certainly affects the performance of the 'adage.

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Key words

Classical ballet, adapted PCEM.

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Protective effect by Vitis vinifera L. extract after UVA irradiation in human endothelial cells EAhy.926: genotoxical and morphological analysis

Laura Marabini[1], Giulia Lombardo[2], Laura Cornaghi[2], Stefano Piazza[3], Marina Marinovichv[3] and Elena Donetti[2]

[1] Università degli Studi di Milano, Department of Environmental Science and Policy, Milan, Kenya
[2] Università degli Studi di Milano, Department of Biomedical Science for Health, Milan, Italia
[3] Università degli Studi di Milano, Department of Pharmacological and Biomolecular Sciences, Milan, Italia

Dermal microcirculation can be affected by the ultraviolet (UV) component of solar radiation (wavelength 100 - 400 nm). In particular UVA is the most penetrating radiation (320-400 nm) and UVA exposure can induce several types of DNA damage in skin cells through an oxidative mechanism. The generated reactive species (ROS) lead to oxidative base modifications with single and/or double strand breaks (SSB and DSB). Flavonoids (flavonols and anthocyanins) can exert a ROS scavenging action or counteract ROS damage. For this reason, we aimed at investigating the UVA effects in an endothelial cell line, EAhy.926, as a first approach to mimic dermal microcirculation in order to preliminary elucidate the possible UVA effect. The aims of our study were i) to characterize UVA damage from morphological and genotoxic point of view and ii) to evaluate the protective action of Vitis vinifera L. water extract from dried leaves after UVA irradiation. The treatment with the extract of Vitis Vinifera L. (100 μg/ml, 1h in serum-free media), particularly rich in flavonoids, was followed by the exposure to UVA (2.5-5-10-20 J/cm2) radiation in PBS. Pre-treatment with the extract before UVA exposure restored almost completely the subcellular organization. On the whole, these data suggest that Vitis Vinifera L. extract can revert UVA damage not only by acting as a scavenger, as evidenced by the reduced production of ROS, but probably by activating the cellular detoxifying enzymatic system antioxidant. The next step will be the use of primary dermal endothelial cells to strictly reproduce the physiological environment.

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Cartilage repair by amniotic fluid stem cell exosomes

Tullia Maraldi, Manuela Zavatti and Francesca Beretti

University of Modena and Reggio Emilia, Dip Department of Surgical, Medical, Dental and Morphological Sciences with interest in Transplant, Oncology and Regenerative Medicine, Modena

Mesenchymal stromal cells from the amniotic fluid (AFSCs) have been shown to favour tissue repair and regeneration after transplantation in rodent models of inflammatory-based disease. The benefits of transplanted amniotic cells were observed despite cell engraftment in injured tissue, thus suggesting these cells produce bioactive factors able to mediate these effects through paracrine signaling. It is increasingly more evident that the bioactive factors included into extracellular vesicles produced by amniotic cells (i.e. exosomes) can trigger tissue repair and regeneration through the resolution of inflammation by acting on different inflammatory mediators. Articular osteochondral defects are often associated with severe joint pain and progressive loss of joint function. The cartilage tissue engineering associated to stem cell-related therapies is becoming very interesting since adult articular cartilage has limited intrinsic capacity for regeneration upon injury.

Based on this state of the art, the main aim of this study was to explore the efficacy of the secreted exosomes, compared to their AFSC source, in animal model of osteoarthritis to mimic a chronic and degenerative process, a paradigm condition in which immune-related mechanisms are prominently involved and lead to irreversible joint damage.

In this study, osteochondral defects were created on rats knees with monoiodoacetate injection. After 3 weeks, the defect was treated with 100 μg exosomes or 5*105 cells derived from three amniocentesis. After 2 weeks exosome treatment was repeated. Analyses were performed by histology, immunohistochemistry, and behavioral scoring up to 4 weeks after the treatment.

Generally, exosome-treated defects showed enhanced behavioral test and improved histological scores than the AFSC-treated defects. Indeed by 4 weeks, TGFβ- rich exosome samples induced a complete restoration of cartilage with characteristic features including a hyaline cartilage with good surface regularity, showing extracellular matrix with GAG and collagen II, and complete bonding to adjacent cartilage. In contrast, there were only fibrous repair tissues found in the PBS-treated defects and initial cartilage close to fibrous tissues in AFSC-treated samples.

This study demonstrates for the first time the efficacy of human AFSC exosomes in cartilage repair, and a positive correlation with the content of TGFβ.

Key words

Exosomes stem cells, osteochondral defects.

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Reappraising the microscopic anatomy of human testis: identification of telocyte networks in the peritubular and intertubular stroma

Mirca Marini[1], Irene Rosa[1], Daniele Guasti[1], Mauro Gacci[2], Eleonora Sgambati[3], Lidia Ibba-Manneschi[1] and Mirko Manetti[1]

[1] University of Florence, Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, Florence
[2] University of Florence, Department of Minimally Invasive and Robotic Urologic Surgery and Kidney Transplantation, Florence
[3] University of Molise, Department of Biosciences and Territory, Pesche (Isernia)

Telocytes are a recently described stromal cell type widely distributed in various organs including the female and male reproductive systems. This study was aimed to investigate for the first time the existence, distribution and characteristics of telocytes in normal human testis by an integrated morphological approach (immunohistochemistry, immunofluorescence and transmission electron microscopy). We found that telocytes displaying typical long and moniliform prolongations and coexpressing CD34 and platelet-derived growth factor receptor-α formed networks in the outer layer of peritubular stroma and around Leydig cells and vessels in the intertubular stroma. Testicular telocytes were immunophenotypically negative for CD31, c-kit/CD117 as well as α-smooth muscle actin, thus making them clearly distinguishable from myoid cells/myofibroblasts located in the inner layer of peritubular stroma. Transmission electron microscopy confirmed the presence of cells ultrastructurally identifiable as telocytes (i.e. cells with telopodes alternating podomers and podoms) in the aforementioned locations. Intercellular contacts between neighboring telocytes and telopodes were observed throughout the testicular stromal compartment. Telopodes intimately surrounded and often established close contacts with peritubular myoid cells, Leydig cells and vessels. Extracellular vesicles were also frequently detected near telopodes. In summary, we demonstrated that telocytes are a previously neglected stromal component of human testis with potential implications in tissue homeostasis deserving further investigation.

Key words

Telocytes, stromal cells, human testis, microscopic anatomy.

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Ascending aorta phenotypic and genotypic changes in bicuspid aortic valve disease

Antonella Marino Gammazza[1], Calogera Pisano[2], Rosario Barone[1], Emanuele Lentini[3], Alessandro Ricasoli[3], Giovanni Ruvolo[2], Vincenzo Argano[3], Felicia Farina[4], Francesco Cappello[1], Francesca Rappa[1]

[1] Dipartimento di Biomedicina Sperimentale e Neuroscienze Cliniche, Università degli Studi di Palermo, Palermo, Italia
[2] Ospedale Universitario Tor Vergata, Ospedale Universitario Tor Vergata, Roma, Italia
[3] Dipartimento di Cardiochirurgia, Università degli Studi di Palermo, Palermo, Italia
[4] Dipartimento di Cardiochirurgia, Dipartimento di Biomdedicina Sperimentale e Neuroscienze Cliniche, Università degli Studi di Palermo, Palermo, Italia

Bicuspid aortic valve (BAV) with left-right (L-R), right-non coronary (R-NC) and left-non coronary (L-NC) cusp fusion represents distinct pathological entities and the rate of aortic enlargement varies according to the pattern of cusps fusion [1]. Here, we investigated the histological features of aneurysms associated to different BAV phenotypes and we looked for specific microRNAs (miRNA) as biomarkers of medial degeneration severity.

Aortic specimens and blood were obtained from BAV patients treated surgically for the repair of thoracic aortic aneurysm and were divided into two groups: low grade medial degeneration (LGMD, n=10); high grade medial degeneration (HGMD, n=10). A control group (CN, n=10), with tricuspid aortic valve not associated to aortopathy, was also involved in the study. We performed commonly used morphological staining to evaluate medionecrosis, fibrosis, elastic fragmentation and mucoid material accumulation. We detected MMP9 and MMP2 immunoreactivity and tunel assay. Moreover, we measured the expression patterns of miR-122, miR-130, miR-718, miR-486 by RT-qPCR.

MMP2 and MMP9 expression increased in HGMD compared to LGMD and control group. Apoptotic cells were observed in the sub intimal region of the media in HGMD group. The expression levels of miR-718 and miR-122 in aortic specimens significantly decreased in LGMD and HGMD groups compared to CN and negatively correlated with the ascending aorta wall score. Moreover, the expression levels of miR-130 significantly decreased in LGMD group compared to CN. HGMD group showed a significant increase of miR-486 expression levels compared to CN and LGMD. Plasma expression levels of miR-718 significantly decreased in LGMD compared to CN. Interestingly, miR-486 expression levels significantly increased in HGMD group compared to the CN and positively correlated with the ascending aorta wall score.

Our work suggests miR-718 and miR-486 might be considered as new non-invasive biomarkers of aorta wall degeneration in BAV due their association with the morphological features of the vessel. A significant dysregulation of these biomarkers might be associated with high risk of dissection and rupture.

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Key words

BAV disease, microRNA.

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Dental adhesive effects on human gingival fibroblasts: viability, extracellular matrix and inflammatory gene expression

Lorella Marinucci[1], Egidia Costanzi[1], Stefania Balloni[1], Stefano Cianetti[2], Guido Lombardo[2], Maria Bodo[1] and Stefano Pagano[2]

[1] Università di Perugia, Dipartimento di Medicina Sperimentale, Perugia, Italia
[2] Università di Perugia, Dipartimento di Scienze Chirurgiche d Biomediche, Perugia, Italia

The development of aesthetic dentistry has led to an increase of composite materials and dental adhesives, both in restorative and prosthetic practice [1]. Dental adhesives are resinous biomaterials used for bonding the composite material to dental tissues (enamel or dentin). These materials consist of an organic matrix of methacrylates, like BisGMA, HEMA, TEGDMA, UDMA, EGDMA, DEGDMA and other additives as benzoylperoxide and camphoroquinone [2,3]. Some studies have focused on the toxicity of materials with metacrilic monomers, responsible for cytotoxic, local inflammatory and allergic reactions (1). This study investigated the effects of two self-etching systems, Adhese Universal (Ivoclar, USA) and Optibond (Kerr, USA), on viability and on extracellular matrix (ECM) proteins and inflammatory cytokine expression in human gingival fibroblasts, involved in ECM remodelling, immune reaction and inflammation. Cell viability was assessed up to 72h in presence of adhesive extracts at different dilutions (from 3,125% to 100%) by MTT assay. ECM proteins and cytokines gene expression was analyzed by RT-PCR [4] after 1, 3 and 48h of exposition to 100% adhesive extracts. It was observed a significant stimulation of proliferation, especially for Universal adhesive in the short times (up to 24h), while no proliferation was observed at 48 and 72h. The 3,125% dilution had no effects at all times for both adhesives. Adhesive extracts induced an early or late upregulation of collagen type I (1h) and fibronectin (48h), respectively, to support of enhanced proliferation. The increased VEGF expression at 1 or 3h was another signal of active cell growth. In concert with higher metabolism, it was observed high MMP1 expression with Universal. Finally, the cytokine pattern analysis showed an upregulation of IL-1b by both adhesives; on the contrary, IL-6 and IL-8 were upregulated by Universal and Optibond adhesives, respectively. In conclusion these findings showed that used adhesives stimulated the proliferation with the unbalance of cytokine signaling and ECM synthesis and degradation, with some difference due probably to their composition.

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Key words

Dental adhesive, gingival fibroblasts, proliferation, gene expression.

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Evaluation of the effect of exosomes isolated from stem cells in in vivo model of ALS

Raffaella Mariotti, Roberta Bonafede, Chiara Buoso, Ilaria Scambi, Lorenzo Schiaffino

Università di Verona, Dipartimento Neuroscienze Biomedicina e Movimento, Verona, Italia

Amyotrophic lateral sclerosis (ALS) is a late-onset fatal neurodegenerative disease: its peculiarity is represented by the progressive loss of the upper and lower motor neurons (LMNs) at the spinal or bulbar level. The ALS sporadic form affects 90-95% of the cases, while the remaining 5-10% are familiar. The superoxide dismutase 1 (SOD1) gene was the first identified gene to be correlated with familiar form.

An effective treatment is currently not available; some drugs seem to modestly slow down the disease progression, influencing minimally the survival of the patients. A promising therapeutic approach for ALS is represented by mesenchymal stem cells, in particular, adipose stem cells (ASC). The beneficial effect of these cells seems to be due to a paracrine action via the release of exosomes (ASC-exosomes).

Exosomes are small vesicles (30-100 nm) containing lipids, proteins, and nucleic acids related to the type of cell that secretes them. Exosomes, through the release of their content, enhance the repair of the damaged area and could be used as a novel cell-free therapeutic approach, avoiding all the risks associated with the use of cells, there are also evidence in in vitro model of ALS.

On this basis we wanted to assess the efficacy of ASC-exosomes in in vivo model of ALS, the SOD1(G93A) mice. We injected ASC-exosomes intravenously, every four days, from the onset of the animals until the end stage. The progression of disease was monitored through the behavioural motor test and the evaluation of neurological score. Despite we did not observe a postponement of the treated animal's survival, we show that the treatment delays the symptoms progression of the disease of the treated animals compared to the control group. Moreover, the evaluation of the motoneurons number and the inflammatory state, through the assessment of astrocytes and microglia activation, is ongoing. In addition, in order to identify some of the molecular pathways by which exosomes could exert the neuroprotective effect on motoneurons, we performed the protein content characterization of ASC-exosomes. These data suggest that ASC-exosomes exert a neuroprotective role in in vivo model of ALS, underlining a possible therapeutic use of exosomes in this neurodegenerative disease and also suggest molecules that could be responsible for these neuroprotective effect in order to potentiate their effect in the future.

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Reparative human dentin: immunohistochemical localization and quantification of Small Integrin-Binding LIgand N-linked Glycosproteins (SIBILINGs)

Desiree Martini[1], Gabriella Teti[1], Maria Menetti[1], Sandra Durante[2] and Alessandra Ruggeri[1]

[1] Università di Bologna, Dipartimento di Scienze Biomediche e Neuromotorie (DIBINEM), Bologna, Italia
[2] Università di Bologna, Giorgio Prodi Cancer Research Centre, Bologna, Italia

Human dentin is formed during the process of dentinogenesis synthesized by odontoblasts that, in addition to type I collagen, also secrete a number of non-collagenous proteins (NCPs) into extracellular matrix (ECM) during the process of dentinogenesis. The Small Integrin-Binding LIgand, N-linked Glycoprotein (SIBLING) family is one category of NCPs characteristic for dentin and bone including dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP), bone sialoprotein (BSP), and osteopontin (OPN) [1].

Tertiary dentin is produced in reaction to external noxious stimulus/injury, such as trauma ordental caries and based on the type and extent of external stimuli or injuries, is further classified into reactionary dentin (RD) and reparative dentin (RepD).

Aim this study was to compare pattern distribution and quantification of SIBILINGs in reparative and reactionary dentin matrix, in response to stimulus, vs human sound dentin.

Ten carious human molars and ten sound human molars were selected for the study, demineralized, fixed, and processed for immunohistochemical approach to detect SIBILINGs. In particular specimens were submitted to an immunolabeling technique by using primary antibodies anti dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP), bone sialoprotein (BSP), osteoponti (OPN), observed at light microscopy and then submitted to quantitative analysis.

Results indicate that the region exposed to carious lesion, SIBILINGs formed a layer of reparative dentin bridge sealing cavity formed by carious lesion and pulp chamber.

In response to the injury, the newly differentiated odontoblast-like cells makes adjustments to meet the challenges by altering the production of these dentinogenesis-related molecules, attempting to produce a hard barrier formation in a very rapid process.

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Key words

Human reparative dentin, SIBILINGs, immunohistochemical technique, light microscopy.

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Induction of leukemic myeloid progenitor cell death by a combination of ER and oxidative stress

Silvia Masciarelli[1], Ernestina Capuano[1], Tiziana Ottone[2], Mariadomenica Divona[2], Serena Lavorgna[2], Alessandra Picardi[2], Maria Teresa Voso[2], Francesco Lo-Coco[2] and Francesco Fazi[1]

[1] Sapienza University of Rome, Department of Anatomical, Histological, Forensic & Orthopedic Sciences, Section of Histology & Medical Embryology, Rome
[2] University of Rome Tor Vergata, Department of Biomedicine and Prevention, Rome

The clonal expansion of hematopoietic myeloid precursors blocked at different stages of differentiation characterizes the acute myeloid leukemia (AML) phenotype characterized by the expression of fusion or mutant proteins that cause impaired differentiation and enhanced proliferation and survival. We previously showed that APL cell lines and primary blasts induced to differentiate by RA become highly sensitive to amounts of ER stress not detrimental for the same cells in the absence of Retinoic Acid (RA) [1]. Furthermore the same cells resulted sensitive to a combination of ER stress inducers with Arsenic Trioxide (ATO) that generates oxidative stress. Importantly we observed that ER stress caused increased amounts of disulphide-bound high molecular weight aggregates of PML-RARα and PML, exacerbating the alteration of cellular proteostasis already generated by induction of ER stress. This observation provides the rationale to translate the findings we observed in APL to other types of AML characterized by fusion or mutant proteins. The presence of mutant proteins that are easily prone to aggregation or mis-folding, because of their mutant structure or because of mis-localization, could render the cells sensitive to levels of ER and oxidative stress that could be recovered in their absence. We first tested a panel of AML cell lines characterized by different oncogenic fusion or mutant proteins and we found that ML-2 cells, bearing the MLL-AF6 fusion protein, and MV-4-11 cells, expressing the fusion protein MLL-AF4 and FLT3-ITD are highly sensitive to the combination of sub-lethal amounts of RA, Tm and ATO. In the cells undergoing ER and oxidative stress in combination, we found prolonged activation of the antioxidant response and of the unfolded protein response (UPR), activated by ER stress, as indicated by the expression of HMOX, CHOP, BiP and sXBP1. Importantly, the combination of ER and oxidative stress significantly reduces the colony forming capacity of primary leukemic blasts isolated from the bone marrow of FLT3-ITD positive patients. Altogether our data suggest that the combination of ER and oxidative stress leads to apoptosis rather than recovery, achieved instead when the same stresses are induced alone.

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Key words

ER stress, oxidative stress, myeloid progenitors.

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Anatomical and surgical insights for hypogastric nerves preservation during pelvic retroperitoneal dissection

Manuela Mastronardi[1], Anna Maria Billi[1], Giulia Adalgisa Mariani[1], Sara Mongiorgi[1], Desiree Martini[1], Mohamed Mabrouk[2], Renato Seracchioli[3], Lucia Manzoli[1]

[1] Dipartimento di Scienze biomediche e neuromotorie, Università di Bologna, Bologna, Italia
[2] Ginecologia e Fisiopatologia della Riproduzione Umana, Policlinico S. Orsola Malpighi, Bologna, Italia
[3] Ginecologia e Fisiopatologia della Riproduzione Umana - DIMEC, Università di Bologna, Bologna, Italia

During several gynecological retroperitoneal pelvic surgeries, portions of the pelvic autonomic nervous system can be accidentally damaged, in particular hypogastric nerves, leading to significant visceral dysfunctions, dramatically affecting woman's quality of life. The aims of this study were to clarify the relationship of hypogastric nerve with definite anatomical landmarks and to assess any anatomical differences between the two sides of the pelvis.

Detailed pelvic retroperitoneal dissection was performed in 5 nulliparous embalmed female cadavers and in 10 nulliparous women during in vivo laparoscopic surgery for rectosigmoid endometriosis without parametrial infiltration or radical hysterectomy (B1 according to Querleu-Morrow) for cervical cancer. On both hemipelvis, the closest distance between HNs and ureters, midsagittal plane, midcervical plane or uterosacral ligaments were documented. Comparison of anatomical data of the two hemipelvis were conducted.

On cadavers and in vivo dissection, a right and left hypogastric nerves, covered by pre-hypogastric fascia, were identified in all specimens. Irrespective of the side, a wide anatomical variability was reported. Regarding differences between the two hemipelvis, we found that the right hypogastric nerve was further to the ureter and closer to the midsagittal plane than the left one. Mid-cervical plane was found 2.7 mm to the left of the midsagittal one. Right hypogastric nerve was found closer to mid-cervical plane and utero-sacral ligament than the left one.

An accurate knowledge of the pelvic retroperitoneal anatomy and differences between the two sides of the pelvis are essential to preserve hypogastric nerve during surgical dissection. Because of the wide anatomical variability, the use of an interfascial approach between fascia propria recti and pre-hypogastric fascia could help to perform an efficient nerve-sparing surgery.

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Modulation of the LC3B and p62 expression in conjunctival fibroblasts by pro-inflammatory macrophages

Elena Mattiuzzo[1], Elena Tarricone[1], Andrea Leonardi[2], Antonino Di Stefano[3] and Paola Brun[1]

[1] University of Padova, Department of Molecular Medicine, Padova, Italia
[2] University of Padova, Department of Neuroscience, Ophthalmology Unit, Padova, Italia
[3] Fondazione S. Maugeri, IRCCS, Istituto Scientifico di Veruno, Veruno, Italia

Autophagy is a key regulatory process involved in many biological aspects, including cell survival, tissue regeneration and homeostasis. Recent studies demonstrated that de-regulated autophagy is implicated in some inflammatory diseases. Previous data obtained in our laboratory demonstrated that some autophagic markers such as LC3B, cathpesin D, Beclin-1 are over-expressed in a severe inflammatory disease such as vernal keratoconjunctivitis (VKC).

In the present study VKC conjunctival cell cultures were exposed to the inflammatory media of activated U937 monocytes to explore the role of inflammatory factors in the induction of autophagy.

Macrophage differentiation of U937 was induced by LPS and PMA and then incubated with fresh DMEM and 10%FBS to produce the conditioned medium. qPCR analysis of the activated cells revealed that IL1beta, TNFalfa were overexpressed. Primary conjunctival cell cultures were then treated with the inflammatory medium conditioned by activated U937and analysed for expression of some autophagic markers at 4, 10 and 24 hours after exposure. qPCR results demonstrated that LC3B, Beclin-1, LAMP1 and p62 increased from 4 to 24 hours. Western blotting analysis revealed cleavage and lipidation of LC3B quantified as an increased LC3BII/LC3BI ratio.

In conclusion, our data demonstrated that the environment created by inflammatory macrophages enahances autophagy in VKC conjunctival fibroblasts.

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Molecular mechanism of PACAP-induced EGFR transactivation in an in vitro model of ALS

Grazia Maugeri[1], Agata Grazia D'Amico[2], Daniela Maria Rasà[1], Concetta Federico[3], Salvatore Saccone[3], Sebastiano Cavallaro[4], Velia D'Agata[1]

[1] University of Catania, Section of Human Anatomy and Histology, Department of Biomedical and Biotechnological Sciences, Catania
[2] University of Rome, San Raffaele Open University of Rome, Department of Human Science and Promotion of quality of Life, Roma
[3] University of Catania, Section of Animal Biology, Department of Biological, Geological and Environmental Sciences, Catania
[4] National Research Council, Institute of Neurological Sciences, Catania

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting both upper and lower motor neurons. By analyzing the transcriptional profile of motor cortex samples from sporadic ALS (SALS) patients, we have previously selected two patient subgroups, known as SALS1 and SALS2. Since Cu/Zn-superoxide dismutase-1 (SOD1) mutation is involved some cases of familial ALS, we have also compared the gene expression profile of motor cortex from SALS patients and SOD1 G93A mice through a meta-analysis study [1]. From this analysis, 19 statistically significant genes emerged as deregulated both in mice and SALS patients, comprising: pituitary adenylate cyclase-activating polypeptide (PACAP), epidermal growth factor receptor (EGFR) and matrix metallopeptidase 2 (MMP-2) [2]. Considering the functional link between PACAP and EGFR, already described both in neurons and in cancer cells, here we have investigated the involvement of PACAP, EGFR and MMP-2 genes in this neurodegenerative disease.

The study has been performed in NSC34 motor neuronal cell lines expressing G93A SOD1.

Our data have showed that PACAP is able to rescue cells degeneration following growth factors deprivation. Its effect is induced through EGFR transactivation mediated by protein kinase A stimulation. Moreover, EGFR phosphorylation triggers the activation of MEK/ERK1/2 survival signaling pathway and increased MMP-2 expression, drastically reduced by serum starvation.

In conclusion, our findings suggest that a deeply characterization of the mechanism linking PACAP/EGFR/MMP-2 axis to SOD1 mutation may open a new perspective for ALS therapy.

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Key words

ALS, PACAP, EGFR, MMP-2.

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Amnion derived epithelial stem cells for the treatment Osteochondritis in a equine model

Eleonora Mazzotti, Angelo Canciello, Luca Valbonetti and Aurelio Muttini

Università degli Studi di Teramo, Teramo, Italia

Repair of articular cartilage defect, occurring as a result of osteoarthritis, fracture, fragmentation, or surgical debridement of Osteochondritis dissecans (OCD) lesions or subchondral bone cysts, represents a significant challenge for orthopaedic surgeons.

Articular cartilage regeneration, following injury is impaired by inherently poor vascular supply and the limited cellular content of hyaline cartilage. Greater Challenges present with larger defect and with subchondral bone involvement and currently no "gold standard" technique exists for repair of such defects.

OCD, a disruption of endochondral ossification, is a common orthopaedic developmental disease in many species, including humans and horses, and results in separation and instability of the overlying articular cartilage.

Equine models are currently recommended for preclinical assessment of new strategies for cartilage repair as they provide the closest approximation to humans in terms of cartilage thickness.

Amnion-derived epithelial stem cells (AECs) are considered a promising source for the treatment of orthopedic diseases. AECs demonstrated to be very effective in tendon tissue regeneration both in experimental and clinical studies, but a direct response of the synovial joint to intra-articular injection of eterologous AECs was never been performed. Furthermore, recent studies demonstrated the low immunogenicity of these cells and their immunomodulatory effect. We tested and studied the clinical response to repeated intra- articular injection of AEC in a 3 year-old saddle horse with spontaneus bilateral OCD of the knee. Clinical signs included joint swelling andlight bilateral lameness; radigraphs swowed a typical ODC picture with involvement of the femoral troclear ridges and of the patella. After aseptic preparation of the knees, an aliquot of 10^6 AECs in 500 μl of αMEM was injected into each joint. The procedure was repeated after two months. Neither adverse reactions nor signs of discomfort were noted following the injection. Clinical and radiographic details showed a significant improvement during the year after the treatment. Actually the horse is used for pleasure and jumping activities with satisfaction of the owner. The repeated injection of ovine AECs into the joints of a horse did not cause any negative reaction but rather a clinical improvement and this confirms the immunomodulatory properties of these cells. The clinical and radiographic data suggest that repeated intra-articular injection of AECs could help the recovery of OCD affected joints.

Further studies are needed but these promising results could demonstrate the effective clinical use of this cell-based therapy and could shows potential for clinical translation to human patients.

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Trans-passing Achilles Tenotomy: A New Improved Experimental Surgical Technique in Rats

Eleonora Mazzotti, Valentina Russo, Paolo Berardinelli, Annunziatina Mauro, Mohammad El Khatib, Luca Valbonetti and Barbara Barboni

Faculty of Bioscience and Agro-food and Environmental Technology, Università degli Studi di Teramo, teramo, Italia

Tendinopathy is a debilitating musculoskeletal condition which can cause significant pain and can lead to a complete rupture of the tendon. These disorders continue to be a clinical challenge, although a range of different biomaterials have been developed to fabricate biomimetic scaffolds for tendon regenerative medicine. Thus, there is now the realistic potential for new technologies to significantly improve the clinical outcomes of this challenging pathology.

Animal models are often used in this field of research as they offer an attractive framework to examine the cascade of processes that occur throughout both tendon pathology and repair. Additionally, animal models provide the ability to reproduce consistent and repeatable injuries that can be treated in a controlled and quantifiable manner and also allow the evaluation of invasive treatments and assessment that would be unethical with human subjects.

The aim of this study was to identify, in rats, a new surgical experimental model that allows to test the safety, biocompatibility and teno-regenerative potential of tendon biomimetic scaffolds for a future in vivo treatment of damaged tendons.

Surgical procedures were performed in 10 cadaver Wistar rats under stereomicroscope. A posterior longitudinal incision, approximately five millimeters in length, was made. The Plantaris tendon was recognized and retracted medially, under a stereomicroscope, which allowed recognition of the Achilles tendon. A longitudinal and trans-passing cut was created in the middle of Achilles tendon, medial to the plantaris tendon, which was carefully preserved. The incision was filled with a biomimetic scaffold and closed using 2 cross stitches using dissolvable sutures.

The improved surgical technique used, for the tenotomy of the Achilles tendon in rats is simple and quickly done and could be used as an easily reproducible and validated model of experimental tendon lesion to test the biocompatibility of tissue specific scaffolds. Indeed, this technique would give in the future the main advantage to verify in vivo if the inserted scaffold within the produced experimental lesion would integrate, and if it is safely tolerated within the host tissue, as well as if it would have any teno-regenerative potential, which could be determined ex vivo on tissue explants.

Moreover, this technique, thanks to the use of tendon specific scaffolds and to this innovative surgical approach, could reduce the possibility of post-surgery adhesions and tendons' scars, which could encourage translation researches about this common tendon injury in humans.

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Expression Of Collagen Type I And Type Iii Proteins As New Markers For Post Mortem Interval Estimation In Human Gingival Tissue

Maria Carla Mazzotti[1], Desiree Martini[2], Laura Ingrà[2], Paolo Fais[1], Susi Peloti[2], Mirella Falconi[2]

[1] Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche, Bologna, Italia
[2] Università di Bologna, Dipartimento di Scienze Biomediche e Neuromotorie, Bologna, Italia

Estimating the time since death is still a crucial aspect in forensic sciences and it is pursued by a variety of methods. However, most of these applied methods have practical limitations and provide insufficient results under certain circumstances.

After death, connective tissues undergo several morphological changes involving both the cellular components and the extracellular matrix. Indeed, proteins are subjected to increasing decay over the time, resulting in a modification of their concentrations in different tissues as well as in an increase of post-translational modifications.

In our previous study [1], we combined morphological and immunoistochemical analyses in post mortem gingival tissues to reach a more detailed knowledge on tissue organization and degradation after death, identifying typical morphological changes as well as different pattern of expression of HIF1alpha protein that correlate to the time of death.

In this study, we aimed at reaching a more accurate estimation of the post mortem interval by assessing the immunohistochemical detection of the extracellular matrix proteins collagen type I and collagen type III in post mortem gingival tissues at different PMIs.

Fragments of gingival tissues were collected from 20 cadavers at different post-mortem intervals during medico-legal autopsy and they were subsequently immunostained with anti-collagen type I and type III antibodies, in order to establish a correlation between the proteins presence and the time of death.

Results showed a progressive reduction in immunostaining with time, reflecting a significant variation in staining positivity that correlate to the different time of death.

In conclusion, although information on collagen degradation time is limited and further studies are needed, in our study degradation processes of sub oral connective tissues of gingival samples revealed to exhibit a discrete dependence upon time intervals after death demonstrating the applicability of immunolabeling techniques for PMI determination in forensic sciences.

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Key words

Post-mortem interval, immunolabeling, collagen fibers, ultrastructural morphological changes, gingival tissues.

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Histological and ultrastructural analysis of the knee anterolateral ligament

Selenia Miglietta[1], Roberto Matassa[1], Andrea Redler[2], Michela Relucenti[1], Edoardo Monaco[2], Matthew Daggett[3], Andrea Ferretti[2] and Giuseppe Familiari[1]

[1] Sapienza Università di Roma, Dipartimento di Scienze Anatomiche, Istologiche, Medico-Legali e dell'Apparato Locomotore, Roma, Italia
[2] Sapienza Università di Roma, Azienza Ospedaliera Sant'Andrea, Roma, Italia
[3] Kansas City University, Kansas City University, Kansas City Mossouri, Stati Uniti D' America

The Anatomy of the lateral aspect of the knee has been increasingly debated in recent literature, the morphology and function of anterolateral ligament (ALL) are not clearly understood even today (1, 2). The aim of present study was to provide for the first time a detailed ultrastructural characterization of the ALL and its ultrastructure collagen arrangement using the light microscopy (LM), the transmission electron microscopy (TEM) and variable pressure scanning electron microscopy (VP-SEM). Eight paired samples from four fresh-frozen males cadavers were used for the study. Samples were harvested from the ALL, from the anterolateral capsule and from the medial collateral ligament (MCL). All samples were treated for microscopy techniques. Histological analysis showed similar morphology features between ALL and MCL sectioning, but clear differences are evident in comparison to the knee capsule. At TEM the collagen fibers of ALL and MCL showed similarity in the ultrastructural morphology, both collagen fibers have parallel alignment mainly orientated longitudinally. Significant structural difference of the tissue of the fibrous capsule compared to the ligaments may be observed. The VP-SEM highlighted that ALL and MCL morphology has shown aligned arrangements of fiber bundles densely packed, completely different assembling organization of the fibers were observed in fibrous capsule. Data from this study demonstrate that the ALL and MCL have comparable ultrastructural properties. It can further lead us to speculate that the ALL is a ligamentous structure distinct from the knee capsule.

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Key words

Anterolateral ligament, Electron Microscopy, Ultrastructure.

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Upper limb disability, quality of life and self-reported active life-style behavior in breast cancer survivors following a structured adapted physical activity intervention

Daniela Mirandola[1], Guido Miccinesi[2], Maria Grazia Muraca[2], Marco Monaci[1], Eleonora Sgambati[3], Mirko Manetti[1] and Mirca Marini[1]

[1] University of Florence, Section of Anatomy and Histology, Department of Experimental and Clinical Medicine, Florence
[2] ISPRO, Oncological network, Prevention and Research Institute, Florence
[3] University of Molise, Department of Biosciences and Territory, Pesche (Isernia)

Growing evidence indicates that person-tailored physical activity interventions may improve the health outcomes (i.e. decreased shoulder range of movement, muscle weakness, pain, and psychological distress) of breast cancer survivors. However, knowledge about either longitudinal benefits on shoulder mobility and quality of life or physical activity maintenance among survivors after adapted physical activity (APA) interventions remains limited. Here, we evaluated the maintenance of a physically active lifestyle in breast cancer survivors after ending a specific APA intervention and examined longitudinally the possible implications in preserving the physical and mental benefits achieved with APA. The study included 112 breast cancer survivors recruited at the Cancer Rehabilitation Centre in Florence. Nine months after ending a supervised APA protocol, breast cancer survivors were interviewed on their participation in regular physical activity, drop-out reasons and surgical shoulder-arm symptoms, using a structured questionnaire. At 1.5-year post-APA follow-up to assess long-term effects, survivors were again evaluated as at baseline/post-APA by fitness tests (i.e. shoulder-arm mobility, range of motion, back flexibility) and Short Form-12 and numerical rating scale questionnaires to assess quality of life and pain intensity on back and surgical shoulder, respectively. Questionnaires on physical activity participation and upper limb function (QuickDASH) were also administered. Our findings indicated that long-term practice of physical activity was poorly maintained among breast cancer survivors resulting in an overall decrease in post-APA achieved benefits. Generalized physical activity does not seem appropriate to improve upper limb disability secondary to breast cancer treatments. In conclusion, the practice of general physical activity by breast cancer survivors seems not able to preserve overtime the physical and mental benefits achieved following a structured APA intervention, suggesting that participation in structured APA protocols should be maintained overtime. The findings of this study may help to plan future APA-based strategies in order to better manage the breast cancer survivor who is experiencing decreased function of the affected upper limb.

Key words

Breast cancer, adapted physical activity, survivorship, quality of life, upper limb disability, active lifestyle.

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Long-term follow up of the impact of dragon boat racing on lymphedema in breast cancer survivors: the Florence Dragon Lady experience

Daniela Mirandola[1], Guido Miccinesi[2], Maria Grazia Muraca[2], Alessandro Piccardi[3], Marco Monaci[1], Giulia Micheli[1], Eleonora Sgambati[4], Mirko Manetti[1] and Mirca Marini[1]

[1] University of Florence, Section of Anatomy and Histology, Department of Experimental and Clinical Medicine, Florence (1)
[2] ISPRO, Oncological network, Prevention and Research Institute, Florence
[3] A.S.D., Canottieri Comunali Firenze, Florence
[4] University of Molise, Department of Biosciences and Territory, Pesche (Isernia)

Upper limb lymphedema, due to lymphatic drainage interruption because of axillary lymph node dissection and/or radiation, is a dreaded chronic complication in breast cancer survivors. Upper limb swelling can cause pain, discomfort, heaviness and decreased movement thereby affecting the quality of life. Numerous lifestyle factors have been implicated in the development of lymphedema, and guidelines for the prevention of this complication have been developed. There were previous concerns in the medical community that vigorous upper-body exercise could lead to the development or worsening of lymphedema. However, the practice of dragon boat racing by breast cancer survivors has challenged the traditional advice about limiting upper extremity activity to prevent lymphedema. Dragon boat is a discipline of ancient Chinese origin, practiced in boats of standardized dimensions by crews of 20 athletes who paddle to the beat of a drum. Paddlers sit in twos side-by-side and use a single-bladed paddle. A team also has a steerer holding the direction. Currently, there are more than 140 breast cancer survivors' dragon boat teams worldwide with well-documented physical, psychological, and social improvements. Here, we assessed the longitudinal effects of dragon boat practice on lymphedema in breast cancer survivors from the Florence Dragon Lady team. This team, formed in February 2006, has 60 athletes training for 1 hour/day twice a week at the Canottieri Comunali on the Arno River and regularly participating in dragon boat racing. The difference between affected and unaffected limb circumference was assessed overtime from 2006 to present at the Oncology Rehabilitation Center by multiple measurements at several anatomical points. Our findings showed that in the majority of breast cancer survivors the difference between limbs remained stable or improved. Only five participants were found to have an onset of mild lymphedema with an increase in circumference difference of about 0.5 cm at one measurement point (i.e. axilla), while a participant who had undergone a mastectomy due to disease recurrence reported moderate lymphedema. In conclusion, these findings contribute to a growing body of literature supporting the value of dragon boat racing as a viable physical activity intervention to enhance physical outcomes on upper limb in breast cancer survivors.

Key words

Breast cancer survivors, dragon boat, upper limb disability, lymphedema.

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Acute effect of Whole Body Vibration on postural control in a Parkinson's Disease: A Case Report

Stefano Moffa[1], Angela Lucariello[1], Antonio Ascione[2], Antonio De Luca[3], Germano Guerra[1] and Domenico Tafuri[2]

[1] Università degli Studi del Molise, Dipartimento di Medicina e Scienze della Salute "Vincenzo Tiberio", Campobasso, Italia
[2] Università degli Studi di Napoli "Parthenope", Dipartimento di Scienze Motorie e del Benessere, Napoli, Italia
[3] Università della Campania "Luigi Vanvitelli", Dipartimento di Salute Fisica e Mentale e Medicina Preventiva, Napoli, Italia

Postural instability and resulting falls are major factors determining quality of life, morbidity, and mortality in individuals with Parkinson's disease (PD) (1); the ability to control balance is different in people with Parkinson's Disease (PD). The application of Whole Body Vibration (WBV) at low frequencies in a patient with PD influences physiological and psychological functions. The purpose of this preliminary study was to explore the acute effects of whole body vibration (WBV) on balance and stabilometric parameters, in a patient with PD, during the 20-min post-intervention. Postural sways of the participant was assessed with a stabilometric platform before and after WBV exposure to. Motor symptoms were assessed by the UPDRS (Unified Parkinson's Disease Rating Scale) motor score. The patient was subject to a whole body vibration session (5 set of 1 minute with 1 minute rest) at low frequencies on Double Vibe Bosco Platform. The vibration frequency used was 5 Hz. The assessment of the balance was performed at the baseline (T0), immediately after the exposure (T1), after 10 minutes (T10) and 20 minutes (T20), with Cyber Sabot Plate System. The stabilometric survey was carried out with open eyes. The postural parameters that were analyzed were the Area of the Pressure Center (AREA), Center of Pressure Total Distance (LENGTH) and Variance of Speed (S.VAR). Repeated Measures Analysis of Variance (RM-Anova) was performed to evaluate significant differences among the different variables (Area,Length and S.Var), no significant differences were found for the variables analyzed. Whole body vibration (WBV) training has emerged as an alternative and effective method which allows greater short term improvement in strength and balance with less time of application. No impairments in static balance were found after an acute bout of whole body vibration at low frequency in a patient with PD, consequently, whole body vibration may be considered as a safe application in individuals with PD.

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Key words

Sensory Information, Vibration Training, Postural Sway, Sport Performance.

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Role of Nuclear PI-PLCbeta 1 during Azacitidine-induced Myeloid Differentiation

Sara Mongiorgi[1], Alessandra Cappellini[2], Eric Owusu Obeng[1], Marilisa Quaranta[1], Carlo Finelli[3] and Matilde Y. Follo[1]

[1] Università di Bologna, Dip Scienze Biomediche e Neuromotorie, Bologna, Italia
[2] Università di Cassino, Dip Scienze Sociali Umane e Salute, Cassino, Italia
[3] Policlinico S. Orsola Malpighi, Istituto di Ematologia L. e A. Seràgnoli, Bologna, Italia

Phospholipase Cbeta1 (PI-PLCbeta1) 1 is a key enzyme of the phospholipase family, a class of molecules involved in the lipidic cell signaling that play central roles in cell proliferation and differentiation. PI-PLCbeta 1 has two splicing variants: 1a, with cytoplasmatic and nuclear localization, and and 1b, which is mainly nuclear.

Myelodysplastic syndromes (MDS) are a heterogeneus group of hematological diseases characterized by a stem cell defect that causes anemia, cytopenia and leucopenia.

MDS can evolve into acute myeloid leukemia (AML). In patients in which the probability is higher, called high risk MDS, the treatment aims to delay the tumor progression and restore the hematopoietic differentiatin.

As the most frequent alterations in high risk MDS cells that cause AML evolution are of epigenetic nature, the therapeutic approach for high risk MDS is based on demethylating agents, like azacitidine. [1] Furthermore, there is a statistical significant increase in the levels of expression of nuclear PI-PLCbeta1 in MDS patients responding to azacitidine [2].

Stemming from these data, here we further analyzed the involvement of PI-PLCbeta1 at different stages of azacitidine-induced hematopoietic differentiation, with particular reference to the myeloid lineage.

Due to the limits of a heterogeneous population in patient blood samples, we firstly studied the azacitidine effect on AML cell lines, using cells at different stages of hematopoiesis. First, by enzymatic method, we showed that azacitidine induces PI-PLbeta1 demethylation. Then, by flow cytometry, we demonstrated not only that azacitidine affects cell cycle and apoptosis, but also that it changes the expression of specific surface myeloid markers. Subsequently, we studied the azacitidine effect on nuclear PI-PLCbeta1 and other enzymes involved in phosphoinositide signaling, firstly by Real Time PCR, then by Western Blotting and immunocytochemistry analyses, showing that azacitidine can specifically change the expression and localization of specific inositide players.

All in all, our results show that azacitidine induces a myeloid differentiation in AML cell lines, particularly rapid in those already showing a myeloid committment (e.g. HL60 promyelocytic cells), and a late one in cells with a higher stem cell population (i.e. KG1 AML cells). More importantly, we demonstrated that it is the nuclear isoform of PI-PLCbeta1 to play a pivotal role in myeloid induction, particularly in the first phases of differentiation.

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Key words

Nuclear PI-PLCbeta 1, Myeloid Differentiation, Cellular Localization.

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Inflammation in white adipose tissue of obese rats: effects of seeds and juice of prunus cerasus L

Michele Moruzzi[1], Nora Kloeting[2], Matthias Blueher[2], Matthias Kern[2], Consuelo Amantini[3], Ilenia Martinelli[1], Seyed Khosrow Tayebati[1], Maria Vittoria Micioni di Bonaventura[1], Maria Elena Giusepponi[1], Carlo Cifani[1], Giulio Lupidi[1], Francesco Amenta[1] and Daniele Tomassoni[1]

[1] School of Medicinal and Health Products Sciences, University of Camerino, Camerino, Italia
[2] Department of Medicine, University of Liepzig, Leipzig, Germania
[3] School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italia

Tart cherries (Prunus Cerasus L.) are a rich source of anthocyanins, phytochemical flavonoids found in red-, blue- and purple-pigmented fruits and vegetables. The components of these plants can modify lipid metabolism in vitro and reduce hyperlipidemia in vivo. Dyslipidemia, hypertension, impaired glucose tolerance, insulin resistance often accompany obesity, in which adipose tissue accumulation and metabolic changes increase the incidence of heart failure and stroke. Visceral adipose tissue (VAT) has emerged as a major player in driving obesity-related inflammatory response. In obesity, chronic infiltration of macrophages in adipose tissue mediates local and systemic inflammation. Transient receptor potential (TRP) proteins are members of a superfamily of cation channels playing a role in the pathophysiology of different systems. They are implicated in inflammatory responses, via their functions in pro-inflammatory immune cells either resident or infiltrating. This study has investigated the potential positive effects of tart cherries on rats with Diet-Induced Obesity (DIO) on the inflammatory status of the VAT. Rats had for 17 weeks a hypercaloric diet with the supplementation of tart cherries seeds powder (DS) and seeds powder plus tart cherries juice containing 1mg of anthocyanins (DJS). DIO rats were compared to the control rats with standard diet (CHOW). In VAT, expression of TNF, CcL2, CD-68, and TRP-channels were measured by qRT-PCR, western blot, and immunohistochemistry techniques. All DIO rats groups increased significantly their body weight compared to CHOW rats. No difference in VAT weight was found in DS and DJS rats compared to age-matched DIO rats. In perigonadal and retroperitoneal AT, an increase of inflammatory markers was observed with a different modulation in DIO rats tart cherries supplemented. Furthermore, TRP channels are modulated with increasing expression of TRPV channels and decreasing of TRPC channels. Tart cherries supplementation regulated in different ways the TRP channels expression. These results suggest that tart cherries enriched-diet, although did not modify the accumulation of visceral fat, it decreased inflammation markers in the VAT. This supplementation could be therefore useful, in combination with healthy lifestyles, to modify adipose tissue cells metabolism and to limit secondary organ damage in target tissues of obesity.

This study was supported by a Grant of University of Camerino.

Key words

Obesity, Visceral adipose tissue, inflammation, tart cherries.

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Rest-activity circadian rhythms and fat mass percentage in men with metabolic syndrome

Antonino Mulè[1], Lucia Castelli[1], Letizia Galasso[1], Eleonora Bruno[2], Patrizia Pasanisi[2], Andrea Caumo[1], Eliana Roveda[1] and Angela Montaruli[1]

[1] Università degli Studi di Milano, Dipartimento delle Scienze Biomediche per la Salute, Milano, Italia
[2] IRCCS Istituto Nazionale Tumori, Dipartimento di Medicina Preventiva e Predittiva, Milano, Italia

Metabolic syndrome is a cluster of risk conditions such as abdominal obesity, dyslipidemia, high blood pressure and high fasting glycemia. These factors generated an increase of risk of cardiovascular diseases and type 2 diabetes mellitus. Furthermore, it has been shown that there is a correlation between metabolic syndrome and disruption of circadian rhythms. The circadian rhythms produce 24-hour oscillations of several physiological variables and any irregularity of these rhythms exposes the subject to an increased risk of metabolic syndrome [1]. Aim of the study was to investigate a possible relation between the percentage of fat mass (FM%) and rest-activity circadian rhythm (RAR) in men with metabolic syndrome. We recruited 36 men with metabolic syndrome in care at Fondazione IRCCS, Istituto Nazionale Tumori. All participants underwent a continuous 7-day actigraphic monitoring (MotionWatch 8®, CamNtech, Cambridge, UK) to record the activity levels. Then participants were divided into 2 groups referring to their median FM%: group 1, with FM% <29.2 (n=19) and group 2, with FM% >29.2 (n=17). The actigraphic activity data were analyzed by single cosinor method to obtain MESOR (M), amplitude (A) and acrophase (Ø) of each subject. In addition, we applied the population mean cosinor method to evaluate the RAR parameters of each group. The results show a trend to have a reduction of MESOR and Amplitude in relation to FM%, even if we didn't find statistically significant differences (MESOR: group 1=207.5 vs group 2=194.7; Amplitude: group 1=158.4 vs group 2=145.3) between group 1 and 2 by Hotelling test.

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Key words

Metabolic syndrome, actigraphy, circadian rhythm, women, body mass index, physical activity levels.

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Circadian Tipology and physical performance in adolescent soccer players

Antonino Mulè[1], Lucia Castelli[1], Letizia Galasso[1], Eleonora Bruno[2], Eliana Roveda[1], Andrea Caumo[1] and Angela Montaruli[1]

[1] Università degli Studi di Milano, Dipartimento delle Scienze Biomediche per la Salute, Milano, Italia
[2] IRCCS Istituto Nazionale Tumori, Dipartimento di Medicina Preventiva e Predittiva, Milano, Italia

The innate circadian rhythms influence our daily behavior, and people typically display preferences for activity at certain time of day. These differences may be classified with the concept of Circadian Typology (CT): Morning, Evening and Neither Types (M-types, E-types, and N-types). The propensity toward diurnal or evening preferences can vary in different life periods and typically a shift towards eveningness occurs during adolescence [1]. In this period, football is one of the most practiced sports, and generally soccer workouts take place during the afternoon or evening. Many variables related to sports performance are linked to the CT [2]. Therefore, if the physical exercise is practiced in a favourable circadian period, it could determine an improvement in performance.

Aim of the study is to verify whether there are performance differences relating to CT in adolescent soccer players. We recruited 80 male soccer players that filled in the Morningness-Eveningness Questionnaire (MEQ) for the assessment of CT. 39 participants, subdivided in M-types (n=13), E-types (n=13) and N-types (n=13), performed three tests (Sargent Jump Test, Illinois Agility Test and 6 Minutes Run Test) at two different times of the day, at 9.00 am and at 6.00 pm. The data, analyzed by Mixed ANOVA, show statistically significant differences between the three CT and time of the day (Sargent Jump, $p<.05$; Illinois Agility $p<.01$; 6 Minutes Run $p<.01$). In particular, for the three tests, E-types show a higher performance during the evening than the morning session. By contrast, M-types performed better in the morning than in the evening session.

The results show that CT is able to influence the performance in adolescent soccers.

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Key words

Circadian typology, chronotype, MEQ, body mass index, physical performance.

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Immunostaining for tyrosinase and nestin in melanocytic nevi as a model for melanocyte differentiation and nevogenesis

Daniela Murtas[1], Cristina Maxia[1], Andrea Diana[1], Luca Pilloni[2], Caterina Ferreli[3], Laura Casula[4], Sara Tomei[5], Franca Piras[1], Paola Sirigu[1] and Maria Teresa Perra[1]

[1] University of Cagliari, Department of Biomedical Sciences, Section of Cytomorphology, Cagliari, Italia
[2] University of Cagliari, Department of Surgical Sciences, Section of Pathology, Cagliari, Italia
[3] University of Cagliari, Department of Medical Sciences and Public Health, Dermatologic Clinic, Cagliari, Italia
[4] University of Cagliari, Department of Medical Sciences and Public Health, Cagliari, Italia
[5] Sidra Medical and Research Center, Omics Core and Biorepository, Doha, Qatar (5)

Histological analysis allows an accurate classification of most melanocytic lesions as benign or malig-nant. However, a challenging diagnosis can be faced when differentiating a nevus from a melanoma, mostly due to the heterogeneous histological appearance of melanomas. It is thus necessary to use immunohistochemistry as a complementary tool. The immunohistochemical expression of tyrosinase, the key melanogenic enzyme in melanocytes, has often been useful in formulating a differential diagnosis thanks to the peculiar staining pattern in nevocytes compared with melanoma cells. The expression pattern of tyrosinase in nevi appears to parallel the cytoarchitectural changes typically observable within the lesion: nevus cells in the epidermis or in the superficial dermis are more likely to be larger and strongly express melanocytic differentiation antigens, such as tyrosinase, compared with deeper nevocytes [1]. Our study aimed to evaluate the immunohistochemical expression pattern of the tyrosinase antigen (clone T311) in different histological types of acquired dysplastic melanocytic nevi, including junctional, compound, and intradermal nevi, as well as in a panel of normal skin tissues. Moreover, to evaluate whether the expression of tyrosinase by nevus cells, pointing out the acquisition of melanin-producing capabilities, was associated with the differentiation state of nevocytes, we likewise investigated the immunohistochemical expression of the two markers of pluripotency, CD34 and nestin. Our results revealed a prominent immunoreactivity for tyrosinase in junctional and superficial dermal nevocytes and a decreasing gradient of staining in dermal nevocytes, up to become negative in the deeper dermis. All junctional and dermal nevocytes were negative for CD34. Nestin immunostaining showed an opposing pattern compared with tyrosinase, leading us to look into the melanocytic nevus as a "histopathological model" to trace the final stages of the differentiation pathway that neural crest-derived melanocyte precursors undertake toward their ultimate anatomical and functional site into the epidermis, consistently with Cramer's dermal precursor model of melanocytic origin and the stem cell-based concept of nevogenesis [2]. Both CD34 and nestin intensely stained the small aggregates of cells, resembling "niches", adjacent to the bulge area of hair follicles, that may represent the reservoir of neural crest-derived melanocyte stem cells residing in the dermis.

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Key words

Tyrosinase, nestin, dysplastic melanocytic nevi, immunohistochemistry, nevogenesis, neural crest.

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Modification of vastus lateralis muscle homeostasis after an acute bout of unaccustomed exercise

Kathryn Myburgh[1], Ashwin Isaacs[1], Felicia Farina[2], Giovanni Zummo[2], Francesco Cappello[2], Valentina Di Felice[2] and Filippo Macaluso[3]

[1] Department of Physiological Sciences, Stellenbosch University, Stellenbosch, Sud Africa
[2] Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, Palermo, Italia
[3] SMART Engineering Solutions & Technologies (SMARTEST) research center, eCampus University, Novedrate (CO), Italia

The purpose of this study was to investigate skeletal muscle changes during rhabdomyolysis induced by an acute bout of jumping exercise in sedentary individuals. Healthy volunteers (N=26) did a bout of jumping exercise (10 sets of 10 squat jumps to maximal jump height rest between sets was 1 min). Blood samples were drawn immediately before the exercise intervention and 6, 24, 48 and 72 hours after. The vastus lateralis was biopsied before (9 or 4 days) and 3 days post-intervention. Subjects were divided into two groups on individual CK responses on day 3 after exercise: high (n=10) and low responders (n=16). The cut-off limit used to diagnose rhabdomyolysis after exercise was CK≥1000 U/L. Structural (dystrophin staining) and ultrastructural (transmission electron microscopy) analysis of muscle fibres did not evidence any difference between high and low responders despite that the high responders perceived more muscle soreness than low responders. Moreover, high responders presented significantly higher muscle myeloperoxidase (MPO) levels at both baseline and postexercise biopsies compared to the baseline of the low responder group. The results seems to indicate that rhabdomyolysis after exercise is caused by secondary inflammatory damage.

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Key words

Rhabdomyolysis, skeletal muscle, jump exercise.

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Role of Engineering in Forensic Clinical Anatomy

Arturo Natali[1], Veronica Macchi[2], Andrea Porzionato[1], Raffaele De Caro[1]

[1] Center of Mechanics of Biological Materials, Università di Padova, Padova, Italia
[2] Istituto di Anatomia Umana, University of Padova, Padova, Italia

Forensic Engineering is an engineering branch that works in the prevention of damage, highlighting any defects in design or construction. In the field of medicine it investigates the technical problems that can occur during or after the surgical procedures. The aim of this paper is to present the role of Engineering in Forensic Clinical Anatomy, as the basis for precision medicine, presenting a series of examples of collaboration between the Institute of Anatomy and the Center of Mechanics of Biological Materials (CMBM) of the University of Padua. Forensic Clinical Anatomy may be defined as the practical application of Clinical Anatomy to the ascertainment and evaluation of medical-legal problems. In particular, individual anatomy (normal anatomy, anatomical variations, age-, disease, or surgery-related modifications) can acquire significant relevance in various fields of legal medicine. Engineering can actively collaborate in defining the most suitable type of prosthesis, taking into account not only the physical characteristics of the subject but also his individual anatomy. For example, in hemodynamic interventions a designer should provide the greatest possible safety margin when specifying catheter diameters or in orthopedics can design and adapt the prosthetic substitute to be implanted. On the other hand, the validation of artificial tissues passes through a complex validation process that can move from a standard validation to an individual valuation based on the anatomical data of the subject for a state-of-the-art health service.

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Morphological and functional characterization of IL-12 Receptor b2 chain on intestinal epithelial cells: implications for local and systemic immunoregulation

Claudio Nicoletti[1], Mari Regoli[2], Alessandra Pacini[1], Jacopo Junio Valerio Branca[1], Angela L. Man[3], Gabriele Morucci[1], Arjan Narbad[4], Massimo Gulisano[1], Eugenio Bertelli[2]

[1] Università degli Studi di Firenze, Dip Medicina Sperimentale e Clinica, Firenze, Italia
[2] Università degli Studi di Siena, Dip Medicina Molecolare e dello Sviluppo, Siena, Italia
[3] Earlham Institute, Dept of Biology, Norwich, Regno Unito
[4] Quadram Institute Bioscience, Gut Health programme, Norwich, Regno Unito

Interaction between intestinal epithelial cells (IECs) and the underlying immune systems is critical for maintaining intestinal immune homeostasis and mounting appropriate immune responses. We have previously showed that the T helper type 1 (TH1) cytokine IL-12 plays a key role in the delicate immunological balance in the gut and the lack of appropriate levels of IL-12 had important consequences for health and disease, particularly with regard to food allergy. Here we sought to understand the role of IL-12 in the regulation of lympho-epithelial cross talk and how this interaction affects immune responses locally and systemically. Using a combination of microscopy and flow cytometry techniques we observed that freshly isolated IECs expressed an incomplete, yet functional IL-12 receptor (IL-12R) formed solely by the IL-12Rb2 chain that albeit the lack of the complementary IL-12b1 chain responded to ex-vivo challenge with IL-12. Furthermore, the expression of IL-12Rb2 on IECs is strategically located at the interface between epithelial and immune cells of the lamina propria (lp) and using in vitro co-culture models and primary intestinal organoids we showed that immune-derived signals were required for the expression of IL-12Rb2 on IECs. The in vivo biological relevance of the IEC-associated IL-12Rb2 was assessed in vivo in a mouse model of food allergy characterized by allergy-associated diminished intestinal levels of IL-12 and in chimeric mice that lack the IL-12Rb2 chain on IECs. These experimental models enabled us to show that the anti-allergic properties of orally delivered recombinant Lactococcus lactis secreting bioactive IL-12 (rLc-IL12) were reduced in mice lacking the IL-12b2 chain on IECs. Finally, we observed that the oral delivery of IL-12 was accompanied by the down-regulation of the production of the IEC-derived pro-allergic cytokine thymic stromal lymphopoietin (TSLP). However, further analysis of intestinal levels of TSLP in IL-12Rb2-/- mice suggested that this event was not directly linked to the IEC-associated IL-12Rb2 chain. We interpreted these data as showing that IEC-associated IL12Rb2 is a component of the cytokine network operating at the interface between the intestinal epithelium and immune system that plays a role in immune regulation.

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The endocrine disruptor Bisphenol A affects cell proliferating ability in PHA-stimulated peripheral lymphocytes exerting a biphasic effect

Stefania Lucia Nori[1], Raffaella D'Auria[1], Mario Capunzo[1], Andrea Viggiano[1], Antonietta Santoro[1] and Vanessa Nicolin[2]

[1] Università di Salerno, Dipartimento di Medicina, Chirurgia e Odontoiatria "Scuola Medica Salernitana", Baronissi
[2] Università degli Studi di Trieste, Dipartimento CSID, Trieste

In the last two decades, a growing number of studies highlighted the importance of the interaction between the estrogen-receptor pathway modulation and immune cell activity in human metabolic regulation [1]. It is recognized that steroid pathways can regulate immune cell metabolism and thus the intacrine-paracrine effects of steroids could be implicated in promoting immune-mediated steroid-dependent cancers and autoimmune diseases. Moreover, it has been shown that both monocytes and T-lymphocytes express estrogen receptors (ERα and ERβ) [2], therefore it is plausible that environmental pollutants interfering with steroid pathways, such as Bisphenol A (BPA) -a widespread contaminant of plastic, epoxy resins, toys and electronics with structural and functional similarities to steroids - could influence immune cell activity leading to cancer, autoimmune disease and neurological disorders. To gain insights into this issue, we studied the effects of BPA in Phytoemagglutinin (PHA)-stimulated Peripheral Blood Mononuclear Cells (PBMCs) from healthy donors examining cell survival by MTT assay, cell proliferation by BrdU assay, and cell cycle progression by cytofluorimetric analysis at different time points (24-72 hs) and concentrations (ranging from 5 nM to 200 μM).

Results show that BPA does not induce apoptosis or necrosis, at all time and doses tested, instead promotes cell proliferation at lowest concentrations. Cytofluorimetric analysis by Propidium Iodide staining also indicates that BPA is able to emphasize PHA-induced effects in enhancing cell proliferation at concentrations ranging from 25 to 100 nM. However, the compound markedly inhibits cell cycle progression at concentrations greater than 25 μM causing a G1 cell cycle arrest starting from 24 hs of treatment. On the other hand, BPA was unable to induce any effect in resting PBMCs, suggesting that BPA -induced biphasic effect in proliferating cells could be the result of mechanisms other than its estrogen-like behavior.

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Key words

Bisphenol A, PBMCs, cell cycle progression.

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Cell damage due to amyloid aggregation process on cell membrane involves membrane ganglioside GM1

Daniele Nosi[1], Monica Bucciantini[2] and Manuela Leri[2]

[1] University of Florence, Department of Experimental and Clinical Medicine, Firenze, Italia
[2] University of Florence, Department of Experimental and Clinic Biomedical Science, Firenze, Italia

The term amyloidosis describes a wide group of pathologies occurring in various tissues, and characterized by the presence of proteinaceous deposits grown from different peptides/proteins, yet sharing similar structural features, the cross-beta structure. Amyloid aggregates may interact with a wide variety of lipid or protein molecules on cell surface, thus inducing membrane destabilization and cell damage. Such surface molecules may interact with monomeric or oligomeric amyloid species, enhancing aggregate nucleation. It is known that the amphipathic structure of amyloid peptides may prompt them to aggregate and eventually to interact with different molecules to gain structural stability in the fibrillar form. Therefore, the cytotoxic properties of fibrillar assemblies may differ on the basis of the physicochemical properties of the interacting molecules. Among the membrane molecules that interact with amyloid fibrils, the GM1 ganglioside raised great interest and contradictory data emerge from literature. In fact, cytotoxic effects of the interaction between amyloid aggregates and GM1 on cell membranes have been demonstrated [1]. On the other hand GM1 appears to contrast amyloid toxicity [2]. In this study, GM1 interaction with various amyloid intermediates at the cellular level were analyzed. To this purpose, amyloid aggregation process of Sup35 was studied by using confocal microscopy, transmission electron microscopy, and spectroscopy techniques. Sup35 is a yeast translation termination factor that has no homologous endogenous proteins in mammalian cells, and therefore allows to analyze structural and molecular basis of amyloid cytotoxicity in the absence of any physiological interference with cell elements. In vitro- experiments were performed on a murine endothelioma (H-END) cell line using the same amyloid peptides.

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Key words

Amyloidosis, aggregation, GM1, Sup35.

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Role of the microenvironment in the imbalance between Th1/Th17 and Th2 cytokines of Mesenchymal stem Cells derived from psoriatic skin

Monia Orciani[1], Giulia Sorgentoni[1], Elisa Molinelli[2], Miriam Caffarini[1], Anna Maria Offidani[2] and Roberto Di Primio[1]

[1] Università Politecnica delle Marche, Dipartimento di Scienze cliniche e moleoclari- Istologia, Ancona, Italia
[2] Università Politecnica delle Marche, Dipartimento di Scienze cliniche e moleoclari- Dermatologia, Ancona, Italia

Psoriasis is an inflammatory and immuno-mediated disease [1] characterized by the over-expression of several Th1-Th17 cytokines, which are able to maintain a low-grade inflammatory status. The expression and secretion of Th1, Th2 and Th17 cytokines has been extensively evaluated in differentiated skin cells of psoriatic patients, while little is known about their production by MSCs in psoriatic skin. Indeed, it is known that the psoriatic microenvironment influences the MSCs phenotypic profile [2]. This works aims to evaluate the immunobiology of psoriatic MSCs (PsO-MSCs) and the potential paracrine effect that can be exerted by Healthy MSCs (H-MSCs) on PsO-MSCs.

To assess these questions, MSCs were isolated from skin of psoriatic and healthy subjects. Subsequently, indirect co-culture of H-MSCs with PsO-MSCs was performed; effects on proliferation and expression of cytokines linked to Th1/Th17 and Th2 pathways were assayed before and after co-culture.

The results show that before co-culture, proliferation of PsO-MSCs was significantly higher than H-MSCs ($p<0.05$) and the levels of secreted cytokines confirmed the imbalance of Th1/17 versus Th2 axis.

After co-culture of H-MSCs with PsO-MSCs, healthy MSCs seem to exert a "positive" influence on PsO-MSCs driving the inflammatory phenotypic profile of PsO-MSCs towards a physiological pattern. The proliferation rate decreased, towards values nearer to those observed in H-MSCs and the secretion of the cytokines that mostly identified the inflammatory microenvironment that characterized psoriasis, such as IL6, IL12, IL13, IL17A, TNFα, and GCSF, is significantly lower in co-cultured PsO-MSCs than in individually cultured PSO-MSCs (p at least <0.05).

In conclusions, our preliminary results seem to provide an intriguing molecular explanation for the ever increasing evidence of therapeutic efficacy of allogeneic MSCs infusion in psoriatic patients.

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Key words

Mesenchymal stem cells (MSCs), psoriasis, inflammation.

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The hunt for peripheral chemoreceptors in an unusual species

Matteo Maria Ottaviani[1], Chiara Ippolito[2], Cristina Segnani[2], Fabio Bernini[1], Elisabetta Giannessi[3], Silvestro Micera[4], Fabio Recchia[1], Amelio Dolfi[2], Nunzia Bernardini[2]

[1] Scuola Superiore Sant'Anna, Institute of Life Sciences, Pisa, Italia
[2] University of Pisa, Unit of Histology, Department of Clinical and Experimental Medicine, Pisa, Italia
[3] University of Pisa, Department of Veterinary Science, Pisa, Italia
[4] Scuola Superiore Sant'Anna, The BioRobotics Institute, Pisa, Italia

The carotid bodies (CBs) are small organs localized bilaterally at the bifurcation of the common carotid artery into the internal and external carotids. They work as peripheral chemoreceptors that sense changes in arterial blood O2, CO2 and pH levels and activate sympathetic-mediated cardiorespiratory reflexes thought their sensory nerve, the carotid sinus nerve (CSN) to restore the altered parameters1. Across all mammalian species, CBs are mainly formed by chemoreceptor cells (glomus cells) surrounded by glia-like sustentacular cells in small highly vascularized cell clusters called "glomoids". A variable quantity of connective tissue surrounds glomoids and defines the capsule of the CB, giving to the organ a discrete and compact aspect in some species whilst in others is rather diffuse2,5. CBs have been recently addressed as peripheral metabolic sensors involved in glucose homeostasis and sympathetic drive control in metabolic diseases like type II diabetes3.Despite the pig represents one of the best pre-clinical model of type II diabetes available4, surprisingly a proper anatomical characterization of pig CBs is lacking in this species5.In the present work we provide a detailed and updated anatomical characterization of pig CBs as a fundamental step for further studies in this species. We firstly focused on the surgical identification of the CBs in pigs (n=7) followed by classic haematoxylin-eosin histological analysis of carotids bifurcation sections. We identified the CB bilaterally as a small compact corpuscle attached to internal carotid wall in the proximity of the carotid bifurcation. We observed a lobular structure where it was possible to recognize glomus and sustentacular cells in clusters resembling the glomoids as described in other species5. Neurofilament immunolabeling revealed a rich amount of neural fibers arising from the CBs that were dispersed in the surrounding adipose tissue without forming a well identifiable nerve structure. Further studies beyond routine histological preparations will be carried on for a deeper characterization of CBs. We aim at giving a proper anatomical basis for future functional studies of this intriguing organ in a key animal model of metabolic diseases like the pig.

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Key words

Carotid bodies, pig, type II diabetes.

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Biliary tree stem cells and peribiliary glands are involved in primary sclerosing cholangitis and cholangiocarcinoma

Diletta Overi[1], Guido Carpino[2], Romina Mancinelli[1], Vincenzo Cardinale[3], Tom Hemming Karlsen[4], Domenico Alvaro[5] and Paolo Onori[1]

[1] Sapienza Università di Roma, Dipartimento di Scienze Anatomiche, Istologiche, Medico Legali e dell'Apparato Locomotore, Roma, Italia
[2] Università degli Studi di Roma "Foro Italico", Dipartimento di Scienze Motorie, Umane e della Salute, Roma, Italia
[3] Sapienza Università di Roma, Dipartimento di Scienze e Biotecnologie Medico-Chirurgiche, Latina, Italia
[4] Oslo University Hospital Rikshospitalet, Division of Cancer, Surgery and Transplantation, Department of Transplantation Medicine, Oslo, Norvegia
[5] Sapienza Università di Roma, Dipartimento di Medicina Interna e Specialità Mediche, Roma, Italia

Peribiliary glands (PBGs) represent the niche of biliary tree stem/progenitor cells (BTSCs) [1]. BTSCs are multipotent stem cells able to differentiate into hepatocytes, cholangiocytes, and pancreatic islets. Primary sclerosing cholangitis (PSC) is a chronic inflammation involving extra-hepatic biliary tree, and is complicated by the risk of cholangiocarcinoma (CCA) development [2]. We aimed to evaluate the involvement of PBGs and BTSCs in PSC and their role in CCA insurgence [2]. Specimens from normal liver (N=5), PSC (N=20), and CCA arising in PSC patients (N=20) were included and processed for histology, immunohistochemistry and immunofluorescence. In vitro experiments were performed on human BTSCs and primary CCA cell cultures. PSC-affected ducts were characterized by the activation of BTSCs and by PBG hyperplasia. In PSC, ducts showed higher microvascular density around PBGs compared to normal ducts. In CCA arising in PSC patients, PBGs showed dysplastic and neoplastic aspects. Compared to non-cancerous ducts, neoplastic ducts showed higher inflammation, wall thickness, and PBG activation. CCAs were characterized by higher expression of epithelial-to-mesenchymal transition (EMT) traits in PBGs and by the absence of primary cilia in BTSCs compared to control ducts. In vitro study confirmed that human BTSCs, under inflammatory milieu, increased proliferation rate and expression of EMT traits, and lost primary cilia compared to control conditions. In conclusion, patients affected by PSC are characterized by PBG involvement and activation of BTSC niche; the insurgence of CCA was characterized by involvement of PBG niche, suggesting a key role of this cell compartment in progressive tumorigenesis.

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Key words

Peribiliary glands, stem cells, angiogenesis, primary sclerosing cholangitis.

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Cilioretinal arteries: incidence in a 1110 patients'sample

Luigi Pannarale[1], Loredana Arrico[2], Martina Formisano[2]

[1] Dipartimento SAIMLAL, Università degli Studi di Roma Sapienza, Roma, Italia
[2] Dipartimento Organi di Senso, Università degli Studi di Roma Sapienza, Roma, Italia

The central retinal artery, supplies retinal vasculature. In some cases, a part of retinal circulation is supplied by a cilioretinal artery [1]. Cilioretinal arteries take rise from a posterior ciliary artey [2]. If retinal vascular occlusion occurs, the presence of a cilioretinal artery can be a significant factor influencing visual morbidity [3]. Studies on the frequency of this anatomical anomaly are limited in number and most of them take into consideration a small number of subjects. Largest two studies are Wang et al. (1991) [4], on 2050 patients, published only in Chinese language, and Liu et al (2011) on 2500 patients [5].

We examined the fundus photographies of 1110 patients between the age of 7 and 100, with an average of 51 years old.Observations were made through the use of a high definition confocal scanner fundus camera (Eidon). This instrument provides high resolution images in a short time even through an undilated pupil. These characteristics have been advantageous for obtaining viable images of the fundus oculi even in the pediatric patients. Cilioretinal arteries were found in 380 patients (34.2%). Among 380 patients, 178 (47%) were males and 202 (53%)were females. Of these patients, 97 (25,5 %) presented this anatomical variant in both eyes and 283 (74,5%) in only one eye. This variant was mostly present in the right eye (64,7 %of the right eyes and 35,3%of the left eyes), and 95 patients (25%) had more than one cilioretinal artery. Finally, in 330 cases (87%) the vessels were temporal, in 19 (5%) were only nasal and in 30 cases (8%) the patients had both temporal and nasal cilioretinal arteries. This is one of the largest studies in literature. Our results are obtained with a non invasive technique. Nevertheless, incidence of cilioretinal arteries in our study is comparable with that obtained by fundus fluorescein angiography, normally considered the most accurate method to visualize retinal vessels. Our non invasive approach is a base for further investigations.

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Key words

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Estradiol and progesterone regulation of neural circuits controlling reproduction

Giancarlo Panzica[1], Stefano Gotti[2], Mariangela Martini[3], Marilena Marraudino[2]

[1] Università degli Studi di Torino, Dipartimento di Neuroscienze, Torino, Italia
[2] Università di Torino, Dipartimento di Neuroscienze, Torino, Italia
[3] North Carolina State University, Department of Biological Sciences, Raleigh, Stati Uniti D' America

In rodents, female reproductive behavior is strongly influenced by estradiol (E2) and progesterone (P) whose levels fluctuate during the cycle. P and E2 work in synergistic fashion to facilitate female receptivity. Some neural circuits implicated in the control of sexual behavior and/or reproductive physiology (the nitrergic and the kisspeptin systems) also show changes during the estrous cycle. Nitric oxide (NO) is a gaseous neurotransmitter playing an important role in the regulation of sexual behavior in rodents. NO is produced by the enzyme neural NO synthase (nNOS) whose expression is influenced by gonadal hormones. The kisspeptin system (clustered in two groups of cell bodies in the periventricular region, RP3V and in the arcuate nucleus, ARC) is also modulated by gonadal hormones during the cycle and sends fibers mainly to the GnRH neurons and in a few other locations, including the paraventricular nucleus, PVN. Previous studies were unable to distinguish among the role played by P or E2 in inducing these changes. In the present study, we investigated the effects of E2 and P (alone or together) on the neural circuits of gonadectomized female mice, following a timing of administration that emulates the different phases of estrous cycle, for two cycles of 4 days.

The quantitative analysis of nNOS-ir system demonstrated a statistically significant variation in the number of positive cells in the bed nucleus of the stria terminalis, the arcuate nucleus and the medial preoptic area, with the highest number of positive neurons observed in E2 + P group. In physiological conditions, the two main groups of kisspeptin neurons respond in different way to the fluctuations of E2: the highest expression being in estrus in RP3V and PVN (positive feedback), and during the diestrus in ARC (negative feedback). As expected, the two cell groups were differentially affected by E2; the RP3V group was positively influenced by E2 (alone or with the P), whereas in the ARC the administration of E2 did not affect the system. However P (alone) induced a rise in the kisspeptin immunoreactivity. All the treatments significantly affected the kisspeptin innervation of the PVN, with regional differences, suggesting that these fibers arrive from both RP3V and ARC nuclei.

In conclusion, our data suggest that, in addition to E2, also P may have an important role in the regulation of neural circuits controlling reproductive behavior.

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A new generation of three-dimensional anatomical atlases, obtained through a process of acquisition and combination of images coming from three different technologies

Ferdinando Paternostro, Giacomo Gelati, Alessandra Pacini and Piergiorgio Francia

Università degli Studi Di Firenze, DMSC, Firenze, Italia

The anatomical representation of the human body deals with a burden throughout the centuries: the gap between the iconographic representation and the real object. The drawings and the watercolour plates are schematizations, so they are deeply far from the real object, apart from the accuracy. The photographic images allow to fix the image of anatomical preparations with high fidelity to particulars, bypassing the aforesaid artistic representation by the author, but they are static. The modern virtual graphic synthesis images are three-dimensional, so they can be observed from every point of view, but they still remain schematizations influenced by the artistic representation of the graphic designer. Moreover, they lack real colour and real light. Nevertheless, it is possible to reduce the aforesaid gap to minimal levels through a process of acquisition and combination of anatomical images conceived by the first of the authors and protected by patent. The images are obtained from non-contact surface scanning, computerized tomography and high resolution photography. The non-contact surface scanning, made through laser scanner or structured-light scanner, generates a cloud of points of the surface of the examined object and each one is identified by specific coordinates. Starting from the cloud of points it is possible to reconstruct a three-dimensional model of the scanned surface, characterized by very high definition and measurable (accuracy of micron). The CT images allow to observe and to analyse in depth the object under examination, according to the sequential sagittal, frontal, or transverse planes. The scanned images can be processed through specific software, in order to obtain a 3D reconstruction, which offers a global three-dimensional overview of the anatomical structure. The object under examination can be photographed in controlled ambient light conditions, in order to obtain high resolution images. A scale superimposition of the three-dimensional model obtained from the non-contact surface scanning, of the 3D reconstruction from CT images, and of the high definition photos, leads to the complete finished model, observable both on the surface and in depth, which can work as an Anatomy Atlas if some hyperlinks are added in order to tag the anatomical details. In this case the observer has the opportunity of analysing not simple schematization, but a model made through an objective instrumental data acquisition process, not falsifiable, highly corresponding in colours, in light and in morphology, from the macroscopic aspect to the under-millimetre details, rotable, observable from every perspective and carefully measurable. Moreover, the finished product can be processed in order to be observed in 3D through virtual reality visors or augmented reality visors.

Three-dimensional files processing and texture mapping: Filippo Sessa, Andrea Di Savino, Adriano D'Elia, Rossana Afeltra. Instruments and technological support: Flavio Biagini.

Key words

Research Methods, Laser Scanner, Morphometry.

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Effect of Silicon food supplement on bone tissue healing: histomorphometric and EDS analysis in human

Gaia Pellegrini

Università degli Studi di Milano, Dipartimento di scienze biomediche chirurgiche e odontoiatriche, Milano

Introduction: Few clinical trials reported beneficial impact of Si on bone metabolism, but no study on alveolar bone healing has been published [1]. Aim of this study was to assess if daily supplementation of Si: i) increases amount of Si within the newly formed alveolar bone, ii) induces histomorphological changes of newly formed alveolar bone. Materials and Methods. 20 systemically healthy individuals requiring premolar tooth extraction were included and immediately after the tooth extraction were randomly assigned to group A (1 tablet/day for 4 months containing Si) or to group B (1 tablet/day for 4 months containing placebo). At 4 months post-op, a bone core biopsy was harvested from each healed site, processed for ground sections and stained. For morphological and histomorphometric analyses, slides were observed using a light microscope equipped with a digital camera and photos were acquired at magnification of 100X. Stereological analysis was done to obtain the proportions of the specimen occupied by every regenerated tissue: lamellar bone, woven bone, osteoid, medullary spaces. Sections were observed using a BSE-SEM system without additional fixation and previous coating of carbon film to assess level of mineralization of regenerated tissue. Slides were observed at energy dispersive spectroscopy to assess levels of silicon in regenerated tissue (% Mass). In both groups, mean and standard deviation were calculated for clinical data, percentage of connective tissue, osteoid and mature bone, % Mass of Si. Inferential statistics was also done. Results. Two patients withdrew from the study. Bone sample harvesting was done on 8 patients of group A and 10 patients of group B. The mean Si content in samples of group A was 0.9% and in samples of group B was 0.2% (no significant differences). Si concentration appeared higher in medullary spaces than in the bone. Si concentration in the mature bone appeared lower than in the bone in the phase of mineralization and in the medullary spaces. Volume fraction of lamellar bone was significantly higher in group A than in group B, and volume fraction of osteoid matrix was significantly lower in group A than in group B ($p<0.05$, Wilcoxon rank sum test). Newly formed blood vessels were 9.75% ± 1.56 for group A and 9.53% ± 3.24 for group B (no significant difference). Conclusions. Supplementation seems to increase Si levels in healing bone tissue and seems to accelerate maturation process of mineralized connective tissue.

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Key words

Bone healing, Silicon, BSE-SEM.

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Effects of different extracts of curcumin on TPC-1, a papillary thyroid cell line

Angelica Perna[1], Angela Lucariello[1], Pasquale Tammaro[2], Antonio Ascione[3], Germano Guerra[4], Domenico Tafuri[3], Bruno Varriale[2], Teresa Esposito[2] and Antonio De Luca[1]

[1] Università degli studi della Campania "Luigi Vanvitelli", Anatomia Umana, Napoli, Italia
[2] Università degli studi della Campania "Luigi Vanvitelli", Medicina sperimentale, Napoli, Italia
[3] Università Parthenope, Sport, salute, benessere, Napoli, Italia
[4] Università del Molise, Medicina e salute, Campobasso, Italia
[5] Università della campania "Luigi Vanvitelli", Medicina sperimentale, Napoli, Italia

The thyroid gland is one of the largest endocrine glands in the body. Thyroid cancers are the most common endocrine tumors and are more prevalent in women and elderly individuals. In particular, papillary thyroid carcinoma is often non-enveloped and multifocal, i.e., it simultaneously affects different parts of the thyroid gland, and spreads mainly because of lymph nodes.

Curcumin possesses a wide variety of biological functions, and thanks to its properties, it has gained considerable attention due to its profound medicinal value. We have undertaken the present work in order to define the possible role of curcumin in modulating the genetic expression of cell markers and to understand the effectiveness of this nutraceutical in modulating the regression of cancer phenotype.

As a template we used the TPC-1 cells treated with the different extracts of turmeric, and examined the levels of expression of different markers (proliferative, inflammatory, antioxidant, apoptotic).

Our data show for the first time that curcumin-enriched compounds are able to decrease TPC1 cell survival and this occurs through the induction of apoptosis mainly by significantly reducing the accumulation of Bcl2 and cyclin D1 and the levels of p21 and p53. Besides, β-catenin, involved in cell growing, is also reduced by these curcumin-enriched compounds. It is also noteworthy that Nrf2, a downstream target of p21, is also affected.

Key words

Curcumin, Thyroid, TPC-1 cells, Anti-oxidant, Nutraceutical.

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The Auricular Neuromodulation as a noninvasive neurostimulation method for the treatment of pain

Alessio Pirino, Giancarlo Bazzoni, Pasquale Bandiera, Maria Alessandra Sotgiu, Andrea Montella

CERNATEC, Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italia

The Auricular Neuromodulation (ANM) is a therapeutic technique based on the stimulation of the outer ear through different methods (microneedles, beads, electrical stimulation); it can be considered the modern and scientific evolution of the auricular acupuncture. The ANM is scientifically based on the particular innervation of the ear, mainly supplied from the cranial trigeminal and vagus nerves and from the cervical plexus [1, 2]. Therefore, through the ANM we can get a noninvasive vagal, trigeminal and spinal stimulation-modulation, which allows to treat several diseases or functional disorders.

In order to further validate the effectiveness of auricular stimulation in the treatment of pain, a study on 100 patients (20-50 y.o., 58 m and 42 f) was conducted at CERNATEC – Research Center on Auricular Neuromodulation and Complementary Therapies of the University of Sassari. The patients, suffering from acute pain in the upper limb, were divided by computerized randomization into 2 groups of 50. All the participants received auricular stimulation by a single needle positioned on a single ear point, homolateral to the pain site.

In the patients of the group A the point was identified as the most sensitive to pressure (Pressure pain test) in the scaphoid fossa (auricular area corresponding to the upper limb).

In the patients of the control group B, the needle was placed on an "ineffective" point on the lobe.

The pain was evaluated by utilizing the Numerical Rating Scale. The NRS was administered 5 minutes before the Pressure Pain Test (T0) and at the times T1, T2 and T3(1 hr, 12 hrs and 24 hrs after the auricular stimulation). Significant differences in pain score were observed at T2 and T3; pain reduction is present in both groups, but is significantly higher in the group treated on the effective ear point. This shows that the stimulation of the auricle always evokes a neuromodulatory response, but the result is much more effective if the stimulation is carried out in well selected areas of the auricle. The study also confirms the efficacy of auricular stimulation in the treatment of non-neuropathic nociceptive pain.

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Key words

Auricular neuromodulation, noninvasive neurostimulation, ear innervation, pain therapy.

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Histological study on the interactions between an agarose gel filler and the human skin: observations within a year

Alessio Pirino[1], Giorgio Maullu[1], Paolo Cossu Rocca[2], Andrea Montella[1]

[1] CRISMENC, Department of Biomedical Sciences, University of Sassari, Sassari, Italia
[2] Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italia

At the CRISMENC, Centre for Research and Development in Aesthetic Medicine, Nutraceuticals and Cosmetology of the Department of Biomedical Sciences, the present study has been conducted in order to evaluate the biological interactions between an agarose gel filler and the human skin structures, as there are currently no studies on humans in the indexed scientific literature, where just two histologic studies conducted on rats can be found [1, 2]. The research has been performed through histological observations on biopsies conducted over a 1 year period. 12 healthy female subjects, 35 to 50 y. o., were selected. A 1,5% agarose gel filler was injected in the superficial hypodermis of the upper medial gluteal region by linear retrograde technique. Five biopsies were done: before the implant (T0), after 1 month (T1), 3 months (T2), 6 months (T3) and 1 year (T4) from the implant. Biopsies were fixed in 10% formalin, paraffin embedded, cut, stained and observed by light microscope. After the injection an histiocytic foreign body reaction was observed, basically characterized by macrophage elements and some polynucleated giant cells. The filler at the times T1 and T2 was gradually included in the connective tissue, with an increase of fibroblastic elements and deposition of collagen. No granulomas, fibrotic encapsulation of the implant and lymphocytic infiltrate were observed. At the time T3 a quantitative reduction of the filler was detected, while the cellular and tissue characteristics previously observed remain. After one year (T4) no more filler was present and a marked increase of the collagen fiber bundles remains, which in some areas are not yet well organized. Above all, a complete absence of an immunological reaction is observed. The results of this study confirm the biocompatibility of an agarose gel filler in the human skin until one year after the injection.

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Key words

Human skin, agarose gel filler, histology, biopsies.

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Human dental pulp stem cells and their application to an animal model of stress urinary incontinence

Alessandra Pisciotta[1], Alessio Zordani[1], Laura Bertoni[1], Daniela Giuliani[2], Giampaolo Bianchi[1], Anto de Pol[1] and Gianluca Carnevale[1]

[1] Università di Modena e Reggio Emilia, Dipartimento Chirurgico Medico Odontoiatrico e di Scienze Morfologiche con Interessere Trapiantologico, Oncologico e di Medicina Rigenerativa, Modena, Italia
[2] Università di Modena e Reggio Emilia, Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Modena, Italia

Stress urinary incontinence (SUI), the most common type of urinary incontinence, is defined by an involuntary leakage of urine due to physical stress involving an increase in bladder pressure. It is associated with life quality issues, depressive symptoms and social discomfort. The pathophysiology is represented by tissue damage of the external urethral sphincter affecting both muscle and nerve tissues. The current therapies are mainly based on rehabilitating methods, pharmacological and/or surgical treatments. However, these therapies cannot resolve the primary cause of incontinence, indeed only symptoms can take relief by such treatments [1]. Mesenchymal stem cells might represent an alternative tool for therapy of SUI. The aim of the study was to evaluate the regenerative potential of human dental pulp stem cells (hDPSCs) in an animal model of SUI. As reported in literature, hDPSCs are easily accessible during routine tooth extraction procedures, own a wide differentiation potential and do not present ethical issues [2, 3]. The first phase of the study demonstrated that hDPSCs were able to reach the myogenic commitment in vitro; then, in the second phase, after surgically inducing urinary incontinence in female rats, we injected pre-differentiated hDPSCs in the urethral sphincter. Four weeks after cell injection the sphincter thickness was almost recovered, hDPSCs engrafted in the external urethral sphincter, committed towards myogenic lineage in vivo and promoted neo-angiogenesis. The urodynamic study showed an appreciable recovery of the continence in rats treated with hDPSCs which, interestingly, were also detected within the nerve, thus suggesting their participation in re-innervating the formerly injured nerve. Our findings, combined with further investigations on paracrine and immunomodulatory effects of hDPSCs, might allow to propose them as a promising tool for future alternative therapies in the treatment of SUI.

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Key words

External urethral sphincter, human dental pulp stem cells, urinary incontinence.

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Analysis of gene-gene interactions among patients with endometriosis

I.V. Ponomarenko[1], E.N. Krikun[2], M.I. Churnosov[1], R.F. Kapustin[3], M.V. Masnaya[2]

[1] Department Biomedical Sciences, Belgorod National Research University, Belgorod, Russia
[2] Belgorod National Research University, Medical College, Belgorod, Russia
[3] Department of Morphology and Physiology, Belgorod State Agrarian University Named After V.J. Gorin, Maiskii, Russia

Endometriosis is a process when benign growth of tissue is found outside of the uterus, the morphological and functional properties of such are alike endometrial [1-4]. The purpose of the study is an analysis of the role of a combination of four genes rs1514175, rs2241423, rs13111134 and rs5930973 in the formation of endometriosis among the population of the Central Chernozem Region of Russia.

Material and methods: 1376 individuals were involved in the study group: 395 patients with endometriosis and 981 female control group. Women of Russian nationality who are native of the Central Chernozem Region of the Russian Federation and who aren't relatives were included in the samples of patients and controls. Venous blood was material for the study in a volume of 6 ml taken from the median cubital vein of the proband. The allocation of genomic DNA from peripheral blood was carried out by phenol-chloroform extraction. Study of the polymorphism was carried out using the method of polymerase chain reaction using appropriate primers and probes on the thermocycler IQ5. Results: genotyping of the four studied molecular-genetic markers was conducted. Studying of the distribution of genotypes at the studied loci among the patients with endometriosis and in the control group revealed that Hardy-Weinberg equilibrium ($p>0.05$) is performed for them. It was found that among patients with endometriosis was the lowest frequency of combinations of genetic variants with rs1514175 with A rs2241423 with A rs13111134 and G rs5930973 (of 8.55%) compared to the control group (13,54%, $p=0.006$; OR=0,60, 95% CI: 0,40-0,89). Conclusions. In the study it was found that the combination of genetic markers with rs1514175 with A rs2241423 with A rs13111134 and G rs5930973 (OR=0,60) reduces the risk of developing endometriosis among women of the Central Chernozem Region of Russia.

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Key words

Endometriosis, gene-gene interactions.

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Intratracheal administration of clinical-grade mesenchymal stem-cell-derived extracellular vesicles reduces lung injury in a rat model of Bronchopulmonary Dysplasia

Andrea Porzionato[1], Patrizia Zaramella[2], Arben Dedja[3], Diego Guidolin[1], Kelly Van Wemmel[4], Veronica Macchi[1], Marcin Jurga[4], Giorgio Perilongo[5], Eugenio Baraldi[2], Maurizio Muraca[6] and Raffaele De Caro[1]

[1] Sezione di Anatomia Umana, Dipartimento di Neuroscienze, Università degli Studi di Padova, Padova, Italia
[2] Unità di Terapia Intensiva Neonatale, Dipartimento della Salute della Donna e del Bambino, Università degli Studi di Padova, Padova, Italia
[3] Dipartimento di Scienze Cardiache, Toraciche e Vascolari, Università degli Studi di Padova, Padova, Italia
[4] The Cell Factory BVBA, (Esperite NV), Niel, Belgio
[5] Clinica Pediatrica, Dipartimento della Salute della Donna e del Bambino, Università degli Studi di Padova, Padova, Italia
[6] Laboratorio di Cellule Staminali e Medicina Rigenerativa, Dipartimento della Salute della Donna e del Bambino, Università degli Studi di Padova, Padova, Italia

Mesenchymal stem cells (MSCs) prevent the onset of bronchopulmonary dysplasia (BPD) in animal models, an effect that seems to be mediated by their secreted extracellular vesicles (EVs). The aim of this study was to compare the protective effects of intratracheally (IT)-administered MSCs vs. MSC-EVs in a hyperoxia-induced rat model of BPD.

At birth, rats were distributed as follows: animals raised in ambient air for 2 weeks (n=10); and animals exposed to 60% oxygen for 2 weeks and treated with IT-administered physiological solution (n=10), MSCs (n=10), or MSC-EVs (n=10) on postnatal days 3, 7, and 10.

The sham-treated hyperoxia-exposed animals showed reductions in total surface area of alveolar air spaces, and total number of alveoli (Nalv), and an increased mean alveolar volume (Valv). EVs prompted a significant increase in Nalv ($P<0.01$), and a significant decrease in Valv ($P<0.05$) compared with sham-treated animals, while MSCs only significantly improved Nalv ($P<0.05$). Small pulmonary vessels of the sham-treated hyperoxia-exposed rats also showed an increase in medial thickness, which only EVs succeeded in preventing significantly ($P<0.05$).

In conclusion, both EVs and MSCs reduce hyperoxia-induced damage, with EVs obtaining better results in terms of alveolarization and lung vascularization parameters. This suggests that IT-administered EVs could be an effective approach to BPD treatment.

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The -2518 A/G single nucleotide polymorphism of MCP-1 in myelofibrosis: functional characterization on ex-vivo patient cells

Giulia Pozzi[1], Elena Masselli[1], Cecilia Carubbi[1], Giuliana Gobbi[1], Daniela Galli[1], Prisco Mirandola[1], Cristina Micheloni[1], Maria Luisa Arcari[1], Franco Aversa[2] and Marco Vitale[1,3]

[1] University of Parma, Unit of Biomedical, Biotechnological and Translational Sciences (S.Bi.Bi.T), Department of Medicine and Surgery, Parma, Italia
[2] University of Parma, Unit of Clinical and Experimental Medicine, Hematology and BMT Unit, Department of Medicine and Surgery, Parma, Italia
[3] CoreLab, Parma University Hospital, Parma, Italia

Host genetic variations have an essential role in the mutational landscape of Philadelphia-negative MPN [1,2]. However, the contribution of inherited factors in disease phenotype and evolution is poorly characterized. In MPNs, chronic inflammation triggers neoplastic transformation and catalyzes clonal evolution toward end-stage disease [3]. We recently demonstrated that the -2518 A/G SNP of the Monocyte Chemoattractant Protein-1 (MCP-1, rs1024611) is an inherited host genetic factor associated with secondary myelofibrosis (sMF) and a biomarker of disease severity in MF [4]. Here we aimed to characterize MCP-1 expression in MF according to patients' genotype, and the potential cellular source(s) of this chemokine. For this study, 15 therapy-naïve MF patients were recruited, 4 healthy subjects and 4 apheresis bags were utilized as controls (CTRL). MF were stratified according to their rs1024611 genotype in A/A (wild type), A/G and G/G (polymorphic). Peripheral blood mononuclear cells (MNCs) were isolated by Ficoll-Hypaque gradient, in part pelleted (resting, T0) and in part seeded in RPMI-1640 medium with 1.1 ng/ml of IL-1 β for 20 hrs (T1). T0 and T1 cells were processed for RNA extraction. $CD34^+$-cells were purified from MF peripheral blood and from apheresis bags by immunomagnetic selection and differentiated toward the MK lineage as previously described [5]. Mature $CD41^+$ MKs were then processed for RNA extraction. MCP-1 expression was evaluated by real-time PCR. We demonstrated that MF-MNCs significantly over-expressed MCP-1 as compared to CTRL-MNCs at basal state. Upon IL1β stimulation, we observed a dose-dependent effect of the -2518 A/G SNP on MCP-1 expression, with polymorphic patients displaying a >100-times higher fold-increase (T1 vs. T0) in MCP-1 levels as compared to A/A. MF-MKs also showed a significantly higher expression of MCP-1 as compared to CTRL. Finally, MF-$CD34^+$-cells from A/G+G/G patients displayed impaired MK differentiation compared to A/A, as indicated by a significantly lower number of $CD41^+$-cells obtained in culture. Our data show that circulating MNCs and $CD34^+$-derived MKs are a major source of MCP-1 in MF. Polymorphic MF patients, who cluster with adverse hematologic characteristics, display here a higher capacity to over-express MCP-1 under an inflammatory stimulus and an impaired megakaryocytic differentiation potential. Further studies to better define the role of MCP-1 on $CD34^+$-cells differentiation in the context of MF are desirable.

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Key words

Myelofibrosis, MCP1, -2518 A/G SNP.

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Phospholipase C-β1 in Adipose Derived Stem Cell osteogenic differentiation

Giulia Ramazzotti[1], Francesca Chiarini[2], Roberta Fiume[1], Isabella Rusciano[1] and Irene Faenza[1]

[1] Università di Bologna, Dipartimento di Scienze Biomediche e Neuromotorie, Bologna, Italia
[2] CNR, Istituto di Genetica Molecolare, Bologna, Italia

Human Adipose Derived Stem Cells (ADSCs) are mesenchymal stem cells isolated from adult lipoaspirates collected during liposuction procedures. These cells have been reported to differentiate down many different lineages including chondrogenic, osteogenic, adipogenic and neural[1]. We have previously investigated the role of Phospholipase C-β1 (PLC-β1) signaling both in C2C12 murine myoblast differentiation and osteogenic transdifferentiation, showing a remarkable increase in PLC-β1 mRNA and protein expression in both the processes[2,3]. Here we present data on the role of PLC-β1 in osteogenic differentiation of ADSCs. Notably, PLC-β1 expression varies during ADSC osteogenic differentiation: at first, its expression is reduced then it shows a marked increase. Nonetheless, PLC-β1 overexpression and silencing experiments demonstrated than its expression is essential for the differentiation process to take place. Since PLC-β1 expression fluctuates during osteogenic differentiation, we investigated if these variations were similar to cyclins expression pattern. Unexpectedly, we found that not only PLCβ-1 expression varies with cyclin E expression but also that the two proteins interact during osteogenic differentiation. This study provides molecular evidence for future therapeutic strategies for bone regeneration by targeting PLC-β1 signaling pathway.

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PLC-β1, Stem cell differentiation, osteogenesis.

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Effect of a collagen-based medical device on morpho-functional properties of cultured human tenocytes

Filippo Randelli[1], Alessio Giai Via[1], Manuel Mazzoleni[1], Marco Brioschi[1], Alessandra Menon[2] and Nicoletta Gagliano[3]

[1] I.R.C.C.S Policlinico San Donato, Centro di Chirurgia dell'Anca e traumatologia, San Donato Milanese - MI, Italia
[2] Azienda Socio Sanitaria Territoriale Centro Specialistico Ortopedico Traumatologico Gaetano Pini-CTO, 1° Clinica Ortopedica, Milano, Italia
[3] Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute, Milano, Italia

Tenocytes are specialized fibroblasts playing a key role in the maintenance of tendon extracellular matrix (ECM) homeostasis and, therefore, determining the tendon ability to resist mechanical forces and repair in response to injury [1, 2]. A medical device containing collagen type I (MD-Tissue, Guna) has been released on the market with the ambition to counteract the physiological and pathological degeneration of tendon connective tissue.

In this study we aimed at characterizing the effect this medical device on cultured human tenocytes, especially focusing on the collagen turnover pathways, in order to understand how the medical device could influence tendon biology.

For this purpose, gluteal tendon fragments were obtained from 8 healthy patients (mean age 64,8 ± 7,2 years) undergoing total hip replacement through an anterior approach, and tenocytes were obtained by outgrow from tendon fragment. Cell proliferation and migration were investigated by growth curves and wound healing assay, respectively. The expression of genes and proteins involved in collagen turnover were analysed by real time PCR, Slot blot and SDS-zymography.

Our data show that tenocytes cultured on MD-Tissue have increased proliferation rate and migration potential. MD-Tissue induced collagen type I (COL-I) synthesis, the main protein of tendon ECM, but matrix metalloproteinases (MMP) 1 and 2 involved in collagen degradation were not affected, suggesting that tenocytes cultured on MD-Tissue have an anabolic phenotype.

Considered as a whole, our results suggest that MD-Tissue could favour tendon repair by inducing tenocyte proliferation and migration, and stimulating COL-I synthesis and deposition.

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Key words

Tendon, collagen turnover, matrix metalloproteinases.

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Effects of Pleurotus eryngii var. eryngii in "in vitro" and "in vivo" cancerogenetic models

Francesca Rappa[1], Rosario Barone[1], Maria Letizia Gargano[1], Celeste Caruso Bavisotto[1], Felicia Farina[1], Filippo Macaluso[2], Claudia Campanella[1], Daniela D'Amico[1], Eleonora Trovato[1], Valentina Di Felice[1], Francesco Cappello[1], Giuseppe Venturella[3], Antonella Marino Gammazza[1]

[1] Dipartimento di Biomedicina Sperimentale e Neuroscienze Cliniche, Università degli Studi di Palermo, Palermo, Italia
[2] SMART Engineering Solutions & Technologies (SMARTEST) research center, eCampus University, Novedrate (CO), Italia
[3] Dipartimento di scienze agricole e forestali, Università degli studi di Palermo, Palermo, Italia

Heat shock proteins (Hsps) are highly expressed in a variety of cancer types contributing to tumor cell propagation and protection against apoptosis [1]. The current anti-cancer therapy is not always target specific and often is associated with complications for patients, Therefore new effective, specific and less toxic therapeutic approaches are needed. Medicinal mushrooms have emerged as wonderful source of nutraceuticals, anti-oxidants, anticancer, prebiotic, anti-inflammatory, cardiovascular, anti-microbial, and anti-diabetic. The ongoing research projects are aimed to promote mushrooms as new generation "biotherapeutics" [2]. The aim of this study was to evaluate whether the cold-water extracts of Pleurotus eryngii var. eryngii can affect Hsp90, 70, 60 and 27 levels in an in vitro model of colon cancer (C26 cells). Cell viability was evaluated using MTT assay after treating the cells with different concentrations of extracts (0-1.9 $\mu g/\mu l$) in the culture medium for 24 and 48 hours. Hsp90, 70, 60 and 27 levels were measured using western blotting and immunofluorescence analysis. Moreover, we evaluated the anticancer effect of the P. eryngii var. eryngii extract in an animal model of ectopically-implanted C26 colon carcinoma, widely used as an experimental model of cancer cachexia. We prepared a mixture of lyophilized P. eryngii var. eryngii with the mice standard diet and the animals were daily fed with ~4g of the mix until they died to draw a survival curve. We sampled the neoformations grown after implantation e on these we performed an immunohistochemistry for Hsp60. Our results showed that the extract significantly decreased cells viability at 0.48 $\mu g/\mu l$ after both 24 and 48 hours of treatments. Western blotting analysis and immunofluorescence showed that Hsp60 protein levels were down-regulate at 24h of treatment but increased after 48h. On the contrary, Hsp90, 70 and 27 protein levels did not changed. In the in vivo model, P. eryngii var. eryngii in the diet significantly extended the median survival compared to untreated mice. The immunoistochemical experiments suggested that Pleuery significantly affected the increase of Hsp60 protein levels. These preliminary results are promising for further studies to better understand the potential effects of P. eryngii var. eryngii on cancer progression especially regarding Hsp60 role.

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Key words

Hsp60, Pleurotus eryngii, cancer.

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Molecular chaperones expression levels and localization in non-tumoral and tumoral thyroid tissues

Francesca Rappa[1,2], Celeste Caruso Bavisotto[1,2], Antonella Marino Gammazza[1,2], Fabio Bucchieri[1], Everly Conway de Macario[3], Alberto J.L. Macario[2,3], Calogero Cipolla[4], Giovanni Tomasello[1], Francesco Carini[1], Daniela Cabibi[5], Claudia Campanella[1,2], Felicia Farina[1], Francesco Cappello[1,2]

[1] Section of Human Anatomy, Department of Experimental Biomedicine and Clinical Neuroscience, University of Palermo, Palermo, Italy
[2] Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy
[3] Department of Microbiology and Immunology, School of Medicine, University of Maryland at Baltimore-Institute of Marine and Environmental Technology (IMET), Baltimore, Maryland, USA
[4] Department of Oncology, Division of General and Oncological Surgery, University of Palermo, Palermo, Italy
[5] Pathology Institute, Department BG. D'Alessandro^, University of Palermo, Palermo, Italy

Papillary thyroid carcinoma (PTC) is the most frequently occurring subtype of thyroid cancer. Exosomes (EXs) secreted from cells to the extracellular environment play an important role in intercellular communication in normality and pathology. Recent data indicates that tumor cells-derived EXs contribute to cancer progression through the modulation of tumor microenvironment [1]. Heat Shock Protein (HSPs) are often overexpressed during carcinogenesis and different studies shown that they can be released by tumors cells and that the mechanism of release is mediated by EXs pathway. In this project we performed an immunomorphological study to investigate Hsp60, 90,70,27 levels expression profile in thyroid tissue from patients with benign goiter (used as benign desease) and patients with PTC. Moreover for each patient, blood samples were collected before and a one week after surgery, to obtain EXs. We performed Western Blotting analysis to verify the presence and the levels of the same HSPs. The immunoistochemistry shown an overexpression of Hsp60,90 and 27 in the PTC cases comparison with peritumoral tissue and with goiter cases. Instead the Hsp70 levels showed no significant changes. In particular Hsp60, 90 and 27 were visible at cytoplasmic and membrane levels. Data regarding exosomal fraction assessment by standard methods (TEM, and WB analysis for Alix) to identify exosomes confirmed their identity. The levels of Hsp60, 90,27 in the exosomes of patients with PTC before surgery were significantly higher than in the exosomes from the same patients after surgery. The data obtained shown that, as demonstrated in other cancer type [2], the HSP levels studied increased in PTC specimens respect to goiter specimens. Moreover the membrane localization of these HSP suggested a their release in tumor microenviroment, in fact we observed exosomal HSP before surgery in PTC patients. The HSP decreases after surgery indicated that if disease recurrence occurs, HSP levels will increase again. For this reason we hipotized that chaperonins could be good candidates as biomarkers of PTC.

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Key words

Papillary thyroid carcinoma, exosome, hsp.

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Ultraviolet A (UVA) and G alfa q/11-mediated signal transduction in uveal melanoma

Stefano Ratti[1], Jessica Scales[2], Lucio Cocco[1], Lucia Manzoli[1], Elena Oancea[2]

[1] Dipartimento Di Scienze Biomediche E Neuromotorie Università Di Bologna, Bologna, Italia
[2] Oancea Laboratory, Department of Molecular Pharmacology, Physiology and Biotechnology, Brown University, Providence, Stati Uniti D' America

Uveal melanoma (UM) results from the transformation of melanocytes in the uveal tract of the eye and is the most common intraocular malignancy in adults, with >50% chance of forming highly aggressive metastases for which no effective treatment exists. Unlike cutaneous melanoma, UM harbors somatic, mutually exclusive mutations in Guanine nucleotide-binding protein G(q) subunit a (GNAQ) and its paralogue GNA11, which encode closely related members of the Gaq/11 family of G proteins that operate downstream of G protein-coupled receptors (GPCRs) to activate Phospholipase Cb4 (PLCb4), leading to an increase in intracellular Ca2+. Approximately 95% of GNAQ and GNA11 mutations in UM encode the Q209L mutation that results in constitutive activity of the GTP-ase and melanocyte transformation. Recent data revealed additional mutations in PLCb4 and Cysteinyl leukotriene receptor 2 (CYSLTR2) mutually exclusive with Gaq/11 mutations, suggesting that UM is defined by activating mutations in the Gaq/11 pathway1. Uncovering the mechanisms involved in UM requires a thorough investigation of Gaq/11 signaling in uveal melanocytes and understanding whether the activating mutations are necessary and sufficient for UM development. The Oancea lab has recently discovered in human epidermal melanocytes a Gaq/11-mediated pathway activated by physiological doses of UVA2a protective response mediated by epidermal melanocytes, chronic exposure can lead to skin cancer and photoaging. However, the molecular mechanisms that allow human skin to detect and respond to UVR remain incompletely understood. UVR stimulates a retinal-dependent signaling cascade in human melanocytes that requires GTP hydrolysis and phospholipase C \u03b2 (PLC\u03b2. Our preliminary results show that this pathway is conserved in 4 different UM cell lines and reveal significant differences between the UM cells that harbor the Q209L mutation compared to the ones that express the wild type Gaq/11. To understand the function of Gaq/11 in non-transformed cells, we extracted and cultured primary uveal melanocytes from the choroid, ciliary body and iris of cow eyes. In addition, the RNAseq data analysis performed on 80 UM patients highlighted that several G-proteins and GPCRs are highly expressed suggesting a possible correlation with the tumor genesis. Our goal is to develop a functional uveal melanocyte culture based on cow eyes and to compare UVA and other signaling pathways mediated by Gaq/11 in primary cells and UM lines. As a first step, we will determine the baseline and UVA-evoked levels of Ca2+ and ROS, two important second messengers that control many signaling pathways downstream of Gaq/11. The results of these studies will significantly advance our understanding of how signal transduction pathways are altered in UM and will reveal novel potential targets for UM treatment.

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Key words

Uveal Melanoma, GNAQ, GNA11, Signal transduction.

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Circumpherential growth of cartilage anlagen and comparison with grow plate cartilage

Marcella Reguzzoni[1], Andrea Salvi[2], Lavinia Casati[3], Daniela Quacci[1], Marina Protasoni[1] and Ugo E. Pazzaglia[2]

[1] Dipartimento di Medicina e Chirurgia, Università degli Studi dell'Insubria, Varese, Italia
[2] Dipartimento di Specialità Medico-Chirurgiche, Scienze Radiologiche e Sanità Pubblica, Università degli Studi di Brescia, Brescia, Italia
[3] Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Milano, Italia

Mineral deposition in cartilage matrix always occurs prior to that in osteoid in order to provide the substrate for osteoblasts apposition. Different architectural patterns characterize different bone anlage developmental phases or those of other cartilaginous structures such as the laryngeal cartilages, so that both structural and morphological differences can be expected in different anatomical sectors of the same bone even if resulting from a substantially similar calcification mechanism.

The primary ossification center of the human metacarpal diaphysis has never been considered for a comparative study of the mineral deposition process in cartilage matrix and osteoid. The two territories are well distinguished and can be studied during a limited period of fetal anlage development. Mineral deposition occurs in the avascular, hypertrophic cartilage mass, where there is no free fluid exchange between the hypertrophic chondrocytes and the circulating blood flow until the marrow vessels seeps into the ossification center. Therefore, this model can provide the basis for a quantitative analysis of mineral deposition in a much larger surface of the inter-territorial cartilage matrix than that of the metaphyseal growth plate intercolumnar septa.

Aim of our study was to compare the morphology, morphometry and progression of mineral deposition in cartilage and in bone matrix, processes contextualized in the primary ossification center model than in the metaphyseal growth plate cartilage. In order to describe this processes we apply an enlarged, methodological approach combining standard histology, SEM/EDAX and analysis of the tissue mineral phase with heat deproteination.

It has been possible to examine the progression of the calcification process, which leads to the complete calcification of the matrices involved in endochondral ossification (cartilage and osteoid) and to consider how specific anatomical and structural conditions can modify the process of evolution.

Our observations can be integrated to form part of the current knowledge of the cellular mechanisms controlling calcium and phosphate concentrations, ion transport pathways and the specificity of the collagen layout where the mineral deposits are settled. The different morphology and dynamics of the calcification process in cartilage and bone matrix can be explained by the anatomical and environmental conditions where the two phases of endochondral ossification develop.

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Ultrastructural characterization of human colon cancer stem cell-derived spheroids and xenograft in a mouse model

Michela Relucenti[1], Paola Contavalli[2], Selenia Miglietta[1], Ezio Battaglione[1], Vito d'Andrea[3], Federica Francescangeli[2], Ann Zeuner[2], Giuseppe Familiari[1]

[1] La Sapienza Università di Roma, Dip. SAIMLAL, Roma, Italia (1) –
[2] Istituto Superiore di sSnità, dipartimento di Oncologia e Medicina molecolare, Roma, Italia (2)
[3] La Sapienza Università di Roma, dip Scienze Chirurgiche, Roma, Italia

Colorectal cancer is the third most common malignancy diagnosed worldwide and one of the major cause of cancer death in developed countries, with broad diffusion and increasing incidence. Despite emerging therapies and advances reached in the last years more than 30% of patients relapse and develop metastasis for acquired resistance. Cancer stem cells represent the population of the tumor responsible for recurrence of the disease, metastatic spread and are resistant to currently available therapies. Human colorectal cancer biopsies, obtained during surgical procedures after patient informed consent, were cultured in a selective medium to enrich a line of colon cancer stem cells (CCSCs) multicellular spheroids (CCSC-L1). Some multicellular spheroids were fixed and stored at 4°C in glutaraldehyde 2,5% for electron microscopy study and other were subcutaneously injected in in 5 immunocompromised NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice. Mice were sacrificed after 3 weeks, when cancer stem cell-derived xenograft reached dimensions of about 100mm3 . Samples were fixed immediately post recovery in glutaraldehyde 2,5% in pbs and were then prepared for conventional scanning and transmission electron microscopy. CCSCs spheroids were observed by means of scanning and transmission electron microscopy, they were formed by 8-10 cells. In these multicellular structures colon cancer stem cell, mitotic figures and differentiated enterocytes were observed. No goblet cells or enteroendocrine cells were found. CCSCs-derived xenograft showed the same morphology of colon cancer, it appeared well vascularized and innervated with a connective tissue envelopment rich in fibroblast. The xenograft showed mainly differentiated enterocytes but also stem cells and cells that are under epithelial mesenchymal transition. No goblet cell or enteroendocrine cells were observed. This is the first ultrastructural study of CCSCs multicellular spheroid and their xenograft from the cellular line CCSC-L1. Cancer stem cells and fully differentiated enterocytes were observed in both spheroids and xenograft, as well as goblet cells and enteroendocrine cells were absent in both samples. Epithelial mesenchymal transition instead was observed only in the xenograft, which is enveloped by connective tissue, innervated and vascularized, this underline the importance of a supportive in vivo microenvironment, whose influence is absent in multicellular spheroids.

Con il contributo del Ministero degli Affari Esteri e della Cooperazione Internazionale, Direzione Generale per la Promozione del Sistema Paese

Key words

Colon cancer, stem cells, electron microscopy, epithelial mesenchymal transition, metastasis.

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Association of polymorphic markers of the functional state of the fetus of pregnant women with preeclampsia

E.A. Reshetnikov[1], E.N. Krikun[2], M.I. Churnosov[1], R.F. Kapustin[3], M.V. Masnaya[1]

[1] Department Biomedical Sciences, Belgorod National Research University, Belgorod, Russia
[2] Medical College, Belgorod National Research University, Belgorod, Russia
[3] Department of Morphology and Physiology, Belgorod State Agrarian University Named After V.J. Gorin, Maiskii, Russia

Preeclampsia is a multisystem pathological condition that occurs in the second half of pregnancy (after 20 weeks), characterized by arterial hypertension in combination with proteinuria (≥3 g/l, in daily urine), often with edema and signs of organ/multisystem dysfunction. Preeclampsia (PE) is a serious complication of pregnancy, resulting in the onset of premature labor, detachment of normally situated placenta. The purpose of this study was to examine the Association of gene polymorphisms of the renin-angiotensin-aldosterone system with the functional status of the fetus of pregnant women with preeclampsia [1-4]. The study group included 132 pregnant women with preeclampsia. The average age of women surveyed was 27.98±5.29 years. To assess the functional state of the fetus is determined by uterine-fetal-placental blood flow (index of resistance (IR) left and right uterine artery and umbilical artery) and basal heart rate of the fetus. All pregnant women carried out the typing of genetic polymorphisms of the renin-angiotensin-aldosterone system: angiotenzinoguena (-6A/G AGT) and angiotensin-converting enzyme (I/D ACE). For the description of indicators were used the median (Me) and interval scale (Q25-Q75), Mann-Whitney test. As a result of the study revealed that pregnant women with genotype-6AA AGT, have lower basal heart rate of the fetus (median 137.0 BPM, interquartile range 130.0-146.0 BPM), in comparison with women with the genotype -6AG and -6GG AGT (median 143.0 beats/min, lower quartile – 136.0 beats/min, upper quartile – 152.0 beats/min, p=0.02). Reliable associations of polymorphism I/D ACE with the functional status of the fetus were not detected (p>0.05). Thus, when examining the associations consider genetic polymorphisms of the renin-angiotensin-aldosterone system with the functional status of the fetus the connection of genotype-6AA AGT with lower basal heart rate of the fetus had been established.

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Key words

Preeclampsia, fetus.

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Did the representation of brain convolutions seen in the wax of Susini-Boi, now in Cagliari and dated 1803, influence Luigi Rolando?

Alessandro Riva[1], Alessio Pirino[2], Francesco Loy[1], Marcello Trucas[1], Maria Alessandra Sotgiu[2], Andrea Montella[2]

[1] Dipartimento di Scienze Biomediche, Università di Cagliari, Monserrato, Italia
[2] Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italia

Luigi Rolando (1773-1831) discovered several features of the central nervous system and, in particular, the paired central gyri on either side of the central fissure which is named after him. In his time, the convolutions were called enteroid processes according to Erasistratus (II C. B.C.) who did assimilate them to the mobile intestinal loops. Rolando was the first to deny such belief, stating in 1828 that convolutions have specific shapes and positions [1]. In 1804, Rolando left for Sardinia having been offered by Vittorio Emanuele I the Chair of Anatomy of the University of Sassari. However, owing to an epidemic of yellow fever in the harbor of Livorno, he went to Florence. There he met the old Felice Fontana who had been the founder of La Specola Museum. He became friends with Paolo Mascagni, the Chairman of the Anatomy School of the Hospital of S. Maria Nuova and principal anatomist of La Specola. Thanks to the latter, he worked in both institutions, not only improving his knowledge of anatomy, but also learning the art of engraving and of ceroplastics, under guidance of Clemente Susini. In the years of Rolando permanence, Susini was collaborating with the Sardinian anatomist F. Boi who, since 1801, was working in Mascagni's lab. Boi had been ordered by Carlo Felice of Savoy to commission to Susini, head modeler of La Specola, a collection of anatomical waxes for his museum in Cagliari. Thus, it is very likely that Rolando was acquainted with the models of Susini-Boi since, by the end of 1804, several waxes were completed, including those on the nervous system. Surely, Rolando saw the one contained in box XIII, dated 1803, where there is a correct representation of the brain convolutions with the pre- and post-central gyri and the intervening fissure [3]. Moreover, a further evidence of Rolando's knowledge of Susini's and Boi work results from the wax molds of the superior part of brains, transversally sectioned at the level of the start of the lateral fissure, which are exhibited in Sassari Anatomical Museum since his stay (1807-1814) there. They were casted by Rolando himself and show convolution patterns similar to those of the wax of Cagliari.

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Key words

Luigi Rolando, cerebral convolutions, Clemente Susini, Francesco Boi.

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Sarcoglycan subcomplex and Alpha-Dystroglycan in human digestive tract: immunofluorescence analysis

Giuseppina Rizzo, Giuseppe Santoro, Grazia Giulia Pantè, Claudia Previti and Angelo Favaloro

University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia

The sarcoglycan complex (SGC) is a multimember transmembrane complex consisting of six glycosylated transmembrane proteins (α,β,δ,γ,ε,ζ). These proteins, primarily expressed in skeletal muscle fibers, interact with other member of dystrophin-glycoprotein complex (DGC), dytrophin, dystroglycans and syntrophins, in order to provide a mechano-signaling connection from the cytoskeleton to the extracellular matrix in myocytes and to stabilize the sarcolemma during contraction and release cycles in the muscle tissue. Our previous investigations have shown that sarcoglycans are not only muscle-specific but they are also present in the epithelial tissues, such as gingival, prostatic, respiratory and digestive, and also in the adipose tissue, demonstrating that these proteins are involved in cell-cell and cell-matrix interactions [1]. In order to verify the presence of sarcoglycans in the digestive epithelium and their interaction between α-dystroglycan, we performed immunofluorescence reactions on biopsies of normal sigmoid colon obtained from 10 subjects who underwent for other pathological reasons. Moreover, in the same samples, also we carried-out immunofluorescence reactions testing mucins. Mucins are a superfamily of highly glycosylated protein, they are part of mucus. The main roles of mucus are to protect and lubricate the underlying epithelia by injuries like enzymes, pH, bacteria and viruses. Mucins, also, lead to coordinate the apoptosis among cellular responses playing a key role as biomarkers for cancer and inflammatory diseases [2]. For the first time, our results show that: (i) sarcoglycans are expressed in the basal, lateral and apical cell's sides; (ii) sarcoglycans colocalize in the apical region with mucins and α-dystroglycan; (iii) α-dystroglycan colocalizes with mucin in the cellular apical region. Our results suggest that a interactions between these sarcoglycans and mucus exists and, in our opinion, α-dystroglycan can play a key role in this interaction. On the basis of our results, we hypothesize that α-dystroglycan and sarcoglycans may have a role in the determination of the cell's polarity, supported by the colocalization of mucins and dystroglycans in the apical area.

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Key words

Sarcoglycan, alpha-dystroglycan, mucin, epithelium.

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Sarcoglycans in the adipose organs during trans-differentiation by genistein

Giuseppina Rizzo[1], Francesco Squadrito[2], Alessandra Bitto[2], Cecilia Spoto[1], Giovanna Vermiglio[1], Grazia Giulia Pantè[1], Emanuele Magaudda[1] and Saverio Cinti[3]

[1] University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia
[2] University of Messina, Department of Clinical and Experimental Medicine, Messina, Italia
[3] Università delle Marche, Department of Experimental and Clinical Medicine, Ancona, Italia

The adipose organ is a highly dynamic organ, and it is formed by white and brown adipocytes. This organ is allocated in multi-depots, which can be found in the subcutaneous and visceral areas. These depots include the two different types of cells: white and brown adipocytes. These areas can be called 'brownish' area or brown adipose tissue (BAT) and white area or white adipose tissue (WAT) by the predominant parenchymal cell with different vascularization and innervation. BAT is highly vascularized and innervated, instead, WAT has lower vascularization and innervation. White and brown adipocytes have also different functional roles. The white adipocytes stores energy in fatty acid because they have one single lipid droplet, to maximise the volume and minimise the space occupation. The brown adipocytes burn fatty acid and glucose to produce heat having more lipid droplets, to enhance the thermogenesis. Recent studies have shown that the adipose organ can trans-differentiate from WAT to BAT and possibly from BAT to WAT. Trans-differentiation can proceed with multiple types of stimuli like cold exposition, or by stimulation of the adrenergic or estrogenic receptor. Previous our studies demonstrated the presence of sarcoglycans, glycoproteins connecting the cytoskeleton to the extracellular matrix in the skeletal muscle, also in the adipose organs showing an increase of all sarcoglycans in cold exposure experiment [1]. On this basis, here we studied the sarcoglycans in adipose tissue, performing an immunostaining labeling by cell culture, with genistein, an isoflavone binding to the Estrogen Receptor Beta (ERb); moreover, this protein can also acts as browing agent to induce white-to-brown adipocyte trans-differentiation. In particular, 3T3-L1 cell cultures were differentiated and treated with genistein at 50mM for up to 24hrs in order to induce the trans-differentiation. Cell cultures were fixed and processed for immunolabeling. Our results showed that sarcoglycans were increased in brown adipocytes than in white adipocytes. These data, similar to our previous results by cold trans-differentiation, showed that sarcoglycans, in trans-differentiation culture cells by estrogenic stimulation, could play a key role in modulation of differentiation during the "browing".

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Key words

Adipose organ, adipocyte, sarcoglycans, browning.

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Delayed peripheral nerve repair: description of degenerative and regenerative processes

Giulia Ronchi, Stefania Raimondo, Benedetta Elena Fornasari, Stefano Geuna

Università degli Studi di Torino, Dipartimento di Scienze Cliniche e Biologiche, Orbassano (TO), Italia

Nerve fiber regeneration and complete functional recovery after peripheral nerve injury do not always occur and can be influenced by patient age, gender, lesion site, injury severity, size of the gap between damaged nerve stumps and time interval that elapses before performing surgical repair.

The poor outcome occurring after a long delay can be due to loss of the neuron ability to regenerate, loss of the Schwann cell ability to support regeneration and, of course, progressive muscle atrophy.

The aim of this study was to investigate the degenerative processes of the denervated distal nerve stump in order to understand which role they can have during delayed nerve regeneration.

Morphological and biomolecular analyses carried out on degenerated nerves showed several collagen fibers and fibroblasts, atrophic Schwann cells and a significant reduction of soluble Neuregulin1 (NRG1, an important factor for the survival and activity of Schwann cells) already after 3 months of degeneration.

Moreover, functional, morphological, morphometrical and biomolecular analyses were carried out on regenerated distal nerve stumps 6 months after nerve repair (immediate or 3 and 6 months delayed). A rat surgical model of delayed nerve repair consisting of a cross suture between the chronically degenerated median nerve distal stump and the freshly axotomized ulnar proximal stump was used.

Functional recovery analysis shows that only the group repaired immediately and not the two delayed-repaired groups, recovered partially. Moreover, quantitative analysis shows that the delayed groups have fewer and smaller myelinated fibers compared to the immediate repair group. Finally, biomolecular analysis performed on the 6-months delayed group shows that soluble NRG1 maintains a low expression also after 6 months of regeneration.

These results demonstrate that, despite a delay of 3 or 6 months, the fibers are still able to regenerate, even if they are fewer and smaller than the immediate repaired group. Moreover, the analysis of the NRG1/ErbB system shows a significant decrease of soluble NRG1 in both degenerating and delayed-repaired nerves.

Our results suggest that NRG1 plays an important role in Schwann cell activity after denervation, therefore its manipulation could be a good strategy to improve the outcome after delayed nerve repair.

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Cardiac development and remodelling in Magic-F1 transgenic mice

Flavio Lorenzo Ronzoni[1], Gabriele Ceccarelli[1], Laura Benedetti[1], Maria Gabriella Cusella De Angelis[1] and Maurilio Sampaolesi[2]

[1] Università di Pavia, Human Anatomy Unit, Dept. Public Health, Experimental and Forensic Medicine - Center for Health Technologies (C.H.T.), Pavia, Italia
[2] Università di Pavia, Human Anatomy Unit, Dept. Public Health, Experimental and Forensic Medicine - Center for Health Technologies (C.H.T.) - Stem Cell Biology and Embryolo, Pavia, Italia

MAGIC-F1 (Met Activating Genetically Improved Chimeric Factor 1) is a human recombinant protein, derived from dimerization of the receptor-binding domain of hepatocyte growth factor (HGF). Previous experiments demonstrated that skeletal muscle specific expression of Magic-F1 can induce constitutive muscular hypertrophy, improve running performance and accelerate muscle regeneration after injury in hemizigous transgenic mice [1]. Furthermore, the microarray analysis of Magic-F1+/+ satellite cells showed transcriptomic changes in genes involved in the control of muscle growth, development and vascularisation [2].

In this study we demonstrate that Magic-F1 mice show an alteration of the heart morphology. Morphometric analysis and three-dimensional reconstruction of the hearth revealed that MAGIC-F1 paracrine effect is able to induce a robust remodelling of the left ventricle chamber in transgenic mice. Interestingly, we found in Magic-F1 hearts an alteration of Phd2 and HIF1 protein levels. These two oxygen sensors are found dysregulated in cardiac ischaemic conditions, where generalised hypoxia causes functional impairments in cardiomyocytes and structural tissue damage [3-4]. These preliminary results support the involvement of oxygen sensors in Magic-F1-induced cardiac hypertrophy and dilation. In addition, Magic-F1+/+ mice can be used as non-pressure overload model to further investigate the role of oxygen-sensors in ischaemic heart disease. To better understand the biological effects of MAGIC-F1 on the morphology and function of cardiac muscle, more detailed studies are required. It could be also interesting to have a longer follow-up of the homozygous animals, to investigate the progression of the cardiac remodelling upon a double dose of MAGIC-F1.

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Key words

Magic-F1, recombinant proteins, cardiac hypertrophy, oxygen sensors, heart remodelling, transgenic mice.

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Exploring the role of ghrelin peptides in sarcopenia development during aging

Flavio Lorenzo Ronzoni[1], Gabriele Ceccarelli[1], Laura Benedetti[1], Nicoletta Filigheddu[2], Simone Reano[2], Maria Gabriella Cusella De Angelis[1] and Maurilio Sampaolesi[1]

[1] Universita di Pavia, Sanità Pubblica, Medicina Sperimentale e Forense / Center for Health Technologies (C.H.T.), Pavia, Italia
[2] Universià del Piemonte Orientale, Medicina Traslazionale, Novara, Italia

Sarcopenia is a complex syndrome defined as the irreversible loss of skeletal muscle mass and functionality in aged individuals that results in frailty, mobility disorders, and loss of independence [1]. The pathology is characterized by muscle atrophy and impaired muscle regeneration. The mechanisms involved in its development are not fully understood, although hormonal changes, inflammation, insulin resistance and nutritional deficiencies are surely involved in. In addition, we and other authors showed that aging affect progenitor myogenic cells, including mesoangioblasts (adult vessel-associated stem cells) [2] unable to counteract sarcopenic phenotype. Due to the increase of the elderly population, sarcopenia has an important social impact, greatly affecting the quality of life of aged people and impacting government health care costs. Therefore, therapeutic strategies aimed at preventing and/or counteracting sarcopenia are of pivotal importance.

Acylated and unacylated ghrelin (AG and UnAG, respectively) are circulating peptides codified by the ghrelin gene. By acting through its receptor GHSR1a, AG stimulates appetite, adiposity, a strong release of growth hormone (GH) and has a broad anti-inflammatory activity. UnAG does not bind to GHSR1a however, similar to AG has a direct anti-atrophic effect on skeletal muscle [3].

Our preliminary results show that murine mesoangioblasts treated with recombinant UnAG or AG were able to differentiate spontaneously forming myotubes. In addition, in murine embryonic stem cells and human mesodermal induced pluripotent stem cells subjected to myogenic differentiation, the presence of recombinant proteins resulted in improved myogenic commitment.

Taken together our results candidate AG and UnAG as potent myogenic inducers, able to modulate the gene expression profile in myogenic progenitors, affecting positively the muscle differentiation process.

This work was supported by grant CARIPLO Foundation #2015_0634.

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Key words

Mesoangioblasts; Embryonic stem cells; IPS; Sarcopenia; Ghrelin; Myogenic differentiation.

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Lipid signalling via phosphoinositides in autosomal dominant leukodystrophy with autonomic disease (ADLD)

Isabella Rusciano[1], Eric Owusu Obeng[1], Sabina Capellari[2], Giovanna Calandra Buonaura[2], Stefano Ratti[1]

[1] Università degli Studi di Bologna, Dipartimento di scienze biomediche e neuromotorie, Bologna, Italia
[2] Università degli Studi di Bologna, Dipartimento di scienze biomediche e neuromotorie; IRCCS, Bologna, Italia

Autosomal dominant leukodystrophy with autonomic disease (ADLD) is an extremely rare and late onset letal progressive neurological disorder. It is characterized genetically by Lamin B1 gene duplication [1] and clinically by autonomic abnormalities and age associated demyelination in the central nervous system (CNS), without any effective treatment up to date. Myelin preserves the integrity of nerve fibers and influences the transmission of impulses in both peripheral nervous system (PNS) and CNS. Interestingly, lipids play active roles in myelination: aberrant expression of lipids are evident in various pathologies such as Alzheimer's and Huntington's disease. For this reason, our study is focused on a specific group of lipids, i.e. Phosphoinositides (PI), and on PI metabolizing enzymes known as Phospholipases (PLCs). PI are highly expressed in the brain, they mediate both cytoplasmic and nuclear signaling associated with brain function [2,3]. In this study, we have investigated a panel of PLCs (mainly PLC-β1a, PLC-β1b and PLC-γ1) in fibroblasts from ADLD patients using Real-Time PCR for evaluation of mRNA expression, Immunocytochemistry and Western blot for protein expression and Flow Cytometry for cell cycle analysis in comparison to control human fibroblasts. Furthermore, we have created an ADLD experimental model using MO3.13 and U87-MG cell lines, with oligodendrocytic and astrocytic origins respectively, to reproduce in vitro the effects of abnormal Lamin B1 expression in the cells that are typically involved in CNS myelination processes. With Lentiviral transduction, we overexpressed Lamin B1 in our cell line models and human fibroblasts to study the PI network at mRNA and protein level. Moreover, Flow Cytometric analysis were performed to study the effects of Lamin B overexpression on the cell cycle of ADLD cell line models. Our preliminary data suggest that PI might play regulatory roles in ADLD disorder.

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Key words

ADLD, Lamin B[1], Phopsphoinositides, Cell signaling.

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Rapamycin promotes trans-differentiation while inhibiting mTOR activity in glioblastoma cells

Larisa Ryskalin[1], Fiona Limanaqi[1], Maria C. Scavuzzo[1], Federica Fulceri[2], Michela Ferrucci[1], Paola Lenzi[1], Marco Gesi[1], Francesco Fornai[1], Francesco Fornai[1,3]

[1] University of Pisa, Human Anatomy, Department of Translational Research and New Technologies in Medicine and Surgery, Pisa, Italia
[2] University of Pisa, Department of Clinical and Experimental Medicine, Pisa, Italia
[3] IRCCS INM Neuromed, via Atinense 18, 86077 Pozzilli, Unit of Neurobiology of Movement Disorders, Isernia, Italia

Glioblastoma multiforme (GBM; grade IV glioma) is the most common and highly malignant primary brain tumor [1]. GBM cells feature mammalian target of rapamycin (mTOR) up-regulation which relates to biological properties of normal stem cells, such as self-renewal, pluripotency and marked proliferation. Thus, they are key in tumor initiation, relapse and resistance to standard treatments [2-4].

Therefore, in the present study we show the effects of different doses of rapamycin on (i) the phenotype of different GBM cell lines; (ii) the number and the ultrastructural morphology of mitochondria. By means of genetic, immunoblotting and morphological analysis at light and electron microscopy, we demonstrate that rapamycin reduces the stem-like phenotype, promotes the neuronal differentiation of GBM cells, and increases the amount of mitochondria by enhancing the mitochondrial fission and mitochondriogenesis. This induced a marked reduction of the stemness marker Nestin, while stimulating gene transcription related to neuronal differentiation, namely the early (beta-III tubulin) and late (NeuN) neuronal markers. No effects were produced for GFAP glial marker. Remarkably, in these experimental conditions, cell phenotype shifts towards a pyramidal neuron-like shape owing long branches.

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Key words

Rapamycin, mTOR, stem cells, neuronal differentiation, transmission electron microscopy.

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Asbestiform zeolite fiber internalization in a human cell model in vitro

Sara Salucci[1], Michele Betti[1], Michele Mattioli[2], Laura Valentini[1], Matteo Giordani[2], Giuseppe Pignataro[1], Debora Burini[1], Pietro Gobbi[1]

[1] Università degli Studi di Urbino Carlo Bo, Dipartimento di Scienze Biomolecolari, Urbino, Italia
[2] Università degli Studi di Urbino Carlo Bo, Dipartimento di Scienze Pure ed Applicate, Urbino, Italia

Exposure to fibrous zeolite erionite, a strong mutagen, more carcinogenic than asbestos fibers in man and rodents, may induce chronic respiratory diseases, including malignant pleural mesothelioma.

Here, erionite effect, at different time points and dosages, has been studied in a human monocyte cell line [1].

Morphological characterization of erionite, using an Environmental Scanning Electron Microscope equipped with an Energy Dispersion Spectroscope, confirmed the expected distribution of the fiber size and the mean diameter because of their potential carcinogenic risk. Erionite is characterized by an extremely fibrous habit with wooly fibers, having a diameter of about 1 μm and variables lengths, with fibrils of about 0.1 μm diameter. Their small size could favor the deep penetration in the biological system. The found Si/(Si+Al) ratio is in the range 0,77-0,78 is slightly higher than the interval for the erionite from basaltic cavities.

TEM observations reveal cell ability to internalize fibrous mineral which shows low cytotoxicity at the lowest times and concentrations. After 36/48h of treatment at the highest dosages, erionite can be found both in the cytoplasm and in the nucleus, where it appears as curvilinear fragments, located mostly in vacuoles. In these experimental conditions, a diffuse number of long microvillous processes with frequent branching or plasma membrane protrusions can be observed. Moreover, mitochondria alterations and a thickening of the nuclear envelope, if compared to control cells, can be revealed. Few cells in secondary necrosis or necrotic death appear. In conclusion, erionite shows low cytotoxicity for what concerns cell death induction. However, a significant number of dysfunctional mitochondria can be detected after fiber exposure, suggesting an oxidative stress involvement which will be further investigated.

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Key words

Erionite cell internalization, morphological analyses.

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Tyrosol prevents glucocorticoid-induced skeletal muscle damage

Sara Salucci, Sabrina Burattini, Davide Curzi, Debora Burini, Giovanni Zappia and Elisabetta Falcieri

University of Urbino Carlo Bo, Department of Biomolecular Sciences, Urbino, Italia

Excessive oxidative stress is linked to the pathogenesis of a variety of skeletal muscle disorders [1]. Therefore, natural antioxidants could play a relevant role to counteract skeletal muscle damage. In particular, Tyrosol, a flavonoid present in virgin oil and known for its protective effect against oxidative injury in various cell models [2], could be active in skeletal muscle too, even if, until now, its effective antioxidant actvity, both in vitro and in vivo, has not been extensively studied in this tissue.

Here, Tyrosol action has been investigated, through morpho-functional approaches, in C2C12 myotubes exposed to dexamethasone, a molecule usually used to mimic muscle wasting in vitro [3].

Dexamethasone-treated cells show a diffuse damage and, in particular, a reduced fiber size, if compared to control condition. In fact, if long and confluent myotubes progressively forming a larger fiber can be observed in control samples, those exposed to dexamethasone appear as immature, smaller syncytia. Moreover, differently from control cells, treated-myotubes show mitochondria alterations, characterized by disorganized cristae and loss of mitochondrial membrane potential and mass. Tyrosol administration before glucocorticoid treatment prevents muscle wasting and improves mitochondrial morphology and functions.

Therefore, these preliminary data encourage the use of this natural antioxidant as "mitochondrial nutrient", able to delay mitochondrial dysfunctions and to prevent glucocorticoid-induced muscle atrophy. Further studies are in progress to highlight tyrosol molecular pathways involved in muscle mass preservation.

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Key words

Muscle wasting, mitochondrial damage, natural antioxidant.

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Activation of Erk and catalase restores a redox equilibrium in DPSCs grown onto Hydroxyapatatite/Alginate composite scaffolds for bone tissue engineering

Silvia Sancilio[1], Monica Rapino[2], Valentina Di Valerio[1], Viviana di Giacomo[3], Susi Zara[3], Marialucia Gallorini[3], Guya Diletta Marconi[3] and Amelia Cataldi[3]

[1] Università G. d'Annunzio, Dipartimento di Medicina e Scienze dell'Invecchiamento, Chieti-Pescara, Italia
[2] Istituto di Geneitca Molecolare, CNR, Unità di Chieti, Università G. d'Annunzio, Chieti-Pescara, Italia
[3] Università G. d'Annunzio, Dipartimento di Farmacia, Chieti-Pescara, Italia

Tissue engineering has been widely recognized as a promising strategy for bone repair and reconstruction and scaffolds consisting in biodegradable polymers are very promising constructs. Our group has previously demonstrated that hydroxyapatite/alginate (HAp/Alg)-based composites scaffolds efficiently support biomineralized matrix deposition and osteogenic differentiation of human dental pulp mesenchymal stem cells (DPSCs) [1]. Cells on HAp/Alg scaffolds express proteins related to osteogenesis like the non-collagenous bone sialoprotein II (BSPII) mainly after 7 and 14 days of culture. Most important, the increased matrix deposition is related to redox homeostasis controlled by the activation of catalase which enhances cell survival as an enzymatic antioxidant. Since the redox equilibrium is crucial for cell survival and osteogenic differentiation of DPSCs [2], we afterwards investigated a plausible molecular pathway underlying cell response to oxidative stress during cell commitment to osteogenesis. Activation of mitogen-activated protein kinase/extracellular signal regulated kinase (Erk) pathway is known to be an hallmark for cell proliferation and survival and it has been found activated by reactive oxygen species during inflammation [3]. In our HAp/Alg scaffold/DPSCs experimental model, pErk increases in a time-dependent manner, registering a peak after 14 days of culture. In parallel, the expression of the inducible Cox (Cox2) dramatically raises up after 7 days, whereas it starts to be downregulated on day 14. Evidences shown here confirm catalase increased activity in DPSCs cultured onto HAp/Alg scaffolds, being the expression of Cox2 significantly decreased in parallel with the boost of the antioxidant activity of the enzyme. Furthermore it is plausible to assume that cells escape inflammation activating Erk, thus balancing redox homeostasis.

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Key words

Oxidative stress, catalase, Erk, hydroxyapatite/alginate scaffolds, Cox2, BSPII.

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In vitro effects of curcuma longa on human keratinocytes derived from ophthalmic pterygium

Silvia Sancilio[1], Gianmarco Stati[1], Mariangela Basile[1], Lucia Centurione[1], Maria Antonietta Centurione[2] and Roberta Di Pietro[1]

[1] "G. d'Annunzio" University", Department of Medecine and Aging Sciences, Chieti-Pescara, Italia (1)
[2] "G. d'Annunzio" University, Genetic Molecular Institute of CNR, Unit of Chieti, Chieti-Pescara, Italia (2)

Pterygium is an ophthalmic pathology characterised by fibro-vascular overgrowth arising from sub-conjunctiva tissue that migrates toward the cornea. Ocular pterygium is usually a bilateral pathology of bulbar conjunctiva, generally located at the nasal side and, occasionally, at the temporal side of conjunctiva [1]. Despite its benign nature, due to its progressive growth the pterygium can invade cornea, reduce visual function leading eventually, to blindness [2]. Currently, the only treatment available is surgical. It has been demonstrated by our research group that low doses of Curcuma longa are capable of inhibiting proliferation of keratinocytes migrated from explants of human pterygium cultured in vitro. To evaluate the in vitro effects of Curcuma longa on human pterygium-derived keratinocytes, pterygium explants were placed in a 6-well plate in complete medium and migrated keratinocytes were treated with an alcoholic extract of 1.3% Curcuma longa in 0.001% Benzalkonium Chloride for 3, 6, and 24 h. Cultured cells were examined for CAM5.2 (anti-cytokeratin antibody) and CD140 (anti-fibroblast transmembrane glycoprotein antibody) expression between 3th and 16th passage to assess cell homogeneity. TUNEL technique and Annexin-V/PI staining in flow cytometry were used to detect keratinocyte apoptosis. We showed that Curcuma longa exerts a proapoptotic effect on pterygium-derived keratinocytes already after 3 h treatment. Moreover, after 24 h treatment, Curcuma longa induces a significant increase in TUNEL as well as Annexin-V/PI positive cells in comparison to untreated samples. Our study confirms previous observations highlighting the expression, in pterygium keratinocytes, of nuclear VEGF and providing the evidence for the first time to the expression of nuclear and cytoplasmic VEGF-R1. These findings suggest that Curcuma longa could have some therapeutic potential in the treatment and prevention of human pterygium.

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Key words

Human pterygium-derived keratinocytes, Curcuma longa, VEGF, VEGF-R1, apoptosis.

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Involvement of BDNF and trkB in the limbic system of Roman High and Low Avoidance rats that show different copulatory patterns

Fabrizio Sanna[1], Maria Pina Serra[2], Laura Poddighe[2], Marianna Boi[2], Francesco Sanna[3], Jessica Bratzu[1], Maria Giuseppa Corda[3], Osvaldo Giorgi[3], Maria Rosaria Melis[1], Antonio Argiolas[1], Marina Quartu[2]

[1] Università di Cagliari, Dipartimento di Scienze Biomediche, sezione di Neuroscienze e Farmacologia Clinica, Monserrato (CA), Italia
[2] Università di Cagliari, Dipartimento di Scienze Biomediche, sezione di Citomorfologia, Monserrato (CA), Italia
[3] Università di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente, sezione di Scienze Farmaceutiche, Farmacologiche e Nutraceutiche, Cagliari, Italia

Roman High- (RHA) and Low-Avoidance (RLA) outbred male rats differ for a respectively rapid vs. poor acquisition of the active avoidance response in the shuttle-box. When put in the presence of a sexually receptive female rat, Roman rats display major differences in sexual activity that concur with the distinctive behavioural traits of the two lines [1]. Thus, sexual motivation and copulatory performance, usually higher in RHA vs. RLA rats, are clear in naïve rats (which copulate for the first time), persist when sexual experience has been acquired, and involve activation of limbic brain areas [Sanna et al., 2014]. Mood disorders show reduced neuronal plasticity whose neurochemical and anatomical ground may reside in the impaired brain-derived neurotrophic factor (BDNF)-trkB signalling as shown in the hippocampus of Roman rats [2]. To clarify the possible role of BDNF in mesolimbic neuronal plasticity, here we report on the immunochemical presence of BDNF and trkB in ventral tegmental area (VTA), nucleus accumbens (Acb) and medial prefrontal cortex (mPFC) of control (no sexual behaviour), sexually naïve and experienced (exp) RHA and RLA rats. As a general rule, BDNF and trkB relative expression levels changed differentially, often conversely, in the VTA, Acb and mPFC of naïve and exp vs. control Roman rats. Thus, for example, after the first copulation BDNF increased in the Acb core and shell in RHA rats displaying an opposite trend in RLA rats, while in sexually exp rats increased only in the VTA of RHA rats. TrkB changes were similar to those of BDNF in the Acb shell, while were opposite in the VTA and mPFC. Our findings highlight a role for the BDNF-trkB trophic system in modulating the activation of neuronal circuits of motivation and reward related to sexual activity in the Roman rat lines.

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Key words

RHA and RLA rats, depression, limbic system, sexual behavior, sexual experience, BDNF, trkB, Western Blot, immunohistochemistry.

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The SOD1, SOD2 and GSTO2 are active and expressed in human sperm: their involvement in the physiopathology of varicocele-associated male infertility

Marta Santoreo[1], Vittoria Rago[1], Rocco Malivindi[1], Daniela De Rose[1], Rosario D'Agata[2], Giuseppina Peluso[3], Giampiero Russo[4], Francesca De Amicis[1], Saveria Aquila[1]

[1] Department of Pharmacy and Sciences of Health and Nutrition, University of Calabria, Cosenza, Italia
[2] Department of Clinical and Experimental Medicine, University of Catania, Catania, Italia
[3] Unit of Physiopathology of Reproduction, Annunziata Hospital, Cosenza, Italia
[4] Family Counseling Center (ASP Cosenza), Health Center-University of Calabria, Cosenza, Italia

Testicular varicocele is strictly associated to male infertility. Nevertheless, the mechanism/s by which varicocele affects fertility remain undetermined. Recently, we showed that varicocele damages male gamete at molecular level, opening a new chapter in the already multifactorial physiopathology of varicocele.

Considerable evidence showed that the retinoic acid receptor α (RARα) and its all-trans retinoic acid (ATRA) ligand, the active form of vitamin A, play key roles in sperm maturation. Previously, we showed in varicocele sperm a reduced RARα expression and that ATRA influence sperm performance. To further define vitamin A significance in human sperm and in the varicocele physiopathology, we tested for the first time ATRA action on the antioxidant defense systems. Poor sperm quality compromises the fertilization process and one of the most important cause is the oxidative stress elicited by excessive ROS generation from sperm and/or by the disruption of the antioxidant defense systems in the male reproductive tract. Recently, many clinical trials have been performed to examine potential therapies for oxidant stress-induced infertility.

Herein, ATRA induced the superoxide dismutase and glutathione transferase activities, while it reduced the malondialdehyde and ROS production both in healthy and varicocele sperm. Interestingly, for the first time we showed that SOD1 and SOD2 have been localized in the acrosome and midpiece, GSTO2 in the acrosome, equatorial and subacrosomial regions. By Western Blotting analyses SOD1, SOD2 and GSTO2 expression were significantly lower in varicocele with respect to healthy sperm. Further, we showed that basal ROS production is elevated in varicocele with respect healthy sperm, and it decreased after ATRA treatment.

Sperm plasma membranes are particularly susceptible to oxidative stress owing to their high levels of polyunsaturated fatty acids that readily undergo lipid peroxidation, affecting the fluidity of the sperm plasma membrane and thus causing functional defects during capacitation, acrosome reaction and sperm-oocyte fusion.

In conclusion, our study describes a novel retinoids action as modulators of antioxidant defense systems in human sperm. Nonetheless, despite the open question of clinical efficacy for these anti-oxidant therapies, their low cost and toxicity, could offer a great advantage for both patients and clinicians, opening the possibility to consider this agent as potential therapeutic tool to ameliorate sperm performance also in pathological samples.

These novel findings further confirm the importance of vitamin A in male fertility and add new insights into the retinoids complex biological framework. Collectively, ATRA administration in procedures for artificial insemination or dietary vitamin A supplementation might represent a promising therapeutic approach for the management of male infertility.

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Effects of physical exercise on metabolic syndrome-associated hypothalamic and testis alterations in the rabbit

Erica Sarchielli[1], Giulia Guarnieri[1], Paolo Comeglio[2], Sandra Filippi[2], Gabriella Barbara Vannelli[1] and Annamaria Morelli[1]

[1] University of Florence, Section of Anatomy and Histology, Department of Experimental and Clinical Medicine, Florence, Italia
[2] University of Florence, Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Florence, Italia

Metabolic Syndrome (MetS) is a cluster of clinical conditions, associated to an increased cardiovascular (CV) and metabolic risk along with hypogonadism (HG). Lifestyle modifications (including physical exercise, PhyEx) are well-known treatments for this condition [1]. We previously established a rabbit model of MetS that recapitulates the human phenotype, including HG [2]. We now report studies on the effects of PhyEx on hypothalamus-pituitary-testis (HPT) axis. MetS was induced in adult male rabbits fed a high-fat diet (HFD). Rabbits fed a regular diet were used as controls (RD). RD and HFD rabbits were exercise-trained to run on a treadmill for 12 weeks (RD + PhyEx and HFD+ PhyEx). HFD rabbits showed typical metabolic and CV features of MetS along with hypogonadotropic HG (reduced testosterone and LH plasma levels). Within the hypothalamus (preoptic region) a significant reduction of GnRH- and KISS1R-positive neurons, along with the increase of genes related to inflammation (COX2, IL6, CD68), glucose metabolism (GLUT1, GLUT4, IRS-1) and estrogen action (ERb, GPR30) was detected in HFD rabbit, as compared to RD group. Immunohistochemistry analysis confirmed the HFD-induced hypothalamic inflammation. Interestingly, genes encoding for inhibitory factors for GnRH, such as NPY, were also increased in HFD hypothalamus. Within the testis, HFD down-regulated LH receptor and all the steroidogenic enzymes leading to T synthesis. PhyEx completely restored T and LH plasma levels and GnRH/KISS1R immunostaining. All the aforementioned HDF-induced increase of inflammatory markers were significantly reduced in HFD+ PhyEx, with the exception of IL6. Moreover, at hypothalamic level, PhyEx decreased orexigenic and GnRH-inhibiting factors (dinorphin and its receptors OPRD1 and OPRK1), whereas increased anorexigenic ones (POMC). Within the testis, genes related to T formation (17βHSD3) and metabolism (5α-reductase 1) were increased by PhyEx. In conclusion, in this experimental model, endurance training (PhyEx) completely reverted MetS-induced hypogonadotropic hypogonadism, exerting beneficial effects on the HPT axis. Particularly, in the hypothalamus PhysEX reduced HFD-induced inflammation. Hence, aerobic exercise training can be regarded an interesting strategy to combat MetS-associated alterations of the HPT axis.

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Key words

GnRH neurons, hypogonadism, inflammation, hypothalamus.

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Evidence supporting the preventive effect of Platelet-rich plasma (PRP) on TGFβ1-induced fibroblast/myofibroblast transition via the involvement of VEGF receptor-1 (FLT-1) signaling

Chiara Sassoli[1], Flaminia Chellini[1], Alessia Tani[1], Larissa Vallone[1], Paola Pavan[2], Franco Bambi[2], Sandra Zecchi-Orlandini[1]

[1] Università di Firenze, Dipartimento di Medicina Sperimentale e Clinica, Firenze, Italia
[2] Ospedale Meyer, Medicina Trasfusionale, Firenze, Italia

Platelet-rich plasma (PRP), defined as a plasma fraction with platelet concentration higher than the baseline concentration in whole blood, represents a cost-effective reservoir of numerous platelet-derived biologically active molecules including growth factors and cytokines, holding a strong potential for improving tissue healing and regeneration. Many studies have demonstrated that the contribution of PRP to the morpho-functional recovery of different damaged tissues/organs depend on its ability to modulate inflammatory responses, promote re-vascularization and stimulate the endogenous mechanisms of tissue repair/regeneration by influencing the cell fate of local stem cells progenitors [1-4]. The positive role of PRP in reducing fibrosis in different damaged and/or diseased organs has also been observed. However, the antifibrotic potential of PRP is still controversial [5,6]. Moreover the bioactive factors contained in PRP, as well as their cellular targets and molecular mechanisms of action need to be clearly identified. On the basis of these considerations, the aim of the present study was to examine the effect of PRP on the in vitro transition of fibroblastic NIH/3T3 cells into myofibroblasts, considered as the key cell effectors of tissue scarring and to investigate the underlying molecular mechanisms. Our results showed that PRP inhibits fibroblast/myo-fibroblast transition promoted by the pro-fibrotic agent TGF–β1, as judged by reduction of stress fibres formation, vinculin rich focal adhesion clustering, α-smooth muscle actin (sma) and type-1 collagen expression. Interestingly we found that VEGF receptor-1 (VEGFR-1/Flt-1) pathway was implicated in PRP-mediated inhibition of fibroblast/myofibroblast transition based on: i) VEGFR-1 expression was reduced by the administration of TGF-β1 as compared with the control cells and PRP was able to prevent this TGF-β1 induced reduction; ii) the selective pharmacological VEGFR-1 inhibitor, KRN633 prevented the effect of down regulation of α-sma expression promoted by PRP, iii) the addition to the differentiation medium of soluble VEGF caused a marked decrease of α-sma expression in TGF-β1-treated fibroblasts , iv) the expression of Smad3, the TGF-β1 downstream signaling molecule, appeared downregulated in fibroblasts cultured in the presence of TGF-β1 +PRP or TGF-β1+ soluble VEGF. Conversely, the addition of KRN633 to TGF-β1-stimulated cells in the presence of PRP determined an increase of Smad3 expression levels. Altogether these findings demonstrated that PRP counteracted the fibroblast/myofibroblast transition by interfering with the TGF-β1-mediated intracellular signaling possibly via VEGFR-1 mediated activation.

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Key words

Fibroblasts, myofibroblasts, fibrosis, regenerative medicine, TGF-β1/smad3 signaling, Platelet-Rich Plasma, VEGF receptor-1.

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Nutritional strategies to counteract the loss of muscle mass and function characteristic of senescent muscle

Bianca Maria Scicchitano, Silvia Sorrentino, Alessandra Barbiera and Gigliola Sica

Istituto di Istologia, Università Cattolica del Sacro Cuore, Roma, Italia

During aging, multifactorial events such as activation of inflammatory pathways and mitochondrial dysfunction lead to the onset of sarcopenia, which is characterized by a gradual loss of muscle protein component. It is well known that changes in the quantity and the quality of dietary proteins, as well as the intake of specific amino acids or antioxidants supplementation, counteract some physiopathological processes related to the progression of the loss of muscle mass and may have beneficial effects in improving the anabolic response of muscle in the elderly.

Taurine is a non-essential amino acid expressed in high concentration in several mammalian tissues and particularly in skeletal muscle where it is involved in the modulation of intracellular calcium concentration and ion channel regulation and where it acts as an antioxidant and anti-inflammatory factor.

Here, we evaluated whether the intraperitoneal administration of taurine in aged mice counteracts the catabolic process related to sarcopenia. We showed that, in injured muscle, taurine enhances the regenerative process as demonstrated by the presence of central nucleated fibers, less amount of inflammatory cells and fibrosis, if compared to the control. Moreover, taurine stimulates the PI3K/Akt signaling leading to an inhibition of FOXO transcription factors thus promoting protein synthesis. These results suggest a role of taurine as a promising nutritional agent to counteract the development and progression of sarcopenia.

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Key words

Sarcopenia, taurine, nutrition, aminoacids.

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Tunneling nanotubes as mediators of Neuron-Mesenchymal Stem Cell interaction

Arianna Scuteri[1], Marianna Monfrini[1], Mario Bossi[2], Valentina Carozzi[1], Mariarosaria Miloso[1], Gabriella Nicolini[1], Guido Cavaletti[1], Paola Marmiroli[1]

[1] Università Milano-Bicocca, School of Medicine and Surgery, Monza, Italia
[2] University Milano Bicocca, School of Medicine and Surgery, Monza, Italia

During the last two decades Mesenchymal Stem Cells (MSCs) have been proposed for the treatment of several neurological diseases, such as Alzheimer's disease or Parkinson's Disease [1], initially with the aim to replace the damaged neuronal cells, and later to cure, rather than to replace the neuronal cells. In particular, previous studies demonstrated that MSCs directly co-cultured with sensory neurons were able to strongly increase the neuronal survival, and to protect them from different toxic stimuli [2; 3], thus theoretically being useful to really change the course of all the diseases affecting sensory neurons. Anyway, it is mandatory to understand the mechanisms involved in such an interaction. Aim of this work is to investigate the different interaction manners, and the identification of the molecules used by MSCs and neurons to communicate.

In particular, by Immunofluorescence and Electron microscopy analysis, we observed the formation of gap junctions and tunneling nanotubes, cellular structures potentially allowing the flow of cellular stuff (4). In addition, with the diffusible fluorescent dye Calcein, we demonstrated the flux direction from MSCs to neurons. We then analyzed the nature of the exchanged materials, and we observed an involvement of exosome and more in general vesicular structures, and even subcellular components as mitochondria. All these molecules and structures may be used by MSCs to cure neurons. As a proof of concept, we will expose neurons to the putative protective MSC-derived molecules, to determine if they are sufficient to achieve a positive effect.

On the basis of the identified interactions and the pivotal molecules exchanged, it will be possible to enhance the MSC protective effect on neurons by exploiting the identified key molecules.

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Key words

Tunneling nanotubes, gap junctions, mesenchymal stem cells, sensory neurons, mitochondria, neuroprotection.

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Differential expression of the immediate early genes c-Fos, DFosB and Arc in the limbic system of the Roman High and Low Avoidance rat lines during the acquisition of sexual experience

Maria Pina Serra[1], Fabrizio Sanna[2], Laura Poddighe[3], Jessica Bratzu[2], Marianna Boi[3], Maria Antonietta Piludu[4], Maria Giuseppa Corda[4], Osvaldo Giorgi[4], Maria Rosaria Melis[2], Antonio Argiolas[2], Marina Quartu[3]

[1] Università degli Studi di Cagliari, Dipartimento di Scienze Biomediche, Sezione di Citomorfologia, Monserrato, Italia
[2] Università degli Studi di Cagliari, Dipartimento di Scienze Biomediche, Sezione di Neuroscienze e Farmacologia Clinica, Monserrato, Italia
[3] Università degli Studi di Cagliari, Dipartimento di Scienze Biomediche, Sezione di Citomorfologia, Monserrato, Italia
[4] Università degli Studi di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente, Sezione di Scienze Farmaceutiche, Farmacologiche e Nutraceutiche, Cagliari, Italia

Male Roman High (RHA) and Low Avoidance (RLA) rats display major differences in sexual behaviour, since RHA rats exhibit higher motivation and better copulatory performance than RLA rats [1]. Such differences are very evident in sexually naïve rats (which copulate with a receptive female rat for the first time), and persist, after five copulatory tests, when sexual experience has been acquired [2]. Since sexual activity is a natural reward that involves activation of limbic brain areas, we studied whether the differences in sexual activity between the two rat lines are accompanied by changes in the expression of the immediate early genes (IEG) cFos, ΔFosB and Activity regulated cytoskeleton-associated (Arc) protein as indicators of neural activation and synaptic plasticity. By means of Western blot and/or immunohistochemistry, we investigated their expression in ventral tegmental area (VTA), nucleus accumbens (Acb) and medial prefrontal cortex (mPFC) of control (no sexual behaviour), sexually naïve and experienced (exp) RHA and RLA rats. Expression levels of selected IEG in the Roman lines have been compared with concomitant changes in sexual motivation and copulatory performance in relation to the level of sexual experience. The results show that cFos, ΔFosB and Arc increased differentially in the VTA, Acb (core and shell) and mPFC of RHA and RLA rats. In both rat lines, the increases were very evident in naïve rats, tended to disappear in exp rats, with the exception of ΔFosB which tended to accumulate with sexual experience, and were usually higher in RHA than RLA rats. These findings confirm that sexual activity induces neural activation in limbic brain areas involved in motivation and reward, thereby leading to changes in the mechanisms controlling neural plasticity with the acquisition of sexual experience and imply that changes in these mechanisms may also depend on specific biobehavioural traits.

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Key words

RHA and RLA rats, sexual experience, limbic system, cFos, ΔFosB, Arc, Western Blot, immunohistochemistry.

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Interaction between Sphingosine Kinase/Sphingosine 1 Phosphate and Transforming Growth Factor-β/Smads pathways in experimental intestinal fibrosis: an in vivo immunohistochemical study

Roberta Sferra[1], Simona Pompili[1], Giovanni Latella[2], Giulia Sabetta[1], Amarildo Smakaj[1], Gloria Stati[1] and Antonella Vetuschi[1]

[1] Università degli Studi, Dipartimento di Scienze Cliniche Applicate e Biotecnologiche, L'Aquila, Italia
[2] Università degli Studi, Dipartimento Medicina Clinica, Sanità Pubblica, Scienze della Vita e dell'Ambiente, L'Aquila, Italia

Intestinal fibrosis is characterized by an abnormal deposition of Extracellular Matrix (ECM) produced by activated myofibroblats. Despite many biological mediators are implicated in ECM proteins accumulation, a pivotal role is certainly played by TGF-β that acts mainly through Smad proteins (1). Recently, it has been thought that different molecules could be involved in TGFβ-dependent fibrotic signaling (2) and for this reason, aim of this study was to evaluate the involvement of Sphingosine kinase/Sphingosine 1 phosphate in an experimental mice model of intestinal fibrosis induced by oral administration of DSS. 20 mice were divided into 2 groups: control (H2O) n=5 and DSS n=15. Histological and immunohistochemical evaluation using TGF-β, p-Smad3, Collagen I-III, α-SMA, SPHK1, RhoA, PI3K, Akt, p-Akt and p-mTOR were performed. In DSS mice histological analysis assessed in H&E and Masson's Trichrome showed marked signs of inflammation and fibrosis. Immunopositivity for canonical TGF-β/Smads pathway molecules TGF-β, p-Smad3, Collagen I-III and α-SMA resulted mild expressed in control mice, while there was a significant increase in DSS group. Immunohistochemical analysis for non-Smad TGF-β pathway proteins SPHK1, RhoA, PI3K, Akt, p-Akt and p-mTOR showed a high positivity in DSS mice compared to untreated group. These preliminary results demonstrated the hypothesis that the development of intestinal fibrosis could be influenced not only by TGFβ/Smad pathway but also by a crosstalk between TGFβ/SPHK1/S1P signaling that could represent a new crucial driver in colonic fibrosis. Development of molecules able to control the synthesis of S1P, through the regulation of its kinase SPHK1, could provide a novel attractive therapeutic target to control fibrogenic process.

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Key words

Fibrosis, TGF-β, S1P.

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Stair climbing performance in assessing donor-site morbidity following osteocutaneous free fibula transfer: a preliminary study

Chiarella Sforza[1], Giada Anna Beltramini[2], Alessandro Remigio Bolzoni[3], Giada Ballabio[4], Tiziana Miraglia[4], Giacomo Tassone[4], Ilaria Annese[5], Michela Bisighini[6], Anna Cazzaniga[6], Giuditta Dalai[6], Ariana del Carmen Mousa Urbina[6], Manuela Galli[6] and Matteo Zago[6]

[1] Università degli Studi di Milano, Dip Scienze Biomediche per la Salute, Milano, Italia
[2] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Maxillofacial and Dental Unit, Milano, Italia
[3] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Maxillofacial and Dental Unit, Milano, Italia
[4] Università degli Studi di Milano, Dip Scienze Biomediche per la Salute, Milano, Italia
[5] Politecnico di Milano, Department of Electronics, Information and Bioengineering (DEIB), Milano, Italia
[6] Politenico di Milano, Department of Electronics, Information and Bioengineering (DEIB), Milano, Italia (6)

Autologous free fibula flap (FFF) is among the most used techniques to reconstruct facial bone defects [1]. Donor site morbidity has been quantified mostly with over ground gait analysis, and no longitudinal investigations of stairs climbing have been performed. Only a pilot cross-sectional investigation reported minimal disturbances [2]. We longitudinally assessed the gait kinematic parameters during stairs ascent and descent after a vascularized FFF removal for facial reconstruction in 15patients (7 men, 8 women; mean age 49±16 y; height 167.5±8.8 cm; body mass 69.7±19.2 kg): a first evaluation was made before surgery and a second 6-months after surgery. In both assessments the patients ascended and descended a three-step wooden staircase (rise height 16 cm, tread length 30 cm) at self-selected, comfortable speed. Kinematic variables were measured through optical gait analysis. The patients approached the stair from farther away, and stair negotiation was done with both the healthy and the operated side. Spatiotemporal parameters and the Range of Motion (ROM) of lower limb joints were obtained. In particular, step duration, cadence, stance, swing and double support duration; step width, velocity; ankle (dorsi-plantar flexion; inversion-eversion), knee (flexion-extension), hip (flexion-extension, abd-adduction) and pelvis ROM (inclination, rotation, tilt) were computed separately for the ascent and descent phases, for the operated and healthy limb, and for the pre- and post-surgical assessments. Data were compared by a 2-way ANOVA (sidetime) with repeated measures on the time factor. No significant effects of side or of time were found (all p values > 0.05) for both the ascent and descent phases. In sum, no functional limitations during gait performance were detected: in our patients, FFF harvest was generally associated to successful functional outcomes of the donor site. While previous studies found some differences in the lower limb function [3,4], this is the first longitudinal investigation focused on stairs climbing. Further studies with an increased sample size and a longer follow up are necessary to draw general conclusions.

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Key words

Free fibular flap, morbidity, gait analysis, longitudinal, stairs.

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Differentiation of dendritic cells from different human circulating progenitors and effect of PPAR-gamma stimulation

Angela Silvano[1], Sara Paccosi[2], Maria Ida Bonetti[3], Astrid Parenti[2], Alberto Bosi[1] and Paolo Romagnoli[1]

[1] Università degli Studi di Firenze, Dipartimento di Medicina Sperimentale e Clinica, Firenze
[2] Università degli Studi di Firenze, Dipartimento di Scienze della Salute, Firenze
[3] Azienda Ospedaliero Universitaria Careggi, Centro Diagnostico di Citofluorimetria e Immunoterapia, Firenze

Dendritic cells (DCs) of the immune system include - among others - Langerhans cells ($CD1a^+$, langerin/$CD207^+$) and connective tissue DCs (DC-SIGN/$CD209^+$). These cells can be generated in vitro from different precursors, but the results have been inconsistent and the differentiation of specific subtypes has been hard to achieve. Although DCs express PPAR-gamma, the expression of this receptor by DC precursors and the effect of its stimulation on DC differentiation are poorly known. This study has addressed the differentiation potential into DCs of different precursors collected from peripheral blood of healthy adult human donors and the effect of rosiglitazone, an agonist of PPAR-gamma, on that differentiation. Upon immunomagnetic separation, $CD14^+$ monocytes and $CD34^+$ and $CD133^+$ progenitors were cultured with cytokines for 8 d ($CD14^+$) or 18 d ($CD34^+$ and $CD133^+$); rosiglitazone (1 μmol/l) was added in some experiments. All precursors generated HLA-DR^+(high) DCs; those from $CD34^+$ and $CD133^+$ precursors were in part large and more rich in organelles, in part medium-sized and less rich in organelles. A proportion of cells, varying with the precursors ($CD14^+$<$CD34^+$<$CD133^+$), were $CD1a^+$ and $CD207^+$; in cells derived from $CD34^+$ and $CD133^+$ progenitors they were among the large ones. Many cells from any culture expressed CD209, also together with CD207. Rudimentary Birbeck granules were observed in few DCs from any precursor. Variable percentages of DCs, highest among those from $CD133^+$ precursors, expressed CD80, CD83 and CD86. Rosiglitazone led to significant increase in $CD207^+$ DCs among cells generated from $CD133^+$ precursors; DCs derived from $CD133^+$ precursors stimulated the proliferation of $CD4^+$ lymphocytes much more than that of $CD8^+$ ones and such proliferation was significantly reduced if DCs had been generated in the presence of rosiglitazone. Freshly isolated CD133+ cells showed a number of copies of mRNA for PPAR-γ higher than $CD14^+$ and $CD34^+$ cells. The results indicate that: the differentiation potential of hematopoietic cells into DCs with different phenotypes depends on the step reached by the precursors in vivo; the orientation towards Langerhans cells can begin very early; the differentiation in vitro does not mimic entirely that in vivo; the most immature progenitors, $CD133^+$ cells, express PPAR-gamma; stimulation of these receptors may play a role in modulating DC differentiation.

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Targeting the phosphatidylinositol 3-kinase/Akt/mechanistic target of rapamycin (PI3K/Akt/mTOR) signaling pathway in B-lineage acute lymphoblastic leukemia

Carolina Simioni[1], Simona Ultimo[1], Alberto Maria Martelli[2], Giorgio Zauli[1], Silvano Capitani[1] and Luca Maria Neri[1]

[1] University of Ferrara, Department of Morphology, Surgery and Experimental Medicine, Ferrara, Italia
[2] University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italia

Constitutive activation of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) network is a common feature of Acute Lymphoblastic Leukemia (ALL), and is frequently observed in the B-ALL subtype, where it plays important roles in the pathophysiology, maintenance and progression of the disease. Aberrant activation of this signaling cascade portends a poorer prognosis in both pediatric and adult B-ALL patients. Promising preclinical data on PI3K/Akt/mTOR inhibitors have documented their anticancer activity and some of these novel drugs entered clinical trials as they could lead to a longer event-free survival, reduce therapy-associated toxicity and provide an important preclinical rationale for the use in combination with BCR-ABL Tyrosine Kinase Inhibitors (TKIs) in Philadelphia positive (Ph+) B-ALL, evaluated by cell viability reduction as well as apoptosis and autophagy induction. The importance of new personalized and targeted therapeutic protocols against the PI3K/Akt/mTOR signaling pathway may impact on microRNA (miRNAs) modulation. miRNAs are involved in the lymphopoietic process, in the control of gene expression of several transcription factors essential for the commitment, differentiation, and apoptosis of hematopoietic stem cells and are frequently localized in common breakpoint regions related to tumors or in fragile sites. Preliminary data showed that treatment of B-ALL cells with PI3K/mTOR Small Molecule Inhibitors (SMI) significantly down-regulated the expression of some onco-miRNAs (miR-150, miR-210 and miR-221) described frequently altered in B-ALL. miRNAs could therefore be considered as promising molecular biomarkers of cancer with prognostic implications and as predictive biomarkers of treatment response, allowing the development of new clinical and personalized protocols.

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Key words

BCR-ABL1, PI3K/Akt/mTOR signaling, B-Acute Lymphoblastic Leukemia, targeted therapies, miRNAs.

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Role of anti-PD-1 antibody-Fc/FcR interaction on macrophages in inducing hyperprogressive disease in non-small cell lung cancer

Michele Sommariva[1], Giuseppe Lo Russo[2], Massimo Moro[3], Valeria Cancila[4], Mattia Boeri[3], Monica Ganzinelli[2], Claudia Proto[2], Diego Signorelli[2], Sabina Sangaletti[3], Chiara Storti[1], Lucia Sfondrini[1], Alberto Bardelli[5], Silvia Marsoni[6], Andrea Anichini[3], Licia Rivoltini[3], Claudio Tripodo[4], Mario Paolo Colombo[3], Andrea Balsari[1], Gabriella Sozzi[3], Marina Chiara Garassino[2]

[1] Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute, Milano, Italia
[2] Fondazione IRCCS – Istituto Nazionale dei Tumori, Dipartimento di Oncologia Medica I, Milano, Italia
[3] Fondazione IRCCS – Istituto Nazionale dei Tumori, Dipartimento di Ricerca, Milano, Italia
[4] Università degli Studi di Paermo, Scienze per la Promozione della Salute e Materno Infantile "G.D'Alessandro", Palermo, Italia
[5] Università degli Studi di Torino, Dipartimento di Oncologia, Torino, Italia
[6] IFOM - The FIRC Institute of Molecular Oncology, Precision Oncology, Milano, Italia

Immune checkpoint inhibitors (ICI) targeting PD-1/PD-L1 axis have made a breakthrough in the treatment of non-small cell lung cancer (NSCLC) [1]. However, a paradoxical boost in tumor growth was reported in a fraction of NSCLC patients after ICI administration; a novel pattern of cancer progression defined "hyperprogression" (HP) [2]. Because the mechanism of HP onset is still unknown, aim of this study was to investigate this phenomenon. Among a cohort of NSCLC patients treated with ICI at Istituto Nazionale dei Tumori in Milan, cases with HP were identified according to clinical and radiological criteria. Among patients evaluable for clinical response (152/187), we identified 4 categories: Responders (21%), Stable Disease (27.7%), Progressors (25.7%) and HP (25.7%). Pre-treatment histological samples were evaluated by immunohistochemistry (IHC) for immune cell infiltrate. Tissue samples from all patients with HP showed tumor-infiltration by M2-like CD163+CD33+PD-L1+ clustered epithelioid macrophages. To validate these findings in preclinical models, we utilized immunocompromised mice that, lacking T-cells that may cloud the results, represent a suitable model to evaluate the role of macrophages in determining HP. Immunodeficient mice were injected with human NSCLC cells and patient-derived xenografts (PDXs), treated with anti-PD-1 antibody and tumor growth was assessed. Anti-PD-1 treated NSCLC-bearing mice showed HP-like tumor growth with dissemination to lung and iliac lymph node metastases, as well as an increase in tumor-associated macrophages (TAMs) aggregating in fibrotic-like areas. Interestingly, in these in vivo models, HP-like growth, triggered by anti-PD-1 treatment, was abrogated by using anti-PD-1 F(ab)2-fragments. These results suggest that FcR engagement by ICI on TAMs may determine a functional reprogramming of these immune cells toward a more aggressive and pro-tumorigenic phenotype, eventually inducing HP.

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Key words

Hyperprogression, PD-1, lung cancer, macrophages.

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Long term effects of cigarette smoke extract and nicotine on Nerve Growth Factor and its receptors in bronchial epithelial cell line

Anna Maria Stabile[1], Lorella Marinucci[2], Stefania Balloni[2], Alessandra Pistilli[1], Maria Bodo[3], Mario Rende[1]

[1] Università Di Perugia, Dipartimento Di Scienze Chirurgiche E Biomediche, Perugia, Italia
[2] Università Di Perugia, Dipartimento Di Medicina Sperimentale, Perugia, Italia
[3] Università Di Perugia, Dipertimento Di Medicina Sperimentale, Perugia, Italia

Long-term exposure to cigarette smoke induces severe injuries to respiratory system through several

mechanisms, some of them are well defined, but many others are not yet elucidated. Beside its classical role in nervous system, Nerve Growth Factor (NGF) and its receptors have a crucial role in airway inflammatory diseases [1]. To expand our knowledge about the relevance of NGF and its receptors in airway diseases induced by cigarette smoking, we exposed for 16 weeks the bronchial epithelial cell line BEAS-2B to sub-toxic concentrations of whole cigarette smoke extract or pure nicotine that maintain viable more than 80% of cells [2]. Viability, cell cycle gene expression, cell morphology and migration ability were tested and compared to NGF release and gene expression. Modulation of its receptors TrKA (high-affinity tropomyosin-related kinase A) and p75NTR (low-affinity neurotrophin p75 receptor) was also analyzed. The present study shows that long term exposure of BEAS-2B cells to cigarette smoke extract or nicotine induces: (A) differences: in cell viability, in the expression of cell cycle-related genes, in NGF release and in gene expression of NGF and its receptors; (B) similarities: in morphology and migration ability. Taken together, our data provide new insights about the biological role of NGF and its receptors in airway diseases induced by long-term cigarette smoking and, finally, our data evidence the opportunity not to use nicotine lozenges or e-cigarettes as anti-smoking replacement therapy in patients with a previous airway disease according to the ability of nicotine to increase the amount of the pro-inflammatory cytokine NGF into the bronchial environment.

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Key words

Human bronchial epithelial cells, NGF, p^{75}NTR, TrKA, nicotine, cigarette smoke extract.

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Hormone receptor expression in human fascial tissue and modulation of the extracellular matrix according to the hormone levels

Carla Stecco, Caterina Fede, Lucia Pedrelli, Damiana Incendi and Raffaele De Caro

Università di Padova, Dipartimento di Neuroscienze, Padova, Italia

Many clinical and experimental findings point to sex differences in myofascial pain, demonstrating that adult women tend to have different myofascial problems with respect to men [1]. It is possible that sex hormons can play a role in extracellular matrix and collagen remodeling and thus contribute to functions of myofascial tissue, causing a sensitization of fascial nociceptors. This study was approved by the Institutional Ethics Review Board according to ethical regulations regarding research conducted on human tissues. Immunohistochemical and molecular investigations of relaxin receptor 1 (RXFP1) and estrogen receptor-alpha (ERα) were carried out on samples of human fascia collected from female volunteer patients during orthopedic surgery (age between 42 and 70 yrs, divided into pre- and post-menopausal groups), and in fibroblast cells isolated from deep fascia. Furthermore, an in vitro stimulation was performed with levels of beta-estradiol equal to the follicular phase or to the periovulatory phase, and the matrix was analyzed after Sirius Red staining. RXFP1 and ERα are expressed in all the human fascial districts examined and in fascial fibroblasts culture cells, to a lesser degree in the post-menopausal with respect to the pre-menopausal women. Furthermore, different levels of beta-estradiol modulate the collagen production, that increases when the hormone levels rise up to the periovulatory concentration (~400 pg/mL). Our results demonstrated that the fibroblasts located within different districts of the muscular fasciae express sex hormone receptors and can modulate the extracellular matrix according to the hormone levels, influencing the tissue hydration and the lubrication of sliding surfaces. These results can help to explain the link between hormonal factors and myofascial pain: estrogen and relaxin play a key role in extracellular matrix remodeling by inhibiting fibrosis and inflammatory activities, both important factors affecting fascial stiffness and sensitization of fascial nociceptors [2].

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Isolation and morphological characterization of IFP-derived stem-like cells: investigation on their potential role in osteoarthritis

Elena Stocco[1], Veronica Macchi[1], Elisa Belluzzi[2], Silvia Barbon[1], Marta Favero[3], Paola Tomic[1], Rosa Di Liddo[4], Silvia Schiavon[4], Maria Teresa Conconi[4], Assunta Pozzuoli[2], Andrea Porzionato[1], Pietro Ruggieri[2], Raffaele De Caro[1]

[1] Sezione di Anatomia Umana, Dipartimento di Neuroscienze, Università degli Studi di Padova, Padova, Italia
[2] Laboratorio di Patologia e Oncologia muscoloscheletriche, Dipartimento di Ortopedia e Oncologia ortopedica, Università degli Studi di Padova, Padova, Italia
[3] Unità di Reumatologia, Dipartimento di Medicina, Università degli Studi di Padova, Padova, Italia
[4] Dipartimento di Scienze del Farmaco, Università degli Studi di Padova, Padova, Italia

The extrasynovial adipose tissue Infrapatellar Fat Pad (IFP) is an emerging player in knee osteoarthritis (OA) [1,2]. While the role of constitutive adipocytes in secreting cytokines is known, little awareness on origin/function of the stem cells component exists. This study aims to isolate/characterize IFP-derived stem cells (IFP-dSCs) from OA patients investigating their role in disease development. IFP samples were processed to discard matrix and the adipocyte fraction. The resulting pellet was resuspended in proliferative medium and cultured routinely. IFP-dSCs morphology and ultrastructure were observed by optical microscope and Transmission Electron Microscope (TEM); expansion potential of cells was assessed by a population doubling level assay. IFP-dSCs vitality was assessed using a Apoptotic/Necrotic/Healthy Cells Detection Kit, while the metabolic activity of cell cultures was analysed by MTT assay. Flow cytometry analysis was performed to identify the presence of specific markers; in particular, IFP-dSCs were stained with antibodies against CD73/105/90/44/34/106, IL-6R/1R, VEGFR2. At last, IFP-dSCs plasticity was also assessed, evaluating their commitment towards the adipogenic, chondrogenic and endothelial lineages. IFP-dSCs isolation required 14 h; cells were fibroblast-like and typically spindle shaped at low density, showing a greater polygonal nucleus at high density. Semithin-sections stained with toluidine blue revealed vesicles in the cytoplasm, as confirmed by TEM analysis. These rounded formations of electron-dense material were in proximity of empty round vesicles and in some contact areas a partial fusion between the external membranes was appreciable. An exponential growth during the entire long-term expansion period was observed, with a replication time of 42.7 $\pm$ 3.8 h. From passage (P)8 to P20, cells performed 11.9 $\pm$ 0.9 population doublings. IFP-dSCs immunophenotype was positive for the investigated markers, suggesting a role in modulation of inflammation; interestingly, cells were 100% CD73+bright both at low and high P in colture. Preliminary data about plasticity revealed the ability in differentiating towards adipogenic and endothelial lineages. Further analysis will be required to assess chondrogenic commitment. Experimental evidence on IFP-dSCs seem to correlate histopathological features of IFP in OA (i.e. thickening of interlobular septa, increase in vascularization and innervation) with the stem cell component.

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Key words

Osteoarthritis, infrapatellar fat pad, adipose tissue, stem cells, immunomodulation, IFP histopathological features.

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Exercise and nutrition protective effects on cartilage disorders, muscle wasting and liver diseases

Marta Anna Szychlinska, Michelino Di Rosa, Francesca Borzì, Rosa Imbesi, Rita Reitano, Silvia Ravalli, Francesca Maria Trovato, Paola Castrogiovanni and Giuseppe Musumeci

SIAI, Università di Catania, Dip di Scienze Biomediche e Biotecnologiche, Catania

The beneficial effects of Extra Virgin Olive Oil (EVOO), the main fat source in Mediterranean diet, are widely studied thanks to its anti-inflammatory and antioxidant properties. Lubricin is a chondroprotective glycoprotein, with lubricant properties. A joint injury causes an increased cytokine expression, associated with decreased lubricin synthesis and predisposition to cartilage degeneration [1]. The aim of the study was to evaluate the beneficial role of EVOO-enriched diet and physical activity, on osteoarthritic cartilage of rats. These effects were assessed through lubricin and IL-6 expression in rat joint tissues. Osteoarthritis was induced mechanically by anterior cruciate ligament transection (ACLT). The 48 animals were divided into 6 groups: 1–control; 2–ACLT and common diet (CD); 3–ACLT, CD and treadmill training (TT); 4–ACLT, Sicilian EVOO diet and TT; 5–ACLT, Tunisian EVOO diet and TT; 6–ACLT, Tunisian EVOO and leaves extract diet and TT. We performed histomorphometric, histological, immunocytochemical, immunohistochemical and biochemical analysis on articular cartilage, skeletal muscle, liver and synovial fluid of rats. The results showed the beneficial effect of physical activity and EVOO supplementation on rat tissues. ACLT determined an increase in IL-6 expression and a significant decrease in the lubricin expression in articular cartilage, while physical activity and EVOO diet (especially S-EVOO), determined the return to normal values when compared to control group. Moreover, EVOO does not cause hepatic steatosis and muscle fibers of all groups did not show damaged histological structure and hypertrophy [2]. Our findings suggest that mechanical stimulation is able to increase the release of lubricin in articular cartilage, while the anti-inflammatory properties of EVOO reduces the expression of IL-6. In conclusion, the results showed a beneficial effect of the conjunction of EVOO-based diet and physical activity on the preservation of rat tissues.

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Key words

Lubricin, IL-6, EVOO, Osteoarthritis, ACLT, sarcopenia, steatosis, cartilage, skeletal muscle, liver.

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The mimic effects of knee exercise by mechanobiology technology induce chondrogenesis in MSC

Marta Anna Szychlinska, Michelino Di Rosa, Francesca Borzì, Rosa Imbesi, Rita Reitano, Silvia Ravalli, Francesca Maria Trovato, Paola Castrogiovanni and Giuseppe Musumeci

SIAI, Università di Catania, Dip di Scienze Biomediche e Biotecnologiche, Catania

Mesenchymal stem cells (MSCs) are currently being investigated as a cell source for regenerative medicine approaches for the repair of damaged articular cartilage. It is important to understand how these cells react to the complex loading environment of a joint in vivo, to use them as a source for the cell-based therapy for articular cartilage regeneration. In addition to investigate alternative MSC sources, it is also important to study the structure of tissue-engineered constructs and their organization within them. A custom-built bioreactor was used to expose human MSCs to a combination of shear and compression loading. The MSCs were either evenly distributed throughout fibrin-poly(ester-urethane) scaffolds or asymmetrically seeded with a small proportion seeded on the surface of the scaffold. The effect of cell distribution on the production and deposition of cartilage-like matrix in response to mechanical load mimicking in vivo joint loading was then investigated. The results showed that asymmetrically seeding the scaffold led to markedly improved tissue development based on histologically detectable matrix deposition [1]. Consideration of cell location, therefore, is an important aspect in the development of regenerative medicine approaches for cartilage repair. This is particularly relevant when considering the natural biomechanical environment of the joint in vivo and patient rehabilitation and adapted physical activity protocols.

This study was supported by the University Research Project Grant (Triennial Research Plan 2016-2018), University of Catania, Italy.

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Key words

MSC, articular cartilage, bioreactor, chondrogenesis, mechanobiology.

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The kidney in an animal model of metabolic syndrome: A morpho-functional study

Seyed Khosrow Tayebati[1], Ilenia Martinelli[1], Michele Moruzzi[1], Enea Traini[1], Daniele Tomassoni[2] and Francesco Amenta[1]

[1] Università di Camerino, Scuola di Scienze del Farmaco e dei Prodotti della Salute, Camerino, Italia
[2] Università di Camerino, Scuola di Bioscienze e Medicina Veterinaria, Camerino, Italia

Metabolic syndrome (MetS) is characterized by obesity, insulin resistance, dyslipidemia, hyperglycemia, and hypertension. Obesity is a chronic pathological condition characterized by an accumulation of adipose tissue associated with an increase in morbidity and mortality. Adipose tissue is a primary source for the production and secretion of leptin. Plasma concentrations of leptin increase parallel to fat mass augmentation. This regulates food intake and energy expenditure to maintain body fat deposits. In this work, the obese Zucker rat (OZR, fa/fa) which show several metabolic dysfunctions were investigated. The mutation (fa/fa) of the gene that codifies for the leptin receptor causes hyperphagia and leads to a marked obesity. In our experiments, OZRs developed simultaneously with hyperphagia, obesity, insulin resistance and arterial hypertension. This highlights the influence of MetS on the kidney and the correlations with degenerative phenomena linked to obesity. OZRs and age-matched lean Zucker controls (LZR) were studied at the age of 12, 16 and 20 weeks. The kidneys were removed and processed for morphological analysis. Furthermore, several inflammatory parameters such as IL-1β and IL-6 and some endothelial markers namely ICAM-1 and PECAM-1 were investigated by immunochemical and immunohistochemical techniques. Morphological changes involving primarily the glomerulus and convoluted tubules were observed in OZR rats of all ages. In the glomeruli, an increase in the glomerular and capsular volume, as well as a more pronounced glomerulosclerosis in older obese rats, were observed. To evaluate oxidative stress, levels of malondialdehyde (MDA) and the oxidation status of plasma proteins were assessed. This analysis revealed an oxidative stress status characterized by an increasing level of oxidated proteins and no changes in the concentration of MDA. An increase of IL-6 expression was noticeable in the proximal and distal convoluted tubules. In summary, the above findings suggest that in the OZRs nephropathy is an extremely complex phenomenon, related both to inflammatory phenomena typical of MetS and to increased oxidative injury. These results can contribute to better characterize end-organ damage in Mets, the relationships of it with chronic kidney disease and to identify therapeutic approaches avoiding renal failure that could result in dialysis or organ transplantation.

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Daily fluctuation of Glia

Chiara Tesoriero, Idris A. Azeez, Filippo Corghi, Federico del Gallo, Giuseppe Bertini, Marina Bentivoglio

Università di Verona, Dipartimento di Neuroscienze, Biomedicina e Movimento, Verona, Italia

We recently reported that the excitatory/inhibitory balance of the innervation of neurons which contain the orexin/hypocretin peptides, located in the lateral hypothalamus, undergoes a remarkable process of daily rearrangement in basal conditions in mice during the periods of the animals' sleep and wake. This finding raises many questions on the regulatory mechanisms. The hypothesis was here tested that daily neuroplasticity could implicate glial cells, since, in the brain, astrocytes are notably key partners of neurons at the synaptic level, and microglia continuously extend and retract their ramifications contacting also synapses. For the present study, unperturbed adult mice and rats were sampled during the day (the period of predominant sleep in these nocturnal animals, as also assessed here with videorecording) or night. In mice, glial cells surrounding orexin neurons were investigated in the lateral hypothalamus. Astrocytes were visualized using glial fibrillary acidic protein as a marker. CX3CR1-GFP mice, in which microglial cells are tagged with green fluorescent protein, were used for the study of microglia. Diurnal changes in microglial morphology and microglia-synapse interactions were investigated in confocal microscopy with multiple immunofluorescence. Three-dimensional reconstructions of glial cells in the lateral hypothalamus revealed striking variations in relation to vigilance state. Astrocytes showed a bushy phenotype, with dense filling of the neuropil, and microglial cells were endowed with highly ramified processes especially at night, when the animals were predominantly awake. Interactions between astrocytic and microglial processes and synapses have been observed during both day and night, and the analysis and quantification of these contacts are currently in progress. Furthermore, to test the activity of microglial cells, microglia-derived microvesicles were quantified in the cerebrospinal fluid of rats, sampled at two time points in antiphase as above. These microvesicles turned out to be significantly more numerous during the period of animals' predominant activity and wakefulness than during the period of predominant rest and sleep. Altogether these sets of data reveal a diurnal fluctuation of the morphology of glial cells in the lateral hypothalamus and of the activity of microglia over the course of the day, opening novel questions on their dynamic properties in relation with behavioural output in health and disease.

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Mesenchymal stromal cell Isolated from healthy and aneurysmal abdominal aortas: a morphological and biochemical study

Gabriella Teti[1], Elio Piccinini[2], Laura Ingrà[1], Alessandra Ruggeri[1] and Mirella Falconi[1]

[1] Università Di Bologna, Dipartimento Di Scienze Biomediche E Neuromotorie, Bologna, Italia
[2] RSH- AUSL Romagna, Ospedale Ravenna, cardiovascular department, Ravenna, Italia

Abdominal aortic aneurysm (AAA) is a common degenerative vascular disorder associated with sudden death due to aortic rupture. The current clinical approaches are to monitor aortic dimensions and to perform an open or endovascular surgical repair when the aortic diameter has attained sufficient expansion, condition that predispose to a high likelihood of aortic rupture.

Although several studies have identified many potential mechanisms involved in AAA pathogenesis, a clear depth understanding is still lacking and further studies are needed to facilitate development of effective therapies. Recent discoveries have demonstrated the presence of mesenchymal stromal cells (MSCs) in human aortic layers. These cells possess high proliferative capacity and potential to generate endothelial, smooth muscle, hematopoietic and mesenchymal cell progeny. Nevertheless, any defect of the proliferation and/or the differentiation process of vascular stem cells may determine the development of human vascular diseases. The aim of this study was to demonstrate the presence of senescent MSCs residing in human abdominal aortic wall, which could have a role in the AAA pathogenesis.

MSCs isolated from healthy (HAA - MSCs) or aneurysmal abdominal aortas (AAA – MSCs) were characterized for their proliferation rate, ultrastructural morphology, senescence-associated β-galactosidase activity and differentiation properties.

Results showed low growth potential, high senescence-associated β-galactosidase activity, an increased cell surface area, a reduced amount of autophagic and lysosome vesicles in AAA – MSCs compared to HAA - MSCs, thus indicated a senescent phenotype in AAA MSCs.

Vascular wall-resident MSCs are deeply involved in the process of vascular remodelling, that is a dynamic and strictly regulated process of structural changes occurs as a result of a pathological vascular trigger. The presence of a senescent population of AAA MSCs in vascular wall could have implications in the genesis and progression of vascular diseases, such as AAA.

Key words

Mesenchymal stem cells, aneurysm, vascular wall, senescence.

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Argonaute 2 drives miR-145-dependent gene expression program influencing epithelial to mesenchymal transition in breast cancer cells

Claudia Tito[1], Teresa Bellissimo[1], Federica Ganci[2], Andrea Sacconi[2], Silvia Masciarelli[1], Natale Porta[3], Mirko Cirenza[3], Melissa Sorci[4], Luciana De Angelis[1], Alessandro Fatica[4], Vincenzo Petrozza[3], Giulia Fontemaggi[2], Giovanni Blandino[2] and Francesco Fazi[1]

[1] Sapienza University of Rome, Department of Anatomical, Histological, Forensic & Orthopedic Sciences, Section of Histology & Medical Embryology, Rome
[2] IRCCS - Regina Elena National Cancer Institute, Oncogenomic and Epigenetic Unit, Rome
[3] Sapienza University of Rome, Department of Medico-Surgical Sciences and Biotechnologies, Pathology Unit, ICOT, Latina
[4] Sapienza University of Rome, Department of Biology and Biotechnology "C. Darwin", Rome

To perform their regulatory functions, microRNAs (miRNAs) must assemble with any of the four mammalian Argonaute (Ago) family of proteins, Ago1–4, into an effector complex known as the RNA-induced silencing complex (RISC) [1]. While the mature miRNA guides the RISC complex to its target mRNA, the Ago protein represses mRNA translation [2]. The specific roles of the various Ago members in mediating miRNAs activity, however, haven't been clearly established.

In this study, we investigated the contribution of Ago2, the only human Ago protein endowed with nuclease activity, to the function of tumor-suppressor miR-145-5p in breast cancer (BC). We show that miR-145-5p and Ago2 protein are concomitantly downregulated in BC tissues and that restoration of miR-145-5p expression in BC cells leads to Ago2 protein induction through the loosening of Ago2 mRNA translational repression. Functionally, miR-145-5p exerts its inhibitory activity on cell migration only in presence of Ago2, while, upon Ago2 depletion, we observed increased miR-145/Ago1 complex and enhanced cell motility. Profiling by microarray of miR-145-5p target mRNAs, in BC cells depleted or not of Ago2, revealed that miR-145-5p performs Ago2-dependent and -independent activities. Our results highlight that the Ago2 protein in cancer cells strictly dictates miR-145-5p tumor suppressor activity.

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c-FLIP is involved in autophagosome biogenesis and regulates autophagy-dependent cell death

Luana Tomaipitinca[1], Simonetta Petrungaro[1], Pasquale D'Acunzo[2], Antonio Filippini[1], Francesco Cecconi[3], Elio Ziparo[1] and Claudia Giampietri[1]

[1] Sapienza Università di Roma, Dipartimento di Scienze Anatomiche, Istologiche, Medico-Legali e dell'Apparato Locomotore, Roma, Italia
[2] IRCCS Bambino Gesù, Dipartimento di Ematologia e Oncologia pediatrica, Roma, Italia
[3] Università degli Studi di Roma "Tor Vergata", Dipartimento di Biologia, Roma, Italia

In the present study we investigate the role of c-FLIP in autophagosome biogenesis. c-FLIP is an apoptosis modulator [1] and plays a complex role in cellular homeostasis [2]. In the last few years a cross-talk between autophagy and apoptosis has been highlighted [3], but this complex mechanism still remains partially unknown. In c-FLIP-/- MEFs (mouse embryonic fibroblasts) compared to WT MEFs we analysed two well-known autophagy markers, LC3 and p62, under different autophagy-inducing stimuli (torin 1, starvation and tunicamycin). We found a strong reduction of the autophagic flux in c-FLIP-/- MEFs. We then studied the activation state of specific markers at each stage of the autophagic process and c-FLIP was found to participate in the nucleation stage and to bind key factors in the autophagosomes nucleation. Then we analysed the autophagic flux at increasing times and doses after treatment with autophagic inducers. A positive correlation was observed between death increase and autophagic flux induction in WT MEFs. Cell death was partially reversed by combining drug treatments with autophagy inhibitors. On the contrary, autophagy inhibition did not affect the basal low-level death of c-FLIP-/- MEFs. Further experiments are currently ongoing to better characterize the involved mechanisms and we can conclude that c-FLIP protein absence reduces autophagy-dependent cell death.

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Key words

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Effects of a tart cherry enriched diet on obese rats brain

Daniele Tomassoni[1], Seyed Khosrow Tayebati[2], Ilenia Martinelli[2], Michele Moruzzi[2], Consuelo Amantini[3], Maria Vittoria Micioni di Bonaventura[2], Maria Elena Giusepponi[2], Carlo Polidori[2], Giulio Lupidi[2], Gabriella Gabrielli[1], Carlo Cifani[2], Francesco Amenta[2]

[1] School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italia
[2] School of Medicinal and Health Products Sciences, University of Camerino, Camerino, Italia
[3] School of Biosciences and Veterinary Medicines, University of Camerino, Camerino, Italia

Increased food intake, reduced physical activity, and altered metabolic processes are the variables that affect energy balance inducing obesity. High Body Mass Index is associated with the development of cardiovascular risk factors such as hypertension, dyslipidemia, insulin resistance and diabetes mellitus leading to cardiovascular and cerebrovascular diseases promoting cognitive decline. Obesity has been suggested as a risk factor for Alzheimer's disease and vascular dementia and has been associated with poorer cognitive performance in population-based studies. Evidence suggests that overconsumption of high-energy foods and the associated obesity negatively influence brain function. This study has evaluated the potential effects of tart cherries (Prunus Cerasus L.) on the brain rats with Diet-Induced Obesity (DIO). They were fed for 17 weeks with a hypercaloric diet with the supplementation of tart cherries seeds powder (DS) and seeds powder plus tart cherries juice (DJS). DIO rats were compared to the control rats feed with standard diet (CHOW). Food consumption, body and fat mass weight, fasting glycemia, insulin, cholesterol, and triglycerides levels were measured. Immunochemical, immunohistochemical and qRT-PCR techniques were used to determine neuronal and glial alterations. No differences in body weight were found in treated rats compared to control group. In DS and DJS a decrease of blood pressure and glycemia and of serum levels of thiobarbituric reactive substances were found. Glial fibrillary acid protein expression decreased in the hippocampus and in the frontal cortex of treated rats. A reduction of microglial activation was also found. Moreover, neurofilament increased in treated rats compared to DIO. Tart cherries did not modify synaptic protein and TRP channels expression in DIO rats. Vascular tree and blood-brain barrier were also affected in DIO rats, with modulation in DS and DJS. These findings indicate that tart cherries, although did not affect body weight values prevent the development of related risk factors. In the brain tart cherries reduced inflammation. The results may represent the first step to clarify the possible use of tart cherries supplementation to prevent obesity-induced brain damage. Further studies are needed to better understand the specific mechanisms of action of tart cherry components.

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Key words

Obesity, high-fat diet, brain damage, antioxidants, tart cherries.

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"The motor paradox": Abnormal postural sway and gait disturbances in schizophrenia spectrum disorders

Matteo Tonna[1], Francesca Paraboschi[2], Filippo Marsella[2], Valeria Lucarini[2], Laura Galuppo[3], Giuliana Gobbi[4], Marco Vitale[5], Carlo Marchesi[2]

[1] Azienda Unità Sanitaria Locale, Ospedale Maggiore di Parma, Parma, Italia
[2] Dipartimento di Neuroscienze, Psichiatria, Università di Parma, Parma, Italia
[3] Dipartimento di Medicina e Chirurgia (DiMeC), Università di Parma/Laboratory of Movement analysis and posture, Parma, Italia
[4] Dipartimento di Medicina e Chirurgia (DiMeC), Università di Parma, Parma, Italia
[5] Dipartimento di Medicina e Chirurgia (DiMeC), Università di Parma/Laboratory of movement analysis and posture, Parma, Italia

Subtle motor abnormalities have been described in schizophrenic patients since the first descriptions of the disease [1,2] and are now properly conceptualized as early endo-phenotypes of schizophrenia. [3] Despite the role of psycho-motor disturbances as endo-phenotipic markers of schizophrenia spectrum disorders, very few studies have investigated the locomotor pattern of gait in schizophrenia. The present study aimed to detect the presence of gait disturbances and postural anomalies by means of "Gait Analysis System", in order to identify specific underlying endophenotipic deficits in motor control . 21 patients and 14 healthy subjects have been analyzed in gait and postural sway by classic full Gait Analysis system. Schizophrenic patients showed a longer gait cycle compared to controls (cycle duration sx=1,11±0,09 vs 1,04±0,06; cycle duration dx=1,10±0,09 vs 1,04±0,06). This difference (sx 0.065, 95%CI [0.12-0.05]) (dx 0.065,95%CI [0.12-0.003]) was significant (sx t(35)2.2,p=0.03) (dx t(35)2.15,p=0.03). Moreover, schizophrenic group had greater sway area with open eyes (sway area OE 107,5±89.8 vs 57,2±31.8). This differences (51.54, 95%CI [97.28-5.20]) was significant (t(35)=2.3, p=0.03). Finally, patients had more postural stability following the removal of visual input, as demonstrated by more length of the curve(464.9±180.68 vs 345.4±54.17)(difference 119.43, 95%CI[95.18+9.4]; t(35)=2.7,p=0.01) with no significative differences in the sway area, compared to controls.

According with current evidence, schizophrenic patients show a different locomotor pattern and specific postural sway abnormalities compared to controls. [4,5,6] Particularly, the present study found a "motor paradox" in the control of posture and balance in schizophrenia: notably, patients exhibit more postural instability with open eyes, as due to an interference of visual input; with postural stabilization following the removal of visual input.

The present findings would support the hypothesis of an information processing disorder, as a core feature of schizotaxic vulnerability, associated to subtle deficits in basic motor control of postural stability. [4]

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Key words

Schizophrenia, posture, sway, gait analysis, information processing.

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Randomized controlled didactical trial to evaluate usefulness of interactive media to teach anatomy in a university setting. A pilot study

Cinzia Tortorella, Rafael Boscolo Berto, Andrea Porzionato, Carla Stecco, Anna Rambaldo, Veronica Macchi, Raffaele De Caro

Istituto di Anantomia, Dipartimento di Neuroscienze, Università degli studi di Padova, Padova, Italia

Anatomy has traditionally been taught via dissection and didactic lectures [1]. The rising prevalence of informatics play an increasingly important role in medical education [2], potentially representing an integrative system to be combined with the dissection [3].

We hypothesized that new technologies can express added value to the dissection, which remains the classical method of teaching anatomy.

We investigated this question in the optional anatomic dissection course organized for the second year medical students (n 30). After a preliminary questionnaire aimed to collect student's personal data, we randomized them to a first group, which applied to an interactive media for 20 minutes, and a second one applied to textbooks of topographical anatomy for 20 minutes. The topic of interest was stated as being the forearm (bones, muscles, vessels and nerves). Following this preliminary step, they all applied to the gross dissection of a human forearm, subsequently surveyed by a test aimed to evaluate their retained information with regard to 2D and 3D anatomical structures other than basic anatomical knowledge. The return rate questionnaire was 76.7%. We found a comparable performance in terms of basic anatomical knowledge regarding bones, vessels, nerves but not muscles and 2D reporting of anatomical structures, for which the interactive media were of benefit. Likewise, the group which used interactive media showed a better 3D reporting of overall anatomical structures, among which muscles location. By logistic regression adjusted for confounding factors, we showed the independent role in predicting the highest results (scores ≥70%) of both interactive media and highest academic scores for anatomy.

The overall evidence was in support of the use of interactive informatics to integrate the learning of human anatomy. This would be of benefit with particularly reference to the understanding of 3D spatial relationships between anatomical structures, by allowing the student to both deconstruct (i.e. virtually dissect) and reconstruct discrete regions of the body, hence visualizing and manipulating complex anatomical structures using 3D models. Nevertheless, these upcoming anatomy applications serve as useful integrative learning tools, when used in conjunction with traditional practical dissection.

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Key words

Education, cadaver dissection, interactive media, randomized trial.

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Morphological and biochemical techniques to detect localization and possible role of ciliary neurotrophic factor in normal and cancer prostate

Giovanni Tossetta[1], Sonia Fantone[1], Martina Senzacqua[1], Roberta Mazzucchelli[2] and Daniela Marzioni[1]

[1] Università Politecnica delle Marche, Department of Experimental and Clinical Medicine, Ancona, Italia
[2] Università Politecnica delle Marche, Department of Biomedical Sciences and Public Health, Ancona, Italia

Ciliary neurotrophic factor (CNTF) belongs to the hematopoietic cytokine superfamily including leukaemia inhibitory factor (LIF), interleukin-6 (IL-6), IL-11, and oncostatin. CNTF gene is localized to chromosome 11q12 in humans [1]. The receptor of CNTF (CNTFR) was initially found to be distributed in neural tissue, later it was also found in skeletal muscle, adrenal gland, liver, and other tissues. Because it does not have a transmembrane or cytoplasmic region and it is anchored to the cell surface membrane by glycosylphosphatidylinositol linkage, CNTFR can produce its effect in either membrane-bound form or soluble form [2]. Since it has been demonstrated that IL-6 is a mediator of prostate cancer morbidity [3]. We have hypothesized that also CNTF could be involved in prostate cancer development. The expressions of CNTF and CNTFR have been evaluated in benign and neoplastic prostate tissues by immunohistochemistry and their possible role in three prostate cell lines including normal human primary prostate epithelial cells PVR1E, human prostatic cancer cell line LNCaP, and human prostate cancer cell line castration resistant 22Rv1. Our findings indicate that CNTF and CNTFR are present in almost tissues analysed and show a localization in the basal cells and not in the luminal cells in benign prostate while a moderate staining of luminal cells was observed in adenocarcinoma sections. In addition, western blotting and cellular immunofluorescent staining analyses showed that all three cell lines expressed both CNTF and CNTFR. Our preliminary data on PVR1E treatment by CNTF suggest that this factor could be involved in prostate cell growth, in particular by negatively modulating cell proliferation.

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Key words

CNTF, CNTFR, prostate, cancer.

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Effect of enhanced cholinergic challenge on brain atrophy in Alzheimer's disease

Enea Traini[1], Anna Carotenuto[2], Angiola Maria Fasanaro[3], Francesco Amenta[4]

[1] School of Pharmacy and Health Products, University of Camerino, Camerino, Italia
[2] Alzheimer Evaluation Unit, National Hospital, "A. Cardarelli",, Napoli, Italia
[3] Alzheimer Evaluation Unit, National Hospital, "A. Cardarelli", Napoli, Italia
[4] School of Pharmacy and Health Products, university of Camerino, Camerino, Italia

Cerebral atrophy is a common feature of neurodegenerative disorders. In Alzheimer's disease (AD) a loss of gyri and sulci in the temporal lobe and parietal lobe, and parts of the frontal cortex and cingulate gyrus has been reported.

In 56 patients, participating to the trial ASCOMALVA [Effect of association between a cholinesterase inhibitor (ChE-I) and choline alphoscerate on cognitive deficits in AD associated with cerebrovascular injury] and reaching the third year of observation, brain MRI were analyzed by voxel morphometry techniques. The purpose was to assess if a combined therapy using a cholinergic precursor (choline alphoscerate) and a cholinesterase inhibitor (donepezil) may have an effect on slowing the volume loss typical of AD brain.

After three years of treatment, in patients treated with donepezil plus the cholinergic precursor choline alphoscerate, the volume loss of the gray matter (with the concomitant increase of the volume of the ventriculi and space of the cerebrospinal fluid) was countered compared to the reference group, treated with donepezil only. The areas, in which brain atrophy was more limited, were the frontal and temporal lobes, hippocampus, amygdala and basal ganglia. Morphological data were also confirmed by neuropsycological assessment done along the course of the trial.

These findings have shown that cholinergic precursor loading strategy with choline alphoscerate associated to cholinesterase inhibition with donepezil counters to some extent the atrophy occurring in some brain areas of AD patients. The observation of a parallel improvement of cognitive and functional tests in patients treated with choline alphoscerate plus donepezil versus donepezil alone suggests that morphological changes observed may have functional relevance.

Key words

Alzheimer's disease, Cerebral atrophy, choline alphoscerate, association, cerebrovascular injury, choline alphoscerate, donepezil.

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Giovanni Falconi (1817-1900) and the influence of Bartolomeo Panizza in the teaching of anatomy in the University of Cagliari

Marcello Trucas

Università degli Studi di Cagliari, Dipartimento di Scienze Biomediche, Cagliari, Italia

Giovanni Falconi, a pupil of Francesco Antonio Boi (1767-1855) [1] graduated in surgery in 1843 and in Medicine in 1850 [2]. He taught Anatomy for 44 years in the University of Cagliari and became also well known in Italy and abroad, for having invented in 1841 a smallpox vaccination needle, the "Falconian Needle", which had a great impact in spreading vaccination in the XIX century [2]. By a search in Cagliari Archives I have found the manuscript, dated 1885, of the Treatise with his lectures that Falconi, had to deliver to the "Magistrato sopra gli Studi". It is a large textbook (672 pp.) that deals not only with gross and microscopic anatomy, but also with neuroanatomy, experimental physiology and surgical physiopathology. Of particular interest are the references to the studies of Bartolomeo Panizza (1785– 1867) [3], pupil and successor of Antonio Scarpa in the University of Pavia. Panizza was a tireless experimenter and performed clinico-pathological studies on both animals and humans [3], regarding vascular absorption, the origin of nerves envelope, the central nervous system, the avascular structure of the hairs and of the Malpighian layer of the epidermis [2]. Panizza was the one who established the first course of microscopic anatomy in Italy [3]. To demonstrate their close relationship, is the fact that Falconi bought in 1864 the first microscopes for his anatomy lab and spent 170 pages (21%) of the Treatise to neuroanatomy and description of cranial nerves [2]. He introduced the didactic approach of Panizza, creating a link between Pavia and Cagliari by carrying on microscopic research on salivary glands and neuroanatomy. Giuseppe Marci, assistant of Falconi, in 1852 was sent to Pavia, for a 6-month internship in Panizza's lab and, in 1859, the student Ettore Lucchi came to Cagliari from Pavia [2]. Finally, it must be worth noting that the relationship with Pavia is still active nowadays in that, since 1963, the chair of anatomy is held by Anatomists belonging to the school of Antonio Pensa.

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Key words

Falconi, Panizza, microscopy, Cagliari, Pavia, smallpox needle.

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My-AHA: my Active and Healthy Ageing

Alessandro Vercelli[1] and Innocenzo Rainero[2]

[1] Università di Torino, Neuroscience Institute Cavalieri Ottolenghi, Orbassano (TO) Italia
[2] Università di Torino, Dipartimento di Neuroscienze, Torino, Italia

Stemming from a holistic view of interrelated frailties, cognitive decline, physical frailty, depression and anxiety, social isolation and poor sleep quality, My-AHA proposes an ICT platform for early detection of pre-frailty and intervention to sustain active and healthy ageing and slowing or reversing further decline.

The main aim of My-AHA is to reduce frailty risk by improving physical activity and cognitive function, psychological state, social resources, nutrition, sleep and overall well-being in older adults with pre-frailty symptoms. It will empower older citizens to better manage their own health, providing new ways of health monitoring and disease prevention through individualized profiling and personalized recommendations, feedback and support. An ICT-based platform will detect defined risks in the frailty domains early and accurately via non-stigmatising embedded sensors and data readily available in the daily living environment of older adults. When risk is detected (pre-frail), My-AHA will provide targeted ICT-based interventions. These interventions will follow an integrated approach to motivate users to participate in physical exercise, cognitively stimulating games and social networking to achieve long-term behavioural change, sustained by continued end user engagement with My-AHA. A randomized controlled study (RCT), involving 300 subjects receiving intervention, and 300 controls from many EU and non EU countries, to evaluate intercultural aspects, is ongoing in order to evaluate efficacy of the my-AHA platform. The ultimate aim is to deliver significant innovation in the area of active and healthy ageing through cooperation between European health care organizations, SMEs, and NGOs.

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The Giocampus project and it's social, health, educational and scientific impact

Elio Volta[1], Prisco Mirandola[2], Giuliana Gobbi[2], Daniela Galli[2] and Francesca Scazzina[3]

[1] MIUR, Ufficio Scolastico Regionale Emilia Romagna and Curriculum of Sport Sciences University of Parma, Parma, Italia
[2] University of Parma, Sport and Exercise Medicine Centre (SEM) and SBiBiT Unit of Department of Medicine and Surgery, Parma, Italia
[3] University of Parma, Department of Food and Drug, Parma, Italia

Giocampus® is a unique multidisciplinary educational project developed in Parma (Italy) dedicated to children and young people with the primary aim of promoting correct lifestyles through structured paths of motor, food and environmental education. The project, structured according to a public-private educational alliance, includes the Municipality of Parma, the University of Parma, the Coni - Emilia-Romagna Regional Committee, the Regional School Office for Emilia Romagna, CUS Parma and Barilla G&R as founding partners, and other private Companies and Institutions as members and supporters of the principles of the Educational Alliance for future generations. Besides the strong social support of the population, over the years the project has also collected the recognition of the scientific community at the international level. Every year, Giocampus involves 15,000 children and hundreds of professionals working on the project: 100 operators (including university students from Scienze Motorie and Scienze Gastronomiche) work on the success of Giocampus School (20 hours per year of Food Education for the third, fourth and fifth classes and for all classes 60 hours a year of Motor Education), 60 for Giocampus Neve (for children aged 9 to 14 to learn and improve their skiing skills) and over 200 contribute to the success of Giocampus Estate (for children aged 5 to 14 a path of experimentation of different sports and movement disciplines) [1-4]. Within the School and summer projects, the Giocampus Insieme project is also active, a program dedicated to the inclusion through the game of children / young people with disabilities and children / teenagers with difficulties from behavioral and environmental point of view. The important scientific path accomplished and the results that support the theories underlying the project led to the definition of the Educational Model, made official in 2014 with the publication of the Giocampus Methodology signed by the Scientific Committee. The results achieved are the product of the important, constant and continuous work of a great Educational Alliance composed of public institutions and the private world.

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Key words

Giocampus, children, sport.

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Assessment of growth and nutritional status in preschool children from Albanian nationality

Biljana Zafirova, Biljana Trpkovska, Marija Papazova, Julija Zivadinovik, Niki Matveeva, Elizabeta Cadikovska, Biljana Bojadzieva and Ace Dodevski

Department of Anatomy, University Ss Cyril and Methodius, Skopje

Growth monitoring and promotion of optional growth are essential components of primary health care for children. Serial measurements of weight, height/length, for all children and measurements of circular and transversal parameters compared with growth of large sample population help to confirm a child's healthy growth and development [1]. It also allows early identification of potential nutritional or health problems and enables prompt action before a child's health is seriously compromised [2]. The aim of the study was evaluation of sex-specific differences of anthropometric parameters as indicator of growth and nutritional status in preschool children from Albanian nationality. Ten anthropometric parameters were measured on healthy children, defining longitudinal, circular and transversal dimensionality of the skeleton using standard technique and instruments. The following indices were calculated: weight-for age; height for age and body mass index (BMI). The majority of anthropometrical parameters have shown significant age and sex specific differences in favour of male subjects. The height-for-age index values corresponding to the 50th percentile showed slightly higher values in our female subjects 110 cm than in our male subjects 107.1 cm. The values of 50th percentile of BMI in our male subjects were 16.7 kg/m", whereas in our females were 16.2 kg/m" .These results show that obesity prevention is recommended, and detected values could be applied for evaluation of deviations in growth and nutritional status in preschool children from Albanian nationality.

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Key words

Preschool children, anthropometry, growth, nutritional status.

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Evaluation of sex-specific differences of anthropometric parameters that were used as indicators of nutritional status in children

Biljana Zafirova[1], Marija Papazova[2], Julija Zivadinovik[2], Niki Matveeva[2], Elizabeta Chadikovska[2], Biljana Bojadzieva[2], Biljana Trpkovska[2] and Ace Dodevski[2]

[1] Department of Anatomy, University of Ss Cyril and Methodius, Zafirova
[2] Department of Anatomy, University of Ss Cyril and Methodius, Skopje

Childhood overweight and obesity are major public health problems worldwide [1].Anthropometric parameters play a central role in identifying children that are overweight or obese, or at risk of becoming so [2]. The aim of our study was evaluation of sex-specific differences of anthropometric parameters that were used as indicators of nutritional status in children. The study included 220 healthy children (110 boys, 110 girls) aged 7 from Macedonian nationality. With standard methodology (IBP) were taken following body measurements (body weight, height, circumferences (mid-upper-arm and waist) and skin-folds thickness (triceps and sub scapular). According to standard formulas were calculated: weight-for-age (BW), height-for-age (BH), body mass index-for-age (BMI), mid-upper-arm-circumference-for-age (MUAC), waist circumference-for-age (WC), and skin-folds thickness-for-age (triceps-SFTr and sub scapular-SFSc). The 7 year old boys have slightly higher mean values for (BH, BW, BMI, MUAC, WC) than girls, but sex-specific differences were not significant, except for the BW. On the other hand, skin-folds thickness (SFTr and SFSc) were significantly higher in girls. Values of the 50th percentile in boys were as follows:26.5 kg for BW, 125.5cm for BH, 16.73 kg/m2 for BMI, 17.2 cm for MUAC, 57cm for WC, 5.2mm for SFSc and 8.2 mm for SFTr. The values of these parameters in girls were: 25 kg for BW, 124.5 cm for BH, 16.35 kg/m2 for BMI, 17.1 cm for MUAC, 55.8 cm for WC, 5.8 mm for SFSc and 9 mm for SFTr. These results can be used as criteria for the assessment and detection of deviations in the nutritional status in children age 7.

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Key words

Children, anthropometry, nutritional status.

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The effect of fatigue on body kinematics during simulated flat water kayak

Matteo Zago[1,2,3], Francesco Raniolo[1], Andrea Pilotto[2], Davide Aliprandi[3], Filippo Bertozzi[4], Nicola Lovecchio[5], Simone Porcelli[2], Mauro Marzorati[2], Manuela Galli[6] and Chiarella Sforza[5]

[1] Department of Electronics, Information and Bioengineering (DEIB), Politecnico di Milano, Milano, Italia
[2] Istituto di Bioimmagini e Fisiologia Molecolare, Consiglio Nazionale delle Ricerche, Segrate (MI), Italia
[3] Circolo Sestese Canoa e Kayak, Sesro Calende, Sesto Calende (VA), Italia
[4] Corso di Laurea in Scienze e tecniche delle attività motorie preventive e adattate, Università Cattolica del Sacro Cuore, Milano, Italia
[5] Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute, Milano, Italia
[6] Politecnico di Milano, Department of Electronics, Information and Bioengineering (DEIB), Milano, Italia

Flat-water kayak requires both technical skills and muscle strength. Previous studies conducted during simulated kayaking suggest that different kinematic variables (joints range of motion [RoM], symmetry, paddle position) are related to performance improvements [1,2] and may distinguish athletes of different competitive levels. On the other hand, muscle fatigue negatively affects both upper and lower limbs performance [3], but its effect on kayaking technique and body arrangement has not been analyzed yet. In this study, we examined changes of 3D kinematics through a simulated 500-meter kayak sprint. Ten athletes (7 M, 3 F, mean age 17±2 y) performed on a kayak ergometer: 1) an incremental test (INCR) to detect peak oxygen uptake (O2peak); 2) a 500-meter sprint trial (TT). During TT, besides collecting cardiometabolic response to exercise and blood lactate concentration ([Lapeak]), a motion capture system sampled the 3D coordinates of 40 cutaneous markers. For each athlete, joints RoMs (trunk, pelvis, lower and upper limbs) were obtained together with symmetry indices. Stroke frequency was computed from the wrist landmarks trajectory. To assess the effect of fatigue on kinematics, data obtained at the beginning of TT (11th-20th rowing cycles) were compared with that obtained at the end (last 10 cycles). During INCR, O2peak was 3.36 L/min. TT lasted 130.1±9.2 s, and paddle frequency was 1.1±0.2 s-1. During TT, in all subjects O2 value was higher than 90% of O2peak, whereas [Lapeak] was 10.6±3.1 mM, thus indicating a large contribution of both aerobic and anaerobic energy systems. Fatigue influenced joints RoM, with an increment in lower limbs joints (significant hip rotation, effect size [ES] 0.60), and a decrement in dominant side shoulder (rotation and adduction; ES 0.58). Trunk inclination also increased (ES 0.63). The first part of TT was performed with a higher shoulder rotation asymmetry (larger dominant limb RoM) than the last part. Results suggest that fatigue affects kinematic variables related to technical skills, where increased trunk and lower limb RoMs may compensate reduced upper limb movements and symmetry. If confirmed, these data can provide useful information to optimize training programs.

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Key words

Kayak, fatigue, range of motion, kinematics.

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Gata1 low mice as a model for multi-organ fibrosis

M. Zingariello[1], L. Sancillo[2], F. Martelli[3], P. Verachi[4], C. Bardelli[4], M. Valeri[5], A.R. Migliaccio[4], R.A. Rana[2]

[1] Unit of Microscopic and Ultrastructural Anatomy, Dept of Medicine, University Campus Bio-Medico Rome, Italy;
[2] Dept. of Medicine and Ageing Sciences, University G. d'Annunzio, Chieti-Pescara, Italy
[3] and [5] National Centre for Drug Research and Evaluation and National Centre Animal Research and Welfare, ISS, Roma, Italy
[4] Dept. of Biomedical and Neuromotorial Sciences, Alma Mater University, Bologna, Italy

In previous studies, we have shown that mice carrying the hypomorphic mutation which reduce the expression of the transcription factor GATA1 in Mk , the GATA1low mutation, develop myelofibrosis, a phenotype which resemble primary myelofibrosis, the most severe of the myeloproliferative neoplasms. The phenotype is driven by high levels of TGF-beta and the bone marrow from Gata1low mice, as those from the patients, is characterized by a strong activation of non-canonical TGFβ signaling including high level of expression of the transcription factor c-Jun which regulates myelo-monocytic maturation. More recently, over-expression of c-Jun has been described to be responsible for multi-organ fibrosis in mice [1]. The strong c-Jun signature suggested to us that also Gata1low mice may develop multi-organ fibrosis with age. This hypothesis was tested by histo-pathological analyses of bone marrow, spleen, liver, skin, lungs, heart and kidney of Gata 1low mice and of their wild-type littermates at 1-, 8- and 15-months of age. Cellular organization was detected by hematoxylin-eosin staining while fibrosis was detected by Gomory and Mallory staining. In addition to bone marrow and spleen, fibrosis was detected in skin, lung, heart and kidney but not in liver. At 1-month, fibrosis was detected only in skin and showed a tissue distribution resembling that observed in Scleroderma. At 8-months, fibrosis was detected in bone marrow, lung and heart. In the lung, the alveoli has thickened walls and fibers bundles were observed on the bronchus walls. In the heart, collagen fibers appeared of variable thickness and the cardiomyocytes had abnormal morphology which a strong reduction of intercalar disks. In the kidney, fibrosis was observed at 15-months and was localized in the medullary and nephron region. In conclusion, in addition to myelofibrosis, Gata1low mice may represent models for scleroderma, idiopathic pulmonary fibrosis and heart and kidney fibrosis, depending on age.

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VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI, 2017

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Verbale della seduta amministrativa e dell'assemblea generale dei soci della Società Italiana di Anatomia e Istologia (SIAI) tenutasi presso l'Hotel San Domenico di Taormina

In data 22 Settembre 2017, alle ore 16.00, in seconda convocazione, si è svolta l'Assemblea Generale dei Soci della Società Italiana di Anatomia e Istologia (SIAI), presso l'Hotel San Domenico di Taormina, in occasione del 71° Congresso Nazionale SIAI, con il seguente Ordine del Giorno:

1. Comunicazioni del Presidente.
2. Approvazione del verbale della seduta precedente.
3. Commemorazione dei Soci scomparsi.
4. Relazione del Tesoriere sul rendiconto finanziario del 2016 e sulla previsione finanziaria per il 2018. Relazione dei Revisori dei Conti.
5. Attività dei Collegi dei Docenti di Anatomia e di Istologia ed Embriologia: Relazione dei Presidenti o loro Delegati.
6. Assegnazione Premio Ricercatore under 40.
7. Assegnazione Premio alla Carriera.
8. Assegnazione Premi Poster.
9. Assegnazione Premio Migliore Comunicazione Orale.
10. Prossimi Congressi nazionali della SIAI e Congressi nazionali ed internazionali previsti per l'anno 2018; proposte di temi di relazione.
11. Problemi relativi all'Italian Journal of Anatomy and Embryology: relazione dell'Editor in Chief, Prof. Paolo Romagnoli.
12. Proposta di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.
13. Risultati elettorali e composizione del Consiglio Direttivo per il triennio 2018-2020.
14. Varie ed eventuali.

Presiede la riunione il Prof. Eugenio Gaudio, Presidente della SIAI; funge da Segretario Verbalizzante la Prof. Gigliola Sica, Segretario della SIAI.

1. Comunicazioni del Presidente.

Il Presidente della SIAI, Prof. Eugenio Gaudio, apre i lavori e a nome di tutti ringrazia calorosamente i Presidenti del Congresso, Proff. Giuseppe Anastasi e Domenico Puzzolo, nonché i loro Collaboratori, per l'impegno profuso nell'organizzazione del 71° Congresso Nazionale SIAI.

2. Approvazione del verbale della seduta precedente.

Il Presidente propone all'Assemblea l'approvazione del verbale della seduta precedente e l'Assemblea approva all'unanimità.

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3. Commemorazione dei Soci scomparsi.

Il Prof. Vincenzo Benagiano commemora il **Prof. Glauco Lucio Ambrosi**, scomparso nel mese di Ottobre 2016. Il Prof. Giuseppe Anastasi commemora il **Prof. Agatino Santoro**, scomparso nel mese di Gennaio 2017. Il Prof. Fabrizio Michetti commemora il **Prof. Nicolò Miani**, scomparso nel mese di Maggio 2017.

4. Relazione del Tesoriere sul rendiconto finanziario del 2016 e sulla previsione finanziaria per il 2018. Relazione dei Revisori dei Conti.

Il Presidente legge il verbale stilato nella riunione dei Revisori dei Conti, Prof. Paolo Romagnoli, Prof. Raffaele De Caro e Prof. Amelia Cataldi.

"Il giorno 21 Settembre 2017 si è riunita la Commissione dei Revisori dei Conti designata in seno alla Società Italiana di Anatomia e Istologia e costituita dai Proff.: Paolo Romagnoli, Raffaele De Caro e Amelia Cataldi.

Dopo aver valutato attentamente il conto Consuntivo relativo all'anno 2016 e il conto di previsione relativo all'anno 2018 presentati dal Tesoriere, Prof. Amelio Dolfi, la suddetta Commissione approva all'unanimità i conti esaminati."

Il Presidente dà la parola al Prof. Amelio Dolfi, che illustra il rendiconto finanziario del 2016, qui di seguito riportato assieme alla relazione di accompagno.

Bilancio consuntivo anno 2016

Causale delle entrate	Entrate	Causale delle uscite	Uscite
Quote sociali incassate nel corso dell'anno 2016 (n° 447) incluse le quote arretrate, le quote incassate non al netto e in attesa di integrazioni e le quote non riconducibili allo stato di alcun socio	**€ 26.849,04**		
		Elenco spese per attività statutarie	
Quote versate in contanti (n°1)	**€ 60,00**	Contributo I.J.A.E., anno 2015	**€ 4.000,00**
		Quote di Iscrizione al Congresso SIAI 2016 di due Soci vincitori dei premi poster, anno 2015	**€ 1.024,80**
		Contributo per l'organizzazione del 70° Congresso SIAI, anno 2016	**€ 5.000,00**
		Contributo per l'organizzazione del Convegno G.I.S.N, anno 2016	**€ 500,00**
		Premio alla Carriera, anno 2016	**€ 579,50**
		Spese varie (mantenimento conto corrente postale e bancario, spese bollo e commissioni bancarie ecc., anno 2016)	**€ 736,67**
		Rimborso spese per partecipazione alla riunione EFEM e alla riunione per Terminologia Anatomica, anno 2016	**€ 1.467,39**

		Spese impreviste: storno somme erroneamente versate a SIAI, anno 2016	**€ 30,00**
		Premio "Giovane Ricercatore" anno 2016"	**€ 2.000,00**
		Iscrizione della SIAI a IFAA, anno 2016	**€ 363,87**
		Iscrizione della SIAI a EFEM, anno 2016	**€ 500,00**
		Premio alla migliore comunicazione presentata al congresso nazionale SIAI 2016	**€ 1.000,00**
		Elenco spese di funzionamento	
		Compenso per Consulenza Commercialista relativa alla stesura del bilancio consuntivo anno 2015 e bilancio previsionale 2017	**€ 1.500,60**
		Versamento deleghe fiscali per compensi Commercialista, anno 2015	**€ 280,80**
		Spese relative all'utilizzo del server UNIFI per il sito web SIAI, anno 2015	**€ 272,06**
		Spese per il funzionamento del Consiglio Direttivo, anno 2016	**€ 687,04**
		Spese per il funzionamento della Tesoreria, anno 2016	**€ 53,20**
Totale entrate	**€ 26.909,04**	**Totale uscite**	**€ 19.995,93**
Avanzo d' esercizio finanziario 2016	**€ 6.913,11**		
Saldo Conto corrente Bancario al 31/12/2015	**€ 11.035,88**		
Saldo Conto corrente postale al 31/12/2015	**€ 4.980,89**		
Totale saldo finanziario al 31/12/2015	**€ 16.016,77**		
Avanzo dell'esercizio finanziario 2016	**€ 6.913,11**		
Saldo finanziario al 31/12/2016	**€ 22.929,88**		
Stanziamenti impegnati al 31/12/2016	**Euro**		**Euro**
Accantonamento premi poster e comunicazione anno 2016 (rimangono da erogare premi per migliori poster anno 2016)			**€ 1.000,00**
Contributo all'It. J. Anat. Embryol., anno 2016			**€ 4.000,00**

Spese per il sito web della Società, anno 2016		**€ 300,00**
Spese per ECM, anno 2016		**€ 530,00**
Spese per il funzionamento della Presidenza, anno 2016		**€ 1.000,00**
Spese per il funzionamento della Segreteria, anno 2016		**€ 1.000,00**
Compenso per Consulenza Commercialista relativa alla stesura del bilancio consuntivo anno 2016 e bilancio previsionale, anno 2018		**€ 1.780,00**
Totale impegno di spesa		**€ 9.610,00**
Saldo disponibile	**€ 13.319,88**	

Relazione di accompagno al rendiconto economico e finanziario per l'anno 2016

Come risulta dal bilancio consuntivo il saldo finanziario al 31/12/ 2016 è pari ad **€ 22.929,88** ed è costituito dal saldo finanziario al 31/12/2015 pari a **€ 16.016,77 sommato all'avanzo dell'esercizio 2016** pari a **€ 6.913,11**.

A tale importo devono essere sottratti **€ 9.610,00** impegnati nel bilancio previsionale del 2016, ma non ancora effettivamente utilizzati alla data del 31/12/2016, per le seguenti voci di spesa:

- **Accantonamento premi poster e comunicazione anno 2016: € 1.000,00;**
  Per questa voce risultano stanziati, nel previsionale del 2016, **€ 2.000,00** che in parte (**€ 1.000,00**) sono stati utilizzati nel corso del 70° Congresso Nazionale della Società del 2016 per il premio alla migliore comunicazione e nella parte rimanente (**€ 1.000,00**) saranno utilizzati per il pagamento delle quote di iscrizione al 71° Congresso Nazionale della Società del 2017 di due Soci risultati vincitori dei premi poster nel Congresso societario del 2016;
- **Contributo all'It. J. Anat. Embryol.,** anno 2016: **€ 4.000,00;**
- **Spese per il sito web della Società,** anno 2016: **€ 300,00;**
- **Spese per ECM,** anno 2016: **€ 530,00;**
- **Spese per il funzionamento della Presidenza,** anno 2016: **€ 1.000,00;**
- **Spese per il funzionamento della Segreteria,** anno 2016: **€ 1.000,00;**
- **Consulenza Commercialista,** anno 2016: **€ 1.780,00.**

Pertanto l'anno 2016 si chiude con un saldo disponibile di **€ 13.319,88**.

Durante il 2016 le quote associative incassate sono state **447** comprese alcune quote arretrate ed integrazioni di versamenti di quote non corretti, per un totale di **€ 26.909,04** che, sommate al saldo finanziario al 31/12/2015 (**€ 16.016,77**), hanno dato la disponibilità di **€ 42.925,81**.

Le voci relative alle competenze di liquidazione del conto Bancoposta e del conto corrente Unicredit sono risultate negative e sono considerate nel totale della voce **spese varie** (**mantenimento conto corrente postale e bancario, ecc.**).

Le entrate hanno permesso di coprire le spese previste e non previste, includendo i fondi impegnati e non erogati.

La rispondenza dei Soci ai solleciti da parte del Tesoriere in merito alla regolarizzazione dei pagamenti delle quote associative si è rivelata discreta, comunque, al 31 dicembre 2016, rimane ancora un numero significativo di Soci che debbono regolarizzare la loro posizione; da questo fatto deriva la impossibilità di effettuare previsioni fondate. Il Tesoriere sottolinea che l'eventuale recupero delle quote arretrate consentirebbe alla SIAI di effettuare una adeguata programmazione delle attività statutarie e di intraprendere nuove iniziative.

Il Presidente, nel ringraziare il Prof. Dolfi per l'accuratezza del rendiconto, pone in votazione il Bilancio Consuntivo 2016.

L'Assemblea approva all'unanimità.

Il Presidente dà quindi la parola al Tesoriere per illustrare la Previsione Finanziaria per il 2018, qui di seguito riportata assieme alla relazione di accompagno.

SOCI NEL 2016:	**534**
SOCI NEL 2017:	**518**
SOCI ORDINARI 2017:	**488**
SOCI DIMISSIONARI 2017:	**16**

ENTRATE

Quote sociali anno 2017 **(n. 488)**	€	29.280,00
Quote sociali arretrate 2007 - 2015	€	6.000,00
Totale entrate	**€**	**35.280,00**

USCITE

Contributo al Convegno Nazionale 2018, atti di convegni, altri contributi a convegni, partecipazione a convegni, organizzazione eventi scientifici, borse di studio, etc.	€	12.000,00
Accantonamento per premi poster dell'anno 2017 e per premio comunicazione assegnato nell'anno 2018	€	2.000,00
Accantonamento per premi SIAI (Premio alla Carriera e Premio Ricercatore under 40), anno 2018	€	4.000,00
Contributo all' Italian Journal of Anatomy and Embryology, anno 2018	€	4.000,00
Spese per sito web della Società, anno 2018	€	300,00
Spese per ECM, anno 2018	€	500,00
Spese per la partecipazione Meeting Comitato Internazionale per la Terminologia Anatomica e Istologica, FICAT, anno 2018	€	3.000,00
Quota adesione all'European Federation for Experimental Morphology, EFEM, anno 2018	€	520,00
Quota adesione all'International Federation of Anatomical Associations, IFAA, anno 2018	€	380,00

Spese varie (bancarie, postali, necrologi, etc.), anno 2018	€	1.000,00
Spese impreviste, anno 2018	€	1.000,00
Totale spese per attività statutarie	***€***	***28.700,00***
Spese per il funzionamento della Presidenza, anno 2018	€	1.000,00
Spese per il funzionamento della Segreteria, anno 2018	€	1.000,00
Spese per il funzionamento della Tesoreria, anno 2018	€	1.000,00
Spese per il funzionamento del Consiglio Direttivo, anno 2017	€	1.800,00
Spese per consulenza Commercialista, anno 2017	€	1.780,00
Totale spese di funzionamento	***€***	***6.580,00***
Totale uscite	**€**	**35.280,00**

Relazione di accompagnamento alla previsione finanziaria per l'anno 2018

La chiusura del bilancio consuntivo del 2016 con un saldo disponibile di **€ 13.319,88** ha permesso al Tesoriere di sostenere alcune spese indicate nella previsione finanziaria del 2017. Il Tesoriere, nel corso di questo anno, oltre a cercare di riscuotere le quote associative del 2017, ha continuato l'azione di recupero di quelle arretrate. Al 31 agosto 2017, sono state incassate 69 quote sociali comprensive di quelle relative all'anno 2017 e arretrate. È probabile che in questo periodo altri Soci abbiano provveduto al pagamento, ma al momento non siano stati considerati in questo resoconto.

Il totale delle entrate è attualmente pari a **€ 15.369,32** e comprende le quote riscosse finora. Comunque il piano previsionale del 2017 prevedeva entrate pari a **€ 35.820,00** dovute alla riscossione delle quote dell'anno in corso, più una cifra forfettaria concernente il recupero delle quote arretrate. In particolare, in tale previsione, come in quelle degli anni precedenti, è stata indicata questa cifra forfettaria sulla base dell'esperienza relativa alle difficoltà di ottenere il pagamento degli arretrati da tutti i Soci non in regola.

La Società conta attualmente **518** Soci, di cui **488** Soci ordinari e **30** Soci Emeriti o Onorari (esonerati dal pagamento della quota sociale). Nel corso del 2017, 16 Soci ordinari hanno espresso la volontà di rassegnare le dimissioni dalla Società.

Allo stato attuale, dei 488 Soci che sono tenuti a pagare la quota associativa:

- 2 Soci sono in regola fino al 2018
- 55 Soci sono in regola fino al 2017 (di cui 12 nuovi soci dal 2017 devono la quota sociale dell'anno in corso)
- 182 Soci sono in regola fino al 2016 devono la quota 2017
- 33 Soci in pari con il 2015 devono la quota 2016 e la quota 2017
- 39 Soci in pari con il 2014 devono le quote del 2015, 2016 e la quota 2017
- 19 Soci in pari con il 2013 devono le quote del 2014, 2015, 2016 e la quota 2017
- 23 Soci in pari con il 2012 devono le quote del 2013, 2014, 2015, 2016 e la quota 2017

- 35 Soci in pari con il 2011 devono le quote del 2012, 2013, 2014, 2015, 2016 e la quota 2017
- 12 Soci in pari con il 2010 devono le quote del 2011, 2012, 2013, 2014, 2015, 2016 e la quota 2017
- 28 Soci in pari con il 2009 devono le quote del 2010, 2011, 2012, 2013, 2014, 2015, 2016 e la quota 2017
- 22 Soci in pari con il 2008 devono le quote del 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016 e la quota 2017
- 22 Soci in pari con il 2007 devono le quote del 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016 e la quota 2017
- 16 Soci in pari con il 2006 devono le quote del 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016 e la quota 2017.
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Il Tesoriere fa presente che il suo mandato terminerà il 31 Dicembre 2017 e quindi si dichiara certo che il suo successore cercherà di raggiungere la parità di bilancio e di fare previsioni finanziarie quanto più possibile aderenti alla realtà. Riferisce inoltre che nel corso del 2017 alcuni soci hanno risposto positivamente all'azione di richiamo per il recupero delle quote arretrate. Rimane ancora un discreto numero di Soci che non hanno adeguatamente risposto ai solleciti di pagamento e, in base a quanto stabilito nello Statuto e al parere in merito espresso dal Direttivo SIAI, è in atto una revisione dell'elenco dei Soci.

Il Tesoriere ricorda che gli scopi istituzionali della Società Italiana di Anatomia e Istologia sono essenzialmente la promozione della ricerca e della didattica nel campo delle discipline anatomiche e istologiche, pertanto l'incasso puntuale delle quote annuali e il recupero delle quote arretrate permetterebbero alla SIAI di raggiungere al meglio questi scopi.

Il Presidente ringrazia il Prof. Dolfi per la precisione nella rendicontazione dei documenti, per l'impegno che ha dimostrato nell'assolvimento della sua impegnativa carica di Tesoriere e pone in votazione la Previsione Finanziaria per il 2018.

L'Assemblea approva all'unanimità.

5. Attività dei Collegi dei Docenti di Anatomia e di Istologia ed Embriologia: Relazione dei Presidenti o loro Delegati.

Il Prof. De Caro, Presidente del Collegio dei Docenti di Anatomia, presenta una breve relazione:

"Il Collegio Docenti di Anatomia si è riunito mercoledì 20 Settembre 2017 alle ore 15.30. Il Collegio ha continuato la disamina della proposta di Core Curriculum ricevuto dalla Conferenza dei Presidenti di Corso di Laurea, già esaminata nelle Riunioni del 17 Dicembre 2016, 18 Marzo 2017, 20 Luglio 2017. Si è proceduto alla discussione collegiale di ciascuna Unità Didattica Elementare (in particolare dalla 255 alla 280) e si è rimandata ad una riunione successiva la definitiva stesura del Core Curriculum di Anatomia. La prossima riunione è programmata per Dicembre 2017."

La Prof. Sica, Presidente del Collegio dei Docenti di Istologia ed Embriologia, comunica che le riunioni della Giunta e l'Assemblea annuale si sono regolarmente svolte. In particolare, in data 3 Febbraio 2017, durante l'Assemblea si sono tenute le

Elezioni di 3 Membri della Giunta poiché i Proff.: Di Primio, Puzzolo e Romagnoli, in data 24 Gennaio 2017, hanno visto scadere il loro primo mandato.

Sono stati eletti dall'Assemblea per acclamazione i Proff.: Daniele Bani, Michelangelo Cordenonsi e Roberto Di Primio.

In seno alla Giunta del Collegio si è deciso di formare dei gruppi di lavoro che si dedicheranno al reclutamento di nuovi Soci, mantenimento ed aggiornamento del Sito del Collegio, aggiornamento sulle procedure dell'ASN. Relativamente al Core Curriculum, il Presidente ha attivato la revisione delle Unità Didattiche Elementari (UDE) elaborate da una Commissione della Conferenza Permanente dei Presidenti di Corso di Laurea. Infatti le tematiche proposte presentano talvolta delle ripetizioni ed altre volte delle gravi lacune. Inoltre le definizioni richieste sono in qualche caso in contrasto con il grado di conoscenza indicato. Non emergono gli aspetti professionalizzanti relativi alle Discipline inerenti a BIO/17. Il lavoro di revisione, che ha già dato dei risultati discussi nell'Assemblea del 2017, verrà continuato in vista della completa definizione delle UDE e della stesura di un documento relativo. E' stata iniziata un'indagine a livello nazionale sulla posizione dell'Istologia nelle Scuole di Specializzazione.

Il Presidente nel corso dell'anno 2017 ha inoltrato a tutti i Soci del Collegio, i documenti utili, con particolare riferimento all'ASN e alla partecipazione alla Conferenza Permanente dei Collegi di Area Medica (Intercollegio).

6. Assegnazione premio ricercatore under 40.

Il Presidente riferisce che il Consiglio Direttivo della SIAI, su indicazione della Commissione formata dai Proff. E. Gaudio, S. Montagnani e S. Adamo, ha attribuito il premio alla **Dott.ssa Gaia Favero**, Assegnista di Ricerca presso la Sezione di Anatomia e Fisiopatologia del Dipartimento di Scienze Cliniche e Sperimentali, Università degli Studi di Brescia (Indice H: 14), candidata già menzionata durante il 70° Congresso Nazionale della SIAI. La Commissione ha inoltre espresso un sentito apprezzamento per la qualità della produzione scientifica della candidata Selenia Miglietta.

Il Presidente consegna il premio alla Dott.ssa Favero.

7. Assegnazione premio alla carriera.

Il Presidente comunica che il Consiglio Direttivo della SIAI, sulla base delle proposte pervenute, ha all'unanimità deliberato l'attribuzione del premio alla carriera al **Prof. Alessandro Riva**, di cui traccia un breve profilo.

Pertanto, a nome di tutta la SIAI, Il Presidente consegna una Targa d'argento ed una pergamena al Prof. Riva che, con un breve discorso, ringrazia per l'onore riservatogli.

8. Assegnazione premi poster.

Il Presidente riferisce che la Commissione per l'attribuzione dei Premi Poster, formata dai Proff. S. Adamo, G. Cavaletti e M. Grano e nominata dal Consiglio Direttivo, nel congratularsi per l'elevato livello scientifico raggiunto dai vari gruppi di ricerca, dopo un'attenta valutazione, ha deciso di assegnare i premi della SIAI ai seguenti Poster:

- *Muscle hypertrophy and vascularization induction using human recombinant proteins.*
  Flavio Lorenzo Ronzoni, Gabriele Ceccarelli, Laura Benedetti, Riccardo Bellazzi, Maria Gabriella Cusella De Angelis, Maurilio Sampaolesi (Pavia, Leuven).

- *TLQP peptides in Amyotrophic Lateral Sclerosis*
  Carla Brancia, Barbara Noli, Marina Boido, Alessandro Vercelli, Paolo Bongioanni, Gian Luca Ferri, Cristina Cocco (Cagliari, Torino, Pisa).

La Commissione ritiene anche di dover fare una particolare menzione per i poster:

- *The myotendinous junction plasticity following aerobic exercise.*
  Davide Curzi, Sara Salucci, Pietro Gobbi (Urbino).
- *Gross anatomy study on isolated formalin-fixed anatomical preparations.*
  Rosa Vaccaro, Nicola Roberto Pepe, Fabiano Svolacchia, Lorenzo Fumagalli (Roma).

Il Presidente comunica che la premiazione dei Premi Poster SIAI avverrà alla fine del Congresso e il verbale della Commissione Poster verrà spedito a tutti i Soci SIAI.

9. Assegnazione premio migliore comunicazione orale.

La Commissione, nominata dal Direttivo della Società nelle figure dei Proff. L. Cocco, S. Miscia e B. Nico, moderatori delle tre sessioni di Anatomia Clinica, tematica con il più alto numero di comunicazioni, primariamente ha espresso un giudizio estremamente lusinghiero sulla qualità scientifica di tutte le presentazioni, evidenziando come le tematiche morfologiche siano state affrontate con metodologie appropriate e innovative e come i dati siano stati trattati con notevole spirito critico.

Dall'esame comparativo la Commissione unanime ha identificato nella comunicazione della Dott.ssa Romina Mancinelli intitolata **"Knock down of hepatic GnRH reduces liver fibrosis in a mouse model of PSC"** quella che ha particolarmente mostrato una rilevante qualità per quanto riguarda la impostazione metodologica, la interpretazione critica dei risultati e la valenza scientifica degli stessi anche nell'avanzamento delle conoscenze dei settori morfologici.

Pertanto ha proposto che il Premio di Euro 1.000,00 sia assegnato alla Dott.ssa Romina Mancinelli.

Il Presidente, nell'accogliere la proposta della Commissione, comunica che alla Dott.ssa Mancinelli verrà anche donata una copia completa della collana Netter, offerta dalla EDRA S.p.A.

Il Presidente procede con la premiazione.

10. Prossimi Congressi nazionali della SIAI e Congressi nazionali ed internazionali previsti per l'anno 2018; proposte di temi di relazione.

Il Presidente ringrazia i Proff. Giuseppe Anastasi e Domenico Puzzolo che hanno dato la loro disponibilità ad organizzare per il 2017 il 71° Congresso Nazionale della SIAI nella sede di Taormina e ricorda che il 72° Congresso Nazionale della SIAI si terrà a Parma.

In merito ai temi di relazione, il Presidente riferisce che in seno al Direttivo, relativamente alle relazioni da tenere al 72° Congresso Nazionale SIAI, viene confermata per il 2018 la relazione del Prof. Antonio Filippini. Quella del Prof. Saverio Cinti, che è stata proposta dopo quella del Prof. Filippini, viene rinviata al 2019. Per la seconda relazione del Congresso 2018 il Presidente auspica di continuare nella linea sinora seguita dal Direttivo, ossia far ricadere la scelta su un relatore straniero. Il Prof. Cocco ha suggerito di contattare la Prof. Sue Black, Direttrice del Centro di Anatomia Macroscopica dell'Università di Dundee.

11. Problemi relativi all'Italian Journal of Anatomy and Embryology: relazione dell'Editor in Chief, Prof. Paolo Romagnoli.

Il Prof. Gaudio dà la parola al Prof. Romagnoli, il quale riferisce che nel corso del 2017 è stato possibile ripristinare l'indicizzazione su PubMed dell'Italian Journal of Anatomy and Embryology. La pubblicazione è ritmica, ma leggermente ritardata rispetto alla data convenuta con la casa editrice, il che dipende in buona parte anche dalla difficoltà di acquisire il pagamento della tariffa da autori stranieri, che sono soprattutto di Paesi africani e asiatici. La tariffa è sempre contenuta, 40,00 euro per pagina stampata più IVA, ma alcuni autori di Paesi asiatici e africani manifestano difficoltà per il pagamento anche di queste cifre e per alcuni di loro sono state coperte le spese per un piccolo numero di pagine con i fondi messi a disposizione dalla Società Italiana di Anatomia e Istologia, in una prospettiva di partecipazione dei costi; in questi casi l'articolo riporta i ringraziamenti per il sostegno ricevuto dalla nostra Società. Altra destinazione per i fondi della Società è la copertura di parte non trascurabile delle spese del supplemento con gli atti del congresso annuale, per le quali non è sufficiente quanto mette a disposizione l'organizzazione del congresso. D'altra parte la pubblicazione degli Atti come supplemento dell'Italian Journal of Anatomy and Embryology giova non solo alla Rivista ma anche agli Atti, che così sono diffusi in rete e mantenuti disponibili nel tempo con le stesse modalità della Rivista. La Rivista riceve un buon numero di manoscritti, circa una cinquantina l'anno in media, dei quali sono infine accettati tra una metà e due terzi: quelli non accettati comprendono sia i manoscritti rifiutati sia quelli inviati indietro con la richiesta di una revisione maggiore e mai più rispediti dagli autori alla Rivista. L'Italian Journal of Anatomy and Embryology è censito da SCImago; i dati per la Rivista sono reperibili all'indirizzo web http://www.scimagojr.com/journalsearch.php?q=9500154001&tip=sid&clean=0. Risulta un rango SJR (analogo all'Impact Factor di Web Of Science) di 0,24 e un indice H 25, quest'ultimo in progresso rispetto all'anno precedente quando era 23. Per confronto, l'Anatomical Record per il 2017 ha un SJR di 0,31 e un indice H 79. Tra i manoscritti prevalgono quelli di Anatomia e di Storia della Anatomia: c'è da augurarsi che aumentino quelli di Istologia e Biologia cellulare e facciano la loro comparsa articoli di Embriologia. Un vivo ringraziamento va ai colleghi Italiani che hanno scelto l'Italian Journal of Anatomy and Embryology per pubblicare i loro studi.

12. Proposta di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.

Sono pervenute 24 domande di ammissione a Socio SIAI da parte di:

1. Alberti Paola
2. Bruno Silvia
3. Bertacchini Jessika
4. Branca Jacopo Junio Valerio
5. Cacciola Giorgio
6. Campagnolo Luisa
7. Carubbi Cecilia
8. Ceccarelli Gabriele
9. Cecchini Maria Paola
10. Di Rosa Michelino
11. Flace Paolo
12. Malivindi Rocco

13. Mariotti Raffaella
14. Migliaccio Anna Rita
15. Morucci Gabriele
16. Nicoletti Claudio
17. Passaretta Francesca
18. Rago Vittoria
19. Ronzoni Flavio Lorenzo
20. Salucci Sara
21. Scicchitano Bianca Maria
22. Sommariva Michele
23. Szychlinska Marta Anna
24. Trucas Marcello

Come previsto dallo Statuto, tutte le domande sono corredate dalla firma di presentazione da parte di due Soci.

Il Presidente comunica che il Direttivo ha deciso all'unanimità che i vincitori del Premio alla Carriera:
1. Mario Molinaro (2014)
2. Francesco Osculati (2015)
3. Nadir Mario Maraldi (2016)
4. Alessandro Riva (2017)

vengano insigniti del titolo di Socio Emerito.

In merito ai Soci Onorari, il Presidente comunica che il Direttivo ha deciso all'unanimità di insignire del titolo di Socio Onorario il Prof. Bernard J. Moxham, Immediate Past President dell'Executive Committee dell'International Federation of Associations of Anatomists (IFAA).

L'Assemblea approva all'unanimità tutte le proposte sopra riportate.

13. Risultati elettorali e composizione del Consiglio Direttivo per il triennio 2018-2020.

Il Presidente ringrazia la Commissione Elettorale, composta dai Proff. Saverio Cinti (Presidente), Chiarella Sforza e Angelo Favaloro, e procede a comunicare l'esito delle votazioni.

Hanno votato **153** Soci; **150** sono state le schede valide, **2** le schede bianche e **1** scheda è risultata nulla.

Hanno ottenuto voti:

Giuseppe Anastasi, Presidente, 136 voti
Roberto Di Primio, Segretario, 136 voti
Gianpaolo Papaccio, Tesoriere, 130 voti

Lucio Cocco, Consiglio Direttivo, per l'Anatomia, 132 voti
Stefania Montagnani, Consiglio Direttivo, per l'Anatomia, 128 voti
Carlo Tacchetti, Consiglio Direttivo, per l'Anatomia, 126 voti
Sandra Zecchi, Consiglio Direttivo, per l'Anatomia, 128 voti

Marina Bentivoglio, Consiglio Direttivo, per l'Istologia, 118 voti
Amelio Dolfi, Consiglio Direttivo, per l'Istologia, 129 voti
Antonio Filippini, Consiglio Direttivo, per l'Istologia, 121 voti
Gigliola Sica, Consiglio Direttivo, per l'Istologia, 127 voti.

Il Prof. Gaudio ricorda che le nuove cariche saranno effettive a partire dal 1° Gennaio 2018.

14. Varie ed eventuali.

La Prof. Sica propone che i verbali siano approvati seduta stante dopo ciascuna Assemblea, invece che nel corso dell'Assemblea successiva.

L'Assemblea approva all'unanimità.

L'Assemblea approva il presente verbale seduta stante.

Il Presidente ringrazia i presenti anche a nome del Consiglio Direttivo e, alle ore 18.30, dichiara conclusi i lavori dell'Assemblea.

Il Presidente	Il Segretario	Il Tesoriere
Prof. Eugenio Gaudio	*Prof. Gigliola Sica*	*Prof. Amelio Dolfi*

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[1] Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia, Viale Europa 11, 25123 Brescia, Italy
[2] Clinica Medica, Department of Clinical and Experimental Sciences, University of Brescia, Italy
[3] Division of Medicine, Istituto Clinico Città di Brescia, Brescia, Italy

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[1] Università di Salerno, Dipartimento di Medicina, Chirurgia e Odontoiatria "Scuola Medica Salernitana", Baronissi
[2] Università degli Studi di Trieste, Dipartimento CSID, Trieste

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[1] Unit of Microscopic and Ultrastructural Anatomy, Dept of Medicine, University Campus Bio-Medico Rome, Italy
[2] Dept. of Medicine and Ageing Sciences, University G. d'Annunzio, Chieti-Pescara, Italy
[3-5] National Centre for Drug Research and Evaluation and National Centre Animal Research and Welfare, ISS, Roma, Italy
[4] Dept. of Biomedical and Neuromotorial Sciences, Alma Mater University, Bologna, Italy

Paolo Romagnoli, Direttore responsabile
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