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COMMEMORAZIONE DELLA NASCITA DI GABRIELE FALLOPPIO (MODENA, 1523)

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500th anniversary of Gabriele Falloppio's birth

Alessandro Vercelli

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Gabriele Falloppio (1523-1592), from Modena of noble origins, lost his father Geronimo at the age of just 10, and due to his family's financial problems he had to become a priest, inheriting the canonary with a financial income from his uncle. However, he never exercised his function and after a few years he abandoned the ecclesiastical condition. He studied first in Modena, then in Ferrara and finally in Padua, where it is not certain that he was directly a pupil of Vesalius or rather belonged to his school. He became professor of Pharmacy in Ferrara, then instructor of Anatomy in Pisa and, in 1555, professor of Anatomy, Surgery and Botany in Padua. Finally, he obtained a professorship in Bologna, where, however, he was unable to move and died in the meantime, it seems probably of tuberculosis. His favorite pupil was Hieronymus Fabricius (Girolamo Fabrici d'Acquapendente).

Of him we remember the enthusiasm and skill of the teacher, and his important clinical activity as a surgeon. His work as an anatomist left an indelible mark on all anatomy books. We owe him the description of the uterine tube, the ileocecal valve, the canal of the facial nerve and the hiatus of the superior petrosal nerve (which took their eponym from him). In addition, he is owed some medical terms such as vagina, placenta, cricoid cartilage and tympanum. We owe him one of the first descriptions of the arterial polygon at the base of the brain (which will go down in history as the circle of Willis).

His main work, published at his expenses, "Observationes anatomicae", has no drawings, as it is a commentary on Vesalius' treatise "De humani corporis fabrica", of which some anatomical "errors" are corrected. His contributions to Anatomy are fundamental and range over all systems and organs.

In addition to his teaching activity, Falloppio was an original doctor, and his treatment of nasal polyps is remembered and, shortly before his death, he was the first to have proved the importance of the use of condoms in preventing venereal diseases, after having tested in 1,100 subjects, in a real clinical trial in which none of the subjects fell ill with syphilis, published posthumously in 1594. In reality, although it is one of the first descriptions of the condom, its use was post-coitum, in order to disinfect the organ. He used a small linen cap soaked in a solution of salt and herbs, and sometimes milk, to cover the glans and under the foreskin, held in place by a pink ribbon. A way to block the disease that had taken away his father when Gabriele was a child.

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Keywords: Fallopian tube; canal, hiatus; valve; history; Renaissance; syphilis; condom

INVITED LECTURES

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The Translational Value of Morphology at The Basis of Innovative Therapies for Diabetes, Obesity, Osteoporosis and Breast Cancer

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WHITE ADIPOSE TISSUE

White adipocytes are spherical cells whose volume is 90% composed by a single fat vacuole used to provide energy to the organism in the intervals between meals (1). They also produce hormones that acting on brain induce behaviours for food search (leptin) and ingestion (asprosin) (2, 3).

BROWN ADIPOSE TISSUE

Brown adipocytes are smaller than white and contain several small lipid droplets used to dissipate energy under the form of heat. This is due to the activity of their specific mitochondria protein UCP1 (4).

UCP1 is a protonophore uncoupling fatty acids oxidation from ATP synthesis. Thus, most of the intrinsic energy of fatty acids is dissipated as heat. Of course, physiologic energy consumption became an interesting tool for the treatment of obesity and related disorders.

THE ADIPOSE ORGAN

Organs are dissectible structures composed by at least two different tissues cooperating for a specific physiologic role. WAT and BAT form a unitary dissectible structure fitting the organ definition both in mice and humans (5). Considering the opposite functions of WAT and BAT which cooperative function allows the organ definition?

TRANSDIFFERENTIATION

After chronic cold exposure white convert into brown adipocytes to increase thermogenesis. In chronic positive energy balance brown convert into white adipocytes to allow increase of energy storing capacities because a fasting period is not predictable.

These data, while answering the question suggest also a new basic property of cells: transdifferentiation (6).

Reinforcing this concept, we found that during pregnancy subcutaneous white adipocytes convert reversibly into epithelial cells of alveolar glands producing milk. These cells contain large lipid droplets, thus fitting the adipocyte definition. White adipocytes occupy the white part of the organ, brown adipocytes the brown part, thus we nominated these cells pink adipocytes because of the color of the organ during pregnancy (7).

THE OBESE ADIPOSE ORGAN

Obese, hypertrophic adipocytes produce chemoattractants attracting macrophages and causing chronic low-grade inflammation (8, 9). A large proportion of these macrophages form characteristic histopathology figures that we nominated crown-like structures (CLS) where residual lipid droplets from dead adipocytes are surrounded by actively phagocytic macrophages (10).

This inflammation plays a role linking obesity to type2 diabetes because active macrophages produce substances that interfere with the insulin receptor physiology causing insulin resistance ending, in the long run, in type2 diabetes (11).

TRANSLATIONAL CONCLUSIONS

Considering all together the above-described data open new therapeutic strategies for obesity and consequently of type 2 diabetes (12). Furthermore, considering the white-pink transdifferentiation and chronic inflammation of obese fat several physiopathology implications in breast cancer are becoming more and more evident (13).

Physical activity induces production of a new discovered hormone by skeletal muscles and BAT: irisin (14). We found that the main target of this hormone is bone. Treatment with this hormone of mice is able to treat induced osteoporosis (15).

Keywords: white adipocytes, brown adipocytes, pink adipocytes, transdifferentiation, obesity, type2 diabetes, breast cancer

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The PI 3-Kinase and AKT Signaling Pathway in Cell Biology and Disease: From Discovery to Therapeutics

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The PI 3-kinase (PI3K) and AKT signaling pathway plays a critical role in regulating all aspects of normal cellular physiology, and is also frequently deregulated in human pathophysiologies, most evidently in cancer and diabetes. Growth factors and hormones stimulate PI3K leading to the biosynthesis of the lipid-derived second messenger PIP3. In turn, PIP3 elicits the membrane recruitment of the protein kinase AKT, originally discovered in 1987 by Staal and colleagues as v-Akt, a transforming oncogene. In the early 1990s, three independent groups cloned and described the cellular homolog c-AKT, a serine/threonine protein kinase with a high degree of homology to other AGC family protein kinases. In the ensuing three decades, the mechanisms by which AKT transduces signals to cell growth, proliferation, motility and metabolism were uncovered. Three AKT isoforms exist in humans encoded by distinct genes (AKT1, AKT2, AKT3), and although originally thought to function redundantly, many studies have shown that AKT isoforms have non-overlapping and unique roles in both normal physiology and disease. Similarly, genetic lesions in the PI3K and AKT oncogenes have been described, and many of the genes that contribute to PI3K/AKT pathway activation and also signal termination have been found to be altered in human cancers. Numerous drugs that inhibit PI3K as well AKT have been developed for therapeutic use in patients, and many of these are being evaluated in late-stage clinical trials. During the lecture, I will highlight the major advances in PI3K and AKT field over the past 30 years, with a focus on mechanistic insight into this ubiquitous lipid signaling pathway. Genetic lesions in the PI3K/AKT pathway in human cancers will also be discussed, as well as efforts to target this pathway therapeutically. The second part of the lecture will focus on recent efforts in our laboratory to uncover novel mechanisms of AKT signaling and biology, with an emphasis on breast cancer and with a focus on metabolic reprogramming mediated by AKT. I will also present recent efforts aimed at targeting AKT with novel therapies, including degrader technologies and how these have illuminated novel aspects of AKT biology.

Comunicazioni orali

Neuroscienze

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High resolution MRI in exploring peripheral nerve anatomy and axonal damage in rodent models

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Preclinical animal models are crucial to understand pathogenesis and devise novel treatments for peripheral neuropathies. To meet this goal, highly translational outcome measures are needed. High resolution diffusion MRI could be a surrogate, translational, biomarker to characterise early morphological changes as axonal damage ensues.

We aimed at characterising MRI changes in a consistent model of axonopathy. We exploited a neurotoxic chemotherapy drug, paclitaxel (PTX) to induce a relevant nerve damage (*Pozzi et al., IJMS 2023*). We compared 2 groups (n=12 each, female Wistar rats). Vehicle treated (VEH, iv) and PTX treated (10mg/kg, 1qwx4, iv). At the end of treatment, neuropathy was assessed via a multimodal approach: mechanical allodynia (Dynamic test), nerve conduction studies (NCS) and light microscopy of the caudal nerve. In a *proof-of-concept* and a *feasibility* setting, 7T MRI was performed on rat tails (collected after sacrifice and formalin-fixed, n=3/group) to study caudal nerves and the whole tail anatomy. High resolution anatomical images were acquired by means of a T1w sequence with a voxel size of 50x50x50 µm3. Diffusion weighted images were acquired in five b-shells: b of 500, 2000, 4500, 6000, 8000 sec*mm-2 with 15, 24, 33, 42, 51 isotropically distributed gradient directions and a voxel size of 125x125x125 µm3. Diffusion data were fitted with the Diffusion Tensor Imaging (DTI) classical model and Fractional Anisotropy (FA), Axial, Radial and Mean Diffusitìvity (AD, RD and MD) were computed.

Behavioural test (dynamic test), NCS and histopathology confirmed a relevant axonopathy was induced in PTX group. For what regards MRI, DTI showed a decrease of FA (by 5%) and an increase of diffusivity, being the more relevant variation in RD (by 15%), in the PTX group, if compared to VEH group. These preliminary results may sustain the hypothesis that PTX-related axonal damage leads to an increased water diffusivity in the tissue microstructure. We provided preliminary promising results suggesting the possible role of the 7T MRI to detect axonal damage in rodent models. This technique has a high translational potential and, if confirmed in further studies, may have a prompt clinical translation.

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Keywords: MRI; peripheral neuropathy; peripheral nerve; axonal damage; caudal nerve

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A motor neuron-like 3D model for *in vitro* evaluation of neurodegeneration

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Three-dimensional spheroid models bridge the gap between standard monolayer cultures and *in vivo* tissue environment by means of self-clustering cells to mimic the extracellular matrix (ECM). The NSC-34 cell line, created by fusing mouse spinal cord motor neurons and neuroblastoma cells, is a widely accepted model for studying motor neuron diseases, however, the characteristics of this cell line in 3D context have not been thoroughly investigated. In this work, we generated NSC-34 spheroids using a non-adhesive hydrogel-based micro-wells template. We characterized the formation and development of spheroids over five days by light and electron microscopy imaging and western blot analysis. After 5 days, spheroids showed a diameter size of 377±13 μm, high circularity (0.91±0.07) and a viability of ~92%. At difference with 2D cultures, characterized by adherent cells with heterogenous neuron-like morphology and protrusions, scanning electron microscopy showed that NSC-34 cells in 3D adopt and maintain a round-like shape, enabling a stable structure with membrane connections among cells interspersed by pore-like cell clusters. Ultrastructural analysis of subcellular organelles revealed alterations in mitochondria morphology and positioning as well as the presence of peculiar U-shaped nuclei, suggesting that NSC-34 cells are affected by 3D context. Indeed, when we investigated how the 3D environment shapes the expression of TAR-DNA-binding protein 43 (TDP-43), a known marker of ALS pathology, immunoblot analysis showed reduced expression of the full length TDP-43 in the 3D model, with the appearance of 35 kDa truncated fragment (TDP-35). Our results highlight a different behavior of the NSC-34 cell line in 3D environments and suggest that further studies are needed to explore the potential of NSC-34 spheroids for motor neuron diseases and associated pathologies.

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Keywords: 3D Cell Model; Motor Neurons Diseases; TDP-43

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Effect of age on metabolomic changes in a model of paclitaxel-induced neurotoxicity

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Chemotherapy-induced peripheral neuropathy (CIPN) is one of the most common dose-limiting side-effects of paclitaxel (PTX) treatment. Many age-related changes have been hypothesized to underlie susceptibility to damage or impaired regeneration/repair after nerve injury. The results of these studies, however, are inconclusive and other targets, which might be used as potential biomarkers of nerve impairment, need to be investigated.

Twenty-four young (2 months of age) and 24 adult (9 months of age) Wistar male rats were randomized to either paclitaxel (PTX) treatment (10 mg/kg i.v. once/week for 4 weeks) or vehicle administration. Neurophysiological and behavioral tests were performed to investigate nerve damage at baseline, after 4 weeks and the 2-week follow-up period. Skin biopsies from sacrificed animals were examined for intraepidermal nerve fiber (IENF) density assessment. Blood and liver samples were collected for targeted metabolomics analysis using Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS/MS).

At the end of treatment, the neurophysiological studies revealed a reduction in sensory nerve action potential amplitude ($p < 0.05$) in the caudal nerve of young PTX-treated animals, and in both the digital and caudal nerve of adult treated animals ($p < 0.05$). Behavioral tests revealed a significant decrease in the mechanical threshold in young PTX-treated animals ($p < 0.001$), while adult treated rats showed no significant difference in mechanical threshold compared to controls. Concerning IENF assessment, both young and adult PTX-rats had reduced IENF density ($p < 0.0001$), which persisted at the end of follow-up. Targeted metabolomics analysis showed significant differences in the plasma metabolite profiles between PTX-treated animals developing peripheral neuropathy and age-matched controls, with triglycerides, diglycerides, acylcarnitines, carnosine, long chain ceramides, sphingolipids, and bile acids playing a major role in the response to PTX administration.

Our study identifies for the first time multiple related metabolic axes involved in paclitaxel-induced peripheral neuropathy, and suggests age-related differences in CIPN manifestations and in the metabolic profile.

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Unveiling the network geometry of the human cerebellum structural connectome

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Studying the brain as a network of interconnected nodes and the recent developments of network theory contributed to unveil the key structural principles underlying the topology of the healthy human brain connectome at the macroscale level. However, despite the increasing advances in connectomics and network neuroscience, disentangling the structure and the hidden organization of the cerebellum network is still challenging. Herein, we applied a new class of dimensionality reduction techniques[1,2] in order to explore the hidden network geometry of cerebellar structural connectomes reconstructed from diffusion MRI data of 100 unrelated healthy individuals of the Human Connectome Project repository. Our findings suggest that the cerebellar networks tend to segregate into two distinct sections representing the left and right cerebellar hemispheres. Another noteworthy result was the perfect angular ordering of the nodes in the hyperbolic representation, which closely matched those of the anatomical regions in the flat representation of the human cerebellum[3]. This remarkable alignment highlights the strong agreement between the inferred geometric coordinates and the actual anatomical distribution of cerebellar lobules. Furthermore, the radial coordinates of the cerebellar nodes showed a high correlation with the hierarchical functional organization of the cerebellum as estimated by within-cerebellum connectivity gradients of resting state functional MRI data. Interestingly, the most radial and therefore central nodes were the lobules Crus I and Crus II, highlighting the fundamental role and extreme level of information processing of these non-motor regions. Overall, our findings provide compelling evidence for the accurate representation of cerebellar networks in the embedded geometric space. To our knowledge, this is the first study to reveal the hidden network geometry of the human cerebellar structural connectome.

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Keywords: Brain; connectomics; network neuroscience; neuroimaging; tractography

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The nigro-thalamic dopaminergic pathway in the human brain: a multi-scale and integrated study

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The subcortical network of the substantia nigra pars compacta (SNc), one of the main dopaminergic nuclei of the brain, is well known and mainly represented by the nigro-striatal pathway that exerts a regulatory function on the basal ganglia circuitry. However, dopaminergic innervation of other brain centers has been only investigated in non-human primates and never characterized in humans. The impossibility of tract-tracing studies in humans has boosted advanced magnetic resonance imaging (MRI) techniques that have shed new light on the whole brain connectivity, in particular diffusion tensor imaging (DTI).

We aim to dissect the dopaminergic innervation of the human thalamus using multi-scale and integrated analyses.

First, consecutive human thalamic sections will be processed for immunohistochemistry (IHC) and stained for morphological analysis (Nissl, Haematoxylin and eosin) and for the main dopaminergic markers (TH, VMAT-2, DAT, AADC).

Second, high-resolution MRI segmentation of the SN (that allows the identification of the postero-medial SNc from the gabaergic anterolateral SN pars reticulata - SNr) and multi-shell high-angular resolution diffusion MRI (MS-HARDI) (that allows the tractographic reconstruction of the SNc) will be performed in a group of 10 healthy subjects (age 25-30, sex-matched, 5M, 5F). Finally, PET-FDOPA data coming from healthy subjects (n=20) will be evaluated for both qualitative and quantitative analysis of the thalamic region.

Our preliminary MS-HARDI results performed with two previously validated diffusion MRI schemes on 10 healthy subjects demonstrate a reproducible structural connectivity between the SNc and the thalamus, with up to an average of ~19% of the total number of streamlines encompassing the SNc and the thalamus, with no other major subcortical structures involved (and without necessarily reaching the cortex).

To the best of our knowledge, this is the first report of a direct nigro-thalamic dopaminergic projection, with a multi-scale and integrated approach. The significance and the characterization of these connections, however, is still under investigation. Understanding of these new pathways will lead to the optimization of the treatments for dopaminergic-related disorders, paving the way for targeted and personalized therapies.

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Amyotrophic lateral sclerosis: CXCR2 involvement in motor neurons degeneration

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the degeneration of motor neurons (MNs). Previous transcriptomic analysis conducted in a subset of sporadic ALS patients as well as SOD1G93A mice showed an up-regulation of C-X-C motif chemokine receptor 2 (CXCR2)-mRNA. This receptor and related ligand, CXCL8, is known to be involved in various biological events including apoptotic cell death. Previous papers have demonstrated CXCR2 expression in human ALS cortex and in SOD1G93A mice brain. The aim of the present study was to investigate the functional role of this receptor on motor neuron degeneration during ALS progression. The expression of CXCR2 was evaluated in hSOD1-G93A mice spinal cord. Subsequently, we investigated CXCR2 role and its related ligands in motor neuron-like cells overexpressing hSOD1-G93A, representing a model *in vitro* of ALS. The results showed an increased expression of CXCR2 in G93A-SOD1-expressing cells with respect to wild-type control. Furthermore, CXCR2 activation by GROα and MIP2α, two murine homologs of CXCL8, reduced cellular viability and triggered apoptosis in a dose dependent manner. The treatment with a CXCR2 inhibitor, reparexin, significantly counteracted GROα and MIP2α induced cell death. Overall, the present data confirm the involvement of CXCR2/CXCL8 axis in MNs degeneration, opening a new perspective to ALS therapy.

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Anticancer activity of novel heme oxygenase-1 inhibitors: focus on glioblastoma aggressiveness

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The heme oxygenase-1 (HO-1) is an enzyme that catalyzes the degradation of heme, which is an iron-containing prosthetic group found in proteins that play critical roles in oxygen transport, energy production, and enzymatic reactions. Its aberrant expression is associated with progression and chemoresistance occurring in several cancers. In particular, much evidence has suggested the inhibition of HO-1 as a possible antitumor strategy [1].

The aim of the present study was to identify a new molecule able to interfere with cancer progression through inhibition of HO-1 activity.

In this work we tested the effect of novel acetamide-based HO-1 inhibitors in different *in vitro* models of cancers. Firstly, we detected the basal expression and distribution of HO-1 in prostate (DU145), lung (A549) and glioblastoma (U87MG, A172) cancer cells. Subsequently, we tested the effect of six novel inhibitors on viability and HO-1 protein expression in these cells. Among the synthetized inhibitors, *N*-((4-chlorophenyl)(phenyl)methyl)-2-(1*H*-imidazol-1-yl)-*N*-methylacetamide (VP18/58) was selected to deepen investigation since it was able to significantly reduce viability and HO-1 expression in U87MG cells. To verify the anticancer efficacy of this molecule in glioblastoma, we evaluated VP18/58 effect on cell invasion and neo-angiogenesis process, both events associated with tumor malignancy.

Results obtained have demonstrated that VP18/58 treatment significantly reduced the percentage of cell migration as well as vascular endothelial growth factor (VEGF) intracellular expression and release in the culture medium of U87MG cells. Since VEGF secretion in tumor microenvironment is responsible for aberrant neovascularization [2], we have further investigated the effect of VP18/58 in this process. To this end, we have tested its effect by using endothelial H5V cells that are able to form a network of tube-like structures, mimicking neovessel formation. These cells were cultured with conditioned medium (CM) derived from U87MG cells treated with vehicle (CM1) or VP18/58 (CM2) for 48h. Cells cultured in CM2 showed a lower number of tube-like structures compared to the control group. Overall, the present data suggested that this compound might be useful to counteract glioblastoma aggressiveness through the inhibition of HO-1 activity.

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Effect of aging on the blood-brain barrier

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The blood-brain barrier (BBB), an anatomical and functional structure located at the cerebral microvessels of the central nervous system (CNS), plays a key role in maintaining the specialized microenvironment of neural tissue. The BBB, and more generally the components of the neurovascular unit (NVU), consists mainly of endothelial cells (ECs) sealed by tight junction (TJ) proteins that establish the first barrier between the peripheral circulatory system and the brain parenchyma. As a result of medical advances and improved quality of life, longevity has increased, and it is estimated that the elderly will make up a large percentage of the world's population. Regarding the brain, normal ageing can be defined as a deterioration of bodily activities without cognitive disorders and dementia. Recent studies have shown that the normal ageing process is characterized by BBB disruption and neuroinflammation that further cause neurodegeneration and cognitive disorders. To investigate the possible involvement of the BBB and NVU cell and molecular components in normal ageing and neurodegeneration, we analyzed the expression pattern of TJs proteins (claudin-5 and occludin), vascular basal lamina molecules and neuroinflammation markers (Iba1 and CD45) in the brains of 2-, 7- and 12-month-old mice by immunofluorescence confocal microscopy. The results confirmed the disruption of the BBB together with moderate astrogliosis and a neuroinflammatory state. In conclusion, ageing appears to impair the morpho-functional characteristics of the BBB, which may lead to general cerebral dysfunction. A precise analysis of the brain vasculature and the factors involved in the maintenance and disruption of the BBB could be a valuable contribution to understanding the pathogenetic mechanisms of neurodegenerative diseases.

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Autophagy improves the mitochondrial status by regulating prion protein expression in glioblastoma cells

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Glioblastoma (GBM) features up-regulation of the mechanistic Target of Rapamycin (mTOR), which leads to a number of detrimental effects on disease course. Among the variety of intracellular pathways affected by mTOR hyperactivity, suppression of autophagy is relevant for the neurobiology of GBM. In fact, in GBM cells autophagy inhibition correlates with increased infiltration and increased stemness, while autophagy stimulation halts cell proliferation [1], occludes the expression of stem cell markers [2], hampers cell migration while increasing cell adhesion [2], and clears cells from misfolded proteins and altered mitochondria. In line with this, alpha-synuclein is overexpressed and accumulates within GBM cells, leading to alterations in non-tumoral astrocytes when co-cultured with GBM cells [3,4].

A key protein in the biology of GBM cells is the cellular prion protein (PrPc) [5,6]. In fact, PrPc in GBM PrPc is elevated, it induces proliferation of GBM stem-like cells and contributes to tumorigenesis by impairing mitochondrial biogenesis. This is in line with impaired mitochondrial turnover, which occurs in prion disease [7].

In the present study we demonstrate that in GBM cells mTOR inhibition by rapamycin produces a sudden and concomitant activation of autophagy, which was assessed by measuring autophagy flux, the colocalization of microtubule-associated protein 1A/1B-light chain 3 (LC3) with cathepsin D immunofluorescence, and the merging of autophagosomes with lysosomes using gold standard transmission electron microscopy. This is accompanied by cell cycle arrest at the G_1 phase, as determined by flow cytometry, and a simultaneous decrease in the levels of PrPc immunopositivity. Remarkably, by combining immunofluorescence, immunoelectronmicroscopy and ultrastructural morphometry we demonstrate that autophagy-induced suppression of PrPc by rapamycin persistently increases mitochondrial fission, mitochondrial removal and mitochondria biogenesis.

These data shed new light on the close relationship between autophagy, PrPc expression and mitochondrial status in GBM neurobiology.

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Keywords: rapamycin; LC3, Cathepsin D; Pink1; Parkin; mitochondria biogenesis; mitochondria fission, fluorescent microscopy; immunoelectronmicroscopy

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An immunohistochemical study on the neurotensinergic system in the human dentate nucleus

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Neurotensin (NT) is a neuropeptide distributed in the central and peripheral nervous systems (CNS and PNS). In the CNS, NT is involved in dopamine neuromodulation mechanisms[1]. A role of NT in dopamine-related brain disorders [1,2,3] has been demonstrated. Although, we have recently evidenced the presence of NT and NT receptor subtype 1 (NTR_1) immunoreactive neurons in the human cerebellar cortex [4]. The cerebellum is not considered a neurotensinergic area. In fact, in the cerebellum, only few neurotensinergic extrinsic fibers, and the receptors NTR_2 and NTR_3 have been detected. Therefore, the aim of this study was to evaluate in the human dentate nucleus the presence of an intrinsic neurotensinergic neuronal system. The study was carried out on fragments of postmortem human dentate nucleus 36-48h after death. Each fragment was fixed in an aldehyde and picric acid solution, embedded in paraffin, cut into 5µm sections, and subjected to light microscopy immunohistochemical procedures using goat and rabbit polyclonal antibodies respectively against NTR_1 and NT. For positive controls, fragments of rat intestine subjected to the same experimental procedure were used. In the dentate nucleus, the NT and NTR_1 immunoreactivity were detected in neuronal cell bodies and processes of different neuron types, such as the small associative neurons, large projective neurons, and perivascular neurons. Moreover, in the dentate nucleus the NTR_1 immunoreactivity in form of fine 'puncta' (putative axon terminals) in the neuropil and in close relationship to the wall of microvessels, was also observed. The present study demonstrates in the human dentate nucleus the existence of an intrinsic neurotensinergic neuronal system, which could be involved in neuromodulation mechanisms of intrinsic cerebellar circuits, in the microvascular innervation, and in projective cerebellar circuits with the midbrain dopaminergic areas. In the midbrain the neurotensinergic neuromodulatory action is known. Moreover, we plan to carry out further studies to evaluate the dopamine-neurotensin co-transmission mechanisms in cerebellar circuits. Finally, this study suggests a role of the neurotensinergic cerebellar system in dopamine-related brain disorders and may also lead to apply new transcranial magnetic stimulation therapeutic protocols[5,6].

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The neuroanatomy of Locus Coeruleus in normal ageing and in Alzheimer's Disease

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The Locus Coeruleus (LC) is the main noradrenergic (NA) nucleus of the brain, and plays a crucial role both in brain homeostasis and in the modulation of neuronal networks [1]. LC degeneration represents the earliest pathological feature of Alzheimer's Disease (AD), and it has been associated with several pathogenic mechanisms and pathophysiological pathways involved in AD [2]. A recently developed neuroimaging tool has allowed researchers in the field to assess *in vivo* the integrity of the LC, profiting from specific sequences of Magnetic Resonance Imaging (MRI) [3]. In this study, we used LC-MRI to evaluate the integrity of the LC in a cohort of cognitively intact elderlies and of patients belonging to the AD clinical *continuum*, to dissect the morphological features of the LC during healthy ageing and the alterations it suffers from in the pathological one.

Cognitively intact elderlies (HC), patients affected by AD dementia (ADD) and Mild Cognitively Impaired individuals (MCI) underwent a thorough neurological and neuropsychological evaluation and were submitted to LC-MRI. Then, they were followed-up for 2.5 years, in order to monitor the conversion of MCI individuals to full-blown dementia, dividing them into converters (cMCI) and non-converters (ncMCI). LC images were analyzed using a state-of-the art approach [4] and LC integrity was expressed using two parameters: LC Contrast Ratio (LC_{CR}), a proxy of neuronal density, and LC-belonging voxels (LC_{VOX}), an indirect estimation of LC volume. A sub-group of patients (30 MCI and 13 ADD) underwent also a Brain [18]Fluorodeoxyglucose (FDG) PET, and the association between cortical metabolism and LC integrity was explored using voxel-wise regression.

We included in the final analysis 53 HC, 77 MCI (45 ncMCI, 32 cMCI) and 34 ADD. Significant differences were found among diagnostic groups, mainly driven by the rostral part of the left LC; ADD and cMCI subjects showed significantly lower values of LC_{CR} ($p = 0.042$ and $p = 0.033$, respectively) and LC_{VOX} ($p = 0.018$ and $p = 0.015$, respectively). The same difference was not detected in ncMCI. LC signal was indirectly associated with FDG uptake in the frontoparietal cortex, with left-hemispheric prevalence.

We successfully detected the LC signal in the brainstem acquisitions, located under the floor of the fourth ventricle, and the post-acquisition reconstruction matched the anatomical data about size and length of the LC [5]. We showed that during normal ageing LC does not degenerate significantly. Even more interestingly, we showed *in vivo* that the loss of integrity of the LC occurring in the AD clinical *continuum* mainly involves its rostral subregion. This is in line with anatomical literature, which reports that LC-NA neurons projecting to the entorhinal and limbic cortex are placed in the cranial part of the nucleus [6]. We also found an association between LC integrity and the metabolism of frontoparietal cortices, which are strongly innervated by the LC itself [7]. Applying state-of-the-art neuroimaging technique and using AD as a "lesion model", we indirectly confirmed some of the key anatomical features of the

LC-NA system, observing *in vivo* what had been previously studied only *post-mortem*.

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Environmental pollutants affect the hypothalamic GnRH system development

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Several environmental pollutants may act as endocrine disruptor chemicals causing adverse effects on many physiological functions, including reproduction. Indeed, there is growing evidence that hazardous environmental contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals may affect neuroendocrine circuits controlling the reproductive axis. In particular, the hypothalamic gonadotropin-releasing hormone (GnRH) system appeared as a critical target. Using human fetal GnRH neuroblasts (FNCB4), we already demonstrated that benzo(a) pyrene (BaP), a prototypic PAH, affects their migratory properties, thus interfering with a crucial step of GnRH neuron maturation [1-2]. Moreover, BaP altered the ability of these neurons to respond to kisspeptin, the main physiological regulator of GnRH neuron activity. Here, we extended our studies analyzing the effect of cadmium (Cd), the main heavy metal environmental toxicant of anthropogenic origin, on FNCB4 maturation. Cd (10μM, 24h) induced oxidative stress and COX2 mRNA increase in FNCB4, as well as altered cell membrane properties and excitability. As a functional effect, Cd significantly reduced FNCB4 migration, and the expression of genes crucially involved in this process during fetal development. Cd-treated FNCB4 also exhibited cytoskeletal F-actin disassembly and a significantly increased NCAM1 mRNA expression, while Cd exposure significantly reduced GnRH and kisspeptin receptor (KISS1R) expression. Cd also affected the formation of primary cilium, a sensory no-motile organelle required for neurogenesis and KISS1R signaling in GnRH neurons. In conclusion, our data suggest that exposure to environmental toxicants, especially during fetal development, may influence human reproductive function.

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Autosomal Dominant Leukodystrophy: Deciphering the new role of astrocytes

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Autosomal Dominant Leukodystrophy (ADLD) is an ultra-rare and fatal late-onset neurodegenerative disorder that affects the central nervous system myelination and lacks effective therapy. The disease is caused by lamin B1 (*LMNB1*) gene alteration that leads to demyelination with the disease mechanisms remaining unknown. Although oligodendrocytes are responsible for myelination, astrocytes and ADLD patients' cells overexpressing *LMNB1* have displayed nuclear alterations with activation of proinflammatory and oxidative stress mechanisms that were absent in oligodendrocytes[1,2]. The present study involved the characterization of astrocytes overexpressing lamin B1 and the elucidation of their new role in demyelination. Human astrocytes (HA) were transfected with *LMNB1* and sorted for two assays: 1) characterization of astrocytes for expression of proinflammatory markers, and 2) myelination assay on 3D microfiber co-cultures with oligodendrocyte precursor cells (OPCs). For the characterization, immunocytochemical analysis displayed nuclear localization of NFAT4 (nuclear factor of activated T cells 4) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) suggesting astrocytic activation and inflammation. The activation was also confirmed with proteome array of the transfected HA supernatants. For the myelination assay the sorted HA were co-cultured with human OPCs on a microfiber scaffold for two weeks. It was displayed that OPCs were unable to produce myelin basic protein when grown with HA overexpressing *LMNB1* indicating the crucial role of astrocytes in supporting myelination. Overall, the study elucidated that *LMNB1* overexpression leads to astrocyte activation that consequently triggers inflammatory states and hinders myelination. Thus, these novel findings could place astrocytes at the epicenter of ADLD demyelination and drug development studies.

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Keywords: Lamin B1; ADLD; astrocytes; neurodegeneration; demyelination; inflammation

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The kisspeptin system inhibits neurogenesis in peripubertal rats by modulating the Transient Receptor Potential Vanilloid 1 (TRPV1)

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Increasing evidence shows that Hypotalamic-Pituitary-Gonadal Axis (HPG) circuits and adult neurogenesis (Trova et al., 2020) interact each other, and HPG axis participates to the fine-tuning of neural plasticity through mechanisms that are not yet defined. Since the kisspeptin system (KpS) is the gatekeeper of HPG axis, we studied the localization and expression of kiss1 and kissR in the hippocampus of peripubertal male rats and analysed the effects of Kiss treatment (Kp-10 peptide) on adult neurogenesis. Male rats (n=4/group, PND38) were injected (i.p.) with a single dose of BrdU (300 mg/kg b.w.), then Kp-10 was administered for other three weeks. Brains were removed, fixed and coronal sections were sampled from bregma -2.04 to -5.04 mm for immunohistochemical analyses. Results showed that Kp-10 treatment reduced the number of BrdU/NeuN positive cells in the DG and this was associated with increased Kiss1 expression in the sub granular zone (SGZ) of the DG but not to significant variations of KissR expression. Since it is well known that the endocannabinoid system (ECS) plays a role in adult neurogenesis (de Oliveira et al., 2019), we also analysed if ECS could be targeted by Kp-10 treatment. We analysed the possible variations in the expression of the most expressed ECS receptors in the hippocampus, the CB1 receptor (CB1R) and the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor which are both involved in neuronal proliferation, differentiation and plasticity. Results indicated that CB1R expression was not affected upon Kp-10 treatment while TRPV1 expression was highly induced in the cell bodies and dendrites of both DG (and CA3). WB analysis of hippocampal proteins also showed that Kp-10 induced BDNF and SIRT1 expression while reducing pERK 1/2, thus suggesting a role for these proteins in the Kp-10 mediated reduction of neurogenesis. These data suggest that the kpS could modulate neurogenesis interfering with the ECS and its downstream regulated pathways.

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Keywords: kisspeptin; endocannabinoid system; TRPV1 receptor; neurogenesis

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Microtubule stabilisation and mitochondrial dysfunctions as axonal degeneration mechanisms in bortezomib-treated sensory neurons

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The causal mechanisms underlying chemotherapy-induced peripheral neuropathy (CIPN) are not yet fully understood, but primary afferent neurons have emerged as a vulnerable initiating pathophysiological target. Bortezomib (BTZ) is a proteasome inhibitor, which results in axonal degeneration, producing in patients a disabling painful peripheral neuropathy that undermine its therapeutic efficacy.

In the current study, we examined whether treatment with BTZ for 24 hours would affect microtubule (MTs) stability as well as axonal transport and oxidative phosphorylation or mitochondrial bioenergetics in primary cultured dorsal root ganglion (DRG) sensory neurons. MT stability was indirectly measured by western blotting and quantitative immunofluorescence microscopy of delta2 tubulin and tubulin acetylation, while axonal mitochondrial trafficking was evaluated by time-lapse confocal microscopy and kymograph analysis. To evaluate the effects of BTZ on the generation and regulation of cellular bioenergetics, mitochondrial oxidative phosphorylation (OXPHOS) and fusion/fission balance were described via western blot analysis of key molecular markers, and real-time oxygen consumption rate (OCR), an indicator of mitochondrial respiration, was obtained by Seahorse bioanalyser.

BTZ-treated sensory neurons induced an approximately 2.5-fold increase of delta2 and acetylated tubulin levels, which occurred at the onset of axonal degeneration. Furthermore, DRG axonal mitochondrial motility was decreased by BTZ. Finally, BTZ treated DRG neurons had no differential protein expression of OXPHOS subunits compared to untreated DRG neurons, whereas increased Mitofusin-2 protein expression and bioenergetics deficits were reported.

In summary, our results provide support for the role of MT stability and mitochondrial dysfunction in the development of BTZ mediated neuropathy. Understanding these pathways may provide therapeutic targets for the treatment of this debilitating complication.

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Keywords: neurotoxicity, bortezomib, microtubule stability, bioenergetics, mitochondrial trafficking, confocal microscopy

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Dissecting glioma microenvironment: validation of a new protocol for secretome extraction from glioma patients' tissue

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Glioma microenvironment refers to the complex cellular and molecular surroundings in which glioma cells reside, encompassing interactions with immune cells, blood vessels, extracellular matrix components, and signalling molecules, all of which contribute to tumour progression and therapeutic responses [1]. In particular, the tumour *secretome* refers to the set of proteins, growth factors, cytokines, and other molecules that are actively secreted by the tumour mass. Characterizing glioma *secretome* is essential as it provides valuable insights into the communication occurring within the tumour mass, aiding in understanding glioma pathogenesis, identifying potential therapeutic targets, and developing personalized treatment approaches. However, current research on glioma *secretome* is based on 2D cultures and co-cultures that possess evident liabilities, first and foremost the inability of recapitulating a comprehensive picture of all the cellular populations composing the tumour mass. Under this light, we adapted and validated a method to extract the *secretome* directly from glioma patients' tissue. This method, based on the physiological stimulation of surgical biopsies to induce *secretome* spontaneous release [2], is currently validated on intestinal mucosal tissue [3], but was never tested before on brain tissue. Thanks to the set-up of a fast and standardized workflow between the operation room and the research laboratory, it was possible to successfully extract the tumour *secretome* from three patients. Finally, as proof of concept, a preliminary profiling of the *secretomes* was performed using a proteome array detecting 84 different cancer biomarkers simultaneously. These preliminary data demonstrate that the direct extraction of the *secretome* from glioma tissue is not only possible, but can also enable further patient profiling, thus providing personalized, comprehensive and reliable information regarding glioma microenvironment.

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Keywords: glioma; tumour microenvironment; secretome extraction; personalized medicine

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The pivotal role of astrocytes and their interaction with endothelial cells in blood-brain barrier formation and function

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The blood-brain barrier (BBB) is an essential cellular structure owning the role of strictly select the molecules that can enter the brain parenchyma, thus maintaining the homeostasis within the central nervous system keeping out toxic substances [1]. In order to comply with this physiological function, brain endothelial cells are closely sealed to each other by tight junctions (TJ). Moreover, other two cellular types, astrocytes and pericytes, key components of the neuro-vascular unit [2], are required to generate a structurally and functionally complete BBB.

In order to study the structural features of the BBB, various *in vitro* models have been used, even if little attention has been paid to the correct subcellular distribution of TJs. In order to study the morphological and molecular patterns of these proteins, in the present research we used two rat cell lines, brain endothelial cells (RBE4) and astrocytes (DITNC1), in order to highlight their role in the establishment of an intact BBB.

The cells were cultured from 3 to 7 days in different conditions. The RBE4 cells, when cultured alone, were stimulated with 1 and 5 μM retinoic acid (RA), a well known molecule synthetized by astrocytes and pivotal for BBB development [3,4]. The RBE4 were cultured with the DITNC1, both through the interposition of a transwell or in direct contact. The expression and the correct localization of claudin 5 was examined.

The western blotting analysis clearly showed that, in the absence of RA, RBE4 express claudin 5 but the immunofluorescent analysis failed to evidence its subcellular distribution. On the other hand, when cells were treated with RA, immunofluorescence shows a scattered spot distribution, very different from the normal localization on the cellular perimeter.

Interestingly, only when the RBE4 cells were co-cultured with DITNC1 the claudin 5 was correctly expressed and distributed alongside the perimetral surface of the endothelial cells, distinctly in the contact co-cultures.

Even if the RBE4 cell line provides a validated *in vitro* BBB model, the presence of astrocytes plays a key role both in inducing the expression of the tight junction proteins such as claudin 5, and the correct distribution in the perimeter of the cells in order to seal each other, thus highlighting the capacity of DITNC1 astrocyte in the contribution of BBB components.

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Keywords: astrocytes; brain endothelial cells; blood-brain barrier; tight junctions; ZO-1; claudin 5

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ActR-Fc-nLG3: a novel biological to increase neuromuscular junction stability and endurance in old sarcopenic mice, by synergistically acting on myostatin and agrin pathways

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Sarcopenia is the primary cause of impaired motor performance in the elderly, also responsible for increased morbidity and mortality. The current prevailing approach to counteract such conditions is increasing muscle mass through inhibition of the myostatin system. However, this strategy is not able to sustain per se the innervation of the hypertrophic muscle, and only moderately improves muscular strength, causing a progressive worsening of the motor performances. In this scenario, we have synthetized a novel biological compound, named ActR-Fc-nLG3, by combining the soluble activin receptor (a strong myostatin inhibitor) to the C-terminal agrin nLG3 domain. This compound has the potential of reinforcing neuromuscular stability to the hypertrophic muscle. Indeed, we have previously demonstrated that young mice treated with ActR-fc-nLG3 are capable of remarkably enhancing the endurance of their motor tasks (rotarod), with a modest gain of muscle mass, compared to common myostatin inhibitors (ActR-Fc). Now we extended this observation by demonstrating that also in aged (2 years-old) mice, long-term administration of ActR-Fc-nLG3 increases in a sustained way both muscle strength and endurance, compared with the administration of the ActR-Fc alone and the control vehicle (PBS). Histological data showed that the effects of this new biological are due to the improvement in neuromuscular stability, with preservation of endplate's innervation and changes in neuromuscular junctions (NMJs) morphology and dimensions, inducing only moderate hypertrophy. The presence of increased membrane folds in the postsynaptic site offers a possible explanation to the increased endurance as a result of improved efficiency of the neurotransmission at the NMJ level. Thus, our peptide may represent a valid option for treating disorders of the striatal muscle tissue, together with muscle dysfunction caused by altered neuronal input to the muscle, raising the hope that a therapy may be developed not only for sarcopenia but also for other neuromuscular disorders.

Keywords: aging; sarcopenia; neuromuscular junction; muscle innervation; endurance

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Volumetric MRI study of Virchow Robin spaces: a potential biomarker of glymphatic system dysfunction

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Perivascular spaces (PVS), also known as Virchow-Robin spaces, are located around penetrating arteries and are enclosed by astrocytic endfeets expressing aquaporin-4 (AQP4). AQP-4 channels help cerebrospinal fluid move into the brain and towards lymphatic drainage. This network, called the glymphatic system (GS), helps to maintain fluid transportation and waste removal in the brain. A rater uses established visual rating scales to grade the severity of PVS enlargement. Algorithms can automate the segmentation and labelling of PVS structures, saving time and effort on manual labelling. Using one of these automatic algorithms, we conducted a single-centre case–control study on PVS in children with autism spectrum disorder (ASD). We examined the medical records of patients with ASD and those without. We used a 1.5 Tesla Philips MRI scanner to perform standard MRI scans. PVS were defined as "enlarged" when there were at least 3 PVS whose diameter perpendicular to the PVS' main axis was above 3 mm. We used a multi-modal approach combining T1 and T2 images to quantify PVS. An algorithm was used, and the images were adaptively filtered to remove non-structural high-frequency noise. To normalize the PVS volume value, we used the individual's white matter amount (WM-PVS).

A total of 136 patients were evaluated. The median age in the total cohort was 4 years. We found that males had higher WM-PVS volume than females ($p = 0.01$). When categorising children with ASD according to age, those younger (below 4 yrs) had higher median WM-PVS volume and more frequent WM-PVS grade 4 compared to those older (at least 4 yrs). Our findings indicate that ASD male patients commonly exhibit PVS dilation as a neuroimaging feature, especially in those who are younger than 4 years old. PVS could serve as a biomarker for the identification of the impairment of GS.

Keywords: Virchow Robin spaces; perivascular spaces; glymphatic system

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Nanocomposite conductive nerve conduits for peripheral nerve injury recovery, a comparative *in vitro* and *in vivo* study

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Management of severe peripheral nerve injuries is a challenge in clinical practice as none of the commercially available devices distinguishes over autografts. Currently, intense research is devoted towards nerve conduits (NC) improvement and introduction of electrical cues seems to be extremely appealing. In consideration of that, this work aims to analyze, through in vitro and preclinical studies, the effectiveness of vanguard nanocomposite conductive NC based on the new polymer oxidized polyvinyl alcohol (OxPVA) [1] and multiwalled carbon nanotubes (CNT). The OxPVA+CNT hydrogel was prepared and then assayed for ultrastructure/in vitro electroconductivity/bioactivity/cytotoxicity/in vivo biocompatibility. Then, derived OxPVA+CNT conduits were investigated for ultrastructure and mechanical behaviour prior to be implanted in an animal model of disease (Sprague Dawley rat; sciatic nerve, gap: 5 mm). Four experimental groups were compared (reverse autograft/Reaxon/OxPVA/OxPVA+CNT) and their effectiveness was verified after 6 weeks through histological/immunohistochemical analyses/morphometric studies. Gathered data highlighted that hybridization with CNTs conferred to OxPVA a rougher surface than CNT-free OxPVA; an increased superficial electroconductivity was detected and potential CNT-related toxicity was excluded. No thick fibrotic capsule was identified surrounding subcutaneous implants, suggesting biocompatibility. All NCs supported nerve regeneration (S100/β-tubulin) without severe inflammatory reaction (CD3/F4/80). Morphometric studies showed higher cross-section area and fasci area for RA>OxPVA+CNT>Reaxon>OxPVA; epineurium thickness was higher for RA>OxPVA>Reaxon>OxPVA+CNT; density of myelinated axons was higher for OxPVA+CNT>OxPVA>RA>Reaxon; myelinated axons total number was prevalent in RA>OxPVA+CNT>Reaxon>OxPVA. The entire cohort had operated limb gastrocnemius atrophy. Incorporation of CNT may be an appealing strategy to improve OxPVA devices outcomes.

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Keywords: peripheral nerve injury; nanocomposite nerve conduits; oxidized polyvinyl alcohol; multiwalled carbon nanotubes

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Functional anatomy of the insula and of the von Economo neurons

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The human insula is hidden in the depth of the cerebral hemisphere by the overlying frontal and temporal opercula, and consists of three cytoarchitectonically distinct regions: the anterior agranular area, posterior granular area, and the transitional dysgranular zone; each has distinct histochemical staining patterns and specific connectivity. Even though there are several studies reporting the functional connectivity of the insula with the cingulated cortex, its relationships with other brain areas remain elusive in humans. From the functional point of view, it is involved in interoception, the feeling of physiological conditions of the whole body (not only viscera).

von Economo's neurons (VENs) are large, spindle-shaped projection neurons in layer V of the frontoinsular (FI) cortex, and the anterior cingulate cortex. VENs have been identified in humans, chimpanzee, bonobos, gorillas, orangutan and, more recently, in the macaque. The phylogenetic distribution may suggest a correlation among the VENs, brain size and the "social brain." VENs may be involved in the pathogenesis of specific neurological and psychiatric diseases, such as autism, callosal agenesis and schizophrenia. VENs are selectively affected in a behavioral variant of frontotemporal dementia. On the contrary, a higher density of VENs is found in superagers, compared to elderly controls and people affected by mild cognitive impairment.

Our findings document two major complementary networks involving the ventral-anterior and dorsal-posterior insula: one network links the anterior insula to the middle and inferior temporal cortex and anterior cingulate cortex, and is primarily related to limbic regions which play a role in emotional aspects; the second links the middle-posterior insula to premotor, sensorimotor, supplementary motor and middle-posterior cingulate cortices, indicating a role for the insula in sensorimotor integration. The clear bipartition of the insula was confirmed by negative correlation analysis. We have recently shown that the salience network is altered in patients affected by schizophrenia compared to healthy controls, i.e. VEN-enriched cortical areas are associated with an altered resting-state brain activity in people with SZ.

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Keywords: Insula; interoception; resting state; von Economo neurons

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Neuroinflammation on hippocampus of hypertensive rats: possible protective activity of antioxidant and cholinergic cognitive enhancing compounds

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Several well-known risk factors, including hypertension, obesity, high cholesterol, dyslipidemia, and diabetes, are recognized in the pathogenesis of cerebrovascular disease and cognitive impairment[1].

Arterial hypertension is accompanied by high levels of cellular reactive oxygen species, cholinergic pathways dysfunction, and neuroinflammation. These changes contribute to the development of brain disorders [2]. In spontaneously hypertensive rats (SHR), brain white matter atrophy and neurovascular unit (NVU) dysfunction accompanied by gliosis and neuroinflammation are noticeable.

Our study has investigated if long-term treatment with CDP-Choline (CDP) and (+)-thioctic acid (TIO), alone or in association, could induce neuroprotection. CDP shows potential nootropic activity, increases acetylcholine synthesis/release, and is involved in preserving cell membrane phospholipids. TIO acts as a free radical scavenger, repairing oxidative damage and regenerating endogenous antioxidants.

24-week-old SHR rats were treated for four weeks with CDP and TIO alone or in combination. Wistar Kyoto rats were used as a normotensive reference. Western blot (WB) and immunohistochemistry (IHC) of neuronal, glial, and inflammatory markers were performed in the hippocampus as the main brain area correlated with cognitive functions.

Treatment with CDP and TIO alone or in association slightly decreased systolic blood pressure. WB and IHC results showed that CDP and TIO countered gliosis and microglial activation, decreased the level of interleukin-1 beta and tumor necrosis factor-alpha possibly related with modulation of alpha-7 nicotinic acetylcholine receptor.

Our findings can contribute to better defining the role of the inflammatory processes of NVU in brain disorders characterized by vascular impairment. Moreover, the use of cholinergic neurotransmission enhancers associated with an antioxidant molecule could represent a therapeutic strategy worth to be investigated in further preclinical and clinical studies.

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Keywords: hypertension; neuroinflammation; cholinergic cognitive enhancer; thioctic acid

Cellule staminali,
dalla biologia cellulare alle prospettive terapeutiche

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Perinatal Stem Cell Spheroids: Insights into Immunomodulation for Type 1 Diabetes Therapy

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Type 1 diabetes mellitus (T1DM) is a complex metabolic disorder characterized by a substantial depletion of insulin-producing cells. The underlying cause of this cell destruction is attributed to an autoimmune response. Currently, the only efficacious treatment represents the daily administration of exogenous insulin. On the other side, there are no effective treatments targeting the underlying immunological cause of T1DM, then considerable research attention has been directed towards stem cell therapy. Specifically, the utilization of three-dimensional (3D) cell cultures to better recapitulate *in vivo* conditions would increase the immunomodulatory and differentiative capacity of cell therapy.

The goal of this study is to provide a reliable cellular model that could be investigated for regenerative medicine applications and evaluate its immunomodulatory capacity as a possible cell therapy for T1DM. To pursue this aim we created a co-culture spheroid of amniotic epithelial cells (AECs) and Wharton's jelly mesenchymal stromal cells (WJ-MSCs) in a one-to-one ratio. The resulting co-culture spheroids were analyzed for viability, extracellular matrix production, and hypoxic state. Moreover, the immunomodulatory ability of spheroids was evaluated by co-culturing with activated PBMCs and T cells. Our results suggest that co-culture spheroids are stable in long-term culture and are still viable with a consistent extracellular matrix production evaluated with immunofluorescence staining. Our model is also able to modulate PBMCs and T cells by reducing both their proliferation and activation. In conclusion, our co-culture model may potentially be applied for regenerative medicine applications in T1DM.

Keywords: type 1 diabetes; amniotic membrane; umbilical cord; perinatal cells; immunomodulation; Wharton's jelly; cell therapy; regenerative medicine; 3D culture

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Molecular profiling of PLCs and cytokine secretion after PLCB1 modulation in hematopoietic cells and bone marrow microenvironment

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Nuclear Phospholipase C (PLC) β1 plays an important role in the progression of Myelodysplastic Syndromes (MDS) to Acute Myeloid Leukemia (AML)[1]. Hematopoietic Stem Cells (HSCs) usually live within the bone marrow (BM) microenvironment, where the presence of mesenchymal stromal cells (MSCs) regulates the hematopoiesis by interacting directly with HSCs or secreting specific molecules[2]. Here we investigated the role of PLCβ1 modulation in leukemic cells, analyzing PLCs expression and myeloid differentiation markers. Moreover, we studied the interactions between leukemic cells and MSCs, focusing on the BM response, in terms of cytokine secretion. THP-1 cells were induced to overexpress PLCβ1 and both wild-type or PLCβ1 overexpressing cells (THP-1 OV) were also co-coltured with HS-5 cells (i.e., MSCs) for 96 hours. Our results showed, in THP-1 OV cells, a reduction of PLCγ1 and PLCγ2 genes and a decrease of myeloid differentiation markers. However, when THP-1 OV cells were co-cultured with HS-5 cells, no significant differences were observed in the expression of PLCs and myeloid markers of THP-1 cells. Instead, after co-culture with THP-1 OV cells, HS-5 cells showed a significant increase of PLCβ1 and PLCγ2 expression, suggesting that leukemia cells expressing high levels of PLCβ1 could activate specific signalling pathways, that are normally silent, within the mesenchymal counterpart. Moreover, THP-1 OV cells showed inhibition of IL-8 secretion, while in co-colture, regardless of PLCβ1 expression levels, THP-1 OV cells showed a selective secretion of IL-1β and the absence of IL-8. All in all, our results show that PLCβ1 may be involved in leukemic transformation and tumor microenvironment mechanisms, providing new hints to identify new therapeutic targeted therapies. However, further studies are needed to analyse also MDS or AML patients, to confirm these preliminary data and better understand the role of PLCβ1 in MDS to AML progression using primary samples.

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Keywords: Myelodysplastic Syndromes (MDS); Acute Myeloid Leukemia (AML); Hematopoietic stem cells (HSCs); Phospholipase C; Myeloid differentiation; Cytokines

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Mitochondria alteration in Mesenchymal Stem Cells: another participant in the onset of psoriasis

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Psoriasis is an immune-mediated skin disease characterized by inflammation, oxidative stress, deregulated proliferation and abnormal differentiation of keratinocytes. To date, the causes that lead to the development of the disease are still unclear and current therapies do not lead to a definitive recovery. Moreover, not much is known yet about the behavior of mitochondria in psoriasis, despite their direct involvement in oxidative stress and inflammation.

Therefore, understanding the molecular mechanisms involved in the pathogenesis is essential for the development of new more targeted therapies. Our previous works demonstrated that mesenchymal stem cells (MSCs) isolated from psoriatic skin showed pathological features related to psoriasis, such as hyperproliferation, Th1-Th17 imbalance and oxidative stress. Consequently, the hypothesis that psoriasis could start at staminal level opens to other remarkable evidence: MSCs could become the real pharmaceutical target and only their treatment will allow to reach a lasting result.

In this work, we have investigated morphological and functional aberrations of mitochondria in psoriatic mesenchymal stem cells (PSO-MSCs) and their behavior following oxidative and pro-inflammatory stimulations. Our results show that PSO-MSCs present more elongated and interconnected mitochondria, mainly due to downregulation of the fission factor dynamin related protein-1 (DRP1), and a greater resistance to oxidative and inflammatory stress than MSCs isolated from healthy subjects (C-MSCs). These morphological features result in a higher mitochondrial calcium uptake and ROS production compared to C-MSCs. Furthermore, our results demonstrate that mitochondrial dysfunctions predispose psoriatic cells to apoptosis induced by oxidative stress or pro-inflammatory stimuli.

Treatments of PSO-MSCs with adalimumab, an anti-TNF-alpha human monoclonal antibody, restore mitochondrial morphology and functions in resting conditions, but has limited effects in counteracting the damage induced by pro-inflammatory and oxidative stimulations.

These findings designate that mitochondrial alteration in psoriasis could represent a new interesting starting point for new innovative researches on the pathogenesis molecular mechanisms and support the key role of MSCs in the onset and development of psoriasis.

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Keywords: Mesenchymal stem cells (MSCs); psoriasis: mitochondria; oxidative stress; inflammation

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Antenatal exposure to Bisphenols and Perfluoroalkyls: implication for development and fertility

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Bisphenols and Perfluoroalkyls are chemical compounds widely used in industry to produce plastics, food packaging and very common household items. They are known to be endocrine disruptors (EDs), indeed, once ingested through contaminated aliments, they mimic the activity of endogenous hormones leading to a broad spectrum of diseases. Due to the extensive use of plastic in human life, particular attention should be paid to antenatal exposure to Bisphenols and Perfluoroalkyls since they cross the placental barrier and accumulate in fetal serum, amniotic fluids and placental tissues. Here we investigated the effects of Bisphenol-A (BPA), Bisphenol-S (BPS), perfluorooctane-sulfonate (PFOS) and perfluorooctanoic-acid (PFOA), alone or combined, on human-induced pluripotent stem cells (hiPSCs) that share several biological features with the pluripotent stem cells within the blastocysts. Our data show that the EDs affect hiPSCs inducing great mitotoxicity and dramatic changes in genes involved in the maintenance of pluripotency, germline specification, and epigenetic regulation. We also evidenced that these chemicals, when combined, may have additive, synergistic but also negative effects. All these data suggest that antenatal exposure to these EDs may affect the integrity of stem cells in the developing embryos, interfering with the critical stages of early human development that might be determinant for fertility. The observation that the effects of exposure to a combination of these chemicals are not easily foreseeable further highlights the need for wider awareness of the complexity of the EDs effects on human health and of the social and economic burden attributable to these compounds.

Keywords: human induced pluripotent stem cells; endocrine disruptors; pluripotency genes; germline specification; DNA methylation; bisphenols; perfluoroalkyls

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Modulation of the inflammatory response in mesenchymal stem cells induced to osteogenic differentiation by composite biomaterial for bone tissue regeneration

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Bone repair after fracture is a complex process that leads to new bone formation through sequential cellular and molecular events regulated by systemic and local factors [1]. Inflammation is an important biological process that should be considered to develop successful biomaterial-based therapeutics, since prolonged inflammation can result in delayed wound healing or, in some cases, biomaterial rejection and additional tissue damage [2,3].

This study aims to assess the potential of mesenchymal stem cells (MSCs) in modulating the inflammation during osteogenic differentiation induced by the hydroxylapatite (HA)/Collagen composite biomaterial, which is currently used in maxillofacial surgery. MSCs were cultured on the biomaterial for 21 days. Upon differentiation, cells were tested for matrix mineralization and gene expression profile, using the Real-Time PCR Arrays for osteogenesis and inflammation. MSCs grown on the biomaterial produced more mineralized matrix than those grown on tissue culture polystyrene vessels, used as control. Among Differentially Expressed Genes (DEGs) involved in osteogenic differentiation (n=31), the transcription factor SP7, secreted phosphoprotein 1 (SPP1) and bone gamma-carboxyglutamate (gla) protein (BGLAP) were over-expressed in human MSCs grown on the biomaterial compared to control. Moreover, n=24 DEGs involved in inflammation were detected in human MSCs, compared to control. Interestingly, the anti-inflammatory interleukin IL-10, was over-expressed, whereas several pro-inflammatory cytokines and chemokines, including interleukin IL-6, were down-expressed in MSCs grown on the biomaterial, compared to control. In conclusion, this investigation indicates that MSCs combined with the composite biomaterial may enhance bone wound healing, possibly lowering the inflammation burden. Therefore, autologous MSCs implanted with the composite biomaterial could successfully be used for bone tissue regeneration in maxillofacial surgery.

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Keywords: Stem cell; osteogenic differentiation; inflammation; biomaterial; bone

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Reassembling *in vitro* the niche of Parvovirus B19 infection: a stem cell co-culture model

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Bone marrow erythroid progenitor cells (EPCs) are one specific target of infection by the Parvovirus B19 (B19V), a human pathogenic virus that can cause serious anemia in immunodeficient people. Moreover, characteristics of B19V include the ability to cross the placenta, infecting the fetus, and persist in several other tissues. The bone marrow hematopoietic niche, including the compartment of mesenchymal stromal cells (MSCs), is an important component in defining the tropism of this virus, thus a co-culture model based on the interaction between EPCs and MSCs would reveal new insight into the viral pathogenesis.

We aimed to investigate the dynamics of B19V infection in different cell culture conditions: monocultures of MSCs or co-cultures of MSCs with EPCs generated *in vitro* from PBMCs.

By studying adult and perinatal sources of MSCs, we found that MSCs constitute a non-productive environment with no significant viral replication; however, they can serve as a reservoir of B19V DNA.

In the co-culture system including Bone Marrow MSCs and EPCs, we identified two sub-fractions of EPCs that we indicated as adherent or not adherent cells. These distinct cell populations sustained B19V infection at a different extent, suggesting a distinct pattern of permissiveness. Viral transcription and protein expression analysis confirmed the diverse permissiveness observed among co-cultured EPCs.

Considering these findings, our study could have fundamental implications for safety in transplantation biology and in cellular therapy strategies involving MSCs.

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Keywords: stem cells; bone marrow; MSCs; perinatal cells; viral infection; parvovirus; co-culture; host pathogen interaction

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Progenitor cell niches in the duodenum and pancreatic duct system and pancreatic endocrine fate commitment

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The liver, biliary tree and pancreas derive embryologically from a common precursor in the primitive duodenum. Niches of endoderm progenitors remain in specific anatomical locations in adult organs. Our aim was to study the phenotype of progenitor cell niches in duodenum and bilio-pancreatic system, and their pancreatic endocrine differentiation potency.

Human organs from control (n=5) or type 2 diabetes mellitus (T2DM: n=5) subjects were studied. *In vitro*, progenitor cells were isolated from human duodenum and cultured in a well-defined pancreatic differentiation medium (PDM). In mice (n=10), pancreatic islets were damaged by streptozotocin (STZ) injection. Samples were examined by immunohistochemistry, immunofluorescence and RT-qPCR.

In normal organs, cells with a progenitor-like phenotype were present in duodenal submucosal glands (dSG), pancreatic duct glands (PDG), and peribiliary glands (PBG). Insulin+ cells were rarely found in PDGs and PBGs, but not in dSGs. In T2DM, the β-cell loss was associated with a higher proportion of central islets, an increased proliferation of PDGs, and the appearance of pancreatic islet-like structures within pancreatic duct walls. Moreover, insulin+ cells appeared within PBGs and, rarely, in dSGs. Isolated dSG cells from normal human duodena showed *in vitro* capability to commit toward endocrine pancreatic fate when cultured in PDM. STZ mice were characterized by an overall reduced islet area, by an increased proportion of central islets, and by modifications in PDG and dSG cell compartment.

Progenitor cells in adult bilio-pancreatic duct system and in dSG glands have the capability to commit towards an endocrine pancreatic fate. Our findings could contribute to defining the role of progenitor cell compartments in islet regeneration.

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Keywords: pancreas; duodenum; regeneration

Dalla morfologia alla patologia molecolare

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PTX3 shapes profibrotic immune cells and epithelial/fibroblast repair and regeneration in a murine model of pulmonary fibrosis

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The long pentraxin 3 (PTX3) is protective in different pathologies but was not analyzed in-depth in Idiopathic Pulmonary Fibrosis (IPF). Here, we have explored the influence of PTX3 in the bleomycin (BLM)-induced murine model of IPF by looking at immune cells (macrophages, mast cells, T cells) and stemness/regenerative markers of lung epithelium (SOX2) and fibro-blasts/myofibroblasts (CD44) at different time points that retrace the progression of the disease from onset at day 14, to full-blown disease at day 21, to incomplete regression at day 28. We took advantage of transgenic PTX3 overexpressing mice (Tie2-PTX3) and Ptx3 null ones (PTX3-KO) in which pulmonary fibrosis was induced. Our data have shown that PTX3 overexpression in Tie2-PTX3 compared to WT or PTX3-KO: reduced CD68+ and CD163+ macrophages and the Tryp-tase+ mast cells during the whole experimental time; on the contrary, CD4+ T cells are consistently present on day 14 and dramatically decreased on day 21; CD8+ T cells do not show significant differences on day 14, but are significantly reduced on day 21; SOX2 is reduced on days 14 and 21; CD44 is reduced on day 21. Therefore PTX3 acts on the pro-immune and fibrogenic microenvironment to prevent fibrosis in BLM-treated mice.

Keywords: CD44; immune cells; long pentraxin-3; macrophages; m/ast cells; pulmonary fibrosis; regeneration; T cells; SOX2

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Histopathological studies on human lymphatic collecting vessels from lymphedema patients

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The lymphatic vasculature starts with blind-ended initial lymphatics in the connective tissue surrounding blood vessels. Initial lymphatics have a thin wall composed only of endothelial cells with a typical oak-leaf shape with overlapping flaps allowing fluid entry. Initial lymphatic vessels merge into precollectors, sparsely wrapped by smooth muscle cells and precollectors drain into collecting vessels that have a continuous muscular coat and are divided by unidirectional valves into pumping units called ''lymphangions".

Lymphedema is a progressive condition of the lymphatic system characterized by an abnormal accumulation of interstitial fluid with subsequent inflammation, adipose tissue hypertrophy, and fibrosis. Lymphedema may be primary, including congenital and idiopathic forms, or, more frequently, secondary to lymph node ablation in cancer treatment.

Previous studies on the histopathological alterations of lymphatic vessels in secondary lymphedema have shown that after an initial phase of ectasia, worsening of the disease is associated with wall thickening (contraction and sclerosis vessel types) accompanied by a progressive loss of the endothelial marker podoplanin.

In this study we enrolled 17 patients with primary and 29 patients with secondary lymphedema who underwent lymphaticovenous anastomoses surgery. Histological sections were stained with Masson's trichrome, and immunohistochemistry was performed with antibodies to podoplanin (lymphatic endothelial cells marker), smooth muscle α-actin (α-SMA, smooth muscle cells and myofibroblasts marker), and myosin heavy chain 11 (MyH11, smooth muscle cells marker). In secondary lymphedema, as expected, we found ectasis, contraction, and sclerosis vessel types. In primary lymphedema however, most vessels were of the sclerosis type, with no contraction vessels. The endothelial marker podoplanin showed a variable level of expression unrelatedly with the morphology of the vessel type. Finally, in both primary and secondary lymphedema not all α-SMA-positive cells were also positive for MyH11, suggesting their transformation into myofibroblasts.

Keywords: lymphedema; lymphatic vessels; lymphaticovenous anastomosis; LVA; podoplanin; α-SMA, MyH11

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The therapeutic effects of MR-409, a GHRH agonist, in an experimental mouse model of Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a severe neuromuscular disease affecting children and young adults. It is caused by the mutation of the Survival Motor Neuron 1 gene (SMN1), leading to reduced SMN protein levels and consequent degeneration of lower alpha motor neurons (αMNs), associated with muscle atrophy, as well as other peripheral alterations. Nowadays the investigation of SMN-independent treatments is spreading ever more to overcome the limits of the already available therapies. Here we evaluated the role of MR-409, a growth hormone-releasing hormone (GHRH) agonist, which has been shown to exert protective effects on experimental models of muscle atrophy, cardiomyopathies, ischemic stroke, and inflammation.

MR-409 has been administered to SMNΔ7 mice, a widely used model of SMA. Daily subcutaneous treatment with MR-409 (1 or 2 mg/kg), from postnatal day 2 (P2) to sacrifice (P12), increased body weight and improved motor behavior in SMA mice, particularly at the highest dose tested. In addition, MR-409 reduced atrophy and ameliorated trophism in quadriceps and gastrocnemius muscles, as determined by an increase in fiber size, as well as upregulation of myogenic genes and inhibition of proteolytic pathways. MR-409 also promoted the maturation of neuromuscular junctions, by reducing multi-innervated endplates and increasing those mono-innervated. Finally, treatment with MR-409 delayed αMN death and blunted neuroinflammation in the spinal cord of SMA mice.

In conclusion, the present study demonstrated that MR-409 has protective effects in SMNΔ7 mice, suggesting that GHRH agonists are promising agents for the treatment of SMA, possibly in combination with SMN-dependent strategies.

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Keywords: GHRH agonists; alpha motor neurons; neuroinflammation; neuromuscular junction; spinal muscular atrophy

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Progression of micro-anatomical, molecular and metabolic changes affecting the liver of patients with TYMP derived mitochondrial disease

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Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a rare fatal disease caused by mutations in TYMP gene, which leads to toxic nucleoside accumulation and mtDNA damage. Liver transplant, used to replace TYMP, is demonstrated to be a life-saving approach for MNGIE patients [1]. Liver has not been considered a target organ of the disease, thus the excised MNGIE liver will possibly be considered for a domino liver donation. Our study aimed to fully characterize MNGIE liver at different stages of the disease. Liver tissue biopsies were collected from n=9 MNGIE and n=7 controls. FFPE sections were used to detect architectural changes, localize and quantify fibrosis, quantify immune mediators, detect hypoxia and assess mtDNA depletion. Snap frozen tissues were used for metabolomic (NMR), proteomic (mass spectrometry) and transcriptomic (RNA-seq) assessment. The combined analysis of transcriptome, proteome, and metabolome has shown that MNGIE liver is in a hypoxic state, with a downregulation of Krebs cycle and oxidative phosphorylation, as confirmed by COX-SDH assay and HIF-1α positive staining. Glucose and glycogen metabolism were upregulated. A metabolic rewiring of lipid metabolism, mediated by the upregulation of ATP citrate synthase, was supported by the detection of steatosis accumulation during the disease progression. The detected fibrosis and septa formation seem to be mediated by the activation of NfKB, SERPINE1 and MYC pathway. The vascular and biliary branches were altered as indicated by VEGF upregulation and localization. Microdissection of hepatocytes and portal spaces revealed that the number of mtDNA copies decreased with the progression of the disease. Immune response was activated and immune cells localized at tissue level. In conclusion, our data, showing marked morphological, molecular, and progressive metabolic changes, indicate that the liver is a target organ in MNGIE, thus a domino liver intervention should be carefully discussed.

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Keywords: Liver abnormalities; Mitochondrial neurogastrointestinal encephalomyopathy; lipid metabolism

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Possible role of Fragile X Mental Retardation Protein in the regulation of cholangiocarcinoma cell plasticity, invasiveness and progression

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Intrahepatic cholangiocarcinoma (iCCA), occurring at the level of the intrahepatic bile ducts, is one of the most aggressive neoplasms with the worst prognosis worldwide. Some clinicopathological prognostic parameters have been found but the identification of molecular prognostic markers is still far off [1]. Fragile X mental retardation protein (FMRP), a missing or mutated RNA-binding protein in patients with fragile X syndrome (FXS) has recently been implicated in cholangiocarcinoma in which FMRP is suggested playing a role in promoting invasiveness of iCCA cells by acting at the level of the leading edges modulating the plasticity of plasma membrane [2]. The present study aims to evaluate the expression of the FMRP protein in the progression of iCCA (non-tumor tissue, intraductal papillary neoplasm of the bile duct (IPNB), primary cancer, locoregional or distant metastases). FMRP is overexpressed in the tumour tissue compared to non-neoplastic bile ducts and IPNB. In metastatic iCCA cell lines, the silencing of FMRP influences adhesion, migration and cellular invasion suggesting a role of FMRP in iCCA progression and it has been found to be localized in cytoplasmic RNA granules accumulating in the protrusions of the plasma membrane binding mRNAs encoding protrusion-related proteins. Metalloproteinase (MMP)9 was found to be the most overexpressed gene during iCCA progression and was identified as a target mRNA of FMRP in iCCA and correlated with its expression. In conclusion, FMRP-dependent regulation of MMP9 seems to play a significant role in the pathogenic progression of iCCA, and these two proteins could be investigated as new potential prognostic markers of disease progression.

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Keywords: Fragile X mental retardation protein; liver malignancies; tumoral invasion

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Characterization of cholangiocarcinoma heterogeneity: integration of digital histologic whole slide imaging with spatial molecular profiling

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Cholangiocarcinomas (CCA) are a heterogeneous group of malignancies arising from the biliary tree. The tumour heterogeneity at anatomical and histological levels hampered the research for effective early diagnosis strategies and treatment options, leading to poor outcomes for the patients. Our aim was to combine digital pathology with spatial molecular profiling for characterizing tumour phenotypes with relevant clinical value.

CCA samples (n=140) were obtained from different European centres joining the European Network for the Study of CCA. Extensive histological/immunohistochemical characterization of tumours has been performed. Slides were digitalized by Aperio Scanscope CS2. Spatial profiling was performed by using Nanostring GeoMx DSP.

CCAs were divided in intrahepatic (n=85), perihilar (n=41) and distal (n=14) based on their anatomical location. Intrahepatic (i) CCAs were histologically distinguished into small (37%) or large (47%) bile duct (BD) types. Large BD type iCCAs were characterized by a higher mucin content ($p=0.021$) and more frequent perineural invasion ($p<0.001$) compared to small BD type. In a subgroup of cases (n=10), spatial molecular analysis was performed by selecting the CD45-positive cell fraction in the tumour centre or at invasion front, revealing CD68 cells at invasion front as significantly associated to prognosis. In the entire iCCA cohort, a lower number of CD68+ cells at the invasion front resulted associated with a poor prognosis in terms of overall survival [HR:3.43 (1.62-7.27), $p=0.028$].

These results represent a proof-of-concept that the combination of digital histology with spatial molecular profiling could represent an integrative approach for tumour characterization and for development of a personalized approach.

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Keywords: digital histology; tumour microenvironment

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Beyond the virus: investigating alternative mechanisms of lung damage in Covid-19 fatalities through ultrastructural analysis of cryobiopsies

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In-depth ultrastructural examination of post-mortem specimens from COVID-19 patients frequently encounters significant structural deterioration, potentially attributed to the extended time gap between the individual's death and proper fixation of the sample. To enhance tissue structure preservation, we collected samples from intubated patients through a transbronchial "cryobiopsy" approach within 30 minutes post-mortem, followed by immediate fixation for electron microscopy. We systematically analyzed thin-section electron microscopy images of samples from six COVID-19 patients with documented histopathology. The diverse specimens and regions examined demonstrated ultrastructural associations for various phases of diffuse alveolar damage, encompassing alveolar epithelium detachment, type 2 cell hyperplasia, exudation, and extracellular material accumulation, such as hyaline membranes and fibrin. Macrophages and neutrophilic granulocytes were consistently identified. Endothelial structural integrity remained unaltered in areas where alveolar epithelium detachment had occurred. No aggregates of erythrocytes, leukocytes with fibrin, or thrombocytes were observed. We report that coronavirus particles were detected exclusively in and around a very limited number of cells in only one of the six patient samples. The specific cell type and origin could not be ascertained, although the overall structural preservation of the samples allowed pulmonary cell type identification. Our data suggest that the observed alveolar damage does not correlate with viral presence or structural impairment due to continued replication during the disease's later stages in fatal cases. This finding implies that lung damage in these patients may be perpetuated through alternative mechanisms, potentially involving an inadequate immune or stress response.

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Identification of a novel Cord Blood NK cell subpopulation expressing functional Programmed Death receptor-1

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Natural Killer cells (NKs) represent the innate counterpart of T lymphocytes and are characterized by a high anti-tumor and an anti-viral cytotoxic activity. Recently, it has been demonstrated that NKs can express PD-1 as an additional inhibitory receptor. Specifically, PD-1 was identified in a subpopulation of terminally differentiated NKs from healthy adults with previous HCMV infection. So far it is unknown whether PD-1 appears during NK-cell development and whether this process is directly or indirectly related to HCMV infection.

In this study, we analyzed the expression and function of PD-1 on Cord Blood derived NKs (CB-NKs) on a large cohort of newborns through multiparametric cytofluorimetric analysis.

We identified PD-1 on CB-NKs in half the newborns analyzed. PD-1 was present on CD56dim NKs, and particularly abundant on CD56neg NKs, but only rarely present on CD56bright NKs. Importantly, unlike in adult healthy donors, in CB-NKs PD-1 is co-expressed not only with KIR, but also with NKG2A. PD-1 expression was independent of HCMV mother seropositivity and occurs in the absence of HCMV infection/reactivation during pregnancy. Notably, PD-1 expressed on CB-NKs was functional and mediated negative signals when triggered.

To our understanding, this study is the first to report PD-1 expression on CB derived NKs and its features in perinatal conditions. These data may prove important in selecting the most suitable CB derived NK cell population for the development of different immunotherapeutic treatments.

Keywords: Programmed death receptor 1; human Natural Killer cells; cord blood; Killer Ig-like receptors; NKG2A; NK cell maturation; immune checkpoints; human Cytomegalovirus

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Crosstalk between bone marrow stromal cells and AML cells is critical for the response to proteotoxic stress-induced AML cell death

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The bone marrow niche, and in particular the bone marrow stromal cells (BMSCs) play a pivotal role in nurturing hematopoietic stem and progenitor cells. Acute myeloid leukemia (AML) cells modify BMSCs activity to their advantage and indeed BMSCs are a main determinant of resistance to AML therapy[1,2]. We previously devised a strategy based on proteotoxic stress by combining low doses of the differentiating agent retinoic acid (R), the proteasome inhibitor bortezomib (B), and the oxidative stress inducer arsenic trioxide (A), which exerts strong cytotoxic activity on FLT3-ITD$^+$ AML cell lines and primary blasts isolated from patients, due to ER homeostasis imbalance and generation of oxidative stress. Indeed, FLT3-ITD mutant protein is partially retained in the endoplasmic reticulum (ER) and generates intrinsic proteotoxic stress. However, AML cells become completely resistant to the combination RBA when treated in co-culture with bone marrow stromal cells (BMSC). Nonetheless, we could overcome such protective effects by using high doses of ascorbic acid (Vitamin C) as an adjuvant to exacerbate oxidative stress induction. Importantly, the combination RBA plus ascorbic acid significantly prolongs the life span of a murine model of human FLT3-ITD$^+$ AML without toxic effects. Furthermore, we show for the first time that the cross-talk between AML cells and BMSC upon treatment involves disruption of the actin cytoskeleton and the actin cap, increased thickness of the nuclei, and cytoplasmic localization of the transcriptional co-regulator YAP in the BMSC. Our findings strengthen our previous work indicating induction of proteotoxic stress as a valid strategy in FLT3-ITD$^+$ AML therapy and open to the possibility of identifying new therapeutic targets in the crosstalk between AML cells and BMSC, involving mechanotransduction and YAP signaling.

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Keywords: bone marrow niche; AML; tumor microenvironment; proteotoxic stress; YAP

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The rs4073 SNP of *CXCL8* as novel potential host genetic determinant of bone marrow fibrotic morphological grading in myelofibrosis

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Several pro-inflammatory cytokine axes - namely CCL2/CCR2, IL-1/IL-1R, CXCL8/CXCR-1/2 and IL-4/IL-13[1-4] - have been recently proved to be relevant in the pathophysiology of myelofibrosis (MF), a chronic, progressive myeloproliferative neoplasm typified by extensive deposition of reticulin and collagen fibers in the bone marrow (BM). MF is classified in pre fibrotic primary form (prePMF) and overtly fibrotic primary (overtPMF) or secondary (sMF) forms[5].

It has been recently demonstrated that the frequency of CXCL8 secreting CD34+ cells correlated with bone marrow fibrosis and that CXCR2 blockage ameliorated the fibrotic phenotype of MF murine models[2,6].

The rs4073 SNP of *CXCL8* has been extensively investigated in chronic inflammatory conditions, including cancer. The T allele, more prevalent Caucasians, accounts for increased production of CXCL8[7].

We analyzed genotypic and allelic frequency of the rs4073 SNP of CXCL8 by allele-specific PCR in a cohort of 99 MF Caucasian patients (57.6% males, median age at diagnosis 68 yrs, range 29-84, median follow-up 8 yrs) of which 45 prePMF, 27 overtPMF and 27 sMF, and 309 matched controls. Genotype-phenotype correlation was performed according to a dominant genetic model (AT+TT *vs*. AA). No significant differences in genotypic and allelic frequencies between MF and controls were detected, but, focusing on MF, we found that both overtPMF and sMF patients were significantly enriched in polymorphic genotypes (25/27, 92.6% in both categories) as compared to prePMF (32/45, 71.1%, P=0.037 in both comparisons). In line with these findings, the presence of the T allele was associated with higher grading (≥II) of bone marrow fibrosis (P=0.014). Intriguingly, all prePMF evolving into overtPMF during the follow-up were polymorphic for the *CXCL8* SNP. We also found that presence of the SNP was associated with leukocytosis in MF (12.6±9.7 *vs*. 8.0±3.2x 10^9/L, P=0.017). These results are consistent with the recent literature[2], in which a higher number of CXCL8 secreting cells correlated with the degree of reticulin fibrosis and leukocytosis in MF. Therefore, germline predisposition to produce more CXCL8 (due to the rs4073 SNP) may configure as a host genetic determinant that favors fibrotic progression in MPN.

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Keywords: CXCL8; bone marrow fibrosis; chronic inflammation; myelofibrosis

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An ultrastructural investigation of adult IDH wild-type Glioblastoma suggests a possible correlation between morphological biomarkers and the Ki-67 index

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Glioblastoma, a highly aggressive brain tumor, typically results in a life expectancy of 14 to 16 months following diagnosis. The Ki-67 labeling index, a measure of cellular proliferation, is emerging as a prognostic indicator in glioblastoma. This study aimed to explore the detailed structure of glioblastoma tissue from 9 patients sharing the same molecular characteristics (adult IDH wild-type glioblastoma, wild-type ATRX, positive for TP53 expression, GFAP expression, and EGFR overexpression). Our objective was to identify potential ultrastructural characteristics that could serve as biomarkers, specifically correlated with the only differing parameter among our samples, the Ki-67 labeling index. Our morphological findings suggest that both the presence of microglia-secreting extracellular vesicles (MsEVs) and microglia-storing lipid vesicles (MsLVs) could be significantly associated with the Ki-67 labeling index. Specifically, we observed an increase in the number of MsEVs and MsLVs in samples with a high Ki-67 labeling index. The statistical analysis of our data indicated that the amount of MsEVs and MsLVs is directly related to a high Ki-67 index, having a potential as a biomarker for predicting glioblastoma prognosis. Our ultrastructural analysis of glioblastoma blood vessels evidenced and documented the presence of small blood vessels with a double-layered basement membrane, where fenestrations allowed astrocyte foot processes to come close to endothelial cells as they extended long processes toward astrocytes. This finding illustrates the anatomical basis for molecular communication between endothelial cells and astrocytes. Our *in situ* study results suggest further investigation of the role of vessel modifications, and mechanisms involved in MsEVs genesis, activity, and their EVs, release. Moreover, the role of MsLVs, the type of LVs content, and storage mechanisms have to be further investigated, to provide viable targets for developing novel therapies.

Keywords: Microglia; glioblastoma; extracellular vesicles; biomarker; Ki67; lipid vesicles

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Morphological comparison between *in vitro* and *ex vivo* models to evaluate the direct keratinocyte responses to a psoriatic proinflammatory milieu

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Considering the psycho-socioeconomic impact of psoriasis, affecting 2-3% of the whole population, the need to understand the pathogenetic early epidermal events is still acute. The development of experimental models easy to use, but also able to reproduce the main psoriatic features is a compelling challenge. In this study, we compared the keratinocyte response after 24 hours to a proinflammatory milieu composed of TNF-alpha, interleukin (IL)-17A, IL-22, and IL-23 (MIX) in i) a 2D model of *in vitro* keratinocytes (HaCaT cells) induced to differentiate with $CaCl_2$ 1.8 mM for 4 days and ii) in an *ex vivo* 3D model of healthy human skin obtained after aesthetic surgery (n=5). Based on the hyperproliferative feature and the impairment of terminal differentiation reported in the psoriatic plaque, we evaluated cell proliferation analyzed as 5-bromo-2'-deoxyuridine incorporation and keratinocyte differentiation by indirect immunofluorescence in both models. Keratin (K) 10/K14 were considered as cytoskeletal markers of suprabasal and basal layers, respectively, and claudin 1/zonula occludens (ZO)-1 components of tight junctions (TJs). In HaCaT cells, both cell proliferation and K14 immunostaining increased as early as 24 hours, while in bioptic fragments keratinocyte proliferation was decreased at the same time point. MIX treatment always reduced K10 distribution. Claudin 1 fluorescence intensity was reduced after MIX incubation in the uppermost differentiated epidermal layers and HaCaT cells, while ZO-1 immunoreactivity was redistributed in the epidermal compartment and resulted fainter in MIX-incubated HaCaT cells. The modulation of TJ composition and the impairment of terminal differentiation are psoriatic events occurring earlier than the proliferation impairment, the latter representing a "response to injury" when the epidermis faces an inflammatory milieu. In conclusion, our observations are relevant not only as it applies to general skincare, but also to clinics.

Keywords: HaCaT cells; psoriasis; cytokines; tight junctions

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Retinoic gene signature in Merkel cell carcinoma

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The identification of the origin cell of Merkel cell carcinoma (MCC), a rare skin tumor, is prevented by the limited molecular knowledge of its two subsets, namely Merkel cell Polyomavirus-positive and -negative MCC (MCCP/MCCN). Given the expression of neuroendocrine, epithelial, fibroblast and B-cell markers in MCC, a multilinear origin has been assessed. However, the existence of two subsets may reflect oncogenic events occurring in two distinct origin cell types. Retinoic signaling activation has been reported to inhibit the epithelial-to-mesenchymal transition (EMT), while altered retinoic-associated genes have been identified in MCC. However, the role of the retinoic signaling in MCC is unclear. Retinoic gene signature was investigated herein in MCCP, MCCN and in epithelial/fibroblast control cells to elucidate the heterogeneous nature of MCC. Hierarchical clustering and Principal Component Analysis were conducted to categorize cells according to the retinoic gene signature. MCCP and MCCN cells were hieratically clusterizable from each other and from epithelial/fibroblast cells, according to their retinoic gene signature. MCCP *vs* MCCN differentially expressed genes (n=43) were identified. Among these, ten hub genes were identified *via* protein-protein interaction network. In particular, SOX2, ISL1, PAX6, FGF8, ASCL1, OLIG2, SHH and GLI1 were upregulated, whereas JAG1 and MYC were downregulated in MCCP compared to MCCN. Enrichment analyses indicated that differentially expressed genes are predominantly related to DNA-binding/transcription factors involved in development, regionalization, pattern specification and morphogenesis. MCCP-associated hub genes are transcription factors active in neurological development, stemness and EMT, thus suggesting that dysregulated retinoic gene expressions might confer a more pronounced invasive/transformation potential in MCCP than MCCN. Our findings on retinoic signature in MCC may suggest the neuroendocrine origin of MCCP.

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Biological consequences of SARS-CoV-2 viral infection on breast cancer cells: a role of estrogen receptor?

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COVID-19 pandemic, caused by SARS-CoV-2 virus, had significant consequences for individuals already dealing with various pathological conditions, including breast cancer [1]. Emerging evidence suggests that SARS-CoV-2 infection may directly affect breast cancer cell biology, even though a deeper understanding of the effects of the virus on tumor cells is still not completely achieved [2]. The present study aimed at examining the molecular events taking place in three breast cancer cell lines expressing different levels of the SARS-CoV-2 entry receptor ACE2, as evaluated by immunofluorescence and western blot analysis, upon SARS-CoV-2 infection. Specifically, MCF7, MDAMB231, and HCC1937 cell lines, representing luminal A (estrogen receptor positive), basal B/claudin low and basal A (both estrogen receptor negative) molecular subtypes respectively [3], were infected with SARS-CoV-2 and viral replication was monitored over-time. Our data indicated that MCF7 cells exhibited the highest permissiveness to the virus, a finding also corroborated by electron microscopy. Furthermore, gene expression profile analysis was conducted at 24 hours and 7 days post infection. In line with our previous observations, functional genomic analysis revealed the enrichment of several pathways associated to Coronavirus infection, viral recognition, immune activation and estrogen receptor signaling in infected MCF7 cells at 7 days post-infection. Interestingly, a metagene based on genes found to be up-modulated in all the three cell lines by SARS-CoV-2 identified a subgroup of pre-menopausal estrogen receptor positive breast cancer patients experiencing poor prognosis. Since it has been reported that SARS-CoV-2 can interact with the estrogen receptor and modulate its biological functions [4], our findings suggest a possible involvement of this protein in sustaining virus replication in malignant cells. Accordingly, pharmacological inhibition of estrogen receptor activity by tamoxifen treatment was able to reduce SARS-CoV-2 replication rate in MCF7 cells. Given the ongoing spread of the SARS-CoV-2 within the population and the high rate of breast cancers expressing estrogen receptor, it is crucial to gain a better comprehension of its direct impact on breast cancer and to assess the long-term consequences of COVID-19 on breast cancer outcomes.

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Keywords: SARS-CoV-2; Breast cancer; Estrogen receptor

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Genetic Architecture of the 3D-Fibrin Meshwork in Pregnancy Maintenance

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Unexplained miscarriage is a frequent pregnancy complication. The aetiology includes genetic abnormalities, infections, implantation process dysfunctions and immunological disorders, uterine/endocrine abnormalities, lifestyle risk factors and maternal age. Given this complexity, the pathophysiological mechanisms and the dynamic changes at maternal-foetal interface have not been clearly stated. Following the similitude between embryo implantation process and wound-healing phases, tissue remodeling, controlled inflammation and angiogenesis are needed to preserve the regenerative/repair processes of the modifying uterine walls, tuned by an appropriate anti-inflammatory milieu when there is no need of further actions. In this line, maternal fibrin 3D organization and fibrinogen levels play a key role in embryo implantation and pregnancy maintenance.

To explore the genetic architecture of the 3D-fibrin meshwork at the maternal/foetus interface, we investigated coagulation Factor XIII (*F13A1* and *F13B*) and fibrinogen cluster (*FGA* and *FGB*) gene variants in a group of women who experienced spontaneous early pregnancy loss (EPL, n=123) and women who undergone voluntary pregnancy interruption (VPI, n=107). Principal Component Analysis accounting for genetic and biochemical variables and logistic regression have been performed to disclose positive/negative associations. Finally, 3D-architecture of fibrin scaffolds obtained from selected "extreme genotypes" were analysed by SEM analysis.

Single gene analyses found statistical significance in *F13A1* V34L (P=0.045), *F13B* H95R (P=0.035) and *FGB* -455GA (P=0.04) gene variants by comparing EPL versus VPI ascribing different risk scores. PCA analyses confirmed significant interactions among FXIII and Fibrinogen (gene variants and levels) ascribing significant risk scores: $OR_{F13B,FGB,PLT,PT}$=0.6; 0.46-0.84;P=0.002, and $OR_{F13A,FGB,age,Fib}$=0.5; 0.40-0.73;P<0.0001. Finally, we reported significant differences in the nanofibers organization (i.e. SEM analysis of pore size, fibres length and diameter, number of branch-points) according to different gene variants and cases subgroups.

Genetically driven 3D-fibrin architecture, by influencing secretome niche, angiogenesis and inflammation may in turn predispose to pregnancy maintenance and outcome.

Keywords: Coagulation Factor XIII; Fibrinogen; Fibrin; SNPs; 3D fibrin meshwork architecture; SEM analysis; Pregnancy maintenance

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Defective immune-megakaryocytes driven by high Sp1 expression as possible drivers of idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive form of pneumonia associated with fibrosis. Insufficient understanding of driver mutations and poor fidelity of currently available animal models has limited the development of effective therapies. Previous studies suggesting that megakaryocytes (MKs) sustain pulmonary fibrosis in the bleomycin mouse model [1] and the recent discovery of a novel MK subpopulation (immune-MKs) exerting immune functions in the lung [2,3] lead us hypothesize that abnormalities in immune-MKs may be involved in the pathogenesis of IPF. This hypothesis was tested in our hypomorphic *Gata1*^{low} murine model of myelofibrosis, whose MKs exhibit high levels of several proinflammatory proteins (TGF-β, CXCL1, P-SEL) that have been implicated also in the development of IPF [4]. We discovered that with age *Gata1*^{low} mice develop fibrosis in the lung with features that recapitulate those observed in IPF in patients: *Gata1*^{low} mice progressively develop fibrosis in the subpleural region of the basal lobes as they age, recapitulating the chronic nature of IPF, and fibrosis is not triggered by inflammatory insults. Moreover, the lungs from *Gata1*^{low} mice, as those from the IPF patients, contain numerous GATA1neg immune-MKs. Therefore, *Gata1*^{low} mice are the first genetic-driven model for IPF and provide a link between abnormal immune-MKs and lung fibrosis. Since immune-MKs differentiate in the bone marrow (BM) from hematopoietic stem cells (HSCs) under the control of the transcription factor PU.1 (Spi1) [5], we further characterized the abnormalities of the immune-MKs in the lung from *Gata1*^{low} mice by comparing the cells present in a knock-in mouse line with reading frames for enhanced yellow fluorescent protein (eYFP) knocked into the gene loci for PU.1[6] carrying the *Gata1*^{low} mutation (PU.1-eYFP/*Gata1*^{low} mice). By flow cytometry analysis, the levels of eYFP expression divides the MKs (CD41^{pos}) of the BM and lungs into four classes: cells not expressing eYFP or expressing low, medium or high eYFP levels. Of note, the frequency of immune-MK (CD41^{pos} CD53^{pos}) and the levels of eYFP expression were higher in the lungs from the double mutants than in the BM. Since high PU.1 levels has been linked to HSCs fate in inflammatory stress conditions [7], we assume that this transcription factor may specifically drive MKs abnormalities in the lungs of *Gata1*^{low} mice.

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Keywords: Idiopathic pulmonary fibrosis (IPF); GATA1; PU.1; Megakaryocytes (MKs)

Istogenesi, funzioni e disfunzioni dell'apparato muscolo-scheletrico

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Protein Kinase C epsilon fosters myogenic differentiation *via* SOD2-mediated downmodulation of ROS levels

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Reactive oxygen species (ROS) are recognized as key drivers of several physiological processes. Myogenesis is accompanied by metabolic remodeling and changes in the activity of antioxidant enzymes that lead to changes in ROS levels. In turn, fluctuations in intracellular ROS during myogenic differentiation appear as a myogenesis-regulating factor, despite the molecular mechanisms still need to be elucidated[1-2]. Protein Kinase C (PKC) epsilon (PKCe) promotes muscle stem cell differentiation and skeletal muscle regeneration after injury[3]. PKC plays a tissue specific role in redox biology and specific PKC isoforms may represent both a target of ROS as well as an up-stream regulator of redox signaling[4,5]. Therefore, we hypothesized that PKCe represents a molecular link between redox homeostasis and myogenic differentiation. We used the *in vitro* model of mouse myoblast cell line (C2C12) to study the PKC-redox axis. We demonstrated that the transition from myoblast to myotube is typified by increased PKCe expression and decreased ROS, suggesting that the antioxidant signaling activation is required to prevent ROS accumulation and allow cell differentiation. Indeed, we found that the expression of the antioxidant enzyme, superoxide dismutase 2 (SOD2), is significantly higher in the late phases of myogenic differentiation, mimicking PKCe protein levels. Furthermore, we demonstrated that, while SOD2 silencing did not affect PKCe expression, a forced *in vitro* PKCe down-regulation was able to induce a reduction of SOD2 coupled with significant increase of ROS, suggesting that the kinase could be an up-stream regulator of SOD2. We also identified a plausible molecular link between PKCe and SOD2 represented by Nrf2, a well known SOD2 activator, by demonstrating the formation of the PKCe-Nrf2 complex in C2C12 cells. Overall, our results indicate that PKCe is capable of activating the antioxidant signaling preventing ROS accumulation in myotube, eventually promoting myogenic differentiation.

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Keywords: PKCepsilon; SOD2; antioxidant; reactive oxygen species; skeletal muscle; myogenesis

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Condylar hyperplasia: new histological data

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Unilateral condylar hyperplasia (CH) is characterized by slowly progressing and enlarging condyle along with elongation of body of mandible, which results in a shift in the midline to the contralateral side, ultimately leading to facial asymmetry, occlusal disharmony and joint dysfunction. It is suggested that the first report of this condition in the English language was made in 1836 by *Robert Adams (Adams*1873). Afterwards many authors began to study CH although to this day the aetiopathogenesis is still unclear, but certain theories have been suggested which include trauma, hormonal imbalance, infection, arthrosis, hypervascularity and possibly genetic role. The typical features include enlargement of mandibular condyle, condylar neck and excessive growth of the body of mandible. This pathology is treated with a double clinical approach: surgical and orthodontic, but it has been seen that years after the resolution of the problem, it can reappear. Histopathological examination reveals excessive formation of articular cartilage, with thickened proliferation zone and manifests itself with the characteristic infiltration of the bone tissue as "islands of cartilage". The aim of our study was to create a multifactorial approach to try to better investigate the nature of the pathology. For this study we used 5 patients with condylar hyperplasia (4 female and 1 male) who were treated with proportional condylectomy and a control patient. The samples were observed under the optical microscope, the SEM and the confocal laser microscope, we used inflammatory markers, type I and II collagen fiber markers and fibroblast markers. The results obtained confirm that the progressive growth of the mandibular condyle is due to a pathological hyperactivity of the cartilaginous tissue, suggesting that this condition depends on a metabolic disorder.

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Keywords: Condylar Hyperplasia; ATM; Cartilage; Immunoistochemical

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Engagement of RAGE at myofiber level is a key event in cancer-induced muscle wasting

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Cachexia is a multifactorial syndrome affecting most advanced cancer patients, characterized by loss of body weight and skeletal muscle tissue, and responsible for about 20% of all cancer deaths. The receptor for advanced glycation end-products (RAGE) sustains cancer cachexia in animal models, increasing inflammation and muscle wasting. Indeed, mice lacking RAGE (*Ager*$^{-/-}$ mice) showed increased survival and delayed loss of muscle mass and strength. To unravel the specific contribution of RAGE to cachexia in the tumor environment and skeletal muscles, we injected LLC (Lewis lung carcinoma) clones stably transfected with full-length RAGE, RAGEΔcyto (lacking the cytosolic and transducing domain) or empty expression vector in C57Bl/6 WT and *Ager*$^{-/-}$ mice. We found that RAGE overexpression sustained the tumorigenic and cachectic potential of LLC cells in WT mice, but not in *Ager*$^{-/-}$ mice, which maintained their body weight and muscle masses in the presence of tumor and did not activate the ubiquitin-proteasome system in muscles regardless of the injected LLC clone. We also generated a conditional mouse model (*Ager*^{mKO} mice) in which the RAGE gene is selectively deleted in skeletal muscles, by crossing *Ager*$^{flx/flx}$ with tamoxifen-inducible HSA-MerCreMer mice. *Ager*^{mKO} mice showed resistance to LLC-induced loss of body and muscle weights, although to a lesser extent than *Ager*$^{-/-}$ mice, and an increased survival compared with LLC-bearing control (*Ager*$^{flx/flx}$) mice. Altogether, our results suggest that overexpression of RAGE in LLC cells is not sufficient per se to induce muscle atrophy, and that RAGE engagement at myofiber level is a key event in inducing muscle wasting in cancer conditions. Since we observed the highest protection against cancer-induced muscle wasting in the presence of total ablation of RAGE, molecular targeting of this receptor might represent a promising approach to counteract the cachectic syndrome in cancer patients.

Keywords: Cachexia; muscle wasting; RAGE

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Altered COL6-NG2 binding impairs pericyte regenerative potential in COL6-related myopathies

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Collagen VI (COL6) is a ubiquitous extracellular matrix (ECM) protein that plays a crucial role in organizing and anchoring the fibrillar collagen network in various tissues, including skeletal muscle[1]. Mutations in genes encoding the three major α-chains of COL6, COL6A1, COL6A2 and COL6A3, are responsible for a group of inherited disorders called COL6-related myopathies. COL6 deficiency affects ECM structure and biomechanical properties, resulting in impaired muscle regeneration and eventually progressive muscle weakness and wasting, and joint contractures.

Recent evidence demonstrated that pericytes, a type of mesenchymal stem cell closely associated with the endothelial cells of blood vessels, contribute to muscle regeneration by releasing trophic factors and increasing myogenic differentiation[2]. Pericytes are quiescent cells but, in response to muscle injury, they become more reactive and differentiate into muscular cells to enhance tissue healing.

Therefore, to gain insight into the involvement of pericytes in the pathogenesis of COL6-related myopathies, we explored cellular and molecular mechanisms coordinating regenerative potential of pericyte of affected patients compared to healthy donors.

Firstly, we evaluated the expression and distribution of COL6 and its cell surface receptor Neural/glial antigen 2 (NG2) in primary pericyte cultures displaying the COL6 defects and an aberrant NG2 expression in affected patients.

Interestingly, our results revealed that the impaired COL6-NG2 binding could reduce the regenerative capacity of pericytes in response to muscle injury due to an irreversible quiescent state of pericytes, as demonstrated by the inverse correlation between proliferative signaling pathways and quiescent markers expression.

Taken together, our findings highlight pericytes as crucial players in maintaining tissue homeostasis in COL6-related myopathies, emphasizing their importance in contributing to muscle repair in response to injury.

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Keywords: COL6-related myopathies; pericytes; extracellular matrix; cellular quiescence; muscle regeneration

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The amazing tenocyte: new insights about cell trafficking and immunomodulation during tendinopathies

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Tendinopathies are common diseases and increase in incidence as people age. To date, there is no established therapeutic strategy for tendinopathies due to a lack of evidence about their exact intrinsic pathogenic mechanisms and the absence of characteristic molecular markers. In general, the main effectors of inflammation upon tendinopathies are myeloid cells, most notably monocytes and macrophages. However, the discovery of inflammation markers classically related to myeloid cells on tendon-derived cells has broken new ground for the understanding of molecular pathways underlying tendinopathies. Our group has previously highlighted the involvement of the redox-sensitive transcription factor Nrf2 (nuclear factor erythroid 2–related factor 2) in the counteraction of H_2O_2-induced cytotoxicity in tenocytes upon HA (hyaluronic acid) administration [1]. In parallel, we have demonstrated that oxidative stress-induced apoptosis in tenocytes is driven by caspase 3 and 7, and this condition could be counteracted by the administration of HA [2]. Moreover, it has been reported that tenocytes are capable of restoring their intracellular redox homeostasis by modulating the NF-κb nuclear translocation and the expression of iNOS (inducible nitric oxide synthase). Finally, we highlighted the involvement of the macrophage-related marker CD200 during rotator-cuff tendon injury/repair in a model of primary tendon-derived cells $CD146^+$, stimulated by IFNγ and TNFα *in vitro* [3]. Current proteomic studies show the upregulation of downstream protein involved in the ROS scavenging and inflammation in primary tendon-derived cells stimulated by LPS.

The present findings demonstrate that not only myeloid cells, but also tendon-resident cells play a role upon immunomodulation during tendinopathies. Gaining a deeper insight into the nature and function of tendon-resident cells in tissue homeostasis and disease lays the ground for developing new treatment strategies for tendinopathies.

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Keywords: tendons; inflammation; tendinopathies; Nrf2; caspase 3/7; hyaluronic acid; iNOS; ROS scavengers; CD200; CD146; IFNγ; TNFα

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Can ultrasonographic evaluation of soleus fascial thickening be a useful predictor for Achilles tendinopathy?

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The Achilles tendon (AT), which represents the conjoined tendon of the triceps surae muscles complex, is one of the largest, and strongest tendons of the human body [1]. However, as it serves as the primary plantarflexing mechanism of the ankle, the AT is also one of the most common sites of rupture. In the last years, the incidence of AT injuries has significantly increased within the middle-aged physically active population due to the growing popularity of some recreational sports [2]. Increasing evidence suggests that alterations in the muscle-tendon complex in response to altered loading and/or muscle contraction may not be restricted to the muscle alone but can also affect the fascial tissue. Ultrasonography (US) is currently considered a clinically reliable and non-invasive tool for delineating tendon, muscle, and fascial echotexture while detecting structural alterations, including thickening and echogenicity abnormalities [3].

In the present study, a comparative US imaging evaluation of textural features of the suro-Achilleoplantar complex was performed both in healthy control and symptomatic subjects with mid-portion Achilles tendinopathy. Preliminary data demonstrate that, in symptomatic subjects, ultrasonographic alterations are not restricted to paratenon and intratendinous areas, but also affect upstream structures along the myofascial chain, resulting in thickening of the fascia interposed between medial gastrocnemius and soleus muscles. Moreover, positive correlations were found between soleus fascia thickening and abnormalities in AT, paratenon, and symptom severity. Interestingly, suro-Achilleo alterations comparable to those occurring in the symptomatic group were also found in a subgroup of asymptomatic subjects, suggesting that US myofascial alterations may be clinically significant in predicting a clinical outcome.

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Keywords: Achilles tendinopathy; musculoskeletal ultrasound; ultrasonography; imaging of crural fascia; triceps surae muscle

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Impact of Drp1 activation and mitochondrial fission in the pathogenesis of Duchenne Muscular Dystrophy

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Loss of function mutations in DMD gene encoding for dystrophin protein causes Duchenne Muscular Dystrophy (DMD), a severe progressive neuromuscular disease. Despite remarkable progress has been made in genetic approaches to restore dystrophin, or its function, new therapeutic strategies are needed. In this view, muscle weakness in DMD is thought to be dependent, at least in part, on damaged mitochondria and compromised bioenergetics. Consistently mitochondria are an attractive target for therapeutic interventions. Dystrophic fibers show marked mitochondria fragmentation; however, few studies have addressed the relevance of mitochondrial shape in the muscle damage progression. Accordingly, we generated a DMD mouse model with intrinsically fluorescent mitochondria, the mdx-PhAM mouse, to precisely define mitochondrial dynamics during DMD progression and we confirmed the existence of a less interconnected mitochondrial network in mdx single fibers by 3-dimensional reconstruction. In agreement, western blot experiments showed a significant upregulation of pro-fission proteins, Drp1 and its receptors, in mdx muscles starting from 3 months of age, suggesting the shifting of mitochondrial dynamics towards Drp1-mediated mitochondrial fission. This can potentially contribute to DMD pathological fibrosis and inflammation by triggering the activation of specific signaling pathways, such as inflammation by DAMPs (mtDNA) release and UPR response. Therefore, to assess the relevance of Drp1-dependent fission enhancement in DMD pathogenesis we treated mdx mice with MDIVI-1, a specific Drp1 inhibitor. We have obtained encouraging results as for muscle functionality and phenotype, thus confirming the relevance of Drp1 as a therapeutic target in DMD.

Keywords: Duchenne Muscular Dystrophy; Mitochondria; mdx mice

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Machine learning accurately classifies phenotypes of human stem cell-derived midbrain dopaminergic neurons exposed to endocrine disruptors based on morphological features

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Parkinson's disease (PD) is the second most common neurodegenerative disease, and it is characterized by the loss of midbrain dopaminergic neurons. Endocrine disruptors (EDs) are active substances that leak from plastic, and they are known for their detrimental action on the endocrine system. Data from animal studies show that EDs exposure may deteriorate the dopaminergic system, but no data are available about the effect of EDs on human models of the nervous system. The aims of this study were i.) to analyze the effect of selected EDs on the phenotype of human midbrain dopaminergic neurons (mDANs) using high content microscopy and ii.) to develop a prediction model able to classify control vs EDs-exposed mDANs using machine learning (ML) algorithms applied to morphological features. mDANs were treated for 72h with the selected EDs (e.g., bisphenols and perfluoroalkyles) at different concentrations and then stained with specific mDANs markers such as MAP2, alpha-synuclein (aSyn), and tyrosine hydroxylase (TH). EDs had a detrimental effect on mDANs, inducing a significant decrease of neurite length and branching. Moreover, a significant increase of aSyn expression and TH redistribution within the neurons were observed. The ML approach showed a high performance in classifying cell phenotypes based on the treatments, with very high accuracy (0.92). Here, we demonstrate that EDs negatively affect human mDANs and shift their phenotype increasing hallmarks of PD. Also, the applied ML algorithm can identify these phenotypic changes that define EDs effects, thus resulting in an instrumental future approach to the evaluation of pathological state and drug screening.

Keywords: Dopaminergic neurons; Parkinson's Disease; Endocrine disruptors; High-content microscopy; Machine learning

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Uniqueness matters: morphological assessment of maxillary sinuses for personal identification

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Paranasal sinuses are known as highly individualizing structures in the human body and this property has been already assessed for possible applications to personal identification, especially for what concerns frontal and sphenoid sinuses (1,2). However, the uniqueness of maxillary sinuses has still to be quantified, as well as their possible use for personal identification.

One hundred head CT-scans (equally divided among males and females) were extracted from a hospital database. Maxillary sinuses were twice segmented from each CT-scan through ITK-SNAP software and the correspondent 3D models were automatically superimposed to obtain 100 matches (if belonging to the same person) and 100 mismatches (if extracted from different individuals), both for the right and left side.

Average RMS (root mean square) point-to-point distance was then calculated for all the superimpositions: differences according to sex, side and group (matches and mismatches) were assessed through three-way ANOVA test with Bonferroni correction ($p<0.017$).

On average RMS value was lower in matches (0.26±0.19 mm in males, 0.24±0.18 mm in females) than in mismatches (2.44±0.87 mm in males, 0.20±0.73 mm in females) with a significant difference ($p<0.001$). No significant differences were found according to sex or side ($p>0.017$).

The study verified that maxillary sinuses, similarly to the frontal and sphenoid sinuses, may be reliably used for personal identification in forensic context.

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Keywords: personal identification; maxillary sinus; CT-scan; individual anatomy; 3D-3D superimposition

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Romina Strawberry Extract: the effect of phytochemical compound on 3D uterine leiomyosarcoma cells

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Uterine leiomyosarcoma is a rare but aggressive uterine smooth muscle cell-derived cancer recognized and diagnosed by histological criteria of hypercellularity, severe nuclear atypia, and high mitotic rate (>15 mitotic figures per 10 high-power fields) [1-3]. Over the years, our research group has developed several *in vitro* studies using phytochemicals on uterine leiomyoma cells, obtaining excellent results, and proposing the use of strawberries as a method of prevention and/or therapy [4-6]. In the present study, we observed the effect of treatment with strawberry extract of Romina cultivar *in vitro* study on leiomyosarcoma spheroids formed using an agarose matrix. Strawberries are the most common fruit in the Mediterranean diet; several *in vitro* and *in vivo* studies have shown that, among other foods of plant origin, strawberries can be a valuable ally in the prevention and reduction of the risk of developing common diseases, including cancer; in particular, most intervention studies focusing on strawberries demonstrated promising effects on oxidative stress, inflammatory state, blood lipid size or distribution, insulin response, and insulin sensitivity, while other studies found no effects on these parameters [7]. We studied what effect of treatment with Romina strawberry extract could have on three-dimensional cultures of leiomyosarcoma cells. Three-dimensional cultures were formed using agarose gel. Subsequently we observed the effect of the strawberry extract through a morphological observation, noting thanks to a cell count a decrease in the number of spheroids following treatment after 24 and 48 hours with 250 µg/mL of strawberry extract of the Romina cultivar. The observation was then confirmed by histochemical techniques such as DAPI (DNA binding fluorescence), hematoxylin and eosin staining and Masson's trichrome stain. Finally, we evaluated through a molecular study with the real time PCR the expression of the genes of the extracellular matrix, and observed a significant decrease after the treatment with the strawberry extract.

From this study it emerged that the extract of this strawberry cultivar could be indicated as a therapeutic agent for the treatment of uterine leiomyosarcoma.

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Keywords: leiomyosarcoma; phytochemicals compound; strawberry; 3D cultur

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Exploring the features of Sertoli cells as anti-infective and drug delivery agents: *in vitro* performances on *Candida tropicalis* biofilms

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Based on our previous observations pointing to the promising features of SCs as anti-infective and drug delivery agents[1], our study sought to investigate the intrinsic *in vitro* performances of naïve porcine prepubertal Sertoli cells (SCs) or SCs pre-loaded with blank poly(lactic acid) microparticles (MP) and Amphotericin B poly(lactic acid) microparticles (AmB-MP) against *Candida tropicalis*, one of the most prevalent pathogenic non-*albicans* species[2], to assess the effect on biofilm development and the pathways potentially involved in cell response.

$1x10^5$ SCs /cm^2 were preloaded with blank MP or AmB-MP, produced by a Mini Spray- Dryer Model B-290, by exploiting their innate phagocytic capacity and then placed in direct contact with the biofilm of *C. tropicalis* at 37 °C for 24, 48, 72, and 96 hours.

TEM analysis showed that SCs were able to internalize C. *tropicalis* cells, maintaining the viability as demonstrated by the presence of perfectly intact organelles such as an RER particularly developed and numerous mitochondria. The biofilm inhibition test confirmed the anti-infective action of naïve SCs reaching values above 70%, which grew to 80-90% in synergy with AmB-MP.

The interaction between naïve SCs or SCs pre-loaded with AmB-MP and *C. tropicalis* induced the activation of MAPK, AKT, NF-kB signal transduction pathways, which led to an increased gene expression of innate immunity factors, including MHC-II, TLR-4, TGF-β, IDO, and β-defensin 123[3].

This study confirmed the surveillance role of SCs against infections and their ability to transport drugs. Furthermore, we reported how the strategy of SCs was to establish a state of tolerance at the fungus/host interface rather than aggressively tackling microbial populations, providing significant insights into how microbial infections might be managed and controlled thus proposing a new concept of antibiotic therapy.

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Keywords: Sertoli cells; Candida tropicalis; microparticles; drug delivery

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Generation of artificial intelligence software for the identification of oocytes competent for embryonic development

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In the antral compartment of the ovary of many mammals (including humans), there are two types of oocytes characterized by a different ability to support complete embryonic development. The SN type (Surrounded Nucleolus; 70-80% of the antral population), if properly matured and fertilized *in vitro*, completes embryonic development up to the blastocyst stage; the NSN type (Not Surrounded Nucleolus; the remaining 30%) inexplicably stops development at the 2/4-cell stage. To date, SN and NSN are selectable only by the presence or absence of a Hoechst-positive heterochromatin ring around the nucleolus and a different cytoplasmic organization. Morphological analyses with TEM, molecular and proteomic approaches have shown that structures and factors fundamental for the correct embryonic development, such as cytoplasmic lattices, lipid droplets, and some proteins responsible for the Subcortical Maternal Complex (i.e., MATER and FILIA) are respectively, absent, abundant and under-expressed in NSN-type oocytes. To find non-invasive methodologies to classify only human antral oocytes competent for development (the SN type; due to the therapeutic advantage that *in vitro* fertilization procedures would get), we have developed an artificial intelligence (AI) software trained to select the two types of oocytes without nucleolar heterochromatin staining or manipulation of any kind. The preliminary analysis of AI conducted on 116 murine oocytes allows the identification of SN and NSN oocytes starting from phase contrast images with an accuracy close to 90%. This result opens the door to developing AI models for SN oocytes' *in vitro* maturation and fertilization procedures. This means an increase of at least 30% in the number of zygotes competent for embryonic development, with a consequent decrease in the number of ovarian stimulation cycles of the patients in therapy.

Keywords: Oocytes; embryos; artificial intelligence; *in vitro* fertilization

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Morphological investigations of spheroid cultures of human leiomyosarcoma cell lines

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Uterine leiomyosarcoma is a rare entity among malignant gynecologic tumors with a very unfavorable prognosis and the highest prevalence (60-70%) in the pre- and peri- menopause. It represents the main entity of the heterogeneous group of uterine sarcomas (1). Leiomyosarcoma is often diagnosed accidentally, and correct diagnosis is sometimes tricky as the tissue may have leiomyoma-like or STUMPSs (smooth muscle tumors of uncertain malignant potential) characteristics. Surgery is the basis of therapy, and it should be done in order to remove the uterus intact (2). At the macroscopic level, hemorrhagic and necrotic areas can be found, and typically leiomyosarcoma shows characteristic hypercellular spindle cells, diffuse moderate to serious cell atypia, a high mitotic index > 15/10 HPF (high power field), atypical mitosis and tumor cell necrosis (3). Although the exact etiology remains unknown, it is known that two molecular mechanisms are involved in this tumour progression: fibrosis, characterized by a large amount of extracellular matrix (ECM) produced, and metastasis (4, 5). In the literature the model systems for studying uterine fibrotic disease are limited to primary cell culture in monolayers, xenografts, and transgenic mouse models, but with some limitations (6). To deepen the study of the pathogenesis of the leiomyosarcoma as well as to test therapeutic compounds, we established a three dimensional cell model based on the use of agarose. In fact, 3D tumor models are useful both for the more accurate understanding of the pathogenesis, as well as for performing solid-cancer related *in vitro* assays (migration, invasion, radiation or drug testing) (7). Also, the agarose is a natural biodegradable, non-adhesive, and non-toxic polysaccharide derived from seaweed. It has high porosity, it is an optical transparent material, it solidifies in molds at room temperature, and that makes it suitable for creating 3D cell culture models (8). We aimed to characterize the morphology of the spheroid 3D formation. Using the 2% of agarose we inoculated 10^6 cells of the leiomyosarcoma cell line, obtaining visible spheroids under a phase contrast optical microscope. We characterized the growing rate of spheroids at different times (48h, 72h, 7 days, 10 days and 14 days), and the ECM formation by histological staining, such as Haematoxylin and Eosin, and Masson's trichrome, and by scanning electron microscopy (SEM) technique. Since leiomyosarcoma is a malignant tumor, it was found performing in terms of growth rate and development of the 3D cellular structure, in fact over time the cells that self-organized into this complex structure, assumed larger dimensions and the production of collagen fibers characterizing the extracellular matrix could be noted. This sustainable spheroids culture that emulates the tumoral conditions through the accumulation of extracellular matrix and successfully high rate of cellular proliferation can represents a good alternative model for a better understanding of tumoral pathophysiology, etiopathogenesis, and for the study and testing alternative preventive drugs.

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Keywords: Leiomyosarcoma; extracellular matrix (ECM); spheroids; agarose

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Ultrasound Anatomy in Aesthetic Medicine

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The knowledge of normal Anatomy is the basis of safe and effective treatments in the field of Aesthetic Medicine. Since the majority of treatments, conducted above all on the face, involves the injection of fillers of different nature in the various layers of the skin, carefully avoiding accidental intravascular injection, and of botulinum toxin in the mimic muscles, it is extremely important to have a perfect anatomical knowledge of the area to be treated, in order to obtain optimal results while avoiding adverse effects. For this reason, the use of the ultrasound study of the patient's face is increasingly developing, with the aim of studying the anatomy of the single individual, evaluating both the precise position of the vessels and their possible variations and the organization of the skin structures, to ensure the precision of inserting the filler in the right skin layer.

The non-invasiveness of the ultrasound procedure and the current availability of portable ultrasound probes, small in size and equipped with wi-fi, make the ultrasound study easy to carry out.

Based on these considerations, in the second level Master's degree in Aesthetic Medicine belonging to the Department of Biomedical Sciences - section of Anatomy of the University of Sassari, a didactic module relating to the study of the Anatomy of the face with ultrasound has been added. In this way, students learn to recognize the various anatomical structures of the face and to plan interventions in a safe and conscious way.

Furthermore, at the CRISMENC, the Research Center in Aesthetic Medicine always pertaining to the Human Anatomy section, in collaboration with other Italian centers, the use of ultrasound anatomical study is being developed in the understanding and treatment of adverse effects secondary to injection procedures.

In conclusion, thanks to an advanced but relatively easy-to-use imaging technique, ultrasound Anatomy is establishing itself as a fundamental discipline in the field of Aesthetic Medicine, which will have to involve anatomists in an indispensable educational training activity.

Keywords: Ultrasound; Anatomy; Face; Aesthetic Medicine

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3D pathophysiological cell systems to model aberrant cardiac trabeculation

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Regenerative medicine is based on stem cells (1) which could be a promising treatment for patients suffering from muscle disorders, including Duchenne muscular dystrophy (DMD). Recently many research groups are relying on pluripotent stem cells and their derivatives, including extracellular vesicles, thought to be used to deliver targeted drugs, including small non-coding RNAs to muscle cells. However, no convincing results were obtained in stem-cell based clinical trials so far, supporting the idea that more fundamental research studies are necessary to better understand the DMD pathogenesis. We generated cardiac organoids from Duchenne patient-derived induced pluripotent stem cells (DMD-COs) and CRISPR/Cas9 isogenic-corrected controls (DMD-Iso-COs) and provided evidence that DMD-related cardiomyopathy and disease progression occur in the organoids upon long-term cultures (2). Recently, we employed DMD-COs and DMD-Iso-COs as the perfect pair of control and mutant cardiac cell types for drug screening. Utilizing a set of novel NOX4 inhibitors (3), targeting a key enzyme in the DMD cardiac pathogenesis, differences in cell viability and calcium transients could be detected. Nonetheless, despite displaying relevant features, cardiac organoids show heterogeneity in the cytoarchitectures and cardiac cell sub-types and lack of the endocardium layer, where the pathological trabeculation remodelling occurs. Endocardial identity is established during early somitogenesis by BMP signalling acting upstream of ETS Variant Transcription Factor 2 (ETV2) gene. Through the use of doxycycline-inducible ETV2-hiPSC line and a specific medium supplemented with BMP10, bFGF and doxycycline, a highly efficient differentiation protocol was designed for endocardial cells capable of inducing a phenotype switch towards trabecular myocardium. Upon fusion of COs with ETV2-endocardial organoids we generated cardiac assembloids, showing increased spatial organization of trabecular markers. Moreover, marked differences in gene expression of trabecular and compact markers between dystrophic and healthy cardiomyocytes have been observed. Finally, the survival and cardiac differentiation capacity of DMD- and DMD-Iso-hiPSCs encapsulated in GelMA hydrogel was evaluated and confirmed for its future application in the generation of cardiac disease modeling using 3D bioprinting techniques.

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Keywords: stem cells; organoids; cardiac trabeculation; Duchenne muscular dystrophy

Invecchiamento e patologie degenerative

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Induction of vesicular trafficking and apoptotic signalling in mononuclear leukocytes sign the "immuno-proteostasis" response to uremic proteins

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Uremic retention solutes have been alleged to induce cell death in different cell types, including peripheral blood mononuclear leukocytes (PBL), which may contribute to uremic leukopenia and immune dysfunction. The molecular effects of these solutes were investigated in uremic PBL (u-PBL) and mononuclear cell lines (THP-1 and K562) exposed to the high-molecular-weight fraction of uremic plasma (u-HMW; prepared 50 kDa micro-concentrator ultrafiltration).

u-PBL show reduced cell viability and increased apoptotic death compared to healthy control PBL (c-PBL). u-HMW induce apoptosis both in u-PBL and c-PBL, as well as in mononuclear cell lines, also stimulating cellular H_2O_2 formation and secretion, IRE1-α-mediated endoplasmic reticulum stress signaling and JNK/*c*-Jun pathway activation. Also, u-HMW induce autophagy in THP-1 monocytes. u-PBL were characterized by the presence in their cellular proteome of the main proteins and carbonylation targets of u-HMW, namely albumin, transferrin and fibrinogen, and by the increased expression of RAGE, a scavenger receptor with promiscuous ligand binding properties involved in leukocyte activation and endocytosis.

In conclusion, large uremic solutes induce abnormal endocytosis and terminal alteration of cellular proteostasis mechanisms in PBL, including UPR/ERSR and autophagy. These are potent triggers of JNK-mediated apoptotic signaling. The findings in this study describe at the molecular level the suicidal role of immune cells in facing the uremic disturbances of systemic proteostasis, a process that we define as the immuno-proteostasis response of uremia.

Keywords: leukocytes; ER stress; JNK; apoptosis; autophagy; uremia; uremic toxins; protein carbonyls; oxidative stress; immuno-proteostasis response

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Expression of APE1 endonuclease in primarily cultured aortic valve interstitial cells undergoing spontaneous senescence

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Apurinic/apyrimidinic endodeoxyribonuclease 1 (APE1) is the major enzyme involved in the base excision repair pathway working on DNA damages mainly caused by oxidative stress. Namely, APE1 hydrolyses the phosphodiester bond at the abasic sites generated by DNA glycosylases, creating the substrate for DNA polymerase β and DNA ligase IIIa which terminate the reparative process [1]. APE1 also seems to play a role in cell senescence maintaining telomere stability and size in interaction with specific telomere-protective proteins [2]. Here, aortic valve interstitial cells (AVICs) isolated from healthy bovine valve leaflets were cultured under normal conditions for up to 90 days to achieve spontaneous cell senescence. Time-dependent increase in β-galactosidase activity, a marker of cell senescence, was paralleled by a remarkable decrease of APE1-expressing AVICs starting from day 60, as immunocytochemically revealed. Quantitative Western blot analyses also showed a drop of APE1 protein content at day 60, whereas RT-PCR analyses revealed a mild increase of the enzyme mRNA over time. Ultrastructurally, AVICs appeared well preserved up to 30-day-long culturing. Conversely, starting from day 60, cells showed non-lysosomal autophagocytosis features mainly consisting of a hypertrophic rough endoplasmic reticulum engulfing suffering mitochondria [3]. Cytoplasm vacuolization due to large organelle degeneration was also clearly appreciable. In conclusion, decreasing APE1 expression over time is supposed to contribute to AVIC decay in a model of spontaneous cell senescence.

Keywords: APE1; cell senescence; aortic valve interstitial cells; autophagocytosis

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GNG13 Is a Potential Marker of the State of Health of Alzheimer's Disease Patients' Cerebellum

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Brain regions such as the cerebellum (CB) and Purkinje cells (PCs) have been neglected for a long time in the study of Alzheimer's disease (AD) pathogenesis (1). Purkinje cells (PCs) are among the largest neurons in the brain located in the cerebellum (CB). In reference to a new emerging hypothesis according to which there is an altered cerebellar synaptic processing in AD (2), we verified the possible role played by new biomarkers in the CB of AD patients compared with not-demented healthy control subjects (NDHS). For this purpose, we consulted and processed the data from several online sources such as GEODataSet, "The Human Protein Atlas" (HPA), BioDBnet, and GENESAT online web utility tool. The analysis of logical relations between three brain transcriptome dataset highlighted two genes, guanine nucleotide–binding protein (GNG13) and centrosomal protein 76 (CEP76) potentially expressed on PCs. The GNG13 expression was for the first time associated with PCs, at the contrary CEP76 had already been described. The mouse brain cell population transcriptome, the in situ hybridization techniques in transgenic C57BL/6 mouse during embryonic development, the bright-field images of EGFP immunohistochemistry (GENESAT), and the protein immunohistochemistry analysis using HPA, have confirmed the GNG13 detection in the CB and in particular in PCs. Furthermore, we have collected several microarray datasets and obtained 626 cerebella sample biopsies belonging to subjects who did not die from causes related to neurological diseases and 199 cerebella belonging to AD. We have correlated GNG13 expression levels with already widely existing bibliography of PC marker genes, such as Purkinje cell protein 2 (PCP2), Purkinje cell protein 4 (PCP4), and cerebellin 3 (CBLN3). We showed that expression levels of GNG13 and PCP2, PCP4, and CBLN3 were significantly correlated with each other in NDHS and in AD and significantly reduced in AD patients compared with NDHS subjects. In addition, we highlighted a negative correlation between the expression levels of PC biomarkers and age. From the outcome of our investigation, it is possible to conclude that the identification of GNG13 as a potentially biomarker in PCs represents also a state of health of CB, in association with the expression of PCP2, PCP4, and CBLN3.

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Keywords: GNG13; Purkinje cells; cerebellum

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A tunable *in vitro* Caco-2/HT-29 cell co-culture mimicking the intestinal barrier aging: a morpho-functional study

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The intestinal epithelial barrier (IEB) of elderlies undergoes a myriad of changes due to the microbiota and intestinal cell alterations, which drive the feed-forward cycle of leaky gut, inflammaging, dampened cell renewal, and defective mucus production [1]. Despite different *in vivo* and *ex vivo* studies [2] have shed some light on the age-associated impairment of IEB integrity and cytokine production, the involved molecular mechanisms are not completely understood. Thus, there is a critical need to develop an *in vitro* study model of the IEB reproducing the interactions between the absorptive and the secreting cells related to aging.

The present study aimed at characterizing the morphology and the physiology of the aged IEB through an *in vitro* model constituted by a co-culture of Caco2 and HT-29 cells, differentiated in absorptive and mucus-secreting phenotypes respectively [3], and sub-cultivated both in standard condition and for a longer time so that cell aging is gradually induced in a "physiological" way, avoiding the administration of exogenous stimuli. Preliminary results evidenced in the aged co-culture i) a diminished epithelial electrical resistance (TEER); ii) an increased paracellular permeability; iii) a slight decrease in cell proliferation; iv) a less homogeneous distribution of the membrane-associated claudin-1 immunostaining. Transmission electron microscopy (TEM) analysis revealed that the intracellular mucus and desmosomes were less represented in the aged co-culture, together with underdeveloped apical microvilli. Taken together, these preliminary results suggest the presence of impaired barrier integrity associated with a modulation of the morphological features mimicking the leaky/aged gut as reported in clinics. Future experiments could ascertain the use of the aged *in vitro* Caco-2 and HT-29 cell co-culture as a useful model for both studying the molecular process and testing potential drug/nutraceutical treatments to ameliorate gut aging.

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Keywords: leaky gut; TEER; cell permeability; TEM; tight junctions

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Clusterin in Parkinson's disease pathology: a study in human brain

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Deposition of β-sheet-rich amyloid aggregates composed of disease-specific proteins is a feature shared by many neurodegenerative disorders. Misfolding and aggregation of these proteins play a key role in neurotoxicity. In this context, molecular chaperones are key effectors of proteostasis, assisting protein folding and liaising with degradative pathways. Clusterin (Clu) is a multifunctional protein that owns chaperone-like properties and is highly expressed in the brain. Variations in CLU gene are risk factors for late-onset Alzheimer's disease. Clu mediates both amyloid beta and tau deposition and clearance, but whether this modulation is neuroprotective or neurotoxic is still controversial. Very recently, Clu has also been associated with the regulation of α-synuclein (αSyn) biology in Parkinson's disease (PD). On one hand, Clu binds αSyn and inhibits its αSyn aggregation. On the other hand, Clu limited the uptake of αSyn fibrils by astrocytes, thus possibly contributing to αSyn spreading. In this work we aim to better elucidate the understudied role of Clu in PD pathology in *post-mortem* human brains using immunohistochemical technique, proximity ligation assay and high-resolution confocal microscopy. First, we confirmed the presence of Clu in Lewy bodies and neurites, and we found extracellular Clu accumulation in PD brains. Moreover, we evaluated whether a differential Clu distribution exists between healthy subjects and PD patients. In particular, we explored Clu expression in different neuronal populations and glial cells. Our preliminary data show a vesicle-shaped staining of Clu in the cytoplasm of nigral neurons, but also astrocytes. Interestingly, we observed an increased staining for Clu in astrocytes of PD *post-mortem* human brains. This study will help to clarify the interplay between Clu and PD pathology, which would be key in the perspective of developing a therapeutic strategy.

Keywords: Clusterin; α-synuclein; Parkinson's disease

Meccanismi molecolari di controllo della crescita cellulare

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Endolysosomal two-pore channel 2 plays opposing roles in primary and metastatic malignant melanoma cells

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The ion channel two-pore channel 2 (TPC2) localized on the membranes of acidic organelles such as endolysosomes and melanosomes and known to control a variety of physiological functions, has been shown to play a role in relevant pathologies, e.g. tumour metastasis and viral infection and to control neo-angiogenesis. We have recently demonstrated that NAADP/TPC2/Ca^{2+} signalling is involved in melanoma progression and metastasis. Using the murine melanoma B16F0 primary cell line and its subline B16F10, which have metastatic traits, we have now characterized the relevance of TPC2-regulated signalling at different stages of tumour progression. By comparing the impact of TPC2 knock-down (TPC2-KD) and/or knock-out (TPC2-KO) on parameters of aggressiveness in these two cell samples, we have detected surprisingly different responses suggesting that, within the same tumour type,TPC2 can play a pro- or an anti-metastatic role, depending on the stage of the malignancy. TPC2 silencing resulted in increased migration and increased the epithelial-to-mesenchymal transition in the metastatic samples, but abated them in the silenced primary ones. Following TPC2 gene inhibition we have observed an increase in the autophagic flux in B16F10 melanoma cells, and a decrease in the autophagic flux in the primitive B16F0melanoma cells. We have found that only in the metastatic subline TPC2 silencing reduces binding to collagen type I matrix. In contrast to the B16F0 melanoma cells, only in B16F10 did TPC2 silencing result in increased levels of the mesenchymal markers vimentin and N-cadherin. Interestingly, while TPC2 inactivation failed to affect markers of proliferation in both samples, it strongly enhanced the migratory behaviour of B16F10 cells, again suggesting that in the more aggressive phenotype TPC2 plays a specific anti-metastatic role. In line with this, overexpression of TPC2 in B16F10 cells resulted in phenotype rescue i.e. a decrease in terms of migratory ability, adhesion to collagen type I, and expression of epithelial-to mesenchymal transition markers, thus collectively resuming traits of the B16F0 primary cell line. In conclusion, our research shows a novel role of TPC2 in melanoma that is intriguingly different at the late stages of cancer development, compared to that during the initial stage of carcinogenesis.

Keywords: lysosomal calcium channels; two-pore channels; TPC2, melanoma; primary melanoma; metastatic melanoma; aggressiveness

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Different lipid nanovesicle preparations containing *C. jejuni* CDT lysate differently inhibit proliferation in tumor intestinal epithelial cells.

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Cytolethal distending toxins (CDTs) constitute a family of bacterial protein toxins that are produced by various Gram-negative bacteria. CDT creates double-strand breaks (DSBs), cell cycle arrest at the G2/M phase, thereby inducing cell distension and ultimately cell death. Recently, different authors proposed CDT as a potential tool to be used in anti-tumor strategies [1]. Indeed, our group [2], demonstrated that Extracellular Vesicles (EVs) from *Campylobacter jejuni* CDT-treated Caco-2 cells inhibit proliferation of tumour intestinal Caco-2 cells and myeloid U937 cells. In our system CDT targets the endo-lysosomal compartment, partially evading lysosomal degradation and exploiting unconventional secretion (EV release). CDT-like effects are transferred by Caco-2 cells to uninfected heterologous U937 and homologous Caco-2 cells: the CDT G2/M blocking effect is particularly evident in homologous Caco-2 cells in respect of the U937 ones, demonstrating both a different uptake degree by cells from different lineages, and different CDT effect, depending on tissue and cell type. In order to confirm if our data have the potential to represent a future bacterial-related biotherapeutic, we provided to construct lipid nanovesicles extruded with *C. jejuni* lysates, containing the active CDT. Our preliminary data demonstrate that these different lipid formulations (lipid nano Vesicles-LNVs) 1 and 2, that can mimic EVs, can rapidly enter epithelial intestinal tumor cells and exert a strong antiproliferative effect, with differences related to the specific formulation of the –LNVs. Indeed, the presence of active CDT is demonstrated by both low proliferation rates with the characteristic G/M blocking, and hypodiploid peak, indicating the induction of a moderate apoptotic process. These findings represent a good foundation to consider these formulations as a potential tool for anti-tumor strategies.

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Regulation of signal transduction pathways in Myelodysplastic Syndromes (MDS): balance between hematopoietic stem cell proliferation and differentiation

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Nuclear inositide signaling pathways revolving around Phosphoinositide-specific Phospholipases C (PI-PLCs) are implicated in the regulation of proliferation and differentiation of hematopoietic stem cells (HSCs). Indeed, PI-PLCbeta1, PI-PLCgamma1 and PI-PLCgamma2, but also the PI3K/Akt/mTOR pathway, play essential roles in the pathogenesis of Myelodysplastic Syndromes (MDS) and their progression to Acute Myeloid Leukemia (AML)[1]. Notably, the AML secondary to MDS are particularly aggressive and resistant to therapy, and their dysplastic HSCs are characterized by increased cell proliferation[2].

Even drug-induced modulation of nuclear inositide signalling is associated with cell proliferation and differentiation in MDS/AML[3]. Indeed, MDS cells obtained from patients losing or lacking response to epigenetic therapy, thus showing an increased blast proliferation, can acquire common mutations on 3 inositide-specific genes (PLCG2, AKT3, PI3KCD)[4]. Interestingly, the same refractory patients also showed specific microRNA deregulation[5], particularly affecting miR-192-5p, a target of inositides that, in turn, specifically targets and inhibits BCL2, thus possibly affecting cell proliferation. All in all, the regulation of nuclear inositide pathways in MDS is important to disclose the molecular mechanisms underlying normal HSC proliferation and AML progression, as well as lead to the identification and development of new targeted therapies.

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Keywords: Phospholipase C; Hematopoietic Regulation; Myelodysplastic Syndromes; Cell Proliferation; Cell Differentiation

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Mechanisms of resistance to last generation EGFR Inhibitors: hijacking NRG1/ERBB3 axis

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EGFR-mutated non-small cell lung cancer (NSCLC) is successfully treated with small molecules TKI inhibitors. However, tumor relapse occurs and the underlying mechanisms of drug resistance remain largely unexplored. In this vein, the efficacy of antibody poly-therapy has been successfully demonstrated in preclinical models. The complete abrogation of EGFR axis, achieved through the combination of osimertinib and cetuximab, leads to the activation of the parallel pathways HER2 and HER3[1–3] along with the upregulation of the HER3 ligand, NRG1. In line, NRG1 when overexpressed increases proliferation and invasion of cancer cells conferring resistance to EGFR/HER2-targeted therapy[4].

Interestingly, EGFR resistant patient-derived cell lines, confirm the strong activation and overexpression of HER2 and HER3, which appear as bypass pathways in the acquisition of Osimertinib resistance.

HER3 is the preferred dimerization partner of HER2. The evaluation of mRNA levels of its ligand, NRG1 support the hypothesis of a correlation between osimertinib resistance and HER3 activation.

Proliferation assays performed both in monolayer and in 3D growing conditions on sensitive cells shown that NRG1 strongly impairs osimertinib response, as tested by cell proliferation and invasion in gelatin degradation assays.

Conversely, employing a monoclonal antibody anti-NRG1 we observed a reduced gelatin degradation in resistant cells, confirming the key role of NRG1 in cell invasion and metastasis. Finally, *in vitro and vivo* data confirmed that, when combined with osimertinib and cetuximab, the anti-NRG1 antibody strongly inhibited cells and tumor growth in mice, preventing TKIinduced up-regulation of HER3 and its activation.

Collectively, these findings support the hypothesis that NRG1 may represent an escaping mechanism to EGFR inhibition and its neutralization will effectively impair ERBB signaling through both HER2, HER3 and HER4 parallel pathways activation.

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Keywords: NSCLC, Resistance, Osimertinib, EGFR, NRG1.

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MDM2 inhibitors counteract inflammation by stabilization of ACE2 *in vitro*

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Angiotensin-converting enzyme 2 (ACE2) is a cell surface receptor that converts angiotensin II to angiotensin-(1-7), which has been identified for its anti-inflammatory activity. In agreement, ACE2 has been reported to be downregulated under inflammatory conditions associated with cytokines storm, such as during Covid-19 disease and sepsis.

Since inflammation is known to induce endothelial cell dysfunctions associated with vascular complications in patients, the aim of our work was to investigate an efficient treatment to restore ACE2 levels and ameliorate endothelial functions.

In a model of alveolar epithelium (A549-hACE2), we preliminarily observed, by western blotting and immunofluorescence analysis, an efficient upregulation of ACE2 protein after treatment with the MDM2-inhibitor Nutlin-3a, contrasting MDM2-depending ubiquitination of ACE2 protein.

In a model of endothelium (HUVEC), we furthermore tested Nutlin-3a both in presence and in absence of inflammatory stimuli induced by TNFα, high glucose or LPS to mimic acute or chronic pathologies. To this end, cells were treated with several concentrations of Nutlin-3a alone or in presence of inflammatory stimuli. After 24 and 48 hours of treatments, cells were collected for analysis of ACE2 levels by western blotting and biological effects mediated by Nutlin-3a-dependent p53 induction (cell viability, cell cycle, apoptosis and protein levels of p53-gene targets). In parallel supernatants were collected for secreted cytokines analysis.

Results showed a concentration-dependent upregulation of ACE2, p53 and MDM2 proteins in Nutlin-3a treated cells together with induction of a cell cycle block, low levels of apoptosis, both in physiological and inflammatory environments. Moreover, Nutlin-3a mitigates inflammation by the reduction of IL-6 release respected to cells exposed to inflammatory stimuli.

These results indicate an efficient ACE2 stabilization and control of inflammation associated with MDM2 inhibitors, suggesting an efficient tool with therapeutic potential for vascular disorders associated with inflammation.

Keywords: ACE2; Nutlin-3; Inflammation

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Phospolipase C family enzymes as prognostic and predictive biomarkers in lung cancer

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Lung cancer (LC) is the most frequently diagnosed cancer in both males and females (11.6% of all cases) and represents the primary cause of cancer-related mortality worldwide (18.4% of all cancer-related deaths). LC therapies have undergone significant improvements with the advent of immunotherapy; however, the effectiveness of the available treatments remains insufficient due to the presence of therapy-resistant cancer cells[1]. The identification of novel prognostic molecular markers is crucial to understand the underlying mechanisms of LC initiation and progression. The potential role of phospholipase C (PLC) in tumor growth and metastatic process has been recently suggested by some researchers[2]. Here, the involvement of PLC isoforms has been evaluated both in patients' biopsies and immortalized lung cancer cell lines. Gene and protein expression has been carried as well as PLC cellular localization. Real time PCR analysis evidences an overexpression of PLCB1 gene in most considered patients. PLCB2, PLCB4 and PLCE1 genes appear reduced mainly in adenocarcinoma samples. PLD4 gene seemed to have an histotype-specific regulation.

Gene expression of PLCs in lung cancer cell lines shows an evident deregulation of PLCB1 and PLCD4 which appears highly expressed in some lung cancer cells and localized both in the cytoplasm and in the nucleus. These findings demonstrate the involvement of PLC family enzymes in LC, suggesting for these molecules an emerging role as potential biomarkers. Further studies are needed to establish how phospholipase family enzymes regulate cancer-associated cellular processes and in particular their influence on cell motility and cell proliferation in specific lung cancer types.

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NFATc1 and NFATc2 transcription factors drive the resistance to glucocorticoids in Acute Lymphoblastic Leukemia pediatric patients

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Around 25% of pediatric patients with T-cell acute lymphoblastic leukemia (T-ALL)[1] exhibit glucocorticoid (GC) resistance and encounter a poor prognosis[2-4]. It is therefore mandatory to identify new molecular mechanisms responsible for GC resistance that can represent novel therapeutic targets for the development of alternative therapeutic strategies to improve the prognosis of these patients. In a previous study we demonstrated how LCK kinase hyperactivation contributes to GC resistance and how its inhibition, both *in vitro* and *in vivo*, sensitizes T-ALL cells to the pro-apoptotic action of GC[5]. In line, we recently observed that the NFATc1 and NFATc2 transcription factors, downstream of LCK, are more expressed at diagnosis in GC-resistant T-ALL patients and that their specific gene silencing restores dexamethasone response by re-establishing GC receptor transcriptional activity. Conversely, their overexpression confers resistance to GCs. Of note, NFATc1 gene silencing decreases the intracellular cholesterol levels and consequently the abundance of lipid rafts (LR), as well as the expression/activation of the LCK kinase that is anchored to LR. In line, exogenous cholesterol supplementation in NFATc1-silenced T-ALL cells restores GC resistance. Additionally, NFATc2 gene silencing reduces the Wnt/β-catenin pathway and the expression of stem cell-related markers, enhancing T-ALL cell differentiation. Finally, *in vitro* inhibition of cholesterol biosynthesis by simvastatin or of the Wnt/β-catenin pathway by the inhibitor ICG-001 sensitizes resistant T-ALL cells to GCs. Collectively, these results suggest that NFATc1 and NFATc2 control cholesterol biosynthesis and the maintenance of a stem-like phenotype respectively in T-ALL GC resistant cells, both processes known to be involved in chemotherapy resistance[6-8]. Thus, the pharmacological inhibition of these signaling pathways could represent new therapeutic options for GC-resistant T-ALL pediatric patients for whom no alternative therapeutic approaches are currently available.

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The RNA binding protein QKI guides a pro-mesenchymal splicing program driving a subtype switch and limiting outcome in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by an extremely poor prognosis. Transcriptomic analyses have identified two molecular PDAC subtypes, one of which, namely the basal-like subtype, is associated with chemoresistance and worse clinical outcomes. Splicing dysregulation is known to contribute to PDAC malignancy. However, its involvement in subtype specification remains elusive to date. Herein, we have identified a subtype-specific splicing signature strongly associated with prognosis in PDAC patients. Functional studies in PDAC cell lines identified the splicing factor QKI as a key determinant of the basal-like splicing signature, which is associated with increased tumor aggressiveness. QKI represses splicing events associated with the classical subtype and improved clinical outcome, while promoting basal-like events associated with shorter survival. Analyses of single cell sequencing data further confirmed that QKI is a reliable marker of the basal-like phenotype. Experiments using PDAC patient-derived organoids and cell lines showed that QKI promotes a plastic, quasi-mesenchymal phenotype that supports migration and chemoresistance of basal-like PDAC cells. Thus, we have identified a splicing signature that molecularly and clinically defines distinct PDAC subtypes. Furthermore, we provide evidence that the splicing factor QKI promotes the acquisition of an undifferentiated and plastic phenotype, which renders PDAC cells chemo-resistant and responsive to environmental cues.

Keywords: Pancreatic cancer; organoids; splicing; gene expression; tumor progression

Anatomia e movimento

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Wild trekking as an opportunity for rapidly improving anthropometrics, cardiorespiratory and muscular performance in active older adults: the Sardinia "Selvaggio Blu" experience

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To test the safety and feasibility of a challenging wild trekking 5-day experience, and its acute effects on anthropometrics and cardiorespiratory and muscular performance in healthy older adults with mountaineering expertise.

Twelve healthy active volunteers in their sixth decade of life of comparable training status, mountaineering experience and age were recruited. Before and after engaging in a 5-day wild trek ("Selvaggio Blu", Sardinia, Italy), participants underwent a comprehensive evaluation of anthropometrics, cardiorespiratory fitness, muscle strength and flexibility, balance, and lipidic profile.

The wild trekking was well tolerated by all participants who, however, reported moderate pain at the ankle joint (mean: 59.2±13.1 mm). Following the trek, sovrailiac fold decreased both in men (-21.9%; p=0.006) and women (-34.5%; p=0.007). Fat mass was also significantly reduced (men: -8.5%; p=0.001; women: -6.6%; p=0.014). Gender-based differences emerged for energy expenditure, with women displaying higher caloric expenditure to complete the trek (women: 12.88±3.37 kcal/hour/kg; men: 9.27±0.89 kcal/hour/kg). Likewise, VO_2 peak increased only in women (+12%; p=0.013). Centre of pressure sway area during closed eyes single stance was found increased, i.e., worsened, both in men (+251%; p=0.027) and women (+71%; p=0.043).

With all due caution, especially for balance and proprioception, wild trekking routes offer unique opportunities to rapidly improve anthropometrics, cardiorespiratory and muscular performance. For older adults with technical and physical preparedness for this type of demanding activity, wild trekking has the potential to evolve from a niche activity for alpine mountaineers into a safe and feasible recreational activity and leisure pursuit.

Keywords: mountaineering; hiking; trekking; energy cost; gender differences; balance

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Ponticulus posticus and Malocclusion: a pilot morphological study in a Southern Italian pre-orthodontic cohort

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Ponticulus Posticus (PP) is a bony protrusion located in the cervical spine, between the posterior portion of the superior articular process and the posterolateral portion of the superior margin of the posterior arch of the atlas vertebrae (C1). It is observed in about 15-20% of the population and has been associated with various clinical conditions, such as headache, neck pain, diplopia, dysarthria, dysphagia, and vertigo, due to vertebral artery compression. The embryological origin of PP is unclear, but it may originate from the dorsal arch of proatlas or protect the passage of the vertebral artery during cranial and neck movements. This study aimed to investigate the presence of different types of PP in a Southern Italian pre-orthodontic cohort and its association with skeletal disorders and malocclusion with the purpose to improve its clinical and therapeutic evaluation. A total of 212 lateral cephalograms were analyzed, and PP was detected in 89.7% of the cases. The prevalence of PP was higher in subjects with skeletal Class I malocclusion compared to Class II and Class III malocclusion. Females had a higher prevalence of PP, but the difference between genders was not statistically significant. PP was also associated with dental anomalies, such as hypoplasia, agenesis, and displacement of mandibular molars. The findings of this study suggest that PP should be considered when evaluating and treating individuals with skeletal discrepancies and dental anomalies. It may have implications for orthodontic treatment planning, especially in Class II individuals. However, further research is needed to understand the exact mechanism behind the association between PP and dental anomalies and its impact on craniofacial growth and morphology. Clarifying this correlation will contribute to comprehensive orthodontic treatment planning.

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Keywords: Atlas; malocclusion; vertebral anomaly; ponticulus posticus; bony protrusion

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Pilates mat training improves flexibility in young volleyball players

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The Pilates method stretches and strengthens muscles, improving muscle elasticity and joint mobility. The effectiveness of this method, with reference to the Abdominal Resistance and Joint Mobility in a male population, was investigated. The effect on abdominal resistance, on the mobility of the shoulder, hip and spine joints was studied with bodyweight training (Mat Class). The hypothesis was that participants would achieve improvements on each of these variables after a 28-week biweekly training period. The study was conducted at the Pilates Studio "La Via del Pilates" of Isernia Fitness gym (Isernia, Italy), from October 2018 to May 2019. The purpose of this study is to evaluate the influence of the practice of Pilates on the Range Of Motion (ROM) of the spine, the flexibility of the lower limbs and the abdominal strength, evaluating in particular the lumbar flexion, the extension and rotation of the spine.

Forty young athletes who played volleyball from the University Sports Center of Campobasso were selected and were equally randomized into two groups (Pilates group and control group). The athletes performed the following tests: Modified Schober test for flexion and trunk rotation, Modified fingertip to floor test, straight leg raise test and popliteal angle and half sit-up.

In the Pilates Group, the results from the modified Schober test in flexion statistically varied 12 weeks after the start of the training protocol ($p<0.05$), but not in the Control Group. Regarding trunk rotation, there was a statistically significant improvement in the Pilates group ($p<0.05$) while not in the control group ($p=0.07$). In both groups, for the FTF test the values improved with statistically significance 12 weeks after the start of the study protocol (Pilates group vs control group, $p<0.05$).

Pilates mat training can improve flexibility in young volleyball players. Pilates mat training could be an effective way to improve flexibility in young volleyball players. Coaches and trainers should consider incorporating Pilates into their training programs.

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Keywords: Pilates Training; Pilates-Based Exercises; Flexibility; Pilates mat exercises

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Preclinical anatomical evaluation of the application of a galeo-pericranial flap in oral cavity defects reconstruction: vascular study and surgical procedure simulation on a revascularized donor cadaver

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Defects of the oral cavity occur after resection of lesions limited to the mucosa, alveolar gum or minimally affecting the bone. Aiming at an esthetical and functional improvement of intraoral reconstruction, the possibility of harvesting a galeo-pericranial free flap was explored. The final objective of this study is to assess the technical feasibility of the flap harvesting through anatomical dissections and surgical procedure simulations.

Dissections on ten (n=10) cadaveric anatomical head and neck specimens were undertaken bilaterally to simulate the surgical procedure and evaluate the vascular calibers of the temporal and cervical vessels. The surgical procedure was therefore reproduced on a revascularized and ventilated donor cadaver: an anastomosis between superficial temporal and facial vessels was performed and blood-mimicking fluid presence and entity were evaluated using doppler ultrasound.

Anatomical dissections demonstrated that mean cervical vascular calibers are compatible with superficial temporal ones, proving to be technically adequate for anastomosis. Perforating branches of the superficial temporal vascularization nourishing the pericranium were identified in all the cadavers dissected. The presence of blood flow was recorded after anastomosing superficial temporal and facial vessels in a revascularized donor cadaver using SimLife® technology, demonstrating both the technical feasibility of this procedure and the potential revascularization of the flap, nourishing both the galeal and pericranial layers.

Galeo-pericranial pedicled flap harvesting has been proven to be a safe and effective procedure for intraoral reconstruction in previous clinical studies. The good intraoral healing and remucosization, together with minimum donor side morbidity and no permanent complications, have encouraged the evaluation of its potential as a free flap for defects that affect mandibular alveolar gum or the lower oral mucosa. This preclinical study demonstrated the technical feasibility of the free flap harvesting procedure and of the potential flap revascularization through vascular anastomosis between superficial temporal and cervical vessels.

Keywords: galeo-pericranial flap; surgical simulation; vascular study; cadaveric dissection

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Non-Invasive Procedures for the Evaluation and Monitoring of Adolescent Idiopathic Scoliosis and Adapted Physical Activity Treatment

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Adolescent idiopathic scoliosis (AIS) is a type of spinal deformity characterized by a three-dimensional alteration in the structure of the spine, causing vertebrae to rotate and deviate from their normal alignment. The conventional method for diagnosing scoliosis involves the use of X-rays, which can pose potential health risks and are sometimes ordered before scoliosis is even suspected. The fixed plumb line, rasterstereography and Infrared Thermography (IRT), represent valuable non-invasive tools for evaluating and monitoring scoliosis. This study aimed to investigate new non-invasive methods for the assessment and monitoring of adolescents with scoliosis in order to identify the best suitable adapted physical activity treatment. Forty-eight adolescents were recruited, 24 of whom had AIS diagnosed by X-rays. Subjects were divided into scoliosis and non-scoliosis groups. The participants were asked to stand still with the FPL touching the most prominent point, either the occipital prominence or the reference point for the kyphosis. Afterwards, the patient underwent a 3D analysis of the back with the Spine 3D system. For the thermographic imaging, the trapezius (TM), latissimus dorsi (LDM), and quadratus lumborum (QLM) muscles were considered. For the fixed plumb line measurements, the scoliotic group showed a C7 depth of 42.06 ± 18.96, T12 depth of 38.82 ± 14.85, and L3 depth of 39.71 ± 16.72. The non-scoliotic group showed C7 depth of 32.69 ± 20.88, T12 depth of 36.92 ± 21.46, and L3 depth of 37.69 ± 20.27. For the Spine 3D measurements, the scoliosis group showed a cervical depth of 38.33 ± 17.52, lumbar depth of 46.73 ± 21.44, and coronal imbalance of -1.53 ± 12.58. The non-scoliotic group presented a cervical depth of 43.85 ± 16.40, lumbar depth 40.39 ± 9.52, and coronal imbalance of -4.76 ± 8.64. Finally, for the thermography imaging, the scoliosis group showed a mean temperature of the TM 33.7 ± 0.94, LDM 32.9 ± 1.1, and QLM 32.2 ± 1.2. The non-scoliotic group presented mean temperatures of the TM 34.1 ± 2.6, LDM 33.3 ± 1.6, and QLM 32.7 ± 1.8. The results of this study confirm that the combination of these non-invasive methods can be effective procedures for evaluating and monitoring adolescents with AIS, supporting the identification of the best-adapted physical activity treatment.

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Keywords: Adolescent idiopathic scoliosis; Fixed plumb line; Rasterstereography; Infrared Thermography

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Digital media exposure and physical and mental health in children

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The exposure to digital media has a growing trend in children and it has an impact on their physical and mental health. Additionally, the lockdown periods due to COVID-19 pandemic further exposed children to digital media for e-learning activities. Therefore, the aim of this study was to systematically review the effect of digital media on physical and mental health in children. Moreover, the effect of COVID-19 pandemic was further investigated.

Scientific databases were referred for studies selection. Screening process followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement and flow diagram. The Joanna Briggs Institute (JBI) Checklists were used for quality score assessment.

Forty studies were included, mainly cross-sectional and rated with moderate quality. The included studies were discussed according to five identified outcomes: 1) physical activity and body composition, 2) motor skills and posture, 3) sleep, 4) behaviour, and 5) COVID-19 and vision function.

The frequent and prolonged exposure to screens reduced the physical activity amount in children, with adverse effects on their body composition. Behaviour and sleep habits changed in children overexposed to digital media (i.e., emerging feelings of anxiety, poor adaptive skills and control abilities, later bedtimes, sleep quality reduced). Poor academic performance and learning development were reported in children with a higher number of (and time spent on) digital devices. Finally, the exposure to digital screens during COVID-19 pandemic resulted in poor vision and ocular condition (i.e., myopia).

Different domains of health in children can be impaired by excessive exposure to digital media. Considering that screen time recommendations and guidelines are provided, parents and adults involved in children's care should be mainly aware of the exposure risks and should differently influence digital media behaviour towards a positive and healthy lifestyle of children.

Keywords: body mass index; adiposity; screen time; quality of life; sleep disturbances

Morfologia, attività settoria e strategie didattiche

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Pterygomandibular raphe influence in Mandibular Advancement of Obstructive Sleep Apnea Syndrome treatment: an anatomical and morphology study

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Obstructive sleep apnea syndrome (OSAS) is a respiratory sleep disorder and consists of recurrent episodes of total or partial blockage of the passage of air. A noninvasive and an alternative surgical procedure, as maxillomandibular advancement or uvulopalatopharyngoplasty, is represented by a treatment using an oral mandibular advancement devices (MAD) MAD treatment increases airway space by a mandibular advancement modifying lateral walls of the pharyngeal airway and their oral and pharyngeal muscular parameters. The aim of this study was identified on ten cadaveric anatomical bilaterally head and neck specimens, obtained by donors, pterygomandibular raphe tendinous (PMR), in particular its presence or none, related with the clinical outcome of MAD treatment. Moreover, evidencing microstructure of tendinous tissue and measuring stretch of the lateral airway tissues, considering PMR as a tendon less extensible than muscle.

The hypothesis is that participants with tendinous PMR presence would have less lateral expansion of the upper airway stimulating by mandibular advancement by using MAD, its efficacy on oropharyngeal space augmentation and a negative consequence on OSAS treatment.

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Keywords: Obstructive sleep apnea; mandibular advancement; pterygomandibular raphe; morphology

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Clinical anatomy of the spina musculi recti lateralis

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The spina musculi recti lateralis (SMRL) is a bony process reported as located along the lower margin of the superior orbital fissure (SOF) at the junction of the wider posterior and the narrower anterior portions of the fissure. It is known to have a certain variability in shape but it still awaits a proper characterization. In this study we studied the clinical anatomy of the SMRL.

Orbits from 291 adult dry skulls and from 60 computed tomography (CT) scans of different age and sex were analyzed in order to measure the distance between the SMRL and the superior (SOF) or the inferior orbital fissures (IOF) as well as SMRL height, width and orientation. Foetal skulls were also observed for comparison with adult samples.

Results indicated that a SMRL has been found in 41% of dry skulls and 43.3% of CT orbits analyzed. Results obtained by CT measurements were similar to those assessed by direct measurement: on average, SMRL were orientated almost along the transverse plane and showed implant bases as wide as 141.9° or as narrow as 36.8°. Though mostly horizontally aligned, orientation of the SMRLs can be markedly different, even vertical in one orbit. The functional meaning of a different orientation is unknown, but it is possibly related to the anatomical structures that take insertion into the spine.

SMRLs were close to the infero-posterior angle of the orbital plate of the sphenoid, 1.21 ± 0.84 mm in front of the SOF, 5.8 ± 1.9 mm above the IOF and 12 ± 2.3 mm from the anterior end of the SOF. They were 1.58 ± 0.64 mm high and did not show any age or sex-related prevalence. SMRLs showed wide morphologic variations ranging from triangular acuminated spines with a base of implant of variable size, to spines with a smoothened or truncated apex. Most of the SMRLs appear as relatively large structures as the angle between their two margins was more frequently obtuse. By CT, the SMRL appeared as the insertion site for the lateral rectus, for tendinous ring and, sometime, for the inferior rectus.

These results showed how the SMRL is a process of the sphenoidal orbital plate rather than a feature of the SOF and, when present, it is also a reliable landmark for the insertion of the tendinous ring and lateral rectus. Orbital surgeon should be aware of this common variant of the orbital apex.

Keywords: spina musculi recti lateralis; sphenoid; orbital apex; annulus of Zinn

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The surgical simulation for urology residents. Preliminary data on the educational impact of cadaver-labs

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The practical activity of surgical residents requires them to try their hand at surgeries of increasing complexity and difficulty during their education. The learning of the surgical procedure by the neophytes is not free from technical mistakes or quality results below the standard, despite the supervision of the tutors. Creating a surgical gym is extremely important in this setting, to help residents train safely with simulated surgery cases within a body donation program.

The results of a preliminary study involving urology residents operating on donated bodies from the Body Donation Program of the University of Padua are reported. The surgical realism perceived, the educational usefulness of a simulated setting, and the emotional involvement perceived by the learners were investigated.

It has been shown that residents' expectations have been exceeded regarding the realism of the surgical setting, the superiority of the simulation compared to the alternatives practiced today, and an emotional involvement clearly present and significant during surgical procedures. Urology residents gained an educational benefit, improving manual skills in safely carrying out the planned interventions, with a positive propensity to participate in future similar training opportunities.

A realistic surgical setting simulation by implementing cadaver labs is an utmost positive training option for surgical residents, in terms of notions, procedures, and emotional involvement, to benefit their future patients.

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Keywords: Cadaver lab; Body donation; Surgical simulation; Education

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The Use of Anatomage Table in Legal Medicine: Forensic Pathology and Odontology applications

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The Anatomage Table (Anatomage, Inc. in Santa Clara, CA) is a life-sized digital tool enhanced by an interactive touchscreen, designed for studying human anatomy. It enables students to virtually dissect cadavers and observe specific structures by applying arbitrary clipping planes in multiple directions. This interactive experience enhances their understanding of anatomical relationships among structures. The Anatomage Table is utilized in undergraduate medical education, offering access to an extensive library of CT and MRI scans, including 4D scans.

Here, we propose an innovative application of the Anatomage Table as a digital aid for legal medicine, and forensic pathology and odontology training programs. By uploading total body CT scans onto the table, it enables virtual autopsies and dissections, facilitating the learning process of postmortem radiological data collection, measurements, and analysis. This can be done both onsite and remotely. Postgraduates can benefit from the high-resolution observation of the entire body, gaining valuable experience in autopsy dissection through special virtual reversible cuts that are otherwise impractical. Furthermore, every tissue can be dissected, allowing for detailed observation of any anomalies. Digital analytical tools can be used in specific cases of identification of victims through analysis of the jaw and teeth.

It is important to note that while the Anatomage Table offers significant advantages, it should be considered as an adjunct training tool in forensic pathology programs. It cannot replace the in-situ examination of cadavers and traditional autopsies. However, its future applications could extend to real forensic casework, seeking second expert opinions, or conducting virtual autopsies on infected or CBRN-exposed (chemical, biological, radiological, and nuclear) cadavers.

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Keywords: Anatomy; Legal Medicine; Odontology; Forensics; 3D Models

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Putting the vertebrae together: a screening method based on 3D morphometric features of the atlantoaxial joint to apply to commingled remains contexts

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Re-associating skeletal remains is challenging and needs methods based on morphometric correspondences to match skeletal pairs and joint elements [1-4]. Re-associating the cranium to the post-cranium is crucial as it allows to properly profile the remains, mainly where the skull is disjointed from the rest of the body. Yet often the skull is still jointed to atlas which is instead the one disarticulated from the rest of the body. If disarticulation occurs at the atlantoaxial joint, the correct match of atlas and axis is auspicial to assembly the entire body. Though the importance of this joint, no study has so far explored methods to match atlas with axis.

We explored the potential of re-associating atlas and axis through a 3D approach testing the congruency of their articulating surfaces. The superimposition of the articular facets of the two 3D vertebrae models acquired by a laser scanner was performed to measure the geometrical congruency in terms of point-to-point distance (Root Mean Square, RMS) of two jointing elements either from the same individual (matches) and from different individuals (mismatches). We verified the RMS values for both groups and their differences with Mann-Whitney test, thus providing a threshold value for discriminating between matches and mismatches. Atlas and axis from 46 skeletal remains were superimposed for making all possible combinations: 41 matches and 1851 mismatches. No sex-related significant differences were found for matches and mismatches (respectively $p=0.27$ and $p=0.21$), allowing to group together the two sexes in each group. RMS values ranged between 0.41mm to 0.77mm for matches and between 0.37mm and 2.18mm for mismatches. Significant differences were found comparing the two groups ($p<0.001$) and the highest RMS of matches (0.77mm) used as the threshold value for discriminating matches from mismatches, so reaching a sensitivity of 100% and a specificity of 41%.

We cannot consider the 3D superimposition of the atlanto-axial articular facets as a re-association method *per se*, but as a screening one with a negative predictive value of 100%. The 3D superimposition performed better in case of atlantoaxial joint if compared with others, as the atlantooccipital and the temporomandibular ones [1,5]. Further studies on other skeletal traits might provide answers to this complex issue.

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Keywords: forensic anatomy; commingled remains; 3D analyses; atlanto-axial joint; bones re-association; 3D morphometric analysis; point-to-point distances

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Relationship between 3D morphometric facial features and other systemic manifestations in Marfan syndrome: an exploratory study

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Marfan syndrome (MFS) is a rare hereditable connective tissue disorder due to mutations in the fibrillin-1 gene *FBN1*, with clinically heterogeneous manifestations including distinctive facial features; early recognition of MFS is essential to prevent its major cardiovascular complications. In a previous study, we pointed out 3D morphometric facial features in a group of adult patients with MFS [1]. In this study we enlarged the sample size and explored the relationship between facial dysmorphism and other systemic manifestations of MFS.

Facial linear distances and angles of 104 adult Caucasic subjects with MFS (42 males, 37±15 yrs; 62 females, 42±16 yrs) were computed from the 3D coordinates of soft-tissue landmarks obtained by stereophotogrammetry. Corresponding z-score values were calculated comparing patients with 665 healthy reference subjects matched for sex and age; their statistical significance was evaluated by Student's t-test. Principal component analysis (PCA) was applied to facial variables to reduce dimensionality; the relationship between the generated components and other manifestations of MFS was evaluated by multiple logistic and stepwise regression analyses.

All the quantitative facial abnormalities identified in the previous study were confirmed; in addition, consistently with the increased middle third of the face, subjects with MFS showed increased nose and ear lengths ($p<0.001$). PCA generated four components that explained 75.32% of the total variance, representing facial heights, facial depths, facial widths and palpebral fissure abnormalities respectively. The former component was positively associated with ectopia lentis ($p=0.004$), systemic score (a value summarizing the systemic features of MFS, particularly the skeletal ones; $p=0.008$) and mitral valve prolapse (MVP; $p=0.047$); the last component was positively associated with MVP ($p=0.018$). Interestingly, among the diagnostic criteria of MFS skeletal features and MVP are considered together.

In addition to confirming that a detailed 3D morphometric analysis of facial appearance may help clinicians in suspecting MFS, the promising findings of the study suggest the existence of easily detectable markers on the surface of the face with potential predictive value for less visible but characters of the syndrome and encourage further in-depth investigations.

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Keywords: face; anthropometry; Marfan syndrome (MFS); stereophotogrammetry; facial morphology; clinical anatomy

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Bifid ribs in ancient human populations: a systematic reassessment

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Bifid ribs (also known as "bifurcated ribs") are a rare congenital anomaly that accounts for ca. 28% of described rib abnormalities. Their presence in modern populations has been reported in both anatomical and radiological studies, but this anomaly's antiquity has never been subjected to a systematic assessment, it often being merely mentioned anecdotally.

The present study consists of two parts:

a. a morphological and radiological examination of cases from 3 different sites, each one distant no more than 1.5 km from one another and all of them located around the city of Brześć Kujawski, north-central Poland, in the historical land of Kujawy. The three populations date back to the Neolithic (4600-4000 BCE), the Middle Ages (12th-16th centuries CE), the Modern Era (15th-19th centuries CE). All analyzed skeletal series came from the collections of the Department of Anthropology, University of Łódź and the Museum of Archaeology and Ethnography in Łódź. The possession and the analysis of the samples were in accordance with the legal status of archeological human remains in Poland.

b. a comprehensive reassessment of the published literature based not only on traditional biomedical databases (Scopus, Web of Science, Pubmed, etc.) but also archeological monographs and posters.

The frequency of bifid rib in the studied populations is 0.4% (3/752 individuals). The lesions were found in typical ribs: the fourth left rib of a male from the Neolithic population, the fifth right rib of an individual of unknown sex from the medieval population and two right ribs (the third and the fourth) of a male from the Early Modern series. Their appearance differs from a barely visible dichotomy with poorly marked independent surfaces on sternal end of rib, to a clear division (approx. 3 cm) with asymmetry in the size of each bifurcated part.

Finally, the presentation offers a comprehensive geographical distribution of this anomaly and practical suggestions on how to avoid misinterpreting it in the anthropological record.

Keywords: bifid rib; congenital anomaly; genetics; history; morphology; radiology; anthropology; Poland

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Does gross anatomy have nothing more to say?

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Since Vesalius, medical research and education have had a strong emphasis on anatomy, being its core pillar for centuries, with pivotal discoveries. In the second half of the last century, gross anatomical observations reduced, and education became the primary mission of anatomy departments. There is also a conservative tendency in anatomic descriptions, with a seeming reluctance of acknowledged anatomists to explore new theories by returning to direct studies of gross anatomy. Although gross anatomy is considered an undisputed core component of the medical curriculum, anatomic research and education have reduced in size and status to accommodate other disciplines [1]. While microscopic and ultramicroscopic anatomy are expanding more and more to explore the microworld at a molecular level, gross anatomy appears a fundamental discipline which however has nothing more to say. Nevertheless, in the last years, some gross anatomical discoveries have been reported, which overturned the long existing dogmas about morpho-functional aspects. For example, the anatomy of a previously unrecognized, though widespread, macroscopic, fluid-filled space within and between tissues, that is a novel expansion and specification of the concept of the human interstitium, was described. The mesenteric continuity, as well as the novel concept of glymphatic system, have been reported [2,3]. Gross anatomy of myofascia is terminologically evolving with the institution of a Fascia Nomenclature Committee [4]. A new field of research is exploring how gut microbiota acts as an "invisible organ" to modulate gastrointestinal functions as well as the general homeostasis of the body [5]. Apart from the sensational discovery of "new organs", gross anatomy research remains an important tool to correct or redefine classic concepts and to ascertain anatomical varieties. Independently of the approach used (dissection or *in vivo* imaging techniques), it represents the unique way to explore the human body. Cadaveric dissection presents several advantages: gross anatomy research; development of new dissection and preservation techniques; forensic medicine research; creation of virtual anatomy software; surgical training; enrichment of anatomic museums with specific preparations (third mission of the university).

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Keywords: gross anatomy; anatomical teaching; anatomical research

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What to keep and what to return from blended anatomical education: an assessment from sport sciences students

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Gross anatomy classes are considered a crucial component of biomedical education worldwide. However, the most effective way to teach anatomy to students is still up for debate. It is important to evaluate the curriculum, teaching methods, delivery quality, and infrastructure to improve the teaching and learning experience. In recent years technical improvements changed how anatomy is delivered to students and questioned the possible overcoming of dissection as the main instrument in anatomical education. Traditional approaches like lectures and gross dissection courses that focused on topographical structural anatomy have been replaced with a variety of study modules, including problem-based learning, plastic models, computer-assisted learning, and curricula integration.

At the end of 2019, the Covid-19 pandemic spread led to a heated debate, especially as the lockdown measures, physical distancing regulations, and restrictions severely affected higher education and brought about sudden changes in anatomical teaching methods. As a result, universities shifted to distance learning and increasingly relied on technology to teach anatomy. In 2021, most institutions adopted a blended approach. This study examines how effective this approach was in sports sciences anatomical education and provides insights for students in less familiar and experienced curricula. First-year students were anonymously interviewed using a semi-structured questionnaire, and the collected data were analyzed. After extensive discussion between team members, open codes were developed, and themes and subthemes were generated and discussed. Themes' analysis demonstrated that it might be useful to customize different teaching modalities in those classes where cadaver dissection might not align with the goals of specific curricula, such as sports sciences.

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Keywords: Anatomical education; sports sciences curriculum; blended; qualitative analysis; COVID-19 pandemic

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Ultrasound Imaging of Thoracolumbar Fascia Thickness: Chronic Non-Specific Lower Back Pain versus Healthy Subjects; A Sign of a "Frozen Back"?

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The role of the thoracolumbar fascia (TLF) in causing lower back pain (LBP) is significant. Recent studies have shown that patients with LBP have an increased thickness of the TLF, which leads to limited movement of the fascia.

The aim of this research was to use ultrasound (US) imaging to measure and compare the thickness of the TLF at the L3 level of the lumbar spine, both longitudinal and transversal scans, in individuals with chronic non-specific LBP and in those without any back issues.

We collected anamnestic data of enrolled chronic non-specific LBP and healthy volunteers. A cross-sectional study was conducted involving 92 participants: 46 with chronic non-specific LBP and 46 healthy individuals.

We assessed the TLF thickness performing a series of TLF ultrasound scans in longitudinal and transversal axes.

The results regarding TLF thickness showed statistically significant differences ($p < 0.05$) in both longitudinal and transversal scans measurements between the two groups. Additionally, the healthy group exhibited a statistically significant difference between longitudinal and transversal scans measurements ($p = 0.001$ for the left side and $p = 0.02$ for the right side), whereas this difference was not observed in the LBP patients.

The study carried out shows that LBP patients experience a loss of TLF anisotropy, with the fascia becoming uniformly thicker and less adaptable in the transversal direction. The US imaging evaluation suggests that the behaviour of TLF thickness indicates abnormal fascial remodelling compared to healthy individuals, resembling a condition of a "frozen back."

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Keywords: thoracolumbar fascia; Low Back Pain; deep fascia; ultrasonography; thickness

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Whole-body donation: *dissecting* the key sociological aspects and increasing citizenship awareness

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The donation of bodies for medical research and education is crucial, particularly in the field of human anatomy teaching through cadaver dissection, which forms the foundation of medical training worldwide[1]. However, in Italy, the practice of whole-body donation is limited due to various factors such as age, education, religion, culture, personal characteristics, and attitudes towards death. Additionally, potential donors lack awareness about this option, needing for informative campaigns to raise awareness. In order to address this issue, an interdisciplinary team comprising sociologists, physicians, and artists has initiated an experimental project called Pro.dono at the University of Bologna. The project aims to utilize Graphic Medicine as a means to effectively communicate information about body donation, emphasizing its significance and sensitivity to support medical education and research. This initiative builds upon a previous pilot experience in which students from the School of Medicine and Surgery at the University of Bologna explored the potential use of Graphic Medicine in relation to body donation, resulting in the development of five graphic projects[2]. To gain insights into the practices, dynamics of knowledge, and relationships associated with cadaver dissection, an ethnographic observation was conducted. The fieldwork involved a five-month observation period supplemented by ethnographic interviews with 40 individuals. Drawing from these firsthand accounts and fieldwork notes, the students from the Academy of Fine Arts of Bologna will create a graphic novel that will ensure the practical application of the project's outcomes beyond its scope.

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Keywords: Whole-body donation; graphic medicine; medical education

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Teaching microscopic anatomy with an active and engaging learning approach

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Teaching human anatomy, including microscopic anatomy, is a crucial aspect of medical education. In Italian universities, like the University of Pisa, traditional methods involve using classic microscopic slides to study the structure of human organs. These slides are examined under light microscopes during classroom activities, providing a unique opportunity to directly observe human tissues before final exams. However, recent technological advancements have allowed the digitization of these slides, making them easily accessible through online platforms on various devices. Many academic institutions provide open access to these digital anatomical structures, benefiting students, teachers, and biomedical professionals worldwide. Taking advantage of this opportunity, we conducted a pilot session for medical students at the University of Pisa. Each student independently accessed websites of selected US universities, using their own devices as virtual light microscopes to examine tissues and organs at different magnifications. To assess the effectiveness of this virtual approach, we conducted anonymous pre- and post-learning session polls. Analysis of the responses indicated that students found virtual images to be valuable and useful tools for studying microscopic anatomy. They also believed that interactive tools enhanced their independent learning. These preliminary findings suggest that the virtual light microscope could serve as a valuable teaching tool in anatomy courses, fostering student engagement, deep learning, and improved performance, aligning with student-centered strategies.

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Keywords: anatomical teaching; virtual anatomy; microscopic anatomy

Medicina rigenerativa

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Pro-regenerative potential of a leukocyte-fibrin-platelet membrane for the morpho-functional restoration of articular cartilage tissue

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Osteoarthritis is a chronic joint disease which still represents a clinical challenge due to the low regenerative potential of articular cartilage. Platelet-rich blood components have been recognized as cutting-edge biomedical devices to convey regenerative elements to the damage site and stimulate cartilage restoration. In this context, a new autologous Leukocyte-Fibrin-Platelet membrane (LFPm) enriched with growth factors/cytokines/stem cells was conceptualized for the orthopaedical treatment of cartilage lesions [1, 2], but scant pre-clinical data supporting its efficacy are currently available. Hence, this work performed a series of in vitro/in vivo studies to support the rationale for LFPm clinical use in cartilage Tissue Engineering.

First, viability assay on the bone marrow stem cell line HM1-SV40 showed that cell proliferation was enhanced when populations were cultured with 10% and 20% LFPm releasate, being the pro-proliferative effect proportional to supernatant concentration and time of release.

Specific growth factor secretion was assessed by ELISA, highlighting that LFPms are able to sustain the release of FGF and VEGF up to 21 days in vitro. In parallel, in vitro wound healing test showed that LFPm releasate induced cell migration for scar closure.

Finally, the LFPm was prepared as a homogenized matrix and combined with a synthetic polymer, oxidized Polyvinyl Alcohol (OxPVA), to fabricate innovative hybrid scaffolds for cartilage repair. The LFPm improved OxPVA bioactivity by promoting HM1-SV40 cell adhesion and proliferation. In addition, after omental implantation into the abdomen of Sprague Dawley rats, the bio-hybrid devices investigated by histological and immunohistochemical analyses showed high biocompatibility, eliciting no severe immune response by the host.

Overall, promising pre-clinical evidence was collected to support the regenerative potential of LFPm and its possible use for autologous treatment of osteoarthritis.

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Keywords: Platelet-rich blood components; Leukocyte-Fibrin-Platelet membrane; osteoarthritis; cartilage regeneration; tissue engineering

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Protective effect of Oral Stem Cells Extracellular Vesicles on Cardiomyocytes in Hypoxia-reperfusion

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Hypoxia signaling plays an important role in physiological and pathological conditions. Hypoxia in the heart tissue can produce different consequences depending on the duration of exposure to the hypoxic state. While acute hypoxic exposure leads to a reversible acclimatization in heart tissue with normal systemic oxygen supply, chronic hypoxia exacerbates cardiac dysfunction, leading to a destruction of the tissue. Extracellular vesicles (EVs) are small membrane vesicles that act as mediators of intercellular communication. EVs are secreted by different cell types and those produced by oral cavity- derived mesenchymal stem cells (MSCs), including human gingival MSCs (hGMSCs), have pro-angiogenic and anti- inflammatory effects and showed therapeutic role in tissue regeneration. The aim of the present work was to evaluate the potential protective and regenerative role of EVs produced by hGMSCs in an *in vitro* model of hypoxia-conditioned HL-1 cardiomyocytes. Immunofluorescence and Western blot techniques were applied to evaluate the expression of following inflammatory, oxidative stress, angiogenesis, cell survival and apoptotic markers: HIF1α, P300, NFκB, NRF2, and VEGF. Results showed that hGMSCs-derived EVs exerted protection of HL-1 cardiomyocytes exposed to both pre and post hypoxic conditions. Moreover, modulation of Caspase-3 expression demonstrated that EVs reduced apoptosis. The analysis of microRNAs in EVs derived from hGMSCs was performed to assess the epigenetic regulation of the presented markers. The following microRNAs: hsa-miR-138-5p, hsa-miR-17-5p, hsa-miR-18a-5p, hsa-miR-21-5p, hsa-miR-324-5p, hsa-miR- 133a-3p, hsa-miR-150-5p, hsa-miR-199a-5p, hsa-miR-128-3p and hsa-miR-221-3p can directly or indirectly target the studied genes by determining their modulation obtained in our study. The data from this study suggested that EVs obtained from hGMSCs may be considered for the cell free treatment option in hypoxia-driven cardiac tissue dysfunction.

Keywords: extracellular vesicles; human gingival mesenchymal stem cells; cardiomyocytes; acute hypoxia

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Improving regenerative capabilities of peripheral nervous cells with chitosan microstructured and functionalized membranes

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The treatment of localized prostate cancer is radical prostatectomy with frequent iatrogenic damages to the periprostatic neurovascular bundles (NVB) responsible for erectile dysfunctions. Our team previously demonstrated the neuro-regenerative effect of chitosan membrane on *ex vivo* cultures of autonomic ganglia and its anti-proliferative effect on metastatic prostatic cancer cells.

The aim of the present study was to test *in vitro* and to develop a functionalized microstructured chitosan membrane to support and promote the main regenerative mechanisms, underlying nerve outgrowth and glial cell survival and proliferation for an *in vivo* use, to repair a lesion affecting the cavernous nerve. The chitosan was blended with 5-15% glycerol and micropatterned to obtain the well-established gratings (GR) and the asymmetric pattern with scalene triangles (SCA). Moreover, the controlled release of phosphodiesterase inhibitors (PDEI) was designed to chemically promote nerve regeneration and functional recovery.

The results of *in vitro* and *ex vivo* direct cultures on microstructured chitosan membranes demonstrated the oriented growth of neurons, a very important step in making regeneration more effective. The *in vitro* protocol for the administration of PDEI (sildenafil-PDE5I and rolipram-PDE4I) was developed and for both stimulations an interesting gene regulation linked to the neuroprotective brain-derived neurotrophic factor (BDNF) in immortalized cultures of sensory and motor neurons was observed. In glial cell cultures, the administration of PDEI resulted in up-regulation of the transcription factor Krox20, which can positively influence the expression of myelin genes, of the proangiogenic Vascular endothelial growth factor (VEGF) and in a decrease of cell migration. Furthermore, the administration of Sildenafil and Rolipram increased the neuritic extension in neuronal populations. Further investigations are underway to deepen the study of the effect of PDEI administration on organotypic cultures of somatic and autonomic ganglia, where neuronal and glial cells co-exist, similarly to what happens *in vivo*.

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Keywords: contact guidance; nerve regeneration; neurons; glial cells; prostate cancer

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Strategies to improve cavernous nerve regeneration after radical prostatectomy

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Prostate cancer is the most frequent cancer among men and the current treatment is radical prostatectomy (RP). Unfortunately, iatrogenic damage to the periprostatic neurovascular bundles (NVB) occurs, leading to erectile dysfunction. Recently, new strategies to improve the regeneration of the prostatic nerves are arising, among these, the application of chitosan membrane has been shown to promote nerve regeneration thanks to its useful properties. For this purpose, *ex vivo* experiments performed on autonomic explant ganglia have been shown the neuro-regenerative effect of a flat chitosan membrane reporting a higher neurite outgrowth. At the same time, in vitro experiments on metastatic cancer cell lines displayed a lower proliferation rate when cultured with chitosan coating and dissolution products.

The safety and the ability of the flat chitosan membrane to promote nerve regeneration was also tested in clinical resulting in higher potency recovery rate in patients that have undergone RP.

To improve the regenerative performance achieved by the flat membrane, nanostructured membranes with grating arrangement and a zig-zag pattern were used to repair cavernous nerve on adult male rats: 3 mm of nerve was bilaterally transected and repaired with chitosan membranes. 60 days after the surgical procedure, samples were harvested and processed for "iDISCO" technique that allowed to detect the pathway of nerve fibers on the whole membranes.

These *in vivo* results provide the first experimental evidence supporting the ability of the chitosan membrane to allow axonal regeneration demonstrating the safety of the device for clinical use and supporting its application in the urological field.

Keywords: prostate cancer; chitosan; nerve regeneration

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Urine-derived Renal Epithelial Cells (URECs) from transplanted kidneys as a promising immunomodulatory cell population

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Kidney transplant is a lifesaving procedure for patients with end stage kidney disease (ESKD). As other surgical procedures, kidney transplant presents several side effects. Organs derived from cardiac, or brain death donors are constantly increasing, due to the high need of kidney donors. However, they are more exposed to the onset of ischaemia-reperfusion (IR) and Acute Kidney Injury (AKI) events, compared to living donors. These phenomena increase kidney cell turnover, to replace damaged cells with functional ones. This results in an increased exfoliation of cells lining kidney tubules, with exfoliated cells being voided in urine. Urine-derived Renal Epithelial Cells (URECs) are rarely present in the urine of healthy subjects, and their loss has been associated with several kidney disorders. The present study aimed to characterize the phenotype and potential applications of URECs voided after transplant. The results indicate that URECs are highly proliferating cells, expressing several kidney markers, including proximal tubules markers and markers of kidney epithelial progenitor cells. Since the regulation of the immune response is crucial in organ transplantation and new immunoregulatory strategies are needed, UREC immunomodulatory properties were investigated. During the co-culture with Peripheral Blood Mononuclear Cells (PBMCs) derived from healthy volunteers, URECs strongly reduced CD4 and CD8 T Lymphocyte proliferation, while exerting an anti-apoptotic effect on PBMCs. Moreover, URECs increased T regulatory (Treg) cells and reduced T helper 1 (Th1) cells producing IFN- . These results suggest that URECs from transplanted patients may represent a promising cell source for the investigation of regenerative processes occurring in kidneys, and for cell therapy applications based on the regulation of the immune response.

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Keywords: chronic kidney disease; kidney transplant; ischaemia-reperfusion; acute kidney injury; urine cells; urine-derived renal epithelial cells; kidney progenitor cells; immunomodulatory capacity; lymphocytes; T regulatory cells

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The inflammatory milieu in natural ovarian aging: how to counteract it

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Natural ovarian aging represents one of the earliest phenomena characterizing female aging. It is likely that this process depends on multiple intraovarian and extraovarian factors whose respective contribution have not been fully characterized [Camaioni *et al*., *J Assist Reprod Genet.* 2022].

The aim of the present study is to describe the changes of the ovarian stroma by age in order to verify whether the *in vivo* injection in old mice of "secretome" present in media conditioned (CM) by human mesenchymal stromal cells derived from adipose (ASC-CM) and dental tissues (DPSC-CM) is able to counteract the observed changes.

Recently, an age-associated increase in inflammation and fibrosis has been documented in the mammalian ovary, accompanied by a shift in the immune cell profile. In line with these studies, we found higher levels of transcripts of pro-inflammatory genes *Il6*, *Il1*β, and *Tnf*α in the ovaries of old (O = 8-months) in comparison to young (Y = 2-months) females of Sv/129 mouse strain. (*Il6*: Y = 1 ± 0.04; O = 4.6 ± 0.3; *Il1*β: Y = 1 ± 0.1; O = 3.1 ± 0.3; *Tnf*α: Y = 1 ± 0.01; O = 1.8 ± 0.1).

Moreover, we verified the presence in the aged ovaries of a unique population of multi-nucleated macrophage giant cells often observed in close proximity to fibrotic foci. We also observed vacuolar changes in the aged ovary, consisting of foamy cells, probably a different macrophage population, or a different stage of the same population. These cells have been described in the ovary as age-associated multinucleated macrophages that are swollen with lipids. Their presence indicates a pro-inflammatory microenvironment, due to high levels of tissue dynamics that could be ascribed to follicle atresia, cell apoptosis and wound healing, main traits of folliculogenesis and ovulation.

Next, we examined the number of mast cells and verified a significant increased at 8-months compared to 2-months old females. We hypothesize that all these changes in the stroma microenvironment can have a significant impact on the quality of follicles and oocytes. In fact, the *in vivo* injection in old females of CM was able to counteract and/or mitigate most of the observed stromal changes and, more important, increase the pregnancy rate (CTRL = 8.3%±5.3; DPSC-CM = 25.0%±9.1; ASC-CM = 60.7%±10.3) and prolong the reproductive life, indicating an improvement on the overall body health.

Keywords: Ovarian aging; Inflammation; Stroma; Mesenchymal stromal cells; Cell-free therapy

Tessuti epiteliali e connettivi. Transizione epitelio mesenchima nell'organogenesi e nella carcinogenesi

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Surgically oriented orbital floor anatomical measurements: a study on 33 cadavers related to the placement of zygomatic implants

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Malar bone could be a source of anchorage for zygomatic implants in the fixed rehabilitation of the fully edentulous and extremely atrophic upper jaw.[1] However, their placement requires the careful respect of the orbital cavity. The implant penetration in the orbit is reported as a severe complication that occurred in up to 6% of the cases.[2] To measure distances with the close anatomical structures and depths of the anterior orbital floor, to guide the surgeon in a correct and safe zygomatic implant insertion avoiding ocular complications. Human cadaver heads were bilaterally dissected. The main anatomical landmarks taken into consideration were: infraorbital foramen (IF), anterior border of the orbital floor (OF) and the anterior end of the zygomatic arch (A). The following points were moreover identified: "IF" projection on the external orbital border (Point 1), "A" projection on the external orbital border (Point 5), midpoint between point 1 and 5 (Point 3), midpoint between point 1 and 3 (Point 2) and midpoint between point 3 and 5 (Point 4). The following linear measurements were taken with a digital caliper from each side: distance IF-Point 1 and Point 1-Point 5. With a periodontal probe the anterior orbital floor depths were taken posterior to the internal orbital rim at Points 1 to 5. A total of 66 sides from 33 Caucasian cadavers were evaluated: 8 (24.2%) were female and 25 (75.8%) male. The mean age was 81.0 ± 9.3 years. IF-Point 1 mean distance was 7.60 ± 1.43 mm, while Point 1-Point 5 was 23.98 ± 0.74 mm. Mean anterior orbital floor depths were 2.78, 2.86, 3.65, 4.24 and 3.31 mm at Point 1, 2, 3, 4 and 5, respectively. Cadaver anatomic study and dissection are of paramount importance to guide the clinician in this approach. The surgeon should be aware of the anatomical shape and dimensions of the anterior orbital floor in order not to invade that noble structure during surgery.

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Keywords: Orbital floor; complications; zygomatic implants; cadaver study; oral surgery

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Phenotype-dependent role of Vav1 in down-modulating Akt in invasive breast tumor cells

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Vav1 is one of the signaling proteins normally restricted to hematopoietic cells that results ectopically expressed in solid tumors, including breast cancer, in which it shows a peculiar localization inside the nucleus of tumor cells. High amounts of nuclear Vav1 are positively correlates with low incidence of relapse and distant metastasis, regardless phenotype, and molecular subtype of breast neoplasia [1]. In breast tumor cells, nuclear Vav1 affects the expression of genes involved in epithelial-to-mesenchymal-transition (EMT), invasion and/or metastasis, including genes encoding for specific isoforms of Akt. Using *in vitro* and *in vivo* models, we demonstrated that the role of Vav1 in down-modulating Akt is dependent on tumor subtype [2]. In cells from triple-negative breast cancer (TNBC), that represents the most aggressive breast tumor, showing a high intrinsic variability in terms of both histopathological features and response to therapies, we demonstrated that the over-expression of Vav1 reduces the levels of Akt2, acting at the post-transcriptional level through the up-modulation of miR-29b. The Vav1/miR-29b dependent decrease in Akt2 is correlated with a reduced lung colonization of circulating tumor cells and occurs only in some molecular subtypes of TNBC tumors [3, 4].

Despite 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 make it difficult to select the inhibitors for each breast tumor subtypes. Our findings suggest that the activation of the Vav1/miR-29b axis, precisely regulating the amount of an Akt isozyme crucial for tumor dissemination, could have great potential for driving more accurate therapies to TNBCs, often not eligible or resistant to treatments. In this context, identify substances capable of increase the levels of Vav1 could constitute an innovative approach to improve outcome of some breast cancers for which target-based therapies are not currently available.

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Keywords: breast cancer; Vav1; Akt; miR29b

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Interactions between melatonin and estrogen may regulate cholestatic liver phenotype in female Mdr2-/- mice

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Estrogens are steroids hormones involved in genomic and non-genomic effects by binding estrogen receptor α/β (ERα/β) and G protein-coupled estrogen receptor 1 (GPER), respectively. The molecular functions of ERα/β in specific organs are not yet well understood. Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by ductular reaction, biliary damage and inflammatory infiltrate (1). We have shown that: (i) cholangiocytes expressed ERα and β in male bile duct ligation (BDL) rats and (ii) melatonin administration ameliorates liver phenotype in male cholestatic model (2). Since melatonin has been shown to act both on ER by inhibiting their gene expression (3), we aimed to investigate the possible inhibitory effect of melatonin on estrogen signalling in cholangiocytes of female *Mdr2*$^{-/-}$ mice (PSC murine model). Female FVB/NJ and *Mdr2*$^{-/-}$ mice had access *ad libitum* to water with/without melatonin for 12 wk. We evaluated: (i) estrogen levels in serum of both human and murine experimental models, (ii) ERα/β in both human PSC and murine samples, (iii) liver damage by histomorphological staining and by measuring ALKP, ALT and AST in the experimental groups, (iv) small and large bile duct mass by staining for CK19 and (v) inflammation by staining for F480, CD3 and CD20 that are markers for Kupffer cell, T and B lymphocytes, respectively. Both human PSC and *Mdr2*$^{-/-}$ mice showed elevated expression of estrogen levels in serum and immunoreactivity of nuclear receptor ERα/β. Melatonin treated *Mdr2*$^{-/-}$ did not show change in estrogen serum levels and ERβ expression when compared with *Mdr2*$^{-/-}$ but showed a decrease of ERα immunoreactivity. This suggested that melatonin may influence the estrogen signalling by inhibiting the action of ERα. Treatment with melatonin improves liver damage in cholestatic murine model and its administration may reduce the estrogen action in female PSC modulating the cholestatic liver disorders.

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Keywords: biliary epithelium; cholangiocytes; melatonin; estrogen

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Role of TGF-ß as a potential molecule for Schwann cell-induced Cholangiocarcinoma progression

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Cholangiocarcinoma (CCA) is a class of epithelial malignancies derived from the biliary tree, characterized by an aggressive behavior and a high neurotropism. In case of documented perineural invasion, a form of metastasis through which cancer cells penetrate and move along peripheral nerves, prognosis gets significantly worse[1], but little is known about the cellular and molecular processes underlying such worsening. Recently, Gundlach et al. reported the presence of Schwann cells in human CCA specimens, in association with a worse prognostic trend as compared to patients whose lesions were negative to SC markers[2]. Increasing evidence acknowledges SC as key regulators of PNI and cancer progression in various cancer models, especially pancreatic ductal adenocarcinoma[3], a tumor which shares many similarities with CCA [4]. We hypothesized that the worse prognosis of patients showing PNI or SC within their lesions is due to soluble factors released by SC, activated to repair nerves damaged by PNI, which may foster cancer progression. We investigated the crosstalk between intrahepatic CCA cell lines (HuCC-T1 and Oz cells) and primary human SC (HSC) *in vitro*. We observed increased motility and invasiveness in CCA cell lines treated with HSC conditioned medium (HSC cm), associated with an epithelial-mesenchymal transition. In addition, cells exposed to HSC cm show increased viability and clonogenicity and proliferate more respect to the controls. To better understand the effects of HSC cm on CCA cell lines, we performed mass spectrometry on lysates obtained from HuCC-T1 single cultures or indirect HuCC-T1/HSC cocultures (transwell inserts, 3um pores) and found the modulation of 20 key proteins, suggestive of the involvement of TGF-ß. We then decided to test the putative role of TGF-ß for the pro-tumoral effects of HSC cm on CCA cell lines. By the use of SB-431542 (a specific Tß1R antagonist) we demonstrated clearly the reversion of the effects observed, proving the mechanistic role of this cytokine in HSC dependent CCA progression. The evidence of a pro-tumoral effect of SC-derived TGF-ß opens the perspective to investigate such mechanisms in other solid cancers such as melanoma.

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Keywords: cholangiocarcinoma; perineural invasion; Schwann cells; epithelial-mesenchymal transition

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Lactose-modified hyaluronic acid molecules induce anti -inflammatory and anti-oxidative effects on bronchial fibroblasts of smokers

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Smoking related pathologies in the population are characterized by macrophage-mediated inflammation that correlates with the severity of inflammation The major cytokines released by macrophages are interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) and and galectins, molecules that bind specifically to β-galactoside sugars.

The aim of this study was to evaluate the anti-inflammatory and anti-oxidant effects of hyaluronic acid modified molecules with different amounts of lactose derivative residues (HYLACH®) on an "in vitro" model of macrophage-mediated inflammation. The best HYLACH ligands for galectins were selected *in silico* and subsequently inflamed with the conditioned medium of activated U937 monocytes. Changes in cell viability, ROS generation, pro-inflammatory mediators and MMPs expression, at both gene and protein level, were analysed.

The *in silico* results show that HYLACH with a percentage of lactosylation up to 10-40% are the best ligands for Gal-3 and the *in vitro* study revealed that the compounds with 10, 20 and 40% lactosylation (HYLACH-1-2-3) administered to inflamed cell cultures exerted higher anti-inflammatory and anti-oxidative effects than hyaluronic acid, restoring gene and protein expression for inflammatory cytokines, galectins and metalloproteinases to near baseline values.

These data may be relevant in terms of prevention of pulmonary deterioration and progression of pulmonary diseases and provide an initial step toward the development of new therapeutic treatments suitable for chronic smoking related pulmonary diseases and smoking related diseases.

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Differential expression of epithelial-to-mesenchymal transition markers in 2D and 3D cell cultures of melanoma cells

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Melanoma is a tumor characterized by high metastatic potential favored by the epithelial-to- mesenchymal transition (EMT), a process leading cells to the loss of epithelial features and the acquisition of a mesenchymal phenotype [1,2].

Although melanoma cells are not of epithelial origin, they exhibit a spectrum of typical EMT markers. The present study is aimed at analyzing the expression of EMT markers in 2D melanoma cell cultures compared to 3D spheroids that better mimic the tumor architecture [3].

For this purpose, A375 and BLM melanoma cell lines were cultured in 2D monolayers and 3D spheroids and analyzed by morphological and molecular methods.

Gene expression analysis revealed that E-cadherin is expressed in BLM cells whilst almost undetectable in A375 cell line. An opposite pattern was evident for N-cadherin, while vimentin was similarly expressed in both cell lines. Interestingly, mRNA levels for the adhering cell junctions' molecules and vimentin were significantly induced by 3D arrangement, and this pattern was also confirmed by confocal microscopy analysis.

SDS-zymography, to investigate the invasive potential, revealed that MMP-2 activity is similar in A375 2D and 3D cell cultures while is significantly reduced by 3D arrangement in BLM cells. Moreover, MMP-2 activity was significantly decreased in BLM compared to A375 3D spheroids. Conversely, MMP-9 activity is similar in 2D and 3D cell cultures, but significantly lower in BLM compared to A375 cells.

Overall, these findings suggest that A375 and BLM cells possess a hybrid phenotype in relation to the expression of EMT markers thus contributing to the characterization of the role of EMT in melanoma cells and, finally, confirm that a 3D cell culture model could provide deeper insight into the understanding of the biology of melanoma.

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Keywords: EMT; melanoma; 3D spheroids

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The emerging role of ferroptosis in course of liver fibrosis

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Ferroptosis is an iron-dependent regulated cell death characterized by iron accumulation, lipid peroxidation and the production of ROS depending on the activity of NADPH oxidase (1). Patients with chronic liver disease may exhibit hepatic and splenic iron loading, usually inside Kupffer cells. Recent studies emphasized the role of low levels of hepcidin in various liver diseases as implicated in both iron deposition in hepatocytes and participation in stellate cell activation and liver fibrosis (2). Hepcidin represents the main regulator of ferroportin, that binds the cytoplasmatic divalent iron, limiting iron export to the blood (3-4). The involvement of ferroptosis in liver fibrosis was recently investigated at the level of hepatocytes, Kupffer cells and hepatic stellate cells, but not in biliary epithelium, for that reason we aimed to investigate it in the most used experimental rat models of hepatic fibrosis: treated with carbon tetrachloride (CCl_4) or with bile duct ligation (BDL) at different times. The first one is commonly used to study acute liver injury and advanced fibrosis, whereas BDL induces cholestatic disease and progresses into acute liver injury following liver inflammation and fibrosis. In these samples, firstly we evaluated the presence of pre-ferroptotic markers, such as hepcidin, ferroportin (Fpn), ferritin (Ftn) and transferrin, then the proper ferroptosis markers such as interleukin 6 (IL-6), cytochrome P450 reductase and the enzyme glutathione peroxidase 4 (GPX4). *In vitro*, we stimulated primary murine cholangiocyte cell lines (mCHOL) with erastin, a typical ferroptosis inducer, to study similar markers. Data showed lower levels of hepcidin that can cause iron overload and increased oxidative stress, consequently Fpn is downregulated and Ftn upregulated. Moreover, we found an increase in all the ferroptosis markers. Targeting ferroptosis in liver fibrosis may represent a novel target to better study the mechanisms during the fibrotic process.

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Keywords: Biliary epithelium; cholangiocytes; cell death; ferroptosis

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Acute and chronic cannabidiol treatment: *in vitro* toxicological aspects on human oral cells

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In recent years there has been an increase in marijuana use[1], a product of the Cannabis plant. There are many varieties of the Cannabis plant and recently many countries in the world have seen a gradual legalization of Cannabis Sativa for medicinal purposes and personal use, mainly in its Light variant characterized by a very low concentration of THC (psychoactive) and a high concentration of Cannabidiol (CBD) thanks to genetic modification. CBD is gaining increasing interest in literature for its potential anti-inflammatory, pain-relieving, immunomodulatory, antidepressant, antiepileptic, relaxing, antioxidant and antineoplastic effects[2].

The aim of this study is to investigate the biological effects of acute and chronic CBD administration on gingival fibroblasts and oral keratinocytes. In particular cell viability was assessed by MTT assay, morphology by SEM, migration by Scratch assay, apoptosis and cell cycle by flow cytometry, expression of related genes (p53, Bcl2, p21 and BAX) and of endocannabinoid system receptors (CB1, CB2 and GPR55) by RT-PCR and DNA damage with phospho-γ-H2AX immunofluorescence detection. In acute treatment cells were continuously exposed to CBD for 24h and 72h, in chronic CBD treatment the administrations were carried out 3 times a day (30 min each) for 1 day and 3 days. Acute treatment cytotoxicity testing used concentrations between 100μM and 0.001μM and three concentrations were selected for subsequent analysis: 50μM, as toxic dose, 25μM, viability promoter and 1μM, nontoxic.

Acute treatment reveals significant effects compared to chronic, in particular in fibroblasts: concentrations ≥ 50μM are highly cytotoxic, with increased apoptosis and reduced migration, confirmed by morphological alterations and reduction of filopodia and lamellipodia. Cell death correlates with increased p53 and BAX, followed by arrest in G0/G1 phase, as evidenced by elevated p21 levels. This suggests a time- and dose-dependent damage. An increase in phosphorylation of H2AX was observed with 25μM and 50μM, while 1μM was biocompatible. Keratinocytes appear less affected by the cytotoxic effect than fibroblasts. In general, induced cell damage appears to be dose- and time-related, with less cell damage following chronic treatment. The proliferative effect at 25μM needs further investigation.

To identify a potential biohazard-free therapeutic dose further studies with longer time frames to evaluate the dose- and time-dependent effects of CBD are of critical importance.

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Keywords: CBD; gingival fibroblast; oral keratinocyte; viability; SEM; apoptosis; DNA damage

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Involvement of PACAP/ADNP Axis in the cornea

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The cornea, unique in its transparency, is the outmost layer of the eye acting as the major refractive element. It is mechanically strong and forms a functional barrier that protects the intraocular elements from the external environment. The cornea is exposed daily to several insults, such as ultraviolet B (UV-B) radiations. Moreover, it can be impaired by various diseases such as diabetic keratopathy (DK) representing the major complication of the cornea characterizing diabetes-affected patients. For these reasons, corneal damage is one of the main causes of blindness in the world.

Several studies have shown the protective role of pituitary adenylate cyclase-activating peptide (PACAP) in different eye diseases. PACAP plays its effects through the activation of G protein-coupled receptors. Moreover, some PACAP effects are mediated by the stimulation of activity-dependent protein (ADNP). However, the role of the PACAP-ADNP axis on the cornea has not been investigated, yet. Therefore, first, we analyzed their expression on the human cornea. Results showed strong expression of PACAP, ADNP, and related receptors in corneal epithelium and endothelium[1]. So, we analyzed the role of ADNP on corneal epithelial cells exposed to UV-B radiations, and in an *in vitro* model of DK. Our results showed that the treatment with ADNP mimicking peptide, NAP, decreases ROS production, inflammatory cytokines expression, and enhanced cell viability and corneal epithelium wound healing, by counteracting apoptotic cell death induced by UV-B-rays or hyperglycemia[2]. Overall, these data suggested that PACAP or NAP might represent a valid strategy for the treatment of some corneal diseases.

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Keywords: cornea; PACAP; ADNP; UV-B; diabetic keratopathy

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Mast cells in superficial fascia as modulators of tissue healing

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The recent findings showed that the superficial fascia is a fibrous layer in the middle of hypodermis, richly innervated and vascularized, more complex than so far demonstrated. This study showed the presence of mast cells in the superficial fascia of the human abdomen wall of three adult volunteer patients (mean age 42 ± 4 years; 2 females, 1 male), by Toluidine Blue and Safranin-O stains and Transmission Electron Microscopy. Mast cells are distributed among the collagen bundles and the elastic fibers, near the vessels and close to the nerves supplying the tissue, with an average density of 20.5 ± 4/mm^2.

The demonstration of the presence of mast cells in the human superficial fascia highlights the possible involvement of the tissue in the inflammatory process, in tissue healing and regeneration processes. A clear knowledge of this anatomical structure of the hypodermis is fundamental for a good comprehension of some fascial dysfunctions and for a better-targeted clinical practice.

Keywords: Fascia; Connective tissue; Mast cells

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The sarcoglycan sub-complex in adipose cells

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Sarcoglycans are transmembrane proteins that modulate the cell-extracellular matrix interactions in muscle and not muscle tissues such as the adipose tissue [1]. These proteins have been found to be expressed at mRNA level in white adipose tissue; in particular, their absence has been correlated to insulin resistance and glucose intolerance. Our previous data have shown that all sarcoglycans are expressed both in white and brown adipocytes and they are more expressed in brown adipocytes if compared to the white ones. Although that, the role played in adipose tissue is still unknown. The aim of the present study was to verify the possible sarcoglycans involvement during trans-differentiation processes from white to brown adipocytes. Culture of 3T3L1 cells were induced to transdifferentiate using agonists of β3-receptors and the cells were processed by immunofluorescence, RT-PCR and western blot techniques. Our results have shown that all sarcoglycans are expressed in white adipocytes and their expression increases after trans-differentiation from white to brown. These data confirm that sarcoglycans are not muscle specific and suggest their involvement in the transdifferentiation process in adipose tissue.

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Ethosomes nanodelivery of the MDM2 inhibitor Nutlin-3a efficiently induces cells death in melanoma

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To solve the solubility issues of Nutlin-3a, a known inhibitor of MDM2 and activator of the p53 pathway, which limits its bio-distribution and therapeutic efficacy *in vivo*, we studied a new nanodelivery system based on ethosomes, and applied against melanoma. In this study we used two *in vitro* models of melanoma: the HT144 cell line, expressing wild-type p53 and therefore sensitive to Nutlin-3a, and the SK-Mel-28 cell line, expressing mutated inactive p53 and therefore resistant to Nutlin-3a. Nutlin-3a-loaded ethosomes were physic-chemical characterized and used to treat melanoma cells at different concentrations, in parallel to Nutlin-3a alone and empty ethosomes as control. 24 and 48 hours after treatments, we evaluated the activation of the p53 pathway through protein analysis of its target genes, the effects on viability, cell cycle phases and apoptosis rate through flow cytometry, and the effects on cell morphology and migration through xCELLigence real-time analysis. In addition, we studied TRAIL-R2 and NOTCH1, respectively by flow cytometry and western blotting, as specific markers regulated by p53 and key molecules involved in survival/apoptosis whose expression is altered or dysfunctional in a large percentage of melanomas. The results indicated that ethosomes are efficient delivery systems that keep Nutlin-3a functionally and biologically active. In fact, Nutlin-3a-loaded ethosomes inhibited MDM2, as revealed by the increase of p53 level and its transcriptional targets on p53 wild-type cells. In addition, they activated the biological effects of p53 including cell cycle block and apoptosis. Interestingly, the ethosomes significantly reduced melanoma cell migration, both alone or carrying Nutlin-3a, indicating a possible anti-metastatic role. In addition, delivery with ethosomes was significantly advantageous, compared to treatment with Nutlin-3a alone, in the up-regulation of surface TRAIL-R2 and in down-regulation of active NOTCH1 (NICD). Since a high percentage of melanomas is resistant to TRAIL, TRAIL-R2 overexpression together with down-modulation of NICD, and to the apoptosis directly induced by Nutlin-3a, may help in controlling the resistance to TRAIL and increase the cytotoxic effects of chemotherapy.

Keywords: nanodelivery; ethosomes; MDM2 inhibitors; cell cycle; p53; Notch1; melanoma

POSTER

Neuroscienze

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Structure and function of the human pulvinar: a track-weighted dynamic functional connectivity-based parcellation study

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The human pulvinar is considered a prototypical associative thalamic nucleus as it represents a key node in several cortico-subcortical networks. Through this extensive connectivity to widespread brain areas, it has been suggested that the pulvinar may play a central role in modulating cortical oscillatory dynamics of complex cognitive and executive functions. Additionally, derangements of pulvinar activity are involved in different neuropsychiatric conditions including Lewy-body disease, Alzheimer's disease, and schizophrenia [1]. Anatomical investigations in non-human primates have demonstrated a topographical organization of cortico-pulvinar connectivity along its dorsoventral and rostro-caudal axes; this specific organization shows only partial overlap with the traditional subdivision into subnuclei (anterior, lateral, medial, and inferior) and is thought to coordinate information processing within specific brain networks [2]. However, despite its relevance in mediating higher-order cognitive functions, such a structural and functional organization in the human brain remains poorly understood. Track-weighted dynamic functional connectivity (tw-dFC) is a recently developed technique that combines structural and dynamic functional connectivity, allowing the identification of white matter pathways underlying the fluctuations observed in functional connectivity between brain regions over time [3,4]. Herein, we applied a data-driven parcellation approach to reveal topographically organized connectivity clusters within the human pulvinar complex, in two large cohorts of healthy human subjects. Unsupervised clustering of tw-dFC time series within the pulvinar complex revealed dorsomedial, dorsolateral, ventral anterior, and ventral posterior connectivity clusters. Each of these clusters shows functional coupling to specific, widespread cortico-subcortical white matter brain networks. Altogether, our findings represent a relevant step towards a better understanding of pulvinar anatomy and function, and a detailed characterization of his role in healthy and pathological conditions.

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Keywords: connectivity; functional MRI; parcellation; thalamus; tractography

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Neurotoxic effects of Coronavirus: potential implications in Alzheimer's onset and progression

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The COVID-19 coronavirus disease, caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), has generated a pandemic in recent years involving every aspect of our globalized society. Although the respiratory tract is the one most affected by SARS-CoV-2, it is emerging that the virus, reaching the central nervous system, can lead to severe neurological disorders.

In particular, patients diagnosed with Alzheimer's disease (AD) would be a high group risk of contracting COVID-19, developing more serious forms with worse relapses. On the other hand, the contraction of the virus could be the cause of an acceleration of the process neurodegenerative. Therefore, understanding the connection between the two pathologies could help in the development of new therapeutic approaches to counter them.

We used the SH-SY5Y cell line differentiated into neurons, as widely used in studies of AD if supplemented with exogenous fibrillary β-amyloid (Aβ). Since the role of cells of macrophage origin is crucial in both the pathogenesis of AD and in the inflammatory process that occurs in SARS-CoV-2 infection, as a glial counterpart, a microglia line (HMC3) and an astrocytic line (D54MG) were used to create co-cultures with neurons *via* transwell systems. In these experimental models, we generated infection with the OC43 version of human Coronavirus (H CoV-OC43), a low-risk model of SARS-CoV-2.

Our results suggest that the infection with H CoV-OC43 leads to a neurotoxic effect not depending on an already present event of Aβ deposition.

Indeed, unlike microglia, neurons and astrocytes are susceptible to infection and even if the infection has not shown a cytotoxic effect in the neurons, at least in the first few days, significant alterations at a biochemical and morphological level have been observed, suggesting that the neurons are reacting to a stressful condition, which includes the prodromal and neurodegenerative features of AD.

Interestingly, the interaction of astrocytes with the cell line of infected neurons resulted in development in the manifestation of signs of neurodegeneration, such as Aβ deposition.

By using exogenous fibrillary Aβ, as an AD in *vitro* model, preliminary data suggest that there is an aggravating effect both on the infection itself and on the disease progression.

In conclusion, the results of this study suggest a causal interconnection between H CoV-OC43 and neurological diseases and demonstrate that the co-presence of CNS cell populations is the necessary condition to study the pathogenic effects *in vitro* as a whole.

Keywords: coronavirus; neurodegeneration; Alzheimer's disease

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Melatonin ameliorates hippocampal inflammation and redox balance in mouse model of autism

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder mainly identified by its two core symptoms: an impairment in common social interactions and the engagement in repetitive behaviors. Current literature clearly establishes melatonin supplementation as a safe and effective long-term treatment for the sleep problems frequently experienced by ASD patients [*1*]. Conversely, its impact on ASD core symptoms remains only partially characterized [*2*]. Many studies have linked abnormalities in hippocampal development and functional connectivity with the onset and intensity of core symptoms in ASD patients [*3*]. Recently, inflammation and mitochondrial dysfunction, leading to reactive oxygen species (ROS) accumulation, have been recognized as key elements in the onset of ASD-linked alterations in the hippocampus [*4*]. Extensive research has evaluated melatonin antioxidant and anti-inflammatory properties in addition to its effectiveness as mitochondrial activity modulator in many organs and tissues. Therefore, the aim of our study is to evaluate the potential for melatonin to ameliorate hippocampal inflammation and redox balance in BTBR mice, a well characterized and widely employed idiopathic ASD animal model. Therefore, we treated C57BL/6 and BTBR mice with melatonin for 8 weeks. Subsequently, we observed in melatonin-treated mice brain sections, through immunohistochemical staining, an increase in nuclear localization of nuclear factor erythroid 2-related factor 2 (NRF2) and in the expression of superoxide dismutase type 1 (SOD-1), proteins central to the maintenance of a correct redox-balance. Furthermore, we detected a downregulation of inflammatory markers such as the nuclear factor kappa B (NFKB) and the NLR pyrin domain containing 3 (NLRP3) in the hippocampus of melatonin treated BTBR mice. Moreover, the employment of a DAB-enhanced Perls reaction protocol allowed us to hypothesize a deficiency in iron homeostasis in BTBR mice, which was ameliorated by melatonin treatment. Our data suggest melatonin could potentially benefit ASD patients beyond what already achieved with sleep regulation.

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Keywords: autism; hippocampus; BTBR mice; melatonin; inflammation; redox balance; ferroptosis

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Distribution and characterization of α-syn and VIP immunoreactivity in the enteric nervous system of human small intestine

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The enteric nervous system (ENS), our "second brain", consists of a complex network of neurons and glial cells located within the gastrointestinal (GI) tract. ENS interacts through numerous neurotransmitters, and it can work independently of the central nervous system (CNS), modulating several functions within the GI tract (1). The ENS has two ganglionated plexuses, the myenteric and submucosal plexuses. There is increasing evidence that several neurodegenerative diseases are not confined to the CNS but also to the peripheral nervous system (PNS), including the ENS (2). In 2003, Braak et al. theorized that the biological process underlying Parkinson Disease (PD) may originate in the ENS and may lead to CNS impairment (3). Alpha-synuclein (α-syn), a small presynaptic protein involved in the pathogenesis of PD, is abundantly expressed in the CNS, but also in the peripheral nerves of PD patients (4). In the normal gut, distribution of α-syn has been reported in the nerve fibers of the lamina propria, sub-mucosa, and in the ganglia (5). Dysfunctions of vasoactive intestinal peptide (VIP) have been associated with impaired motility in inflammatory bowel diseases (6). The present study aims to characterize the presence of α-syn and VIP in the normal human jejunum. Specimens of proximal jejunum were collected from patients and sections underwent immunohistochemical procedure using antibodies for α-syn and VIP. Alpha-syn immunoreactive (ir) structures were detected along both 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 in the axis of the villi. The distribution pattern of α-syn and VIP has been compared. Our preliminary observations of co-distribution of α-syn and VIP may elucidate their physiological role in the ENS and can shed light on how their structural alterations could contribute to the visceral pathogenesis of neurodegenerative disease.

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Keywords: enteric nervous system; intestinal inflammation; α-synuclein; VIP

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Diagnostic value of salivary biomarkers in Parkinson's Disease

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Parkinson's Disease (PD) is characterized by the accumulation of misfolded alpha-synuclein (a-syn) and by the activation of different molecular pathways, converging in neuronal death and synaptic loss. Clinical diagnosis and treatment of PD are hampered by the progressive deterioration of target neuronal circuits and by the mismatch between clinical and neuropathological onset. Molecular biomarkers are of unreplaceable importance to couple neuropathological and clinical features. Salivary glands are richly innervated and nervous fibres enter in contact with serous and mucous adenomeres, reversing neuronal-derived extracellular vesicles (EVs) in saliva. For this reason, saliva has recently demonstrated a great potential as a source of biomarkers for PD.

ELISA analysis [1,2] and Real-Time Quaking Induced Conversion (RT-QuIC) assays [3] have been applied to detect a-syn aggregates, tau and phosphorylated aggregates, as well as inflammation and autophagy biomarkers, in the saliva of PD patients and healthy subjects. Molecular data have been correlated with clinical features of PD patients and used for molecular clustering through principal component analysis (PCA). Immunofluorescence for a-syn and phosphorylated a-syn have been employed on skin biopsies of PD patients and healthy subjects and analysed by confocal microscopy to correlate molecular alterations in saliva with nerve fibres degeneration.

Reduced levels of total a-syn and increased levels of a-syn aggregates have been demonstrated in the saliva of PD patients by ELISA. RT-QuIC assay demonstrates seeding competent a-syn species in the saliva of PD patients and RT-QuIC kinetic parameters correlate with disease severity. Autophagic and inflammatory markers were detected in the saliva of PD patients and were responsible for their molecular clustering. In skin biopsies, a-syn aggregates were detected in sensory intra-epidermic amyelinic fibres and in autonomic fibres around sweating glands and pilo-erector muscles and correlated with a-syn in saliva.

Saliva represents a key biofluid candidate for the detection of biomarkers in PD and could be also used for clustering different PD subtypes, improving molecular diagnosis and follow-up.

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Keywords: alpha-synuclein; saliva; neurodegeneration; Immunofluorescence; protein misfolding

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Olfaction and Gustation in Blindness: the "Tasting in the dark" experience

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Evolutionarily, smell and taste are the oldest of our senses, greatly differing when compared to the younger senses as vision and audition. Interestingly though, vision is the most powerful sense to interact with the environment and for a very long time, such that it has been hypothesized that congenital blindness people develop better abilities in making use of the other senses, due to a supposed compensatory plasticity of the visual brain areas in the processing of non-visual information. To date, there are quite a few studies assessing olfaction and gustation in blindness and only two works reported sensory food experience in blind individuals. Indeed, flavour is among the most complex and powerful of all human sensations, involving, besides smell and taste, vision, audition and somatosensory modalities. Here, we would like to report our experience as an "on the fringes" event of the Neuroscience Congress in Milan, named "Tasting in the dark" at Villa della Torre, Verona, Italy. Three groups of people were involved: congress participants (non-expert/normal sighted, n=28), experts (wine producers/sommeliers, n=17) and late onset blind participants (non-expert, visually impaired/blind, n=8). Participants, all blindfolded, had to taste six different brands of Amarone Valpolicella wine. Before, some of them underwent smell-validated tests (Threshold, Discrimination, Identification). In particular, during the identification test (16 different odours) every participant had to identify each odour before and after the presentation of four written options *per* odour each time. From this pilot experience, we found a significant interaction between wine descriptors X group, p=0.013 and the multiple comparisons showed, particularly for the blind group, the ability to discriminate among the descriptors of the different wines. Within the olfactory and taste *status*, we found one close to be significant correlation (p= 0.056) only for blind people, considering the test for smell identification without options. Thus, instead of sensory compensation, blind individuals could rely on different cognitive strategies involving a better use of verbal memory processes. Future research, including cognitive assessment as well as brain-imaging investigation, could help for a better understanding of multisensory systems integration and how is the brain reorganized when a perceptual source of information is impaired or temporarily unavailable.

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Keywords: blindness; olfaction; gustation

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Oxytocin-Leptin Crosstalk in the Regulation of the Energy Balance

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Oxytocin (Oxt), peptide produced by the supraoptic (SON) and paraventricular (PVN) hypothalamic neurons, closely collaborates with the fat-derived hormone Leptin (Lep) to regulate the energy balance. Intracerebroventricular (icv) Lep administration activates Oxt PVN neurons which partly mediate Lep anorectic effect. On the other hand, exogenous Oxt induces satiety and promotes lipolysis in white adipose tissues where the expression of its receptor is the highest. Nonetheless, the effect of peripheral Lep on Oxt neurons is unknown, and possible sex and age-dependent variations in the crosstalk between these two systems have not been investigated yet.

In this study we explored the Oxt-Lep crosstalk assessing the effect of intraperitoneal (ip) Lep injection (0.5 μg/g) on Oxt neurons in male and female C57BL/6 mice at postnatal day 21 (p21, weaning) and p60 (adult).

A different age- and sex-dependent trend in the number of Oxt neurons was revealed by morphometry: while it was higher in males SON and PVN at p21 compared to p60, it had the opposite trend in females, overall resulting in a greater Oxt neurons population in males and females at p21 and p60, respectively. These data suggest that the Oxt system is shaped by sex-hormones during the postnatal development.

We then performed immunofluorescent double staining to establish whether peripheral Lep targets Oxt neurons. While we did not detect p-Stat3 (Lep downstream) staining in Oxt neurons following Lep ip injection, cFos positivity (marker of neuron activation) was observed in 28% and 21% of Oxt neurons in females SON at p21 and p60, respectively. Only a non-significant trend for cFOS+ was detected in males in the same area, while no activation was observed in the PVN in both sexes.

As Lep anorectic effect is achieved through POMC neurons activation in the arcuate nucleus (ARC), we used our C57BL/6J-Tg(Pomc EGFP)1Low/J model to assess POMC projections to Oxt neurons. POMC projections to the PVN were evidenced in both adult females and males, while POMC fibers were observed in the SON only in the former.

Collectively, these data suggest that female SON Oxt neurons may be activated by peripheral Lep indirectly through POMC projections from the ARC, highlighting the existence of a novel, sex-dependent pathway possibly involved in the regulation of the energy balance.

Keywords: Oxytocin; Leptin; Hypothalamus; Energy Balance

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Understanding and modeling nerve-melanoma interactions

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The tumor microenvironment exerts significant influence over the development and progression of cancer. The peripheral nervous system, as part of this microenvironment, can shape tumor initiation, growth and metastasis. In melanoma biopsies high levels of neuronal genes have been detected and the interaction of melanoma with nociceptor neurons has been demonstrated to enhance neurite outgrowth while suppressing immune surveillance through neuropeptide release (Balood et al., Nature 2022).

We have analysed by immunohistochemistry the expression of neuronal and Schwann cell markers in human melanoma biopsies. We observe that nerve fibers and glial cells are distributed mainly in the peritumoral areas of high-grade melanomas especially in association with lymphocyte infiltrations. Also, benign naevi were included in this study as non-pathologic controls with similar histological origin and similar cutaneous distribution.

An in vitro co-culture model system was developed to analyse soluble factors produced by human primary Schwann cells that can affect proliferation/migration of human melanoma cell lines.

Keywords: melanoma; peripheral nervous system; Schwann cells

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Biological effects of Vitamin E on BV-2 microglial cells

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Neuroinflammation is a protective mechanism designed to sustain the brain in response to inflammatory stimuli, however a prolonged inflammatory status, as well as oxidative stress, creates an environment that promotes the onset and progression of neurodegenerative disorders. Microglial cells are responsible for the defence and homeostasis of the Central Nervous System (CSN) and are activated in response to inflammatory stimuli they are commonly referred to as 'brain resident macrophages'. The pathogenesis of several neurodegenerative diseases is closely linked to the persistent activation of microglia, due to their fundamental role as mediators of inflammation in the brain. The present study is designed to investigate the biological effects of Vitamin E on BV-2 microglia as a potential neuroprotective and anti-inflammatory agent following stimulation with lipopolysaccharide (LPS). Vitamin E represents a family of lipid-soluble compounds consisting of four tocopherols and four tocotrienol derivatives. Vitamin E has both anti-inflammatory and antioxidant properties on CSN, although its neuroprotective effects are clear, the underlying molecular mechanisms remain to be elucidated. Results show that pre-incubation of microglia with vitamin E has neuroprotective effects on LPS-induced microglial activation, preserving the branching morphology typical of microglia in a physiological state, reducing the migratory capacity, the production of pro- and anti-inflammatory cytokines such as TNF-α and IL-10, and finally the activation of receptors such as TRL4 and CD40, which modulate the PI3K-Akt signalling pathway. These results require further understanding and research. However, they open up new future scenarios for the use of vitamin E as an antioxidant to provide greater neuroprotection in vivo for the prevention of possible neurodegenerative diseases.

Keywords: Vitamin E; microglia; inflammation; central nervous system; neuroprotective effects; antioxidant action

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PACAP-ADNP axis prevents death induced by trophic factor deprivation in mutated SOD1 motor neurons in amyotrophic lateral sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration of upper and lower motor neurons. Mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1) account for approximately 20% of familial ALS cases. The pathological mechanisms underlying mutant SOD1-mediated neurotoxicity remain unclear. However, an overproduction of reactive oxygen species (ROS) leading to oxidative stress (OS), has been observed in the spinal cord and motor cortex of ALS patients as well as in cellular and animal models suggesting that OS may play a crucial role in the pathogenesis of ALS. It has been reported that SOD1 mutations reduce the ability of cells to react to oxidative stress by impairing the expression of the nuclear factor erythroid 2-related factor 2 (Nrf2), which plays a crucial role in the protection against OS [1]. Many studies have shown the protective effects of pituitary adenylate cyclase-activating peptide (PACAP) in neurodegenerative disease. Its effects are mediated by the activation of three different G-protein-coupled receptors: PAC1R, VPAC1, and VPAC2. The stimulation of PAC1R triggers different signaling pathways including adenylate cyclase (AC), protein kinase A (PKA), and phospholipase (PLC)/protein kinase C (PKC). Previously it has been demonstrated that PACAP prevents motor neuron death following serum deprivation by increasing EGFR phosphorylation through protein kinase A (PKA) activation [2]. Moreover, some PACAP effects are also mediated by the stimulation of an intracellular factor known as activity-dependent protein (ADNP) through PKC activation [3]. However, the role of PACAP-ADNP axis on mutant SOD1 motor neurons degeneration has not been investigated, yet. The purpose of the present study was to investigate whether the protective effect of PACAP against apoptotic cell death induced by growth factor deprivation is mediated by ADNP activation and the role of the small peptide derived from ADNP, known as NAP to counteract ROS formation. Our data revealed for the first time that PACAP is able to rescue cell degeneration following growth factors deprivation activating PAC1R and increasing ADNP expression via PKC stimulation. We also demonstrated that PACAP/ADNP axis counteracted ROS formation through NRF2 nuclear translocation. In conclusion, our study provides new insights regarding the protective role of PACAP-ADNP in ALS.

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Keywords: ALS; PACAP; ROS, NAP

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Choline alphoscerate and thioctic acid effects on neuroinflammation in lipopolysaccharide-stimulated BV2 microglial cells

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Choline alphoscerate (α-GPC) is a choline-containing phospholipid and an acetylcholine precursor enhancing cognition [1]. Thioctic acid (TIO) is a potent antioxidant with the ability to promote neuronal proliferation, and the eutomer (+)-TIO is more active than the racemic form or (–)-enantiomer to counter neuronal damage [2]. Thus, both these compounds have previously been shown to exhibit neuroprotection [2,3]. Since microglia plays a crucial role in brain development, homeostasis, and disease, this study investigated the effects of α-GPC and the (+)-TIO on the inflammatory response in BV2 culture cells, a cell line of murine microglia origin, stimulated with lipopolysaccharide (LPS). BV2 microglia were treated with or without LPS and were incubated with LPS and α-GPC and (+)-TIO alone or in the association for 24 h. MTT assay, immunocytochemistry, and western blotting methods were utilized. MTT assay did not show significant changes in cell viability after treatments at different concentrations in unstimulated cells. On the contrary, LPS triggered morphological changes and an increase in ionized calcium-binding adapter molecule-1 (Iba-1) expression in BV2 cells. Moreover, an up-regulation of the nuclear factor-kappa B (NF-κB) and the interleukin-1β (IL-1β) were found in LPS-treated BV2 cells. These alterations were reversed by the treatment with α-GPC alone but not with (+)-TIO. The mechanism underlying α-GPC-mediated attenuation of inflammation in BV2 cells appeared to involve the suppression of translocation of NF-κB into the nucleus. In conclusion, we provide evidence that α-GPC attenuates LPS-induced neuroinflammatory responses, and this suggests that it may have therapeutic potential for the treatment of neurodegenerative diseases that are accompanied by microglial activation.

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Keywords: Microglia; Choline alphoscerate; Thioctic acid; Inflammation

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Exploiting lamin A processing: a potential pharmacological strategy to enhance oxidative stress sensitivity in glioblastoma cells

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Glioblastoma is the most lethal brain tumor affecting adults. Despite numerous efforts to seek new therapeutic approaches, the survival rate of patients remains unsatisfactory. This project aims to identify a new potential pharmacological strategy, exploiting the peculiar feature of glioblastoma cells to express lamin A, a key constituent of the nuclear lamina, differently from the healthy nervous tissue[1,2]. It is known that, the compromised maturation of prelamin A, the precursor of lamin A, hinders the proper activation of the response to DNA damage caused by reactive oxygen species (ROS), leading to a state of accelerated cellular ageing, as observed in laminopathies[3]. In this context, it is proposed to investigate the possibility to make glioblastoma cells more sensitive to oxidative stress, after prelamin A accumulation, in order to affect their survival and aggressiveness. To achieve this objective, a combination of two drugs has been suggested. Firstly, Lonafarnib was used to induce prelamin A accumulation. Secondly, Menadione was employed to induce ROS-mediated injury. This study shows how the combined treatment induced morphological changes in glioblastoma cells, resulting in evident nuclear reorganization. Additionally, the proposed treatment reduced the migratory and invasive capabilities of cells, inhibited colony formation, and impaired cell survival and aggressiveness. A deeper comprehension of how the proposed treatment influences the structure of the cell, particularly the interactions between the nuclear lamina and the cytoskeleton, as well as its impact on the associated signaling pathways, could pave the way for new therapeutic approaches for this tumor that is currently incurable.

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Keywords: lamin A; prelamin A; glioblastoma; Lonafarnib; Menadione; cellular ageing

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Potential role of lactoferrin in reprogramming astrocytes by promoting neurogenesis

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Neuroinflammation is a protective mechanism with the aim to clear infective agents and also to repair, regenerate and remove the damaged cells [1]. The chronic inflammation and the resulting prolonged activation of brain cells, however, drive to alterations in neuronal structure and functions leading to neurodegeneration [2].

Upon injury, the astrocytes have the potential to acquire neural stem cell properties to be reprogrammed into neurons [3], but the chronic exposure to inflammatory signals diminishes progressively the neuron's recovery due to the inhibitory environment and the glial scar formation [4].

In this work we investigated the effects of lactoferrin, an iron-binding glycoprotein with known immunomodulatory functions, on the reprogramming properties of DI-TNC1 astrocyte cell line. To mimic a chronic inflammatory environment, the astrocytes culture was treated with lactoferrin (LF) (4μg/ml), or LPS (0,4μg/ml), or LF+LPS, adding LPS 24 hours later the LF treatment. Then, cells were analyzed at three time points (t), respectively, at 2, 9 and 16 days from the first stimulation. We showed that LF boosts the neurogenic properties of the astrocytes at t2 and t9 evidenced by the overexpression of the stem cell markers, SOX-2 and Oct4, and the neural differentiation marker, β-tubulin III. In addition, we also performed a morphological analysis, evidencing that the number of cells with neuronal-like morphology and the length of neurites were increased by the pre-treatment with LF. Interestingly, after 16 days, the LPS treated-cells showed signs of necrosis, whereas the LF+LPS-treated cells exhibited neural morphological features without signs of suffering.

Taken together, these results demonstrate the lactoferrin capability to trigger and boost the astrocytes reprogramming into cells with neural phenotype, suggesting its possible protective effects in a context of chronic inflammation.

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Keywords: astrocytes; lactoferrin; inflammation; neurogenesis

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Histone deacetylases inhibitors to overcome Chemotherapy-induced peripheral neuropathy

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Histone deacetylases inhibitors (HDACi) are a family of epigenetic regulators, able to act on both histones and non-histonic proteins, such as tumor suppressor proteins and oncogenes.

A deregulation of epigenetic control, as occurring after an increased activity of HDACs, is a common early event in tumor development and progression; for this reason, HDACi could have an anti-proliferative action, modulating acetylation by targeting histone deacetylases.

In addition, HDACs could play a crucial role in neurodegeneration, mediating both neurotoxic and neuroprotective effects, therefore HDACi could also have a neuroprotective activity. This point may be particularly useful to fight Chemotherapy-Induced Peripheral Neuropathy (CIPN).

CIPN is a common side effect occurring during the treatment with the mainly used antineoplastic drugs. CIPN onset often forces a reduction of the clinical effective drug dose, or even the anticancer treatment interruption, besides affecting patient quality of life.

In this study, we verified if HDACi may be used in combination with the gold standard anticancer drugs, to enhance their antineoplastic effect and/or to reduce their neurotoxicity.

We first identified the IC_{50} of different HDACi (SAHA, SW100, Panobinostat and Romidepsin) in the colorectal adenocarcinoma-derived cell line, HT-29. Then, we evaluated the effect of the combination of each HDACi with Oxaliplatin (OHP), the gold standard drug used for the treatment of colon cancer. The putative neuroprotective effect was assessed on organotypic dorsal root ganglia (DRG) cultures by neurite length evaluation, a very reliable neurotoxicity assay.

We observed that HDACi slightly enhanced OHP antineoplastic effect, but overall that inhibitors targeting HDAC6 were also able to counteract OHP neurotoxic effect, thus being theoretically useful against CIPN. We are now focusing on the molecular mechanisms potentially involved in such a neuroprotection.

Keywords: Histone deacetylases inhibitors; Chemotherapy-Induced Peripheral Neuropathy; colorectal adenocarcinoma cells; Dorsal Root Ganglia cultures; neuroprotection

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Effect of tail pinch on BDNF and trkB expression in the hippocampus of Roman Low- (RLA) and High-Avoidance (RHA) rats

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The Roman low-(RLA) and high-avoidance (RHA) rat lines are one of the most validated genetic models for the study of fear/anxiety- and stress-related behaviors. We have previously shown that, in RLA rats, an intense acute stressor such as forced swimming (FS) elicits a marked decrease in the level of the brain-derived neurotrophic factor (BDNF) protein in the ventral hippocampus (vHC) associated with an increase in the dorsal HC (dHC), but no significant changes in BDNF levels are observed in both compartments of the HC in RHA rats. Since the modality and intensity of the stressor may impact distinctly on the expression of BDNF and its tyrosine-protein kinase B (trkB) receptor in different brain areas, it was considered of interest to examine the effects of tail pinch (TP), a mild acute stressor on BDNF/trkB signaling in the dHC and vHC of the Roman rat lines.

Using western blot (WB) and immunohistochemistry assays, we show that TP induces distinct changes in the levels of BDNF and trkB proteins in the RHA and RLA rats.

In particular, the WB assays showed that TP increases BDNF and trkB levels in the dHC of both lines but induces opposite changes in the vHC decreasing BDNF levels in RHA rats and trkB levels in RLA rats. These results suggest TP may enhance plastic events in the dHC and hinder them in the vHC. In keeping with WB data, immunohistochemical labeling revealed that TP induces changes in the dHC and particularly in the CA2 subfield, increasing BDNF-like immunoreactivity (LI) in both Roman lines, in the CA3 subfield increasing BDNF-LI in RLA rats, and in the dentate gyrus (DG), increasing trkB-LI in RHA rats. In contrast, in the vHC, TP elicits only a few changes, represented by decreases of BDNF- and trkB-LI in the CA1 sector of RHA rats. Collectively, these results provide additional evidence that the genotypic/phenotypic features influence the effects of an acute stressor, even as mild as TP, on the basal BDNF/trkB signaling, eliciting different changes in the dorsal and ventral subdivisions of the HC and influencing the direction and subregional distribution of the adaptive plastic responses of BDNF/trkB signaling in the HC.

Keywords: BDNF; trkB; tail pinch; stress; depression; Roman high- and low-avoidance rats; hippocampus; western blot; immunohistochemistry

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SMN circular RNAs as potential new targets and biomarkers for the therapeutic response in Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a neuromuscular disease caused by deletions of the human Survival Motor Neuron 1 (SMN1) gene, which encodes the ubiquitously expressed SMN protein. However, reduced expression of SMN primarily affects the viability of spinal motor-neurons, resulting in a severe muscle atrophy. Although several treatments for SMA have been approved and ameliorate the course of the disease in most patients, they do not yet represent a cure. Thus, a deeper knowledge of the molecular mechanism/s underlying SMN expression regulation and the identification of prognostic biomarkers to better stratify SMA patients will improve their clinical management. Our laboratory has recently identified numerous circular transcripts deriving from the SMN locus (SMN circRNAs). Interestingly, for their biochemical properties circRNAs tend to accumulate in long-lived cells (i.e. post-mitotic neurons) and they are abundant in body fluids (i.e. blood). Based on this evidence, we investigated the functional relevance of SMN circRNAs in human SMA fibroblasts and induced pluripotent stem cells (iPSCs). Our results show that SMN circRNAs are ubiquitously expressed in all SMA cell lines tested and that the most of them are predominantly localized into the cytoplasm, with the exception of two SMN circRNAs which are exclusively expressed in the nuclear compartment. Lastly, we evaluated the expression of the most abundant SMN circRNAs in the total extra-cellular vesicles (EVs) extracted from blood of SMA patients. Our results show that one of the SMN circRNAs is abundant and positively correlates with the response to the therapy. Further studies on SMA patients with different clinical responses to therapy will give us information about their potential as prognostic biomarkers.

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Petroclival Clinoidal Folds and their Relationships with Arachnoidal Membranes of the Anteromedial Incisural Space: An Anatomical Dissection

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In this work particular attention was focalized on the anatomy of the area located at the junction of the sphenoid and the basal portion of the temporal bone (petrous bone, petrous apex, upper petro-clival region) encircled by the free edge of the tentorium, the insertion of the tentorium itself to the petrous apex and the anterior and posterior clinoid processes that give rise to three distinct dural folds or ligaments: the anterior petroclinoid ligament, the posterior petroclinoid ligament and the interclinoid ligament. These dural folds constitute the posterior portion of the roof of the cavernous sinus denominated "the oculomotor triangle"(1).

The main purpose of this review study was to describe this anatomical region particularly in the light of the relationships between the anterior margin of the free edge of the tentorium and the above-mentioned components of the sphenoid and petrous bone. The anatomy of this interesting region is studied in the light of the available anatomical and neurosurgical literature.

Keywords: clinoid processes; petroclival folds; oculomotor triangle; anterior petroclinoid ligament; posterior petroclinoid ligament; interclinoid ligament

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Hippocampus volume analysis in Alzheimer's disease

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Cognitive dysfunctions are characterized by a decrease in the weight and volume of the brain, due to cortical atrophy, with widening of the grooves and flattening of cortical convolutions. Brain atrophy that mainly involves the hippocampus is related to the progression of cognitive impairment and the conversion from mild cognitive dysfunction to dementia.

The study of hippocampal atrophy has been shown to be a sensitive and suitable approach to confirm Alzheimer's disease (AD) diagnosis. Hippocampus develops degenerative changes before the onset of cognitive symptoms, and is the first region to degenerate in AD, followed by other brain areas.

The development of morphological models of the hippocampus can represent a starting point in identifying the tools allowing an early diagnosis of neurodegenerative disorders, but also an interesting tool to evaluate the impact of treatments in AD.

This work has analyzed Magnetic Resonance Imaging (MRI) of 56 patients participating in a clinical trial evaluating cholinergic (choline alphoscerate) supplementation in AD with vascular involvement (the ASCOMALVA trial). Data regarding hippocampal morphology were subsequently correlated with cognitive, functional and behavioral performance assessed by neuropsychological testing.

The obtained results showed the strong correlation between hippocampal volume and cognitive performance. A lower correlation was found between neuroimaging and functional tests and no correlation was noticeable between hippocampal volume and behavior. These results have also demonstrated that patients on active treatment with choline alphoscerate experienced less hippocampal atrophy in the first few years of treatment, which translated into a slower decline in cognition.

The study of hippocampal atrophy, in addition to being a powerful predictive tool, may also represent an efficient means to evaluate the efficacy of treatments proposed for AD and other age-related dementia disorders.

Keywords: Alzheimer's disease; Hippocampus atrophy; MRI; Cognition; Choline Alphoscerate

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Expression and topography of α-synuclein strains in the normal human brainstem

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α-synuclein is a neuronal synaptic protein known to regulate neurotransmitter release and vesicle trafficking. In synucleinopathies, such as Parkinson's Disease, insoluble forms of α-synuclein are known to accumulate in neurons and glial cells leading to Lewy Body Pathology. While α-synuclein in synucleinopathies has been widely studied, very little is known regarding its normal subcellular, cellular and topographic expression in the normal human brain. Furthermore, synucleinopathies present specific anatomical vulnerability patterns, so that distinct neuronal populations appear to be particularly susceptible to α-synuclein pathology. Yet, the morphological and biochemical causes of this vulnerability are unknown. Here we investigate the expression of monomeric strands of α-synuclein in the normal human medulla, a region particularly susceptible to synucleinopathies and implicated in the first stages of the disease. To evaluate the specific populations of neurons expressing monomeric strands of α-synuclein, we investigate the co-expression of main Calcium Binding Proteins (CBPs) (Calbindin, Calretinin and Parvalbumin), which are known to serve a neuroprotective role and are downregulated in neurodegenerative diseases.

The brains of 10 neurologically healthy body donors of the Body Donation Program of the University of Padova underwent double label immunohistochemistry to determine Calbindin, Calretinin, Parvalbumin, and monomeric α-synuclein expression within standardized sections of the medulla. Semi-quantitative morphometrical assessment was performed to compare protein expression within the structure.

α-synuclein was found to be predominantly expressed in the reticular formation, tegmental nuclei and olivary nuclei. CBPs appear to have a distinctive pattern of expression throughout the brainstem, with Calbindin having marked reactivity within neurons involved in motor control, respiration and autonomic functions, while Calretinin expression was limited to mostly reticular formation nuclei. Colocalization analysis was performed to assess co-expression of α-synuclein and CBPs.

This study helps better understand the localization and interplay between different groups of CBPs and α-synuclein within the human brainstem advancing the understanding of patterns of anatomical vulnerability in neurodegenerative diseases.

Keywords: Calbindin; Calretinin; Parvalbumin; α-synuclein; Neuropathology; Topography

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The dynamic of glioblastoma-associated glia: evidence from mouse and human tissue

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Glioblastoma multiforme (GBM) is the most common primary tumor of the central nervous system (CNS) with a poor prognosis. GBM cells migration is supported by resident astrocytes and extracellular matrix (ECM) remodeling. GBM cells connect with neuronal networks, destabilize synaptic activity and alter the blood-brain barrier with infiltration of macrophages from the peripheral circulation. Microglia and macrophages are recruited by the GBM for immune mimicry, which ensures tolerance by the adaptive immune system.

To date, there is no approved therapy for blocking GBM progression. The lack of knowledge regarding the CNS remodelling is mainly responsible for the failure of translational research in this area. In the present study we aim to investigate the molecular dynamic of glial cells in the peritumoral tissue from both murine and human organotypic slices.

Human primary GBM cells tagged using lentiviral transduction were injected into the organotypic cortex of whole brain mouse slices or peritumoral tissue from patients affected by glioma (day 0). The activity of the slices was tested before and after the GBM cells injection through a multi electrode array, and the tumor progression was studied at different time points until DIV 14 using pharmacological assays and morpho-molecular techniques.

GBM cells change their morphology with time and form spheroids in the late stage. Molecular targets related to astrocytes (Cx43, GFAP), microglia/macrophages (Iba1) and ECM (CD44, MMPs) revealed differential glial morphology and ECM protein expression patterns in the tumor core and peritumoral tissue, with heterogeneous profiles among the human specimens. The blockage of Cx43 hemichannels modified GBM evolution, glial reaction, and ECM composition, indicating a role for the astrocytic Cx43 in orchestrating the CNS response to the tumor.

Our results shed light on Cx43 dynamic in the peritumoral tissue and the identification of time-dependent targets in the GBM microenvironment that may be crucial for the reinforcement or re-education of the glial cells to counteract the progression of the disease.

Keywords: astrocytes; microglia; invasiveness; extracellular matrix; time-dependent targets

Cellule staminali.
Dalla biologia cellulare alle prospettive terapeutiche

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The CXCR1/CXCR2 Inhibitor, Reparixin Reduces the Emperipolesis Between Neutrophils and Megakaryocytes in the *Gata1*^{low} mice

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Pathological emperipolesis between megakaryocytes and neutrophils is frequently observed in the bone marrow of patients with myelofibrosis (MF), the most severe of the Philadelphia-negative myeloproliferative neoplasms (1) in which it is believed to contribute to increasing the transforming growth factor (TGF)-β microenvironmental bioavailability responsible for fibrosis (2). Using confocal microscopy, we can detect emperipolesis by staining with CD42b, specifically expressed on megakaryocytes, coupled with the Ly6B or Neutrophil Elastase antibodies, that recognize the neutrophils. We first confirmed that the bone marrow from patients with myelofibrosis and that from the *Gata1*^{low} mouse model of myelofibrosis, contains great numbers of neutrophils and megakaryocytes in emperipolesis. Both in patients and in *Gata1*^{low} mice, the emperipolesed megakaryocytes were surrounded by high numbers (5/megakaryocytes) of neutrophils, suggesting that neutrophil chemotaxis precedes the actual emperipolesis event. Because neutrophil chemotaxis is driven by CXCL1 (3), the murine equivalent of human interleukin 8 (IL-8) that is expressed at high levels by the malignant megakaryocytes, we tested the hypothesis that neutrophil/megakaryocyte emperipolesis could be reduced by Reparixin, an inhibitor of the receptors of CXCL1, CXCR1/CXCR2 (4). Indeed, this treatment greatly reduced both neutrophil numbers (by 4-fold), neutrophil chemotaxis (by 4-fold) and emperipolesis with megakaryocytes (by 3-fold) (Figure 1). Since treatment with Reparixin was previously reported to reduce both TGF-β content and marrow fibrosis in *Gata1*^{low} mice (5), these results identify neutrophil/megakaryocyte emperipolesis as the cellular interaction linking IL-8 to TGF-β abnormalities in the pathobiology of marrow fibrosis.

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Keywords: Emperipolesis; Myelofibrosis; Megakaryocytes; IL-8; Reparixin

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The secretome derived from human amniotic fluid stem cells causes an anti-cancer effect on melanoma cell line *in vitro*

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In general, mesenchymal stromal cells (MSCs) show intrinsic antitumor property due to their anti-proliferative activity, induction of apoptosis, and suppression of angiogenesis. However, it is not fully understood how these cells govern their anti-cancer effects. Moreover, the anti-cancer effect of MSCs seems to be cancer type-related. In recent years, the MSCs derived from the amniotic fluid (hAFSCs) and amniotic membrane have been introduced as an attractive and potent stem cell source for clinical application because of their easy, safe, and painless collection procedures with minimized ethical issues. Until now various studies have obtained controversial results and poor understanding of mechanisms behind the effect of hAFSCs on cancer cells. On the contrary, other studies have shown that these cells promote tumour progression and metastasis by enhancing angiogenesis, upregulating Akt/mTOR pathways and promoting metastasis.

Amniotic fluids were obtained from both the second and the third trimester, in order to compare the anticancer potential of different amniotic cell populations. To evaluate the effects of hAFSCs secretome on human melanoma cells (SK-MEL-28) and the possible mechanism involved, cell cycle analysis, western blot and scratch tests were used.

In the presence of hAFSCs secretome, a reduction of G2/M phase was observed. In parallel with the blockage of the cell cycle, an activation of the apoptotic pathway occurred. Moreover, the exposure to the secretome reduced the migration ability of SK-MEL-28 cells through inhibiting the epithelial-mesenchymal transition (EMT) process. A similar trend was observed for the secretome obtained from amniocentesis and caesarium hAFSCs, even if a more evident effect on EMT can be noticed with caesarium hAFSCs secretome.

Our data demonstrated that hAFSCs can release soluble factors in cell culture, causing an efficient anticancer effect inhibiting melanoma cell proliferation, tumour growth and migration, possibly by altering cell cycle arrest and ERK signalling-triggered EMT.

Keywords: stem cells; secretome; cancer

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Trop-2 Expression/Activation in Human Amniotic Epithelial Cells

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Trophoblast cell-surface antigen 2 (Trop-2) is a transmembrane glycoprotein expressed by stem/progenitor-like cells and epithelial cells at various stages of differentiation (1, 2). First identified on the surface of invasive trophoblast cells (3), Trop-2 has emerged as a driver of tumor growth and progression since the discovery of its overexpression in the majority of human carcinomas (2). This makes Trop-2 an attractive target for anticancer therapy, already applied in the clinic with the FDA-approved antibody drug conjugate Sacituzumab govitecan. Recent findings have demonstrated that Trop-2 undergoes proteolytic cleavage at the R87-T88 site, which activates the molecule to promote tumor growth and metastasis dissemination (4). Cleavage/activation is cancer specific, and absent in normal adult epithelia, where Trop-2 is present in its unprocessed full-length form. Given the stem-cell-like properties of human Amniotic Epithelial Cells (hAECs), we used them as a model to investigate Trop-2 expression/activation in stem cells. Based on our reported subdivision of human amniotic membrane regions (5), we also aimed at detecting whether this expression/activation could belong to specific areas of the amniotic membrane. Placentas were collected from healthy women after caesarean section at the Hospital SS. Annunziata of Chieti, and OCT-embedded. Frozen tissue samples were analysed by confocal multicolor immunofluorescence using anti-Trop-2 antibodies directed against either an immunodominant pan-Trop-2 epitope or distinct cleaved/activated form, followed by Western blotting analyses. This approach allowed us to show that Trop-2 is expressed by hAECs, with a specific pattern of expression/activation that was heterogeneous across the 4 distinct areas of the amniotic membrane.

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Keywords: Trop-2; human amniotic epithelial cells; stem cells; cancer growth; metastasis; immunofluorescence

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Chemical environmental pollutant BPA is involved in angiogenesis VS carcinogenesis

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Every day many polluting substances are released into the environment by industrial and agricultural factories, which can potentially represent a risk to human health, and due to their ubiquity, their exposure is difficult to avoid. Particular concern derives from those substances defined as "endocrine disruptors", which can interfere and mimic the actions of natural hormones leading to a range of developmental, reproductive, immune, neurological, or metabolic diseases in humans and animals. In recent years, among the potentially contaminating substances involved in endocrine disruption, Bisphenol A (BPA) has received increased attention due to its adverse effects on health, particularly in promoting carcinogenesis. In the current study, an *in vitro* model of human periodontal ligament stem cells (hPDLSCs) differentiated into endothelial cells (e-hPDLSCs) has been developed for the evaluation of the potential role of BPA on on endothelial differentiation and its potential carcinogenic effects. In our experimental model, hPDLSCs were treated with BPA at 10 μm during endothelial differentiation (e-hPDLSCs), promoting an increase of the expression of angiogenesis markers such as PECAM-1, VEGF, VEGFR, and vWF. Moreover, through the Next Generation Sequencing technique (NGS), it was possible to evaluate differentially expressed genes during endothelial differentiation which were characterized by expression profiles related to the induction of cancer growth and metastasis. The results obtained suggested that may BPA represent a risk for human health since it can induce upregulation of angiogenic factors and, as a consequence, neoplastic formations and tumor progression, highlighting the directly proportional relationship between angiogenesis and carcinogenesis.

Keywords: Bisphenol A; human periodontal ligament stem cells; angiogenesis; carcinogenesis; endocrine disruptors

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The multidomain protein Vav1 in the differentiation of pluripotent stem cells to insulin-producing cells

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Type 1 diabetes (T1D) is a complex metabolic pathology characterized by the autoimmune destruction of pancreatic β cells resulting in decrease of insulin production [1]. In recent years, the therapeutic approaches to T1D were aimed to restore fully functional insulin producing cells and, in addition to pancreatic islet transplantation, various strategies have been implemented to obtain high number of β-like cells by differentiation of pluripotent or multipotent stem cells. Despite the differentiation of human embryonic stem cells is considered the gold standard, obtaining insulin producing cells from adult induced pluripotent stem cells (iPSC) opened new horizons in regenerative medicine of diabetes [2].

During the complex differentiation process of iPSCs to β cells, several pathways are hierarchically modulated, and specific markers are evaluated during the maturation stages. To obtain fully functional β cells, a better knowledge of the intracellular signaling involved in the differentiation process is required.

In the differentiation of the multipotent human biliary tree-stem cells (hBTSCs) to β cells, a crucial role was revealed for the multidomain protein Vav1, whose expression in mature pancreatic islet is limited to cells showing low levels of insulin [3]. Vav1 also sustains the expression of insulin in the partial trans-differentiation to β cells of pancreatic ductal adenocarcinoma-derived cells [3].

Here, we explored the expression of Vav1 in the different stages of a six-step differentiation process of iPSCs to insulin producing cells [4]. Because in other cell models Vav1 participates in modulating specific Akt isoforms [5,6], we also evaluated the levels of the members of this protein family, a critical determinant of β cell mass and function [7]. We revealed that Vav1 is expressed in iPSCs and dynamically decreases during the differentiation, with a slight recovery in the last maturation stage (β cells). Both expression and activation of Akt inversely correlated with Vav1 levels, suggestive of a relationship between these molecules.

Even if preliminary, these data identify Vav1 as a new potential marker of specific stages of the differentiation of multipotent precursors into insulin producing cells, and indicate its possible role in modulation of Akt signaling, a promising therapeutic target for T1D.

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Keywords: Diabetes mellitus; iPSCs differentiation; β cells; Vav1; Akt

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CXCL1 is a potential biomarker for the variegation in the levels of fibrosis present in the bone marrow of animal models of myelofibrosis

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Myeloproliferative neoplasms (MPNs) are clonal disorders driven by the somatic mutations JAK2^{V617F}, MPL^{W515L} and CALR^{del} at the level of the hematopoietic stem cell. Myelofibrosis (MF) is the most severe MPN and is characterized by bone marrow failure and fibrosis, increased hematopoietic stem/progenitor cell trafficking, extramedullary hematopoiesis in spleen with splenomegaly. The serum level of the pro-inflammatory cytokine interleukin-8 (IL-8) expressed by the malignant megakaryocytes has been proposed as a possible marker to predict adverse prognosis in MF (1). In previous studies, we demonstrated that megakaryocytes from the *Gata1^{low}* mouse model of MF express high levels of CXCL1, the murine equivalent of human IL-8 and that their bone marrow has a CXCR1/CXCR2, the receptors for CXCL1, activated signaling. Furthermore, treatment with the CXCR1/CXCR2 inhibitor Reparixin reduces bone marrow fibrosis in this model. (2, 3,4).

To further validate CXCL1 as a marker for myelofibrosis, we correlate the level of fibrosis and those of CXCL1 expressed by several mouse models of the disease. This study included analyses of *Gata1^{low}* mice (at pre-MF and MF stage), and MPL^{W515L} mice and JAK2 ^{V617F} (early and later stages of the disease) and WT controls. Variegation was observed both in the level of fibrosis and CXCL1 with the (m)CXCL1 antibody, expressed by the bone marrow from the different models.

In the old control WT mice for all the models, the level of fibrosis in the bone marrow was barely detectable (Figure 1A). In *Gata1^{low}* mice, fibrosis raised from 7.8% at the pre-MF to 16.1% at the MF stage; in MPL^{W515L} mice, it went from 2.1% at three weeks post-transplant to 12.7% at six weeks post-transplant and in JAK2^{V617F} mice, it went from 2.8% at 2 months to 7.8% at the 6 months. The CXCL1 levels in the bone marrow from the WT controls were also barely detectable (Figure 1B). In *Gata1^{low}* mice, they went from 56.5AU in the pre-MF to 146.2 AU at the MF stage. In MPL^{W515L} mice, CXCL1 levels went from 49.8AU at three weeks post-transplant to 89.7AU at six weeks post-transplant stage and in JAK2^{V617F} mice, they went from 91.3 AU at 2 months to 96.0 AU at six months. Overall, the levels of fibrosis and CXCL1 observed in the different models were directly correlated (Figure 1C). These data provide a spectrum of the severity of fibrosis detected in the animal models most frequently used for preclinical validation studies and further support the role of IL-8 as the driving pro-inflammatory cytokine of myelofibrosis.

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Characterization and secretome evaluation of human Mesenchymal Stromal Cells derived from Adipose Tissue and Dental Pulp: an innovative therapy to counteract pathophysiological processes

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Mesenchymal Stromal Cells (MSCs) derived from Adipose Tissue (ADSCs) and Dental Pulp (DPSCs) were obtained from healthy patients in order to evaluate their use in regenerative medicine.

ADSCs and DPSCs were isolated by two methods, one based on enzymatic digestion and the other on mechanical fragmentation. Among the DPSCs, more than 90% were Nestin$^+$ and less than 10% αSMA$^+$, while the ADSCs showed an increased positivity to αSMA (~30%) and negativity for Nestin (<1%) compared to the DPSCs. Interestingly, the doubling time of DPSCs was significantly lower in DPSCs compared to both ADSCs. Multivariate analysis by PCA for the expression of 16 surface markers by flow cytometry showed that the organ source (dental pulp *vs* adipose tissue) was the most critical factor responsible for cell clustering. Then, we collected conditioned medium (CM) from the various stem cell populations after three days of culture and evaluated the presence of 27 analytes and total miRNAs. The analyte composition of CMs from different types of DPSCs appeared very similar while the method of isolation was responsible for differences between CMs from ADSCs. PCA analysis on miRNAs showed that these molecules clustered into two distinct groups based on the organ source. The interest in miRNAs arises from recent evidence that these molecules can promote tissue regeneration and decrease inflammation, oxidative stress, and fibrosis, processes that characterize aging as well as later stages of many human diseases. Recent studies conducted in our laboratory, showed that CMs from ADSCs and DPSCs can recover age-related ovarian dysfunction and we hypothesise that miRNAs may represent major players in this process. To validate whether such media were able to protect ovarian tissue from oxidative damage, we tested *in vitro* the ability of CMs to preserve murine granulosa cells viability following treatment with H_2O_2. Preliminary results suggest a protective action by all conditioned media compared to controls, with DP-CM being the most effective. All together, these results indicate that MSC populations show different phenotypic and functional signatures depending on tissue source and isolation method that need in-depth studies in order to differentiate the use of them and of their products as next-generation therapy in the different pathophysiological processes.

Keywords: Mesenchymal stromal cells; Adipose tissue; Dental pulp; Secretome; miRNome; Cell-free therapy

Dalla morfologia alla patologia molecolare

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Altered morphology of mitochondria in cardiomyocytes as an early sign of gut dysbiosis: an ultrastructural study

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In humans several diseases are associated to a microbiota dysbiosis. Since every organ has a distinct pattern of microbial population, recent clinical and experimental evidence have demonstrated that an inter-organ translocation of microbial communities could play a key role in driving pathological processes. affect

Recent advances in human microbiota studies shed light on the relationship between the gut microbiota and the cardiovascular system, revealing its contribution to the development of several cardiovascular diseases (CVDs).

In this context, we recently investigated in an *in vivo* experimental model the effects of gut microbiota homeostasis alteration, induced by the administration of an antibiotic dissolved in drinking water, on the cardiac muscle tissue. Light microscopy analysis did not reveal any morphological alteration and tissue structure was preserved. However, immune profile analysis suggested an impairment of innate immune response in antibiotic-treated mice.

The aim of the present study is to investigate, in the above described experimental setting, the murine cardiac muscle tissue morphology by transmission electron microscopy (TEM), aiming to define the effect of dysbiosis on the myocardium at the ultrastructural level.

Briefly, at the sacrifice, the hearts of untreated (CT) and vancomycin-treated (VAN) Friend leukemia virus B (FVB) mice were immediately processed for TEM observations. Image analysis of digitalized images of cardiomyocytes showed that the regular arrangement of myofibrils was preserved, but mitochondria in the VAN group were significantly larger and with a more elongated shape compared to CT.

Characterization at molecular level of pathways involved in fission, fusion and biogenesis of mitochondria are ongoing to elucidate the relationship between morphology and function alterations.

Moreover, a slight decrease of immune activation at the systemic level has been confirmed by analysis of immune infiltrate of mesenteric lymph-node.

The mechanisms involving gut microbiota in CVDs are not yet described but our preliminary evidence suggest a role for microbiota signalling to mitochondria, which indeed it was previously demonstrated to affect mitochondrial metabolism and immune cells.

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Effects of garlic extract on breast tumor cells: a phenotype dependent modulation of morphology, cell cycle and invasive potential

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Breast cancer is the most common neoplasia in women of industrialized and developing countries, and includes tumor subtypes showing intrinsic morphological, molecular, and clinical heterogeneity that often lead to the failure of even the most advanced therapeutic strategies (1). In this context, the use of natural agents to integrate conventional therapies was proposed to improve breast cancer approach (2).

In recent years, garlic (Allium sativum) has been used as a healthy functional food due to its great content of active phytochemicals, as organosulfur compounds, and antioxidants. High garlic consumption is protective against various solid tumors and its anti-cancerous potential has been validated by preclinical studies also in breast tumors, in which garlic derivatives act at distinct stages of carcinogenesis and interfere with cell proliferation and motility (3). Our research group firstly revealed anti-cancer properties of garlic in non-invasive breast tumor cells, demonstrating a protective role of this natural product against the malignant progression of breast neoplasia induced by low oxygen availability (4).

In this study we explored the role of a garlic extract on malignancy of invasive breast tumor cells, revealing specific and phenotype-dependent effects on their morphology, cell cycle, motility, and invasive potential. Antitumor role of garlic was confirmed in breast tumor cells showing a Luminal or Basal-like phenotype, in which it promotes an epithelial-like phenotype, but garlic extract induced a mesenchymal phenotype in HER2 positive cells and improved their invasive potential. These effects are correlated with a phenotype-dependent modulation of the Akt isoforms, crucial signaling molecules in solid tumors (5).

Although they need to be confirmed by in vivo studies, our results firstly demonstrate that the effects of garlic on breast cancer cells are not unique and depend strictly on the tumor phenotype. This suggests that a precise knowledge of action mechanisms activated by this natural product in the different tumor subtypes may be at the basis of the use of this natural compound in personalized therapeutic approaches for breast cancer management.

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Keywords: breast cancer; natural compounds; Akt

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Cerebral vascular disorders in sickle cell trait: a morphological study

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Sickle Cell Trait (SCT), besides the increasing evidence of erythrocyte stiffness and the long-term onset of chronic kidney disease in the trait carriers[1], is still considered a benign condition. The transgenic mice developed by T.M. Townes, harboring two sickle *HBB* human alleles ($HBB^{S/S}$), is one of the most used disease models to study Sickle Cell Disease (SCD)[2]. However, there are scanty data on the morphology and histopathology of heterozygous animals ($HBB^{A/S}$ - Sickle Cell Trait).

Using this model, we have recently demonstrated the existence of ultrastructural vascular modifications that could be responsible for possible multi-organ damage in the carrier of SCT[3]. To date no confirmed data exist regarding chronic ischemic disease of the nervous central system associated with the SCT trait. On this topic, the literature is fragmentary and rather contradictory; thus, while among African Americans, one of the most SCT-affected populations, SCT appears not to be associated with the incidence of ischemic stroke[3], other studies showed silent cerebral infarcts, linked to the sickle cell trait and associated with impaired cognitive ability. Thus, to extend our previous study, here we present a study of the ultrastructure of cerebral vascular beds of SCT carrier mice.

12 mouse brains, comprising 6 heterozygous Townes mice (i.e. the SCT carriers) and 6 healthy controls were examined by conventional light and transmission electron microscope (TEM). A part of the tissue samples was paraffin-embedded and cut into 10 μm thick sections to perform the histopathological analysis, while another part was rapidly immersion-fixed in glutaraldehyde and embedded into epoxy resin. Ultrathin sections underwent staining with UranyLess and lead citrate for TEM analysis.

The histopathological analysis of the brains of the SCT carrier mice revealed a normal parenchymal structure except for mild congestion of the vessels associated with the choroid plexuses. In the semithin sections, a slight tortuosity of the cerebral vessels was observed in heterozygous mice. At the ultrastructural level, the cortical capillaries of the SCT mice showed a three-times thicker basal lamina compared to control mice. Myelination and oligodendrocytes at the cortex-white matter boundary were conserved in both control and SCT mice.

The results obtained in the brain, added to previous multi-organ data, point out that, besides their ubiquitous presence, the chronic vascular alterations could affect long-term organ function also in heterozygous mice brains whose molecular nature requires further insights.

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Deciphering the hepatic role of Interleukin-13 expression in pre-clinical models of organ fibrosis

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Repeated liver injury has the potential to lead to liver fibrosis in which the regenerative capacity of the organ in the context of chronic inflammation results in excessive extracellular matrix deposition and, over time, impaired liver function [1]. A dysregulated type 2 immune response, promoted by IL-13, is implicated in myelofibrosis [2] and it has been recently claimed as pivotal in liver fibrosis too [3]. The aim of the present study is to compare the role of IL-13 expression in sustaining bone marrow and liver fibrosis. We investigated liver fibrosis in the following mouse models of myelofibrosis: Jak2$^{+/V617F}$Vav-Cre+ mice, at baseline and overexpressing murine IL-13 (56.6 vs 564.5 pg/ml of plasma, respectively) [2], and *Gata1^{low}* mice (b.d. levels of IL-13 in plasma). Mild to moderate liver fibrosis was observed in Jak2$^{+/V617F}$Vav-Cre+ mice with and without IL-13 overexpression in terms of increased reticular fibers, collagen III deposition and activation of hepatic stellate cells (HSCs). By contrast, in *Gata1^{low}* mice, fibrosis with high number of HSCs was observed at 10 months but not at 15 months, despite persistence of HSCs. Of note, by 15 months the liver of *Gata1^{low}* mice became a site of extramedullary hemopoiesis with a large number of megakaryocytes (MKs) in close contact with HSCs, some of which acquired features of degenerated cells. These results suggest that interaction between HSCs and MKs in presence of low IL-13 tissue content, reduces the pro-fibrotic activity of HSCs in the liver of *Gata1^{low}* mice. Further studies which will include a deeper molecular characterization of HSCs and MKs are underway to clarify the complex interplay leading to spontaneous liver fibrosis resolution in *Gata1^{low}* mice and whether this mechanism may pave the way for innovative approaches in the treatment of liver fibrosis in humans.

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Keywords: Liver fibrosis; Interleukin-13; Hepatic Stellate Cells

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Visualizing the subcellular action of anti-HER2 therapeutics at molecular nanoscale in breast cancer cells

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ERBB2/HER2+ breast cancer affects around 20%–30% of patients and is characterized by amplification of the ERBB2/HER2/Neu gene or overexpression of the ERBB2 receptor. Neratinib (NE) is an irreversible pan-ERBB tyrosine kinase inhibitor that is currently approved only for the treatment of ERBB2+ breast cancer. NE exerts its anti-cancer effects by inhibiting ERBB2 downstream signaling kinases, such as pERK and pATK, which are critical for cancer cell survival. Interestingly, NE also affects autophagy and mitochondrial homeostasis, two physiological processes that cancer cells exploit to survive. By combining high resolution microscopy and molecular approaches, we found that NE inhibited kinases involved in cancer cell survival after 2 hours of treatment, while kinases involved in DNA damage response were inhibited after 72 hours. NE was also found to transiently enhance autophagy, a process by which cells degrade and recycle damaged cellular components, and to affect mitochondrial dynamics and energy metabolism. Specifically, NE increased the expression levels and nuclear localization of the TFEB and TFE3 transcription factors, resulting in increased autophagic flux. Additionally, NE increased the release of extracellular vesicles (EVs) with reduced ERBB2 positivity. In conclusion, NE is a potent activator of TFEB and TFE3, acting through ERBB2 and/or other kinases to suppress cancer cell survival by promoting autophagy, unbalancing mitochondrial function and response to DNA damage, as well as reducing ERBB2 dissemination through EVs.

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Deacetylases inhibition in cellular models of Fanconi Anemia: effects on antioxidant responses and on mitochondrial network

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Fanconi anemia (FA) is a rare genetic disease characterized by defective DNA repair and oxidative stress accumulation due to a defect in aerobic metabolism related with the mitochondrial network alteration[1]. This redox imbalance is not counteracted by endogenous antioxidant defenses, whose expressions and activities are lower than in healthy cells[2-3]. Since the reduced expression of these proteins might depend on the hypoacetylation of the respective genes, we investigated the effect of deacetylase inhibitors (i-HDACs) in the cellular models of FA, evaluating i) expression and activity of enzymes involved in antioxidant responses and ii) morphology and function of mitochondria. Lymphoblasts and fibroblasts mutated for the FANC-A gene were treated with 3 different i-HDACs: valproic acid (VPA), β-hydroxybutyrate (OHB), or EX527 (a Sirt1 inhibitor) under both baseline conditions and after oxidative stress induction. Under basal conditions, VPA treatment increased both expression and activity of enzymes involved in the antioxidant response. On a metabolic point of view, VPA incubation corrected the metabolic defect resulting in an increase of energy status and in a reduction of oxidative damage. These improvements might depend on a restoration of mitochondrial dynamic due to a decrease in DRP1 expression, which led to a mitochondria reorganization, improving their functional efficiency. By contrast, OHB impaired the mitochondrial function defect, increasing the oxidative stress production, probably also acting as a metabolite of oxidative phosphorylation, which is impaired in FA. Treatment with EX527 showed no effect.

Finally, the different i-HDACs showed the same effects even in the presence of an oxidative insult after the addition of H_2O_2.

In conclusion, the data suggest that VPA could be a promising drug to modulate gene expression in FA cells, confirming that the modulation of the antioxidant response plays a central role in the pathogenesis of FA, acting both on oxidative stress levels and on mitochondrial function and organization.

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Keywords: Fanconi Anemia; deacetylases inhibitors; antioxidants responses; mitochondrial morphology; mitochondrial function

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HSF-1/miR-145-5p transcriptional axis enhances hyperthermic intraperitoneal chemotherapy efficacy on peritoneal ovarian carcinomatosis

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Hyperthermic intraperitoneal administration of chemotherapy (HIPEC) increases locally drug concentrations and reduces systemic side effects associated with prolonged adjuvant intraperitoneal exposure in patients affected by either peritoneal malignancies or metastatic diseases originating from gastric, colon, kidney and ovarian primary tumors. The molecular mechanisms that render HIPEC treatment more effective than systemic therapies have been poorly explored. Herein we documented the role of miR-145-5p tumor suppressor activity during HIPEC treatment in a pilot cohort of patients with ovarian peritoneal metastatic lesions. Low miR-145-5p expression level was closely associated with metastatic tissues of many cancers and poor prognosis of patients.

RNA sequencing from metastatic nodules treated at different times of HIPEC allowed the identification of the mechanism by which HSF-1 transcriptional factor was able to rescue miR-145-5p expression. The restored miR-145-5p levels resulted in the downregulation of its oncogenic target genes such as c-MYC, EGFR, MUC1 and OCT4, revealing that the involvement of heat stress-related genes was able to promote the response to intraperitoneal chemotherapy.

In aggregate, our findings highlight a novel transcriptional network involving HSF-1, miR145-5p, MYC, EGFR, MUC1 and OCT4 whose proper activity contributes to HIPEC anticancer efficacy in the treatment of ovarian metastatic peritoneal lesions.

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Keywords: miR-145; HSF-1; ovarian cancer; HIPEC; metastases

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Ultrastructural analysis of Large Japanese field mice (*Apodemus speciosus*) testes exposed to low-dose-rate (LDR) radiation after the Fukushima accident

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Since the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident, there was great attention to the exposure damages to low-dose-rate (LDR) radiation on the biological systems [1]. Male reproductive functions are sensitive to radiation, with implications connected to infertility [1,2]. This preliminary study aimed to evaluate the effects of LDR radiation on the testis ultrastructure in the Large Japanese field mouse (*Apodemus speciosus*), captured from three different contaminated sites, Tanashio area (low-dose-rate, air dose rate: 0.29 μGy/h), Ide area (intermediate-dose-rate, air dose rate: 5.11 μGy/h) and Omaru area (high-dose-rate, air dose rate: 11.80 μGy/h), in the FDNPP accident ex-evacuation area. Mice captured from the unpolluted site in the Niigata Prefecture were used as a control. Testes were fixed in 2.5% glutaraldehyde/PBS and subjected to the preparative for Light Microscopy (LM) and Transmission Electron Microscopy (TEM) [2,3]. Results showed a good preservation of seminiferous tubule morphology in mice, suggesting a normal process of spermatogenesis compared with the control. Some modifications were noted in the ultrastructure of mitochondria, which appeared sometimes vacuolated; cytoplasmic vacuolization, which seems due to dilatation and vesiculation of the endoplasmic reticulum, was also present. Furthermore, TEM revealed the presence of lipid droplets in the sperm cytoplasm, associated with an increase in the phagocytic activity of Sertoli cells. However, these findings could be correlated with the physiological dynamics occurring during spermatogenesis. In conclusion, although testes are hypersensitive to radiation, LDR radiation exposure associated with the FDNPP accident had no significant effects on the male reproductive organs in Large Japanese field mice. These ultrastructural data may be used for further studies on the male reproductive potential of mammals, inhabiting the Fukushima area and chronically exposed to LDR radiation.

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Keywords: Fukushima; ionizing radiation; ultrastructure; testis; environmental pollution

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Sialylation Profile in Placentas of Pregnancies Affected by SARS-CoV-2 Infection

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Recent investigations have indicated the potential negative consequences of SARS-CoV-2 infection on pregnant women and pregnancy outcomes. Studies have described pathological changes in the placental tissue of SARS-CoV-2-positive mothers, which may or may not be associated with infection severity or the trimester of infection [1]. Among the various molecules involved in the normal structure and functioning of the placenta, sialic acids (Sias) appear to play a significant role. Therefore, our objective was to examine potential alterations in the distribution and levels of Sias with different glycosidic linkages, specifically α2,3 and α2,6 Galactose- or N-acetyl-Galactosamine-linked Sias, as well as polymeric Sia (PolySia), in placentas from pregnant women infected with SARS-CoV-2 during different trimesters of pregnancy [2, 3].

We employed lectin histochemistry, utilizing *Maackia amurensis* agglutinin (MAA) and *Sambucus nigra* agglutinin (SNA) for the evaluation of α2,3 and α2,6 Galactose-linked Sias, respectively. Immunohistochemistry was employed for the detection of PolySia.

The data revealed decreased levels of α2,3 Galactose-linked Sias in the trophoblast and underlying basement membrane/basal plasma membrane in placentas from women infected during the second and third trimesters, in comparison to uninfected cases and those infected during the first trimester. Conversely, higher levels of PolySia were observed in the trophoblast during the second and third trimesters of infection.

Our findings suggest that alterations in the sialylation status of the trophoblast and its basement membrane/basal plasma membrane, in conjunction with other concurrent factors, could underlie the most common placental histopathological changes and gestational complications found particularly in pregnancies with SARS-CoV-2 infection during the second and third trimesters.

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Keywords: Sialic acids (Sias); polymeric Sia (PolySia); α2,3 and α2,6 Galactose- and N-acetyl Galactosamine-linked Sias; placenta; SARS-CoV-2 infection; trophoblast

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Morphological alterations associated to anticancer activity of olive (*Olea europaea* L.) leaf enriched extract in prostate cancer cell lines

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Prostate cancer is one of the most frequent malignancy in men worldwide[1]. Genetic traits, advancing age and differences in social and environmental aspects are well-established risk factors, together with black race/ethnicity[2].The risk of advanced prostate cancer is increased also by lifestyle factors, including obesity[3], diabetes mellitus[4], insufficient physical activity, nutritional factors and smoking[5]. Conventional treatments for recurrent and/or metastatic prostate cancer typically produce side effects and new approaches have been considered to reduce disease progression or recurrence in patients with known disease.

Natural compounds or phytocomplexes with known anticancer properties are often used as dietary supplements[6,7]. Several findings have shown that the secondary metabolites present in the drupe and leaves of *Olea europaea* L. have a protective role on digestive, breast, and prostate cancer[8,9,10].

In this study we analysed the phytochemical profile, the antioxidant activity and the cytotoxic effect of an aqueous enriched extract obtained *Olea europea* L. leaves, collected in the flowering time in Syracuse (Sicily, Italy), on two prostate cancer cell lines (LNCaP, PC3) and on a fibroblast cell line (HFF-1). Our results confirmed the quantitatively and qualitatively high content of phenolic compounds in olive leaves (evaluated by HPLC-DAD analysis, Folin-Ciocalteu method and aluminium chloride method) and their related antioxidant activity (assessed by DPPH test and intracellular ROS and RSH quantification). We also showed a significant cytotoxic effect, confirmed by MTT test, on the two prostate cancer cell lines. Remarkably, the extract resulted non-toxic on HFF-1 cells at the concentrations and exposure times tested, so showing a good selectivity towards tumour cells. The cytotoxicity was associated to evident morphological changes and further investigated by LDH release assay, which allowed to establish necrosis as the main cell death mechanism.

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Free fatty acids cause kidney injury and lipid metabolic memory in obesity related glomerulopathy

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The signaling pathways leading to renal pathology in obesity are not understood well. Growing evidence suggests that FFAs induced oxidative stress plays a role as the critical factors linking obesity with its associated complications. Our study demonstrates that the modified protocol of *Oliveira et al,* for conjugating palmitate with albumin, produces an unbounded palmitate fraction able to cause podocytes dysfunctions similar to the one observed in the ORG.

Conditionally immortalized human podocytes cells line were exposed to different ratios of conjugated PAL / BSA for 24h and then cells were incubated, for an additional two days, in medium without palmitate. Palmitate, at concentrations seen in obese patients, causes ROS overproduction in human podocytes. This oxidative stress induces podocytes dysfunctions such as inflammation, and changes in profibrotic and lipotoxic markers. HMGB1 appears to be the main mediator of ROS damaging action, since its pharmacological inhibition prevents all ROS effects on podocytes. Moreover, palmitate is able to start a feed-back loop that causes a persistent overproduction of ROS and consequently a persistent podocyte dysfunction. Therefore, a "metabolic memory" has been demonstrated after FFAs treatment on podocytes, causing a persisting damage even in presence of a normalized lipid profile. The present findings may provide further insight into the underlying mechanisms that contribute to the pathogenesis of ORG.

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Oxidative/glycative damage and morphological alterations in the PCOS mouse tubal ampulla: the protective effects of L-carnitines

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Polycystic ovary syndrome (PCOS) is a multifactorial metabolic disorder associated with female infertility. After having assessed the metabolic features and morpho-functional effects of this syndrome on mouse ovaries and uteri (Di Emidio *et al*., 2020a; Di Emidio *et al*., 2020b; Palmerini *et al*., 2023), we here analyzed morphological alterations in the third portion of the uterine tubes, the tubal ampullae, in a mouse model of PCOS. Based on the antioxidant effects of carnitines, we also investigated whether L-carnitine (LC) and acyl-L-carnitine (ALC) could improve the PCOS phenotype in a mouse model induced by dehydroepiandrosterone (DHEA).

Adult CD1 mice received DHEA for 20 days alone (DHEA, 6 mg/100 g body weight) or with 0.40 mg LC and 0.20 mg ALC (LC-ALC), by oral gavage. Control animals were untreated. At the end of the treatments, tubal ampullae were collected and subjected to histology, immunohistochemistry, and transmission electron microscopy (TEM).

Respect to well-preserved controls, hematoxylin-eosin, Azan-Mallory and Col1 stainings highlighted hyperplasia, hypertrophy, and hyperfibrosis in the luminal epithelium of DHEA ampullae. MG-AGE accumulation was indicative of glycative stress. A decreased Tomm20 expression associated with increased HNE and 17 β-HSD4 stainings showed mitochondrial and oxidative damages, with altered steroidogenesis. The DHEA group showed by TEM a preserved ampullar epithelium with ciliated and nonciliated cells. However, numerous mitophagic vacuoles, large lysosomes, and vesicles were visible in the apical cytoplasm. LC-ALC administration induced a decrease in hyperplasia and hypertrophy of the luminal epithelium, with ampullae showing a normal collagen distribution. Expression of MG-AGEs, HNE and 17 β-HSD4 were reduced if compared to the DHEA group, while Tomm20 increased. The ultrastructural study evidenced similarities with controls, with an abundant presence of ciliated cells and the absence of mitophagic vacuoles and vesicles; mitochondria appeared well preserved, similarly to controls.

In conclusion, LC-ALC in the murine DHEA model of PCOS decreased glycative and oxidative stress with an evident reduction of ultrastructural damages, thus providing promising therapeutic strategies for L-carnitines.

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Keywords: PCOS; tubal ampullae; mouse; dehydroepiandrosterone; carnitine; TEM

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Variazioni fenotipiche e morfofunzionali nel ciclo vitale delle cellule di Leucemia Linfatica Cronica durante la transizione tra sangue periferico e organi linfoidi secondari

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La leucemia linfatica cronica (LLC) presenta una evoluzione clinica estremamente eterogenea. La definizione delle cinetiche del clone e delle sue sottopopolazioni intraclonali (ovvero delle caratteristiche morfologiche, fenotipiche e funzionali) durante il ciclo di attivazione/proliferazione negli organi linfoidi secondari e successivi cambiamenti a livello periferico) è essenziale per comprenderne la patogenesi, l'evoluzione clinica e sviluppare nuove ed efficaci terapie. Le interazioni con il microambiente (a livello linfonodale, splenico e midollare) determinano nuove mutazioni dovute alla maggiore sopravvivenza e divisione cellulare. Perciò, la possibilità di identificare le cellule leucemiche che hanno subito recentemente un processo di divisione mitotica può consentire di studiare la sottopopolazione (o le sottopopolazioni) più probabilmente responsabile della progressione della malattia e della resistenza alle terapie.

A questo scopo, la cinetica intraclonale delle cellule B della LLC è stata studiata in sottogruppi clonali definiti da livelli reciproci di espressione in membrana di CXCR4 e CD5. In questo modello, sono state identificate tre frazioni: "Proliferative Fraction" (PF: $CXCR4^{Dim}$-$CD5^{Bright}$), "Intermediate Fraction" (IF: $CXCR4^{Int}CD5^{Int}$) e "Resting Fraction" (RF: $CXCR4^{Bright}CD5^{Dim}$).

Le cellule LLC non manipolate, studiate *ex vivo* da 10 pazienti che avevano assunto ^2H_2O (Acqua Pesante) per 4 settimane, sono state selezionate in base alle densità relative di CXCR4 e CD5 per isolare le sottopopolazioni PF, IF e RF e due frazioni non precedentemente caratterizzate, "Double Dim Fraction" (DDF: $CXCR4^{Dim}CD5^{Dim}$) e "Double Bright Fraction" (DBF: $CXCR4^{Bright}CD5^{Bright}$). Per ciascuna frazione è stata misurata la quantità di deuterio incorporato nel DNA cellulare *in vivo*. Coerentemente, la PF conteneva livelli significativamente più elevati di DNA marcato con ^2H e tassi di divisione cellulare più elevati rispetto alla RF e alla IF. È interessante notare che anche la DDF conteneva una quantità significativamente maggiore di DNA marcato con ^2H rispetto alla RF; al contrario, la DBF assomigliava maggiormente alla frazione RF. Il gradiente complessivo di incorporazione di ^2H osservato è stato: PF>DDF>IF>DBF>RF.

Nella LLC, la segnalazione del BCR è fondamentale e la quantità di IgM di membrana (m) è associata alla competenza di segnalazione e all'aggressività della malattia. Inoltre, se attivate indipendentemente, mIgM e mIgD possono portare a sequele di segnalazione diverse. Pertanto, abbiamo analizzato le 5 sottopopolazioni per le densità di mIgM e mIgD. Ciò ha mostrato una distribuzione simile a quella dell'incorporazione di ^2H-DNA: per le IgM: PF=DDF>IF=DBF=RF, e per le IgD: PF>DDF>IF=DBF>RF.

Di conseguenza, abbiamo misurato il ^2H-DNA in sottopopolazioni con livelli bassi, intermedi e alti di IgM e IgD. Ciò ha rivelato una correlazione diretta tra le densità di IG e la sintesi di DNA *in vivo*, coerente con le sottopopolazioni intraclonali con IG elevate che si sono divise più di recente rispetto a quelle con IG basse.

Tuttavia, questi risultati non sono in linea con l'ipotesi che la divisione cellulare sia avviata principalmente dalla stimolazione del BCR, poiché ciò ridurrebbe i livelli di mIgM. Pertanto, abbiamo verificato se la stimolazione attraverso il TLR9 potesse influenzare la densità di mIG sulle cellule LLC. Dopo la stimolazione di 32 cloni di LLC con CpG+IL15, anti-IgM+IL4, anti-IgD+IL4 o anti-IgM-IgD+IL4, si è osservato un aumen-

to significativo delle mIG solo dopo l'attivazione con CpG+IL15; ogni stimolazione anti-IG ha portato a una downregolazione delle mIG.

Infine, ci siamo interrogati sull'efficacia, *in vivo* ed a livello subclonale, degli inibitori delle chinasi coinvolte nella segnalazione del BCR utilizzati modernamente in terapia. I campioni di LLC prelevati dagli stessi pazienti, prima e durante il trattamento con ibrutinib (inibitore della Bruton's Tyrosine Kinase: BTK), hanno mostrato cambiamenti intraclonali nelle densità di mIgM e nelle caratteristiche di dimensioni e complessità morfologica cellulari. Questi ultimi possono essere considerati marcatori di attivazione cellulare e metabolica, collegati ai tassi di proliferazione della LLC *in vivo*. Il trattamento con ibrutinib ha normalizzato le densità intraclonali di mIgM e mIgD e ha portato a una riduzione complessiva delle dimensioni e complessità delle cellule. Gli elementi più grandi e complessi, arricchiti di ^{2}H e con più alta densità di mIG risultavano maggiormente colpiti dalla terapia (PF>DDF>IF>DBF>RF).

Nel complesso, questi risultati suggeriscono che le cellule che hanno avuto una recente replicazione lasciano gli organi linfoidi secondari ed entrano in circolazione come PF, da cui transitano a fenotipi con espressione inferiore di CD5 (DDF) o superiore di CXCR4 (IF e DBF). Ciascuna di queste sottopopolazioni alla fine converge come RF, morfologicamente meno complessa, di dimensioni minori e con caratteristiche funzionali di quiescenza. Inoltre, poiché le densità di mIG nelle popolazioni di recente divisione (PF e DDF) sono elevate, i dati implicano che il successo della divisione cellulare non è solo una conseguenza della stimolazione del BCR. Il coinvolgimento delle vie TLR, in concomitanza o in serie con la segnalazione del BCR, è coerente con i livelli più elevati di mIG, con l'incremento di dimensione cellulare e con la maggiore complessità intracellulare. Infine, il trattamento con ibrutinib sembra colpire preferenzialmente le cellule di recente divisione con elevati livelli di mIG e con caratteristiche morfologiche di cellule attivate.

In conclusione, fenotipi più complessi, che includono le densità di membrana sia di CXCR4/CD5 che di smIG, possono migliorare la nostra capacità di individuare sottopopolazioni leucemiche che differiscono per il tempo trascorso dall'ultima divisione cellulare e per la loro sensibilità ad interventi terapeutici. Pertanto, possono migliorare la nostra capacità di identificare e comprendere i meccanismi biologici alla base della suscettibilità a varie terapie, fondamentale per lo sviluppo di trattamenti specifici e duraturi che agiscano nella proliferazione e nella maturazione delle cellule della LLC.

Keywords: Leucemia Linfatica Cronica, B cell receptor, Toll-like receptor, inibitori delle chinasi

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Cadmium levels in the follicular fluid affect the ultrastructure of the mature human cumulus-oocyte complex

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It is known that exposure to heavy metals impairs female fertility. Particularly, cadmium (Cd) exposure has been associated with an increased risk of endometriosis and breast and endometrial cancer. Cd exposure disturbed follicle development, increasing the number of atresia follicles [Dong et al., 2020; Qu et al., 2022]; in mammals, Cd presence impairs oocyte meiotic maturation rate both in vivo and in vitro and alters the quality of oocytes via oxidative stress leading to a decrease in female fertility. Therefore, the present study aimed to investigate the presence of Cd in the intrafollicular fluid (FF) of women no-smokers but exposed to HMs for professional reasons (garbage women, crossing guard) or living in rural areas near landfills and waste disposal areas and correlated the intrafollicular Cd levels with possible alterations in the ultrastructure of human cumulus-oocyte complexes (COCs). FFs were collected from the two most prominent individual follicles for each ovary, and corresponding oocytes surrounded by cumulus cells (COCs) were subjected to ultrastructural analyses by Transmission Electron Microscopy [Miglietta et al., 2023]. We demonstrate that intrafollicular Cd levels, at nanomolar concentration, may be associated with morphological alteration of the oocyte ultrastructure. Moreover, COCS showed scarce active steroidogenic elements, whereas regressing ones, with cytoplasmic alterations related to Cd, were numerous. According to Cd's endocrine-disrupting activity, the poor steroidogenic activity of cumulus cells might correlate with delayed oocyte cytoplasmic maturation. We conclude that Cd exposure negatively affects oocyte maturation, subsequent fertilization, and embryo development.

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Keywords: oocytes; heavy metals; cumulus oophorus; electron microscopy

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Linking between Telocytes and immune cells in IBD, an immunohistochemical study

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Inflammatory bowel disease (IBD) forms a group of idiopathic immune-mediated inflammatory conditions, associated with various areas of the intestinal tract(1). The main are Crohn's disease (CD) and Ulcerative colitis (UC).

Telocytes (TCs) are a type of stromal cells identified in many human tissues and organs, including the gastrointestinal tract(2). They are characterized by cytoplasmic extensions called telopodes. TCs play a crucial role in the tissue homeostasis and regeneration, they are also involved in the modulating the inflammatory response of many pathologies(3).

S100 protein family represents the largest group of the Ca^{2+}-binding protein (4). It is expressed by a variety of cell types including innate immune cells, epithelial cells, and fibroblasts(5). This multifunctional protein has a regulatory role in a variety of cellular processes as growth arrest, apoptosis, and stimulation of cell proliferation (6); furthermore, S100 protein acts as damage-associated molecular patterns (DAMPs) and interacts with pattern recognition receptors (PRRs) to modulate proinflammatory responses. S100 protein may also have fundamental roles in gut defense or in the pathogenesis of IBD(7).

In this study we evaluated the involvement of telocytes, characterized with vimentin antibody and the expression of S100 in IBD. The results showed numerous immune cells S100 positive in the mucosal surface and in the connective tissue in IBD specimens as compared with control samples; furthermore, we have observed a progressive loss of TCs leading to a reduced number or even absence of TCs in fibrotic areas in both CD and UC, correlating to the severity of the disease. Some telocytes co-localizing with S100 e vimentin, demonstrating their involvement in inflammatory state.

In conclusion, by referring to our previous studies about IBD(8), we can confirm that the crosstalk between different types of cells is crucial, for the maintenance of homeostasis and in the pathogenesis of these diseases.

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Keywords: Inflammatory bowel disease (IBD); telocytes; S100 proteins

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Secosterol-B induces nitrosative stress and UPR activation in human endothelial cells

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The endothelium, consisting of a single layer of endothelial cells, functions as a homeostatic organ that maintains the structure and tone of the vascular system. Oxysterols, products generated by cholesterol oxidation, are involved in endothelial dysfunction and development of atherosclerosis. In the last decade, some studies have investigated the mechanism and *in vivo* relevance of endoplasmic reticulum (ER) stress in the atherosclerotic process. ER redox homeostasis is crucial for the protein folding process and disulfide bond formation representing a potential activator of unfolded protein response (UPR). Recent studies support the view that ER protein folding pathways are highly correlated with nitric oxide (NO) production and oxidative stress conditions. Recent evidence reported that an altered bioavailability of NO causes nitrosative stress (NSS), inducing S-Nitrosylation (SNO) of ER stress sensors, such as IRE1α and PERK [(1)]. Here, we described how 3β-hydroxy-5β-hydroxy-B-norcholestane-6β-carboxaldehyde (Secosterol-B) - an autoxidation derivative of cholesterol found in atherosclerotic plaques - affects ER stress in human primary endothelial cells (HUVEC).

Our findings demonstrated that Secosterol-B triggers ER stress by the activation of ATF4 and XB1s. The enlargement of ER membrane and GRP78 relocalization from ER to the cytosol has been shown by electron and confocal microscopy, respectively. Besides, at early time Secosterol-B is able to induce the increase of ROS and eNOS protein expression followed by increase in intracellular NO production. Moreover, the induction of ER stress promoted by Secosterol-B brings an excessive accumulation of misfolded proteins as observed by confocal microscopy.

Taken together, our findings provide new insights into Secosterol-B induced UPR activation and NSS possibly leading to endothelial dysfunction.

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Keywords: endothelial dysfunction; Secosterol-B; nitric oxide; nitrosative stress; ER stress; s-nitrosylation

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Investigating SARS-CoV-2 Infection in Pregnancy: Clinical and Histopathological Analysis

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During pregnancy, the presence of SARS-CoV-2 infection is associated with various negative outcomes, including an increased risk of pre-eclampsia, preterm delivery, hypertensive disorders, gestational diabetes, and fetal growth restriction due to the development of placental vascular abnormalities [1].

In our study, we examined human placenta samples from uncomplicated, full-term pregnancies that were affected by SARS-CoV-2 infection during the first, second, or third trimesters. Using immunohistochemistry, we analyzed the expression of CD34 and podoplanin (PDPN) as markers of vasculogenesis to identify any differences [2]. Additionally, we investigated the correlation between maternal symptoms and histological changes in the placenta, such as fibrin deposits, lymphocyte infiltration in the villi, edema, and thrombi.

Our findings revealed the presence of PDPN expression around the villous stroma, forming a plexiform network around the villous nucleus of fetal vessels. Notably, a significant down-regulation of PDPN expression was observed in the villous stroma of women infected during the third trimester. However, we did not observe any changes in the expression levels of CD34.

Among the common maternal symptoms experienced during SARS-CoV-2 infection were fever, anosmia, ageusia, and asthenia. The majority of patients were treated with medications such as paracetamol, corticosteroids, and azithromycin. Patients requiring multiple symptomatic treatments displayed a substantial amount of fibrin deposition in the villi.

It is evident that PDPN plays a crucial role in promoting healthy placental vasculogenesis and maintaining proper placental physiology. The altered expression of PDPN due to SARS-CoV-2 infection indicates a disruption in normal placental function. Further research is needed to understand the specific mechanisms that are being affected in order to mitigate potential complications for both the mother and fetus, considering that infections will continue to occur.

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Keywords: COVID-19; pregnancy; placenta; immune cells; placenta vascular abnormalities; placental histological alterations

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Effect of antiretroviral therapy on adipocyte differentiation

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The purpose of the study is to investigate how antiretroviral drugs influence the differentiation of adipocytes using an in vitro adipogenesis model. The researchers focused on four integrase inhibitors (DOL, CAB, DOR, RIL) administered individually or in combination, as well as two nucleoside reverse transcriptase inhibitors (NRTIs) (TAF and TDF) in combination with specific integrase inhibitors.

Were used 3T3-L1 cells, a commonly used cell line for studying adipocyte differentiation [1]. The cells were cultured in differentiation medium for eight days [2, 3]. The antiretroviral drugs, at a concentration of 30 μg/ml, were added to the differentiation medium on the first day and administered daily until the fourth day of differentiation [4]. The differentiation of the adipocytes was assessed using Red Oil O staining to evaluate the accumulation of intracellular lipids, and western blotting to measure the expression levels of specific markers of differentiation (PPAR and C/EBP).

Each integrase inhibitor, when used individually, induced adipocyte differentiation. This was evidenced by an increase in lipid droplet formation and up-regulation of PPAR and C/EBP compared to the control group. Moreover, the combination of integrase inhibitors showed a synergistic effect, resulting in enhanced adipocyte differentiation and morphological changes in the cells indicating a shift towards the adipocyte phenotype. When TAF was combined with RIL, was observed an inhibition of adipocyte differentiation. There was a reduction in lipid droplets and down-regulation of PPAR and C/EBP compared to the control group. A similar, but less pronounced, inhibition was observed when TDF was combined with DOR.

In conclusion, the study demonstrated that both integrase inhibitors and nucleotide analogues can significantly induce adipocyte differentiation in 3T3-L1 cells. Combination treatments showed greater effects than single drug treatments, indicating a synergistic effect. Interestingly, TAF acted as an antagonist when combined with RIL and DOR, inhibiting adipocyte differentiation. These findings emphasize the complex effects of antiretroviral drugs on adipose tissue biology and highlight the need for further research to elucidate the underlying mechanisms of these interactions.

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Keywords: antiretroviral therapy; adipocytes; differentiation; NRTIs; PPAR and C/EBP ; lipid droplet

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NGF and its receptors in human Sperm Cells: novel tools for reproductive fitness

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This study aims to evaluate the Nerve Growth Factor (NGF) levels in human semen and study the localization of its receptors (p75NTR and TRKA) on testis, epididymis and ejaculated sperm suggesting a role of this molecule on human male reproductive function.

Semen samples were collected from 21 individuals (31-40 y) subdivided as following: varicocele (n=7); urogenital infected (n=7) and control group (n=7). Semen samples were microbiologically tested, stained for leukocyte identification, and analyzed for the classical parameters: concentration, motility, morphology, and viability (evaluated according to WHO guidelines). The amount of seminal NGF, IL-1β and F_2-Isoprostanes (F_2-IsoPs) was dosed, and the gene (qPCR) and protein (flow cytometry and western blot) expression of NGF receptors on sperm cells were tested. Immunofluorescence analysis of NGF receptors on ejaculated sperm, testis and epididymal tissues was performed.

As expected, control group showed normal parameters compared to infection and varicocele groups. In addition, significant reduced levels of NGF, F_2IsoP and IL-1β were detectable when controls were compared to pathological groups. Notably, in normal sperm, TrKA and p75NTR were both localized in the entire tail, moreover TrKA in the postacrosomal sheath.

Their localization appeared different in altered sperm, in particular a strong signal was detected in the midpiece, presence of cytoplasmic residue or coiled tails.

These results were also confirmed by other techniques. In line with these findings, NGF receptors were both strongly expressed in epididymis and in interstitial tissue of testis.

These preliminary data may indicate an involvement of NGF and its receptors in human sperm physiology and pathology suggesting a role for new targeted therapies.

Keywords: human sperm; neurotrophins; TrKA and p75NTR

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LINC complex as potential mediator in bone sarcomas differentiation

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Changes in lamin levels and nuclear lamina-associated proteins are associated with poor prognosis in several cancers[1]. The interaction between lamins and LINC (linker of nucleoskeleton and cytoskeleton) complex proteins mediates the transmission of mechanical stimuli from the extracellular matrix to the nucleus, resulting in chromatin reorganization and gene expression regulation[2,3]. This interaction modulates epigenetic mechanisms which controls cell differentiation. Several pieces of evidence identified the LINC complex as a crucial factor involved in mesenchymal stem cells (MSC) commitment[2,4], while lamins are already known to have a role in inducing MSC differentiation towards osteogenic lineage[5,6].

For these reasons our aim was to investigate the role of LINC complex in normal and pathological differentiation in bone sarcomas. We already demonstrated that lamin A levels were significantly reduced in osteosarcoma (OS), compared to differentiating osteoblasts[7], and that the overexpression of lamin A pushes Ewing sarcoma cells to a more differentiated phenotype, rescuing LINC complex[8].

Here, we assessed the expression and localization of LINC complex proteins during human osteoblast differentiation, compared to osteosarcoma. We found a significant increase in lamin A expression in both models during differentiation, accompanied by a strong increase in the expression of SUN1, Nesprin 2, and Emerin. Interestingly, these modulations were observed already after 24 hours of differentiation in normal osteoblasts, while in OS cells, the increase of SUN1 and Emerin was evident only after 7 and 14 days of differentiation, with a partial rescue of the LINC complex. The comprehension of how to repristinate physiological nuclear envelope dynamics will be particularly relevant for these tumors, aberrantly stopped in their process of differentiation and where differentiation-based non-toxic strategies are urgently needed.

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Keywords: Lamins; LINC complex; bone sarcomas; cell differentiation

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The sarcoglycan sub-complex in visceral fat depots of obese rats

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Obesity is a pathological condition involving a high percentage of the world population, which often constitutes an important risk factor for cardiovascular pathologies and diabetes. The induction of the browning process has been identified as a promising anti-obesity therapy. By that, the discovery of new and specific markers for transdifferentiation could be useful for anti-obesity treatment. Sarcoglycans are transmembrane glycoproteins which link each other to form the sarcoglycans sub-complex. Sarcoglycans have been mainly studied on muscle tissue but they aren't muscle specific; in particular, their presence has also been highlighted in adipose tissue. Previous data have shown that the absence of sarcoglycans determines insulin resistance and glucose intolerance. Due to the hypothetical role of sarcoglycans in adipose tissue we aimed to investigate the involvement of these proteins in obesity condition. We tested sarcoglycans expression in adipose tissue of obese Male Zucker Crl:ZUC-Leprfa rats before and after treatment with anti-obesity natural drugs products such as lycopene and hydroxycitric acid obtained from Garcinia Cambocia fruit; samples of visceral adipose tissue were treated for molecular and immunohistochemical techniques. Our results show that all sarcoglycans decrease in adipose tissue of obese mice and increase after anti-obesity treatment. These data suggest us that sarcoglycans could play a role as protective markers against obesity.

Istogenesi, funzioni e disfunzioni dell'apparato muscolo-scheletrico

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Sex-specific impact of Bisphenol A exposure in the embryonic cardiac development

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Cardiac development is a complex process, consisting of heart tube formation, looping and septation. Disruption at any point during primary morphogenesis results in a large spectrum of congenital heart defects[1]. Endocrine disruptors (EDs), such as bisphenol A (BPA) pass the placenta and deposit to embryo and fetal tissues, calling for risk assessment of gestational exposures[2].

Given that it is still difficult to establish a causal link between the BPA exposure during pregnancy and potential effects on embryos development, this study focus on the BPA exposure during the highly sensitive cardiac developmental stages, highlighting sex-related disparities, in order to understand if and how sex-based features could advance the study of cardiovascular chemical-induced diseases.

Pregnant C57BL/6 mice were exposed to BPA (4 μg/kg/day) via gavage administration from embryonic day 0.5 (E0.5) to E14.5. Prenatal BPA exposure leads to an increase of embryo and liver weights in both male and female E14.5 embryos compared to the controls (treated with 0.1% EtOH).

Micro-computed tomography (μCT) showed a decreased heart/embryo volume ratio in female BPA-treated embryos. In particular, female embryos BPA-treated displayed a significant reduction of atria volume and an increase of ventricles volume compared to the female controls. Conversely, male embryos did not show any significant difference in morphological heart parameters, except for a 12% increase in interventricular septum (IVS) thickness similar to what observed for female BPA-treated embryos. These sex-related differences were linked to a specific modulation in heart transcriptome and metabolome in response to BPA exposure.

Based on these preliminary results, this research suggests that prenatal exposure to BPA could specifically lead to morphological sex-specific changes during the embryonic heart development and could clarify the toxic effects of BPA on cardiac development during embryogenesis.

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Keywords: Bisphenol A; cardiac development; congenital heart defects

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Spondylarthritis and city lifestyle: a study case

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In 2007, during urban works in Sassari city centre, in front of San Nicola cathedral, was brought to light a medieval cemetery area, with different multiple deposition. This cemetery has undergone anthropological study, to define biological profile and presence of pathologies, at the paleo-anthropological laboratory of the Department of Biomedical Sciences at the University of Sassari.

A recent analysis of the presence of arthritis in those individuals has brought to light a high incidence of spondylarthritis compared to other anatomical regions. This high incidence had some consequences in upper and lower limbs, but just like a higher presence of enthesis.

The situation appears different compared with sites in the same area in previous age when arthritis of limbs was more frequent. In fact, 5% of cervical vertebrae present were pathological, a higher percentage for thoracic (20%) and lumbar vertebrae (27%) in the medieval age, only 2% of cervical and thoracic and 10% of lumbar vertebrae in the previous age; considering individuals present in cemetery areas, 12% of medieval people were pathologic and only 1% of previous age people were. This change in body region can be related to a dissimilar occupational stress, hypothetically related to a change of lifestyle between urban medieval society and previous non-urban societies in the ancient ages.

This hypothesis can be confirmed by analysing anthropological data that comes from another medieval local site, a cemetery area of San Michele, Alghero, where spondylarthritis is anyway incident.

Keywords: Sardinia; medieval age; paleopathology; spondylarthritis

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Myoblast-derived Galectin 3 impairs osteogenesis during the early steps of osteoblast differentiation

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Galectin-3 (Gal-3) is a pleiotropic lectin that regulates multiple cellular processes in a multitude of tissue types. In bone, depending on its cellular localization, Gal-3 has a dual and opposite role: if on the one hand, intracellular Gal-3 promotes bone formation, on the other, its circulating form antagonizes osteoblast differentiation and increases osteoclast activity. In the present study, we demonstrate that Gal-3 is released into the extracellular environment from myoblast cells during differentiation into myotubes. Moreover, by way of a direct co-culture in vitro system simultaneously combining the growth of myoblast and osteoblast cells, we unveil the Gal-3 role as a mediator of muscle-to-bone crosstalk. Proteomic and biochemical analyses revealed that, at the molecular level, secreted Gal-3 modulated the first biochemical processes occurring in the early phases of bone formation, impairing the activity of STAT3, and PDK1/Akt signaling pathways, and at the same time triggering that one of NOTCH1. Circulating Gal-3 was also able to affect the expression of the most common factors involved in bone remodeling processes, including BMP-2, -6 and -7. Intriguingly, Gal-3 was able to interfere with the ability of differentiating osteoblasts to interact with components of the extracellular bone matrix, a crucial condition required for a proper osteoblast differentiation. All in all, suggesting an antagonizing role for Gal-3 in the early phase of bone remodeling in an *in vitro* model, our evidence lay the foundation for further studies aimed at presenting this protein as a novel putative myokine involved in muscle-to-bone crosstalk.

Keywords: Galectin-3; muscle-to-bone crosstalk; myokine; proteomics; Akt; Notch

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Sarcoglycan subcomplex in cartilage tissue: a preliminary study

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The sarcoglycan subcomplex (SGC) is a transmembrane protein system, important in the interaction between the extracellular matrix and cytoskeleton, SGC plays a key role at the cell membrane and is crucial in maintaining sarcolemma viability in muscle fibers. The sarcoglycan subcomplex (SGC) is a system connected laterally with the dystroglycan subcomplex, and is formed by a group of transmembrane glycoproteins, in which six isoforms are recognized: α, β, γ, δ, ε and ζ. The α, β, γ and δ subunits were discovered first, while the ε and ζ were discovered more recently. Many studies have shown that the subunits of this complex were not all present in the various tissues, for example in skeletal muscle and cardiac muscle, the sarcoglycan complex (SGC) is made up of α, β, γ and δ-sarcoglycans, while in ε-SG smooth muscle replaces α-SG (48,21,8). Furthermore, other studies have been able to refute, for example, that the levels of β-, δ- and ε-SG are elevated in the lungs; modest in brain, heart, muscle and skeletal muscle; poorly expressed in the liver and kidneys. The aim of this study was to evaluate for the first time in the literature the presence of sarcoglycans in cartilage tissue. The following methods were used for this study human primary chondrocytes were obtained from Scien Cell™ Research Laboratories (Carlsbad, CA, USA). Cells were cultured in 75 cm^2 plastic flasks containing 15 ml of the specific chondrocyte medium with 2,5% FBS, l-glutamine (2.0 mM), and a mixture of penicillin/streptomycin (100 U/mL, 100 mg/mL). Cells were incubated at 37 °C in humidified air with 5% CO_2. Experiments were performed using chondrocyte cultures between the third and fifth passages. The data coming from the preliminary results confirm what was stated in our previous works, that sarcoglycans are ubiquitous proteins which, based on their localization, play a specific role.

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Defining the role of HIF-1α and MMP-9 during skeletal myoblast differentiation

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Although the hypoxia-inducible factor (HIF)-1α has been highlighted as a regulator of the functionality of the resident skeletal muscle stem cells namely satellite cells (SCs), its spatio-temporal expression and its role in SCs undergoing myogenic differentiation remain controversial. HIF-1α represents the oxygen-sensitive subunit of the transcriptional factor HIF, that is usually degraded in normoxia and stabilized in hypoxia to translocate to the nucleus and regulate its target gene expression. However, in SCs an oxygen level-independent regulation of HIF-1α expression and activation has been observed, thus suggesting a role of such protein in skeletal muscle physiological functions and homeostasis. Here we aimed to further investigate the role of HIF-1α in murine C2C12 myoblasts and primary SCs undergoing myogenic differentiation under normoxia conditions. Furthermore, based on the reported role of metalloproteinase (MMP)-9 in myogenesis, we evaluated MMP-9 as an HIF-1α downstream effector. We first showed that the cells underwent metabolic reprogramming during differentiation: proliferating myoblasts mainly depends on glycolytic energy production whereas myotubes show a superior aerobic capacity. We found that: i) HIF-1α expression synchronized with that of MMP-9 and of the myogenic activation marker MyoD, increasing after 24 h of differentiation, ii) MMP-9 was a target of HIF-1α and iii) HIF-1α/MMP-9 axis was required for myoblast myogenic commitment, as judged by gene silencing experiments and morphological, biochemical and electrophysiological analyses. In conclusion, we showed HIF-1α/MMP-9 axis involvement in the early phases of skeletal myoblast differentiation.

Keywords: HIF-1α; myoblast differentiation; MMP-9; normoxia; satellite cells

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Inhibiting RAGE (receptor for advanced glycation end-products) as a tool to counteract cancer-associated cachexia

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Cancer cachexia is a paraneoplastic syndrome characterized by skeletal muscle atrophy leading to pronounced weight loss and poor prognosis, and responsible for about 20% of all cancer-associated deaths. Efficacious treatments for cancer cachexia are still lacking. We demonstrated that, in cancer conditions, the overexpression and chronic activation of RAGE (receptor for advanced glycation end-products) sustain all hallmarks of cachexia. Genetic ablation of RAGE (*Ager*$^{-/-}$ mice) translates into reduced serum levels of proinflammatory cytokines, delayed loss of muscle mass and strength, reduced tumor progression, and increased animal survival after tumor injection. Thus, RAGE appears as a potential molecular target to counteract cancer-induced cachexia. We injected Lewis lung carcinoma (LLC) cells in C57Bl/6 mice and treated them with intraperitoneal injections of the RAGE inhibitors, FPS-ZM1, Azeliragon (TTP488), RAP (RAGE antagonist peptide) or papaverine, or the S100B (a RAGE ligand) inhibitor, pentamidine, from the day of tumor appearance until 25 days post-injection. We found that only papaverine was able to strongly prolong animal survival and reduce the loss of body and muscle weights, muscle protein degradation, and activation of the proteolytic systems in LLC-bearing mice. Conversely, Azeliragon and pentamidine showed toxic effects, inducing premature death in tumor-bearing mice; and, FPS-ZM1 and RAP did not show significant effects in terms of protection against muscle wasting. Thus, inhibiting RAGE with the use of papaverine appears as a promising pharmacological strategy for anti-cachectic purposes.

Keywords: cachexia; muscle wasting; RAGE; papaverine; RAGE inhibition

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Skeletal muscle extracellular matrix is remodeled by physical training in a murine model of Down syndrome

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Down syndrome (DS) is a genetically based disease caused by triplication of chromosome 21. DS, which affects approximately 1 in 700 newborns in humans, is characterized by multi-system premature aging associated with severe muscle weakness, deficits in motor coordination, balance and postural control.

The extracellular matrix (ECM) is a dynamic compartment that structurally and functionally supports the myofibers of skeletal muscle. The ECM is involved in muscle development, growth/repair and transmission of contractile force. Therefore, alterations in the normal remodeling of the ECM can have an impact on the functional properties of skeletal muscle.

Using a morphological, morphometrical and immunocytochemical ultrastructural approach, the study investigated the effect of adapted physical training on the ECM characteristics of vastus lateralis muscle of Ts65Dn mouse, a murine model of DS, and whether the forecasted exercise-induced ECM remodeling impacts on sarcomere organization, Z-lines alignment and distribution/abundancy of telethonin (Z-line molecule that anchors titin and maintains sarcomere architecture). Ultrastructural morphometry and immunocytochemistry demonstrated some interesting parallels between trisomy and aging: trisomic sedentary mice showed a thicker basement membrane, larger collagen bundles with increased interfibrillar spacing, as well as irregular registration of sarcomeres and lower telethonin density on Z-lines than their sedentary euploid counterparts. The ECM was remodeled by adapted physical training in both trisomic and euploid mice: collagen bundles enlarged in association with hypertrophy of collagen fibrils and reduced interfibrillar spacing. Moreover, in trisomic mice the sarcomeres realigned and telethonin density increased at the Z-line.

Altogether the findings suggest that physical training may be an effective tool to limit and counteract musculoskeletal anomalies associated with trisomy, and provide a solid experimental background for further study on the possible positive effect of physical training on skeletal muscle performance.

Keywords: skeletal muscle; extracellular matrix; trisomy; aging; transmission electron microscopy

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Characterization of PLC gene expression and intracellular localization in human osteoblasts cultured with and without U73122 inhibitor

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Phosphoinositide-specific phospholipase C (PLC) family groups enzymes that hydrolyze the head group of inositol phospholipids producing two intracellular messengers: inositol-1,4,5-triphospate (IP_3) and diacylglycerol (DAG). Thirteen PLCs are known in mammals, categorized in six isotypes (β, γ, δ, ε, ζ, and η) depending on similar structural characteristics [1]. PLCs usually localize at the cytoplasmic membrane due to the activity of phospholipid hydrolysis. PLCs can also be found within the nucleus, in podosome-like structures and in vesicles [2, 3]. Much scientific evidence suggested that PLC enzymes are involved in human osteoblasts (hOb) differentiation, especially PLC-β1 [4].

In the present study, in order to modulate the PLCs, U73122 aminosteroid was used as a specific molecular inhibitor of PLCs [5]. U73122 bears several off-target effects, as it is involved in several signaling pathways not related to PLCs, such as the extracellular ions afflux or the estrogenic effect, due to the structural similarity of U73122 compared to 17β-estradiol. U-73122 off-target also acts upon the regulation of *PLC* genes' expression, downregulating or upregulating genes depending on the isotype and on the type of tumor, with special regard to human osteosarcoma [6,7].

In order to investigate the effects of U-73122 upon hObs' differentiation, cells were treated with low dose U73122 (10μM), and *PLC* genes' expression and PLCs' intracellular localization were investigated.

Our results confirmed literature data excluding cell toxicity of U73122, and indicated no difference in hObs growth after 10μM U73122 exposure. We observed that hObs expressed all the *PLC* genes, with the exception of *PLCD3* both in freshly thawed and cultured cells. Apparently, U73122 had no ON/OFF effects upon the transcription of *PLC* genes, and affected the intracellular localization of PLC enzymes instead, as PLCs were observed in cell protrusions derived from plasma membranes, and in the endoplasmic reticulum.

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Multiomic deconstruction of (teno)synovial tissue: application of innovative technologies for the prevention of rheumatoid arthritis

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Individuals at risk of developing rheumatoid arthritis (RA) can be identified preclinically by the presence of autoantibodies and a combination of symptoms, while clinical arthritis is still absent (clinically suspected arthralgia, CSA). The presence of subclinical inflammation at the (teno)synovial level is an independent risk factor for the development of RA, but to date no data are available on the cellular and molecular composition of the (teno)synovium of CSA who will develop RA. Only very limited studies have investigated the synovial tissue characteristics of individuals with CSA that showed no evidence of histologically proven synovial inflammation. This study was designed using an innovative methodological approach combining different spatial transcriptomics and multifunctional imaging technologies. Minimally invasive ultrasound-guided procedure was developed to retrieve good quality samples to be used in translational studies and will be used to collect (teno)synovial tissue from patients with CSA and treatment-naïve RA patients. We intend to create a comprehensive cellular and molecular atlas of (teno)synovium in different clinical stages of RA aiming to identify novel potential biomarkers for treatment decision-making in the management of RA pathogenesis[1,2]. Furthermore, the use of novel *in vitro* migration assays will dissect how adhesion molecules, such as certain isoforms of CD44[3-4], are involved in tissue trafficking of immune cells infiltrating (teno)synovial tissue in the preclinical phase of RA. The aim of this methodological approach is to (i) generate a comprehensive cellular and molecular atlas of the (teno)synovium and blood of CSA individuals at risk of developing RA and (ii) discover molecular targets for the development of future therapies to counteract the pathogenic processes leading to chronic inflammation, in the context of the precision medicine.

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Keywords: Synovium; Individuals at risk of developing rheumatoid arthritis; spatial transcriptomic and multifunctional imaging

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Pro-differentiating effects of red photobiomodulation on skeletal myoblasts: morpho-functional evidences

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Adult skeletal muscle possesses the ability to regenerate lost damaged tissue mainly thanks to the activity of a small population of resident stem cells, namely satellite cells (SCs). In the case of chronic or severe damage, the functionality of SCs may be compromised by the occurrence of an aberrant fibrotic reparative response. Strategies aimed to improve the muscle intrinsic regenerative capacity while limiting the excessive deposition of fibrotic tissue may be promising. In this perspective, photobiomodulation (PBM) (*i. e.* application of light with 400-1100 nm wavelength using different laser or LED devices, power density less than 100 mW/cm^2 and energy density less than 10 J/ cm^2 at target) may represent a valid option based on its well-known pro-regenerative effects and increasing evidence of its antifibrotic potential. However, results on the PBM actual benefits on skeletal muscle are controversial and light-tissue interactions need to be better elucidated.

To this aim we evaluated the effects of a treatment of red PBM (laser diode 635±5 nm, energy density: 0.4, 4 and 8 J/cm^2, single exposure) on myoblastic cells undergoing differentiation and on differentiated cells (myotubes). Preliminary results obtained by morphological analyses (cell viability, myogenic markers, myotubes) and electrophysiological investigations (cell membrane passive properties and ion currents) suggest the capability of red PBM to positively affect myoblast differentiation. At the same time, PBM treatment did not alter myotube viability and dimension. Experiments are ongoing to explore the effects of red PBM on mitochondria and on myoblasts induced to differentiate on a liquid crystalline network used as a cell instructive scaffold to support a correct myogenic differentiation.

Keywords: skeletal muscle; satellite cell; photobiomodulation; myoblast differentiation

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V. macrocarpon prevents Western diet- and cancer-dependent detrimental effects in muscles by reducing advanced glycation end-products (AGEs) *in vitro*

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Advanced glycation end-products (AGEs) are non-enzymatic adducts, especially glycosylated proteins, endogenously formed in oxidative stress and aging conditions, or introduced with the diet (dAGEs) by consumption of ultra-processed and high sugar/fat foods typical of Western diet (WD). AGEs damage tissues by altering proteins' function or by interacting with their receptor, RAGE, leading to generation of reactive oxygen species and amplification of inflammation. AGEs/RAGE signaling sustains the progression of many diseases. High levels of AGEs have been associated with muscle wasting (MW) associated with aging and several human diseases, including cancer (cachexia). Here, we report that the consumption of WD containing high dAGEs induces MW in adult mice. Indeed, muscles of WD- *vs* standard diet (SD)-fed mice showed: i) a significant weight reduction concomitantly with high presence of atrophic myofibers; and ii) upregulation of RAGE and the ubiquitin ligase, atrogin-1. In accordance, the principal endogenous AGE, AGE-bovine serum albumin (BSA), and the exogenous AGE, methylglyoxal (MG) induced myotube atrophy *in vitro*. *Vaccinium macrocarpon* (*VM*) standardized dry extract: i) dramatically counteracted AGE formation; ii) increased myotube size and viability; iii) counteracted the reduction of myotube diameter and MyHC-II degradation induced by AGE-BSA or MG; iv) preserved myotube trophism in the presence of pro-inflammatory cytokines (TNF-α), or cachectic factors secreted by Lewis lung carcinoma (LLC) cells, as *in vitro* models of cachexia, by reducing AGEs accumulation. Collectively, these data suggest that *VM* has the property of blunting dAGE formation/activity and might be used as a food supplement to prevent WD-dependent detrimental effects in muscles, also in cancer conditions.

Keywords: AGEs; Western diet; muscle wasting; food supplement

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Equisetum arvense standardized extract delays the onset of osteosarcopenia in geriatric mice

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The age-associated osteosarcopenia (OS) is an emerging geriatric giant syndrome characterized by concomitant deterioration of bone (osteopenia) and skeletal muscle (sarcopenia) tissues increasing the risk of falls, fragility and mortality. A chronic low-grade inflammatory state in aging is a determinant common pathogenetic factor for the progression of sarcopenia and osteoporosis.

An unbalanced bone remodeling process, in which excessive resorption is followed by inadequate deposition of new material, is the main cause of bone microarchitecture deterioration in osteoporosis. Excessive protein breakdown and reduced protein synthesis, especially type II myosin heavy chain (MyHC-II), together with myofiber shift towards oxidative type, are mainly responsible for loss of muscle mass and strength in sarcopenia. *Equisetum arvense* (*EQ*) is traditionally recommended for treating many pathological conditions, due to its anti-inflammatory effects. We administered for 3 months a standardized dry extract of *EQ* in pregeriatric (21-month-old) C57BL/6 WT mice, as an animal model of OS. We found that during aging *EQ*: i) preserves muscle performance and mass, myofiber area, and MyHC-II expression; ii) restrained oxidative shift of myofibers, and muscle inflammation; iii) reduced loss of bone volume and surface, trabecular thickness and connectivity density; and iv) maintains microarchitecture and reduces the levels of proinflammatory cytokines in the spleen. Collectively, our data identify *EQ* as the first natural compound able to preserve both muscle functionality and bone microarchitecture and metabolism during aging by restraining the inflammatory state in muscle and spleen. Thus, EQ might represent a potential intervention for OS, which is an unresolved and urgent health problem due to the growing life expectancy.

Keywords: Osteosarcopenia; muscle atrophy; osteopenia; natural compounds

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Granules *versus* scaffolds: which bioactive glass arrangement can express the highest angiogenic potential *in ovo* for bone regeneration?

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In the last years, the progressive aging of population and the increasing incidence of musculoskeletal diseases has led to solutions for overcoming the limitations of autologous transplantation and bone substitutes. One of the main concerns in the clinical scenario is the disruption of vascularization following critical size fractures that causes the inability to repair spontaneously. In fact, a functional vascular network is a necessary pre-requisite to allow the regenerative processes, particularly osteogenesis. Bioactive glasses (BGs) are a class of synthetic materials that exhibit outstanding properties, such as osteoconductive abilities[1], and anti-bacterial activity. In particular, the 45S5 Bioglass® had stood for its capacity to form a chemical bond with bone, while stimulating osteoprogenitor cells at genetic level. However, 45S5 has the tendency to crystallize during the thermal treatments that are necessary to the fabrication of porous scaffolds for regenerative medicine applications[2]. To overcome this challenge, two novel BGs, namely BGMS10 and Bio_MS, were designed by adding strontium and magnesium. These BGs had demonstrated osteogenic abilities, supporting bone cell adhesion, colonization and differentiation *in vitro* better than 45S5[3,4]. In this study, BGMS10 and Bio_MS, together with 45S5, were tested for their angiogenic potential by means of the Chorio-Allantoic Membrane (CAM) assay. This simple and versatile model, simulating the *in vivo* conditions but in compliance with the 3Rs' principle for animal experimentation (Replacement, Reduction, Refinement)[5], acts as a natural bioreactor that could rapidly predict the angiogenic potential of the grafted materials. The BGs, both in the form of granules and scaffolds, were evaluated at a morphological level (count of the neo-formed vessels and histological evaluations) and molecular level (expression of genes that have a role in angiogenesis): preliminary observations show that granules seem to be more angiogenic than their relative scaffolds.

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Keywords: Bioactive glasses; therapeutic ions; CAM assay; vascularization; bone regeneration

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Histotopographic characterization of the suprapatellar fat pad

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The suprapatellar fat pad (SFP) is an adipose tissue of the anterior knee with a debated role in osteoarthritis (OA). As anatomy drives the function, the aim of this histotopographic study was to elucidate the SFP morphological characteristics *versus* the IFP in absence of OA, for a comparative analysis. Human SFP and IFP tissues underwent microscopical/immunohistochemical staining and transmission microscopy analysis (TEM); thus, vessels and nerve endings, lobuli, adipocytes features, septa, extracellular matrix proteins were focused. Multiphoton microscopy was also adopted to determine collagen fibers orientation. The absence of inflammation was confirmed, and comparable counted vessels/nerve endings were shown. Like the IFP, the SFP appeared as a white adipose tissue with lobuli and septa similar in diameter and thickness, respectively. Tissue main characteristics were also proved by semithin sections and TEM analysis. The SFP showed roundish adipocytes with a smaller area/perimeter/major axis than that of the IFP; the collagen fibers surrounding them showed no significant difference in collagen type I and significantly higher values for collagen type III in the IFP group. As for the septa, elastic fibers were statistically comparable between the two groups, even though more represented by the SFP. Total collagen was significantly higher in the IFP and comparing collagen type I and type III they were similarly represented in the whole cohort, despite collagen type I appeared to be higher in the IFP than in the SFP and vice versa for collagen type III. SHG microscopy and coherency calculation assessed an anisotropic distribution of septa collagen fibers. From a mechanical perspective, the different morphological compositions determine a major stiffness of IFP *versus* the SFP. This study provides, for the first time, a SFP topographic description *versus* the IFP; differences between the two groups may be ascribed to a different anatomical location within the knee.

Keywords: knee; suprapatellar fat pad; infrapatellar fat pad; microscopic anatomy; adipocytes; extracellular matrix; morphometry

Tecnologie innovative, modelli 3D e organoidi per lo studio di patologie e drug discovery

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Single-cell multi-omics characterization of Natural Killer lymphocytes in COVID-19 patients: a clinical case series

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COVID-19 pandemic caused by the Severe Acute Respiratory Syndrome-Coronavirus-2 (Sars-Cov-2 virus) emerged in late 2019 as the biggest global health emergency in recent decades. Patient response to Sars-Cov-2 infection is very diversified and depends on a series of factors (age, gender, the co-presence of cardiovascular pathologies, co-morbidities such as diabetes and obesity). Current immunological research is still trying to understand the reasons for this strong diversification of patient reaction and a key role in the response to infection is thought to be played by the cells of the innate immune system like Natural Killer (NK) lymphocytes. Based on that, this study performed a single-cell multi-omics analysis of transcripts and proteins of NK lymphocytes in COVID-19 patients, for the characterization of the innate immunological response to infection.

NK cells were isolated from peripheral blood samples collected from adult subjects divided into 3 study groups: 1) non-infected subjects (*Naïve group*, n=3), 2) post COVID-19 convalescent subjects (*Healed group*, n=3) and 3) patients that were vaccinated against Sars-Cov-2 (*Vaccine group*, n=3). Cells were then analysed by the BD Rhapsody System for the single-cell multi-omics investigation of membrane proteins and transcriptome. The bioinformatic analysis identified 5 cell clusters which differentially expressed gene/protein markers which defined NK cell subsets as "Active NK cells" and "Mature NK cells". Calculating the relative proportion of each cluster within patient groups, more than 40% of the *Naïve group* cell population was found to belong to Mature NKs, whereas more than 75% of the *Vaccine group* cell population belongs to the cluster of Active NKs. Many differentially expressed genes (DEGs) were detected comparing the profile of the 3 patient groups, suggesting the possibility to identify novel biomarkers for clinical-diagnostic use of non-specific resistance to Sars-Cov-2 infection and/or re-infection.

Keywords: innate immune response; Natural Killer lymphocytes; COVID-19; multi-omics analysis; Rhapsody System

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A fluid dynamic system to test different kinds of compounds on human skin explants

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The study of biological barriers is always of great importance in various research fields: in the study of the impact of pollutants or in the assessment of the bioavailability of compounds for both pharmaceutical and cosmetic use. Of great importance is the morpho-functional characterization of biological barriers in relation to their function and possible pathological alterations. Especially in the biomedical field, the design, synthesis and production of new formulations is focused on delivering drugs to their site of action more efficiently, increasing their bioavailability and bypassing biological barriers, such as the skin. In this study, the ability of different types of compounds to penetrate human skin was tested by exploiting an in vitro model using human skin samples immediately after explantation and incubated in an innovative fluid-dynamic system[1,2]. Different types of compounds were evaluated such as a particular kind of nanovesicles, already characterised [3,4,5] (ethosomes and transethosomes, phosphatidylcholine and polysorbate-based nanoconstructs with average diameters of 100-350 nm) or different formulations of hyaluronic acid. Each compound was applied topically to human skin explants kept in a bioreactor for up to 24, 48 and or 72 hours, a time frame in which tissue integrity is optimally preserved[1,2]. The effectiveness of penetration into the deep layers of the skin was tested with bright-field microscopy and scanning and transmission electron microscope analysis. The results showed that all the different components were able to penetrate the skin while preserving its structural integrity, but reaching different levels of depth. The ability to reach the different skin layers depends essentially on the physico-chemical characteristics of each compound. Therefore, our dynamic skin model not only demonstrated its suitability to act as a biological barrier in vitro, but provided original insights into the efficacy of different types of formulations for topical administration.

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Keywords: fluid dynamic bioreactor; human skin explants; TEM; SEM

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Nanotechnological approach for the treatment of myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is a genetic disorder characterized by a progressive dysfunction of multiple organs and tissues, one of the most severely affected tissues being skeletal muscle. Currently no therapies are available and the treatments are administered to only manage the symptoms. Pharmaceutical research is evaluating the capability of small molecules, such as pentamidine (PTM), to mitigate the pathological hallmarks of DM1. However, despite its promising therapeutic effect, PTM has limited applicability due to its high systemic toxicity. To overcome this limitation, hyaluronic acid-based polymeric nanoparticles (HA-NP) have been developed [1], and their features and effects have been evaluated by microscopy techniques. Morphological analyses performed through transmission electron microscopy (TEM) and cryoelectron microscopy (CryoTEM) showed the rounded shape and size (around 200 nm) of HA-NPs. TEM analysis demonstrated that HA-NPs were rapidly internalized into cultured C2C12 muscle cells via endocytosis, then they escaped endosomes and occurred free in the cytosol without causing cell organelle damage or alteration. Moreover, conventional fluorescence microscopy proved that HA-NPs loaded with PMT are able to reduce nuclear foci (a hallmark of DM1) in C2C12 cells transfected with altered human genes responsible for this dystrophy. Conventional and confocal microscopy were also used to visualize the biodistribution and targeting efficacy of NPs injected into explanted murine skeletal muscles, showing an efficient NPs internalization within skeletal muscle fibers at both short and long incubation time. Furthermore, NPs were found to be homogeneously distributed into the entire muscle suggesting that NPs were able to move into myofibers [2]. The results obtained from this work underline the potential of the nanotechnological approach to mitigate the DM1 pathology.

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Keywords: Drug delivery systems; hyaluronic acid, pentamidine; C2C12 cells; explanted skeletal muscles; muscular dystrophy type 1

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The impact of simulated microgravity on gene expression profiling of keratinocyte cells

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The process of skin wound healing after damages appears to be a complex biological process, which involves the interaction of cells, extracellular matrix and growth factors. It has been demonstrated that simulated microgravity impaired the process of skin regeneration after injury and for this reason astronauts injured in space have a harder time healing [1]. Recent advancement in the field of molecular biology has demonstrated that small non-coding microRNA (miRNA) can have a broad effect on gene expression networks and play a key role in cellular response to environmental stresses [2]. For this reason, we tried to analyse how the simulated microgravity could perturbate the expression of some miRNAs and genes involved in the process of wound healing. Simulation of μg was achieved by a rotary cell culture system (RCCS), in collaboration with ASA Campus (Florence, Italy). A human cell line of keratinocytes (HACAT cells) was exposed to modeled μg for 24 and 72h and the miRNAs analysed were: miRNA 21-5p (involved especially in re-epithelialization), miRNA 31-5p (it acts as an inhibitor of proliferation and migration of keratinocytes) and miRNA 203a (most expressed in the skin). In addition to miRNA previously described, we also performed qRT-PCR on CK1 and CK10 genes, the prominent suprabasal skin differentiation markers. The expression of miRNAs and keratin genes were strongly influenced by simulated μg, in particular at 72h there is a significant up-regulation of miRNA 21-5p and miRNA 203a, and a down-regulation of miRNA 31-5p with respect to HACAT cells exposed for only 24h. Regarding CK1 and CK10 genes, the expression was significantly reduced in HACAT cells exposed to simulated μg for 24 and 72h. Based on these results, we can conclude that simulated microgravity by RCCS can significantly affect the expression of stress-related miRNAs in HACAT cells and of genes involved in cutaneous wound re-epithelialization.

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Keywords: simulated microgravity; wound healing; keratinocytes; miRNAs; gene profiling

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Digital workflow for colour normalization of histological specimens

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The rapid technological progress in the field of image analysis has inspired significant changes in multiple fields of medicine. The evolution and widespread availability of slide scanners capable of producing digitized images of entire histological slides (WSI, whole slide images) now allow the visualization and analysis of histological slides using digital systems, comparable to conventional microscopy. In many contexts, traditional workflows have been enhanced with digital tools and software that facilitate the visualization and analysis of WSI. A crucial step for the effective use of digital slides is colour normalization. Various methods for colour normalization exist, and the method employed in this study is the one proposed by Macenko et al. [1], applied to histological sections of liver tissue stained with haematoxylin-eosin using different staining and mounting protocols, resulting in significantly different colour rendering.

The transition to digital offers tremendous possibilities for pathologists, but also presents new requirements, such as workflow standardization and colour normalization. We propose a workflow that leverages the tools of digital image analysis to establish easily reproducible staining standards that simplify the visualization and interpretation of histological and histopathological preparations.

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Keywords: Colour; normalization; Macenko; WSI; Digital histology

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Digital pre-analysis: quality control of digitised slides using artificial intelligence models

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The preparation of histological specimens follows well-established criteria that have been used for decades, including preparations to distinguish the structures they consist of and benefit the specialist in the evaluation traditionally linked to observation with optical microscopes. Recently, the possibility of digitising histological slides, using appropriately designed scanners, is gaining ground, transforming them into so-called virtual slides or WSI (whole slide images). This innovation, in addition to allowing a more practical visualisation of histological preparations, introduces the possibility of performing computational analyses using tools that help the morphologist maximise the information that can be extracted from both histological and histopathological slides. This new use requires us to investigate the adequacy of traditional technical protocols also in the digital environment.

In the present work, we evaluated the yield of liver tissue sections stained with haematoxylin/eosin and mounted with different mounts, following distinctive protocols.

The slides were digitised with an Aperio AT2 scanner using the same protocol, after which the WSIs were subjected to qualitative analysis using artificial intelligence models designed for this purpose.

The tool used was HistoQC, an open-source tool that performs a quality check not only to identify and delineate artefacts but also to discover outliers at the cohort level, e.g. slides coloured darker or lighter than others in the cohort, focus defects, etc. The system returns a score, based on dozens of features, indicative of the overall quality of the preparation and its usability in the computational domain. [1].

The enormous potential that the conversion to digital offers in the field of microscopic morphological analysis must be confronted with an increase in quality standards. We propose a workflow that allows for serial and automatic quality control to identify inadequate preparations at an early stage.

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Keywords: WSI; AI; HistoQC; Andrew; Janowczyk; Digital pathology

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The Application of a Fluoride-and-Vitamin D Solution to Deciduous Teeth Promotes Formation of Persistent Mineral Crystals: A Morphological *Ex-Vivo* Study

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The use of effective, low-cost, and easy-to-use products for early caries management will avoid loss of dental vitality and impairment in oral function. The ability of fluoride to re-mineralize dental surfaces has been widely reported as well as vitamin D demonstrated to have significant potential in improving the remineralization of early lesions on enamel surfaces(1). The aim of the present ex vivo study was to evaluate the effect of a fluoride and vitamin D solution in terms of formation of mineral crystals on the enamel of primary teeth, and their permanence over time on dental surfaces. Methods: Sixteen extracted deciduous teeth were cut to obtain 64 specimens that were divided into two groups. The first consisted of immersion of specimens for 4 days in a fluoride solution (T1); in the second group, the specimens were immersed for 4 days (T1) in fluoride and Vitamin D solution, and for a further 2 (T2) and 4 days (T3) in saline solution. Then, samples were morphologically analyzed by using Variable Pressure Scanning Electron Microscope (VPSEM) and underwent 3D surface reconstruction(2). Results: After a 4-day immersion in both solutions, octahedral-shaped crystals were formed on the enamel surface of primary teeth, demonstrating any statistically significant differences in terms of number, size, and shape. Moreover, the binding of the same crystals seemed to be strong enough to be maintained until 4 days in saline solution. However, a partial dissolution was observed in a time-dependent manner. Conclusions: A topical application of fluoride and Vitamin D promoted the formation of persistent mineral crystals on enamel surfaces of deciduous teeth and should be further studied to be potentially used as an alternative strategy in preventive dentistry.

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Keywords: primary teeth enamel; dental caries; remineralization; vitamin D; surface analysis; VPSEM

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Crosstalk among neurons, muscle and bone: *in vitro* model of triple culture

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Studying cell interactions in healthy and pathological conditions occurring in musculoskeletal apparatus requires to set-up a new optimized *in vitro* model allowing the isolation of cellular compartments for region-specific analyses. Indeed, the molecular mechanisms that cause both muscle and bone loss, namely osteosarcopenia, are still unclear, and how skeletal muscle cells send retrograde signals to motor neurons (MNs) represents an intriguing field of research.

With the purpose to study the perturbation in neuro-muscle junctions (NMJs) occurring in muscle atrophy, beside bone side defects, an ideal model would contain MNs, myotubes and osteoblasts to better recapitulate the human disease pathology.

Thus, we developed a three culture system exploiting the use of well-known transwell supports, in which osteoblasts could be treated separately from muscle and neuron cells. In this model, precursors of osteoblasts (lower side) were differentiated and then could be induced to osteoporosis with a pharmacological treatment before the insert, carrying both myotubes and neurons, will be put in co-culture into the well. In fact, the insert (put upside down) can be first seeded with a neuroblastoma cell line, then the myoblasts will be put on the other sheet and then a common differentiation medium will be added. Indeed, the first aim of this study was to find a common differentiation medium for both MNs and myotubes and we demonstrated that a medium containing retinoic acid (RA 1μM) and horse serum (HS 2%) is able to drive an efficient differentiation of both cell types.

This model is useful since it allows us to perform several analyses. The conditioned medium can be collected to analyze the modifications in the secretome produced by the cells. Aside from the biochemical interaction, additional modifications in morphology can be explored. All the cell samples can be analyzed with histological stating or immunofluorescence analysis: the osteoblast part are seeded onto coverslips, while the muscle-nervous system can be treated to be included and cut in slices in order to analyze the neurites passing through the transwell net (3 μm diameter) and contacting myotubes. Preliminary experiments demonstrated that the neurites can reach the myotubes passing through the membrane of the insert, recapitulating their physiological interaction.

Collectively, this method will be useful to understand if the modifications induced in osteoblasts during bone disorders have a cascade in the muscle and neuron parts. This system mimics the events occurring during aging, such as osteoporosis arising in females after menopause that leads to muscle weakness and it will also be applicable to identify prospective targets for future interventions.

Keywords: neurons; bone; muscle; osteoporosis

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Evaluation of the combined inhibition of alternative splicing and EZH2 activity as pro-immunogenic treatment for triple-negative breast cancers

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Triple-negative breast cancer (TNBC) is a heterogeneous and highly aggressive breast cancer subtype, for which surgery and chemotherapy remain to date the standard of therapy[1]. Being a high degree of tumor-infiltrating lymphocytes a predictive marker of good prognosis and of lower risk of relapse for TNBC patients, therapies enhancing tumoral immunogenicity represent a promising therapeutic approach for this disease[2].

Neo-antigens expression is a major determinant of tumoral immunogenicity and aberrantly spliced transcripts contribute to their generation. This process can be pharmacologically enhanced by treatment with splicing inhibitors, such as indisulam, which acts by inducing the degradation of the splicing factor RBM39 via DCAF15[3]. Neo-antigens are presented by cancer cells via the MHC-I proteins, whose downregulation is a common mechanism of immune-evasion. In immune desert TNBC, downregulation of MHC-I genes is due to the epigenetic repression operated by EZH2, whose pharmacological inhibition was recently shown to promote an anti-tumoral immune response[4]. Interestingly, analysis of transcriptomic data from TCGA revealed that DCAF15 and EZH2 are highly expressed in TNBCs, suggesting that combining indisulam treatment with EZH2 inhibition might be a promising immune-enhancing therapy. By performing RNA-sequencing of a representative TNBC cell line, MDA-MB-231, we identified several splicing-derived transcripts elicited by indisulam treatment and encoding for potential neoantigens with a high MHC-I affinity. Experiments on both TNBC cells lines and patients derived organoids showed that induction of selected neoantigens is maintained in a combined treatment with indisulam and the EZH2 inhibitor tazemetostat, which concomitantly increases MHC-I proteins expression. Collectively, our observations hint at the combination of alternative splicing and EZH2 inhibition as a putative therapeutic strategy to improve TNBC immunogenicity.

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Keywords: triple-negative breast cancers; patients-derived organoids; alternative splicing; neo-antigens; cancer immune-response

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Computer-Aided Diagnosis Systems for Early Detection of Alzheimer's Disease

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Alzheimer's disease (AD) is its most common type of adult-onset dementia disorder. Although at the present no disease-modifying agents capable of reversing the pathological changes occurring in AD are available, early detection can contribute to delay worsening of the disease and the complete loss of autonomy of patients.

Cognitive neuropsychological tests are the conventional approach to detect AD because they are fast and not expensive. However, these tests have no predictive value, because cognitive impairment develops when brain damage is advanced and becomes irreversible.

Morphological (magnetic resonance imaging, MRI) and liquoral biomarkers can contribute to a more precise and accurate diagnosis of AD, earlier than that obtainable with neurocognitive tests. Computer-aided diagnosis (CAD) systems have emerged as valuable tools for the early detection of AD, leveraging advanced imaging techniques and machine learning algorithms.

In this work a comprehensive analysis of CAD systems designed for the early detection of AD is presented. These approaches can be applied to various imaging modalities, including structural magnetic resonance imaging (MRI), functional MRI (fMRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT).

Our analysis has considered different machine learning algorithms employed in CAD systems, including support vector machines (SVM), random forests (RF), convolutional neural networks (CNN), and ensemble boosting algorithms such as AdaBoost and XG Boost. These algorithms enable the extraction of meaningful biomarkers from imaging data and the development of predictive models for early AD detection. Among the considered algorithms, ensemble boosting algorithms demonstrated the highest accuracy in AD detection. These algorithms combine the predictions of multiple weak classifiers to improve overall performance and achieve superior diagnostic accuracy.

Early detection of AD plays a crucial role in providing timely interventions and improving patient outcomes.

The use on a large scale of the most efficient CAD systems can contribute to an early and accurate diagnosis of AD with the aim of establishing as soon as possible specific treatments to counter or delay the progression of the disease.

Keywords: Alzheimer's disease; Early diagnosis; Ensemble ML algorithms; CAD; Supervised ML modelling

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A new method for oral cancer biomarkers detection with a non-invasive cyto-salivary sampling and rapid-highly sensitive ELISA immunoassay: a pilot study in humans

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Oral squamous cell carcinoma (OSCC) represents approximately 90% of oral tumors and has a five-year mortality rate close to 50%. A significant portion (70%) of all oral tumors is diagnosed at an advanced stage due to the inefficiency of available screening techniques. The standard diagnostic reference is tissue biopsy with histological and immunohistochemical evaluation. However, this method has some limitations: 1) it is an invasive and semi-quantitative examination, 2) highly processed tissues may lead to information loss.

The search for biomarkers on tissue/cell fragments collected with a cytobrush is a highly promising technique for the diagnosis and early detection of OSCC, thanks to its non-invasive sampling and simple collection method. In this study, we analyzed tissue samples obtained with a cytobrush, collected from the oral cavity of 15 patients with already diagnosed OSCC, applying an innovative high-sensitivity ELISA technique. To this end, we selected six biomarkers, already used in clinical practice for the diagnosis of OSCC, or selected based on recent scientific and clinical data indicating their presence or overexpression in transforming cells and their role as potential molecular targets in immune checkpoint blockade therapies.

The selected biomarkers were found to be highly expressed in the tumor core, while they were virtually negative in the healthy tissues of the same patients. These differences were statistically significant and consistent with those obtained using the reference test. These preliminary data suggest that the proposed approach could be useful as a diagnostic and screening tool for the characterization of oral lesions in view of the application of new anti-tumor treatments, such as immunotherapy, targeted to patients with OSCC.

Keywords: oral cancer; screening; tumor biomarkers; natural killer cells; immunotherapy; ELISA immunoassay; cytobrush; Immune Checkpoints

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Detecting the Undetectable: Risks and Mitigation Strategies for Illegitimate Use of AI-Generated Images in Microscopy

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The integration of Artificial Intelligence (AI) techniques in microscopy has revolutionized the field, enabling faster and more accurate analysis of microscopic images. However, the increasing capabilities of AI in generating realistic images have raised concerns about their potential misuse in illegal contexts. This study investigates the risk of illegal use of AI-generated images in microscopy, focusing on the methods and techniques used to generate these images, the consequences of their misuse, and possible solutions to mitigate these risks.

A Google Form survey was conducted using 30 images, including authentic microscopy images, hybrid images combining real and AI-generated content, and false images generated entirely by AI. Participants from the morphologists' community were asked to identify whether the images were real or AI-generated.

The results revealed that over 40% of the manipulated images went undetected by the participants, indicating a significant risk associated with hybrid AI-generated images. While participants could detect authentic and false images at relatively high rates, they struggled to identify hybrid images. The study also found that expertise level and age influenced participants' ability to detect hybrid images.

The implications of these findings emphasize the need for continued research in AI-generated images in microscopy to improve the detection of hybrids and ensure data authenticity. Proposed solutions include developing advanced image-verification techniques, educating researchers and technicians, updating guidelines and regulations, establishing centralized databases, and incorporating blockchain technology for image authentication.

Furthermore, the study highlights the importance of addressing the lack of correlation between skills in light and electron microscopy image detection, suggesting tailored training programs for each modality. The limitations of the classification model used were identified, guiding future improvements.

In conclusion, the study underscores the risks associated with the illegitimate use of AI-generated images in microscopy and calls for proactive measures to protect data integrity. The findings contribute to advancing research and development in this field and emphasize the potential risks posed by future technologies, such as generative adversarial networks (GANs), in generating highly convincing fake images.

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Keywords: artificial intelligence; microscopy; image generation; illegal use; ethical guidelines

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Simple and fast: TEM imaging in a standard SEM

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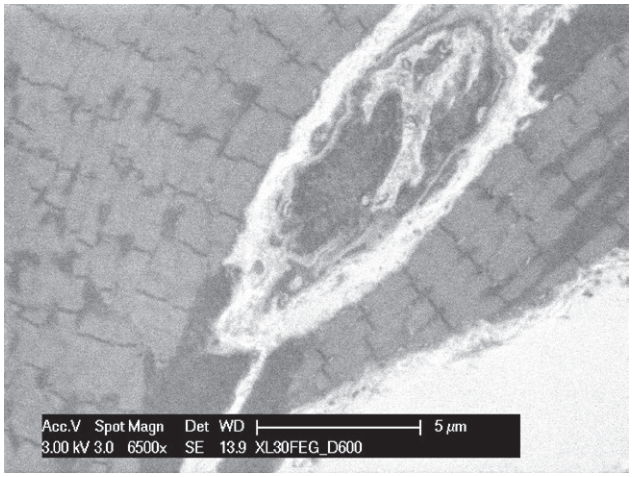
In addition to the usual secondary electrons (SE) and backscattered electrons (BSE) imaging modes, a SEM can also be set up for transmitted electrons imaging. Most SEM manufacturers make a STEM option available as a relatively inexpensive add-on, but even an unmodified scanning electron microscope can provide TEM-like pictures from standard TEM sections (same thickness, same staining) by means of a simple DIY grid adapter.

In this device, low-energy electrons traverse the ultrathin section and impinge upon an angled metal surface where they give origin to conventional secondary electrons. For an optimal yield the surface is gold plated and is directed toward the existing Everhart-Thornley detector, where the secondary electrons are collected as usual. The image is still obtained by scanning the electron beam across the specimen, so the conversion is actually a low voltage STEM. In our case the best results were obtained at 3 to 5 kV: the difference in electron-density caused by the heavy metal staining typical of TEM gives a contrast high enough for a good imaging, but at such low voltages the contrast can be acceptable for unstained specimens as well.

Although the home-made TEM conversion is no match for a real TEM, mainly because of efficiency and signal/noise concerns, it costs literally nothing, can be made and put into operation in an afternoon, and can be a useful shortcut when a real TEM is not at hand.

Below, a micrograph of an unstained thin section of mouse cardiomyocytes in transmitted electrons obtained with a FEI XL-30 FEG SEM operated at 3kV.



Keywords: Electron microscopy, SEM, STEM Conversion

Invecchiamento e patologie degenerative

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Expression of CD44 and its partner Podoplanin in epiretinal membranes

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Epiretinal membranes (ERM)s are membranes composed of cells and extracellular matrix (ECM) that pathologically proliferates on the inner surface of the retina at the vitroretinal interface. Recently we applied a bioinformatics approach on ERM proteins previously identified with an MS-based proteomic analysis. This analysis unveiled interactions occurring among ECM molecules, their cell receptors and intra- or extracellular proteins that may play a role in matrix biology in this special context (Bianchi et al 2022). Among the several proteins identified, we focused on hyaluronic-acid-receptor cluster of differentiation 44 (CD44) which our interactomic analysis highlighted as a central regulator of ERM aberrant dynamics and progression. In the retina, CD44 is expressed by Müller cells and astrocytes, and histopathological studies reported glial cells as the main ERM cell types. Interestingly, CD44 has been demonstrated to interact with podoplanin (PDPN) and this interaction was shown to promote directional migration of epithelial cancer cells. PDPN is a glycoprotein overexpressed in various cancers and a growing body of evidence indicates its relevant function in several fibrotic and inflammatory pathologies.

Therefore, we investigated the expression of PDPN and CD44 in ERM and we found that ERMs express PDPN and the majority of PDPN+ cells also synthesize CD44, thus suggesting their possible interaction and involvement in cell migration during ERM onset and development (Bonente et al 2023). Moreover, PDPN could contribute to ERM formation by promoting epithelial-mesenchymal transition. In support of this, it has been reported that the ectopic expression of PDPN in epithelial cells promotes their switch from an epithelial to a fibroblast-like phenotype.

PDPN expression in ERMs may give us a clue for possible novel pharmacological treatments of ERM progression.

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Keywords: podoplanin (PDPN); cluster of differentiation 44 (CD44); epiretinal membrane (ERM); proliferative vitreoretinopathy

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Phase 1 of HEBE Project: fighting low grade chronic inflammation with physical activity to put out the risk of chronic diseases

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Inflamm-aging is defined as a state of low-grade chronic inflammation underlying the processes of aging and cellular senescence and has been associated with the onset of several pathologies. The identification of markers associated with such processes (both physiological and pathological) and their relationship and interaction with environmental aspects could be fundamental for monitoring the body responses to the onset and progression of chronic-degenerative pathologies. HEBE is a project born within the 2022-2024 University Strategic Plan, the National Recovery and Resilience Plan (PNRR), the National Research Program (PNR 2021-2027) and the UN Agenda 2030 for sustainable development. HEBE is focused on physical activity (PA) (possibly assisted by other nutrition and/or pharmacological interventions), in order to favor the reduction of inflammation and promote healthy aging. Phase 1 of HEBE project, started in 2022, allowed to test the methods of interaction of the consortium's skills and to produce solid preliminary data for the continuation of the project. Phase 1 design consists in 1) Study of population and 2) Personalized medical intervention. Specifically: 1) baseline lifestyle habits (PA, sedentary lifestyle, nutrition, smoking, perception of stress, sleep, alcohol, etc.) and clinical individual characteristics has been assessed trough an anonymous online questionnaire proposed on a voluntary basis via institutional email to all UNIMI employees. Then, healthy-lifestyle recommendations have been proposed, and the changes after 6-months obtained in terms of quality of life and psychophysical well-being has been evaluated using the same questionnaire on more than 1000 subjects. 2) The first 100 eligible subjects who have expressed their availability in the questionnaire were included to undergo a personalized protocol: on the basis of the clinical and objective characteristics, a personalized PA protocol was prescribed, defining modalities, intensity, duration, frequency and progression of exercise. The target population is representative of the general population, and for this reason a stratification has been made according to age (<50; ≥50 years), gender (female; male) and body mass index (BMI<25; ≥25). For each subject, at baseline and after 6-months of PA intervention, whole blood, serum, plasma, urine, buffy coat, nasal swab, saliva and pricked samples are collected to perform the analysis of at least 40 different biomarkers, able to monitor the effect of PA on systemic inflammation.

To date, Phase 1 of HEBE project allowed to enroll 100 subjects; to set up a shared biobank; to set up a shared database; to build the "HEBE" website, with UNIMI domain (www.hebe.unimi.it) containing training/informative material. We developed a new bottom-up approach applied involving the cooperation of 140 researchers from the University of Milan to create a synergy to assess a panel of clinical, biochemical, epigenetic and omic data of inflamm-aging and evaluates its modulation by PA, assisted by lifestyle changes.

Keywords: Inflammation; aging; circulating biomarkers; physical activity; clinical study

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Skin care and aging

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Skin is the largest organ in human body and its main function is to act as a barrier against external stimuli, so it is constantly exposed to the risk of injury and consequently the process of skin care is of primary importance [1,2]. Aging and dermatological diseases can alter skin integrity [3].

The aims of the present study were both extend the actual knowledge on aging-related impaired skin care and also analyze eventual inter-individual anatomical/morphological factors that may influence and modulate the processes of skin aging. The present study was conducted on lower limb specimens (age range from 58-year-old to 90-year-old) subjected to an anatomical dissection at the Anatomical Training Centre "Luigi Fabrizio Rodella", University of Brescia (Italy). The skin biopsies were obtained at the lower limb cleavage lines. Our research group observed, in addition to the significant difference in epidermal thickness between adult and elderly specimens, also that the thickness of the epidermal layer significantly varies among the different sampling sites. Furthermore, the elderly specimens showed disorganized and altered dermal collagen fibers and almost exclusively type I collagen fibers. Also the collagen component changes among the different anatomical bioptic sites of the same donor. The main cause leading to not only degradation, but also to reduced synthesis of dermal collagen fibers is the increased production and activity of metalloproteinases. Our research group observed also that the metalloproteinase 9 is intensely expressed at the level of the dermis of elderly subjects with differences among the bioptic sites.

The above reported data reflect the physiological cutaneous variability in relation to anatomical site and thus to morphological factors and mechanical forces to which the skin is subjected.

Acknowledgements

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Keywords: Aging; Cleavage lines; Collagen; Skin

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Exploring the potential of an olive leaf extract to contrast skin photoageing, using an *in vitro* morphofunctional approach

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Skin photoaging occurs after exposure to UVA rays, which are capable of reaching the dermis causing oxidative stress and inflammation. Deep wrinkles and skin laxity represent the main macroscopical effects and derive from alterations of both cells and extracellular matrix (ECM). The reduction in fibroblast size after UVA exposure is related to decreased production of both fibrillar and amorphous ECM components, regulation of ECM degradation, and an increase in mitochondrial ROS generation [1].

Here we propose an *in vitro* approach testing the efficacy of the olive leaf extract Oleuropein (OLE) in contrasting UVA-induced damage in normal human dermal fibroblasts (NhDFs). OLE was delivered by two different hydrogels, based on low acyl gellan gum (GG) blended with sodium alginate (NaALG). Immunofluorescence, live cell imaging, Western Blotting and RT-PCR were used to assess fibroblast morphology, expression of ECM proteins, and production of antioxidant enzymes and inflammatory-related molecules.

We observed that exposure of fibroblasts to 5 min UVA irradiation determined an enlargement and shortening of cells, which acquired a rhomboid morphology, as well as decreased motility. UVA-damaged NhDFs also showed a reduction in Type I Collagen and Decorin production, reduced expression of Catalase and Superoxide Dismutase 1 (SOD-1) and increased levels of TNF-α and IL-6 mRNAs. We demonstrated that in the presence of OLE delivered by the hydrogels, the UVA-damaged fibroblasts regained their spindle-shaped morphology and motility. Moreover, the treatment with the two different hydrogels delivering OLE was also capable to hamper most of the cell alterations induced by UVA exposure.

Overall, our morphofunctional results showed the properties of the proposed hydrogels to counteract the damages caused by UVA and restore normal dermis composition by recovering normal fibroblast morphology, contrasting oxidative stress and, consequently, inflammation, as well as stimulating the expression of ECM proteins [2]. These fully naturally formulated anti-photoaging materials could be intriguing for potential use as facial masks.

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Keywords: aged skin; dermal fibroblasts; Extracellular Matrix; UVA-induced damage; Oleuropein

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Effects of caloric restriction on the loss of liver function typical of aging evaluated using an animal model

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During the aging process, the body's organs gradually lose the ability to maintain homeostasis, due to structural alteration or dysfunction in cellular metabolism (1). Aging represents a major risk factor for most chronic diseases including ischemic heart disease, cerebrovascular disease, chronic obstructive disability, cancer, and diabetes (2). Aging is associated with gradual alteration of hepatic structure and function, it increases the risks for various liver diseases and plays as an adverse prognostic factor, causing an increased mortality rate. The maintenance of normal body weight through dietary modification or habitual physical activity are both associated with a reduced incidence of serious liver disorders (3,4). In this study, we investigated potential interactions between diet and liver disorders using an animal model. The experiments were performed on 14 (24-month-old) male Sprague-Dawley rats, which at the age of 18 months were divided into two subgroups: 1) Normal diet (ND) (n=7) fed an *ad libitum* of a standard laboratory meal, 2) Low-calorie diet (HD) (n = 7) fed a diet of the same chow limited to 60% until sacrifice (24 months). After the sacrifice, different markers of fibrosis, inflammation and aging were evaluated on the liver samples, by histochemical, immunohistochemical and western blot analysis. Our results showed that caloric restriction (CR) was able to reduce fibrosis, inflammation, and immune cell infiltration on rat old liver. These data strictly suggest that CR protects against fibrosis, inflammation, and liver injury and mitigates the effects of aging.

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Keywords: aging; caloric restriction; rat liver

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Early aging in childhood cancer survivors: role of antioxidant defenses

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Although the survival rate of childhood cancer patients has improved dramatically over the last four decades, it is known that Childhood Cancer Survivors (CCSs) more frequently develop clinical complications associated with chemotherapy/radiotherapy and are consistent with early aging compared to the age-matched healthy population [1]. Recently, we have shown that mononuclear cells (MNCs) derived from the peripheral blood of CCSs are characterized by an alteration in mitochondrial morphology and function that leads to the oxidative damage accumulation that could explain the early aging [2]. However, oxidative stress accumulation could also depend on low antioxidant defense (AO) activity. To verify this hypothesis, we evaluated the expression and activity of catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), and glucose-6-phosphate dehydrogenase (G6PD), the expression of NRF2 and KEAP1 and the content of NADPH, NADP, GSSG, GSH, malondialdehyde in MNCs isolated from 96 CCS aged 5-20 years and from 74 healthy subjects aged 5-106 years, used as controls.

The results show that MNCs isolated from CCSs exhibit lower expression and activity of CAT, GR, GPx, and G6PD than healthy subjects of the same age. This altered activity causes a reduction of NADPH and GSH content. In addition, reduced AO enzyme expression appears to be associated with low expression of NRF2 and KEAP1, two proteins that act as sensors of oxidative stress by inducing AO gene expression. Notably, CCS also show low levels of AO defenses compared to healthy elderly subjects (>70 years), suggesting that the molecular mechanisms underlying the premature aging of CCS are different from those of physiological aging.

In conclusion, the data suggest that the inability to activate AO defenses in response to the oxidative insult associated with altered aerobic metabolism may be one of the underlying causes of early aging in CCS, thus, representing an excellent target for possible therapies to slow its progression.

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Keywords: early aging; antioxidant defenses; oxidative stress; impaired mitochondrial morphology and function; childhood cancer

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Detection of neurodegenerative, oxidative and neuroinflammatory biosignatures characterizing frailty condition

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Frailty is an age-related deficit defined as a clinical state of increased vulnerability in which the ability of older people to deal with everyday stressors is compromised. It was caused by reduced physiological reserve and the decline of multiple physiological systems, all associated with age.

The aim of this study was to identify peripheral biomarkers to generate a biological profile useful both to characterise the frailty condition and to uncover potential targets for the development of effective strategies to counteract, limit, slow down or prevent this syndrome.

We enrolled a group of frail elderly patients (N=15), aged 65 years or older; a group of non-frail elderly healthy subjects (N=15), aged 65 years or older; a group of young healthy subjects (N=15), aged between 18 and 35 years.

The comparison of the molecular features obtained from the frail group with the ones from elderly and young healthy subjects resulted in: i) significantly modified plasmatic levels of NGF and proNGF (ELISA assays); ii) significant modulation of the amount thiols and of the thiolation index (PTI) in plasma (HPLC analysis); iii) significant deregulation of expression levels 152 genes involved to 13 different pathways related to frailty, (functional transcriptomic and bioinformatic approaches); iv) consistent modulation of 27 different pro-inflammatory cytokines and chemokines able to acutely activate T cells (multiplex assay).

In conclusion, our study highlights the complex alteration of the molecular pattern of frailty in the context of ageing. The combination of peripheral protein and gene determinants, occurring with this condition, will be useful to generate oxidative and neurodegenerative and neuroinflammatory biosignatures and will uncover novel potential therapeutic targets for the improvement of management of frailty patients.

Keywords: Ageing; biomarkers; NGF; proNGF; thiols; gene expression; oxidative stress; cytokines

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Selective effect of quercetin on senescent mesenchymal stromal cells

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Mesenchymal stromal/stem cells (MSCs) have shown significant therapeutic potential in preclinical studies of regenerative medicine. In addition to their potential to differentiate into various cell types, their ability to secrete paracrine factors to modulate cell behavior contributes to their utility in treating inflammatory conditions. However, the clinical use of MSCs is relatively underdeveloped due to their heterogeneity, which largely arises from their susceptibility to senescence during aging.

Cellular senescence, a natural response to aging and stress, is defined as a permanent cell cycle arrest in metabolically active cells. While senescence has protective and reparative roles, it can also contribute to the functional decline of tissues. When senescent cells are not cleared, this build-up can lead to aging-related inflammatory conditions. Targeting senescent cells by a selective elimination or blocking senescence-associated secretory phenotypes (SASP) with natural or synthetic compounds has been suggested to improve lifespan.

Flavonoids such as quercetin are emerging as potential therapeutic agents to mitigate senescence. Being mainly studied for their anti-oxidant effects in cancer, allergic reactions, inflammation, and cardiovascular disorders their influence on cellular senescence in still under investigation.

The aim of the study was to evaluate the effect of quercetin as a senolytic molecule in counteracting cellular senescence. Human MSCs were induced to senescence by H_2O_2 treatment. Different concentrations of quercetin were tested to evaluate the effects on the expression of senescent cell cycle arrest related markers p53, p21, and p16. Apoptosis and autophagy markers were also evaluated.

Results showed a strong activation of autophagy followed by apoptosis in senescent cells compared to control ones, underling the potential role of quercetin as a therapeutic drug in selectively targeting senescent cells.

Keywords: cellular senescence, mesenchymal stromal cells, senotherapy, quercetin

Meccanismi molecolari di controllo della crescita cellulare

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Aristolochia Olivieri: a potential chemoadjuvant in the treatment of gastric cancer

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Helicobacter pylori (H. pylori) is one of the most common human infectious agents worldwide. The mean worldwide incidence of H. pylori infection was 58%[1]. Since its discovery, H. pylori has been closely associated with a spectrum of gastrointestinal injuries. Although most infected individuals remain asymptomatic for life, approximately 15% develop peptic ulcer disease, 0,1% develop mucosa-associated lymphoid tissue lymphoma (MALT)[2] and 1-3% progress to gastric cancer[3].

Cisplatin is the one of major chemotherapeutic drugs in gastric cancer, particularly in treating advanced forms. However, neoplastic cells often develop resistance to cisplatin, which seriously affects the efficacy of chemotherapy[4]. In the search for chemopreventive compounds that reduce the risk of cancer relapses, preclinical studies have demonstrated promising results with various vegetables substances[5,6].

Aristolochia Olivieri is a plant belonging to the family of Aristolochiaceae widely used in Kurdish Folk Medicine for the treatment of gastrointestinal ailments.

Here, the Aristolochia extract effect on H. pilory and gastric cancer cells have been evaluated to microbiological, morphofunctional and cytotoxic analyses. Data obtained showed that Aristolochia is a powerful bactericide agent against H. pilory. In addition, Aristolochia has a potent effect as inhibitor of cell viability by inducing apoptosis in different gastric cancer models, as demonstrated by morphological analyses.

These preliminary results suggest that Aristolochia could be used as a chemo-adjuvant in the treatment of gastric cancer.

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Keywords: Aristolochia Olivieri; Helicobacter pylori; Gastric cancer

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Unconventional actions of LH on prepuberal ovary

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In mammals, growing follicles develop from a reserve of primordial follicles (PMFs) constituted early in life. From this pre-established reserve, a second ovarian reserve is formed consisting of gonadotropin-responsive growing follicles that in the adult are cyclically selected for ovulation [1]. PMFs reserve progressively diminishes due to follicle atresia as well as recruitment, maturation, and ovulation. The role of LH in the natural menstrual cycle is undisputed. While the crucial participation of LH in both steroidogenesis and ovulation is well established, other possible functions in follicle dynamics are debated. We demonstrated the presence of LH receptor in early postnatal mice ovaries and that LH treatment can promote PMF survival [2]. Therefore, our aim is to investigate LH potential role in activating signaling pathways associated with follicle activation in the ovarian reserve.

We performed *in vitro* cultures on ovarian fragments from 4 days post-partum (dpp) female mice and treated them with different concentrations of LH (LH 0.2 0.5 and 1 IU) every day for 5 days. The results showed that in the fragments cultured in the presence of 1 IU LH, both the total number of oocytes and the % of those with diameter >40 μm were significantly higher in comparison to CTRL.

LH effects were also investigated *in vivo* by treating 4dpp mice with intra-peritoneal injection once a day for 8 consecutive days, with physiological solution or with LH 1IU. On day 9, ovaries were collected for histological analysis. Follicle count in control ovaries showed a higher pool of PMF (68,22%) than the other follicles, which have as expected undergone activation (growing [GR] 14,18%, and primary [PF] 7,2% follicles). Interestingly, in LH treated mice ovaries, we observed a higher percentage of PMF (78,29%) and a lower percentage of GR (8,56%) and PF (4,05%) than control. Although the percentage of secondary follicles (SEC) was similar in both groups (CTRL 7,6% vs LH 7,35%), in the LH-treated mice SECs showed a thicker layer of granulosa cells (> three layers CTRL 42,8% vs LH 54,6%). Finally, we observed decreased atresia in the LH-treated group (CTRL 2,64% vs LH 1,72%).

In conclusion, our results extended our previous data about the responsiveness of prepuberal ovaries to LH. At this stage, LH seems to favor the maintenance of PMF reserve and to sustain the follicle growth toward the secondary stage upon PMF activation.

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Keywords: Luteinizing Hormone; ovarian reserve; folliculogenesis; mammalian ovary; female fertility

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The endocannabinoid-EGFL7/Notch axis in endometrial preparation to implantation

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In humans, the endocannabinoid system (ECS) plays an important role in several physiological processes, including reproductive system functions (Ligresti et al., 2009; Maccarrone et al., 2010).

The components of ECS comprise two main receptors, CB1 and CB2, two main ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) and their metabolic enzymes involved in their synthesis and degradation (Ligresti et al., 2009; Maccarrone et al., 2010)

The ECS has been shown to regulate pathways involved in the process of implantation and its dysregulation might be a determinant of infertility (Maia et al.;2020). In particular, it has been demonstrated that sustained levels of anandamide (AEA) impair decidualization, a process known to be necessary for endometrial receptivity.

We are currently investigating whether the adverse effect of AEA on decidualization might be a consequence of its negative regulation of the EGFL7/NOTCH axis. The role of the NOTCH pathway in the regulation of endometrial receptivity has been well established (Afshar et al, 2012; Su et al, 2015). We recently demonstrated that EGFL7 can drive the differentiation of human primary endometrial stromal cells towards the decidual phenotype by activating the NOTCH pathway (Lacconi et al., 2023). HESC were decidualized in vitro (DESC) with medroxyprogesterone acetate (MPA), and 8-Bromo-cyclic AMP (cAMP). At 4 and 6 days decidualized cells were treated with AEA (10μM) for 24 and 72h.

qRT-PCR showed that HESC and DESC express the CB1 receptor and the major enzymes involved in AEA metabolism (FAAH). In agreement with data from the literature, AEA culture supplementation downregulates the expression of the decidualization markers IGFBP1 and Prolactin (Almada et al.,2016).

Concomitantly we observed downregulation of the NOTCH target gene HEY1, whose expression is normally increased upon decidualization (Lacconi et al., 2023). As we previously demonstrated decidualization upregulates the expression of EGFL7 in stromal cells, however in contrast with what expected AEA treatment further increased its expression. We hypotheses that AEA-induced EGFL7 excessive increase may negatively impact on the activation of NOTCH signalling, as demonstrated in other biological systems (Nichol and Stuhlmann 2012), this in turn negatively impact on decidualization.

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Keywords: eCB; AEA; decidualization; NOTCH; EGFL7

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Influence of medium composition on the anti-tumoral potential of cold atmospheric plasma: preliminary results

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Plasma medicine is an interdisciplinary field that emerged in the last two decades with the final aim to use cold atmospheric plasmas (CAP) for human therapeutic applications, including antibacterial activity, regenerative medicine and anticancer effects. The effectiveness of CAP as non-invasive anti-cancer solutions is due to the possibility of activating liquids - plasma activated media (PAM) – to use to treat the tumoral cells[1].

HNSCC is the sixth most common type of cancer by incidence worldwide, with an approximate 5-year survival rate of 65%, dropping to an average of 40% for patients diagnosed in advanced stage[2]; this is due to a poor response to the conventional therapies, such as chemotherapy and radiotherapy, and to its heterogeneity, which calls for an urgent need of multimodal therapy.

Therefore, this work is aimed at investigating the anti-tumoral effects of different PAM on two oral cavity squamous carcinoma cell lines, namely CAL-27 and Fadu.

Two different volumes - 2 mL and 5 mL - of the two culture media (DMEM for CAL-27 and MEM for Fadu) were treated using a Jet plasma with air gas, provided by PBRC, Kwangwoon University Seoul, under a Memorandum of Understanding with the University of Chieti-Pescara, for 5, 10 and 20 min at 6 mm working distance between the capillary of the device and the surface of the media.

Fadu cell viability, assessed by MTS assay at 24, 48 and 72 h, showed a decrease in all the experimental conditions, but in particular when cells were exposed to 2 ml PAM. On the other hand, CAL-27 proliferation appeared to be affected just when treated with 2 ml 20 min PAM after 48 and 72 h.

Since the CAP anti-tumoral potential is obtained mainly by the generation of a mixture of reactive oxygen and nitrogen species (RONS), the total ROS and H_2O_2 content was measured

and a certain scavenging activity was found in the DMEM, respect to the MEM. Therefore, in order to elucidate any involvement of the culture medium in the toxicity exerted by CAP, the CAL-27 cells were exposed to 5 ml of MEM treated with plasma for 20 minutes, whereas Fadu cells were kept in DMEM treated with the same experimental settings.

Interestingly, CAL-27 suffered a high reduction of cell viability while Fadu cells appeared to be unharmed by the exposure to PAM up to 72 h.

Many questions arise in light of these results and further studies will be focused on clarifying the role of media and liquid composition on the effects exerted by CAP on tumoral cells.

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Keywords: cold atmospheric plasma (CAP); oxidative stress, scavenging; cell medium; head and neck squamous cell carcinoma (HNSCC); plasma-activated medium (PAM); anti-cancer therapy; RONS

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Characterization of epidermal growth factor-like domain 7 (EGFL7) expression in normal endometrium and in the endometrium of women with poor reproductive outcomes

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Implantation of the embryo into a receptive endometrium is one of the most critical events in human reproduction and occurs in a specific period of the menstrual cycle called "window of implantation". The expression and role of the many modulators (e.g. NOTCH1, PGE_2, hCG) involved in endometrium-embryo crosstalk remain partially explored. Alteration in this complex network of signals might determine pathological conditions, such as implantation failure (RIF) and recurrent pregnancy loss (uRPL) [1,2]. EGFL7 is a secreted factor expressed during embryogenesis by the developing vascular system, mouse blastocyst and mouse and human trophoblast cells [3,4]. To clarify the role of EGFL7 in the regulation of endometrial receptivity, we analyzed EGFL7 expression in endometrial biopsies from fertile women and women undergoing RIF and uRPL. Previous presented data showed higher levels of EGFL7 in samples from the secretory compared to proliferative phase and a novel, not previously reported, expression in endometrial glands and stromal cells. We, now, demonstrated that EGFL7 is significantly reduced in both endometrial glands and stroma in women with uRPL and RIF in secretory phases [9]. Decidualization using cAMP/MPA of endometrial stromal cells (EndSCs) obtained from fertile women showed an up-regulation of EGFL7 expression, whereas cells obtained from women with uRPL and RIF did not [5]. To investigate whether a physiological stimulus might regulate EGFL7 expression, we treated EndSCs with PGE_2, a physiological inducer of decidualization *in vivo* [6,7]. PGE_2 was able to upregulate the expression of EGFL7 in cultures from fertile women but no in pathological samples [5]. Since during implantation a cross-talk between blastocyst and endometrium occurs, we decided to elucidate whether embryo-derived molecular signals regulating endometrial expression of EGFL7. To this purpose, we stimulated EndSCs with hrCG. Even though hrCG alone was not able to induce decidualization, EGFL7 was upregulated in cells from fertile women but not from RIF and uRPL. Our data suggest that EGFL7 is a new player regulating the modifications occurring in the endometrium in preparation to implantation and that its downregulation might be associated with poor reproductive outcomes.

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Keywords: EGFL7; NOTCH signaling; recurrent pregnancy loss; recurrent implantation failure; decidualization; endometrial stromal cells

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Cell cycle block by p53 activation reduces SARS-CoV-2 release in A549-hAce2 cells

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The two last decades have shown great sanitary emergencies due to the pandemic diffusion of SARS-CoV-2 that has presented a new scientific challenge for the search of effective therapies against infection, replication and spreading. One of the targets of the virus that plays an important role both in the mechanisms of innate immunity and in the control of the cell cycle and other pathways that regulate cell replication, damage repair, apoptosis and metabolism is the p53 protein.

SARS-CoV, as observed for other viruses, have evolved specific molecular mechanisms to contrast p53, in order to avoid the host response to infection. For example the stabilization of its inhibitor, MDM2, and the interference with its transcriptional activity, indicating that p53 has a central role in controlling its proliferation in the host.

Following all these evidence and considerations, the aim of the project was to evaluate a new approach against the virus, by using MDM2 inhibitors to effectively raise p53 levels and activate p53-dependent pathways including cell cycle inhibition. Experiments setting was done in the alveolar basal epithelial cell line A549-hACE2 expressing $TP53^{wild\text{-}type}$ and the SARS-CoV2 receptor ACE2. Cells were treated with several concentration of Nutlin-3 or RG-7112 at the time points of 24 and 72 hours post treatment for the instauration of a cell cycle block steady-state condition before and during SARS-CoV-2 infection, and for the evaluation of p53 activation and impact on virus release and related innate immune events.

The results of the project suggest that Nutlin-3, as well as RG-7112, significantly reduced SARS-CoV-2 replication in A549-ACE2 cells and promoted a complete inhibition of IL-6 expression, associated with inhibition of NF-kB and interferon-lambda, important mediators of inflammation. These data indicate that p53 represents an efficient target for new therapies against the virus and that MDM2 inhibitors can be a realistic therapeutic option.

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Novel role of *BCL11A* in the proliferative response to glucocorticoids of erythroid cells

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The effect of Glucocorticoids (GC) on erythroid cells proliferation is well reported[1,2] and a developmental specificity of the GC stimulus was observed previously on immortalized erythroid cells with fetal phenotype[3], hybrid cells generated from human fetal liver erythroid and murine erythroid cells (MEL cells) that accelerates the increase in adult globin in presence of GC[4] and on certain cord blood cells that do not respond to GC in vitro[5]. We reported an increase of the nuclear content of BCL11A in the presence of GC and to demonstrate that this increase was not just associated with GC addition, we studied the responses in patients with BCL11A microdeletions compared to their parents. The CD34+ from those patients were not able to generate as much erythroid cells as the control and, when the patient 2 cells and their mother (as a control) were cultured with or without GC, we observed that the number of erythroid cells from the patients did not change in their proliferation after GC stimulation in contrast from their mother. The same impairment on the GC-induced proliferation was observed in CD34+ with engineered reduced *BCL11A* expression (through shRNA). We observed that the number of colonies from erythroid progenitors from the CD34+ with low *BCL11A* is decreased with respect to the control**,** and those cells do not modify their differentiation upon GC stimulation. Further molecular/mechanistic details uncover that GC induced BCL11A amplification is necessary both for the GC associated response genes(i.e GILZ)[6] as well as cell cycle associated genes(i.e. CDKN2B (log2FoldChange = 2.389, padj < 0.000)[7] and CDKN2D (log2FoldChange = 1.179 , padj < 0.000)[8]), or implicated in the quiescence maintenance (i.e. MEIS1(log2FoldChange= 1.179, padj<0.000), EGR1 (log2FoldChange = 1.523, padj < 0.000), FOS (log2FoldChange = 1.399 , padj < 0.000))[9]. Our data and previously reported *BCL11A* effects on proliferative influence on normal HSCs[9] and on B-cells[10] suggests that *BCL11A* is an important participant in the proliferative response elicited by GC.

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Keywords: BCL11A; Glucocorticoids; Erythroid cells; Proliferation

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Inhibition of endolysosomal two-pore channel 2 (TPC2) activity stimulated osteoblast differentiation and function and impeded the autophagy termination

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Autophagy is a highly conserved catabolic process that is critical for the survival, differentiation, and function of bone cells and its dysregulation leads to the bone disorders [1, 2]. Recently, it has been reported that autophagy is regulated by the endolysosomal two-pore channel 2 (TPC2) which are voltage and ligand gated ion channels that control the release of both Ca^{2+} and Na^{+} in response to NAADP and PI(3,5)2, respectively [3, 4]. Regarding this, we aimed to assess the interaction between autophagy pathway and TPC2 inhibition during osteoblastogenesis. To achieve this goal, bone marrow derived mesenchymal stem cells (MSCs) were treated with two pharmacological TPC2 inhibitors including naringenin and tetrandrine for 21 days. Increased osteoblast differentiation was observed in the presence of both inhibitors as confirmed by alkaline phosphatase staining and RUNX2 expression by western blot. Furthermore, stimulated expression of early autophagy markers (Atg5 and beclin1) was observed and interestingly the expression of LC3II as the marker of late phase autophagy remained unchanged. To uncover the effects of TPC2 inhibition on bone mineralization, the human osteosarcoma cells (SAOS-2) were used and treated with varying concentrations of naringenin and tetrandrine. TPC2 inhibition induced the formation of calcium deposits by SAOS-2 cells as confirmed by both Alizarin red S and Von Kossa staining methods. Interestingly, inhibition of TPC2 activity reduced the expression of LC3II in these cells. It could be concluded that TPC2 inhibition by naringenin and tetrandrinecan enhance the differentiation and function of osteoblasts and impedes the completion of autophagy process probably due to modification in release of Ca^{2+} and Na^{+} and subsequent blockage in autophagosome-lysosome fusion.

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Keywords: Osteoblast; differentiation; TPC2; mineralization; autophagy

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X-ray-induced endothelial damage: sex differences and antioxidants effects

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Cellular alterations induced by ionizing radiation (IR) are associated with some pathological conditions, but information on the influence of sex is scarcely known. Therefore, we investigated if male and female HUVECs responded differently to X-ray exposure and if the antioxidants 10 mM taurine (TAU) and 5 mM N-acetylcysteine (NAC) can prevent IR-induced damage in a sex-dependent way.

In untreated cells, sex differences were observed only in autophagy, which displayed a higher degree in female cells.

TAU and NAC did not modify viability, LDH release, migration, and autophagy. NAC increased MDA levels in female HUVECs.

After X-ray irradiation an increase in LDH release and a reduction of viability and migration was observed both in male and female HUVECs. TAU and NAC did not affect viability while reduced LDH release in irradiated cells: they have the same protective effect in female HUVECs, while TAU was more protective than NAC in male cells. Moreover, TAU and NAC significantly promoted the closure of the wound in both sexes in irradiated cells, but NAC was more effective in female HUVECs in doing this.

TAU did not affect autophagy, while NAC reduced sex differences after X-ray treatment. Finally, MDA decreased in irradiated male HUVECs and increased in irradiated female HUVECs after NAC treatment. In conclusion, female cells seem to be more affected by IR damage and the effects of the two antioxidants present some sex differences, suggesting the need to deeper study the influence of sex in prevention of radiation-induced damage.

Keywords: ionizing radiation; taurine; N-acetyl cysteine; sex differences; HUVECs

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Characterizing possible mechanisms eluding PI3K inhibition effectiveness in AML

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Acute myeloid leukemia (AML) is a malignant disorder of haemopoietic stem cells characterized by clonal expansion of abnormally differentiated blasts of myeloid lineage with acquired genetic changes[1,2]. In the last decades there has been a big urge to continually develop new treatments for this disease. Several pieces of evidence highlighted the pivotal role of PI3K/AKT pathway in leukemogenesis, promoting aberrant cell proliferation and survival[3]. Being this pathway overactivated in 60-70% of AML patients and correlating with a poor outcome, it represented an attractive target for therapeutic strategies[4]. Indeed, the use of PI3K/AKT inhibitors, either individually, or by dual inhibitors, has been widely tested demonstrating cytotoxic activities in AML cells[5,6]. However, despite the efficacy of these small molecules in arresting tumorigenesis in *in vitro* and *in vivo* models, clinical trials for their use as single agents have shown limited effects, resulting in insufficient clinical antileukemia activity when administrated at tolerated doses[7,8]. Our aim was to investigate possible mechanisms of drug ineffectiveness of AML cells treated with PI3K inhibitors.

Our data showed that the treatment of AML cells with the pan-class I PI3K inhibitor (*i.e.,* BKM-120) resulted in a significant arrest of cell proliferation and cytotoxicity in a panel of AML cell lines. These results were also obtained in a co-culture system with human stromal cells, which did not significantly support leukemic cells, and were not affected by the treatment. AML cells also showed a downmodulation of PI3K/AKT, as revealed by RPPA (Reverse Phase Protein Array) analyses. However, mass spectrometry analyses of AML treated with BKM-120, also revealed an upregulation of several molecules. We focused our attention on HDAC1, RAD21, and ABCF2. These preliminary results warrant further investigation to define possible novel combinatorial approaches to target AML efficiently.

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Keywords: Acute Myeloid Leukemia; PI3K inhibitor; drug resistance

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Aptamer to ErbB-3/HER3 as innovative therapeutic agent in HNSCC

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Cetuximab (CTX) treatment induces an acquired resistance in the majority of head and neck squamous cell carcinoma (HNSCC) patients, leading to local and distant treatment failures[1]. The emergence of resistance is associated with compensatory up-regulation of HER3[2], making it a new therapeutic target. In this regard, our approach involves targeting the HER3 receptor axis with an innovative drug, known as aptamer. Aptamers are DNA-based molecule synthesized *in vitro* through SELEX technology. Briefly, a large library of random DNA sequences was incubated with purified IgB-3, a fusion protein combining the extracellular domain of HER3 and the Fc domain of a human IgG1. Any sequences that bounded to the target were retained, amplified by PCR to generate a larger pool of molecules, and lastly subjected to cloning and sequencing.

Using the HNSCC cell line FADU, we conducted experiments to confirm the binding specificity of the selected aptamer (A33) to HER3, comparing it to a control primer.

Furthermore, we observed an increased HER3 protein level by Western Blot already after 24hrs of CTX treatment. These results suggest its potential involvement in the bypass pathway resistance mechanism.

Based on these results, we proceeded to assess the biological function of A33.

Through Western Blot analysis, A33 showed a promising HER3 degradation after 24hrs of treatment with a consistent downregulation of the downstream signaling pathway, pAKT. Using the Livecyte instrument, we observed that incubation with A33 resulted in a decreased cell track speed. Furthermore, when combined with CTX, A33 increased cell doubling time and decreased cell confluence.

Their addictive effect was also confirmed in both monolayer cultures, with the reduced ability of single cells to grow as colonies, and in 3D growing conditions, where there was a decrease in spheroid volume.

Collectively, our promising results shed new light on the potential use of DNA-aptamers as therapeutic agents in cancer therapy.

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Keywords: HER3; Aptamers; HNSCC; Cetuximab; Resistance

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The inhibition of Hedgehog signaling pathway exerts synergy with MK2206 AKT inhibitor in acute lymphoblastic leukemia cells

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In the Hedgehog (Hh) signaling pathway the interaction of the Hh ligand with the inhibitory receptor *Patched1* (*Ptch1*) removes the inhibitory effect that *Ptch1* exerts on another receptor, *Smoothened* (*Smo*), a key positive regulator in signal transduction [1-2]. All these events lead to the activation of the zinc-finger transcription factors of the *Gli family* (*Gli1, Gli2 and Gli3*), with consequent nuclear translocation and transcriptional activity.

Of note, this pathway appears to be involved in the regulation of T cell proliferation, survival, and differentiation. Hh plays a crucial role in the embryonic development, but also in tumorigenesis, as well as in the proliferation and maintenance of cancer stem cells.

The PI3K/Akt/mTOR signaling pathway is also known to be often hyperactivated in T-acute lymphoblastic leukemia (T-ALL) and plays a crucial role in tumorigenesis.

Therefore, using Gant-61 (*Gli transcription factor inhibitor*), GDC and Glasdegib (*SMO inhibitors*) and/or MK-2206 (*Akt inhibitor*) pathway, we investigated in different T-ALL cell lines the effect of these drugs up to 72 hours.

IC50 concentration was assessed by CCK8 assay and the expression of total and phosphorylated downstream proteins of these two pathways (such as Akt, GSK3αβ, p70S6K, Gli) was analyzed. Flow cytometry analysis was used to investigate cell cycle and apoptosis (by means on Annexin V).

Gant-61 showed a significant cytotoxicity, whereas GDC and Glasdegib exerted modest inhibition of cellular viability. In combination with MK2206, Gant-61 showed a relevant synergy. Total *Gli1* protein was reduced and Akt phosphorylation was inhibited by drug treatments.

Moreover, the treatment with single or combined drug administration may have a relevant role in the apoptotic process and in inducing cell cycle arrest in *G0/G1 phase*.

In conclusion, our data suggest that the combination of drugs targeted to Hh and PI3K/Akt/mTOR represents a potential strategy for T-ALL treatment.

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Keywords: GANT61; MK-2206; GLI; Hedgehog signaling; PI3K/AKT/mTOR pathway

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In vitro toxicity of mineral fibres: a possible role in placental development

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Asbestos has been practically used everywhere because of its unique physical-chemical and technological properties. It is known that exposure to asbestos causes serious damage to health. This has led to the prohibition of their use in most countries but, one species, the chrysotile, is still mined, marketed and exported in many countries like China, India and Russian Federation where its "safe use" is allowed. Chrysotile is still used because it is not bio-persistent and considered less toxic than other amphibole asbestos species. It is demonstrated that asbestos fibres were present in placenta tissues. So, the aim of our study was to investigate if placental tissues differentiation and proliferation could be modified in presence of asbestos fibres. We used BeWo cell line, an *in vitro* model that mimics the syncytiotrophoblast (STB), the outer layer of placental villi treated with two kinds of chrysotile fires, long (CHR-L) and short chrysotile fibres (CHR-S). Our data demonstrated that both fibres induced ROS formation and reduced cell proliferation but only CHR-L fibre was able to induce irreversible DNA alterations that carried to cell apoptosis. These data suggest that CHR-L may induce placental villi damage and/or death leading to impaired placental development, which is at the basis of many gestational pathologies. Since gestational pathologies are very dangerous for foetal and maternal life, we suggest to the gynaecologists to carefully evaluate the area of maternal residence, the working environment, the food used, and the materials used daily to avoid contact with these fibres as much as possible during gestation.

Keywords: Asbestos; BeWo cells; Toxicity; Gestation; Pregnancy; Trophoblast; Chrysotile; Wollastonite; ROS; Oxidative stress

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The role of miR-145 in the emergence of adaptive resistance to AKT inhibition in prostate cancer

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The tumor-suppressor miR-145 regulates different cellular processes in prostate cancer (PC) and its loss is involved in the transition from localized to metastatic disease progression. Moreover, low expression of miR-145 is able to predict poor survival of PC patients. A recent and promising therapeutic strategy for PC associates AKT inhibitors to drugs blocking androgen synthesis such as abiraterone. However, AKT inhibition is very well known to trigger a rapid development of resistance due to the interruption of negative feedback circuits or perturbation of pathway homeostasis. Interestingly, in our laboratory we found that AKT modulates miR-145 expression in the prostate cancer cell line PC3. In particular, we observed that AKT inhibition not only downregulates miR-145 but also evokes a dramatic increase (~20 folds) in the expression of a gene target of miR-145, namely the oncogene RAS. Since RAS is a potent activator of the PI3K/AKT pathways, we hypothesized that AKT inhibition could result in AKT reactivation. Confirmation that the observed drop of miR-145 triggers an increase of RAS, both in terms of mRNA and protein, was obtained using PC3 cells engineered by us to transiently silence the 145-5p guide strand of miR-145 following exposure to doxycycline. We further demonstrated that pharmacological inactivation of AKT with capivasertib leads to overexpression not only of RAS but also of another GTPase family member, Rab5, a key regulator of early endosomes formation and maturation to late endosomes [1]. This finding is particularly interesting as Rab5 is a direct target of miR-145 [2]. Moreover, the overexpression and activation of this protein not only has been related to the internalization of growth factor receptors, but is also considered a switch in the process of tumor cell migration, which also involves more targets of miR145 such as N-cadherin and β-catenin. Our finding that N-cadherin and β-catenin expression and intracellular localization is affected by capivasertib is therefore in good agreement with our previous results and pave the way to the identification of markers characterizing patient subgroups that will derive maximal benefit from PI3K/AKT targeting.

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Keywords: miR-145; prostate cancer; AKT inhibitors; adaptive resistance; RAS

Anatomia e movimento

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Relationship between BMI, physical activity and daytime activity levels during a week of a spa stay

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Obesity has become a worldwide issue that can exacerbate some ageing-related comorbidities. Physical activity (PA) can counteract weight gain and obesity [1]. The thermal environment, including facilities and treatments, have been described as increasing PA [2], [3]. The current study aims to assess the PA levels in relation to different BMI categories during a week of spa stay.

137 participants (males=35.8%; 64.3±10.5yrs) staying one week at *GB-Hotels* (Abano Terme, Italy) and undergoing spa treatments filled in the *Godin-Shepard Leisure-Time Physical Activity Questionnaire* (GSL-TPAQ; LSI – as measure unit) both at the beginning and the end of the spa stay in order to evaluate the PA of the previous week and during the spa stay. Actigraph Motion Watch 8 (CamNtech) monitored daytime activity all week long. One-way ANOVA analyses among BMI categories were adjusted for age and sex. FORST funded the current study.

BMI values classified particpants as normal weight (*NW:* 50.4%), overweight (*OW:* 35%), and obese (*OB:* 14.6%). NW participants collected the highest GSL-TPAQ score either the week before (NW=31.1±21.9 LSI; OW=25.7±23.4 LSI; OB=20.5±17.5 LSI), even though without statistical significance (p=0.08), or during the week of the spa stay (NW=44.3±33.1 LSI; OW=36.6±24.4 LSI; OB=27.6±15.9 LSI; p=0.04), with only a tendency to statistical significance between NW and OB in the *Bonferroni post-hoc test* (p=0.06). GSL-TPAQ delta values were higher in NW than in the other two BMI categories, although they did not reach statistical significance (NW=13.1±7.3 LSI; OW=11±6.6 LSI; OB=7.1±4.7 LSI; p=0.8). Thus, NW seemed to increment the PA more during the spa stay. The actigraphic data analysis showed a higher percentage of daytime activity in NW compared to the other two BMI classifications (NW=43±7.6%; OW=40.6±9%; OB=38.5±8.4%) even though without statistical significance (p=0.8).

With a view to promoting an active lifestyle and increasing PA, it seems that a week of spa stay can be effective. The data showed that NW subjects seemed to have more significant benefits from the spa stay to improve PA and, in general, their active lifestyle, compared to the other two BMI categories. The leisure-time PA could be favoured by the facilities offered by the resorts, free time, and a greater predisposition to being active during the spa stay.

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Keywords: Body mass index; body mass; active lifestyle; physical activity; thermal treatments; balneotherapy

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Application of Biomechanical Analysis to Latin Dancing

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Background: It is well known that dance can benefit physical, social, emotional, and cognitive functions both in healthy and pathological elderly subjects. However, most studies on dance techniques lack scientific rigor. A biomechanical approach based on optical stereophotogrammetry, and musculoskeletal modelling can be exploited to improve the quality of dance execution and reduce the risks of potential injuries [1].

Purpose: In this study, we analysed the biomechanical aspects of the so-called "Cuban Motion", a basic step of Latin dancing from bachata to mambo and cha-cha-cha. It is characterized by rotation of the hips around the spine, which is achieved by an alternate bending and straightening of the knees [2].

Material and Methods: The experimental acquisition of the movement, performed by an instructor of the Italian Dance Sport Federation, was conducted at the Biomechanics Laboratory of the "Sport and Anatomy" centre of the University of Pisa equipped with a Vicon analysis system, with eight infrared cameras and two force plates. Additionally, collected kinematic and kinetic data were analysed in OpenSim (an Open source software for musculoskeletal simulations [3]) to investigate muscle recruitment and coordination.

Results and Conclusions: The results showed the role of pelvis axial rotation (+30° fw- -17° bw) and list (-20° +20°) with a knee flexion up to 65°. The most activated muscles were the iliopsoas in the lower limbs and the external oblique for the upper body. A validation by EMG measurement is needed to confirm the main muscles involved during each movement.

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Keywords: Cuban motion movement, dance, biomechanics, muscles recruitment pattern, motion analysis.

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Neuromuscular evaluation of the masticatory muscles in patients with periodontal disease

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Periodontal disease is a chronic infectious and inflammatory condition affecting the supporting structures of the teeth, including gums, periodontal ligament, and alveolar bone. It is caused by bacterial plaque accumulation and, if left untreated, can lead to the destruction of support tissues and tooth mobility. Masticatory muscles, particularly masseters and temporalis, work in synergy with each other and are controlled by the central nervous system through neuromuscular pathways that lead to periodontal receptors. The consequent reduction of periodontal receptors and the impairment of the stability of dental occlusion may affect the function of these muscles, during clenching, chewing and other functions [1]. The aim of the study was to investigate, by means of standardized superficial electromyography (ssEMG) the alterations in neuromuscular activity of the masticatory muscles in patients with tooth mobility and inflammation of periodontal tissue.

In the present observational study, subjects affected by periodontal inflammation, periodontal bone loss, tooth mobility on at least three teeth -one molar- were enrolled. In these patients, the function of masseters and temporalis was screened and analysed in their clenching and kinematic activity with ssEMG. Activation Index, Torque Coefficient, Impact Coefficient, Asymmetry Index, Percentage Overlapping Coefficient (POC%), both for masseters and temporalis were computed and compared to data of healthy subjects from already published studies that applied the same methodology.

Seven subjects, 5 male and 2 female, (mean age 50 years ± 5.4) with stage III, grade B periodontitis were included. At the ssEMG analysis, the variations both in static -reduced clenching impact and poc- and dynamic conditions -decrease in the chewing impact while maintaining the masticatory rhythm and the ellipsis' area – was assessed.

Despite the maintenance of the masticatory rhythm, in patients with compromised periodontal health, the sensory input received from the receptors of the periodontal tissues appears to elicit "protective behaviours" in the masticatory system, both during maximum voluntary contraction and chewing activities (such as Impact and POC). The findings may provide insights into the interplay between the two entities: the condition of the periodontal structures and the generation of the masticatory muscles' contractive signals by the central nervous system. However further studies assessing changing of the neuromuscular activity after treatment of periodontal disease are needed.

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Keywords: ssEMG; masseters; temporalis; periodontal receptors

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Posture and visual effect: variability and correlation of plantar pressure and stabilometric parameters in open and closed eyes conditions

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The "Postural Control System" acts through biomechanical strategies and functional neuromuscular adaptations to maintain body balance in static and dynamic conditions [1]. Postural stability and body weight distribution can be affected by external sensory inputs, such as different visual stimuli. Little knowledge is available on the influence of visual receptors on stabilometric and plantar pressure parameters. The aim of this study was to analyse variability, correlations and changes of these parameters in open (OE) and closed (CE) eyes conditions.

31 stabilometric and plantar pressure parameters [2] were acquired in 20 young and healthy adults during baropodometric examination performed in bipedal standing in both visual conditions. Variability of parameters was evaluated via coefficient of variation, correlation analysis via Pearson R^2 and statistical differences via Wilcoxon test.

High intra-subject repeatability was found for all plantar pressure parameters and Center-of-Pressure (CoP)-speed (CV<40%) in OE and CE, while CoP-sway area (CoPsa) and Length Surface Function (LSF) showed larger variability (CV>50%). Mean and peak pressure at midfoot and total foot load showed the least number of significant correlations with other parameters in both visual conditions, whereas the Arch-Index and rearfoot load showed the largest number of significant correlations. Limb side significantly affected most parameters. A trend for larger LSF and for lower CoPsa, mean and peak pressures on right forefoot side were found in CE.

The present study provides a deeper insight into the associations between postural stability and foot load. Interesting postural adaptations, in particular with respect to different visual stimuli, the effect of the dominant side and the specific role of the midfoot [3] in balance control were highlighted.

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Keywords: posture; postural control; foot; baropodometry; stabilometry; pressure plate; visual control

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Executive Functions Assessment Based on Wireless EEG and 3D Gait Analysis During Dual-Task

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Different levels of cognitive inhibition activation during a dual task (cognitive and motor) execution [1] were detected by means of electroencephalography (EEG)[2]. Lightweight wireless EEG device, with eight channels and dry electrodes, was used to minimize interference during spontaneous walking assessed by 3D gait analysis. Aim of the present study was to investigate different levels of executive functions activation during walking by EEG.

Thirteen healthy subjects are included in this exploratory study, based on previous evidence showing the invariance of neurophysiological features on the activation of cognitive functions in dual-tasks with ageing [3].

Inhibition (Go-NoGo cognitive task) resulted more involved than working memory (NBack cognitive task) during ambulation as revealed by the variation in stride length and foot progression. A significant relation was found between the increase of relative power in the delta band at Fz and inhibition activation levels in both sitting and walking conditions. No significant EEG-trends emerged for working memory during walking. This study reinforces the hypothesis of the prevalent involvement of inhibition with respect to working memory during walking, until now based only on prefrontal functional near infrared spectroscopy (fNIRS) evidence and gait speed. Moreover, the foundations are laid for EEG-based monitoring of cognitive processes involved in gait.

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Keywords: Gait analysis; working memory; inhibition; EEG; dual task

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Physical performance: which factors can influence it?

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Three major chronotypes exist: Morning- (M-types), Neither- (N-types) and Evening-types (E-types). M-types tend to perform better in the morning, while E-types in the evening [1]. It has been shown that bad sleep habits tend to worsen physical performance [2], but it is still unclear the impact that such chronobiological variables could have on neuromuscular performance.

The current study aims to assess if chronotype, time session and sleep can influence physical performance. 13 participants (males=8, 26±4.5 yrs, 26.5±1.7 kg/m^2; females=5, 28.4±4.5 yrs, 26±3.5 kg/m^2) underwent an anthropometric assessment and filled in the reduced Morningness-Eveningness Questionnaire and Pittsburgh Sleep Quality Index. They performed 3 neuromuscular tests (Squat Jump-SJ, Counter Movement Jump-CMJ, Counter Movement Jump with swinging arms-CMJ_{SA}) on a Chronojump jump mat. The tests were carried out at 09:00 am, 01:30 pm and 06:00 pm, following a randomized order in 3 different days. From the 3 tests the jump height was automatically calculated as function of the flight time by the software provided by the jump mat manufacturer. Both the elastic and coordination indices [3] of performance were computed. Mixed ANOVA tested the interactions between chronotype, time session, sleep and physical performance, while a paired t-test assessed the intragroup comparisons.

The total sample (n=13, 26.9±4.8 yrs, 26.3±2.7 kg/m^2) was classified as M-types (n=4), N-types (n=6) and E-types (n=3), and Good Sleepers (n=5) and Bad Sleepers (n=8).

ANOVA analysis did not show any statistically significant difference. Paired t-test showed that, in the total sample, there was a difference between the elastic index obtained at 09:00 am compared to those obtained at 06:00 pm (6.2±6.8 *vs* 12.5±8.4 %, p= 0.005). The same difference was evident in the male (8.4±6.5 *vs* 15.4±8.9 %, p= 0.011) but not in the female sample.

The comparison of SJ, CMJ, and CMJ_{SA} height are worldwide methods to assess neuromuscular capability. The elastic index could be associated with a better ability to use the stretch-shortening cycle, generating more powerful movements. From this perspective, our results may suggest that the elastic index was higher at evening sessions, probably due to the habitual late afternoon/evening training that could act as an environmental synchronizer.

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Keywords: Chronotype; time session; sleep; physical performance; jump; biomechanics; anatomy movement

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Effects of acoustic and visual stimuli on Counter Movement Jump free arms assessed by a digital accelerometer sensor

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The ability to perform maximal vertical jumps significantly affects athletic feat in several sports, such as basketball, handball, and volleyball [1]. A few studies analyzed the effects of different sensory stimuli on Vertical Jump performance [2], [3]. To the best of our knowledge, no study quantitatively assessed the variations of spatial-temporal parameters during Counter Movement Jump (CMJ) free arms execution in different acoustic and visual stimuli conditions.

The aim of the present study is to explore the effects of the incentive and disincentive sensory stimuli on CMJ free arms performance via a digital accelerometer sensor.

Twenty young and competitive volleyball athletes were recruited and assessed using "BAIOBIT" (BTS, Milan) inertial sensor, during 5 trials, consisting of 3 CMJ free arms each. The athletes executed the sets, respectively, without sensory stimulus (NS), with incentive (IAS) and disincentive (DAS) acoustic stimulus, and with incentive (IVS) and disincentive (DVS) visual stimulus. Spatial-temporal parameters were computed. Moreover, statistical analysis was employed to test intra- and inter-subject variability and significant differences between different sensory stimuli.

The results showed low intra- and inter-subject variability for all spatial-temporal parameters (median CV < 10%); in particular, the lowest intra-subject variability was found for mean time of flight phase (CV < 3%) and the largest one for peak speed (5%< CV<10%). As far as the effect of different acoustic and visual stimuli, a trend for larger peak speed was found in IVS condition with respect to NS one (P value= 0.092).

The present study seems to underline a higher influence of visual stimulus on CMJ free arms. These findings may be useful during the training in order to improve the performance in the sport competition.

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Keywords: Accelerometer; Inertial Measurement Unit; Visual stimulus; acoustic stimulus; Counter Movement Jump; Volleyball

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Squat Test: Subjective vs. Objective Analysis

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The Squat Test is a functional movement test that is commonly used among strength and conditioning coaches for screening movement quality. Despite functional movement assessments are routinely used in sports to recognize the quality of movement and to identify athletes predisposed to future injury, however, to date, the visual (subjective) assessments are the most commonly used. The aim of the present study is to compare subjective and objective data obtained from two different squat test variations, namely the third (3P, hands on shoulders) and fifth (5P, overhead squat) position.

Eleven healthy male subjects (25.3 ± 4.5 years, 180.7 ± 6.0 cm height, 77.7 ± 5.4 kg weight) performed five repetitions both in 3P and 5P. Each session was video-recorded. Visual (subjective) data analysis was performed by five expert trainers. For the objective analysis, the Kinovea software and a multi-axis biomechanics force plates were used.

5P was more difficult to perform than 3P squat test variation (-5.8%, $p<0.0001$ total quality of movement subjective analysis), but the former allowed a better hip alignment (+3.1%, $p<0.001$ subjective analysis). In addition, 5P showed a greater knee flexion (+2.1%, $p<0.01$). Visual assessment of total movement quality was strongly correlated with those of knee ($R=0.94$, $p<0.0001$) and ankle movements ($R=0.93$, $p<0.0001$). A slight correlation was observed between visual and objective assessment of the balance of the strength expressed by the right to left lower limb ($R=0.42$, $p=0.0014$). The squat depth did not differ when performing the test either in the third or fifth position. An inverse correlation occurred between squat depth and visual assessment of knee movement ($r= -0.57$, $p<0.0001$).

The present study highlighted some differences between visual (subjective) and objective assessments. Despite the reliability of visual assessment in functional movement tests, such as the squat test, it appears to be increasingly necessary the support of objective assessment methods to improve the screening movement quality.

Keywords: Squat test; Functional movement test

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Outdoor motor activity to counteract sarcopenia in the elderly: protocol proposal

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Sarcopenia is the age-associated decline in muscle mass and strength that begins in about the fourth decade of life and is a major contributor to ill health and disability in the elderly.

Progressive loss of muscle mass and concomitant decline in muscle strength (dynapenia) are associated with a large and diverse group of diseases, including type 2 diabetes mellitus and cardiovascular disease, cause frailty and disability, increased risk of falls and fractures, loss of physical independence, cognitive decline, depression and therefore, in general, lower the quality of life.

The main factor in the sequence of events leading to sarcopenia is lack of physical activity. A high degree of inactivity has been shown to result in significant changes in muscle cross-sectional area, volume and mass, promoting metabolic dysfunction (anabolic resistance) and leading to reduced muscle function [1].

The main tool to counteract the advance of sarcopenia is therefore motor activity, even better if carried out outdoors.

Physical activity outdoors, compared to that carried out in closed spaces, has various benefits referable to the presence of vegetation, sunlight, air and weather conditions and promotes a greater degree of socialisation.

An activity suitable for the elderly, to be carried out outdoors, is Nordic walking.

Nordic walking is an activity involving specially designed poles used to push against the ground with each stride for the purpose of activating the upper body while walking. It is a more intensive form of walking using muscles of upper body and lower body. It is a safe, feasible and beneficial form of exercise training in older adults.

Normal walking utilizes muscles of the lower half of the body while nordic walking is a whole body activity that uses muscles of the back, arms, shoulder and neck. It reduces load on the lower body and helps people remain stable while walking. [2].

A motor protocol that combines aerobic activity, such as Nordic walking, which enhances endurance and muscle strength, and exercises that improve balance skills, lends itself perfectly to helping the elderly to counteract and stem sarcopenia, avoiding problems related to posture and balance which very often result in accidental falls.

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Keywords: Sarcopenia; outdoor; physical activity; nordic walking

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Trunk control may reduce ACL injury risk: kinematic assessments in personalised functional tests

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Anterior Cruciate Ligament (ACL) injury is a high-severity injury in sports, mostly occurring with no contact with other players [1]. During cuttings or landings, the ground impact with extended lower limb and knee dynamic valgus may lead to ACL lesions [2]. Functional landing tests are used to assess players' ACL injury risk by simulating the injury mechanisms. Many studies analysed the lower limb kinematics, not considering the potential influence of trunk on hazardous mechanisms. This study aimed to analyse trunk movements in 5 unilateral landing tests, investigating the influence of initial contact (IC) trunk position on knee kinematics in the first 100 ms after IC, high-risk time window [3].

57 amateur athletes (22.2±2.4 years) performed: drop-landing from a box with maintenance of balance (DL), or a secondary vertical (DV) or forward jump (DF); horizontal hop with maintenance of balance (HH) or with a secondary vertical jump (HJ) (drop-tests height: 20% subject's height, distance: 60% maximal horizontal jump; hop distance: 90% maximal horizontal jump). Trunk and knee kinematics was provided by a 9-camera motion capture system using 38 skin-mounted markers. The influence of IC trunk flexion (TF), lateral flexion (TL) and rotation (TR) on knee peak abduction and flexion (100 ms after IC), was assessed with 10 multiple regression models, 5 tests and 2 knee angles, (trunk angles=independent variables, knee angles=dependent variables, $p<0.05$).

Knee abduction regression models included TL with positive beta coefficient ($\beta>0$), and TR ($\beta>0$) was also included in DF ($R^2_{DL}=0.10$, $R^2_{DV}=0.10$, $R^2_{DF}=0.19$, $R^2_{HH}=0.08$, $R^2_{HJ}=0.09$). In knee flexion models, it has been found statistically significant regression models only for DL and DF ($R^2_{DL}=0.04$, $R^2_{DF}=0.04$), including TF ($\beta>0$), and DV ($R^2_{DV}=0.06$), including TF ($\beta>0$) and TL ($\beta<0$).

Knee kinematics seems to be affected by IC trunk position. Ipsilateral TL, associated to higher knee abduction, moves the ground reaction forces laterally with respect to the knee, increasing the ACL injury risk [4]. In drop-tests, a greater TF was positively associated to a larger knee flexion, reducing the injury risk. The influence of IC trunk on knee kinematics suggests the inclusion of core control exercises in preventive and rehabilitative programs, to avoid risky posture at the ground impact.

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Keywords: Anterior Cruciate Ligament; Landing; Kinematics; Trunk

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The positive effect of physical training on the motor neuron cell nucleus in the Ts65Dn mouse, a model of Down syndrome

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Down syndrome (DS), a disease based on the trisomy of chromosome 21, is characterized by intellectual disability as well as several pathological traits e.g., early aging and altered motor coordination.

Physical training or passive exercise has been found to be able to counteract, at least in part, motor impairment in DS subjects. In this study we used a widely accepted animal model of DS, the Ts65Dn mouse, to explore the ultrastructure of the medullary motor neuron cell nucleus, a marker of the cell functional state, and the effect thereupon of adapted physical training. Using trisomic and euploid TS65DN mice, either sedentary or treadmill trained, we exploited transmission electron microscopy, ultrastructural morphometry, and immunocytochemistry to carry out an in-depth structural investigation of the trisomy-related alteration(s) of nuclear constituents insofar these are known to vary their amount and distribution according to the functional state of the cell nucleus.

Results showed that trisomy alone has a limited impact on nuclear constituents; however, adapted physical training showed the capability to chronically stimulate pre-mRNA transcription and processing activity in motor neuron nuclei of both euploid and trisomic mice, although to a lesser extent in the latter. These findings represent a contribution to the understanding of mechanisms involved in the positive effect of physical activity in DS.

Keywords: Down syndrome; Ts65Dn mouse; cell nucleus; motor neuron; physical exercise; transmission electron microscopy

Morfologia, attività settoria e strategie didattiche

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Clinical Anatomy of Maxillary Sinus: Vascularization and Underwood Septa investigations

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Assessing the vascular supply and the presence of Underwood septa in maxillary sinus is fundamental for surgical intervention on these structures[1,2].

The study aims to provide updated information for clinicians to better understand the vascular and surgical complications associated with the maxillary sinus.

Two groups of cone-beam computed tomographies (CBCT) of patients referred to the Dental Clinic of University "G. d'Annunzio" Chieti-Pescara were collected to study the alveolar-antral artery (AAa) and the presence of Underwood septa in the maxillary sinus. For the evaluation of the AAa, 100 CBCT scans from patients with a clinical condition of edentulism of posterior teeth were analyzed, examining the presence of the AAa in the antral wall of the maxillary sinus, the length of the arterial pathway, the height of the maxillary bone crest, and the size of the branches of the AAa.

The results showed that the intraosseous variant of the AAa was present in 100% of the cases. There were no statistically significant differences between the presence of the AAa on the left and right sides. The length and height measurements varied slightly between the two sides, but the differences were not statistically significant. The average size of the branches of the AAa was around 1.07mm.

Regarding the presence of Underwood septa, a sample of 100 CBCT scans was collected, and a total of 200 maxillary sinuses were evaluated. The results showed that 19% of the examined sample had septa in the maxillary sinus, with no significant gender differences in the presence of septa.

In conclusion, this study provides insights into the clinical anatomy of the maxillary sinus, specifically the arterial blood supply and the presence of Underwood septa. The findings can be valuable for clinicians in understanding the vascular and surgical complications associated with the maxillary sinus and for improving surgical planning in procedures such as sinus floor augmentation and implant placement.

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Keywords: infraortbital artery; posterior superior alveolar artery; Underwood septa

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Anatomical variants of the left vertebral artery: a case report

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During the last academic year, routine dissections on human cadaver for Medical School students of University of Bologna, some anatomical variants affecting the vertebral arteries (VA) were noted.

Vertebral artery anatomical variations in origin, course, size, and branches are quite common.

Regarding the size, the diameter of the left vertebral artery (VA) is slightly greater than the right one. In 20-40% of cases one VA is clearly smaller than the contralateral one. According to literature, VA was defined as hypoplastic when its diameter was less than 2-3.5 mm (1).

The frequency of this last variation is 2-6% (2), but the clinical significance of VAH remains controversial.

Left vertebral artery hypoplasia (VAH) was highlighted in a 78-year-old female Caucasian cadaver, whose cause of death was acute coronary syndrome.

This abnormal artery had an outer diameter of 1 mm whereas the right one had an outer diameter of 5.5 mm. The two arteries joined to form the basilar artery (BA) far above the bulbopontine sulcus probably due to the tortuosity of the right VA.

Concerning the branching, the anterior spinal artery arises from the medial surface of the right VA only and not from the union of two branches of each VA; furthermore, the right posterior inferior cerebellar artery (PICA) was slightly greater than the left.

Some authors considered marked asymmetry of VA as a harmless variant due to the absence of clinical symptoms and signs of vertebrobasilar insufficiency and its frequent association with a compensatory flow in contralateral non hypoplastic VA (3).

Different observations suggested that a hypoplastic VA could be a predisposing factor of posterior circulation stroke (2), even in young patients (4). Other studies reported a positive correlation between VAH/associated alterations in posterior circulation and migraine with aura and vertigo (5,6). Moreover, the tortuosity of BA is related to the development of aneurysms (7).

Concerning the branching, the origin of the anterior spinal artery from the right VA only corresponded to Type II described by Lanzino (8). In this case, the knowledge of the different pattern of origin and course of the proximal portion of the anterior spinal artery is critical for planning and safely performing endovascular and surgical procedures involving the distal VA, the vertebrobasilar junction and the ventral medulla (8).

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Keywords: anatomical variation; vertebral artery; vertebral artery hypoplasia; posterior circulation

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Nephron endowment evaluation in pre-transplantation kidneys: a digital histomorphometric methodology for future machine learning development approach

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Machine learning (ML) could significantly estimate nephron endowment (NE) in kidney assessment for transplantation (1). Knowing NE is crucial for assessing renal function and predicting the risks after transplant. ML algorithms can analyze clinical and laboratory data to predict NE in each kidney before transplantation. This preliminary study aims to measure NE, using digital pathology (2), facilitating the development of future ML algorithms for automatic NE recognition and computation.

40 kidneys removed from 26 brain-dead donors (12 female, 14 male, mean age 62.6 years) were collected. Computed tomography was performed on kidneys to compute stereologically the volume of the cortical portion (3). Tissue sections from 6 standardized kidney areas were harvested and processed for histomorphometric analysis. The stereological method was employed in each section to evaluate the glomerular density, size, and volume. Then, NE was computed for each kidney by multiplying the cortical volume by glomerular density and dividing this result by the average glomerular volume. The number of functional (FG) and atrophic (AG) glomeruli were also assessed.

The mean volume of the cortical portion was 48.2cm^3 ± 17.7. Glomeruli represented 9.53% ± 2.19 of the cortex. The mean glomerular volume resulted in 5.75E-6 ± 2.04E-06 cm^3. The glomerular number was: NE 858.550 ± 373.245, FG 650.606 ± 310.400, AG 207.944 ± 99.837. The number of glomeruli was higher in the superior area of the kidney than in the medium and inferior areas but not significantly.

Cutting-edge computation strategies, based on the foundations of traditional histomorphometry, could be useful to define ML algorithms for enhanced diagnostics and a deeper comprehension of the intricate mechanisms and influential factors underlying kidney transplantation outcomes, improving the process of patient care.

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Keywords: Histomorphometry; machine learning; transplantation; kidney

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The puzzle of facial asymmetry: is the dentition stage a contributing factor?

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Facial asymmetry is intrinsic in all persons since birth. Its degree during growth might vary depending on several factors, and its quantification in healthy is crucial for its understanding. Still, the literature lacks a comprehensive definition of normal ranges.

We analyzed facial asymmetry in three-dimensional (3D) stereophotogrammetric facial models of 122 males of two age groups, 9-14 and 15-20 years old (N=61 for both groups), each chosen as representative of a dentition (mixed and permanent) to provide normal ranges, and to survey the influence of dentition on asymmetry degree. We examined asymmetries of trigeminal thirds (upper-UT, middle-MT and lower-LT thirds) by a 3D superimposition procedure with the VAM software (Vectra 3D Analysis Module): the left side was mirrored and superimposed on the right one and asymmetry expressed as root mean square (RMS). A two-ways ANOVA analysis assessed significant differences ($p<0.05$) between age groups and facial thirds. Pearson correlation was applied to assess the correlation between age and asymmetry.

RMS values ranged from 0.23 to 1.14mm and those of younger proved more symmetrical ($p<0.001$). UT RMS values proved higher, followed by LT and MT but significant differences were found only between UT and MT in both groups. No statistically significant interactions were found between the factors age x facial third ($p=0.92$) while a significant positive correlation ($p<0.001$) was found between ages and asymmetry of any facial third.

Faces of males from 4 to 20 years always show a slight asymmetry degree but its clinical significance is challenging since no agreement on tolerance of asymmetry is reported in literature. In both groups, the greater asymmetry of soft tissues related to mandible and its dentition may reflect its articular mobility, extended post-natal development, and presence of a preferential chewing side.

Back to the starting question, we can only speculate that the dentition type influences facial asymmetry: though a significant positive correlation was found, this might respond to other factors too. Yet, the significant differences among the two age groups give no space to misunderstanding: facial asymmetry increases from 9 to 20 years old. What are the factors influencing this trend, whether dentition or skeletal growth or other, remains an open question.

Keywords: facial asymmetry; three-dimensional imaging; 3D facial analysis; dentition; reference values; anthropometry; pediatric population; stereophotogrammetry

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Anatomical variants of sphenoid bone: is there a correlation among different types of accessory foramina?

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Sphenoid bone is one of the anatomical structures most affected by anatomical variants, especially for what concerns accessory foramina (1,2), such as foramen meningo-orbitale, foramen of Vesalius, canaliculus innominatus and palatovaginal canal. In literature several studies report prevalence of each variant, but very few information is available about the possible correlation among different variants.

Three hundred maxillofacial CT-scans of patients (equally divided among males and females) were retrospectively assessed to verify the presence of foramen meningo-orbitale, foramen of Vesalius, canaliculus innominatus and palatovaginal canal. Possible differences in prevalence of each accessory foramen according to sex and possible correlation among different variants were assessed through Chi-square test ($p<0.01$).

Overall prevalence of foramen meningo-orbitale, foramen of Vesalius, canaliculus innominatus and palatovaginal canal was 30.7%, 67.7%, 14.0% and 35.3% respectively, without any difference according to sex ($p>0.01$). A significant positive correlation was found between foramen of Vesalius and canaliculus innominatus, both in males and in females ($p<0.01$). In detail, subjects with canaliculus innominatus in 85.7%-100.0% of cases show also foramen of Vesalius, independently from sex and side.

The present study for the first time found a correlation between foramen of Vesalius and canaliculus innominatus: results may be useful for improving surgical procedures of the cranial base.

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Keywords: sphenoid bone; foramen meningo-orbitale; foramen of Vesalius; canaliculus innominatus; palatovaginal canal; CT-scan

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The anatomical variations of the sciatic nerve during dissection classes: three different case reports

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The sciatic nerve (SN), also called the ischiatic nerve, is the longest and largest nerve in the human body. This wide nerve originates from the lumbosacral plexus (L4-S3) roots, and exits from the pelvis closely below the piriformis muscle (PM). However, many variations of the SN are present concerning its relationship with the PM. Indeed, in this regard, these anatomical variations were recorded and classified by Beaton and Anson since the first one in 1937 [1].

In the present study, we describe two different case reports of the anatomical variations of the SN and its relationship with the PM, observed during dissection classes at the ICLO Teaching and Research Center (Verona, Italy), both in male and female bodies aged between 62 and 78 years old.

In the first case (a 62 year old male reported), it was observed the SN divided into the common peroneal nerve (CPN) and the tibial nerve (TN) that, respectively, passed between and below the PM. On the other hand, in the second case (observed in a 70 year old female), even if the SN was split into its two components, the CPN passed above the head of the PM, and the TN passed through the PM. Eventually, the third case of a 78 year old female, another case reported the TN emerging below the PM, and the CNP passed through the PM.

The anatomical variability knowledge of any part of the human body, observed by different techniques ranging from imaging to surgery or autopsy, is fundamental. Focusing on the SN and its relationship with the PM, understanding this variability is essential to effectively treat the patient with the appropriate medical approach. For example, piriformis syndrome is a rare syndrome which is one of the main causes of nondiscogenic sciatica, causing severe low back pain due to entrapment of SN, either by hypertrophy or by inflammation of the PM [2]. Thus, the anatomical features of each variation might be useful for the surgical treatment of piriformis syndrome.

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Keywords: anatomical variations; sciatic nerve; ischiatic nerve; tibial nerve; common peroneal nerve; piriformis muscle; piriformis syndrome

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Bland & Altman Plot in facial anthropometry: has the quest for clinical quantification ended?

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Diverse methods are used for facial asymmetry assessment, and comparisons are needed to evaluate their comparability and interchangeability. The concordance of two methods can be assessed by the Bland-Altman plot [1] that needs an *a priori* establishment of the clinical significance of the results, i.e. the amount of "discordance" between the methods we can accept without causing clinically different results.

This study aims at comparing two methods for quantifying facial asymmetry to establish if their measurements are comparable and the methods interchangeable. The sample consists of 134 Caucasoid individuals' 3D stereophotogrammetric facial models (67 males and 67 females, 5 to 95 years old). The hemiface and facial thirds asymmetry, after selection according to two common 3D methods, is assessed as Root Mean Square (RMS).

The first method [2] entails the selection of the two hemifaces and related facial thirds (upper, middle and lower) according to the trigeminal innervation. The left facial area is mirrored and superimposed onto the right one, and the RMS is calculated. The second method [3,4] involves the division into thirds, without splitting the two sides, according to two horizontal planes: one passes through the inner canthi, and the other through the cheilions. The facial areas are mirrored and overlapped on their original version to calculate the RMS.

The concordance between the RMS values obtained with the two methods was evaluated through the Bland-Altman plot by estimating biases, upper (U) and lower (L) limits of agreement (LOA).

A bias of -0.07 mm was found disregarding the facial area, with U-LOA=0.24 mm and L-LOA=-0.39 mm. MT and LT had worse values: bias= -0.22 mm, U-LOA= 0.09 mm, L-LOA= -0.53 mm, for MT; bias= 0.03 mm, U-LOA= 0.33 mm, L-LOA= -0.27 mm, for LT. Hemiface and UT showed better results: bias= -0.07 mm, U-LOA=0.11, L-LOA= -0.26 mm for hemiface; bias= -0.03 mm, U-LOA= 0.20 mm, L-LOA= -0.26 mm for UT.

Literature lacks a definition of the clinically significant limits of facial asymmetry, hindering the practical interpretation of "asymmetric" faces in healthy individuals. As a result, no conclusive remarks are possible on the interchangeability of the two methods but clues on the need to provide clinical practical data first, otherwise statistics might become a trap for research.

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Keywords: 3D analysis; Anatomical structures; Facial asymmetry; Stereophotogrammetry

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Histochemical and immunohistochemical study on an innovative hydrogel-based scaffold for bone regeneration

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Bone regeneration is a challenge in biomedicine to accelerate and promote the patient's recovery after a trauma or a defined pathology involving the skeleton. Bioactive gelatin-chitosan hydrogel scaffolds have been previously evaluated for their ability to support the activation of the bone marrow human mesenchymal stromal cells (BM-hMSCs) to osteogenic lineages [1-3].

The aim of the present research is to evaluate an innovative and promising three-dimensional bioresorbable scaffold of gelatin-chitosan hydrogel with a stiff poly(lactic acid) lattice structure, to ensure temporary mechanical support, as an environment for the proliferation and differentiation of seeded BM-hMSCs in the presence of fetal bovine serum or human platelet lysate with or without the osteogenic medium.

The scaffolds were analyzed after 28 days, included in paraffin, cut at the microtome, and evaluated both histochemically, with Von Kossa staining for calcium deposits and Masson Trichrome for extracellular matrix, and immunohistochemically for Osteocalcin (OSC), Osteopontin (OSP) and Bone Morphogenetic Protein 2 (BMP2), all important molecules in bone matrix deposition.

The results showed the presence of calcium deposits in the mash of the scaffold, the cells appeared well integrated with a positivity for OSC and BMP2, but not for OSP, showing a clear differentiation state as osteoblasts. The positivity for BMP2, in particular, indicated a remodeling activity for the extracellular matrix.

In conclusion, this innovative scaffold could be a promising approach to bone regenerative medicine.

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Keywords: hydrogel-based scaffold; bone regeneration

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Effects of differently treated titanium surfaces on the viability and osteogenic differentiation of hPDLSCs

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Titanium (Ti) is the most commonly used biomaterial for dental implants. Osseointegration is accomplished through three main events: early immune-inflammatory response, angiogenesis, and osteogenesis [1]. The latter consists in the recruitment of mesenchymal stem cells (MSCs) and their differentiation into osteoblasts around the implant surface. The surface properties have a great influence on both attachment and osteodifferentiation of MSCs. In this work, we have determined the viability, morphology and osteogenic differentiation of cells with different Ti surfaces. To this aim, MSCs isolated from human healthy periodontal ligament (hPDLSCs) were seeded on Ti disks (medical grade 5) previously treated with seven different procedures for surface modification: sandblasted with Al_2O_3 particles, polished, anodization-colored yellow and grey Ti, polished and anodized yellow Ti, polished and anodized grey Ti, double acid etched Ti. A 10-mm-diameter Ti bar was machined into 0.6-mm-thick disks. Standard 24-well tissue culture plates (polystyrene) were used as control surfaces.

hPDLSCs were harvested from healthy third molars and then cultured in MesenPro medium with low serum (2% FBS) containing growth supplement. A commercial osteogenic medium was used for osteogenic induction. For each experiment, cells derived from at least three different samples at passages 3–5 were used. hPDLSCs morphology and osteogenic differentiation were assessed by immunofluorescence analysis of cells stained with phalloidin and Collagen I, respectively. Cell proliferation was evaluated by counting the DAPI-stained nuclei.

Our observations show that Ti surface treatments included in this study modulate both cell proliferation and osteogenic differentiation. All Ti surfaces are able to induce the synthesis and the secretion of Collagen I, suggesting the stimulation of osteogenesis. Further analyses are in progress to relate these results to the physico-chemical properties of the Ti surfaces.

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Keywords: periodontal ligament; titanium surface; osteogenic differentiation

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Polylevolysine and Fibronectin-Loaded Nano-Hydroxyapatite/ PGLA/ Dextran-Based Scaffolds for Improving Bone Regeneration: A Histomorphometric in Animal Study

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The regeneration of large bone defects remains challenging and requires biocompatible scaffolds with osteoconductive and osteoinductive properties. This study aimed to evaluate the effectiveness of a nano-hydroxyapatite(nano-HA)/PGLA/dextran-based scaffold (Control scaffold) loaded with Polylevolysine (PLL) (1) and fibronectin (FN) (Test scaffold) for bone regeneration in critical-size tibial defects in the ovine model.

After the physicochemical properties characterization, the scaffolds were implanted in the tibiae of ten adult sheep, creating monocortical defects. The animals were divided into two groups and euthanized at 3 and 6 months post-surgery. The defects were filled with either the control scaffold or the test scaffold. The distal defects served as negative control sites without any scaffold. Histological and histomorphometric analyses assessed bone ingrowth and residual material 3 and 6 months after surgery.

Both scaffolds showed submicrometric crystals on surfaces and within the structures at scanning electron microscopy. The light microscope revealed macroscopic 3-dimensional porous architecture. X-ray diffraction confirmed the presence of highly crystalline nano-HA. Histological and histomorphometric evaluations showed new bone formation and residual biomaterial within the defects at 3 months, with no significant differences between the scaffolds. At 6 months, the defects filled with the test scaffold exhibited significantly higher levels of regenerated bone compared to the control scaffold, while the residual material was higher in the control scaffold.

The nano-HA/PGLA/dextran-based scaffold loaded with PLL and FN shows promise in promoting bone regeneration in critical-size defects, demonstrating a balanced combination of regenerative and resorbable properties that support new bone deposition.

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Keywords: bone regeneration; hydroxyapatite; fibronectin; polylevolysine; critical-size bone defect

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Physiological mechanical stimulation improves the maturation and organization of three-dimensional cardiac bioconstructs *in vitro*

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Cardiac tissue engineering (CTE) aims at restoring cellular and extracellular compartments of myocardium by combining cells and scaffolds. It has been recently demonstrated that decellularized human skin (d-HuSk) can be used to prepare three-dimensional scaffolds that support survival and differentiation of human resident cardiac progenitor cells (hCPCs). We hypothesize that the intrinsic elasticity of the dermal matrix might be exploited in a cyclic stretch bioreactor to further stimulate the maturation of cardiac bioconstruct. To test our hypothesis, we prepared d-HuSk scaffolds that were repopulated with hCPCs and cultured in static conditions for one week. Afterwards, we transferred bioconstructs to a bioreactor applying a cardiac-like cyclic stretch (10% strain at 1 Hz) for seven days. The effects of the mechanical stimulation were then evaluated by SEM, histochemistry, immunofluorescence, and gene expression analyses, using bioconstructs cultured in static conditions as a reference. The histological analysis showed that hCPCs organized into a multilayered tissue on the surface of d-HuSk in both conditions. However, cyclic stretch promoted hCPC migration towards the inner layers of the dermal matrix and a well-ordered alignment of cells, mainly orthogonal to the direction of stretch and parallel to each other. Additionally, gene expression profile including genes typical of main cardiac cell lineages showed the up-regulation of transcripts for cardiac myocytes, smooth muscle, endothelial and mesenchymal cells in hCPCs cultured on d-HuSk in dynamic conditions, thus providing evidence of further maturation of stretched cardiac bioconstructs. Collectively, our results support the evidence that bioconstruct mechanical stimulation boosts the differentiation of hCPCs and strengthen the suitability of d-HuSk as a scaffold for CTE.

Keywords: decellularized extracellular matrix; cardiac progenitor cells; bioreactor; tissue engineering; regenerative medicine

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Polydeoxyribonucleotide-based gel for horizontal bone regeneration: histological assessment of human ground sections

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The combination of hyaluronic acid and polynucleotides (HA-PN) seems to promote cellular vitality, to improve tissue trophism and support more efficient repair [1]. The present study aimed to analyze the morphological and morphometric features of newly formed bone after regeneration using graft particles embedded in HA-PN based gel.

Horizontal bone regeneration of the alveolar ridge was performed. Biopsy samples were taken from 6 patients during implant placement 5.5 months after surgery. Samples were fixed in formalin and treated for histological examination without prior decalcification. Each block was cut longitudinally (Micromet Remet, Bologna, Italy), and two slices were obtained from each sample. Each slice was thinned to about 100μm, polished and stained with Toluidine Blue and Pyronin Yellow. Magnifications were finally acquired with the optical microscope. Histomorphometric analysis was conducted with stereological methodology applying a grid of 100 test points positioned on histological images acquired at a total magnification of 200 X. Tissue was categorized in: 1) lamellar bone tissue, 2) woven bone tissue, 3) osteoid matrix, 4) residual particles of grafting material, and 5) medullary spaces. Each component has been quantified as a percentage relative to the total tissue area present in the section (tissue fractions), providing tissue composition of biopsy samples.

At the morphological observation, the formation of highly mineralized and well-organized vital bone appeared in all specimens. The remnant particles of the grafted biomaterial resulted perfectly integrated in the regenerated tissue. Few resorption lacunae were observed. Numerous large blood vessels were evident in the medullary spaces, and the inflammatory infiltrate was sparse. Histomorphometric analyses showed that on average the 41,2% (SD 12,62%) of the tissue is represented by newly mineralized bone, in more detail 14,3% (SD 9,8%) is lamellar bone tissue and 22,8% (SD 8,3%) is woven bone tissue. The 4,1% (SD 4,3%) of the entire tissue is represented by osteoid matrix, 29.9% (SD 11,5%) is represented by grafting material and 28,9% (SD 5,2%) is represented by medullary spaces.

Data of the present study show that at 5.5 months of healing, the use of demineralized bovine bone particles mixed with hyaluronic acid/polynucleotides for the regeneration of atrophic alveolar crest induces the regeneration of mature and well-organized bone tissue without arising anomalous inflammatory response. Further qualitative and quantitative data are necessary to confirm these preliminary values.

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Keywords: alveolar bone; tissue regeneration; hyaluronic acid; histomorphometry

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In vitro evaluation of the protective activity of T4N5 liposomal lotion on dermal fibroblasts from Xeroderma Pigmentosum

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In the last decade, numerous strategies enhancing the photoprotection against UV-induced damage have been tested in subjects affected by Xeroderma Pigmentosum (XP), a rare autosomal recessive genodermatosis characterized by an enzymatic deficiency of the DNA nucleotide excision repair (NER) system. The onset of symptoms is observed in most cases at the age of 1-2 years, while in 5% of patients, it occurs at 14 years. Initial symptoms include an abnormal reaction to sunlight with the development of severe burns and persistent erythema after minimal exposure to sunlight. Premalignant actinic keratosis, benign and malignant skin neoplasms (melanomas, basal cell or squamous cell carcinomas), ocular (photophobia, conjunctivitis, ectropion, keratitis, and neoplasms) and neurologic (progressive cognitive impairment, sensorineural deafness) abnormalities may develop. To date, there is no specific therapy, and the treatment of XP patients includes i) sun exposure and sun protection practices with sunscreens, sunglasses, hats, suitable clothes, and plastic UV filters; ii) the early detection and removal of skin tumors. Among the medical devices that guarantee photoprotection, the liposome formulation that combines a very high skin protection factor (SPF) (50+) and the enzyme photolyase or T4 endonuclease V (T4N5) has demonstrated great efficacy in the repair of photoproducts DNA [cyclobutane pyrimidine dimer (CPD), (6-4) photoproducts (6-4 PP)]. In the present work, we report the *in vitro* characterization by flow cytometry, confocal microscopy, and Nanolive 3D imaging technology of the biological activity of a custom-made formulation of T4N5-loaded liposomes in cultured dermal fibroblasts isolated from an Italian child affected by XP and protected for four years, starting from the diagnosis of the disease, to a photoprotection based on photolyase and T4N5.

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Resident human cardiac progenitor cells remodel *in vitro* the scaffolds of decellularized human dermal matrix prepared for cardiac tissue engineering

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The role played by the ECM in myocardium integrity and homeostasis is emphasized by cardiac tissue engineering (CTE) focus on biomaterials capable to recapitulate ECM properties. However, since ECM abundance of chemical and physical cues are still an unmet goal, decellularized ECM has rapidly emerged as an attractive biomaterial. We have recently provided evidence in support of the decellularized human skin (d-HuSk) as a promising biomaterial for CTE. Since in vivo ECM is in dynamic equilibrium with cells, the scope of the present study is to evaluate in vitro whether d-HuSk is remodeled by resident human cardiac progenitor cells (hCPCs). To this purpose, human skin from patients undergoing abdominoplasty was decellularized and cryosectioned to prepare three-dimensional scaffolds, while hCPCs were isolated from explanted human hearts, seeded on d-HuSk scaffolds, and cultured for two weeks. Afterwards, hCPCs on d-HuSk were induced to myogenic differentiation and cultured for two additional weeks. The absence of nuclei in H&E staining and a DNA content of 9.138 ± 1.009 ng/mg of tissue in d-HuSk scaffolds confirmed the decellularization efficiency. Real-time PCR analyses revealed a significant up-regulation in transcription of differentiating and mature cardiomyocyte genes, along with retained transcription of mesenchymal, endothelial, and smooth muscle cell genes in hCPCs induced to differentiate. SEM analysis showed a better organized network of elastic fibers in hCPC-d-HuSk bioconstructs than in acellular d-HuSk, which resulted richer in collagen thicker fibers. Furthermore, quantitative analyses of scaffold composition revealed in hCPC-d-HuSk the significant increase of GAGs and decrease of collagen and elastin, along with a remarkable higher fluorescence mean intensity for fibronectin and tenascin, thus confirming the hypothesis that d-HuSk is actively restructured and remodeled by hCPCs.

Keywords: decellularized extracellular matrix; cardiac progenitor cells; tissue engineering; regenerative medicine

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Osteogenic potential of bioactive composite scaffolds for the regeneration of critical-sized mandibular defects

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Mandibular bone has complex anatomy and functions, making it challenging to design bone scaffolds suitable for its regeneration. However, additive manufacturing (AM) is a promising technique, as it provides high control over scaffold architecture, and allows the processing of a variety of osteoinductive materials.

Herein, we developed AM composite scaffolds, made of polycaprolactone (PCL) and a high content (40% w/w) of tricalcium phosphate (TCP). To obtain more bone-like architectures, scaffolds with diamond and star-shaped pores were printed. Interestingly, the diamond shape displayed higher compressive modulus than the star one. The proliferation and differentiation of human mesenchymal stromal cells (hMSCs) were tested *in vitro*, comparing the effect of the two shapes and the two materials (PCL alone vs PCL+TCP). Firstly, the seeding efficiency using a dextran-based seeding method was evaluated, reaching values around 40% for all the samples. DNA quantification also showed good proliferation of the cells after 28 days (3.38 ± 0.15 μg of DNA corresponding to around 4 million cells). Secondly, the expression of osteogenic markers was investigated. At early time points, alkaline phosphatase (ALP) reached values of 300-400 (A.U.)/DNA μg, and immunostaining showed that Runx2 was highly expressed. In both cases, no significant differences between the composite and the polymeric scaffolds were observed. At later time points, collagen production was evaluated with hydroxyproline assay, and SEM images confirmed the deposition of the matrix. Additionally, collagen I and osteocalcin expression was also revealed with immunostaining. Finally, characteristic osteogenic markers (Runx2, ALP, osteocalcin, BMP-2, collagen I) were investigated through qPCR, displaying osteogenic differentiation of cells over 28 days. Our results showed the potential of scaffolds with high ceramic content and biomimetic shapes to promote osteogenic differentiation of hMSCs. In the future, surface treatments will be applied to further enhance the osteoinductivity of the scaffolds, increasing the particle release in the culture medium.

Keywords: mandible; critical-sized bone defects; bone tissue engineering; composite; additive manufacturing; osteogenesis; regenerative medicine

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Innovative 3D-printed titanium specimens favor a balanced modulation of inflammation in DPSCs during liposome-triggered mineralization

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Developing customized titanium specimens, with modified and innovative surfaces, is a suitable strategy to overcome implant failure in regenerative dentistry. Additionally, a faster and more efficient osteogenic commitment assists tissue regeneration. To investigate the interplay between inflammation and differentiation upon implantation, Dental Pulp Stem Cells (DPSCs) were cultured on 3D-printed titanium owning an internal open cell form [1], administering osteogenic factors by a liposomal formulation (LipoMix) [2] compared to traditional delivery with differentiation medium (DM). Osteogenic differentiation, matrix mineralization, angiogenesis, cell adhesion and inflammation responses were evaluated. Our results demonstrate that LipoMix enhances cell proliferation and adhesion, as revealed by in-creased integrin β1 expression. Mineralized matrix deposition, SP7 gene expression, Collagen I release and Alkaline Phosphatase activity all appear significantly increased in LipoMix condition. Additionally, the redox-sensitive transcription factor Nrf2 is overexpressed at the earliest experimental times, triggering the catalase activity. Data reported confirm that internal topography and post-production treatments on titanium surfaces dynamically and positively condition the DPSC progress towards the osteogenic phenotype, moreover, the combination with LipoMix fastens the positive modulation of inflammation under osteogenic conditions. Therefore, the development of customized surfaces with specific porosity and controlled features, along with the administration of differentiating factors enclosed in a liposomal delivery system, could represent a promising and innovative tool in regenerative dentistry.

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Keywords: 3-D printed titanium; Dental Pulp Stem Cells; inflammation; liposomes; mineralization

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Pig decellularized peripheral nerve supports fiber regeneration

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When a peripheral nerve injury with a large defect occurs, end to end suture is not possible and conduit is not enough to obtain good results. Allograft could be an alternative, but nerves from donors frequently cause immunogenic response. Several authors are looking for the correct way to decellularize nerves preserving both the extracellular matrix (ECM) and basal lamina to improve nerve regeneration. Over the past years, the decellularization of peripheral nerves has been used to provide a natural substrate composed of nerve ECM without the resident cells to prevent the host immune response when transplanted in patients.

The aim of this study is to evaluate the efficacy of a new decellularization protocol, that is both easy to prepare and allow to preserve the nerve ECM, on pig nerves, specie that has attracted considerable interest, since some tissues derived from it are already used in clinical practice showing excellent biocompatibility.

Decellularized pig nerves have been analyzed for demonstrating the effectiveness in removing cells and preserving ECM. Then, they have been implanted for repairing rat median nerves demostrating their ability to sustain peripheral nerve regeneration. One month after injury and repair of the rat median nerve, regenerating fibers have colonized the graft suggesting a promising use for repairing severe nerve lesions.

Keywords: acellular allograft; decellularization; large-gap repair; peripheral nerve injuries; pig.

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Treating critical-size bone defects with Pal-OS® powder: exploiting the CAM assay model to evaluate the angiogenic potential of different 3D printed scaffolds for bone tissue engineering

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Critical-Size Defects (CSD) are severe bone lesions in which the natural regeneration system is unable to repair spontaneously due to the dimensions of the bone gap: thus, they still represent one of the major challenges in the orthopedic field. The current approaches do not consider the angiogenic-dependent component of regeneration, which cannot occur without a previous proper development of blood vessels in the site of the lesion. The full reconstruction of a functional vascular network represents a necessary pre-requisite to induce and support reparative osteogenesis. For this reason, the angiogenic potential of different materials was tested by exploiting the chick Chorio-Allantoic Membrane (CAM) assay. This simple, economic and well-known model allowed the selection of the materials with the highest angiogenic response, overcoming the limitations of 2D cell cultures and animal experimentation[1]. In this study, we focused on the Pal-OS® powder, derived from the mechanical fragmentation of the scleral ossicles (SOs), subjected to trademark filing (Pal-OS®) and to patent, respectively. SOs are naturally decellularized bony slice whose cells before undergoing massive apoptosis secrete molecules that remain entrapped into the bone matrix; such bone segments are located in protruding eyeballs of lower vertebrates and could be extracted from poultry waste. Since they had displayed angiogenic properties both *in vitro* and *in vivo*[2], probably due to osteocyte-derived molecules entrapped in the bone matrix, we decided to chop up the SO (obtaining a powder with a defined granulometry) in order to favor the release of the molecules inducing vascular proliferation. Different Pal-OS® powder percentages mixed with different hydrogels were 3D printed by the micro-extrusion technique. Micro-extrusion bioprinting is a versatile solution to blend and print different materials together, while maintaining high precision in the final constructs. The hydrogel scaffolds containing Pal-OS® powder with different percentages were thereby tested *in ovo* to evaluate their angiogenic ability. The final purpose was to select the scaffold composition with the highest vascular response to recover critical-size bone defects, while using a sustainable experimental method (the CAM assay) in line with animal welfare.

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Keywords: Extrusion printing; scleral ossicles; 3D scaffolds; CAM assay; angiogenic potential; bone tissue engineering; critical-size bone defects

Tessuti epiteliali e connettivi. Transizione epitelio mesenchima nell'organogenesi e nella carcinogenesi

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Antisteatotic and antifibrotic activity of a new synthetic GPR120 receptor agonist in mice overfed with a "Western-style diet"

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Several factors such as genetic variants, obesity, and unhealthy lifestyle with unbalanced diets are correlated to the pathogenesis of nonalcoholic fatty liver disease (NAFLD). This is a complex disorder ranging from simple steatosis to inflammation (nonalcoholic fatty liver, NAFL), worsening into fibrosis (nonalcoholic steatohepatitis, NASH). The driving injury is the alteration of lipids homeostasis, leading to an abnormal accumulation of triglycerides and other lipids in the liver parenchyma. Currently, no resolutive pharmacological treatment for NAFLD is available, and the only therapeutic approach is represented by a healthy diet and physical exercise, although many reports suggest the potential druggability of specific receptors for fatty acids to improve liver fitness in many metabolic contexts. In this study, we targeted the G-protein-coupled receptor 120/free fatty acid receptor 4 (GPR120/FFAR4), with a new and specific synthetic agonist, GprA. We monitored the progression of liver damage in mice fed for different periods (26 weeks, NAFL stage; and 30 weeks, NASH stage), with a "Western-style" diet (WSD) and treated daily by oral gavage with 3 doses (30-60-90 mg/Kg) of GprA. The analyses highlighted that GprA is able to reduce signs of steatosis at the two time-point of WSD-fed mice, both at the histological and molecular levels, when used at 90mg/Kg. Furthermore, in NASH-like treated mice, GprA is also effective in the reduction of collagen maturation and deposition, fibrosis development, and connective tissue growth factor (CTGF) expression. Altogether, our data confirm the central role of FFAR4 in the context of NAFL/NASH onset and progression and revealed that GprA could represent an interesting candidate for the development of a new therapeutic approach in NAFL treatment.

Keywords: GPRs; diet; NAFLD; steatosis; liver fibrosis

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Effect of lidocaine on collagen turnover pathways in human tenocytes: an *in vitro* study

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Peritendinous injection of local anaesthetics is commonly performed in the treatment of tendinopathies. Previous studies have shown that local anaesthetics are toxic for many cell types, including neurons, myocytes and chondrocytes [1,2], but there has been little investigation of their effects on tenocytes [3,4].

The aim of this study was to characterize the effect of lidocaine on collagen turnover pathways in cultured human tenocytes.

For this purpose, primary cell cultures were obtained from fragments of human gluteal tendons of 6 patients (mean age 56.2 ± 10.5 years, 3 males and 3 females) without any gluteal tendon pathology and undergoing total hip replacement through an anterior approach. Tenocytes at the 5th passage were treated for 24h with 0.1% lidocaine (LD group) or served as untreated controls (CT).

COL-I gene expression was significantly up-regulated in LD-treated tenocytes but the levels of COL-I secreted in the cell culture supernatants was similar in CT and LD-treated samples. Gene expression for LOX and LH2b, responsible for collagen cross linking, were not affected by LD treatment as well as MMP-1 levels in cell supernatants. By contrast, TIMP-1 and TIMP-2 mRNA levels were induced ($p=0.07$ and $p<0.05$, respectively).

Although previous studies demonstrated that lidocaine exerts a toxic effect on tenocytes by decreasing cell proliferation and inducing oxidative stress, our preliminary results suggest that collagen turnover pathways are not significantly affected by lidocaine administration. However, further experiments using different doses of lidocaine and evaluations at longer time points are needed to confirm this pattern and to understand if the effects of lidocaine at the cellular level could affect tendon homeostasis and biomechanical properties.

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Keywords: tenocytes; collagen turnover

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Liver alterations in the mouse model of autism spectrum disorders: a possible beneficial effect of melatonin

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Autism spectrum disorders (ASD) is a neurodevelopmental disease characterized by repetitive or stereotyped behaviors, impairments in social interaction and restricted interests. In ASD, it has been shown an alteration in the brain development, mood and cognitive functions, as well as a dysregulation of gut-brain crosstalk, that can affect metabolic homeostasis [1]. In addition, the establishment of oxidative stress and inflammatory conditions seem to play a crucial role in the pathophysiology of ASD [2]. The liver is one of the most important organs that helps regulate vital events by detecting and eliminating pathogens that enter the body through the intestines. To date, the liver involvement in the pathophysiology of ASD has been poorly investigated.

We used as autistic model BTBR T+Itpr3tf/J mice (or BTBR mice) treated and not treated by melatonin, an endogenous indoleamine that has many functions [3]. The control group shows a normal liver morphology; the hepatocytes display central nucleus and regular shape with a diffuse localization of the glycogen in the cytoplasm. In opposition, even if the hepatic cytoarchitecture is preserved, BTBR mice hepatocytes showed very low presence of glycogen droplet, an increase of binucleated hepatocytes number and a decrease in the nuclei diameter. In BTBR mice treated by melatonin we observed a decrease in the number of binucleated hepatocytes and an increase in the nuclei diameter compared with BTBR mice. We analysed oxidative stress and inflammation by immunohistochemical evaluation of many related markers such as HO-1, IL-1β, SOD-1 and CAT, and observed variation in the expression of these markers among the different groups of mice. Furthermore, ASD, such as a plethora of liver pathological conditions, should be characterized by an alteration in the lipid peroxidation, so we have also studied molecular pathways through regulatory networks associated with ferroptosis [4].

Evidence obtained from morphological and immunohistochemical analysis suggests liver involvement in autism spectrum disorders and a possible beneficial function of melatonin in improving autistic-induced liver changes in mice. These findings could open the way for new therapeutic strategies.

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Keywords: liver; autism spectrum disorders; oxidative stress; inflammation; melatonin

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Photodynamic Effects of Different Light-Colored Radiations on Mechanobiology of Human Skin Cells: Viability, Migration and Extracellular Matrix Deposition

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Skin cells contain various light-absorbing pigments, known as chromophores, that can absorb different wavelengths of light. In addition, several biomolecules can act as light absorbers, such as nucleic acids and carrier molecules. These biomolecules play crucial roles in various cellular processes and can undergo structural changes or generate reactive species, potentially leading to cellular responses or damage.

Understanding the interactions between light stimuli and cell behaviors is important to study the effects of light on cells and skin tissues, and clarifying the mechanisms provides insights into the potential risks or benefits associated with different wavelengths of light.

The objective of this research was to examine the effects of different light-colored radiations on adult human skin cells and the resulting photodynamic changes. We conducted the study using an in vitro model, which involved a keratinocyte cell line (HaCaT) and a fibroblast cell line (HDF) [1]. Our aim was to explore the potential mechanisms triggered by these radiations, including both degenerative and proliferative processes.

The cells were exposed to two specific light wavelengths: 530 nm and 780 nm, with fluence values of 1.5 J/cm^2 and 3 J/cm^2 respectively [2].

In terms of cell viability, we observed an increase in viability among HaCaT treated with both the wavelengths, compared to the control group. However, no significant changes were observed in HDF cells. Interestingly, HDF cells exhibited an enhanced migration rate when exposed to the 780 nm wavelength and fluence 3 J/cm^2, compared to the control, while the migration rate of HaCaT cells remained unchanged. Regarding cell proliferation, no notable differences were observed in either keratinocytes or fibroblasts.

ECM was also examined. Some of the key components of the ECM are fibronectin and collagen. Using immunofluorescence analysis, we assessed the ECM in HDF cells by examining the levels of type I collagen and fibronectin [3]. The goal of this analysis was to understand the behaviour of irradiated cells and determine whether there was an increase in regenerative capacity, knowing that type I collagen and fibronectin are critical for maintaining the structural integrity of the ECM and promoting cell adhesion and migration, processes that are important for tissue regeneration.

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Keywords: Mechanobiology; color light radiation; remodeling ECM; fibronectin; collagen type I; keratinocyte cell line (HaCaT); fibroblast cell line (HDF)

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Epithelial cell adhesion modulation *via* hsa-microRNA-1249-3p/ Homeobox A13 Axis

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The intercellular adhesion is a key function for epithelial cells. The fundamental mechanisms relying on epithelial cell adhesion have been partially uncovered. Recent evidence indicates this process under the microRNAs (miRNAs) regulation. Hsa-microRNA-1249-3p (hsa-miR-1249-3p) plays a role in the epithelial mesenchymal transition in carcinoma cells, but its biological function in epithelial cells is unknown. The purpose of this study was to investigate the functional role of hsa-miR-1249-3p in epithelial cell adhesion. The hsa-miR-1249-3p expression was evaluated by droplet digital PCR in epithelial HaCaT and NCTC cells and in cervical carcinoma SiHa, CaSki and HeLa cells, used as control. Hsa-miR-1249-3p mimic and inhibitor transfections in HaCaT cells were carried out to assess hsa-miR1249-3p regulatory function on Homeobox A13 (HOXA13) target gene and its downstream gene β-catenin. Upon transfections, cell proliferation, colony forming potential, migration and apoptosis were assessed. The expression of the cell adhesion marker E-cadherin was tested. Hsa-miR-1249-3p expression was higher in epithelial cells compared to carcinoma cells. Hsa-miR-1249-3p inhibition prompted the expression of HOXA13 which, in turn, favored the expression of β-catenin paralleled with a downregulation of the adhesion marker E-cadherin. An increased colony forming capability in miR-inhibited cells was shown, while no change was detected in cell proliferation, migration, and apoptosis. Epithelial cell adhesion might be under regulation of hsa-miR-1249-3p by modulating HOXA13 gene expression and its downstream gene β-catenin. Our study provides novel insights into the miRNA-based mechanisms which control epithelial cell adhesion.

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Immediate Effects of Extracorporeal Shock Wave Therapy in Fascial Fibroblasts: An In Vitro Study

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Extracorporeal shockwaves (ESWs) are acoustic waves that induce a mechanical wave that passes through the cell compartment by cavitational effects, where cellular reply is proportional to the energy used. They are used in the treatment of soft tissue injuries, but their role in the treatment of myofascial pain has not yet been demonstrated.

The aim of this study was to investigate changes in cell biology of fibroblasts derived from deep/muscular fascia following treatment with ESWs.

Primary fascial fibroblasts were collected from small samples of human fascia lata of the thigh of three volunteer patients (two men, one woman) during orthopedic surgery, and put in culture. These cells were exposed to 100 impulses of 0.05 mJ/mm2 with a frequency of 2.5 Hz, using 3D-printed support. This study demonstrated for the first time that ESWs can lead to in vitro production of hyaluronan-rich vesicles immediately after the treatment. At 1, 4, and 24 h after treatment, Alcian blue and Toluidine blue staining; immunocytochemistry to detect hyaluronic acid binding protein (HABP), collagen I, and collagen III; and transmission electron microscopy demonstrated that these vesicles are rich in hyaluronan and collagen I and III.

The diameter of these vesicles was assessed, highlighting a small size at 1 h after ESW treatment, whereas at 4 and 24 h, they had an increase in the size.

According to Dunn's multiple comparisons test, the diameter comparison between the different times showed a statistically significant difference for 1 h vs. 4 h ($p < 0.01$) and for 1 h vs. 24 h ($p < 0.0001$), whereas it did not show a statistically significant difference for 4 h vs. 24 h ($p > 0.05$). Particularly evident was the release of hyaluronan-rich vesicles, collagen-I, and collagen-III starting at 1 h, with an increase at 4 h and maintenance by 24 h.

These findings demonstrate that, in a model of human fascial fibroblasts, ESW treatment can promote ECM remodelling, as well as synthetic activity, over a short time period, with effects visible by the first hour that are maintained for up to 24 h, suggesting that the clinical benefits and timing of this therapy can be explained by enhanced efficacy in ECM remodeling.This study indicates that fascial fibroblasts are metabolically "activated" by ESW treatment and significantly induce the synthesis and release of collagen types I and III and HA, compared with untreated cells, in a fast modality. Future studies may shed more light on how different doses of focused ESW treatment, and other types of ESWs, are able to stimulate fascial fibroblasts, thus modulating the therapeutic effect for musculoskeletal dysfunctions and myofascial pain.

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Keywords: Extracorporeal shockwaves (ESWs); fascial fibroblasts; deep fascia; remodelling; extracellular matrix

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Blood supply to the superficial fascia of the abdomen: an anatomical study

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The superficial fascia of the anterior abdominal wall was the first superficial fascia to be studied. Antonio Scarpa (1752–1832) described it as a membranous layer that extends into the subcutaneous fat tissue in the first and second editions (1809, 1823) of his famous texts regarding the inguinal hernia.

The aim of this study was to examine data demonstrating that Scarpa's fascia, a superficial fascia of the anterior abdominal wall, is a vascularized tissue.

Specimens of the fascia of seven volunteers undergoing abdominoplasty surgical procedures at the Plastic Surgery Unit of the University of Padova Medical Center were collected. Fractal analysis and quantitative assessment of the vascular network of the fascia was carried out, exploiting the presence of blood in the vessels. Each sample was divided and processed for histological/immunohistochemical analysis (into 5 micron-paraffin embedded sections and cryo-sectioned free-floating samples) as well as for electron microscopy study.

A rich vascular pattern forming a fine, dense meshwork with an area percentage of 6.20% ± 2.10% von Willebrand factor stained vessels was noted in all the specimens of the fascia examined; the area percentage of the αSMA-stained vessels was 2.93% ± 1.80%. The diameters of the vessels fell between the 13 and 65 μm range; the network was composed of arteries, veins, capillaries and lymphatic segments.

Topological results showed that the vascular network within Scarpa's fascia is well branched (segments: 6615 ± 3070 and 8.40 ± 3.40 per mm2; crossing points: 3092 ± 1490 and 3.40 ± 1.90 per mm2). Fractal analysis (fractal dimension = 1.063 ± 0.10; lacunarity = 0.60 ± 0.10) revealed that this particular vascular network has an optimal spatial distribution and homogeneity occupying the entire space of the superficial fascia.

In conclusion, the study's results confirm that Scarpa's fascia is a highly vascularized tissue and they have clarified the types of vessels, topological and fractal parameters characterizing the network. Moreover, they contribute to a better understanding of the difference between the vascularization of the superficial fascia and the rest of the subcutaneous tissue and, accordingly, explain the benefits of the preservation of Scarpa's fascia during abdominoplasty. Future studies should continue to examine the vascularization of the superficial fascia given its implications for reconstructive surgery and pain management.

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Keywords: superficial fascia; Scarpa's fascia; vascularization; arteries; veins; capillaries; lymphatics

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PPAR-Gamma coordinates EMT, AGE, and senescence signaling and mitigates the intestinal fibrosis in IBDs

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Inflammatory bowel disease (IBD), represents a wide range of intestinal disorders that commonly lead to an anomalous production and deposition of extracellular matrix (ECM) proteins and consequently to fibrosis. Despite much progress made in the last twenty years in the treatment of IBD, once the fibrogenesis process is onset, the only resolutive treatment for IBD complications is still the surgical approach. The main driver of fibrosis and epithelial-mesenchymal transition (EMT) is the transforming growth factor beta (TGF-β), as shown by the anti-fibrotic action exerted by several molecules able to modulate its signaling such as peroxisome proliferator-activated receptor (PPAR)-γ and its agonists. We investigated the contribution of AGE/RAGE (advanced glycation end products/receptor of AGEs), the senescence pathways, other than the EMT in the context of the complex pathophysiology of IBD. We evaluated human biopsies from control and patients in remission stage of IBD, and a mouse model of colitis dextran sodium sulphate (DSS)-induced, treated or not with GED (PPAR-gamma-agonist), or 5-aminosalicylic acid (5-ASA), a reference drug commonly used in the IBD treatment. In the human samples, we highlighted an increase in EMT markers (β-catenin, E-cadherin, vimentin), AGE/RAGE signaling, and senescence (β-gal, MMP1, IL-1β) pathways, compared to controls. Coherently, we found overexpression of the same pathways in DSS-treated mice. Surprisingly, the GED can reduce all the pro-fibrotic signaling, often more efficiently than 5-ASA. Our data suggest that a molecule able to act simultaneously on multiple pathways involved in the fibrogenesis process could be a valid target candidate for IBD treatment. In this context, PPAR-γ activation, enhanced by an agonist such as GED, may represent a good strategy for mitigating IBD progression.

Keywords: IBD; intestine; inflammation; fibrosis; senescence; β-galactosidase; AGE; RAGE; EMT; PPAR-γ

VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI SIAI, 2022

SIAI Società Italiana di Anatomia e Istologia

VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI DELLA SOCIETÀ ITALIANA DI ANATOMIA E ISTOLOGIA (SIAI) TENUTASI PRESSO LA SALA ELETTRA DEL PALAZZO DELLA SALUTE-MUSME, IN PADOVA, E ONLINE SUL LINK RISERVATO AGLI ISCRITTI.

In data 15 Settembre 2022, alle ore 17:30, in seconda convocazione, ha avuto luogo, in presenza, presso la Sala Elettra del Palazzo della Salute-MUSME e online sul link riservato agli iscritti, l'Assemblea Generale dei Soci della Società Italiana di Anatomia e Istologia per discutere il seguente Ordine del Giorno:

1. Comunicazioni del Presidente.
2. Relazione del Tesoriere sul rendiconto finanziario dell'anno 2021 e sulla previsione finanziaria per l'anno 2023. Relazione dei Revisori dei Conti.
3. Elezione Membri e Presidente del Collegio dei Probiviri.
4. Attività dei Collegi dei Docenti di Anatomia Umana e di Istologia ed Embriologia. Relazioni dei Presidenti o dei loro Delegati.
5. Aggiornamento sull'Italian Journal of Anatomy and Embryology.
6. Aggiornamento sul sito web della SIAI.
7. Assegnazione Premio alla Carriera.
8. Assegnazione Premi Ricercatori under 40.
9. Assegnazione Premio Migliore Comunicazione Orale.
10. Assegnazione Premi Poster.
11. Prossimi Congressi Nazionali SIAI: proposte temi di relazione.
12. Istituzione Borsa di Studio SIAI.
13. Proposte di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.
14. Commemorazione Soci Scomparsi.
15. Varie ed eventuali.

Presiede la riunione il Presidente della SIAI, Prof. Lucio Ildebrando Maria Cocco; funge da Segretario Verbalizzante la Prof. Gigliola Sica, Segretario della SIAI, collegata online.

La Segreteria Organizzativa ha informato il Presidente della SIAI, ai fini della Elezione del Presidente e dei Membri del Collegio dei Probiviri, che i Soci, in regola con il pagamento delle quote associative (e quindi aventi diritto al voto) sono 190 in presenza e 38 online, pari a 228 (**Allegati N.1** e **2**).
Il Presidente dichiara aperta l'Assemblea e procede alla discussione dell'Ordine del Giorno.

1. **Comunicazioni del Presidente.**

Il Presidente saluta i Soci presenti e quelli collegati online. Dichiara che non ci sono comunicazioni e passa alla discussione del secondo punto all'OdG.

2. **Relazione del Tesoriere sul rendiconto finanziario dell'anno 2021 e sulla previsione finanziaria per l'anno 2023. Relazione dei Revisori dei Conti.**

Il Presidente dà la parola al Prof. Gianpaolo Papaccio, Tesoriere della SIAI. Il Prof. Papaccio illustra all'Assemblea sia il Bilancio Consuntivo 2021 che la Previsione Finanziaria dell'anno 2023 con relative Relazioni di Accompagnamento (**Allegati N.3** e **4**). Tali Bilanci sono già stati inviati a tutti i Soci in data 8 Settembre 2022, in modo che essi potessero averne contezza ed analizzarli prima dell'Assemblea. Il Tesoriere informa l'Assemblea che i Revisori dei Conti hanno depositato la loro relazione (**Allegato N.5**) dalla quale si evince che non ci sono irregolarità nei Bilanci e che la SIAI è in attivo.
Dopo aver descritto le varie voci, il Tesoriere si è soffermato sulle parti salienti delle Relazioni di Accompagnamento, sottolineando che l'attivo è stato frutto anche di un lavoro di costante richiamo dei Soci alla puntualità nei pagamenti annuali e che un discreto numero degli stessi ha risposto positivamente.
Inoltre, c'è stato un piccolo numero di riammessi, pari a 4, che hanno pagato tutte le quote arretrate.
Nell'illustrare la Previsione Finanziaria per l'anno 2023, il Tesoriere fa rilevare che compare per la prima volta la voce relativa ai contributi liberali dei Soci per la pubblicazione di lavori sull'Italian Journal of Anatomy and Embryology. Tale voce è stata desunta dall'ammontare dei contributi liberali giunti al 31 Agosto 2022, in quanto il contratto stipulato dall'allora Presidente della SIAI, Prof. Giuseppe Anastasi, con la Firenze University Press (FUP), fa riferimento a due sole tipologie di fascicoli con un ammontare che varia rispetto alle pagine.
Questa fattispecie rende difficile potere prevedere effettivamente quante pagine potranno in un anno essere stampate in quanto il numero dipende ovviamente dai lavori pervenuti.
Pertanto, la medesima somma compare anche negli impegni in uscita, ma potrebbe variare sia in eccesso che in difetto.
Sicuramente, trattandosi di contributi che vengono versati alla SIAI e da questa poi riversati alla FUP, la SIAI non dovrebbe avere alcun problema. Resta il fatto che una definizione più precisa degli esborsi avrebbe aiutato a formulare una previsione più corretta.

Il Tesoriere, infine, si ritiene molto soddisfatto dell'andamento finanziario della Società e fa riferimento al fatto che si è venuto a creare un avanzo di cassa tale da poter permettere alla SIAI di aumentare le premialità nei confronti dei Soci.
Quindi passa all'elenco dei 26 Soci decaduti sulla base dell'Art. 10 del Regolamento (**Allegato N.6**)
Infine, propone di invitare i nuovi Soci a leggere bene Statuto e Regolamento e a non pagare la quota annuale se non a partire dal 1° Gennaio dell'anno successivo alla loro ratifica come nuovi Soci da parte dell'Assemblea annuale.
Altro annoso problema riguarda quei Soci che inviano le quote per il tramite dei Dipartimenti o Atenei: essi vengono invitati a provvedere personalmente indicando nome, cognome del Socio ed anno di riferimento della quota SIAI, ed a farsi eventualmente rimborsare subito dopo. In questo modo si eviterebbe di cercare per conto di chi sia stata pagata la quota e soprattutto si eviterebbero le richieste, da parte del personale amministrativo, di partita IVA e DURC che la nostra Associazione non possiede, in quanto Società Scientifica non a scopo di lucro.
L'Assemblea approva all'unanimità.

3. Elezione Membri e Presidente del Collegio dei Probiviri.

Il Presidente presenta il risultato delle Elezioni per il Collegio dei Probiviri, sulla base del Verbale della Commissione Elettorale, formata dai Proff. Antonella Camaioni, Pietro Gobbi e Ferdinando Paternostro (**Allegato N.7**; **Allegato N.01 e 02 al Verbale della Commissione Elettorale**).
Risultano eletti i Proff.
Raffaele De Caro (Presidente)
Francesco Cappello (Membro)
Marco Artico (Membro)
E' opportuno precisare che, come riportato nel Verbale della Commissione Elettorale, gli aventi diritto al voto erano 229, ma che uno di questi, pur avendo versato le quote associative, non si è presentato al Congresso; quindi, in realtà, gli aventi diritto erano 228.
L'Assemblea prende atto e ratifica i risultati delle Elezioni.

4. Attività dei Collegi dei Docenti di Anatomia Umana e di Istologia ed Embriologia. Relazioni dei Presidenti o dei loro Delegati.

Prende la parola il Prof. G. Papaccio che illustra l'attività del Collegio dei Docenti di Istologia ed Embriologia nel periodo Settembre 2021/Settembre 2022.
Il Collegio e la Giunta dello stesso, durante tale periodo, sono stati impegnati sia sul fronte di leggi e documenti, in particolare Legge Fregolent sui Ricercatori, poi sulla nuova Declaratoria del GSD, sia sul piano scientifico che divulgativo (terza missione).
Sui punti squisitamente accademici la SIAI è stata sempre tempestivamente informata nei vari Consigli Direttivi che si sono succeduti.

In particolare si sono avute:

- Discussioni su modifiche legge 240/2010 (proposte su reclutamento ed interlocuzioni con CUN, CRUI ed Intercollegio);
- Programmazione 2022/23 per SEDI;
- Documenti su PNRR;
- Lettere SIERR e discussione con SIAI-Direttivo;
- Legge Fregolent e poi attuale. Criticità contratti di ricerca;
- Convergenza con altri Collegi e Società Scientifiche su proposte migliorative;
- Accoglimento proposte Ministro, durante l'audizione con lo stesso, e su ASN.

Inoltre, insieme al Past President, Prof. G. Sica, si sta preparando la revisione dello Statuto e del Regolamento del Collegio.

Sul piano scientifico, oltre alla seduta scientifica dopo l'Assemblea annuale, è stato conferito il Premio Monesi-Rizzoli (seconda Edizione) ed i premi in memoria del Prof. Sergio Adamo, recentemente scomparso.

Infine è stata effettuata una giornata divulgativo-scientifica con relatori i Prof. Sen. Elena Cattaneo e Prof. Andrea Grignolio. A tale iniziativa sono stati invitati tutti i Soci SIAI via e-mail.

In itinere vi è l'iniziativa di organizzazione di eventi scientifico-divulgativi a Solomeo in collaborazione con la Fondazione Brunello e Federica Cucinelli.

Prende la parola il Prof. A. Montella che riferisce le ultime iniziative del Collegio dei Docenti di Anatomia Umana.

Il Collegio dei Docenti di Anatomia Umana ha eletto, con votazione a scrutinio segreto, il nuovo Direttivo in occasione dell'Assemblea tenutasi a Roma il 9 Aprile 2022. Sono stati eletti: Andrea Montella, Presidente; Mario Rende, Segretario; Veronica Macchi, Tesoriere; Guido Carpino, Sergio Castorina, Valentina de Felice e Stefano Ratti, Consiglieri. Nella stessa occasione La Prof.ssa Rita Rezzani è stata nominata Vicepresidente.

Nel corso degli ultimi mesi si sono tenute varie riunioni del Direttivo (22 Aprile, 26 Maggio, 7 Luglio, 1° Agosto e 7 Settembre) e, tra le varie attività svolte, si è provveduto a:

- Riordinare l'archivio Soci.
- Rinnovare la scheda di iscrizione al Collegio.
- Aggiornare le pagine web dedicate al Collegio nel sito SIAI.
- Aggiornare i dati relativi alla composizione dei Docenti BIO/16 di tutti le sedi universitarie.
- Trasmettere ai Soci il certificato di iscrizione annuale.
- Elaborare e trasmettere a tutti i Docenti in servizio un questionario per il censimento dell'attività didattica.
- Partecipare alle riunioni dell'Intercollegio di area medica.
- Elaborare la nuova Declaratoria del Gruppo Scientifico Disciplinare Anatomia Umana e Anatomia Clinica.

- Nell'Assemblea del Collegio del 14 Settembre a Padova, in occasione del 75° Congresso SIAI:

  È stata approvata all'unanimità la nuova Declaratoria del Gruppo Scientifico Disciplinare Anatomia Umana e Anatomia Clinica.

  È stato fatto il punto sulla applicazione della legge 10/2020 e sull'accreditamento delle sale settorie.

  Sono stati presentati i dati preliminari del censimento dell'attività didattica di Anatomia Umana.

  È stata avviata la costituzione di alcuni gruppi di lavoro.

5. Aggiornamento sull'Italian Journal of Anatomy and Embryology.

Il Presidente dà la parola al Prof. D. Ribatti, Direttore Scientifico dell'Italian Journal of Anatomy and Embryology,

Il Prof. Ribatti riferisce circa l'attività editoriale dell"Italian Journal of Anatomy and Embryology nel corso dell'anno 2022. Sono stati pubblicati il numero 1 del volume 125 (2021); il supplemento al volume 126 (2022) che raccoglie gli Abstract del Congresso di Padova ed è in corso di pubblicazione il numero 1 del volume 126 (2022). Il Prof. Ribatti ringrazia il Prof. F. Paternostro, Direttore Responsabile della rivista, ed i componenti del Consiglio Direttivo della Società Italiana di Anatomia e Istologia per il continuo supporto.

6. Aggiornamento sul sito web della SIAI.

La Prof. G. Sica aggiorna i convenuti sullo stato del Sito web della SIAI. A seguito di un lavoro molto lungo e meticoloso, si è provveduto all'aggiornamento dell'Elenco dei Soci, con una uniformità di presentazione di tutti i dati utili (qualifiche, affiliazioni, indirizzi, numeri di telefono, e-mail). Sono state inserite alcune parti nuove quali la garanzia di uguaglianza, diversità ed inclusività e promozione dell'eccellenza delle scienze morfologiche nonché alcune informazioni concernenti il Collegio dei Docenti di Anatomia Umana. Sono stati introdotti il Verbale dell'Assemblea Generale dei Soci del 2021, i Bilanci Consuntivi 2019 e 2020 e le Previsioni Finanziarie 2021 e 2022.

La Prof. Sica ricorda ai convenuti che è stato costituito un Comitato di Redazione del Sito, Comitato formato dalla Prof. Zecchi, dalla Prof. Sica e dalle Proff. Francesca Diomede e Giulia Ramazzotti. I Membri più giovani del Comitato di Redazione provvederanno alla traduzione in lingua inglese delle parti più importanti del Sito.

7. Assegnazione del Premio alla Carriera.

Il Presidente, sulla base della Relazione presentata dalla Commissione per l'Attribuzione del Premio alla Carriera, composta dai Proff. Monica Mattioli Belmonte (Presidente), Paolo Onori (Segretario), Carla Palumbo (Membro), e Oriana Trubiani (Membro), (**Allegato N.8**) proclama vincitrice la

Prof.ssa Elisabetta Falcieri. La Prof. Falcieri viene invitata a ritirare la targa ed esprime il suo compiacimento per il riconoscimento ricevuto ed il suo sentito ringraziamento alla Società.

8. Assegnazione Premi Ricercatori under 40.

Il Presidente, sulla base della Relazione presentata dal Comitato Scientifico (**Allegato N.9**) proclama vincitori dei due Premi ai Ricercatori under 40 i Proff. Grazia Maugeri e Stefano Ratti, che, invitati a salire sul podio, ringraziano vivamente la SIAI per il riconoscimento ricevuto.

9. Assegnazione del Premio per la Migliore Comunicazione Orale.

La Commissione per l'Assegnazione del Premio per la Migliore Comunicazione Orale è stata nominata nel corso del Consiglio Direttivo del 14 Settembre ed è composta dai Proff. Guido Macchiarelli, Marco Vitale, Sandra Zecchi, Monica Mattioli Belmonte, Mario Rende, Rita Rezzani, Lucia Manzoli e Stefania Montagnani. I risultati del lavoro della Commissione non sono ancora pervenuti e verranno comunicati successivamente a tutti i Soci.

10. Assegnazione Premi Poster.

La Commissione per l'Assegnazione dei Premi Poster è stata nominata nel corso del Consiglio Direttivo del 14 Settembre ed è composta dai Proff. Giuseppina Cutroneo, Emanuela Marcenaro, Simona Sivori e Claudio Sette. Allo stato, la Commissione non ha ancora concluso i suoi lavori; pertanto, i risultati verranno comunicati successivamente a tutti i Soci.

11. Prossimi Congressi Nazionali SIAI: proposte temi di relazione.

Per l'anno 2023, viene accolta la proposta delle Proff. Carla Palumbo e Sandra Marmiroli di organizzare il prossimo Congresso Nazionale presso l'Università di Modena-Reggio Emilia. La sede sarà a Modena e il Congresso si svolgerà dall'11 al 13 Settembre 2023. Viene anche accolta la proposta della Prof. Palumbo di commemorare con una lettura il cinquecentenario della nascita di Gabriele Falloppia.

Per il Congresso Nazionale del 2024, si propone la sede di Genova.

L'Assemblea approva.

Per quanto riguarda i temi di relazione, facendo riferimento a quanto riportato nel Regolamento, Art. 2 e 3, viene scelto come tema preferenziale: Morfologia traslazionale e clinica. Il secondo tema sarà: Aspetti morfo-funzionali della crescita cellulare.

L'Assemblea prende atto e ratifica.

12. Istituzione Borsa di Studio SIAI.

Nel Consiglio Direttivo del 18 Luglio 2022, è stata presentata ed approvata la proposta di istituzione di una borsa di Studio SIAI, di cui si allega il Bando (**Allegato N.10**). Su proposta dei Past President

SIAI, Proff. Gaudio ed Anastasi, la prima edizione della Borsa viene dedicata al Prof. Francesco Antonio Manzoli. La prossima edizione verrà dedicata al Prof. Giovanni Orlandini.
L'Assemblea prende atto e ratifica.

13. Proposte di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.

La Prof. Sica presenta l'Elenco dei nominativi di coloro che hanno presentato la domanda di ammissione in qualità di Soci Ordinari, domande che sono già state sottoposte al Consiglio Direttivo:

1) **Annese Tiziana**
2) **Barbaro Fulvio**
3) **Barbagallo Federica**
4) **Barbon Silvia**
5) **Brugnoli Federica**
6) **Canonico Barbara**
7) **Casciano Fabio**
8) **Ceci Ludovica**
9) **Chiappalupi Sara**
10) **Chiarini Francesca**
11) **D'Amico Daniela**
12) **De Santis Elena**
13) **De Stefano Alessia**
14) **Di Conza Giusy**
15) **Di Credico Andrea**
16) **Fazio Antonietta**
17) **Fedrigo Pierangelo**
18) **Gaggi Giulia**
19) **Grassilli Silvia**
20) **Isola Michela**
21) **Klinger Francesca**
22) **Mancuso Francesca**
23) **Marcuzzi Annalisa**
24) **Marvi Maria Vittoria**
25) **Masciarelli Silvia**
26) **Melloni Elisabetta**
27) **Neri Irene**
28) **Nittari Giulio**
29) **Overi Diletta**
30) **Perugini Jessica**

31) **Pistilli Alessandra**
32) **Ravera Silvia**
33) **Rimondi Erika**
34) **Romani Arianna**
35) **Romaniello Donatella**
36) **Rosa Irene**
37) **Simeone Pasquale**
38) **Stabile Anna Maria**
39) **Stocco Elena**
40) **Tamma Roberto**
41) **Tossetta Giovanni**
42) **Traini Enea**
43) **Zecca Antonio**

La Prof. Sica ricorda a tutti che il Prof. Roberto Di Primio, che ha ricevuto il Premio alla Carriera nel 2021, è stato inserito nell'Elenco dei Soci Emeriti presenti sul Sito. Nel medesimo Elenco verrà inserita la Prof.ssa Elisabetta Falcieri, Premio alla Carriera 2022.

14. Commemorazione Soci scomparsi.

Il Prof. Sergio Adamo viene commemorato dal Prof. Antonio Filippini. Il Prof. Sergio Morini viene commemorato dal Prof. Simone Carotti. Il Prof. Giovanni Orlandini viene commemorato dal Prof. Ferdinando Paternostro e il Prof. Giancarlo Panzica viene commemorato dal Prof. Alessandro Vercelli.

Nulla al punto **15) Varie ed eventuali**.

Il presente Verbale viene approvato seduta stante dall'Assemblea. La seduta assembleare si chiude alle ore 19:15.

Il Presidente	Il Segretario Verbalizzante	Il Tesoriere
Prof. Lucio Ildebrando Maria Cocco	Prof. Gigliola Sica	Prof. Gianpaolo Papaccio

Elezioni per il Collegio dei Probiviri della SIAI

Carissimi colleghi, riceverete una mail da Polyas E-Voting (voting@polyas.com), intitolata *Il Suo invito al voto: Elezioni dei probiviri della Società Italiana di Anatomia e Istologia di Sabi Work*

Tale mail contiene l'ID elettore e la Password per accedere al sito delle elezioni, il cui link sarà contenuto nella stessa mail.

Si aprirà una pagina in cui immettere ID elettore e la Password.

SIAI Società Italiana di Anatomia e Istologia

Per accedere al sistema inserisci le tue credenziali personali. Le trovi nell'invito al voto.

ID:

Password:

Accedi al sistema di voto

Un consiglio:
utilizza la versione più aggiornata del tuo browser e consenti l'utilizzo dei cookies per garantire un'ottima esperienza di voto. Qui trovi maggiori informazioni sui

Una volta introdotti ID e password, si preme sul tasto verde "Accedi al sistema di voto".

Si viene reindirizzati alla seguente pagina con le istruzioni:

SIAI Società Italiana di Anatomia e Istologia

Benvenuto/a nel sistema di votazione online di POLYAS!

Sei iscritto nel registro degli aventi diritto al voto e nel passaggio successivo hai la possibilità di fare la tua scelta. Dopo aver compilato la scheda o le schede, questa o queste verranno visualizzate in un ulteriore passaggio per la conferma. Questo ti dà l'opportunità di rivedere ed eventualmente correggere la tua selezione. Se non hai ancora cliccato sul pulsante "Esprimi voto definitivo", puoi annullare il tuo voto in qualsiasi momento. In questo caso, la tua selezione non verrà salvata. Potrai quindi registrarti nuovamente e votare in qualsiasi momento durante il periodo elettorale.

Tieni presente che i tuoi dati personali verranno trattati solo in forma anonima durante la votazione, al fine di tutelare la segretezza del voto. Ora verrai **reindirizzato alla cabina elettorale virtuale. Assicurati di votare senza essere osservato.**

Interrompi la votazione

Vota ora

Premendo sul tasto verde "Vota ora" si viene reindirizzati alla scheda di voto:

SIAI Società Italiana di Anatomia e Istologia

Esprimi il voto nel rispetto delle regole elettorali e dei voti a disposizione. Questa informazione è presente sulla scheda elettorale.

Numero massimo di voti esprimibili: 4

Numero massimo di voti esprimibili: 3

Votazioni per i membri del collegio dei probiviri della SIAI (3 voti esprimibili)

Marco Artico

Giacomina Brunetti

Francesco Cappello

Raffaele De Caro

Numero massimo di voti esprimibili: 1

Votazioni per il presidente del collegio dei probiviri (1 voto esprimibile)

Marco Artico

Giacomina Brunetti

Francesco Cappello

Raffaele De Caro

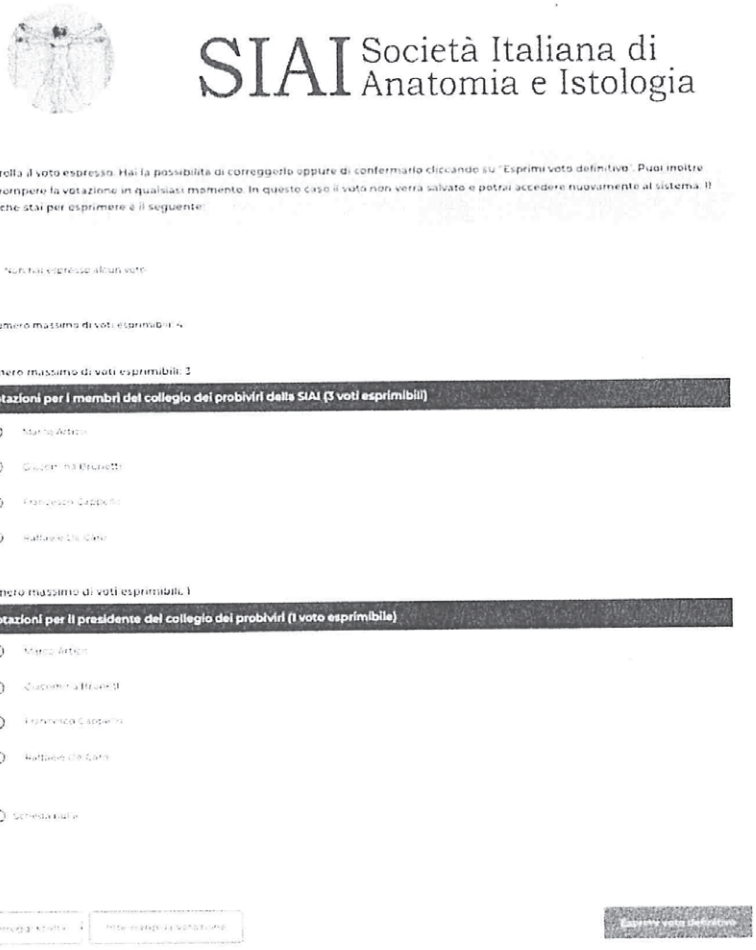
Scheda nulla

Interrompi la votazione

Verifica il tuo esercizio

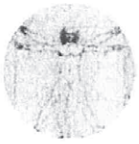
Si ricorda che si hanno a disposizione 3 voti per i membri del Collegio ed uno per il presidente dello stesso. Il presidente deve essere scelto tra i membri scelti per il Collegio.

Cliccando sul tasto "Verifica il voto espresso" si viene reindirizzati ad una schermata di verifica (nel caso qui sotto nessuna opzione è stata scelta).



Se si ravvisa di aver fatto un errore, si può cliccare sul tasto "Correggi scelta", tornando così alla pagina precedente dove si può ripetere la procedura di voto.
Se no, si può premere il tasto verde, concludendo così l'operazione di voto.

Si viene quindi reindirizzati ad una pagina di commiato.



SIAI Società Italiana di Anatomia e Istologia

Ottimo! Il tuo voto è stato registrato in modo anonimo e segreto nell'urna digitale!

Ora puoi chiudere questa finestra

Il risultato dell'elezione sarà comunicato dalla Commissione Elettorale durante l'assemblea generale della SIAI.
Verranno considerati eletti coloro che prendono il numero maggiore di voti. In caso di parità nei voti di preferenza sia per i Componenti che per il Presidente, la Commissione Elettorale, stando ai compiti previsti per il Collegio, prevede che viga il criterio preferenziale dell'anzianità nel ruolo della Docenza Universitaria e, ove ancora risulti una parità, preliminarmente il criterio dell'equilibrio di Genere e, in subordine, l'anzianità anagrafica.

Documentazione elettorale

Sabi Work

Elezioni dei probiviri della Società Italiana di Anatomia e Istologia

Dal 14-set-2022 17.00.00 al 15-set-2022 14.00.00

SIAI Società Italiana di Anatomia e Istologia

Panoramica

L'elezione "Elezioni dei probiviri della Società Italiana di Anatomia e Istologia" si è conclusa con successo. In questo documento troverà i risultati elettorali e le informazioni sulla partecipazione al voto.

TOTALE AVENTI DIRITTO AL VOTO	VOTI ESPRESSI	PARTECIPAZIONE
229	177	77,29%

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34131 Kassel
Germania

Risultati elettorali

Conteggio delle schede

Elettori	229
Schede elettorali consegnate	177
Schede elettorali valide	176
di cui lasciate bianche	0
Schede elettorali non valide	1
di cui non validi per trasgressione delle regole elettorali	0
di cui selezionati come scheda nulla	1

Regole

Numero massimo di voti per scheda: 4
Numero minimo di voti per scheda di voto: 0
Numero minimo di voti per lista: 0
Numero massimo di voti nell'elenco: 3
Numero minimo di voti di lista: 0
Numero massimo di voti per preferenza: 1
Numero minimo di voti per opzione: 0
"Voto non valido" previsto come opzione
Opzione "Astensione" non consentita
Schede bianche considerate valide.
Tipo di scheda elettorale: Elezioni con candidati o votazioni generiche
Non era consentito esprimere troppi voti.
Era consentito esprimere troppi pochi voti.

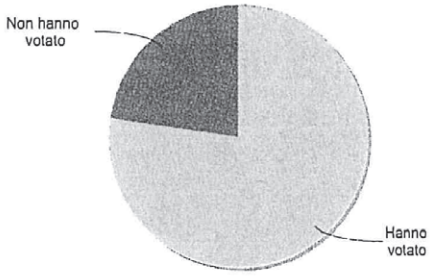
Votazioni per i membri del collegio dei probiviri della SIAI (3 voti esprimibili)	Voti
Marco Artico	97
Giacomina Brunetti	54
Francesco Cappello	114
Raffaele De Caro	140
Voti totali allinterno della lista	405

Votazioni per il presidente del collegio dei probiviri (1 voto esprimibile)	Voti
Marco Artico	9
Giacomina Brunetti	8
Francesco Cappello	48
Raffaele De Caro	108
Voti totali allinterno della lista	173

Partecipazione al voto

Tutti gli elettori

Totale 229
Hanno votato 177
Partecipazione 77,29%



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Germania

Allegato N.1

SOCI IN REGOLA CON IL PAGAMENTO DELLE QUOTE ASSOCIATIVE PRESENTI IN SEDE CONGRESSUALE

1. Alberti Paola
2. Albertin Giovanna
3. Amenta Francesco
4. Anastasi Giuseppe Pio
5. Bandiera Pasquale
6. Bartolini Desireè
7. Battistelli Michela
8. Bernardini Nunzia
9. Bertagnolo Valeria
10. Bertelli Eugenio
11. Bianchi Serena
12. Billi Anna Maria
13. Bonomini Francesca
14. Borsani Elisa
15. Boschetti Elisa
16. Boscolo Berto Rafael
17. Brun Paola
18. Cacciola Alberto
19. Camaioni Antonella
20. Campagnolo Luisa
21. Cappella Annalisa
22. Cappelletti Graziella
23. Cappellini Alessandra
24. Cappello Francesco
25. Carotti Simone
26. Carozzi Valentina Alda
27. Carpino Guido
28. Carubbi Cecilia
29. Caruso Bavisotto Celeste

30. Casini Arianna
31. Castorina Sergio
32. Cataldi Amelia
33. Cavaletti Guido Angelo
34. Ceccarelli Gabriele
35. Centofanti Antonio
36. Chellini Flaminia
37. Ciarmela Pasquapina
38. Cirillo Giovanni
39. Cocco Cristina
40. Cocco Lucio
41. Conconi Maria Teresa
42. Cordenonsi Michelangelo
43. Cutroneo Giuseppina
44. D'Agata Velia
45. De Caro Raffaele
46. De Luca Antonio
47. De Luca Ciro
48. Di Baldassarre Angela
49. Di Felice Valentina
50. Di Giacomo Viviana
51. Di Pietro Roberta
52. Di Sante Gabriele
53. Di Vito Anna
54. Dolci Susanna
55. Donetti Elena Bianca
56. Evangelisti Camilla
57. Fabrizi Cinzia
58. Faenza Irene
59. Fais Franco
60. Falcieri Elisabetta
61. Falconi Mirella
62. Falletta Paola
63. Favaloro Angelo
64. Fazi Francesco

65. Ferrucci Michela
66. Filippini Antonio
67. Fiume Roberta
68. Flace Paolo
69. Follo Matilde Yung
70. Franchitto Antonio
71. Gagliano Nicoletta
72. Geloso M. Concetta
73. Gesi Marco
74. Ghinassi Barbara
75. Giampietri Claudia
76. Gibelli Daniele Maria
77. Giordano Antonio
78. Giorgi Filippo Sean
79. Gobbi Pietro
80. Grimaldi Paola
81. Guerra Germano
82. Guidolin Diego
83. Gulisano Massimo
84. Isola Raffaella
85. Lauriola Mattia
86. Lazzeri Gloria
87. Lenzi Paola
88. Lofrumento Dario
89. Loreto Carla
90. Loy Francesco
91. Lucariello Angela
92. Macchi Veronica
93. Macchiarelli Guido
94. Magaudda Ludovico
95. Mancinelli Romina
96. Manzoli Lucia
97. Maraldi Tullia
98. Marcenaro Emanuela
99. Marchi Saverio

100. Marconi Guya Diletta
101. Mariani Giulia Adalgisa
102. Marmiroli Paola Lorena
103. Marmiroli Sandra
104. Marzioni Daniela
105. Masselli Elena
106. Mattioli Belmonte Monica
107. Maugeri Grazia
108. Michetti Fabrizio
109. Miglietta Selenia
110. Milani Daniela
111. Milardi Demetrio
112. Mirandola Prisco
113. Montagnani Stefania
114. Montella Andrea
115. Morucci Gabriele
116. Natale Gianfranco
117. Nicolini Gabriella
118. Noli Barbara
119. Nottola Stefania Annarita
120. Onori Paolo
121. Orciani Monia
122. Ortolani Fulvia
123. Palumbo Carla
124. Panaro Maria Antonietta
125. Pannarale Luigi
126. Papa Stefano
127. Papa Veronica
128. Papa Michele
129. Papaccio Gianpaolo
130. Paternostro Ferdinando
131. Pellegrini Gaia
132. Pergolizzi Simona
133. Perna Angelica
134. Pierdomenico Laura

135. Porro Chiara
136. Porzionato Andrea
137. Pozzi Giulia
138. Protasoni Marina
139. Quartu Marina
140. Ramazzotti Giulia
141. Rana Rosa Alba
142. Raspanti Mario
143. Ratti Stefano
144. Reguzzoni Marcella
145. Relucenti Michela
146. Rende Mario
147. Reno' Filippo
148. Rezzani Rita
149. Ribatti Domenico
150. Riuzzi Francesca
151. Riva Federica
152. Rizzo Giuseppina
153. Ruffoli Riccardo
154. Ruggeri Alessandra
155. Ryskalin Larisa
156. Salucci Sara
157. Sancilio Silvia
158. Santoro Antonietta
159. Sapte Elena
160. Sassoli Chiara
161. Scalia Federica
162. Scuteri Arianna
163. Secchiero Paola
164. Serra Maria Pina
165. Sette Claudio
166. Severi Ilenia
167. Sforza Chiarella
168. Simioni Carolina
169. Sivori Simona

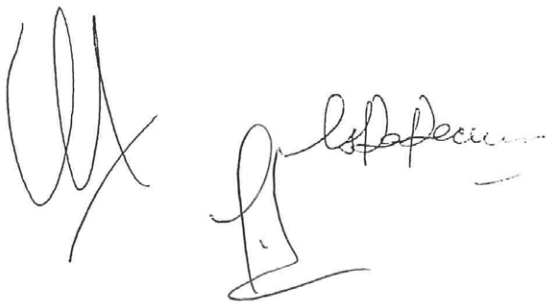
170. Sogos Valeria
171. Soldani Paola
172. Sorci Guglielmo
173. Sotgiu Maria Alessandra
174. Stecco Carla
175. Tacchetti Carlo
176. Tamagnone Luca
177. Tayebati Seyed Khosrow
178. Teti Gabriella
179. Tirino Virginia
180. Tomassoni Daniele
181. Tortorella Cinzia
182. Trubiani Oriana
183. Trucas Marcello
184. Vercelli Alessandro E.
185. Vermiglio Giovanna
186. Vitale Marco
187. Voltan Rebecca
188. Zancanaro Carlo
189. Zecchi Orlandini Sandra
190. Zingariello Maria

Allegato N.2

SOCI IN REGOLA CON IL PAGAMENTO DELLE QUOTE ASSOCIATIVE COLLEGATI ONLINE

1. Arnaboldi Francesca
2. Artico Marco
3. Barbiera Alessandra
4. Bertacchini Jessika
5. Branca Jacopo Junio Valerio
6. Brunetti Giacomina
7. Buffoli Barbara
8. Castrogiovanni Paola
9. Corsetti Giovanni
10. D'Amico Agata Grazia
11. Dellavia Claudia
12. Di Rosa Michelino
13. Favero Gaia
14. Franco Caterina
15. Giunta Salvatore
16. Guarnieri Giulia
17. Heyn Rosemarie
18. Imbesi Rosa Maria
19. Lombardo Claudia
20. Madaro Luca
21. Manetti Mirko
22. Marini Mirca
23. Moscato Stefania
24. Pacini Alessandra
25. Palmerini Maria Grazia
26. Piras Franca
27. Pompili Elena
28. Pompili Simona
29. Ronzoni Flavio Lorenzo

30. Scicchitano Bianca Maria
31. Sferra Roberta
32. Sica Gigliola
33. Sommariva Michele
34. Stacchiotti Alessandra
35. Taurone Samanta
36. Vaccaro Rosa
37. Vetuschi Antonella
38. Viscomi Maria Teresa



Allegato N.3

SIAI Società Italiana di Anatomia e Istologia

Bilancio consuntivo anno 2021

CAUSALE DELLE ENTRATE	ENTRATE Euro	CAUSALE DELLE USCITE	USCITE Euro
Quote sociali incassate nel corso dell'anno 2021 (n°711) 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	**€ 42.686,08**	**Elenco spese per attività statutarie**	
		Quota di Iscrizione al Congresso SIAI 2021 di n.1 Socio vincitore del premio poster	€ 100,00
		Contributo per l'organizzazione del Convegno G.I.S.N. anno 2021	€ 500,00
		Spese varie (mantenimento conto corrente bancario, spese bollo e commissioni bancarie ecc.), anno 2021	€ 609,64
		Pagamento F24 n. 2 quote: anno 2020 (€ 234,00), anno 2021 (€ 224,00)	€ 458,00
		Premio (2) Ricercatore under 40, anno 2021	€ 4.000,00
		Premio "Migliore comunicazione orale", anno 2021	€ 1.000,00
		Contributo Congresso Bologna, anno 2021	€ 5.000,00
		Quote associative (n.2) EFEM, anno 2020 (€ 390,00) ed anno 2021 (€ 390,00)	€ 780,00
		Quota associativa all' IFAA (International Federation of Associations of Anatomists), anno 2021	€ 144,63
		Elenco spese di funzionamento	
		Compenso per consulenza commercialista relativo al bilancio consuntivo 2019 e previsionale 2021	€ 1.200,00
		Spese per il funzionamento del Consiglio Direttivo, anno 2021	€ 2.333,00
TOTALE DELLE ENTRATE	**€ 42.686,08**	**TOTALE DELLE USCITE**	**€ 16.125,27**

AVANZO DELL'ESERCIZIO FINANZIARIO 2021	**€ 26.560,81**		

SIAI Società Italiana di Anatomia e Istologia

CAUSALE DELLE ENTRATE	ENTRATE Euro	CAUSALE DELLE USCITE	USCITE Euro
Saldo Conto Corrente Bancario al 31/12/2020	**€ 32.029,63**		
TOTALE SALDO FINANZIARIO AL 31/12/2020	**€ 32.029,63**		
AVANZO DELL'ESERCIZIO FINANZIARIO 2021	**€ 26.560,81**		
SALDO FINANZIARIO AL 31/12/2021	**€ 58.590,44**		
STANZIAMENTI IMPEGNATI AL 31/12/2021			**Euro**
Spese per rinnovamento integrale sito WEB SIAI, anno 2021			€ 5.000,00
Contributo all'It.J. Anat. Embryol., anno 2021			€ 410,00
Spese per ECM, anno 2021			€ 500,00
Spese per il funzionamento della Segreteria, Tesoreria e Presidenza, anno 2021			€ 2.467,00
Compenso per Consulenza Commercialista relativa al bilancio consuntivo anno 2020 e bilancio previsionale, anno 2022			€ 1.500,00
TOTALE IMPEGNO DI SPESA			**€ 9.877,00**
SALDO DISPONIBILE	**€ 48.713,44**		

SIAI Società Italiana di Anatomia e Istologia

Relazione di accompagnamento al rendiconto economico e finanziario per l'anno 2021

Come risulta dal bilancio consuntivo, il saldo finanziario al 31/12/ 2021 è pari ad **€ 58.590,44.**
A tale importo devono essere sottratti **€ 9.877,00** impegnati nel bilancio previsionale del 2021, ma non ancora effettivamente utilizzati alla data del 31/12/2021, per le seguenti voci di spesa:

- **Spese per rinnovamento integrale sito web della Società anno 2021: € 5.000,00 ;**
- **Contributo all'It.J. Anat. Embryol., anno 2021: € 410,00;**
- **Spese per ECM, anno 2021: € 500,00**
- **Spese per il funzionamento della Segreteria, Tesoreria e Presidenza, anno 2021: € 2.467,00;**
- **Consulenza Commercialista bilancio consuntivo anno 2020 e previsionale 2021 : € 1.500,00.**

Pertanto l'anno 2021 si chiude con un **saldo disponibile** di **€ 48.713,44.**

Durante il 2021, le quote associative incassate sono state 711 comprese alcune quote arretrate ed integrazioni di versamenti di quote non corretti, per un totale di **€ 42.686,08**, che sommate al saldo finanziario al 31/12/2020 pari ad **€ 32.029,63** hanno dato la disponibilità di **€ 74.715,71.**

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 questa volta ottima; comunque rimane ancora un certo numero di Soci che debbono regolarizzare la loro posizione. Il Tesoriere sottolinea che il pagamento delle quote da parte dei Soci deve essere puntuale, ad inizio di ciascun anno solare, tale da consentire alla SIAI di effettuare una adeguata programmazione delle attività statutarie e di intraprendere nuove iniziative.

Il Tesoriere

Prof. Gianpaolo Papaccio

Previsione finanziaria 2023

SOCI NEL 2021:	**395**
SOCI NEL 2022:	**424**
SOCI ORDINARI 2022:	**407**
SOCI DIMISSIONARI/CANCELLATI/DECEDUTI 2022:	**10**
SOCI ORDINARI DIVENUTI EMERITI 2022	**1**
SOCI RIAMMESSI 2022:	**4**
SOCI EMERITI/ONORARI:	**17**

Quote Sociali anno 2023	407	€	24.420,00
Quote Sociali arretrate 2015 – 2022		€	12.780,00
Contributi liberali per pubblicazione lavori scientifici su Italian Journal of Anatomy and Embryology		€	5.500,00
Totale Entrate		€	**42.700,00**

USCITE

Contributo al 76° Convegno Nazionale 2023, atti di convegni, altri contributi a convegni, partecipazione a convegni, organizzazione eventi scientifici, borse di studio, etc.	€	15.000,00
Accantonamento per premi poster dell'anno 2023 e per premio alla migliore comunicazione orale assegnati nell'anno 2023	€	3.000,00
Accantonamento per premi SIAI (Premio alla Carriera e n. 2 Premi Ricercatori under 40), anno 2023	€	4.200,00
Contributo alla Firenze University Press per pubblicazione lavori scientifici su Italian Journal of Anatomy and Embryology, anno 2023	€	5.500,00
Spese per sito web della Società, anno 2023	€	3.000,00
Spese per la partecipazione Meeting Comitato Internazionale per la Terminologia Anatomica e Istologica, FICAT, anno 2023	€	1.000,00
Quota adesione all'European Federation for Experimental Morphology, EFEM, anni 2022 e 2023	€	900,00

	€	400,00
Quota adesione all'International Federation of Anatomical Associations, IFAA, anno 2023		
Spese varie (bancarie, necrologi, etc.), anno 2023	€	700,00
Spese impreviste, anno 2023	€	1 .000,00
Totale spese per attività statutarie	***€***	***34.700,00***
Spese per il funzionamento della Segreteria, Tesoreria, Presidenza e Consiglio Direttivo	€	6.000,00
Spese per consulenza commercialista anno 2022	€	2.000,00
Totale spese di funzionamento	***€***	***8.000,00***
Totale Uscite	€	**42.700,00**

SIAI Società Italiana di Anatomia e Istologia

Relazione di accompagnamento alla previsione finanziaria per l'Anno 2023

La chiusura del bilancio consuntivo del 2021 con un saldo disponibile di € **48.713,44** ha permesso al Tesoriere di sostenere alcune spese indicate nella Previsione Finanziaria del 2022.

Al 31 agosto 2022, sono state incassate 525 quote sociali comprensive di quelle relative all'anno in corso e arretrate (dal 1° settembre 2021 al 31 agosto 2022).

Al 31 agosto 2022, il totale delle entrate è pari a **€ 62.428,58** e comprende le quote riscosse finora.

Il piano previsionale del 2022 prevedeva entrate pari a **€ 32.100,00**, 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 424 Soci, di cui 407 Soci Ordinari e 17 Soci Emeriti o Onorari (esonerati dal pagamento della quota Sociale). Nel corso del 2021 ad oggi (31 agosto 2022), n. 10 Soci sono stati cancellati poiché deceduti o hanno espresso la volontà di rassegnare le dimissioni dalla Società, n.1 Socio ordinario è divenuto Socio emerito e n.4 Soci sono stati riammessi.

Allo stato attuale, dei 407 Soci Ordinari che sono tenuti a pagare la quota associativa:

- 4 Soci sono in regola fino al 2023;
- 184 Soci sono in regola fino al 2022;
- 171 Soci sono in regola fino al 2021, devono la quota 2022;
- 4 Soci sono in regola fino al 2020, devono le quote del 2021 e 2022;
- 17 Soci in pari con il 2019, devono le quote del 2020, 2021 e 2022;
- 9 Soci in pari con il 2018, devono le quote del 2019, 2020, 2021 e 2022;
- 10 Soci in pari con il 2017, devono le quote del 2018, 2019, 2020, 2021 e 2022;
- 5 Soci in pari con il 2016, devono le quote del 2017, 2018, 2019, 2020, 2021 e 2022;
- 3 Soci in pari con il 2015, devono le quote del 2016, 2017, 2018, 2019, 2020, 2021 e 2022.

Il Tesoriere fa presente che cercherà di raggiungere la parità di bilancio e di fare previsioni finanziarie quanto più possibile aderenti alla realtà. Riferisce inoltre che nel corso del 2022, un discreto numero di Soci ha risposto positivamente all'azione di richiamo per il recupero delle quote arretrate. Rimane un piccolo numero di Soci che non ha mai risposto ai solleciti di pagamento; pertanto, in base a quanto stabilito nello Statuto (Art. 15) ed al parere del Direttivo SIAI, si è già provveduto alla revisione dell' Elenco dei Soci che sarà ancora revisionato ove mai tali Soci non provvedessero, secondo le norme statutarie, al pagamento delle quote arretrate.

SIAI Società Italiana di Anatomia e Istologia

In particolare, viene presentato elenco dei Soci morosi decaduti (n=27) in ottemperanza a quanto prescritto dall'Art. 10 del Regolamento della Società.

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 ed il recupero delle quote arretrate permetterebbero alla SIAI di migliorare ulteriormente tali scopi, come peraltro già messo in atto dal Presidente e dal Direttivo tutto.

Il Tesoriere

Prof. Gianpaolo Papaccio

Allegato N.5

Associazione "Società Italiana di Anatomia ed Istologia"

Verbale dei **R**evisori dei **C**onti

L'anno 2022, il giorno 15 del mese di settembre, alle ore 10:30 si sono riuniti i Revisori dei Conti, nominati dal Consiglio Direttivo della Società Italiana di Anatomia e Istologia (SIAI) in data 18/07/2022, nelle persone dei Professori:

Sandra Marmiroli
Stefania Montagnani
Mario Rende

E' stato eletto Presidente della Commissione il Prof. Mario Rende.
La Commissione ha quindi preso visione sia del Bilancio Consuntivo dell'anno 2021 sia della Previsione Finanziaria dell'anno 2023.
L'esame della sue sposta documentazione non evidenzia irregolarità e appare chiara ed esaustiva.
I Revisori approvano all'unanimità l'intera documentazione finanziaria.

Sia il Bilancio Consuntivo dell'anno 2021 sia la Previsione Finanziaria dell'anno 2023 evidenziano che l'Associazione è in attivo.

La seduta viene tolta alle ore 12:00.

Letto, confermato e sottoscritto.

I REVISORI

Sandra Marmiroli

Stefania Montagnani

Mario Rende

Allegato N.6

SOCI DECADUTI

1. Brancia Carla
2. Cacciola Giorgio
3. Capitani Silvano
4. Castellucci Mario
5. Chiarini Annamaria
6. Ciccocioppo Fausta
7. Continenza Cantalini Maria Adelaide
8. Dal Prà Ilaria Pierpaola
9. Ferretti Marzia
10. Gonelli Arianna
11. Iacopino Fortunata
12. Lupo Corrado
13. Mammola Caterina Loredana
14. Mariotti Raffaella
15. Migliorato Alba
16. Palma Antonio
17. Pisani Antonina
18. Previati Maurizio
19. Procacci Patrizia
20. Puzzolo Domenico
21. Renzi Anastasia
22. Robecchi Maria Giuseppina
23. Sirigu Vincetelli Paola
24. Trimarchi Fabio
25. Turci Michela Cristina
26. Vaccarezza Mauro

Allegato N.7

VERBALE DELLA COMMISSIONE ELETTORALE PER L'ELEZIONE DEL COLLEGIO DEI PROBI VIRI SIAI

La Commissione Elettorale, composta dai Professori Antonella Camaioni, Pietro Gobbi e Ferdinando Paternostro, si è riunita in via preliminare, telematicamente, il giorno 12 settembre alle ore 10.30 per verificare, insieme al Prof. Michelangelo Cordenonsi (che ha fornito la più ampia collaborazione per le procedure tecnico-organizzative) la piena funzionalità del sistema di voto e per contribuire alla stesura del tutorial con le istruzioni.

Verificato quanto necessario e stabiliti i criteri di valutazione per eventuali ex-aequo, che sono stati inseriti nel tutorial (ALLEGATO 01), la Commissione ha quindi sospeso i propri lavori.

Durante lo svolgimento del voto (dalle ore 17.00 del giorno 14 alle ore 14.00 del giorno 15 settembre) la Commissione ha sovrainteso telematicamente affinché le operazioni avvenissero in maniera regolare, facendo fronte a sporadici dubbi procedurali.

La Commissione ha continuato i suoi lavori in presenza a Padova il giorno 15, alle ore 15.00 presso la Sede Congressuale. Al termine delle votazioni il sistema informatico ha fornito i dati riguardanti le percentuali di partecipazione e i voti ottenuti da ciascun candidato, per le posizioni di Componente e di Presidente del Collegio (ALLEGATO 02).

La Commissione può quindi dichiarare che i votanti sono stati 177 su 229 aventi diritto, pari a una percentuale di voto del 77,29%.

Hanno ottenuto voti:

- per i Membri del Collegio dei Probi Viri:

Marco Artico	97
Giacomina Brunetti	54
Francesco Cappello	114
Raffaele De Caro	140

- per il Presidente del Collegio dei Probi Viri:

Marco Artico	9
Giacomina Brunetti	8
Francesco Cappello	48
Raffaele De Caro	108

Il Collegio del Probi Viri risulta così costituito dai Prof.ri: Raffaele De Caro (Presidente), Francesco Cappello e Marco Artico.
Alle ore 15.45 la Commissione trasmette il seguente verbale, per via telematica, al Segretario SIAI, affinché ne venga data lettura nel corso dell'odierna Assemblea dei Soci.

Padova, 15 settembre 2022

Firmato
Antonella Camaioni
Pietro Gobbi
Ferdinando Paternostro

Allegato N.8

COMMISSIONE PER L'ATTRIBUZIONE DEL PREMIO ALLA CARRIERA

DELLA SOCIETA DI ANATOMIA E ISTOLOGIA (SIAI)

Il giorno 7 settembre 2022 alle ore 15.00 in modalità telematica (https://meet.google.com/rfv-zhkv-tte) si è riunita la Commissione preposta per l'attribuzione del Premio alla Carriera della Società di Anatomia e Istologia (SIAI) 2022, nominata nel Consiglio Direttivo del 18 luglio u.s.

La Commissione risulta composta dai Proff. Monica Mattioli Belmonte, Paolo Onori, Carla Palumbo e Oriana Trubiani.

Assume la Presidenza la Prof. Monica Mattioli Belmonte. Svolge le funzioni di Segretario il Prof. Paolo Onori.

La Commissione prende atto che entro il termine del 25 agosto 2022 è giunta una sola proposta. Un'ulteriore candidatura è pervenuta, invece, in data 2 Settembre 2022. Essendo questa giunta ben oltre la data di scadenza, non può essere presa in considerazione.

L'unica candidatura idonea, avanzata dal Prof. Pietro Gobbi, è per la Prof. Elisabetta Falcieri, già Ordinario per il SSD BIO/16 - Anatomia Umana presso l'Università di Urbino Carlo Bo.

Dopo aver valutato il CV allegato alla proposta, la Commissione unanime si dichiara favorevole all'assegnazione del Premio alla Carriera della SIAI alla Professoressa Elisabetta Falcieri.

La Commissione conclude i lavori alle ore 15.30, redatto il presente verbale lo trasmette al Presidente della SIAI.

Letto, approvato e sottoscritto seduta stante

07/09/2022

Il Presidente

Prof. Monica Mattioli Belmonte

Il Segretario

Prof. Paolo Onori

Allegato N.9

VERBALE DELLE RIUNIONI DEL COMITATO SCIENTIFICO PER L'ATTRIBUZIONE DI NUMERO 2 PREMI AI RICERCATORI "UNDER 40"

Il Comitato Scientifico, nelle persone di:

Prof.ssa Emanuela Marcenaro (in qualità di Presidente)
Prof.ssa Bianca Maria Scicchitano (in qualità di Componente)
Prof.ssa Matilde Yung Follo (in qualità di Componente)
Prof. Antonio De Luca (in qualità di Componente)
Prof. Guido Carpino (in qualità di Segretario)

si è riunito telematicamente (via TEAMS) mercoledì 7 e giovedì 8 settembre 2022 per valutare le Candidature per l'assegnazione dei 2 Premi ai ricercatori "under 40".
Sono pervenute n. 8 Candidature nelle persone di:

Prof.ssa Celeste Caruso Bavisotto
Prof. Jacopo J.V. Branca
Prof. Giovanni Cirillo
Prof.ssa Luca Madaro
Prof.ssa Grazia Maugeri
Prof.ssa Cristina Meregalli
Prof.ssa Selenia Miglietta
Prof. Stefano Ratti

Il Comitato ha dapprima valutato la posizione dei Candidati rispetto al pagamento delle quote di iscrizione alla SIAI e ha rilevato che tutti i Candidati sono in regola.

Successivamente, il Comitato ha valutato attentamente i curricula dei Candidati, la loro produzione scientifica, il contributo personale nelle pubblicazioni, la congruenza delle tematiche di ricerca con gli SSD di riferimento, la continuità temporale e i loro indici bibliometrici con particolare riferimento agli ultimi anni.

Alla fine di tale valutazione, il Comitato propone di assegnare all'unanimità i 2 Premi a:

Prof.ssa Grazia MAUGERI, la cui attività di ricerca si concentra nel campo delle neuroscienze e in particolare si focalizza sull'identificazione e l'analisi degli aspetti morfologici e molecolari caratterizzanti la rigenerazione tessutale in malattie neurodegenerative, quali la sclerosi laterale amiotrofica e la retinopatia diabetica.

Prof. Stefano RATTI, la cui attività di ricerca si concentra nell'identificazione e analisi di varianti anatomiche di interesse clinico e chirurgico e nello studio morfologico e topografico delle vie di trasduzione del segnale in malattie rare.

Addì, 08 settembre 2022

Prof.ssa Emanuela Marcenaro

Prof.ssa Bianca Maria Scicchitano

Prof.ssa Matilde Yung Follo

Prof. Antonio De Luca

Prof. Guido Carpino

Allegato N.10

BANDO PER BORSA DI STUDIO DELLA SOCIETA' ITALIANA DI ANATOMIA E ISTOLOGIA (SIAI)

Articolo 1

Tema dell'iniziativa

La **Società Italiana di Anatomia ed Istologia (SIAI)** intende **istituire** una **Borsa di Studio** del **valore di € 10.000 di durata semestrale** con inizio a far data dal **1° febbraio 2023, da svolgersi presso prestigiose Istituzioni estere. La SIAI garantirà inoltre una indennità di viaggio fino a € 1000.**

Possono partecipare candidati che posseggano i requisiti esposti nell'**Articolo 2.**

Le tematiche di ricerca che faranno parte del progetto presentato da ciascun candidato dovranno essere coerenti con quelle delle discipline che costituiscono la Società Scientifica (Anatomia ed Istologia). **Qualora vengano prodotte pubblicazioni scientifiche nell'ambito del progetto sarà obbligatorio citare la SIAI nei ringraziamenti.**

Articolo 2

Requisiti per la partecipazione

Possono partecipare alla selezione:
- i dottorandi di ricerca;
-gli assegnisti di ricerca;
-i borsisti;
-i ricercatori di tipologia RTD-A

Essi debbono inoltre possedere i seguenti requisiti:
-essere iscritti alla SIAI ed essere in regola con i pagamenti della quota sociale sino all'anno di emissione del bando;
-avere una età inferiore ai 35 anni;
-prestare servizio in una struttura **universitaria** ed **afferente** alla Anatomia o alla Istologia.

Il candidato deve inoltrare:

Formale lettera di richiesta con allegato CV in formato europeo corredato **dalle pubblicazioni.**

La **richiesta** deve contenere le seguenti informazioni:

- cognome e nome del candidato;
- paese di residenza;
- cittadinanza;
- data di nascita;
- indirizzo e-mail;
- numero di telefono;
- un dettagliato programma di ricerca che si intende effettuare utilizzando anche (e non soltanto) le risorse di cui alla Borsa;
- **lettera formale di accettazione** da parte della Istituzione **ospitante.**

Articolo 3

Esclusione

-Non possono partecipare **al bando** familiari entro il IV grado dei membri del **Consiglio Direttivo** della SIAI;
-I candidati che non posseggano i requisiti esposti nell'Articolo 2.

Articolo 4
Criteri di assegnazione della Borsa di Studio
La Borsa di Studio sarà assegnata a seguito di un **procedimento** di selezione effettuato dal **Comitato Scientifico della SIAI**.
Il Comitato selezionerà e classificherà le candidature pervenute via mail all'indirizzo segreteria.siai@unicatt.it indicando nell'oggetto "Candidatura Borsa Studio" e allegando tutta la documentazione richiesta entro il 30 Ottobre, 2022.
Saranno accettate unicamente le candidature inviate alla suddetta mail ed entro la data suindicata.

Articolo 5
Tempistiche di assegnazione della Borsa di Studio e modalità di comunicazione
Il Comitato Scientifico si impegna a comunicare il nominativo del candidato selezionato entro e non oltre il 31.12.2022 sul sito www.siaionline.it e tramite indirizzo di posta elettronica (indicato nella candidatura) alla persona selezionata.
Qualora il vincitore risultasse impossibilitato ad accettare la Borsa di Studio, essa verrà assegnata ad altro candidato secondo l'ordine di classifica stabilito dal **Comitato Scientifico**.

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