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PRESENTAZIONE

IJAE
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Presentazione

Carissime colleghe, carissimi colleghi,

il 24 e il 25 settembre 2021 si è svolto a Bologna il 74° Congresso SIAI, in una formulazione decisamente atipica: una giornata interamente in remoto e una in presenza, ma con la possibilità di partecipare anche a distanza.

Per comprendere appieno il contesto, bisogna considerare che 74° Congresso SIAI era stato progettato nei primi mesi del 2021, quando chi osava programmare con una prospettiva superiore a qualche settimana veniva considerato quanto meno imprudente…

L'inevitabile sospensione delle attività imposta dall'emergenza pandemica aveva annullato la programmazione del 2020, che avrebbe riportato il Congresso SIAI a Bologna dopo esattamente 30 anni. Nonostante le prospettive altalenanti, abbiamo sentito fortissima l'esigenza di immaginare comunque un momento di confronto e trovare il coraggio di presentare al Consiglio direttivo l'ipotesi di una formula contratta, che consentisse la possibilità di adeguamento in caso di nuova emergenza.

Oggi, a un mese dal Congresso, vogliamo ringraziare di cuore il Presidente, il Consiglio Direttivo e tutti i membri SIAI per l'entusiasmo con il quale è stata accolta la nostra proposta.

Il numero dei contribuiti inviati ha superato la previsione più positiva e il livello scientifico dei contenuti ha confermato il valore di una Società attrattiva per i giovani e in grado di valorizzare discipline che, attraverso la morfologia, sono in grado di dialogare in maniera traslazionale con altre, mantenendo però intatta e riconoscibile la propria identità.

Con questo spirito di condivisione, è un immenso piacere presentare questo numero speciale del 2021 dell' Italian Journal of Anatomy and Embriology che, grazie alla disponibilità dell'Editor-in-Chief Prof. Ribatti, racchiude la forza degli Atti di un congresso atipico e forse anche per questo indimenticabile, che resterà il simbolo dello slancio di ciascuno di noi verso la ripresa e il ritorno alla normalità

Laura Bonsi, Lucio I. M. Cocco, Irene Faenza, Mirella Falconi, Lucia Manzoli

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LETTURE MAGISTRALI

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Quantification of cell - niche interactions by large-volume multicolor 3D single-cell imaging with single molecule sensitivity

Timm Schroeder[1]

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Our understanding of the functional cellular organization in the bone marrow is hampered by technical limitations. Technologies for imaging bone marrow typically only cover small volumes in few colors and without quantitative single-cell analysis of the resulting voluminous electronic imaging data. We therefore develop quantitative multi-color 3D imaging pipelines including the required software for the quantification of very large tissue volumes. With these novel approaches for imaging the highly complex organization of mouse bone marrow, we observed unexpected distributions of hematopoietic stem and progenitor cells and their putative cellular and molecular niches. Quantification of the spatial distribution of chemokines further found the surprising absence of long-range CXCL12 bone marrow gradients. I will discuss our current approach for imaging the tissue-wide location of individual blood and niche cells and individual regulatory extracellular molecules in mouse and human bone marrow and other tissues.

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A reappraisal of the significance and numbering of the pharyngeal arches

Anthony Graham[1]

[1] King's College London

The pharyngeal arches are a prominent and important feature of vertebrate embryos, including humans. These are visible as a series of bulges on the lateral surface of the developing head. There has been much discussion of these structures and their significance for well over a hundred years. It is generally believed that the pharyngeal arches underpin the later anatomy, and that it is within these structure that the adult pharyngeal/laryngeal apparatus is organised. However, in this talk I will make the case that this is overstated. The anterior pharyngeal arches do relate to the later anatomy in simple fashion, but this is not so for the posterior arches, which are transient structures that are of little significance for laryngeal anatomy. Furthermore, in humans, and other amniotes, there are five pharyngeal arches numbered 1, 2, 3, 4 and 6 and this is the standard scheme for the numbering of these structures. I will make the case that this is wrong and propose that the numbering needs revising. We arrive at these conclusions through careful study of the development of the pharyngeal arches in a range of vertebrate embryos, and a consideration of the evolutionary history. I would suggest that the scientific literature and the textbooks need correcting.

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Roles for growth factors in cancer progression and opportunities for patient treatment

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Biological systems robustly integrate metabolic, energy and signaling networks, by maintaining dense webs of control circuits. My lecture will concentrate on systemic defects in signaling networks involved in malignant cell proliferation and migration. As a starting point, I will argue that primordial signaling pathways have been replaced in the course of metazoan evolution by layered signaling networks. Unlike linear pathways, networks can be trained to overcome perturbations, and their control wirings are much more sophisticated. These transitions are relevant to pharmacological attempts to intercept signaling networks, as well as to the excessive reliance of oncogenic networks on 1- 2 essential hubs ('oncogene addiction'). Using the epidermal growth factor receptor (EGFR) and its kin, a kinase called HER2/ERBB2, I will exemplify defects in system control and feedback regulation, and focus on ways to overcome resistance to targeted therapies.

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Germline mutagenesis, automated meiotic mapping, and the discovery of mutations that suppress disease

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Many biological questions are raised and ultimately answered by mutations that perturb normal function: so-called "experiments of nature." Examples would include mutations that cause obesity, immunodeficiency, and neurobehavioral abnormalities. At one time, it was quite arduous to identify which mutations were responsible for strange and intriguing phenotypes. Typically, years were required to track down a causative mutation. During the past several years we have developed a way of finding causative mutations instantaneously in mice, through a technique we call automated meiotic mapping (AMM). This has led us to emphasize the creation of new phenotypes as rapidly as possible, with simultaneous measurement of genome saturation. We have also begun to emphasize the creation and identification of mutations that mitigate disease, known as "suppressor" or "modifier" mutations. Out of ~32,000 germline mutations that change coding sense in mice, we have found two single base-pair substitutions that cause robust resistance to transplantable syngeneic cancers. These mutations operate by different mechanisms, and show considerable potential for translation to new therapies for cancer in humans.

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COMUNICAZIONI

Neuroscienze

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Tubulin and not proteasome as a crucial target for bortezomib-induced neurotoxicity

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Bortezomib (BTZ) and Carfilzomib (CFZ) are proteasome inhibitors (PIs) that represent the gold standard in the treatment of multiple myeloma. BTZ is frequently used as first line therapy, but peripheral neuropathy (PN), occurring approximately in 50% of patients, gets worse their life, representing a dose-limiting toxicity. CFZ, a second-generation PI, determines a significantly less severe PN[1]. In this study, we investigated possible BTZ and CFZ off-targets able to explain the difference in their neurotoxicity profiles.

In order to recognize the possible PIs off-targets we used the SPILLO-PBSS software that performs a structure-based *in silico* screening on a proteome-wide scale[2,3]. Among the top-ranked off-targets of BTZ identified by SPILLO-PBSS we investigated tubulin which, by contrast, did not turn out to be an off-target of CFZ. We hypothesized that BTZ-microtubules direct interaction would inhibit the tubulin alfa GTPase activity, thus reducing the microtubule catastrophe and consequently furthering the microtubules polymerization.

The hypothesis was biologically validated *in vitro* in adult mice dorsal root ganglia (DRG) primary sensory neuron cultures and in a cell free model of tubulin polymerization and depolymerization. NMR binding studies were performed to demonstrate the interaction with the identified off-target.

We compared the neurotoxicity of BTZ (2.8nM) and CFZ (3.2nM). These BTZ and CFZ concentrations result in cytotoxicity for multiple myeloma cells and they inhibit proteasome in the same manner in adult mouse DRG primary sensory neurons. At these concentrations, and in this cellular model, the two drugs showed the same different neurotoxicity profiles observed in clinical practice. In neuron cultures, BTZ, but not CFZ, induced neurotoxicity and increased the percentage of polymerized tubulin. Moreover, in a cell-free model of tubulin polymerization and depolymerization only BTZ slowed down the depolymerization of microtubules and reduced the free phosphate concentration released during GTP hydrolysis. Lastly, NMR binding studies clearly demonstrated that only BTZ is able to directly interact with both tubulin dimers and polymerized form.

In conclusion, our data demonstrate that:

a. BTZ neurotoxicity is not related to its well-known proteasome inhibition
b. BTZ, but not CFZ, is able to directly bind and perturb microtubules

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A dual inhibitor as strategy to overcome glioblastoma temozolomide resistance: *in vitro* study

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Glioblastoma (GBM, grade IV glioma) represents the most aggressive brain tumor[1]. Despite various combinations of surgery, chemotherapy and radiotherapy, GBM is characterized by frequent recurrence and very high lethality. Until now surgical resection followed by radiotherapy and alkylating agent Temozolomide (TMZ) treatment represents the standard strategy for GBM. GBM alkylating drug resistance is largely due to damage repair mechanisms involving principally 06-methylguanine-DNA methyl- transferase (MGMT) and alkylated DNA repair protein 2 (ALKBH2)[2].

The greatest challenge in developing a therapy to arrest GBM invasiveness is represented by GBM stem cells (GSCs), very heterogeneous, tumor initiating cells, and responsible for GBM chemo- and radio- resistance.

We show that the imidazobenzoxazin-5-thione MV1035, synthesized as a new sodium channel blocker, is able to significantly reduce GBM U87 cells migration and invasiveness. Moreover we demonstrate that MV1035 used on both U87 and patient-derived GSC lines, in combination with TMZ, has a significantly synergistic effect in reducing cell viability and sphere formation assays. Unexpectedly, data obtained in several non glioma cancer cell lines demonstrate that MV1035 effect is independent from its ability to block sodium channels.

In order to identify pathways responsible for MV1035 effects we used an innovative powerful software, SPILLO potential binding sites searcher (SPILLO-PBSS), able to recognize targets and off-targets of any small molecule on a proteome-wide scale.

Among the top-ranked off-targets of MV1035, we focused on the RNA demethylase ALKBH5, known to be overexpressed in GSCs and on ALKBH2, known to be overexpressed and to play a key role in GBM.

Our biological and cell free data show that MV1035 is able to significantly reduce U87 cell line migration and invasiveness, inhibiting directly ALKBH5 and consequently reducing the expression of the downstream protein CD73[3].

Moreover we demonstrate that MV1035 inhibits directly the activity of ALKBH2 and induces reduction of MGMT expression in patient-derived GSC lines. Acting in this way, MV1035 could determine the increase in MGMT promoter methylation which would ultimately lead to a reduction of MGMT expression.

Taken together our data confirm the *in silico* predictions and suggest that MV1035 could act as a dual inhibitor overcoming TMZ resistance and reducing GBM migration and invasiveness, specifically on the GSC subpopulation.

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Key words

Glioblastoma, temozolomide, dual inhibitor, *in vitro*, patient derived cell lines.

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Benzo[a]pyrene affects the development of human GnRH neuroblasts

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Endocrine disrupting chemicals (EDCs), such as benzo[a]pyrene (BaP), are widespread pollutants that can interfere with reproductive function and embryo development [1]. To date, the direct effects of BaP on human reproductive axis at the central level are poorly investigated. The central regulation of the reproductive system is mediated by gonadotropin-releasing hormone (GnRH) neurons, which originate in the olfactory placode and, during fetal development, migrate towards the hypothalamus [2]. Given the importance of the migratory process for GnRH neuron maturation, we investigated the effect of BaP on GnRH neuroblasts isolated from the human fetal olfactory epithelium (FNCB4).

FNCB4 cells express the enzymes cytochrome P450 (CYP1A1 and 1B1), required for metabolic activation of BaP and the mRNA expression of both genes was significantly increased by Bap exposure (10μM for 24h). From a functional point of view, we demonstrated that BaP affected FNCB4 migratory properties. In particular, BaP exposure significantly reduced the mRNA levels of FGFR1 and NRP2 genes, both implicated in GnRH neuron migration, and inhibited the cytoskeletal remodeling-based motile phenotype. Accordingly, using endothelin-1 (ET1) as a chemoattractant, the number of migrated FNCB4 cells significantly increased when compared to untreated cells, whereas the pre-incubation of BaP (24h) significantly prevented this effect. Interestingly, BaP exposure did not affect GnRH mRNA expression, while significantly reduced the mRNA expression of kisspeptin receptor (KISS1R) in FNCB4 cells. Since kisspeptin is the main physiological regulator of GnRH neuron function in the mature hypothalamus, we further investigated the effects of BaP in post-migration hypothalamic GnRH neurons isolated from the human fetal hypothalamus (hfHypo). Treating hfHypo with BaP (10μM for 24h) significantly reduced not only KISS1R mRNA levels but also GnRH expression at both gene and protein level.

In conclusion, our findings indicate that BaP may affect the central regulation of the reproductive axis directly interfering with GnRH neuron maturation and function.

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Key words

GnRH neurons; benzo[a]pyrene; EDCs; cell migration; pollution.

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Glymphatic system and neurodegenerative diseases: a systematic review of the literature

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Introduction: Over the last years, perivascular space of Central Nervous System (CNS) was identified as a pathway for the clearance of interstitial solutes (1). Due to its dependence on glial cells and its functional similarity to the peripheral lymphatic system, the term of "glymphatic system" was coined (2). Perturbations of this pathway can take a role in favouring or accelerating neurodegenerative processes within CNS (3). Currently, several and different diagnostic tools are used to identify a glymphatic system impairment in some neurodegenerative and neuroinflammatory disorders, such as Alzheimer disease (AD), Parkinson disease (PD), idiopathic Normal Pressure Hydrocephalus (iNPH) and Multiple Sclerosis (MS). The aim of this study was to perform a systematic review and meta-analysis on the current literature on glymphatic system and the above-mentioned diseases.

Material and Method: The search was restricted to articles published in English language from January 2010 to March 2021. The primary outcome was the observation of glymphatic system impairment in AD, PD, iNPH and MS. Secondary outcomes were the identification of the most effective diagnostic tool in detecting glymphatic system alterations and correlating them with disease progression. A formal meta-analysis was not performed because of the heterogeneity of the retrieved data.

Results: Among the selected studies, 73.3% have used Magnetic Resonance (MR) techniques, 6.6% Positron Emission Tomography and 20% have quantified aquaporins (AQP4). 54.65% of patients were affected by PD, 26.91% by AD, 9.7% by iNPH and 7.6% by MS.

Mini Mental State Examination, MDS-Unified Parkinson's Disease Rating Scale and Expanded Disability Status Scale were the instruments used to evaluate mental and physical disability.

Discussion: The high in-between study heterogeneity was the main issue to identify the most helpful diagnostic tool to detect glymphatic system defects and to perform a correlation with the severity of disease. Data from this study suggest that a combination of multiple diagnostic techniques seems to be the more advantageous for identifying glymphatic system abnormalities.

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Glymphatic system, neurodegenerative diseases, diagnostic instruments.

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Grape pomace extract exerts neuroprotective activity in a rat hypothalamic cell line

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Vitis vinifera (grape) is cultivated worldwide and contains various compounds with acknowledged phytochemical and pharmacological properties, including neuroprotective activity. Among the different parts of the plant, pomace is of particular interest as winemaking industry by-product (1).

A characterization of the water extract from grape pomace from Montepulciano d'Abruzzo variety (Villamagna doc) was conducted and the bioactive phenolic compounds were quantified through HPLC-DAD-MS analysis. Catechins were the prominent phenolic compounds identified in the extract. Focusing on the neuroprotective effects, the hypothalamic cell line HypoE22 was challenged with an oxidative stimulus and exposed to different concentrations (1 μg/mL-1 mg/mL) of the pomace extracts for 24, 48 and 72 h. Cell proliferation was investigated by the MTT assay, and the extract, while not affecting cell proliferation in basal conditions, reverted the cytotoxicity exerted by the oxidative stimulus at all the experimental times in a dose-dependent manner.

Dopamine release from HypoE22 cells was also assayed, through HPLC-EC method. The inhibitory effect induced by pomace treatment on hydrogen peroxide- induced neurotransmitter depletion was observed at any given time points (24, 48 and 72 h). After 48 and 72 h of exposition, the pomace extract was also effective in preventing the downregulation of the neurotrophin brain-derived neurotrophic factor (BDNF) and the concomitant upregulation of ciclooxigenase-2 (COX-2) gene expression induced by the oxidative stress stimulus. In accordance, PGE_2 release, measured by an ELISA kit, was augmented by the oxidative stress conditions and reverted by the administration of the three different concentration of water extract from grace pomace.

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Key words

Neuroprotection, Grape pomace extract, Oxidative stress, Inflamamtion, BDNF, COX- 2, PGE2.

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Oral melatonin administration influences prefrontal cortex and hippocampal neurochemistry in a transgenic murine model of autism

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Autism spectrum disorders (ASD) are characterized by impaired social/communication abilities and stereotyped behaviours. Moreover, ASD patients present brain morphological, physiological and biomolecular alterations. In reference to neuroanatomy, magnetic resonance imaging (MRI) detected brain volume abnormalities also in the cerebrum gray nuclei and the cortex, even if these data are complex and heterogeneous. In particular, the prefrontal cortex and the hippocampus are considered key components in ASD for their involvement in learning and cognition [1,2]. In reference to physiological and biomolecular alterations, sleep disorders are frequently encountered in patients due to an altered pathway of melatonin with a subsequent deleterious effect on daytime behaviours in up to 50-80% of children with ASD. For instance, among the available sleep aids, the indolamine melatonin is the most popular for its ability to promote the normal sleep-wake cycle, however, its administration as alternative natural therapy for cognitive improvement has never been assessed.

The aim of the present study was to evaluate the effect of exogenous melatonin administration on endogenous antioxidant system and synaptic development/plasticity in prefrontal cortex and hippocampus using a transgenic mouse model of autism, immediately after weaning.

The male mice were daily treated *per os* with melatonin (10 mg/ Kg/day) or vehicle for 8 weeks starting from the 6th week of life. At the end of the treatment, social and repetitive behaviours were tested. Moreover, the antioxidant modulation, the GABAergic/glutamatergic impairment and the synaptic remodeling in prefrontal cortex and hippocampus have been evaluated immunohistochemically.

The behavioural results showed no statistical evidence, instead the immunohistochemical data indicated the ability of melatonin to promote the activity of antioxidant system, the GABAergic/glutamatergic equilibrium, and the synaptic remodeling.

In conclusion, the daily administration of melatonin in ASD mice, starting from the first period of life, is able to reduce the redox imbalance promoting a modulation of neural connectivity. These are the first data on this topic suggesting the use of melatonin in ASD as adjuvant therapy and encouraging the onset of clinical trials.

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COVID-19 Neuropathology: evidence for SARS-CoV-2 invasion of anatomically defined regions in the human CNS

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SARS-CoV-2 is a novel strain of Coronavirus with important implications for the CNS. Data deriving from autopsy studies supports the neuroinvasive potential of SARS-CoV-2, even though infection appears to be limited to sparse cells within the brainstem and was not associated with the severity of neuropathological changes. We examined the neuropathological alterations of twenty-four (24) patients who died following a diagnosis of Sars-CoV-2 infection in Padova, during the COVID-19 pandemic, and 10 Age-matched controls with comparable medical conditions.

The brain, cranial nerves, meninges, choroid plexus were sampled and histopathological evaluation was conducted. Immunohistochemistry for GFAP, CD3, CD8, CD20, CD61, CD68 and HLA-DR antibodies was performed. SARS-CoV-2 proteins (Spike Subunit 1 and Nucleocapsid Protein) and RNA (N2 Gene and E Gene) were investigated through immunohistochemistry, RT- PCR, in-situ hybridization and electron microscopy. Digitally assisted quantification of reactive microglia was performed on sections of the medulla, pons and mesencephalon.

Neuropathological alterations include marked astrogliosis at the level of the brainstem, microgliosis and microglial nodules in different sites of the medulla oblongata, substantia nigra and basal ganglia. Haemorragic injury was found in four patients and small vessel thromboses in seven patients. Seven subjects displayed immunoreactive structures within CNS parenchima. Five subjects presented immunoreactive neurons within the anatomically defined boundaries of the solitary tract nucleus, nucleus ambiguus and substantia nigra. Viral RNA was detected through RT- PCR in all the aforementioned IHC Viral protein positive samples. Quantification of reactive microglia revealed an anatomically segregated pattern of inflammation targeting mainly the medulla oblongata and the substantia nigra, and was significantly higher when compared to matched controls.

The study contributes to define the neuroinvasive potential of SARS-CoV-2 within the CNS. Unlike previous findings, we have documented several cases in which viral proteins and RNA were clearly detectable within anatomically defined regions of the CNS, testifying an important neuroinvasive potential by the virus. SARS-CoV-2 direct invasion does not appear to directly correlate with the severity of neuropathological changes. This may be ascribed to the relative short interval between infection and death, and requires further confirmation from other studies.

Key words

SARS-CoV-2, COVID-19, central nervous system, brainstem, microglia, neurotropism, viral infection.

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Early postnatal genistein administration affects neural circuits controlling food intake in cd1 mice

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Genistein (GEN), a phytoestrogen contained in soy and other legumes [1], can act as 'obesogen' and increase the risk of developing metabolic disorders such as diabetes or obesity [2]. Recent studies demonstrated that some of them might induce permanent morphological alterations of estrogen sensitive circuits in adults as the systems that influence food intake [3]. The neuroendocrine control of food intake and energy expenditure is based on many circuits including both orexinergic (Orexin, NPY) and anorexinergic [pro-opiomelanocortin (POMC)] elements that are targets for a series of chemical signals coming from the periphery (as leptin, ghrelin). From the arcuate nucleus (ARC) and the lateral hypothalamic area (LHA) these systems project to many other hypothalamic nuclei. Due to the frequent use of soy milk in the neonatal diet, we aimed to study the effects of early GEN exposure on neural circuits controlling metabolism and food intake.

We analyzed the effects on adult CD1 mice of both sexes of an early postnatal treatment (from PND1 to PND8) with GEN (50 mg/kg body weight dissolved in sesame oil) or with the vehicle (control, CON). In particular, we examined the expression of the POMC neuronal system within different hypothalamic nuclei [ARC, Paraventricular Nucleus (PVN) and Dorsomedial Nucleus (DM)] and the orexin system in LHA.

Early postnatal exposure to GEN, in a dose comparable to the exposure level in babies fed with soy-based formulas, induced sexually dimorphic effects. In fact, only in adult females GEN treatment significantly increased the body weight. POMC immunoreactivity was significantly reduced in adult GEN females compared to CON females only in PVN, while we have not observed any significant difference in DM and ARC, and in males. In addition, we observed an increase of the positive cell number in the inner part of ARC only in GEN-treated females. The orexin system in the LHA is sexually dimorphic in CON mice (having males more cells than females), and this dimorphism was totally reverted in GEN mice: the cell number increased in GEN female and decreased in GEN male.

In conclusion, the early postnatal exposure of CD1 mice to GEN determines long- term sex specific organizational effects on neural circuits controlling food intake and energy metabolism. The increase of body weight as well as the morphological alterations of the two circuits expressing orexin and POMC suggest that the obesogenic effect of GEN is sexually dimorphic and is due, at least partly, to alteration of metabolic regulation.

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Key words

Phytoestrogens, endocrine disruptor, dimorphism, obesity, POMC, orexin.

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Cadmium-induced stress on glial cells: morphological and molecular in vitro study.

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C Cadmium is a toxic heavy metal known as environmental pollutant world-wide distributed. Its toxicity, predominantly due to its long half-life of 15-20 years, affects many organs and tissues including kidney, lung, liver, heart, bone, muscles and the central nervous system (CNS) as well [1]. Our research group previously demonstrated that cadmium is able to alter the blood-brain barrier by modifying the tight junction sealing morphology and to induce oxidative stress, thus leading to neuronal degeneration mainly by oxidative stress [2]. Among the cellular population of the CNS, glial cells own a pivotal role in maintaining the physiological brain homeostasis and their alteration has been demonstrated to indirectly affect neurons [3].

The aim of the present research is to evaluate cadmium toxicity in two glial cell lines (astrocyte DITNC1 and microglia BV-2), at different time and increasing concentration of metal exposure.

Cadmium increasing concentrations (0.1-100 μM) were administered in starvation medium for 8, 24 and 48 hours. Cell viability assay, western blotting analysis, and immunofluorescent staining were employed in order to evaluate the molecular and morphological alterations induced by the heavy metal.

Our results clearly demonstrated that Cd is able to affect the cell viability even at the lowest concentrations and in a time-dependent manner, by altering the oxidative balance (ROS production, cytochrome C expression) and inducing an endoplasmic reticulum stress (GRP78 upregulation).

Moreover, Cd is able to induce glial morphological alterations as well as an overexpression of activation glial markers, such as GFAP, S100β, CD86, and Iba1.

In conclusion, the data obtained clearly demonstrated that Cd toxicity occurs even at low doses and for very short exposure times

Further experiments should be performed in order to evaluate the mechanisms underlying the Cd-dependent activation of glial cells and how this, in turn, induces a neuronal homeostasis alteration.

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Oxidized polyvinyl alcohol-based nerve conduits for peripheral nerve regeneration in severe injury: a comparative pre-clinical study

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The surgical approach to peripheral nerve injuries (PNI) with substance loss depends on injury severity. If end-to-end suture is not possible, interposition of autologous nerve grafts represents the current gold standard [1]; however, growing interest is arousing towards effective comorbidities- free alternatives: nerve conduits (NCs). In this study, different NCs based on the new oxidized polyvinyl alcohol (OxPVA) [2,3] were implanted in a rat model of sciatic nerve transection (gap=5mm). Briefly, 18 animals were randomized to 6 groups implanted with: Reverse Autograft (RA, control); Reaxon® (commercial device made of chitosan); OxPVA; OxPVA+EAK peptide; OxPVA+EAK-YIGSR peptide; OxPVA+Nerve Growth Factor (NGF). The end point considered was 6 weeks. After implantation, both functional recovery assessment (i.e., gait analysis, sciatic functional index, von Frey filaments assay for mechanical sensitivity) and histological (hematoxylin and eosin, toluidine blue staining) and immunohistochemical (CD3, F4/80, S100, β-tubulin) analyses occurred on explants. Eventual fibroconnective infiltrate was also evaluated by Second Harmonic Generation (SHG) microscopy. All the NCs sustained nerve regeneration as demonstrated by functional studies; OxPVA+NGF distinguished for better functional outcomes, like RA. Upon explant, all grafts were clearly recognizable without severe formation of fibrous tissue outside the NC; presence of n=1 neuroma was observed in Reaxon®. Histological/immunohistochemical analyses proved presence of regenerated nerve fibers in the central portion of all grafts (S100, β-tubulin) in absence of lymphomonocytic infiltration (CD3, F4/80). In the centre of the NCs, morphometric analysis showed a total axons number that followed this descending order: OxPVA+EAK- YIGSR> RA>OxPVA>Reaxon®>OxPVA+NGF>OxPVA+EAK; in the distal portion, a decreasing total axon number was progressively displayed by: RA>OxPVA>OxPVA+EAK- YIGSR>OxPVA+NGF>OxPVA+EAK>Reaxon®. SHG microscopy, revealed a less intense signal for: Reaxon®< OxPVA+EAK<OxPVA+NGF<RA<EAK-YIGSR<OxPVA<OxPVA. Coherency analysis, focusing on collagen fibers orientation, showed highly isotropic areas for Reaxon® (0.02 AU); while comparable values for the other groups (range:0.07-0.10 AU). Study results highlight oxidized polyvinyl alcohol as a promising polymer for bioactive NCs, guaranteeing morpho- functional recovery from severe PNI.

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Key words

Peripheral nerve injury, nerve conduits, oxidized polyvinyl alcohol.

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A new potential supportive role of MR409, a GHRH agonist, in an experimental mouse model of Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a pediatric neurodegenerative disease caused by the deletion or mutation of the telomeric gene "survival motor neuron 1" (SMN1), resulting in the loss of α-motor neurons (MNs) in the brainstem and in the spinal cord. Patients show a progressive skeletal muscular atrophy and neuromuscular junction (NMJ) defects, often leading to premature death. Nowadays, despite their effectiveness, SMN-dependent available therapies have different limitations (several adverse effects, high costs, unknown long-term effects and poor efficacy in milder patients or in late-treated people). Moreover, seen the demonstrated involvement of peripheral districts, new therapeutic approaches are emerging beside the SMN-dependent treatments available.

In this scenario, we have investigated the effects of MR409, a growth hormone- releasing hormone (GHRH) agonist that has already shown a remarkable activity in preventing apoptosis and proteolysis in an in vitro model of muscle atrophy (Gallo et al., 2015).

To this aim, from postnatal day 2 (P2) to P12, we daily administered vehicle or MR409 (1mg/Kg and 2mg/Kg) to SMNdelta7 mice (a well-known murine model of SMA). We observed a progressive weight gain, especially with the highest dose, as well as significant improvements in motor behavior. These results positively correlated (in a dose-related manner) with histological and molecular analyses on quadriceps and gastrocnemius muscles, respectively early and late affected by the pathology. Indeed, H/E staining showed a significant increase in the size of the muscular fibers of MR409-treated mice. Moreover, immunofluorescence analyses on NMJs have shown a higher monoinnervation (sign of NMJ maturation) and a reduced denervation of the endplates. Additionally, molecular analyses revealed a significant enhancement in the expression of different isoforms of myosin heavy chains (MYH1, MYH2, MYH7 and MYH8) and of markers of myogenesis and muscular damage repairing (respectively, Myogenin and MyoD1), and a remarkable downregulation of MuRF1 and Atrogin-1 (whose increased expression seems correlated with muscular atrophy). Finally, by performing stereological counts of MNs in the lumbar spinal cord, we also observed a delay in cell death in the treated mice.

Thus, our results suggest MR409 as a new promising therapeutic approach for the treatment of SMA, possibly in combination with SMN-dependent therapies.

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α-Synuclein imbalance as a key player in epileptic synaptic dysfunction

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The term "epilepsy" covers a heterogeneous set of severe neurological diseases, whose hallmark is the presence of recurring seizures, i.e. transient events of abnormal neuronal activity in the brain. The symptoms can be often controlled by antiepileptic drugs, but about 30% of the patients are refractory to pharmacological treatment. The etiological factors can be structural, genetic, infectious, metabolic, or neurodegenerative. In order to develop new therapeutic strategies against epileptogenesis, proteins involved in neurodegeneration, such as α-synuclein (α-syn), have recently gained increased attention (Paudel et al., 2020). α-Syn is the common trait of a group of neurological diseases, called synucleinopathies, such as Parkinson's disease (PD) and dementia with Lewy bodies, that besides α-syn accumulation, showed sleep dysfunction and EEG alterations, frequently becoming epileptic seizures. Therefore, due to the prevalent expression of α-syn in presynaptic terminals, our aim was to analyze its synaptic distribution during development and in adulthood in a murine model of Autosomal Dominant Sleep-related Hypermotor Epilepsy (ADSHE; Becchetti et al., 2015, 2020) and in human PD and epileptic cerebral cortex. In particular, in control and transgenic (TG) mice we analyzed α-syn immunolocalization in four different types of synaptic terminals identified by means of the relative vesicular neurotransmitter transporters (VGLUT1, VGAT, VAChT, VMAT2) in sample cortical and striatal areas, where we had found an imbalance of α-syn expression in TG mice between developing and adult brains. Notably, *striatum* displayed an increasing number of both VGAT+ and VAChT+ synaptic terminals expressing α-syn in adult TG mice, suggesting an alteration of GABAergic and cholinergic circuits, whereas only developing somatosensory cortex showed a decrease of VGAT+ terminals expressing α-syn. Thereafter, *post-mortem* human brain cortical sections from both control subjects, PD patients and post-surgical human tissues from patients with Temporal Lobe Epilepsy due to Hippocampal Sclerosis (TLE-HS) were analyzed showing variable α-syn expression in GABAergic and glutamatergic cortical synaptic terminals. The present study provides a new perspective to understand the histopathological consequences or basis of different types of epilepsy, highlighting the key role of α-syn in the pathological alteration of the synapse.

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On the role of vascularization in pathoconnectomics

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In recent years, pathoconnectomics emerged as an interesting framework for the investigation and better comprehension of disorders affecting the brain. Research in this field has used so far structural, functional, metabolic and genetic data, but limited attention was addressed to the possible role of vascularization. In the present work the following aspects making it a valuable candidate to pathoconnectomics investigation are discussed: i) The vascular system is by its nature a network, endowed with directionality information on the basis of circulation; ii) The current imaging techniques allow in vivo detection of the vascular system to a very good level of detail; iii) The information extracted from this kind of data could interact in a meaningful way with the functional profile of the brain, being the BOLD effect in turn based on blood flow; iv) Further evidence could be found in support of the trophic failure hypothesis; v) Data about vascularization could allow to bring in the pathoconnectomics framework cardiovascular and metabolic disorders affecting the brain. We suggest, therefore, that the evaluation of vascular connectivity could enhance the pathoconnectomics paradigm, and provide new elements towards the understanding of brain disorders.

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Astrocyte and microglia reactivity and interactions in a transgenic mouse model of Alzheimer's disease.

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Astrocytes and microglia in the central nervous system (CNS) perform multiple functions, ranging from tissue maintenance to immune defense. They are major players of the neuroinflammatory response to endogenous pro-inflammatory triggers such as amyloid aggregates and cytotoxic cell-debris in Alzheimer's disease (AD). However, reactive astrocytes and microglia express a broad spectrum of different phenotypes and perform anti-inflammatory as well as pro- inflammatory activities. In chronic neuroinflammation, pro-inflammatory activities of these cells have been associated with significant neurodegenerative effects. An intense heterotypic signaling, involving the different cell types in the CNS plays a key role in determining their phenotypes. Known indicators of neurodegenerative phenotype of astrocytes are an increased expression of glial fibrillary acidic protein and a typical hypertrophic morphology, instead an amoeboid morphology has been associated with neurodegenerative microglia. Although microglia activation is known to precede and promote neurodegenerative shift of astrocytes in neuroinflammation, the functional meshwork of astrocyte processes may be the first glial structure to directly contact pro-inflammatory molecules. We recently demonstrated that early interactions between astrocytes and amyloid peptides induced the distal fragmentation of astrocyte processes (clasmatodendrosis) in the gray matter of different rat models of neuroinflammation [1]. In aged rats, clasmatodendrosis underlaid the disruption of the astrocyte framework, and was correlated with impairment of amyloid clearance, and pro-inflammatory activation of microglia [2]. In this study we extended these observations to a mouse model of Alzheimer's disease. We found that clasmatodendrosis and disruption of the astrocyte meshwork paralleled the appearance of pro-inflammatory microglia, and preceded the onset of the classical reactive morphology of astrocytes, suggesting clasmatodendrosis to be an early indicator of AD neurodegeneration. Moreover, our data indicated that hypertrophic astrocytes actively concurred to the formation of amyloid plaques, gathering cytotoxic molecules and isolating them from the surrounding nervous tissue within a sort of glial scar. These data add new elements to the current view of glial cells reactivity, a complex environment- dependent process involving multiple molecular, morphological and evolutive steps.

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Disentangling the complex anatomy of small fiber bundles of the human diencephalon using track-density imaging

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The human diencephalon is a region of high anatomical complexity, tightly packed with tiny fiber bundles having a complex course and anatomy [1]. Some of these small tracts, such as pallidothalamic [2], pallidosubthalamic [3] , dentorubrothalamic [4] and mammillothalamic tracts [5], as well as their subcortical projection territories [6] are relevant target structures in functional neurosurgery for various brain diseases. Diffusion weighted imaging (DWI)-based tractography has been suggested as a useful tool to map white matter pathways in the human brain in-vivo and non-invasively [7], but the reconstruction of these specific fiber bundles is made challenging by their small dimensions, especially when compared to the usual resolution of commonly available DWI data. Track-density imaging (TDI), a recent imaging technique derived from tractography, exploits the information derived from fiber tracking to obtain a super-resolved, highly detailed reconstruction of white matter structures [8], [9]. In the present work, we apply an optimized, tailored TDI pipeline to both high quality and downsampled, clinical- level data from the Human Connectome Project database [10] to reconstruct the course and anatomy of small diencephalic white matter tracts. We show that this approach can identify and follow across their diencephalic course and up to their termination most of the more surgically relevant white matter tracts, such as ansa lenticularis (AL), fasciculus lenticularis (FL), dentorubrothalamic tract (DRTT), and mammillothalamic tract (MT), and both on high quality and downsampled DWI datasets. In addition, we devise a reliable TDI-based tractography protocol for individualized reconstruction of such white matter tracts. We use this protocol to build a population template of white matter fiber bundles that can be useful for the anatomical characterization of these structures in the living human brain. We suggest that this approach could be useful both in clinical anatomy and functional neurosurgery settings, to improve our understanding of the complex morphology of this important brain region.

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Key words

Tractography, deep brain stimulation, functional neurosurgery, ansa lenticularis, dentothalamic tract.

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Ventral Intermediate Nucleus structural connectivity-derived segmentation: anatomical reliability and variability

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The Ventral intermediate (Vim) thalamic nucleus is the most commonly targeted structure for the treatment of drug-refractory tremors [1]. The identification of "functional targets" putatively corresponding to the Vim using connectivity based parcellation (CBP) of thalamus represents a promising approach for patient- tailored presurgical planning and post-surgery evaluation [2]. Since methodological differences across existing studies are remarkable [3], [4] we tested different parcellation pipelines for tractography-derived putative Vim identification.

Thalamic parcellation was performed both on high quality, multi-shell datasets and on down-sampled clinical-like datasets of 210 subjects from the Human Connectome Project (HCP). CBP has been performed using two different diffusion signal modeling techniques and two different voxel classification criteria thus, implementing four parcellation pipelines. The most reliable pipeline has been picked and parcels putatively corresponding to motor thalamic nuclei have been selected by calculating similarity with a histology-based mask of Vim and spatial proximity with known optimal stimulation points for the treatment of essential tremor. Finally, effect of data quality and parcellation pipelines on a volumetric index of connectivity clusters has been assessed.

We found that the pipeline characterized by higher-order signal modeling and threshold-based voxel classification criteria was the most reliable regardless data quality. The maps putatively corresponding to Vim were those derived by precentral- and dentate nucleus-thalamic connectivity. However, tractography- derived functional targets showed remarkable differences in shape and sizes when compared to a ground truth mask derived from digitalization of a histologic atlas.

Thalamic voxels connected to contralateral dentate nucleus resulted to be the closest to literature-derived stimulation points for essential tremor but at the same time showing the most remarkable inter-subject variability. Finally, the volume of connectivity parcels resulted to be significantly influenced by data quality and parcellation pipelines.

Hence, methodological variables play an important contribution on inter- individual variability of tractography derived thalamic parcellation, thus caution is warranted when performing thalamic connectivity-based segmentation for stereotactic surgery.

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The topological organization of the human cerebellum network

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The human brain is a complex network of interconnected regions [1]. Highly connected and highly central neocortical hub regions play a key role in global information integration between different parts of the network. The potential relevance of these "brain hubs" is highlighted by recent studies showing that disturbances of their structural and functional connectivity profile are linked to neuropathology. However, the growing field of connectomics have poorly focused its attention on the characterization of the cerebello-cerebellar network at the system level, despite the cerebellum acts as a crucial node in the information transfer throughout the whole brain and represents itself a little complex network. Here, we aim at characterizing the cerebellar network topology, mapping the hubs of the cerebellum and their mutual relationship. Whole-cerebellum structural networks of 100 un- related healthy subjects were reconstructed combining diffusion magnetic resonance imaging data with advanced signal modeling and tractography techniques. We demonstrate that the topology of the cerebellum network clusters into modules with high efficiency and short path length, thus reflecting an intrinsic small-world architecture, functionally segregated (local clustering) and integrated (global efficiency). In addition the cerebellum seems to exhibit a rich-club organization, with highly connected and central nodes having a strong tendency to be mutually interconnected, thus constituting a focal point for whole-brain communication.

Mapping the higher-level topology of the cerebellum network is of fundamental importance for better understanding the information process within the cerebellum and may provide new insight into the evaluation of different cerebellar disorders.

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Key words

Brain networks, cerebellum, connectome, network neuroscience, topology.

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Mesenchymal stem cells (MSCs) administration reduces microgliosis and microglial CCL2 release in EAE mice

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In myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), the mouse cerebral cortex shows several areas of demyelination associated with inflammation and blood- brain barrier (BBB) alteration. Mesenchymal stem cells (MSCs) administration has already been demonstrated to be capable of reducing neuroinflammation and astrogliosis in EAE. In order to further examine the pathogenetic mechanisms of gray matter EAE and the effects of MSC administration in this condition, we investigated the cellular sources of CCL2, a chemokine involved in leukocytes recruitment and BBB impairment. In this study macrophage/microglia markers and microglia-specific markers, namely TMEM119 and SALL1, combined with CCL2, were investigated by immunohistochemistry (IHC) and dual RNAscope IHC/in situ hybridization techniques. The results revealed that in EAE-affected mice, the primary source of CCL2 production is represented by hypertrophic microglial cells, which surround neurons of the neocortex and take contact with microvessels characterized by a damaged BBB. In EAE-affected MSC-treated mice, reduced microgliosis, decreased CCL2 expression, and restored BBB features were observed. The findings of this study highlight important pathogenetic mechanisms involved in neocortex neuroinflammation and BBB dysfunction, also revealing ameliorative effects of MSC administration and possible therapeutic implications.

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Key words

Experimental autoimmune encephalomyelitis, neocortex, microglia, CCL2, mesenchymal stem cells.

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COVID-19 and Carotid Body: Histopathological and Virological Analyses of an Autopsy Case Series

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The involvement of the carotid body (CB) in Coronavirus Disease 2019 (COVID- 19), with consequent chemosensitive impairment, has been hypothesized by various authors in the past months [1-5]; direct invasion or indirect effects by systemic stimuli ('cytokine storm', angiotensin-converting enzyme [ACE]1/ACE2 imbalance) have been suggested as the possible mechanisms. However, empirical evidence is limited or partial. Here, we present an integrated histopathological and virological analysis of CBs sampled at autopsy from four subjects (2 males and 2 females; age: >70 years old) who died of COVID-19. Histopathological, immunohistochemical and molecular investigation techniques were employed to characterize Severe Acute Respiratory Syndrome – Coronavirus 2 (SARS-CoV2) viral invasion and inflammatory reaction. SARS- CoV2 RNA was detected in the CBs of three cases through Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR). In these cases, positive immunostaining for Nucleocapsid and Spike protein were also demonstrated, mainly at the level of large roundish cells consistent with type I cells, confirming direct CB invasion. In these cases, T lymphocytes showed focal aggregations in the CBs, suggestive of local inflammatory reaction. Blood congestion and microthrombosis were also found in one of the positive cases. Intriguingly, microthrombosis, blood congestion and microhaemorrages were also bilaterally detected in the CBs of the negative case, supporting the possibility of COVID-19 effects on the CB even in the absence of its direct invasion. SARS-CoV-2 direct invasion of the CB is confirmed through both immunohistochemistry and RT-PCR, with likely involvement of different cell types. We also reported histopathological findings which could be ascribed to local and/or systemic actions of SARS-CoV-2 and which could potentially affect chemoreception.

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Macrophage infiltration contributes differently to axonal degeneration after chemotherapy treatment: an experimental study

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Chemotherapy-induced peripheral neurotoxicity (CIPN) is a serious dose-limiting chemotherapy toxicity which decreases quality of life in cancer survivors[1]. Its pathogenesis and treatment continue to be the subject of debate. Chemotherapy drugs can accumulate in peripheral axons causing neuroinflammation through activation of immune cells, and consequent secretion of mediators that enhance sensory nervous system sensitization. Macrophages comprise a very important component of the innate immune system that play key roles in PNS injury[2]. Despite our increasing understanding of the mechanisms that drive CIPN, the interplay between the immune and nervous systems remains unclear.

In the current study, we investigated neuroimmune changes associated with neurophysiological and morphological evaluations in 4 experimental rat models of CIPN, using different antineoplastic drugs that are known to cause CIPN: bortezomib (BTZ, proteasome inhibitor), cisplatin (CDDP, platinum compound), paclitaxel (PTX, taxane family) and vincristine (VCR, vinca alkaloid family).

The repeated injection of BTZ caused mechanical allodynia in rats from the 3rd week of treatment, which is associated with peripheral neuropathy after 4 weeks of BTZ treatment. Moreover, the morphological analysis described a huge axonal damage and a macrophage infiltration characterized by the pro- inflammatory phenotype M1. In this context, we tested the immunomodulatory effect of human intravenous immunoglobulins (IVIg) in reducing allodynia symptoms and macrophage infiltration in peripheral nerves.

Furthermore, the macrophage infiltrate was already detected from the 1st week of treatment in PTX- treated animals with a progressive increase until the end of the treatment, while chronic VCR-injection caused a mild macrophage infiltration after the 2nd week. Impairments of caudal nerve conduction amplitude were detected from the 2nd week after both anticancer administrations, with a stronger severity in PTX group compared to VCR one. On the contrary, no macrophages were observed in peripheral nerves of CDDP-treated animals, in which only a very mild axonopathy was detected.

In conclusion, our data suggest macrophage infiltration as a key element which could exacerbate both the development and symptoms associated with CIPN. Moreover, further investigation will be directed to define a new therapeutic approach by targeting macrophages in treating CIPN.

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Serotonergic neuronal bodies and processes in the human cerebellum: a light microscopy study

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Recently studies attribute to the cerebellum a critical role in emotional and cognitive functions and in serotonergic related brain disorders. Currently, in the cerebellar cortex and in the deep nuclei, has been demonstrated the presence of extrinsic serotonergic afferents, originated by several serotonergic neuronal groups of the brainstem reticular formation, and the presence of different serotonergic receptor subtypes. Morphofunctional data on a human intrinsic neuronal cerebellar serotonergic system are lacking. Therefore, the aim of this study is to evaluate in the human cerebellum the presence of an intrinsic serotonergic neuronal subpopulation. The study were carried out on postmortem fragments of the human cerebellar cortex and dentate nucleus, fixed in an aldehyde-picric acid solution, embedded in paraffin, cut into 5μm sections and subjected to light microscopic immunohistochemistry with rabbit polyclonal antibody for serotonin (5- HT).

The immunoreactions were revealed by streptavidin-biotin-peroxidase and 3,3-diaminobenzidine tetrahydrochloride (DAB) technique; for positive controls were used duodenum and brainstem fragments of rat subjected to the same experimental protocol.

The immunoreactions revealed 5-HT immunoreactive neurons in the cerebellar cortex (i.e. basket neurons, Purkinje neurons, granules, Golgi neurons, Lugaro neurons, candelabrum neurons, perivascular neurons) and in the dentate nucleus (associative neurons and different projective neuron types, perivascular neurons). This study indicate the existence of a serotonergic neuronal subpopulation, which could be involved in intrinsic and extrinsic cerebellar circuits. Moreover, this study suggest also a direct serotonergic neuronal control of the cerebellar blood flow and of the blood brain barrier, through the 5-HT immunoreactive perivascular neuron types. Finally, open new perspectives on the direct role of the cerebellum in the pathophysiology of neurologic and psychiatric disorders (e.g. ataxias, dystonia and mood disorders), and perhaps, the target for innovative pharmacologic and non-pharmacologic combined therapies.

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Cellule staminali, istogenesi e differenziamento

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Morphological behavior of human periodontal ligament fibroblasts towards the exposition to dentinal derivates biomaterial.

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The histological composition of the dentinal tissue lead researchers to explore its use as autologous grafting materials for regeneration interventions in oral and maxillofacial surgeries. Due to the novelty of the use of this material, few reported cases and few in vitro stud- ies are available in literature. The aim of the study was to evaluate the morphological behaviour of human periodontal ligament fibro- blasts (HPLF) towards different types of dentine derivates grafting material. The study design included the evaluation of mineralized dentine (SG), of deproteinized and demineralized dentine (DDP) and demineralized dentine (TT) as test materials, and the evaluation of deproteinized bovine bone (BIOS) as positive control material in con- tact with the HPLF cell line after 24 h, 72 h and 7 days of in vitro cul- ture. The evaluated outcomes were the morphological characterist- ics such as cellular shape and surface by light microscopy (LM) and scanning electron microscopy (SEM), and the adhesion using confocal microscopy (CLSM). The LM observations showed the pres- ence of densely packed cells, whilst the SEM observations showed how fibroblasts exposed to DDP and TT presented cytoplasmatic ex- tensions, while SG and BIOS presented, in addition to digitations and cytoplasmatic extensions, the thickening of the cellular mem- brane. The CLMS observations showed the expression of cyto- skeletal elements (vinculin, actin and integrin) involved in the adhesion process.

Overall, the experimental materials induced a positive response of the HPLFs in terms of proliferations and adhesion. The knowledge of the effects of the degree of mineralization of the dentine on the cellu- lar behavior will help clinician in the choice of the type of dentine derivates material according to the required clinical situation.

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Key words

Human periodontal fibroblasts, dentinal derivates, regenerative dentistry, dentin.

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Impairment of TLR9 agonist antitumor activity by anti-PD-1 antibody Fc domain: role of Fc receptors expressed by macrophages

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Toll-like receptor 9 (TLR9) agonists have been extensively investigated for their abil- ity to treat different cancer histotypes [1]. However, several inhibitory mechanisms established after TLR9 activation can restrain the efficacy of these immunothera- peutic drug [2]. An example is represented by the up-regulation on immune cells of programmed cell death 1 (PD-1), an inhibitory receptor known to dampen adaptive and innate immune cell function upon interaction with its cognate ligands [2,3]. Therefore, TLR agonists and anti-PD-1 antibodies may be extremely effective when combined. Blockade of PD-1 signaling may enhance the immunostimulatory effects of TLR agonists, and TLR ligation may improve the effect of PD-1/PD-L1 blockade by restoring the effector functions of tumor infiltrating immune cells. In this regard, it has been demonstrated that the combination of CpG-ODN, a TLR9 agonist, and anti-PD-1 antibody has been reported to reactivate T cell function but the effects on innate immune cells has not yet been completely elucidated [4]. To investigate the contribution of the innate immune system to this combinatorial regimens, immuno- deficient athymic nude mice were xenografted with IGROV-1 human ovarian cells and treated with CpG-ODN, anti-PD-1 antibody or their combination. We observed a strong reduction in the antitumor efficacy of a TLR9 agonist upon anti-PD-1 antibody administration. The impairment of CpG-ODN activity by anti-PD-1 antibody was paralleled by an increased infiltration of macrophages, characterized by a M2- like phenotype, in the tumor microenvironment. Moreover, gene expression profile data revealed that that TLR9-stimulated macrophages acquire an immunoregula- tory phenotype through interacting with the fragment crystallizable (Fc) domain of the anti-PD-1 antibody, leading to dampening of CpG-ODN antitumor effect. Ac- cordingly, in vivo macrophage depletion abrogated the detrimental effect exerted by the anti-PD-1 antibody. Since the stimulation of macrophage by TLRs can be achieved not only by synthetic agonists but also by molecules present in the tumor microenvironment, the data we are presenting may represent another possible mechanism of anti-PD-1 antibody therapy resistance. Indeed, it is possible that when delivered as a monotherapy, anti-PD-1 antibody Fc domain may interact with macrophages in which TLR signaling has already been triggered by endogenous ligands, mirroring the biological effects described in the present study.

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An immunohistochemical analysis of TLR4 and TLR7 expression during development in mouse: adrenal gland, liver, and pancreas.

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Toll-Like receptors (TLRs) are the mammalian orthologue of the type-I transmembrane receptor Toll originally identified in Drosophila for its function in embryonic developmental patterning (1). In mammals, TLRs are known to function in innate immunity by recognizing molecular motifs unique to pathogens or injured tissue. However, literature data are emerging about a morphogenetic role of TLRs during development also in mammals. We recently demonstrated the expression of TLR4 (recognizing the membrane component of Gram-negative bacteria LPS) and TLR7 (recognizing single strand viral RNA) in murine peripheral nervous system and in the gastrointestinal tube during the embryonic development (2). The aim of the present work was to proceed our investigation by studying the expression of TLR4 and 7 in the developing adrenal gland, with its medulla being a neural crest derivate together to the peripheral nervous system, and in the glands related to the gastrointestinal tube, such as liver and pancreas. Mouse embryos from stages E12 to E18 were processed for morphological and immunohistochemical analysis on formalin-fixed paraffin-embedded sections. TLR4 and TLR7 immunoreactivity appeared in the developing pancreas and liver already at E12, and, in the subsequent embryonic stages, showed different pattern of localization and intensity in the different glands. At E12, TLR4 and TLR7 expression started to be weakly visible also in the presumptive cortical primordium of the adrenal gland. As development proceeds, TLR4 and TLR7 expression appeared also in some isolated cells in the inner embryonic medulla of the adrenal gland. These data imply that TLR expression is developmentally regulated in mouse and could suggest a role for these receptors not only in the developmental processes, but also linked to the maturation of immunity mechanisms in preparation for birth.

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Key words
Toll-like receptors, development, immunohistochemistry, immune system.

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Differentiating factors loaded liposomes enhance DPSC osteogenic commitment

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Human Dental Pulp Stem Cell (DPSC) differentiation toward the osteoblastic lineage is accelerated and strengthened when culture medium is supplemented with differentiating factors, i.e. ascorbic acid, β- glycerophosphate and dexamethasone [1]. Liposomes, phospholipidic bilayer vesicles, are commonly used as carriers for drugs, growth factors and hydrophobic molecules [2]. This work aimed at speeding up DPSC commitment to osteoblasts by embedding liposomes with differentiating factors.

Firstly, liposomes containing differentiating factors were prepared by rehydrating a phospholipidic thin film, comprising 1-palmitoyl-2-oleoylphosphatidylcholine and cholesterol, followed by sonication. The obtained liposomes were characterized in terms of dimensions by dynamic laser light scattering.

Secondly, liposomes-exposed DPSCs were characterized by their immunophenotypic profile by measuring the expression of mesenchymal CD markers related to osteogenic differentiation. Levels of CD90 are significantly reduced in the presence of liposomes filled with differentiating factors (Lipo Mix) with respect to normal differentiation medium (DM), while CD73 and CD29 expression is increased in Lipo Mix condition, suggesting osteogenic commitment. Moreover, an appreciable inorganic matrix deposition is measurable.

Thirdly, it is evidenced that Lipo Mix formulation slightly increases ALP activity respect to DM, reduces H_2O_2 release triggering a precocious antioxidant cell response and redressing the redox balance required upon mesenchymal stem cell commitment to osteogenesis.

To conclude, it can be argued that Lipo Mix formulation speeding up DPSC osteogenic commitment could represent a valuable tool for clinical regenerative purposes.

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Key words

Liposomes, DPSC differentiation, ascorbic acid, dexamethasone, β-glycerophosphate, oxidative stress.

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Effects of antiretroviral drugs on adipocyte differentiation in an *in vitro* model

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BACKGROUND: The human imunodeficiency virus (HIV) still continues to be a global health problem, counting more than approximately 38 million people with the infection at the end of 2020, and of these, approximately 25.4 million people appear to be currently on treatment (UNIAIDS, December 2020)[1]. The antiretrovi- ral drugs used belong to the class: nucleoside analogues, nucleotide analogues, integrase inhibitors (InInt), protease inhibitors (IP) and non-nucleoside analogues. Side effects of therapy include the development of lipodystrophy, which leads to the accumulation of abdominal and dorsocervical region fat[2].

METHODS: The objective of the study is to evaluate in vitro the morphological and molecular effects on adipogenesis and lipogenesis, following single exposure to some drugs belonging to the class of integrase inhibitors (RAL, DTG, ELV, BIC) and combined exposure of these with tenofovir alafenamide (TAF)[3], nucleotide analogue.

RESULTS: In the case of single drug treatments, differentiation does not appear to be very different from that of control, although increased expression of both PPARγ and C/EBPα is observed. Instead in the combined treatment with TAF, an inhibition of adipocyte differentiation and a morphological change of the cells are evident, especially in combinations BIC+TAF and DTG+TAF; an increase in the level of PPARγ expression is observed in the early stages following induction, with a subsequent down-regulation, while the cells appear with an irregular shape and jagged contours.

CONCLUSIONS: The use of some of these drugs in patients who already have a BMI above normal values and therefore a particularly present fat mass at impendenziometry, could have a role in limiting lipid accumulation and/or the differentia- tion of mesenchymal cells into adipocytes.

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Key words

HIV, cell line 3T3-L1, lipodystrophy, antiretroviral therapy, adipogene- sis.

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CCL2/CCR2 chemokine system is selectively activated in hematopoietic progenitors of primary myelofibrosis and down- regulated by JAK-inhibitors.

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Philadelphia-chromosome-negative myeloproliferative neoplasms are a group of closely related hematopoietic stem cell disorders. Among them, primary myelofibrosis (PMF) is considered the paradigm of inflammation-related cancer development (1). Indeed, although its pathogenesis is triggered by acquired somatic mutations in specific myeloid genes, cell-extrinsic effects exerted by the malignant clone via inflammatory mediators result in systemic complications (2).

CCL2 is one of the most potent immune-modulatory cytokines, exerting its biological effects by preferentially engaging its cognate receptor CCR2, which activates a downstream signaling which includes G-proteins, MAPK/ERK, PI3K/Akt and JAK/STAT pathways (3).

SNPs in the regulatory regions of CCL2 gene account for inter-individual variability in CCL2 expression levels. Specifically, the rs1024611 (A>G) SNP of CCL2 leads to higher chemokine production (4) and is increased in secondary myelofibrosis (5).

Here, we investigated the functional relevance of CCL2/CCR2 axis in PMF, demonstrating that PMF cells overexpressed CCL2 as compared to control healthy subjects (HD). When stratified according to the to the *CCL2* SNP, MNCs from homozygous G/G PMF displayed a significant overexpression of CCL2 as compared to both wild type A/A and heterozygous A/G after ex-vivo stimulation with IL-1β, pinpointing G/G patients as the highest chemokine-producers.

We then asked whether PMF hematopoietic progenitors could be a target of CCL2, and we found that PMF CD34+ cells display > 60-fold-higher expression of CCR2 vs. HD, and ex-vivo stimulation with rhCCL2 determined a significant increase of Akt phosphorylation. Finally, we tested the effects of ruxolitinib, a JAK1/2 inhibitor with well-established immunomodulatory properties, on CCL2/CCR2 expression in PMF cells. MNCs from patients at 1, 3 and 9 months of therapy displayed a significantly reduced capacity to produce CCL2 upon inflammatory stimulus. Interestingly, this effect was more pronounced in G/G PMF. Reduced CCL2 production was coupled with a significant and stable reduction of CCR2 expression on CD34+ cells.

In conclusion, CCL2/CCR2 axis inhibition may be envisioned as a novel therapeutic strategy in PMF. Also, we provided a novel mechanism underlying the anti-inflammatory effects of ruxolitinib, via down-regulation of CCL2/CCR2 chemokine system.

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Regulation of cerebellar nuclei morphogenesis by *Zfp423* / *ZNF423*, a gene mutated in Joubert syndrome and cerebellar vermis hypoplasia

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The *Zfp423* gene encodes a 30-Zn-finger transcription factor that acts as a scaffold for the assembly of complex transcriptional and cellular machineries regulating development of the central nervous system and choroid plexus. Mutations of the human homolog *ZNF423* have been identified in patients carrying cerebellar vermis hypoplasia or Joubert Syndrome (JS), associated with other signs of classical ciliopathy outside the central nervous system (nephronophthisis 14). To further characterize the role of ZFP423 in cerebellar neurogenesis, we have performed morphological and molecular studies on a mutant mouse line carrying a deletion of exons 6 and 7 of *Zfp423*. The truncated protein loses 91 C- terminal amino acids, including zinc-fingers 28-30. Mutant mice exhibited clear defects in the morphogenesis and composition of all cerebellar nuclei (CN). Hence, several markers of the CN were analyzed and some of them were found to be altered, such as Lmx1a, Tbr1 and Brn2. These results highlight the important role played by ZFP423 in CN development and contribute to our understanding of its role as a disease / modifier gene in JS and, possibly, other ciliopathies.

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Faulty expression of *Spo11* splice isoforms predisposes to XY aneuploidy in sperms with a penetrance that depends on genetic background

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Sex chromosomes aneuploidies (SCAs) are among the most common human whole-chromosomal copy number variations [1], and unlike whole-chromosomes aneuploidies of the autosomes, are often of paternal origin [2]. In approximately 50% of males with Klinefelter Syndrome (KS) (47, XXY), the supernumerary X is paternal, due to the lack of crossing-overs (COs) between XY chromosomes [3-9], hampering their proper disjunction and segregation into daughter cells. Turner syndrome (TS) (45, X) also originate from defective segregation of XY chromosomes, as the ma- jority (60%-80%) of the X chromosomes are maternal in origin. In mammals, for- mation of COs between XY chromosomes occurs following double strand breaks (DSBs) formation and processing at the pseudo autosomal region (PAR) a short region of homology between them. DSBs are generated by the Sporulation Protein 1 (SPO11), through a type II DNA topoisomerase-like activity [10]. The *Spo11* gene generate two main slice isoforms: Spo11β and Spo11α. Previous data has shown that the *transgenic* expression of the single Spo11β isoform in mice, predisposes to XY asynapsis and sterility, due to the lack of DSB formation at the PAR [11].

In our study, using a new Spo11β *knock-in* mouse model, we demonstrate that fail- ure in DSB formation in the PAR of Spo11α-only mice varies with mouse genetic background, and that mice expressing only the Spo11β knock-in allele (Spo11αki- only males) are fertile and generates XY aneuploid sperms. In mammals, formation of DSBs requires the assembly onto chromatin of a pro-DSB protein complex, in advance of DSB formation. Key factors of the complex are MEI4, REC114 [12] and the PAR-specific factor ANKRD31 [13]. Here we demonstrate that faulty DSB for- mation in the PAR of males with different genetic backgrounds (C57 Vs. C57 and 129sv mix) is independent from MEI4, REC114 and ANKRD31 foci formation. For- mation of DSBs at the PAR also relies on the structure of DNA loops that extend from chromosome axes at the PAR [11, 12]. Here we provide evidence that genetic background shapes PAR loops length.

A lack of DSB formation in the PAR of Spo11α-only mice has suggested that Spo11β might play a specific function in ensuring DSB formation in the PAR [11]. To test this hypothesis, we generated a Spo11αki-only mouse model. We observed that both males and females were sterile, indicating that Spo11β is not able *per se,* to form DSBs in the whole genome.

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Key words

Spo11, DSBs, XY aneuploidy, genetic background, male sterility.

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Mapk activation drives male and female teratocarcinomas from late PGCs.

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Germ cell tumors (GCTs) represent a model of progressive neoplastic transformation starting from primordial germ cells (PGCs) or from more developmentally advanced germ cells, such as gonocytes, i.e., the male or female fetal gonadal germ cells. GCTs occur mainly in neonates, infants, and adolescents, rarely in the adults. They arise primarily in the testes and ovaries, though they also occur less frequently in extragonadal sites (1). GCTs account for about 98% of testicular cancer cases (2) and for only 2-3% of ovarian cancer cases (3). Testicular germ cell tumors (TGCTs) represent the most common solid malignancy affecting males between the ages of 15 and 35. Ovarian germ cell tumors (OGCTs) account for approximately 1–2% of all ovarian malignancies (4-5). To understand the mechanisms that underlie germ cell transformation, we established a GCT mouse model carrying germ cell-specific *BRafV600E* mutation with or without heterozygous *Pten* deletion. Both male and female mice developed monolateral teratocarcinomas containing embryonal carcinoma (EC) cells that showed an aggressive phenotype and metastatic ability. Germ cell transformation started in fetal gonads and progressed after birth leading to gonadal invasion. Prepuberal testes showed areas of intratubular germ cell neoplasia (ITGCN) together with areas of tumor progression, while ovaries did not present any carcinoma in situ (CIS) formation but showed increased number of follicles, multi-ovular follicles (MOFs) and scattered metaphase I oocytes containing follicles. Our results indicate that Mapk over-activation in fetal germ cells of both sexes can expand their proliferative window leading to neoplastic transformation and metastatic behaviour.

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Key words:
Germ cell tumors, PGCs, BRaf, Pten, mouse model.

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Dysfunctional hepatic lysosomal acid lipase decreases fat disposal and promotes NAFLD progression

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Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Western countries and is correlated with altered lipid metabolism and impaired lipophagy [1]. LAL is a lysosomal enzyme that hydrolyzes cholesterol esters and triglycerides and in general plays an important role in lipid disposal. The impairment of LAL activity was proposed as an underlying mechanism in the pathogenesis of NAFLD [2]. In order to find a correlation between LAL alteration and NAFLD, we examined LAL expression and localization in pre-clinical cellular and animal models of NAFLD and in NAFLD patients. In Huh7 cells exposed to high-glucose/high-lipid (HGHL) medium and in the liver of C57BL/6 mice fed with high-fat-diet (HFD), a reduced LAL activity together with decreased functional/not-ubiquitinated LAL protein levels was evidenced. Moreover, the accumulation of dysfunctional/ubiquitinated LAL, the high rate of ubiquitination and extra-lysosomal localization was demonstrated. Lastly, a lower level of functional/not-ubiquitinated LAL protein correlating with disease progression was detected in NAFLD patients. All these findings strongly suggest that the reduced hepatic fat disposal and NAFLD progression are promoted by impaired LAL function.

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Lysosomal acid lipase, non-alcoholic fatty liver disease.

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Retinoic Acid synergizes with proteotoxic stress to induce the death of FLT3-ITD$_+$ Acute Myeloid Leukemia cells

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Acute myeloid leukemia (AML) is a heterogeneous group of diseases, due to chromosomal abnormalities and gene mutations, causing the impairment of myeloid progenitor differentiation. Since leukemic blasts express mutant proteins and especially fusion proteins which cannot reach a proper conformation, this intrinsic source of proteostasis alteration renders the cells more sensitive to pharmacological induced proteotoxic stress[1]. Indeed, we previously demonstrated that, in the presence of the differentiating agent Retinoic Acid (R), the combination of Endoplasmic Reticulum (ER) stress induced by Tunicamycin (T) and oxidative stress induced by Arsenic Trioxode (A) is effectively inducing the death of AML cell lines and human primary blasts bearing ITD (Internal-Tandem-Duplication) mutations of the tyrosine kinase receptor FLT3 (FLT3-ITD)[2]. In order to increase the potential of a combined treatment as new therapeutic strategy for AML, we replaced the ER stress inducer T with proteasome inhibitor Bortezomib (B), already used in clinical practice, likewise R and A. Our results show that the triple combination Retinoic Acid, Bortezomib and Arsenic trioxide (RBA) is even more efficient than RTA regimen above in causing the death of FLT3-ITD+ AML cell lines and primary leukemic stem cells isolated from patients. We found that cell death induced by RBA combined treatment is mostly accounted by the generation of cellular oxidative stress: in fact, despite ROS production leads to a strong activation of the oxidative stress response, this is not enough to enable leukemic blast survival in these conditions. We furthermore evaluated the efficacy of this novel combined treatment by targeting the bone marrow niche *ex vivo*[3]. We found that murine bone marrow stromal cells are able to protect leukemic cells from the toxic effects of RBA combined therapy. In particular, stromal cells protect AML cells by attenuating the oxidative stress induced by RBA. Thus, we applied high doses of the pro-oxidant agent ascorbic acid (vitC) to tamper with the protective effect of bone marrow stromal cells on AML cells. Indeed, high doses of vitC have been assayed in clinical trials as adjuvant for cancer treatment, without relevant toxicity. Finally, we obtained preliminary evidences *in vivo* confirming the efficacy of the triple treatment RBA combined with ascorbic acid: these results open a new perspective for the potential translational application of this drug combination in AML treatment.

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The inhibitory role of miR-486-5p on the ALDH + and CD44 + /CD326 + of Cancer Stem Cells in Colorectal Cancer

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Globally, colorectal cancer (CRC) is the third most frequent cancer and the second cause of death for cancer worldwide. A precocious diagnosis of CRC is linked to a good outcome, which however remains silent for several years from the onset. Increasing evidence associates the model of Cancer Stem Cells (CSCs) to this tumor, binding them to the most critical aspects of the pathology, such as relapses, resistance to treatments and metastatic dissemination. To have a greater characterization of this cellular phenotype would be a fundamental tool in the fight against cancer. Excellent candidates for this purpose are microRNAs, small sequences of single strand RNA (19- 22nt) very stable in the blood, with an important role in the regulation and cell operation, whose deregulation is associated with the development of cancer diseases and could be linked to the development of CSCs.

In order to understand the involvement of miRNAs in CSCs and CRC metastases, we analyzed miRNoma in the serum of a group of metastatic and non-metastatic CRC patients, leading to the identification of miR486-5p, downregulated in the metastatic group. The expression of mir-486-5p was then analyzed in HCT-116, HT-29 and T-84, CRC models, and compared with the same models enriched in CSCs (ALDH+ and CD44+/CD326+), highlighting a downregulation of miR-486-5p in the latter, corroboring the association between metastases and CSC. The forced induction or inhibition of miR-486-5p expression on the same models allowed to highlight the inhibitory effect of this miRNA on the stem cell factors (Sox2, Oct4, Klf4, cMyc and Nanog) and on some genes involved in Wnt, Hedgehog, Notch, and TGF-β, the four main Pathways of the CSC. These data could allow to interpret the role of miR-486-5p as inhibitory in the CSC phenotype of CRC, indicating it as a candidate for further studies aimed to marker discovery and targeted therapy.

Key words

MiRna, CRC, CSC, marker discovery.

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Enhanced Extracellular Matrix Deposition, Runx2 and VEGF/VEGF-R Expression in Human Periodontal Ligament Stem Cells Seeded on Titanium Implant Surfaces: Cellular and Molecular Responses

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The major challenge for dentistry is provide to the patient an oral rehabilitation maintaining an healthy bone conditions in order to reduce the time for loading protocols (1). The advancement in implant surface design are necessary to favour and promote the osseointegration process (2, 3). The surface features of titanium dental implant, can apply a relevant influence on the morphology and cells differentiation of mesenchymal stem cells promoting the osteoblastic and vascular genes expression and the release of extracellular matrix (ECM) components. The present study aimed at evaluating the in vitro effects of two different dental implant titanium surfaces, TEST and CTRL, cultured with human Periodontal Ligament Stem Cells (hPDLSCs) on the expression of ECM molecules such as Vimentin, Fibronectin, N-cadherin, Integrin beta-1 (ITGB1) and Focal Adhesion Kinase (FAK), the osteogenic related markers as RUNX2 and Alkaline phosphatase (ALP) and vascular related markers as Vascular Endothelial Growth Factor (VEGF) and its receptor (VEGF-R). The TEST implant surface cultured with hPDLSCs demonstrated a better cell adhesion capability as exhibited by Scanning Electron Microscopy (SEM) analysis. The immunofluorescence and the western blot experiments showed an over expression of Fibronectin, Laminin, N-cadherin, RUNX2, VEGF and VEGF-R in hPDLSCs seeded onto TEST implant surface. The gene expression executed by RT-PCR validated the results obtained by protein assays and exhibited an high expression of Vimentin, ITGB1 and F in hPDLSCs seeded on TEST surface compared to the CTRL dental implant surface. Understanding the mechanisms of ECM components release and its regulation, is essential for developing novel strategies for tissue engineering and regenerative medicine. In fact, our results demonstrated that the impact of treated surfaces for titanium dental implants might increase and accelerate the ECM apposition, other than stimulate the VEGF factor to promote the vascularization process and provide the starting point to initiate the osseointegration process.

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A Novel Anti-Inflammatory Strategy on Endothelial Committed Human Periodontal Ligament Stem Cells Treated with Lipopolysaccharide from Porphyromonas Gingivalis

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Curcumin, a yellow polyphenol extracted from the turmeric root is used as a diet supplement. It exhibits anti-inflammatory, antioxidant, and antitumor properties by modulating different intracellular mechanisms (1). Due to their low solubility in water, the curcumin molecules must be encapsulated into liposomes to improve the bioavailability and biomedical potential (2). For the periodontal tissue and systemic health, it is essential to regulate the local inflammatory response (3). In this study, the possible beneficial effect of liposomes loaded with curcumin (CurLIP) in neural crest-derived human periodontal ligament stem cells (hPDLSCs) and in endothelial-differentiated hPDLSCs (e-hPDLSCs) induced with an inflammatory stimulus (lipopolysaccharide obtained from Porphyromonas gingivalis, LPS-G) was evaluated. The CurLIP formulation exhibited a significant anti-inflammatory effect by the downregulation of Toll-like receptor-4 (TLR4)/Myeloid differentiation primary response 88 (MyD88)/nuclear factor kappa light chain enhancer of activated B cells (NFkB)/NLR Family Pyrin Domain Containing 3 (NLRP3)/Caspase-1/Interleukin (IL)-1_ inflammation cascade and reactive oxygen species (ROS) formation. Moreover, the exposure to LPS-G caused significant alterations in the expression of epigenetic modifiers, such as DNA Methyltransferase 1 (DNMT1) and P300, while the CurLIP treatment showed physiological expression. Overall, our in vitro study provides novel mechanistic insights into the intracellular pathway exert by CurLIP in the regulation of inflammation and epigenetic modifications.

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Key words

Curcumin, liposome, human periodontal ligament stem cells, endothelial-differentiation, Porphyromonas gingivalis, reactive oxygen species, inflammation, cardiovascular disease.

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Liver histo-morphology and progenitor cell niche are altered by lipopolysaccharides from gut microbiota in non-alcoholic fatty liver disease.

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Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease (1). Numerous evidence suggested that gut microbiota may be implicated in the pathogenesis of NAFLD (2). small intestinal bacterial overgrowth contributing to increased serum endotoxemia has been described in NAFLD, with Escherichia coli being the most abundant bacterium. The aim of this study is to investigate the mechanisms by which lipopolysaccharides (LPS) from gut microbiota favors the progression of NAFLD and its effects on liver histology and progenitor cell niche. We studied Escherichia Coli LPS in patients with biopsy-proven NAFLD and matched serum samples: 25 simple steatosis (nonalcoholic fatty liver) and 25 nonalcoholic steatohepatitis (NASH). Biopsies were examined by routine histology and immunohistochemistry. In vitro, hepatocytes and macrophages were cultured in media with LPS addition and Toll-like receptor 4 (TLR4) pathway was analyzed by western blot. In mice, NASH was induced by methionine-choline deficient (MCD) and high fat diets; TAK-242, a TLR4 inhibitor was administrated to MCD mice by daily intraperitoneal injection. NASH patients had higher serum LPS and hepatocytes LPS localization than controls, which was correlated with serum zonulin and phosphorylated Nuclear Factor-κB expression. In vitro, LPS can activate TLR4 pathway in hepatocyte and liver macrophages, triggering pro- inflammatory phenotypes. TLR4+ macrophages were higher in NASH than simple steatosis or controls and correlated with serum LPS. TLR4+ portal macrophages are spatially associated with hepatic progenitor cells and correlated with ductular reaction (hallmark of progenitor cell activation). In mice with NASH, LPS serum levels and LPS hepatocyte localization were increased compared with control mice and associated with Nuclear Factor-κB activation. Treatment with TLR4 inhibitor resulted in lower liver inflammation in mice with NASH. In conclusion, In NAFLD, Escherichia Coli LPS may increase liver damage by inducing macrophage polarization, liver inflammation and progenitor cell niche activation through TLR4 pathway.

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Key words

Liver, gut microbiota, macrophages, toll like receptor, stem cells.

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Role of Galectin-3 as modulator of pericytes and endothelial cells interplay during human fetal brain angiogenesis

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During corticogenesis, the human fetal brain is characterized by pronounced angiogenesis, leading to an increasingly dense microvascular network. Angiogenesis is driven by pericytes (PCs) that intimately interact with endothelial cells (ECs), which can be activated in different phenotypic states. Previous studies, carried out *in vitro* and *in vivo* models of angiogenesis, have demonstrated that the PCs/ECs interaction is mediated by the Neural/glial antigen 2 (NG2) proteoglycan, expressed by angiogenic pericytes, the a3b1 integrin, present on the surface of endothelial cells, and the Galectin-3, a member of the lectin superfamily, detectable on the endothelial cell membrane or dispersed in the extracellular matrix. These studies suggested an essential role for lectin in inducing NG2 proteoglycan clustering and a subsequent amplification effect in endothelial cell adhesion and migration. To better understand the tight interaction endothelium-pericyte during normal neural angiogenesis and the exact Galectin-3 localization in cerebral microvessels, fetal mouse brain were processed for immunohistochemistry (IHC) by using CD31 endothelial and NG2 pericyte cell markers combined with Galectin-3 and for dual RNAscope *in situ* hybridization/IHC by CD31 and NG2 antibodies combined with Galectin-3 probe to transcript detection. The results confirmed the expression of Galectin-3 in endothelial cells and revealed Galectin-3 mRNA in pericytes suggesting an additional source of lectin during fetal brain angiogenesis. Overall, these results indicate NG2 and Galectin- 3 as possible regulators of PCs/ECs communication during human cerebral cortex vascularization.

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Key words

Neovascularization Perycytes Galectin-3.

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Thymol and Vitis Vinifera Leaf extract trigger a different response to a single dose of UVB rays in a 3D model of normal human skin.

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In epidermal keratinocytes, tight junctions (TJs) and cytoskeletal keratin (K) filaments represent dynamic scaffolds able to respond to environmental stress as ultraviolet radiations B (UVB). K10 is constitutively expressed in the suprabasal differentiating layers. K16 and K17 are key early barrier alarmins and their upregulation can alter proliferation, cell adhesion, and migration. Up to now, K17 expression in keratinocytes is regulated through STAT3-dependent mechanisms by pro-inflammatory cytokines. UVB-induced epidermal damage can be prevented by dietary polyphenols.

The aim of the present study was to investigate the potential protective effect of a short pretreatment (1 hour) with either thymol (1μg/ml) (T) or Vitis vinifera L. ex- tract (100μg/ml) (V) after a single UVB dose (200 mJ/cm^2) in a 3D model of normal human skin. Samples were harvested 24 hours after irradiation and routinely pro- cessed for paraffin embedding. The expressions of ZO-1, claudin 1, keratin 10, 16 and 17 were analyzed by indirect immunofluorescence. Results were expressed as the percentage of keratin-immunopositive area per mm2 of living epidermis + 1 standard deviation.

UVB irradiation induced i) a statistically significant increase of K16 expression compared to control samples ($p<0.0001$), ii) an increase of K17 expression ($p<0.0001$), accompanied by STAT3 phosphorylation, and iii) a parallel decrease of K10 positivity in the lower/medium spinous layers ($p<0.01$). Only the T pretreat- ment was able in reducing K16 expression comparable to controls ($p<0.001$). In V pretreated samples, K17 immunoreactivity was significantly decreased ($p<0.05$) and K10 expression reached the values of the control group. On the other hand, the T pretreatment was able to inhibit both K17 and pSTAT3 expression, but had no effect on K10 expression.

After irradiation, a faint ZO-1 immunolabelling persisted in the granular layer. The V pretreatment was able to revert its expression throughout the epidermis while the T pretreatment did not. No effect on claudin-1 distribution was observed in all irradiated samples, independently of the treatment.

In conclusion, we demonstrated that, after UVB exposure, V pretreatment triggers the maintenance/restoration of epidermal homeostasis, as to terminal differentia- tion and TJs, while T pretreatment limits the early stress response induced by UVB.

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Unravelling the role of TPC2/calcium signaling-dependent autophagy in melanoma microenvironment remodelling

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The ion channel TPC2, localized on the membranes of acidic organelles such as endo-lysosomes and melanosomes, is known to play a role in relevant pathologies, e.g. tumor metastasis and viral infection. We set out to explore a so far neglected aspect of its functions, i.e. the impact of TPC2 in the cross-talk between tumor cells and tumor microenvironment, an interaction known to regulate tumor aggressiveness. Data showing that TPC2 participatesin the regulation of autophagy and secretionsuggest focusing on these functions and their interrelations. Relevant is the known plasticity of macrophages that, in response to tumor microenvironmental cues undergo a phenotypic switch (polarization) from anti-tumoral (M1-like) to pro-tumoral (M2-like). Our experimental model is represented by malignant melanoma, a highly insidious malignancy in which we have previously characterized the relevance of TPC2-regulated signaling. In particular, using different melanoma cell lines, human peripheral blood mononuclear cells (hPBMCs) and murine bone marrow precursor cells (BMPs), we tested how TPC2 genetic deletion or pharmacological inhibition can modify the secretoma of either cell type, in turn subverting malignancy traits, among which melanoma radioresistance. Aiming to disclose a novel targetable component in the chain of local interactions regulating the aggressiveness of melanoma, our data show that in mouse and human melanoma cells, TPC2-KO and TPC2 pharmacological inhibition impacts on the autophagic process, radiosensitivity and secretoma, in turn affecting macrophage polarization.

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Triple negative-breast cancer translation rewiring shapes invasiveness and niche composition

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Breast cancer (BC) is the deadliest cancer for women worldwide. Despite great progresses in breast cancer treatment, a targeted therapy for Triple Negative Breast Cancer (TNBC), the most aggressive and metastatic subtype, is still an unmet therapeutic need[1].

We previously demonstrated that cancer cells with metastatic potential are capable to survive microenvironmental stress and initiate the transition towards a migratory phenotype by triggering the evolutionarily conserved Integrated Stress Response (ISR)[2]. ISR encom- passes several interconnected signalling pathways, converging on the eIF2α/ATF4 axis and eliciting translation and metabolic rewiring[3]. The mechanism by which the metastatic cells hijack eIF2α/ATF4 pathway and the downstream effect on the cancer niche are yet to be unravelled and may provide promising therapeutic targets for TNBC treatment.

Our aim is to provide a mechanistic understanding of stress-induced translation and meta- bolic reprogramming in TNBC, elucidating how these stress-induced mechanisms are involved in driving the metastatic phenotype and modulate the immune niche composition. Here we show that TNBC bears high levels of ISR, which correlates to high metastatic po- tential, EGFR tyrosine kinase receptor expression levels and macrophagic infiltration, shedding light on a stress-driven and EGFR-enhanced cross-talk between the cancer cell and its immune niche.

Our studies point towards proposing, as an innovative therapeutic approach, that the target- ing of the translation machinery in combination to the EGFR inhibitors already available would be an effective therapy by limiting the capacity of cells to survive stresses in the in- tratumour microenvironment or during metastatic dissemination.

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Triple Negative Breast Cancer, metastasis, Integrated Stress Response, EGFR, macrophages.

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Demonstration of the anatomical pathway of ESP anesthetic block. A pilot study

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Erector Spinae Plane (ESP) block is an interfascial blockade used daily in several clinical scenarios, with variable efficacy and reproducibility [1]. This study investigated the anatomical target of ESP block and its pathway of diffusion.

Experienced anesthetists bilaterally performed the ultrasound-guided ESP block with an injection at the T5 vertebral level [2]. Diluted black tissue marking dye (20 mL) was injected in two fresh frozen bodies within the Body Donation Program of the University of Padova [3]. The gross anatomical dissection followed through a combined posterior plus anterior sequential approach. The histotopographic with morphometric examination completed the investigation.

By gross anatomical dissection, the dye spreading macroscopically was observed on the dorsal side of the chest from T2/3 to T10/11 with an extension up to 10 cm laterally, and on the ventral side of the chest from T2/3 to T9 10. By histotopographic examination, the dye diffused ventrally to the intercostal nerves spaces (2-3 and 5-6 nerves spaces on the right and left, respectively) by following the blood vessels coupled to the dorsal nerve passing through the costotransverse foramen.

The anterior dye diffusion from the injection site within the erector spinae muscles group during an ESP block reached the intercostal nerves. The dye followed the blood vessels and dorsal branches of spinal nerves, suggesting the passing through the costotransverse foramen to reach the anterior paravertebral space and the intercostal nerves, somehow resembling a paravertebral block.

The authors express their deepest gratitude to the people adhering to the Body Donation Program 'Donation to Science' of the University of Padova, thanks to whom this research was possible.

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Key words

Erector spinae plane block, ESP, histotopography, cadaveric study.

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Electron microscopy investigation of the Townes mouse spleen: an example of Translational Anatomy

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Background: the chimera mouse developed by T.M. Townes, expressing human HbS, is one of the most used disease models to study Sickle cell disease (SCD)[1], but an exhaustive analysis of the morphology and histopathology of heterozygous animals (Sickle cell Trait - HbSA) has not been published to date.

Recent data reveal that even in heterozygotes human subjects, Sickle cell Trait (SCT), erythrocytes can undergo changes in shape, plasticity and function if exposed to reduction of plasma pH, oxygenation and temperature, or dehydration. Although only homozygous individuals (HbSS) develop classic multi-organ ischemic disease, some long-term complications are beginning to be well known in Trait subjects, especially African Americans[2]. The mechanisms leading to these phenomena are not well understood, however it has been demonstrated that the erythrocytes of heterozygous human subjects are significantly stiffer than normal[3]. Our preliminary histological observations in 5 spleens of HbSA mice, revealed only a mild vascular ectasia with a surrounding increase in white pulp and some multinucleated giant cells.

Materials and Methods: We analyzed 2 healthy controls homozygous HbAA and 2 HbSA mice. After surgical removal a very rapid immersion of the organ was made in glutaraldehyde, where the reduction took place until obtaining 1x1x3 mm pieces. The immersion in fixative lasted 6 hours. After fixation, the sample was stored inside the vials in fresh phosphate buffer at 4° C. Subsequently, they were postfixed with OSO4, dehydrated on an increasing alcoholic scale and then incorporated into epoxy resin. Ultrathin sections of 50-80 nm were cut and stained with uranyl acetate and lead citrate for Transmission Electron Microscope analysis.

Results: compared to HbAA, HbSA mice showed a thickening of the endothelial basal lamina of the marginal zone vessels. Furthermore, mitochondria projecting into the vascular lumen have been observed in endothelial cells. Another significant finding was the accumulation of collagen in the perivasal spaces.

Discussion: our results suggest a translational value of this heterozygous mouse model to study the mechanisms by which subclinical vascular changes may occur in asymptomatic patients with SC trait. These observations may indicate an increased endothelial adhesion of stiff red blood cells and an increase in tissue stress with the reduction of plasma diffusion and a consequent mitochondrial adaptation.

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Morphological alterations and stress proteins variations in lung biopsies obtained from autopsies of COVID-19 subjects.

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The Coronavirus Disease 2019 (COVID 19) is a complex disease caused by SARS-CoV-2 which, in a short time, has become pandemic. This pathology is mainly characterized by an acute respiratory manifestation but can also affect other organs simultaneously or individually. Molecular chaperones, many of which are heat shock proteins, play a role in cell stress response and regulate the immune system in various ways, including hyperinflammatory/autoimmune reactions. In our study we performed a histological analysis and an immunomorphological evaluation on lung samples from subjects who died for COVID 19. The histological evaluation showed a significantly modified lung parenchyma architecture and abnormal cells with large, irregular, and monstrous nuclei. Immunomorphological evaluations, performed by CKAE1AE3, CK7, CD34, CD61, CD68, Ki67 antibodies, permitted us to better characterize the morphological alterations and the abnormal cells. Concerning molecular chaperones, Hsp60 and Hsp90 immunopositivity was significantly higher in the COVID-19 group compared to the control group, and their immunolocalization was found also in the plasma membrane of the endothelial cells of the COVID-19 subjects. Interestingly, this plasma membrane localization seems to be related to post-translational modifications of these chaperones. Since our group already demonstrated that Hsp60 and Hsp90 share immunogenic epitopes with proteins of SARS-CoV-2, our working hypothesis is that they could be involved in the pathogenesis of COVID-19 by inducing molecular mimicry phenomena.

Key words

SARS-CoV-2, COVID-19, Hsp60, Hsp90, endothelium, molecular mimicry, autoimmunity.

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A comprehensive review of the history of Pott's disease: an osteological perspective

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Tuberculosis (TB) is a communicable pulmonary disease that still nowadays remains one a major cause of ill health, ranking one of the top 10 causes of death worldwide. Also, it is the leading cause of death from a single infectious agent rating above HIV/AIDS. According to the World Health Organization, almost 10 million people fell ill with TB in 2019, with an estimated 1.4 million deaths globally. Unfortunately, the number of infected people decreases slowly every year as well as current therapeutical strategies are showing their limitations due to multi-drug resistant strains. Totally effective vaccines are still not available. Moreover, the origin and the evolution of TB is still not fully understood. Therefore, understanding its origins and history is a relevant issue to assess this medical problem from an evolutionary perspective. The principal causative organism of TB is *Mycobacterium tuberculosis*, an obligate pathogen that is a member of the *M. tuberculosis complex* (MTBC) which is mainly spread by air. The disease typically affects the lungs but can also affect other sites (extrapulmonary TB). Of those, the most common and characteristic are the skeletal changes affecting the spine, such as Pott's disease. The bacilli locate in the anterior portion of the vertebral bodies (mainly at the thoracic tract). Afterward, these lesions can cause vertebral collapse (from one to three in most cases) and fusion of the vertebrae themselves producing the curved spine known as Pott's disease. This condition which results from the exaggeration of the physiological kyphosis of the thoracic spine was named after Sir Percivall Pott (1714-1788) who, as a surgeon at St Bartholomew's Hospital in London, first described it in 1779. Moreover, Pott's disease might be identified studying the morphology and shape of the gibbus in which there is loss of function in the lower limbs due to damage to the spinal column. Therefore, studying the skeletal remains dating back thousands of years could be very important in order to better understand the evolution and the mechanism of infection of TB. Here, we offer a comprehensive review of the history of Pott disease as well a full contextualization of Pott's disease in the ancient times.

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Key words

Tuberculosis, Pott's disease, osteological remains.

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Biomimetic keratin gold nanoparticle-mediated *in vitro* photothermal therapy on glioblastoma multiforme

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Biomedical applications of plasmonic nanoparticles (NPs), have steadily grown in the past 10 years. In particular, the ability of NPs to convert light into thermal energy (photothermal effect) has been exploited to develop a noninvasive, drug-free therapy defined as plasmonic photothermal (PPT). Indeed, when the NP surface, such as gold NPs (AuNPs), is illuminated, the strong absorption of light induces a significant temperature increase thereby achieving temperatures able to induce apoptosis in cancer cells [1]. Among the different proteins used to functionalize the surface of AuNPs, keratin (Ker) is still unexplored and no studies reporting its utilization in the field of PPT-based applications have been found. We tried to validate the exceptional biocompatibility of Ker-AuNPs and to perform PPT experiments on a human cell line (U87- MG) derived from glioblastoma, a brain tumor of glial origin, one of the most aggressive and currently untreatable cancers [2]. For comparison, cells were exposed to various concentrations of Ker-AuNPs (0–50 μM) for 24, 48 and 72 h. The chemical, physical, morphological and photothermal properties of Ker-AuNPs are investigated using dynamic light scattering, ζ-potential, UV–Visible, Fourier transform infrared spectroscopy, x-ray photoelectron spectroscopy and thermography. *In vitro* experiments are performed using viability assays, fluorescence microscopy, cytometric analyses and PPT experiments. Morphological analysis of NPs, cellular uptake of Ker-AuNPs and ultrastructural changes were examined by transmission electron microscopy (TEM). Ultrastructural analysis shows the interactions between the U87-MG cells and the Ker- AuNPs; in particular free Ker-AuNPs appear close to the cell's apical surface, small clusters of Ker-AuNPs associated with the cell membrane invagination and well-packed Ker-AuNPs in the cytoplasm prevalently located in endosomes. Results have evidenced the extraordinary biocompatibility of Ker-AuNPs, even at the highest concentrations (50 μM) and after 72 h of *in vitro* incubation. Cytofluorimetric analysis evaluated both cellular uptake and PPT induced cell death; it turns out that 68% of U87-MG cells have been loaded with Ker-AuNPs, thus generating a reduction in their viability, after performing PPT experiments, of about 30%. The reported structural and functional properties pointed out these Ker-AuNPs as a promising biocompatible photothermal agent for PPT treatments against cancer-related diseases.

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Imaging of Endocytic Trafficking and Extracellular Vesicles Released Under Neratinib Treatment in ERBB2+ Breast Cancer Cells

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Breast cancers (BCa) with ERBB2 amplification show rapid tumor growth, increased disease progression, and lower survival rate. Deregulated intracellular trafficking and extracellular vesicle (EVs) release are mechanisms that support cancer progression and resistance to treatments. Neratinib (NE) is a Food and Drug Administration–approved pan-ERBB inhibitor employed for the treatment of ERBB2+ BCa that blocks signaling and causes survival inhibition. However, the effects of NE on ERBB2 internalization, its trafficking to multivesicular bodies (MVBs), and the release of EVs that originate from these organelles remain poorly studied. By confocal and electron microscopy, we observed that low nanomolar doses of NE induced a modest ERBB2 internalization along with an increase of clathrin-mediated endocytosis and of the CD63+ MVB compartment in SKBR-3 cells. Furthermore, we showed in the culture supernatant two distinct EV subsets, based on their size and ERBB2 positivity: small (30–100 nm) ERBB2− EVs and large (>100 nm) ERBB2+ EVs. In particular, we found that NE increased the overall release of EVs, which displayed a reduced ERBB2 positivity compared with controls. Taken together, these results provide novel insight into the effects of NE on ERBB2+ BCa cells that may lead to a reduction of ERBB2 potentially transferred to distant target cells by EVs.

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Key words

Adjuvant therapy, breast cancer, disease-free survival, electron microscopy, endocytosis, extracellular vesicles, HER2, multivesicular bodies, neratinib, tyrosine kinase inhibitors.

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Visceral Adipose Tissue Inflammation and Fat Embolism in COVID-19 patients with Obesity: a Histomorphologic Cross-sectional Study

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Obesity is a critical determinant of severe coronavirus disease 2019 (COVID-19), as it increases complications and mortality following SARS- CoV2 infection. Studies characterizing features underlying such association are extremely urgent. In this cross-sectional study, we performed a comprehensive histomorphologic analysis of lung, liver and visceral adipose depots (VAT) of 19 patients dead for COVID-19 and 23 subjects dead for other reasons. Mean age was 63±14 years and BMI was 29.0±5.4 kg/m2. Although there were no differences in BMI and adipocytes area between COVID-19 and control patients, we observed higher prevalence of CD68+ macrophages in the VAT of the former compared to the last (4.25±2.1 vs 8.08±5.60 CD68+cells/10 adipocytes; p=0.005) suggesting higher VAT inflammation following SARS-CoV2 infection. Since VAT inflammation is strongly asso- ciated with lipids spill-over from dying adipocytes, we studied lipids pres- ence and distribution in lung and liver of the same patients employing Oil Red-O staining (ORO). Lipids were widely distributed in the lung and liver of COVID-19 patients and were evidenced not only within endothelial cells, macrophages and interstitial spaces, but also inside vessels' lumen, a feature suggestive of fat embolism syndrome (FES), detected in all COVID-19 patients and 53% of controls (p<0.001). Signs of FES were more prevalent among patients with obesity (93%vs63%, p=0.03), suggesting that such con- dition may be peculiar of the disease independently of COVID-19. Interestingly, all COVID-19 patients lungs presented hyaline membranes (HM), which seemed to be lipidic in nature based on ORO. Lastly, to evaluate whether the elevated VAT inflammation among COVID-19 patients could be related to the ability of SARS-CoV2 to infect adipocytes, we infected hu- man multipotent adipose-derived stem cells (hMADs) brought to differenti- ation. SARS-CoV2 was revealed in both, cell pellet and supernatant 96 hours post-infection, timepoint at which adipocytes displayed lower cell viability, pyknotic nuclei and clear signs of death.

Collectively, these data suggest that obesity and VAT inflammation in the context of COVID-19 may result in FES and HM formation, contributing to the negative prognosis documented in this population, and that such phenomenon may be possibly due to obesity-induced adipocytes death, worsened by SARS-CoV2.

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VP-SEM surface evaluation of cholesteatoma-affected ossicles treated by CADiss®

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Introduction: The surgical removal of cholesteatoma from affected ossicles can be performed by combining the manual dissection with the Chemically Assisted Dissection (CADISS®) system, made of Mesna 5% (sodium 2-mer- captoethanesulfonate) that acts by disulfide bonds splitting. The use of this technique allows to loosen the tension between the pathological tis- sue and the healthy ones (held together by cross-linked fibrous proteins with disulfide bonds) along the cleavage planes.

Aim: Our study aims to assess, by VP-SEM (variable pressure scanning elec- tron microscopy), the effectiveness of this combined surgical procedure during middle ear cholesteatoma removal, in terms of the absence of residual pathological tissue.

Materials and methods: 6 ear incus involved in cholesteatoma and re- moved during surgery were randomly divided into two groups: a) 3 after use of the CADISS® system; b) 3 with manual dissection only. The samples were fixed in glutaraldehyde 2.5% in PBS (0.1M, pH 7.4) immediately upon recovery for at least 24 h. Samples were then washed in PBS and underwent OsO_4 post-fixation for 1 h. After washing samples were impregnated with tannic acid 1% for 30 min, then washed, dried on absorbent paper ad directly observed at Hitachi SU 3500 at 30 Pa and 10 kV operating condi- tions. Images were analyzed by the software Hitachi Map 3D advanced 8.2 (Digital surf, France) to provide quantitative measurements of cholesteatoma tissue debris on the incus bones surface.

Results: VP-SEM allowed to observe the samples in a hydrated state, avoiding subjecting them to the long preparation procedures typical of the conventional SEM. Likewise, the use of this technique for evaluating the eventual residual tissue on the ear ossicles would decrease the risk of los- ing the pathological tissue while processing the ossicle. Preliminary results show that incus treated with the CADISS® system presents an almost com- plete absence of epithelial debris on their surface, while in the untreated ones various amounts of residual pathological tissue can be observed.

Conclusion: CADISS® system use could be useful in eliminating residual pathology from the incus bones removed during cholesteatoma surgery, with several clinical advantages such as avoiding their removal, or using them for the reconstructive phase (ossiculoplasty), and reducing the risk of possible disease recidivism.

Key words

Variable pressure Scanning electron microscopy, cholesteatoma, middle ear surgery, ossiculoplasty.

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Re-associating crania to post-crania in commingled contexts: A morphometric and 3D geometrical analysis of the occipital condyles and atlas articular facets

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The morphometric and geometrical study of the joints can provide key clues for the re-association of human remains in commingled scenarios, where bones from different individuals are mixed. In particular, the atlanto-occipital joint is an anatomical part which can permit the re-association of the skull to the entire body if the disarticulation is limited only to this specific joint. Despite the importance such joint can demonstrate for the re-association of crania and atlases in commingled context, an in-depth study of the potentialities of this articulation is still lacking. This study explored whether osteometry and the 3D surfaces of the occipital condyles and atlas articular facets can represent valid insights for this intent.

The dimensional variations among 8 corresponding linear distances were analyzed in crania and atlases of 150 individuals and examined through 6 supervised machine learning approaches attempting to develop classifiers able to identify the elements belonging to the same individual.

The 3D analysis was conducted on the articular surfaces through superimpositions of 46 3D models of occipital and atlas articular surfaces (corresponding and non-, respectively named matches and mismatches), differences in terms of point-to-point distances (expressed in Root Mean Square, RMS) among the superimposed 3D surfaces were explored.

No classifier among the applied approaches was able to correctly detect a satisfying ratio of correspondent specimen pairs in the overall sample by using linear distances while the 3D analysis set a threshold value (0.53 mm) over which almost 35% of the mismatches were identified.

Thus, results highlighted little morphological and metric congruency between the two articulating bones which makes no chance for the re-association of crania to atlases to succeed when linear distances are considered alone. However, the 3D geometrical congruency of the articular surfaces has the potential to screen/exclude incorrect re-associations rather than re-associate correctly crania to atlases. This kind of analysis might provide more successful outcomes when applied to diverse articulations opening a new way to operate for re-associating bones in commingled remains contexts. However, although a certain grade of 3D articular surfaces congruency exists at the occipital- atlantoid joint, the ideal 'fit' for classifying corresponding crania and atlases does not necessarily exist, and the re-association process in this case should be based also on other parameters. (2529 WC).

Key words

Atlanto-occipital joint, 3D analysis, morphometric analysis; commingled remains; forensics; dry bones.

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The effect of age on infrapatellar fat pad adipocytes and extracellular matrix: a comparative analysis

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Osteoarthritis (OA) is a degenerative disorder and a major cause of pain and disability worldwide. For long time it has been considered a cartilage disease; currently, it is best recognized as a whole joint disease whose pathogenesis is not fully understood. Within the knee, the infrapatellar pad (IFP) is a fibro-adipose tissue recently identified as an active player in OA onset/development: together with secretion of bioactive factors, the IFP undergoes itself to structural/ultrastructural modification [1-3]. Thus, for consciousness on IFP effective role in OA, a fine comprehension of its developmental characteristics along with aging, in case of no OA signs, is intriguing. To this purpose, the IFP from young patients undergoing anterior-cruciate ligament (ACL) reconstruction (n

= 24) and elderly cadaver donors (n = 23) were compared. Absence of inflammatory features was confirmed by histopathological score. Hence, the morphometric study focused on adipocytes perimeter and area, equivalent diameter and volume, major and minor axes. Evaluation of the extracellular matrix (ECM) proteins in the pericellular areas and septa also occurred. Specifically, Sirius red and Van Gieson stainings were adopted for collagen subtypes (type I/III) and elastic fibers identification, respectively; fibers organization was then described by Second Harmonic Generation microscopy. Data showed no inflammatory signs in the cohort. As for adipocytes morphometric study, area, perimeter, and equivalent diameter/volume were significantly higher in the elderly group ($p < 0.0001$). Regarding ECM proteins, collagen III displayed significantly higher values in the young group ($p = 0.004$) with a lower total collagen deposition with aging; however, collagen I/III ratio was not affected ($p = 0.157$) as well as samples architecture, as confirmed by coherency images analysis by FFT and OrientationJ. Elastic fibers were highly observed around the adipocytes in the ACL-IFPs ($p = 0.021$) but not in correspondence of the septa in the cadaver donors-IFPs ($p = 0.0872$). Study evidence shows that age affects the IFP tissue causing a variation in ECM proteins' content and specific cells' morphometry; this is particularly intriguing as the 3D microenvironment that cells experience in vivo affects their behavior. Different mechanical stimulation (i.e., mobility), likely depending on age, could have a role in tissue remodelling.

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Key words

Aging, infrapatellar fat pad, anterior-cruciate ligament rupture, cadaver donors, adipocytes, extracellular matrix.

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Imaging of visceral fat in living humans for didactical purposes

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Overweight and obesity are well known risk factors for cardiovascular and dysmetabolic disorders. In particular, the role of visceral fat (VF) in cardiovascular diseases has been strongly emphasized by recent literature. Even if overweight and visceral obesity are extremely frequent in western countries, anatomy textbooks and atlases do not include images showing the morphology and the locations of VF depositions. In turn, current imaging techniques (CT and MR) allow to identify and even quantify the presence and percentage of VF in different districts of the human body.

The aim of this study is to demonstrate how to achieve images of VF, either by CT and MR, suitable to explain to anatomy students the morphology of the different patterns of VF deposition.

CT and MR images from overweight subjects will be compared to those from normal subjects and from traditional and digital atlases. In particular, images of the VF in the epicardial, pericardial and periaortic spaces as well as in the peritoneal and retroperitoneal compartments will be displayed.

Didactical implications will be discussed and a prototype of a lesson dedicated to the anatomy of VF will be finally proposed.

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Training the eye during pandemics with an Art webinar: genetic diseases

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Observation is a key step preceding diagnosis, prognostication, and treatment. Careful patient observation is a skill that is learned but rarely explicitly taught [1]. The emergence of CT scans, MRI, US, and other diagnostic tools revolutionized modern medicine. Nevertheless, there is a negative impact on visual skills, as medical students/physicians are becoming more reliant on technologies to gather patient information rather than direct observation [2]. The Arts or "medical humanities" are increasingly used in medical education, and growing evidence exists that visual arts-based activities can enhance visual skills. During the lockdown associated to the COVID-19 pandemics, within the context of the medical students' cultural project "Art and disease: knowing how to observe to take care", a series of 2-hour webinars (via Zoom) were organized to keep an interactive dialogue among medical students and teachers. Each webinar was promoted in several ways and transmitted live through social and media networks (Facebook, YouTube). Our aim is to summarize one of these webinars, connecting human anatomy with artworks and genetic diseases. Figurative paintings from the 15th to the 20th Centuries depicting some traits of genetic disease (Down, Angelman, Prader-Willi, Noonan, Marfan, Ehler-Danlos syndromes; achondroplasia; pycnodysostosis; Paget's disease, and consanguinity) were selected. Particular attention was given to "Las Meninas", a painting by Diego Velázquez. Following the specific approach to teaching, known as Visual Thinking Strategies -VTS-, a facilitated group discussion was encouraged. Our faculty actively and enthusiastically participated in the process of artistic interpretation with peaks of 200 online presences. Students noted figure position and expression; color nuances; differences in texture; perspective, and shading of the various artworks. The painting became richer because different eyes focused on different things. The process increases analytical thinking as students "decode" the images seen in the paintings [3]. The use of Art impacts on students' diagnostic skills, competence in physical exam ability, empathy, teambuilding facilitation, promoting wellness/preventing burnout, and cultural sensitivity and acceptance of ambiguity; promotes problem solving, communication, thinking creatively, and appreciating other perspectives, all of which are fundamental in the progression of a good physician [2].

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Key words

Art, visual skills, genetic diseases, eLearning, social networks, medical education, webinar.

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Fifty shades of the xiphoid process anatomy: the challenging world of virtual dissections

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Advances in technology have created new teaching tools, such as digital dissection tables, which provide both new learning and research opportunities, conjoining pure and clinical anatomy. Through touchscreen technology, three-dimensional volume rendering (3DVR) images are obtained by the software from DICOM files belonging to real clinical cases contained in the public library image dataset of our digital anatomical table Sectra F18. Starting from the most canonical anatomical description of the sternum, and particularly, the xiphisternum or xiphoid process (XP), our aim was to observe some clinical cases with their anatomical variants. Clinical cases including images of the thorax -and particularly the sternum- were searched within the public library of the table. A total of 39 cases were analyzed, of which 14 men, 11 women and 14 patients with undetermined genre. The following parameters were considered: genre; age (if supplied by the institutions); general conditions of the patient (presence of any pathology); presence or absence of XP; if present, shape, alignment, calcification, and presence of foramina/pseudoforamina were also evaluated. The human XP appeared in morphological diversity, displaying pointed, oval, monofid, bifid (forked, crab-like), and trifid processes. Heterogeneous foramina/pseudoforamina were observed in 13/39 cases. In 3 cases the xiphoid process was absent/not identified. Knowing the postnatal development, maturation, and anatomy of the sternum is important for treating several bone, hematological, and developmental diseases and for planning thoracic surgery, identifying possible perioperative and postsurgery complications, and preventing mediastinal organ injury. In particular, the presence of foramina along the whole sternum may represent a clinical risk. The terminology classifying XP morphology and foramen patterns is not universally accepted [1- 4]. It is also important to consider the risk of sternal fracture during cardiopulmonary resuscitation maneuvers, which may lead to heart tamponade. Anatomic variations may mimic sternal fractures if only plain imaging is considered. Therefore, 3D imaging is useful to display any anatomical variations or abnormalities of the XP, especially considering that axial CT may miss variants or fractures because of the slicing. Color presets are to be adopted and adapted according to the tissues intended to better interpret clinical images. In this way the filter becomes the tailored suit.

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Key words

Imaging, digital table, volume rendering, sternum, xiphoid process, anatomical variants, computed tomography.

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Sars-CoV-2 binding receptors modulation by h2s in human airway epithelium

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COVID-19 pandemic has now affected around 180 million people worldwide, accounting for more than 3.5 million confirmed deaths. Besides ongoing global vaccination, finding protective and therapeutic strategies is an urgent clinical need. SARS-CoV-2 mostly infects the host organism via the respiratory system, requiring ACE2 and TMPRSS2 to enter into target cells. Therefore, these surface proteins are considered potential druggable targets [1-3]. H_2S is a gasotransmitter produced by several cell types and is also part of natural compounds, such as sulfurous waters (SWs) that are often inhaled as low-intensity therapy and prevention in different respiratory conditions. H_2S is a potent biological mediator, with antioxidant, anti-inflammatory and, also anti-viral activities [4].

Considering that respiratory epithelial cells can be directly exposed to H_2S by inhalation, here we tested the in vitro effects of H_2S -donors on TMPRSS2 and ACE2 expression in human upper and lower airway epithelial cells. We show that both fast- and slow-releasing H_2S-donors significantly reduce mRNA and protein expression of TMPRSS2, without modifying ACE2 expression, both in bronchial and pulmonary cell lines and in primary human nasal and lung cells of healthy donors.

Our results provide the first experimental evidence of the H_2S-effects on the expression of SARS-CoV-2 binding receptors by the respiratory cells of host organism. The regulation of protease activity in the respiratory system has been previously proposed as a useful strategy to protect from virus infections [5]. We show that H_2S is a down-modulator of TMPRSS2 expression in respiratory cells, that spares the expression of ACE2. ACE2 is often reported to be negatively associated with lung injury and it is currently held that caution should be taken targeting ACE2 at systemic level [6, 7, 8]. Overall, since: i) SWs exposure has negligible side effects and can be directly supplied to every anatomical level of the respiratory tree; ii) inhalation of SWs are indicated as a beneficial option for the primary and secondary prevention of several respiratory conditions; iii) H_2S selectively down-regulates TMPRSS2 expression in airway epithelial cells; we consider all this as a proof-of-concept that H2S -rich inhalational treatments can contribute to the protection from Sars-CoV-2 respiratory infection, ready to be tested in a dedicated clinical trial.

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Key words

Hydrogen sulfide, SARS-CoV-2, TMPRSS2, ACE2.

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Glutathione S-transferase P interacts with Nrf2 transcription factor in the drug-resistance model of mouse hepatocarcinoma: a first molecular and physical characterization.

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Hepatocellular carcinoma (HCC) is the second leading cause of cancer death worldwide (over 700,000 deaths/year), and it is the first cause of death in patients with chronic liver diseases (CLD). At present, Non-Alcoholic Fatty Liver Disease (NAFLD) represents the most common form of CLD worldwide due to its causal association with highly prevalent conditions, i.e. obesity and diabetes. NAFLD can progress to steatohepatitis (NASH) and eventually to cirrhosis and HCC (NASH- HCC). HCC is highly drug-resistant, a condition that limits the efficacy of available chemotherapy protocols. The expression of the oncogene and drug resistance gene *Glutathione S-transferase P* (*GSTP*) is increased in HCC and is associated with the cancerogenic transformation.

The study hypothesis is that GSTP induction results in a physical and functional interaction with the stress-response transcription factor Nrf2 to provide a cancer- specific mechanism of drug resistance in HCC. This interaction was investigated in mouse models of HCC, including: 1) N-nitrosodiethylamine (DEN) induced liver cancer; 2) choline deficiency-induced cancer (this is a 9-month protocol that recapitulates the human NASH-HCC phenotype); 3) carbon tetrachloride (CCl_4) induced cancer. In all these *in vivo* models, the liver expression of both GSTP and Nrf2 proteins was increased during tumor development. According with the phenotype observed in human HCC cell lines, this results in drug-resistance gene induction and higher resistance to apoptotic stimuli. In DEN-HCC, Nrf2/Keap1

pathway activation was associated with increased expression of β–TrCP, a protease that respond to Nrf2 nuclear translocation and transcriptional activation. Also, GSTP levels nicely correlated with *c*-Jun expression, a JNK-stimulated transcription factor with important role in drug resistance behind Nrf2 activation.

GSTP co-immunoprecipitated with Nrf2 indicating a physical interaction of these hepatic proteins in HCC. The presence of the dimeric form of GSTP in this binary interaction with Nrf2 was cancer-specific and suggests the involvement of the reduced form of this drug-resistance protein.

In conclusion, the oncogene GSTP has been demonstrated to physically and functionally interact with hepatic Nrf2 in mouse models of HCC. The role of this cancer-specific interaction in the drug resistance mechanisms of HCC deserves further investigation in order to develop more efficient therapeutic protocols for this form of liver cancer.

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Key words

Hepatocarcinoma, HCC, GSTP, Nrf2, NAFLD.

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3D-3D facial registration method applied to personal identification from videosurveillance systems: does it work with disguised faces?

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Personal identification of the living from videosurveillance systems is usually based on 2D-3D superimposition, where the images of the culprit is compared with a 3D facial model of the possible suspect[1]. However, the diffusion of modern 3D image acquisition systems will enable operators to reach personal identification through 3D-3D comparison, whose reliability still needs to be verified in case of disguised subjects where only a part of face is visible.

This study aims at testing the reliability of a protocol for 3D-3D registration of facial models applied only to the upper third of faces.

Fifty male subjects aged between 18 and 45 years were randomly chosen from a database of 3D facial models acquired through stereophotogrammetry (VECTRA- 3D® M3: Canfield Scientific, Inc., Fairfield, NJ). For each subject two acquisitions were available; from each model, the upper facial third included between trichion, right and left frontotemporale, exocanthion and endocanthion landmarks was se- lected.

The 3D models of faces were then registered onto other models belonging to the same and different individuals according to the least point-to-point distance on the entire facial surface, for a total of 50 matches and 50 mismatches. RMS value (root mean square) of point-to-point distance between the two models was then calculated through VAM® software (Vectra Analysis Model, Canfield Scientific, Inc., Fairfield, NJ). Possible statistically significant differences between matches and mismatches were assessed through Mann-Whitney test ($p<0.05$).

Average RMS point-to-point distance was 0.32±0.12 mm in matches, 1.40±0.32 mm in mismatches ($p<0.001$). No overlap was found between the group of matches and mismatches.

This study shows that the novel method of 3D-3D superimposition method may provide an important help for personal identification of the living also when only a part of the face is visible in disguised subjects.

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Key words

Personal identification, videosurveillance system, stereophotogrammetry, 3D-3D registration.

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Stili di vita e prevenzione:
scienze del movimento, dalla nutrizione
e del benessere

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Acupuncture or adapted physical activity for the management of lymphedema in breast cancer survivors

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Lymphedema (LE) can cause psychophysical sequelae jeopardizing quality of life (QoL) in breast cancer (BC) survivors. Chronic LE and lack of effective therapies represent a major challenge for healthcare professionals. The aim of our study was to investigate the effects of an intervention featuring acupuncture or adapted physical activity (APA) in LE management. BC survivors (n=28) with chronic LE, recruited at the CeRiOn Center in Florence, were assessed before and after 8-week APA (n=16) or acupuncture (n=12) intervention. Body mass index and bioimpedance parameters were measured. Indirect upper limb volume measurement and ultrasonography were performed. Functional tests (*i.e.* wrist flexion/extension and hand strength) were also executed. QoL, depression/anxiety and pain intensity were evaluated by ULL27, HADS, distress thermometer and NRS questionnaires, respectively. Ultrasound measures revealed a LE reduction in all women, though a statistically significant improvement was observed only in those who received acupuncture treatment. Volumetric measurements showed a trend toward an improvement in both intervention groups. Overall, bioimpedance measurements were unchanged. Wrist mobility, hand strength, pain perception, depression and QoL were all significantly ameliorated after the APA intervention.

In conclusion, BC-related LE requires a multidisciplinary treatment approach involving both cancer care medical/psychological team and APA professionals to improve psychophysical outcomes and QoL.

Key words

Breast cancer survivors, upper limb disability, lymphedema, adapted physical activity.

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Knee injury reduction through forefoot posture training in non-professional runners

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Runner's postural biomechanics is a qualitative aspect of sports performance that must be perfected to increase performance and reduce joint trauma incidence. Specifically, a motor pattern that prefers the rearfoot run over the forefoot is common among western runners. The heel generates a ground reaction force of about 1.5-3 of the body weight in 50 ms from a dynamic perspective, affecting the lower limb joints[1]. In the forefoot run, the ground reaction force develops more slowly, in about 200 ms, to reduce the shock absorbed by the joints[2]. The purpose of this study is twofold: to investigate the biomechanical aspects of non- professional runners and to understand if, following a training based on the acquisition of a correct forefoot posture in conjunction with the learning of the correct running frequency set at 180 steps per minute, spm, they can improve sports and physical performance. The study involved 30 non-professional runners, average age 45.7 ± 6.7, and with the support of a motion analysis system, a treadmill with a markerless 3D camera, the joint biomechanical relationship of running posture was evaluated. A specific 4-week training protocol was then administered to a pilot group aiming to induce the correct lower limb kinematic, learn forefoot running posture, train muscles through the isoinertial system, and run on the correct step frequency at 180 spm with a digital metronome aid.

Standard values of non-professional runners' joint biomechanical relationships were obtained. The 4-week training showed changes in joint values, such as the foot's dorsiflexion from 26° to 2.7°, force spike reduction in the load kinematics, and spatio-temporal parameters related to running as contact time from 0.26 sec to 0.23 sec. A 4-week training based on posture management, step frequency training, and targeted muscle training can induce significant changes in non- professional runners whose running posture is rearfoot, determining a knee injury reduction, reactive force increase, and correct running biomechanics.

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Key words

Running, metronome, step frequency, posture, knee injury, sport injury, forefoot, training.

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Long-term effects of asymmetrical posture in boxing assessed by baropodometry

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BACKGROUND: Asymmetrical posture maintained over long trainingperiods may affect phenotypic plasticity, resulting functional to sporting goal but negative to the locomotor system. Aim of this study was to quantitatively evaluate these long-term effects in competitive boxers.

METHODS: Baropodometric analysis was used to assess 20 competitive boxers and 20 non-sportsmen in upright bipedal posture for 5 s and for 51.2 s with open (OE) and closed (CE) eyes.

RESULTS: The boxers' group (BOX) showed a larger total foot load (TFL) ($p=0.022$) on the right foot and a larger rearfoot load (RfL) ($p=0.011$) on the left foot compared to non-sport controls (CTR). Moreover, a larger forefoot load (FfL) ($p=0.001$) on the right foot respect to left one was found in the BOX group, with the inversion of the RfL to FfL ratio ($p=0.001$) between two feet, while no significant differences were found in the CTR group. These findings, associated to a signifi - cantly larger center of foot angle (COF) in the BOX group, may indicate an anti- clockwise rotation of the anatomical structures above the ankle joint of the right hemisoma respect to the left one, that appears to be consistent with the orthodox stance. Eventually, the BOX group showed a larger centre-of-pressure sway area (COPsa) in the OE condition than what measured in the CE and a significant dif- ference in Romberg Index (BOX< CTR).

CONCLUSIONS: The results of this study seem to confirm the theory of neuro- muscular plasticity imprinted by the repetitive movements and long-lasting pos- tures. Moreover, competitive boxers show an increase of proprioceptive function and a decrease of visual dependence on the postural control.

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Key words

Asymmetrical posture, postural stability, neuroplasticity, bodyweight distribution, baropodometry.

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Histological evaluation reveals widely altered lipid deposition, local and systemic inflammation in normocholesterolemic mice lacking high-density lipoprotein

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It is commonly believed that an unbalanced diet, rich in lipids, is crucial to develop atherosclerosis and to promote an abnormal lipid deposition in various organs and tissues. High-density lipoprotein (HDL) and its major protein component, apolipoprotein A-I, plays a unique role in regulating cell cholesterol homeostasis and in modulating immune cell activation. In the present study, we investigated the impact of genetic manipulation of HDL/apoA-I levels on lipid deposition in skin, lymphoid organs and heart vessels in mice fed a normal laboratory diet (NLD).

ApoE deficient (EKO) mice, apoE/apoA-I double deficient (DKO) mice, DKO mice overexpressing human apoA-I (DKO/hA-I) and C57Bl/6 control mice (WT) were fed NLD diet until 30 weeks of age. Plasma lipids were quantified, atherosclerosis development at the aortic sinus and in coronary arteries was measured, skin ultrastructure was evaluated by electron microscopy. Blood and lymphoid organs were characterized with histological and immunocytofluorimetric analyses.

DKO were characterized by almost complete HDL deficiency and by plasma total cholesterol levels comparable to control mice.

Only DKO mice showed a disarranged subpapillary dermis filled with cholesterol clefts and foam cells were interspersed in the reticular dermis. Neutral lipid deposition was increased in the thickened dermis of DKO mice, compared with the other genotypes.

In addition, features of severe inflammation were detected in the skin-draining lymph nodes of DKO mice: i) accumulation of foamy macrophages surrounded by lymphoid cells; ii) presence of cholesterol crystals and granulomatous reactions in the inner cortex and medulla; iii) dilation of subcapsular, cortico-medullary and medullary sinuses. Moreover, the deposition of neutral lipids in lymph node parenchyma was low and comparable in WT, EKO and DKO/hA-I mice, whereas in DKO mice it was dramatically elevated.

An increased presence of CD4+ T effector memory (TEM) cells was detected in blood, spleen and in the skin-draining lymph nodes of DKO mice. A worsening of atherosclerosis at the aortic sinus and coronary arteries was also observed in DKO vs EKO mice. Human apoA-I overexpression in the DKO background was able to rescue the skin phenotype and to halt atherosclerosis development.

In conclusion, HDL deficiency, in the absence of hyperlipidemia, is associated with severe alterations of skin morphology, aortic and coronary atherosclerosis, local and systemic inflammation.

Key words

Atherosclerosis, xanthoma, lymphoid organs, HDL.

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Fat-shaped microbiota affects microvesicular steatosis and lipid metabolism in malnourished mice

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Protein malnutrition is characterized by stunted growth, hepatic steatosis and a damaged gut mucosal architecture. Since high-fat shaped gut microbiota has an increased ability in providing nutrients and energy from food to the host, the aim of this study was to determine whether such a microbiota could beneficially impact on the consequences of malnutrition.

The cecal content of specific pathogen free C57Bl/6J mice fed a high-fat diet or a low-protein diet was transplanted in two groups of germ-free C57Bl/6J recipient mice, named HFM and LPM respectively, which were subsequently fed a low- protein diet for 8 weeks.

Body weight gain was comparable between the two groups of microbiota-recipient mice.

Plasma lipid levels were lower in HFM compared with LPM mice. In particular, total cholesterol levels were reduced by 12.28% ($p = 0.048$), high-density lipoprotein- cholesterol by 16.01% ($p = 0.018$) and triglycerides by 20.71% ($p = 0.032$).

Liver histology indicated an increased occurrence and severity of microvesicular steatosis in the HFM group. No signs of macrovesicular steatosis were noticed. In order to evaluate glycogen accumulation in livers, the PAS staining was performed and no differences were observed between the two groups. Similarly, the staining for the extracellular matrix with the Masson's trichrome did not show anomalous fibrosis in livers from both HFM and LPM mice.

The length of small and large intestine was comparable in the two experimental groups that received different microbiota. Villi in duodenum, jejunum, and ileum had a normal appearance and showed comparable length and width in both groups. Moreover, the ileal crypts showed the same depth in the two groups, whereas, in the large intestine, crypts of LPM mice were slightly deeper than those of HFM mice.

To evaluate if the two different microbiota had an impact on mucus production, the abundance of neutral and acid mucins produced by goblet cells in the ileum and large intestine was analyzed and no differences were observed. Nonetheless, the expression of antimicrobial genes promoting oxidative stress and immune response at the ileal epithelium (Duox2, Duoxa2, Saa1, Ang4, Defa5) was increased.

In conclusion, the transplant of the caecal microbiota shaped by a high-fat diet in mice fed a low-protein diet represents a noxious stimulus for the ileal mucosa and impairs hepatic lipoprotein secretion, promoting the occurrence of hepatic microvesicular steatosis.

Key words

Steatosis, small intestine, malnutrition, gut microbiota.

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Dynamic Flow Cloud - an Internet of Things solution based on Arduino, which enables easy, secure and scalable re-organization of the space in research laboratories and university departments, to face the new COVID-19 emergency

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Coronavirus Infectious Disease 2019 (COVID-19) caused by the virus SARS-CoV-2 was declared a pandemic by the World Health Organization (WHO) on the 11th March 2020. Starting in Wuhan, Hubei Province, China, the rate of global dissemination has accelerated, and in June 2020 in Italy there were 19,573 positive people, 184,585 healed cases, 34,675 deaths, with 238,833 total confirmed cases.

SARS-CoV-2 has a high viral load at the onset of symptoms that declines up to 5–6 days later. Severe cases of COVID-19 have higher viral loads and excrete virus longer than mild cases. The capsid protein, the spike one, has a high affinity for the receptors on the surface of the cells of the respiratory epithelium, with the capacity to lysate the cells and cause dissemination of the virus in the interstice of the pulmonary alveoli. Dissemination of the virus may cause intravascular disseminated coagulation worsening the clinical picture of virus-induced pneumonia.

SARS-CoV-2 is a Coronavirus which passed from animals to the human. The peculiarity of this virus is that it is new to the human being and the human does not have defenses against it.

Various models applied to early SARS-CoV-2 epidemic data found that an infected person spread the disease to an average of 2.6 people, the basic reproductive rate R0. Thus, after 10 generations of transmission, with each taking about 5–6 days, a single case would expand to more than 3,500 new cases in the course of just two months in the absence of mitigation strategies, as vaccination. But vaccination is not compulsory and the real efficacy of the vaccination against the several variants of the virus coming out is not known. That's why we need an efficient and calculated strategy to avoid dissemination of the virus.

The only way we have to maintain a low number of infections is to maintain distances, to avoid overcrowding, to trace infections registering accesses to all the University facilities and to use individual protection devices whenever necessary (3T:Test, Treat and Track).

The solution to the problem is Dynamic Flow Cloud, a simple and very cheap way to create a virtual and dynamic row and to manage the space to better organize profit esams, frequency of traineeships and research laboratories at university locations.

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Lactobacillus fermentum protection in gut and cerebellum in a mouse model of ethanol-induced damage

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Chronic alcohol consumption induces the alteration of the composition of the intestinal microbiota favoring intestinal permeability and translocation of endotoxins in the systemic circulation. This may lead to the dysfunction of other anatomical structures including those of the central nervous system[1,2]. Here, we have used a mouse model of ethanol abuse to investigate the effects of the probiotic *Lactobacillus fermentum* against alcohol damage in gut and cerebellum. Female 12-month-old mice (BALB/cAnNHsd) were orally fed with ethanol (EtOH) (96% diluted in water) alone (n=5) or in combination with *Lactobacillus fermentum* (10^9 CFU, 20 minute after EtOH, n=5), every day for twelve weeks. Both groups were compared with mice (n=5) fed with the standard diet (Ctrl). Morphological evaluations were performed in the small intestine and cerebellum. Standard histological stainings of the small intestine showed morphological alterations of the intestinal villi in the group fed only with EtOH. The same stainings showed modification in the Purkinje cells morphology in the cerebellum after EtOH assumption. *Lactobacillus fermentum* administration attenuated these modifications. Immunohistochemistry was conducted to determine glial fibrillary acid protein (GFAP), S100 calcium binding protein B (S100β), Hsp60 and Hsp90 levels both in small intestine and cerebellum. GFAP, and S100β immunoreactivity increased after EtOH consumption in both organs while the group fed with the probiotic showed a decrease of these proteins. Finally, Hsp60 and Hsp90 immunoreactivity increased in both small intestine and cerebellum of mice fed with *Lactobacillus fermentum.* The results suggest a protective role of *Lactobacillus fermentum* against inflammation and oxidative stress damage induced by alcohol consumption.

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Key words

Lactobacillus fermentum, gut, cerebellum.

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The activation of NLRP3 inflammasome in tissue-resident macrophages contributes to enteric neuroplastic remodelling in a mouse model of diet-induced obesity

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Introduction. Obese patients experience gastrointestinal disturbances (1). Gut dysbiosis, impaired intestinal epithelial barrier and enteric inflammation contribute to digestive symptoms in obesity (2). In this context, the NLRP3 inflammasome acts as a key immune sensor involved in the pathogenesis of immune/inflammatory responses (3).

Aim. This study examined mucosal permeability, immune/inflammatory responses and colonic motility in mice with high-fat diet (HFD)-induced obesity.

Methods. Wild-type C57BL/6J and NLRP3-KO (NLRP3-/-) mice were fed with HFD or standard diet for 8 weeks. At sacrifice, blood samples were collected to evaluate circulating lipopolysaccharide (LPS) levels by ELISA. The activation of inflammasome signalling [ASC, caspase-1 and IL-1β] in colonic tissues from obese mice was assessed by RT-PCR and ELISA. The alterations of intestinal barrier (epithelial tight junctions) and the activation of resident macrophages were assessed by confocal immunofluorescence as well as the density of eosinophils in colonic

tissues from obese mice. The role of NLRP3 in *in vitro* colonic tachykininergic contractile activity was evaluated. The effect of substance P (SP) on NLRP3 pathway was tested in macrophages.

Results: HFD mice displayed increased body and epididymal fat weight, cholesterol levels, plasma LPS levels, colonic IL-1β levels and ASC and caspase-1 mRNA expression. HFD animals also displayed and increased ASC immunopositivity in F4/80-positive macrophages, eosinophil density along with a decreased claudin expression in epithelial cells. Colonic tachykininergic contractions were increased in HFD mice. NLRP3 gene deletion in HFD mice was associated with lower increase in body and epididymal fat weight, cholesterol levels and systemic and bowel inflammation. The alterations of tachykinergic neuromuscular contractions were normalized in HFD NLRP3-/- mice. In macrophage cell lines, SP induced IL-1β release. Such an effect was abrogated in the presence of caspase-1 inhibitor or NK1 receptor antagonist and was not observed in ASC-/- cells.

Conclusion. In the setting of obesity, the activation of NLRP3 inflammasome in tissue-resident macrophages contributes to colonic dysmotility, through the modulation of tachykininergic neurogenic responses, thus identifying NLRP3 as a therapeutic target for the treatment of bowel symptoms related to obesity. However, the role of NLRP3 signalling in recruited and muscularis macrophages needs to be also investigated.

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Key words

NLRP3 inflammasome, colonic motility, high-fat diet, inflammation, macrophages, obesity, substance P.

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Antiangiogenic potential of kinesin Eg5 inhibitors for gastric adenocarcinoma treatment

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Kinesins are motors proteins involved in different biological processes such as intracellular transport, bipolar spindle assembly and microtubule remodelling. Kinesin Eg5 is a well-known kinesin essential for the bipolar spindle correct formation during mitosis and it is commonly overexpressed in tumours thus representing a new possible target in cancer therapy [1]. The aim of this work is to evaluate the anticancer activity of two newly synthetized Eg5 inhibitors (Cmp 2 and Cmp 41), alone and in combination with the polyphenol Hesperidin (HSD), on a gastric adenocarcinoma cell line (AGS), focusing to a considerable extent on the angiogenetic process [2].

Firstly, western blot analysis reveals that Cmp 2 and 41 determined a significant reduction of PI3K, p-AKT and p-ERK/ERK proteins expression, compared to untreated cells.

Secondly, ELISA test evidences that Cmp 2 and 41 statistically decreased VEGF activity respect to untreated cells, moreover, angiopoietin 2 (ANGPT2) gene expression levels appeared to be markedly increased when AGS are treated with Cmp 41 compared to AGS treated with K858 parental compound.

Thirdly, the combination HSD/Cmp 2 is able to provoke a reduction of p-AKT protein expression along with an increase of ANGPT2 gene expression, compared to Cmp 2 alone, while the combination HSD/Cmp 41 mainly induced p-Erk reduction, respect to Cmp 41 alone.

The obtained results show that Eg5 inhibitors seem to effectively counteract angiogenetic process by recruiting PI3K/AKT/MAPK pathway, reducing VEGF activity and promoting ANGPT2 gene expression even better if combined with HSD. Overall Eg5 inhibitors can represent a promising starting point for additional *in vitro* and *in vivo* investigations aiming at developing a new strategy to counteract gastric cancer.

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Key words

Kinesin Eg5, angiogenesis, gastric adenocarcinoma, polyphenols, cancer treatment.

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Role of FOXO1 mutations as genetic drivers of resistance to BCR inhibition in Non-Hodgkin Lymphomas

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The B-Cell-Antigen-Receptor (BCR) provides survival signals to normal and malignant B-lymphocytes[1,2]. Transformed B-cells that lost BCR expression have been recently identified in different Non-Hodgkin lymphomas (NHLs)[1,3]. Nevertheless, the mechanisms underlying the selection of BCR-defective lymphoma clones are only partially understood. A significant fraction of NHL patients carries missense mutations in Forkhead-box protein-O1 (FOXO1) transcription factor[4], a target of the PI3K/AKT pathway which relays survival signals downstream the BCR. However, the contribution of FOXO1 mutations to the pathogenesis of NHL remains elusive. We propose that FOXO1 mutations are selected to mimic signaling pathways conventionally activated in response to specific immune cues, allowing the cells to efficiently respond when those signals are delivered, or to maintain signaling competence even in the absence of specific immune receptors (i.e. BCR). Under these circumstances, B-cells may be relieved from the selective pressure to maintain BCR expression expanding in the absence of BCR-derived signals. Accordingly, FOXO1 mutations were identified in >20% of lymphomas with undetectable BCR_3, possibly representing a genetic mechanism of resistance to the loss of BCR-expression. Using a FOXO1 mutant mouse model and lymphoma cell lines characterized by the conditional control of BCR1, we report that the expression of FOXO1 mutation in primary B cells leads to abnormal germinal center responses. Moreover, we observed increased resistance to stress-induced apoptosis in FOXO1 mutant lymphoma cells, particularly upon loss of BCR expression. Of note, these phenotypes were associated to signaling adaptations of BCR-dependent and BCR- independent pathways. Overall, our data suggest an unreported role for FOXO1 as a molecular-switch that adapt cellular signaling to sustain the growth of BCR- deficient normal and transformed B-cells. This is relevant considering the growing interest in BCR-targeted therapies, currently proposed as therapeutic strategies for the treatment of several aggressive B-cell malignancies. The identification of compensatory mechanisms that lymphomas adopt to survive in otherwise non- permissive conditions (i.e. BCR loss) represents novel potential therapeutic options for patients diagnosed with aggressive B-cell lymphomas with poor prognosis and dismal outcomes, at high-risk of relapse.

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Morphological analysis in tested SARS-CoV-2 positive and negative patients: role of some host receptors and vascular biomarkers and correlation with viral entrance

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Increasing interest is arousing in the analysis of a possible correlation between SARS- CoV-2, responsible for coronavirus disease (COVID-19), and multi-organ vascular damage. Several scientific reports described how the virus is able to exert its activity in organs other than lung, focusing the relevance of SARS-CoV-2 dissemination in the body associated with peripheral vascular injury.

In particular, several evidences suggest that, in addition to the respiratory tract, also the gastrointestinal tract may be a main site of SARS-CoV-2 infection, possibly associated to poor prognosis.

The structure of blood vessel wall can be strongly altered during SARS CoV-2 in- fection, therefore the identification of morpho-functional alterations of the vessel wall is a very important aspect to characterize the infection. A correlation between autoph- agy and vascular damage has been hypothesized and this process may be involved in the modulation of the vascular process during infection. Recent observations in pregnant SARS-CoV-2 infected women pointed out that also placenta vessels may be affected by viral infection, with consequences to the fetus.

We carried out analysis of tissue, vascular and placenta samples by transmission (TEM) and scanning (SEM) electron microscopy. In addition to the ultrastructural char- acterization of specific tissue organelles comparing non-pathological to pathological tissues, a morphological evaluation of biomarkers associated with vascular damage has been performed, as well as the analysis of the autophagic marker LC3B and the expression of the cellular transmembrane glycoprotein CD147 and ACE2 receptor, that can interact with viral proteins. The data therefore support the existence of a pe- culiar pathogenic process for SARS-CoV-2 infection in multiple tissues, also with an involvement of vascular damage at the endothelial level.

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Key words

SARS-CoV-2, thrombosis, viral entry, inflammation, vascular damage.

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CAPE synthetic derivatives and their potential role in skin regeneration

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Morbidity due to non-healing wounds significantly affects the patient's quality of life which makes long healing process a large and growing interdisciplinary clinical problem [1]. The development of new agents that could accelerate the healing of ulcers, acute and chronic wounds is needed. Apitherapy is an alternative therapy using products coming from honeybees as therapeutic approach in many conditions, including wound care. The caffeic acid phenethyl ester (CAPE), one of the main component of propolis, has antiseptic, anti- inflammatory, astringent, anesthetic and antioxidant activity, and its positive effects on skin regeneration have already been explored [2]. Hence, two different novel series of CAPE derivatives were designed, synthesized and evaluated as potential skin regenerative agents. The first series was designed to have an ester linkage (series A) while the second series would contain an amide linkage (series B) with different heterocyclic rings such as quinoline and isoquinoline. The 12 compounds were administered in different concentrations (0-80 μM) to two different cell types: human gingival fibroblasts (HGFs) and the keratinocyte cell line HACAT, meant to represent epidermidis and derma using both CAPE and caffeic acid as reference compounds. The viability and cell proliferation were evaluated by crystal violet. All the compounds showed no cytotoxicity at the lowest concentrations (0-20 μM), but four of them proved to stimulate cell proliferation, in a manner very similar to the cell exposed to CAPE. The most promising compounds were administered at very low concentrations (0, 0.5, 1 and 5 μM) to both types of cells submitted to a wound healing assay. Interestingly, both HGFs and HACAT treated with two compounds from series A and one compound from series B were more effective than the control cells and the CAPE-treated cells in closing the scratch.

Since an interesting structure-activity relationship (SAR) was found, further studies on the most promising compounds will include investigation of the antibacterial activity, their effects on cells challenged with inflammatory and/or oxidative stimuli, absorption on biopolymers used for wound dressing applications and evaluation of biocompatibility, biodegradability, and toxicity.

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Key words

Caffeic acid, CAPE, skin, regeneration, wound healing, CAPE synthetic derivatives, quinolone, isoquinoline.

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Melatonina e stress ossidativo nella prevenzione delle malattie cardiovascolari

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Oxidative stress and chronic inflammation are well known as potential responsible for many cardiovascular diseases (Lim et al., 2012; Mancia et al., 2013). The clinical trial, approved by the Ethics Committee of the ASST Spedali Civili (code NP 2717, authorized Prot. n. 0042068, approved 04.07.2017), was born for understanding if intervening on the inflammatory and oxidative cascade that seems to result in the development of hypertension, it is possible to restore an oxidative balance leading to positive results even at the endothelial and vascular level. Among all the antioxidant molecules, melatonin was chosen for its well-known role as a powerful antioxidant and anti-inflammatory molecule.

The study involved 23 patients, aged between 45 and 60 years old, with BMI <30 kg/m^2, diagnosed with essential hypertension but no other cardiovascular or autoimmune diseases and conditions or habits that could alter the oxidative background. They were randomly assigned to 2 groups: "melatonin" in which patients have been asked to add to their antihypertensive therapy the intake of 1 mg/day of melatonin for a year and a "control" group in which there were no changes in the therapeutic scheme. Both groups underwent an evaluation before the start (T0) and at the end of the study (1 year later - T1) in which were considered: 1. total antioxidant capacity and melatonin concentration in plasma obtained from venous withdrawal; 2. endothelial dysfunction and 3. arterial stiffness. It was possible to define that at T0 53% of patients belonging to the melatonin group presented with endothelial damage, whereas 40% presented with an altered arterial stiffness. At T1, in the patients supplemented with melatonin, we find out that the arterial stiffness index decreased in a statistically significant way, next to a significant decrease in total antioxidant capacity plasma values.

We hypothesized that a correlation between total antioxidant capacity and arterial stiffness exists and that the combination between antihypertensive therapy and antioxidant supplementation could improve the vascular health and functionality (Vlachopoulos et al., 2015; Tembo et al., 2020).

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Essential Hypertension, oxidative stress, melatonin, endothelial dysfunction, arterial stiffness.

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Diagnostic and prognostic value of three microRNAs in environmental asbestiform fibers associated malignant mesothelioma

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Fluoro-edenite (FE) is an asbestiform fiber identified Biancavilla (Sicily, Italy) (Filetti et al., 2020a). Environmental exposure to FE has been associated with a higher incidence of Malignant Mesothelioma (MM) (Rapisarda et al., 2016).

The present study aimed to validate the predicted diagnostic significance of hsa-miR-323a-3p, hsa- miR-101-3p, and hsa-miR-20b-5p on a subset of MM patients exposed to FE and matched healthy controls. For this purpose, MM tissues vs. non malignant pleura tissues were analyzed through droplet digital PCR (ddPCR) to evaluate differences in the expression levels of the selected miRNAs (Filetti et al., 2020b) and their MM diagnostic potential. In addition, further computational analysis has been performed to establish the correlation of these miRNAs with the online available asbestos exposure data and clinic-pathological parameters (Shirdel et al., 2011; Tokar et al., 2018) to verify the potential role of these miRNAs as prognostic tools.

ddPCR results showed that the three analyzed miRNAs were significantly down-regulated in MM cases vs. controls. ROC analysis revealed high specificity and sensitivity rates for both hsa-miR- 323a-3p, and hsa-miR-20b-5p which thus acquire a diagnostic value for MM. In silico results showed a potential prognostic role of hsa-miR-101-3p due to a significant association of its higher expression and increased Overall Survivor (OS) of MM patients. The computational analysis performed to further establish the functional role of these three miRNAs in MM pathogenesis have showed that these miRNAs can target and modulate both oncogene and tumor suppressor genes.

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Tessuto muscolare e connettivo

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Displaced Myonuclei in Cachexia Suggest the Occurrence of Altered Innervation

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An idiopathic myopathy characterized by central nuclei in muscle fibers (Mazzotti, 2016), a hallmark of muscle regeneration, has been observed in cancer patients. In cancer cachexia skeletal muscle is incapable of regeneration (He, 2013), consequently, this observation remains unaccounted for. In C26-tumor bearing, cachectic mice, we observed muscle fibers with central nuclei in the absence of molecular markers of bona fide regeneration. These clustered, non-peripheral nuclei were present in NCAM-expressing muscle fibers. Since NCAM expression is upregulated in denervated myofibers, we searched for additional makers of denervation, including AchRs, MUSK, and HDAC. This last one being also consistently upregulated in cachectic muscles, correlated with an increase of central myonuclei. This held true in the musculature of patients suffering from gastrointestinal cancer, where a progressive increase in the number of central myonuclei was observed in weight stable and in cachectic patients, compared to healthy subjects. Based on all of the above, the presence of central myonuclei in cancer patients and animal models of cachexia is consistent with motor neuron loss or NMJ perturbation and could underlie a previously neglected phenomenon of denervation, rather than representing myofiber damage and regeneration in cachexia. Motoneuron loss and gliosis typically occur in association with neuromuscular regressive changes during ageing in mice, however there are only two, contradictory reports on altered innervation in cachexia (Boehm, 2020; Daou, 2020). Based on all the above, we propose that, similarly to aging, denervation dependent myofiber atrophy contributes to muscle wasting in cancer cachexia.

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Is the MYH16 still a useful protein in human masticatory muscles?

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The MYH16 gene encodes the myosin heavy chain protein that plays a key role in mammals' muscles. In details, MYH16 is characteristic of temporalis and masseter muscles of the jaw. This protein is functional in non-human primates and it is correlated to the high power of jaw muscles. In human, MYH16 gene is inactivated and it plays as pseudogene because of a mutation that leads to a very low mRNA transcription level. This low level of mRNA in human seems to be responsible of a small size of jaw muscles if compared to the non-human primates (1). The aim of the present work was to analyse the possible expression of MYH16 protein in human masseter and temporalis muscles of subject with different occlusal characteristics and with different type of malocclusion. We have processed muscle samples for microarray, western blot and immunofluorescence techniques. Results have shown that MYH16 protein is still detectable in human masseter and temporalis muscles and that both mRNA and protein level increase in severe malocclusion. On this basis, the present data show that this protein is still expressed at basal level in human masticatory muscles and that their expression could be influenced by the need of muscle tissue.

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Key words

Masticatory muscles, MYH16, malocclusion, masseter, protein expression.

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The involvement of the TGF-β1/miR-31 axis in the modulation of biliary senescence and liver fibrosis during cholestasis

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Cholangiocytes line the three-dimensional biliary system. These cells represent the main target of several chronic cholestatic liver diseases, called cholangiopathies, such as primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC), characterized by the damage/proliferation of cholangiocytes (1). During the progression of these diseases, cholangiocytes acquire neuroendocrine phenotypes secreting different neuropeptides. In detail, α-calcitonin gene related peptide (α-CGRP) and substance P (SP) (2-3). In fact, knockout of α-CGRP improves biliary damage and liver fibrosis by reduced biliary senescence but enhances hepatic stellate cell (HSC) senescence. Moreover, SP stimulates biliary mass/senescence as well as liver inflammation and fibrosis by binding to tachykinin neurokinin 1 receptor (NK1R) expressed by both cholangiocytes and HSCs. On the base of this background and using a well-characterized model of PSC: the $Mdr2^{-/-}$ mouse, we have developed a $NK1R^{-/-}$ /$Mdr2^{-/-}$ model to evaluate changes in biliary mass/senescence, activation of HSCs, liver fibrosis and TGF-β1/miR-31 signaling, important in biliary senescence and activation of HSCs. Studies were performed in male mice: (i) $NK1R^{-/-}$; (ii) $Mdr2^{-/-}$; (iii) $NK1R^{-/-}$ /$Mdr2^{-/-}$; and their corresponding WT controls. Liver tissues and cholangiocytes were collected to discover decreased ductular reaction, liver fibrosis, biliary senescence, and inflammation in $NK1R^{-/-}$ /$Mdr2^{-/-}$ mice compared $Mdr2^{-/-}$ samples. In addition, increased expression of miR-31 was observed in $Mdr2^{-/-}$ mice, but it was lowered in the double knockout model, modulating the TGF-β1 signaling in HSC-mediated liver fibrosis. In the end, we found that SP/NK1R/TGF-β1/miR-31 axis plays an important role in the regulation biliary senescence and liver fibrosis during chronic cholestatic diseases.

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Connexins involvement in a murine model of Ponatinib-induced cardiotoxicity.

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Connexins (Cxs) are mainly involved in the constitution of gap junctions but they also have additional functions partecipating in many intracellular transduction signaling thus affecting cell-to-cell communication as well as other cellular processes as cell growth and differentiation [1].

In cardiac tissue the most expressed connexins are Cx40, Cx43 and Cx45, mainly expressed at the intercalated disks and involved in gap junction formation, and Cx26 that has been recently found mainly expressed in the cytoplasm of cardiomiocytes [2].

Tyrosine kinase inhibitors (TKIs) are drugs used against different types of cancers and Ponatinib (PON) is a multi-TKIs currently approved against different types of leukemia. Trials are in progress to evaluate PON anti-tumorigenic activity also in lung cancer and glioblastoma. Despite the potent PON activity, cardiotoxicity is one of its main side effect, as well as for other TKIs [3].

Aim. The aim of our study was to explore the signaling pathway of PON in a murine model of PON-induced cardiotoxicity focusing on the involvement of Cx43, serine 368 phoshorylated Cx43 (pS368-Cx43) and Cx26 also considering sex-related differences.

Methods. Mice cardiac function was assessed by trans-thoracic echocardiography, while ponatinib induced cardiac damage was assessed by cTn assay. Cxs protein expression was evaluated by western blot methods and proteomic analysis on heart tissue lysates and by immunofluorescence methods on frozen embedded hearts. Samples were obtained from both females and males mice treated or not with PON.

Results. The obtained results demonstrated that Cx43, pS368-Cx43 and Cx26 were more expressed in female compared to male mice under basal conditions and that PON-induced cardiotoxicity influenced Cxs expression in a sex-related mode. Indeed, in PON-treated mice, Cx43 and pS368-Cx43 cardiomiocytes expression was increased more in females than in males and their distribution was disordered compared to the controls. On the contrary, ponatinib induced a Cx26 decrease in female cardiomiocytes only.

Conclusion. Connexins are involved in mice PON-induced cardiotoxicity, showing a sex-related altered expression. Interestingly, proteomic and western blot analysis performed to evaluate Cxs expression gave different results compared to the immunohistochemical analysis, underlining the importance of using different methods for molecular analysis.

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Expression and localization of Phosphoinositide-specific Phospholipases C in cultured and differentiating human osteoblasts

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Homeostasis in the bone tissue primarily depends on the balance of the activities of osteoclasts and osteoblasts, primarily involved in bone forma- tion and turnover (Zaidi 2007; Khosla et al 2005). Osteoblasts maintain the bone mass, and intervene in bone injuries repair. The limited number of therapeutic agents able to promote osteogenesis ingenerated great interest addressed to manipulate the activity of osteoblasts. Insights in the events leading to the differentiation and proliferation of osteoblasts might allow uncover potential molecular therapy targets to control the complex mecha- nisms underlying the skeletal remodeling (Marie 2015; Kawai et al 2011). Oscillations of calcium act crucially during the remodeling of bone, affect- ing both the differentiation and proliferation of osteoblasts. Signal transduction pathways contribute to the differentiation and metabolic activities of osteoblasts, with special regard to calcium-related signaling (Kimple et al 2011, Keinan et al 2014), including the Phosphoinositide (PI) pathway and related Phospholipases C (PLCs).

In order to evaluate the role of PLC enzymes' family in human osteoblasts (HOBs), we analyzed the expression of *PLC* genes and the localization of PLC enzymes both in cultured HOBs and in *in vitro* differentiating HOBs after 3, 10, 17 and 23 days. Our results confirm the transcription of most *PLC* genes and the presence of a number of PLC enzymes in HOBs, differently localized in the nucleus, in the cytoplasm or both, as well as in cell protrusions. The presence of PLC enzymes within the HOBs suggests the activation of the PI nuclear cycle in HOBs. Along both the culture and dif- ferentiation culture periods, transcripts of splicing variants of selected *PLC* genes were detected and the localization of most PLC enzymes varied, with spevcial regard to enzymes belonging to the PLC β, ε and η sub-families. The behavior of selected PLC enzymes will be discussed more in detail. The presented results overall suggest that PLC signaling might provide further insights into the complex signal transduction network in bone remodeling, also representing promising molecular targets.

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Skeletal muscle adaptation gender-dependent: focus on distribution of muscle fibers and PGC1α, Hsp60 and IL-6 expression in response to a single bout of endurance exercise

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The gender dimorphism of skeletal muscle can be stated in the of mass, diameter, metabolism, and fatigue differences of the individual fibers, as well as it can be associated with the distinct hormone levels in males and females. The aim of our study was to show the different distribution of skeletal muscle fibers based on the mRNA and protein expression of the myosin heavy chain (MHC) isoforms in the soleus and extensor digitorum longus (EDL) muscles of adult BALB/c mice. Significant differences between males and females were identified in response to a single bout of endurance exercise. In fact, skeletal muscle adaptation was reflected on the mRNA levels of transcription factor Peroxisome proliferation-activated receptor-γ (PPAR-γ) coactivator-1α (PGC1α), as well as the mRNA levels of Heat shock protein 60 (Hsp60), and interleukin 6 (IL-6). Our analysis focused on gender differences showed that the aerobic muscle soleus in females is rich in type I fibers, while the male one in type IIa fibers. Otherwise, the glycolytic muscle EDL in males showed a high mRNA and protein expression of type IIb fibers, that in females displayed only high mRNA levels of MHC type IIa fibers. The muscle response to a single bout of endurance exercise resulted in an increase expression of genes involved in mitochondrial biogenesis such as PGC1 α1, Hsp60 and IL-6 in the soleus muscle of males, while the expression of PGC1 α2 and α3 was increased in the EDL muscle of both animal sexes. Although these genes are important in the physiological response to exercise, our data are useful for the characterization of the fiber-type distribution in other muscles with different anatomical function and location.

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Perivascular and endomysial macrophages expressing VEGF and CXCL12 promote angiogenesis in anti-HMGCR immune me- diate necrotizing myopathy

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Increasing interest has been devoted to the phenotype of macrophages in Idiopathic Inflammatory Myopathies (IIMs). Besides their role in innate immunity, macrophages are also involved in tissue regeneration and angiogenesis promotion.

The density and distribution of the subpopulations of macrophages (M1, inducible nitric oxide$^+$, CD11c$^+$; M2, arginase-1$^+$), endomysial capillary (CD31$^+$, FLK1$^+$), degenerating (C5b-9$^+$), and regenerating (NCAM$^+$) myofibers were investigated by using immunohistochemistry microscopy in human muscle samples from diagnostic biopsies of a large cohort of untreated patients (n: 81) with anti-3- hydroxy-3-methylglutaryl coenzyme A reductase (anti-HMGCR)$^+$ Immune Mediated Necrotizing Myopathy (IMNM), anti-signal recognition particle (anti-SRP)$^+$ IMNM, seronegative IMNM, Dermatomyositis, Polymyositis, Polymyositis with mitochondrial pathology, sporadic Inclusion Body Myositis, Scleromyositis, and anti-Synthetase Syndrome, compared with mitochondrial myopathy and control muscle samples.

In comparison with the other IIMs and control groups, endomysial capillary density (CD) was higher in anti-HMGCR$^+$ IMNM, where endomysial and perivascular M1 and mainly M2 macrophages were detected by confocal microscopy, accumulated, and expressed angiogenic molecules as VEGF-A and CXCL12. These angiogenic macrophages were preferentially associated with CD31$^+$ FLK1$^+$ microvessels in anti-HMGCR$^+$ IMNM and their M1 and M2 relative densities significantly correlated with CD (p M1: 0.005; p M2: 0.013). WB analyses revealed increased expressions of VEGF-A, FLK1, HIF-1α, and CXCL12 in anti-HMGCR$^+$ IMNM. Both CD and expression levels of these angiogenic molecules were not increased in anti-SRP$^+$ and seronegative IMNM, providing additional, useful information for differential diagnosis among these IIM subtypes.

Our findings indicate that a reciprocal crosstalk occurs between infiltrating macrophages and microvascular cells, suggesting a restorative role for macrophages in modulating muscle regeneration potential and angiogenesis.

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3D collagen type I-based cell free scaffold for joint cartilage regenerative strategy.

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The articular cartilage defects represent a big challenge due to the limited self- healing capacity of this complex tissue. Several approaches in this field obtained partial suitable results. Engineered cartilage, which combines innovative biomaterials and mesenchymal stem cells, emerges as a promising strategy for the cartilage regeneration. The aim of this study was to investigate the collagen I- based cell-free scaffold ability to promote cartilage repair after its *in vivo* orthotopic implantation. The cartilage defects were created at the patellofemoral groove in rat knees, the cell-free scaffolds were implanted into articular defect and maintained for 4, 8 and 16 weeks. No scaffold was implanted for the experimental control group. At each time-point post-transplantation, general features of cartilage repair process are evaluated through morphological, histochemical and gene expression analyses. At 4 weeks, histological analysis shows the formation of fibrous tissue, replaced progressively by a tissue resembling the calcified one at 16 weeks in the experimental control group. In the experimental group receiving cell-free scaffold, progressive replacement of the collagen scaffold with the newly formed cartilage- like tissue is observed, as confirmed by Alcian Blue staining and immunohistochemical and gene expression analyses, which show the expression of typical cartilage markers. The results of this study suggest that the collagen I- based cell-free scaffold is able to recruit host cells from the surrounding joint tissues and to promote cartilaginous repair of articular cartilage defects, suggesting its use as a potential strategy for articular cartilage regenerative approaches.

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Key words

Articular cartilage defect, cartilage regeneration, cartilage tissue engineering, collagen I-based cell-free scaffold, orthotopic implantation.

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In vitro generation of tissue-engineered grafts from decellularized human esophagus

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Reconstruction of esophagus after caustic injuries, perforations, atresia or tumors often requires the autotransplant of other organs (i.e., stomach or colon), with important co-morbidity problems and sub-optimal functional outcomes[1]. The development of a bio-engineered esophagus would represent a valid therapeutic alternative to current surgical techniques[2]. In this work, decellularization of human esophagus was investigated for the first time to obtain non-immunogenic allografts with biological features resembling the native tissue.

Esophageal samples collected from cadavers were decellularized by two detergent-enzymatic protocols involving 1) sodium dodecyl sulfate (SDS) and 2) Tergitol. After decellularization, samples underwent substantial whitening, with tissue homogeneity/manipulability being better preserved by SDS rather than Tergitol. Quantification of residual DNA and histological analyses demonstrated that both protocols efficiently removed cells, DNA (50-60 ng/mg of tissue and fragments <200 bp in length) and muscle fibers, with preservation of collagen/elastin. According to colorimetric assay, 80-90% of the glycosaminoglycan component was preserved after decellularization. Immunohistochemistry showed that the detergent-enzymatic treatments did not affect the expression of specific extracellular matrix (ECM) markers (collagen I and IV, laminin), while leading to the loss of HLA-DR expression to obtain non- immunogenic allografts. Hydroxyproline quantification confirmed the maintenance of the collagen component (3-6 ug/mg of tissue), which was ultrastructurally analysed by Scanning Electron Microscopy. Second Harmonic Generation Microscopy highlighted that the collagen intensity was significantly lowered by Tergitol treatment. Based on uniaxial tensile tests, decellularization significantly affected the mechanical properties of stiffness and strength, with a significant decrease in the Ultimate Tensile Strength and Young's modulus of treated *versus* native samples.

Overall, SDS treatment assured for better preservation of the manipulability and the collagen component of the esophageal samples. Cytotoxicity assay, in vivo subcutaneous implant and scaffold recellularization will assessed the biocompatibility of the acelluar esophageal allografts.

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Molecular and cellular network underlying neurogenic muscle atrophy

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Neurogenic muscle atrophy is a hallmark of several degenerative processes involved in different pathologies, such as Amyotrophic Lateral Sclerosis (ALS). This condition is characterized by progressive motor neurons (MNs) degeneration, which leads to a deterioration of neuromuscular functions, causing weakness, paralysis and atrophy of skeletal muscles. During neurogenic muscle atrophy, the interruption of transmission of neurogenic signals to muscles, caused by loss of neuromuscular junction (NMJ) integrity, activates protein breakdown and reduces protein synthesis, leading to the loss of muscle mass and contractile activity. To dissect the role of muscle resident cells in the maintenance of the cross talk between muscle and nerve, we use next generation sequencing, in-vitro and in-vivo studies. Thanks to these complementary approaches, we identified new cellular and molecular players that underlie the neurogenic muscle atrophy. Perturbations of cellular response, that includes the activation of muscle resident cells (FAPs and Glial cells) that partecipate to neurogenic muscle atrophy has been recently observed (Madaro et al. 2018; Proietti et al. 2021).

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Key words

Muscle Denervation, FAPs, Glia, NGS, single cells RNAseq.

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Paclitaxel alters angiogenesis in the peripheral and central nervous system of neuropathic rats

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Neurotoxicity is the most debilitating non-haematological adverse effect of paclitaxel (PTX) in cancer patients that report typical symptoms of a dose cumulative sensory peripheral neuropathy with paraesthesia, disaesthesia, tingling, and numbness. Many patients develop allodynia and hyperalgesia, experiencing neuropathic pain (NP). NP can originate from a peripheral sensitization then transmitted to the central nervous system where it can determine structural and functional changes. An abundant microvascular angiogenesis was described in the primary somatosensory cortex, specifically on the hindlimb projection of rats with NP of other origin. In this work, we investigated the microstructural vascular anomalies in the central somatosensory pathway and peripheral compartments (dorsal root ganglia, DRG) in rats exposed to chronic PTX treatment.

Twenty-four rats were chronically treated with PTX 10 mg/kg to induce a painful peripheral neuropathy. Animals were tested for neurophysiological abnormalities and behavioral NP and finally perfused with fixative and/or indian ink before collecting samples. Samples have been analyzed at synchrotron radiation resources by X-ray Phase-Contrast Tomography (XPCT) Imaging (Diamond, Didcot, UK and ESRF, Grenoble, France). Volume rendering allowed a detailed visualization of vasculature at the sub micrometric scale. We performed a quantitative and morphological analysis of micro-vascular structures in PNS and CSN of control and NP rats. Histochemical and histological evaluations validated the results obtained by XPCT.

A significant increased number of vessels has been found in NP samples, suggesting an angiogenesis at the capillary level in NP condition. The effect was larger (about +173%) in somatosensory cortex, still relevant in lumbar spinal cord and noticeable in related DRG. Specific analyses indicated that neo-formed vessels were smaller than 15 micron. Moreover, a significant decrement of the number of capillary branch points and tortuosity was evident in NP samples, suggesting an impairment of the normal microcirculation and neuronal activity. These events have been confirmed both by tomato-lectin staining, that showed a vessel neogenesis in all peripheral and central compartments, and by histological observations at light microscopy. These results shed light on new pathogenic mechanisms and potential novel therapeutic approaches for PTX-induced painful peripheral neuropathy.

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Insights into the motor cortex: changes in corticospinal circuits during premovement facilitation in physiological conditions – a TMS study

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Changes in corticospinal excitability have been well documented in the preparatory period before movement, however their mechanisms and physiological role have not been entirely elucidated. We aimed to investigate the functional changes of excitatory corticospinal circuits during a reaction time (RT) motor task (thumb abduction) in healthy subjects (HS). 26 HS received single pulse transcranial magnetic stimulation (TMS) over the primary motor cortex (M1). After a visual go signal, we calculated RT and delivered TMS at three intervals (50, 100, 150 ms) within RT and before movement onset, recording motor evoked potentials (MEP) from the abductor pollicis brevis (APB) and the task-irrelevant abductor digiti minimi (ADM). We found that TMS increased MEP_{APB} amplitude when delivered at 150, 100 and 50 ms before movement onset, demonstrating the occurrence of premovement facilitation (PMF). MEP increase was greater at the shorter interval (MEP_{50}) and restricted to APB (no significant effects were detected recording from ADM). We also reported time-dependent changes of the RT and a TMS side-dependent effect on MEP amplitude (greater on the dominant side). In conclusion, we here report changes of RT and side-dependent, selective and facilitatory effects on the MEP_{APB} amplitude when TMS is delivered before movement onset (PMF), supporting the role of excitatory corticospinal mechanisms at the basis of the selective PMF of the target muscle during the RT protocol.

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Key words

Premovement facilitation, reaction time, transcranial magnetic stimulation, MEP, motor cortex.

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Sodium/calcium exchanger (NCX): axonal damage as a consequence of functional disturbances. A new target for neuroprotection?

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Oxaliplatin (OHP) induced peripheral neurotoxicity (OIPN) is a double folded late toxicity. It comprises a sensory chronic neuropathy and a peculiar acute neurotoxicity syndrome (i.e., axonal hyperexcitability due to voltage-operated ion channel transient disturbances). Our group previously observed a possible causative link between the two syndromes: targeting acute OIPN, we prevented chronic manifestation in a comprehensive OIPN rat model (Alberti P. et al., 2020). Sodium-calcium exchanger family (NCX) might be the pivotal element in converting a transient alteration of ion conductances in an persistent anatomical damage. To validate this hypothesis we combined *in vivo* and *in vitro* observations.

The possible role of NCX2 in OHP neurotoxicity was ascertained *in vitro*, exploiting embryonic rat Dorsal Root Ganglia (DRG) organotypic cultures treated with different concentrations of SEA0400 (SEA), a strong NCX inhibitor. In the *in vivo* setting, we tested a cohort of balb/c mice: we compared a treatment group (OHP 7 mg/Kg, iv, once a week per 8 weeks) with a vehicle treated group (n=8 each). Acute neurotoxicity induction was verified via nerve excitability testing (NET) after the 1st administration. Chronic neurotoxicity was assessed via nerve conduction studies (NCS), behavioural tests and neuropathology (intraepidermal nerve fiber density [IENFD] and caudal nerve morphometry). NCX2 levels (western blot [WB]) on DRG pool were assessed too.

In the *in vitro* experiment, we observed a significant OHP-related neurotoxicity reduction related to NCX inhibition assessing DRG neurite elongation (from 40.8 to 58.4%). In the *in vivo* experiment, NET confirmed acute OIPN was induced and NCS, behavioural tests and neuropathology confirmed chronic OIPN were both fully induced in the treatment group; NCX2 WB analysis showed a significant decrease in OHP group ($p<0.001$).

In the *in vivo* setting we confirmed, first, that our model fully induced both acute and chronic OIPN features, and then that this was matched by NCX2 downregulation. These observations confirmed OHP administration induced an aberrant – despite transient – depolarization in neurons, known to downregulate NCX2. Moreover, we tested the potential benefit of NCX2 inhibition in the *in vitro* setting: a satisfactory neuroprotection in neurons was observed. These findings will give the basis for new neuroprotectant strategies exploiting drug development targeting the NCX family.

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Key words

NCX2, neuropathy, neurotoxicity, neuroprotection, neuropathology, nerve excitability.

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Glioblastoma multiforme: focus on antiangiogenic effect of pacap

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Glioblastoma multiforme (GBM) is a lethal form of brain cancer affecting adults, characterized by poor prognosis. The uncontrolled proliferation of cancer cells produces hypoxic niches in tumor mass. By inducing activation of hypoxia inducible factors (HIFs), hypoxia triggers signaling cascades responsible of uncontrolled cell proliferation as well as release of vascular endothelial growth factor (VEGF), directly responsible of neoangiogenesis. All these factors affect cancer development by promoting malignant progression and recurrence. Assumed the heterogeneity of tumoral mass, the actual therapeutic approach consists in a multimodal treatment consisting in surgery, radiation and chemotherapy with different molecules.

Recent studies have demonstrated the involvement of pituitary adenylyl cyclase-activating peptide (PACAP) in GBM, by showing its anti-invasive effect on GBM cells. The aim of our studies was to investigate the molecular mechanisms underlying the modulatory effect of PACAP on glioblastoma neoangiogenesis. By using frozen sample, we have analyzed the expression of PACAP and its related receptor (PAC1R) in hypoxic as well as non-hypoxic region of GBM sections.

To investigate the functional role of PACAP on neoangiogenesis of hypoxic area we have used human U87MG glioblastoma cells exposed to hypoxia. Our data have showed that PACAP and PAC1R co-localize with HIF-1α suggesting that their tissue expression is related to hypoxia. Furthermore, PACAP treatment modulates neoangiogeneis process as demonstrated by reduced expression and release of VEGF as well as decreased formation of vessel-like structures in H5V endothelial cells cultured in U87MG glioblastoma cells conditioned medium.

Although additional studies are need, the study suggest new insight on modulatory action exerted by PACAP in neoangiogenesis of GBM.

Key words

Glioblastoma multiforme, ADNP, NAP, hypoxic microenvirorment.

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Do choline alphoscerate and thioctic acid prevent the cerebrovascular alterations in spontaneously hypertensive rats?

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Chronic brain vascular injury is a severe risk factor of cerebral dysfunction. Brain hypoperfusion and white matter lesions correlate with the development of cognitive impairment leading to Alzheimer's disease or vascular dementia. Hypertension has deleterious effects on the brain carrying out to cognitive decline and dementia. Concerning the mechanisms, hypertension-induced cerebrovascular alterations are due to increased production of reactive oxygen species and cholinergic pathways impairment [1].

(+)-Thioctic acid or (+)-TIO eutomer is a well-known antioxidant compound showing brain activity, while choline alphoscerate (GPC) improves choline availability and acetylcholine synthesis/release in brain areas [2,3]. Our study aimed to assess if long-term treatment with these two compounds could induce neuroprotection in the brain of spontaneously hypertensive rats (SHR), used as an animal model of cerebrovascular alterations. Male SHR aged 24 weeks and age-matched normotensive Wistar–Kyoto (WKY) rats were treated for 4 weeks with GPC (150 mg/kg/day), (+)-TIO (125mmol/kg/day) alone or in the association. The brains were removed for western blot and immunohistochemical analysis for different neuroinflammatory markers in the hippocampus as it is involved in memory and learning tasks.

Blood pressure (BP) was higher in SHR rats compared to normotensive WKY. The compounds, alone or in the association, were able to reduce systolic BP, while only their association reduced the diastolic BP. Our results showed that GPC, alone, restored the levels of neuronal nuclei protein while injection of (+)-TIO and GPC combination did not prevent the downregulation of microtubule-associated protein-2 on dendritic arborization. (+)-TIO and GPC alone and in association counteracted the astrogliosis and microglial activation and decreased the level of tumor necrosis factor-alpha.

Our findings indicate that treatment with GPC plus (+)-TIO attenuates neural damage and glial reaction in the hippocampus of SHR and thus affords neuroprotection in hypertensive rats. The administration of the two compounds could represent a new potential strategy to prevent the cerebral alterations related to hypertension opening the opportunity to further evaluations in clinical trials.

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Neuroprotection Vitamin C mediate in animal model of PD

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*Chiara Porro and Dario Domenico Lofrumento should be considered joint first author, Chiara Porro will present the abstract

Vitamin C (Vit C) is a water-soluble vitamin, presents in many foods. Vit C is studied for its applications in prevention and management of different pathologies including neurodegenerative diseases.

Neuroinflammation is firstly a defence mechanism of body that initially preserves the brain by promoting tissue repair and removing cellular debris, but persistent inflammatory responses are detrimental and may lead to neurodegenerative diseases. Parkinson's Disease (PD). is the second most common chronic progressive neurodegenerative disorder and oxidative stress is one of the most important factors responsible of its pathogenesis; due to this, the research of antioxidant and anti-inflammatory compounds is an important target for counteract neurodegenerative diseases including PD. In Central Nervous System the presence of Vit C in the brain is higher than in other body districts, but why and how this happened is still unknown. In this research Vit C with its anti-inflammatory and anti-oxidative properties is studied in deeply to better understand its contribute in brain protection; in particular, we have investigated how administration of Vitamin C in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced animal model of PD exerts neuroprotective effects.

First, we observed that Vitamin C significantly reduced the MPTP-induced loss of tyrosine hydroxylase (TH)-positive dopaminergic neuronal cells, microglial cells activation and astrogliosis. Also, gait and spontaneous locomotor activity, evaluated by an automated treadmill and the open field test respectively, were partially ameliorated by Vitamin C treatment in MPTP-intoxicated animals.

In relation to neuroinflammation, results show that Vit C reduced the protein and mRNA expression of inflammatory cytokines such as IL-6, TLR4, TNF-a, iNOS, CD-40 while anti-inflammatory proteins such as IL-10, CD163, TGF-b and IL-4 resulted increased. Vit C was able to attenuate dopaminergic neuronal degeneration in striatum and SNpc. Interestingly, we have demonstrated, for the first time, that Vit C suppresses neuroinflammation, by modulating microglial polarization and astrocyte activation. Moreover, Vit C was able to reduce NLRP3 activation which is linked to pathogenesis of many inflammatory diseases including neuroinflammatory disorders.

In conclusion our study has evidenced that Vit C may represents a new promising food additive for the prevention and alleviating the inflammatory cascade in PD, contributing to neuroprotection.

Key words

Vitamin C, Neuroinflammation, Neuroprotection, Microglia, Parkinson's Disease, Inflammasome.

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The epithelial-mesenchymal transition in glioblastoma: the role of pituitary adenylate cyclase-activating polypeptide

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Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults. Its highly malignant phenotype as well as its resistance to chemo-therapy and radiotherapy is exacerbated by the extensive hypoxic areas in the tumor bulk. The early response to hypoxia is mediated by hypoxia-inducible factor α (HIF-1α) involved in aberrant development of new blood vessels and metastasis by promot- ing epithelial to mesenchymal transition (EMT) in the tumor bulk. Pituitary adenylate cyclase-activating polypeptide (PACAP) exerts different effects in various human cancer. The expression of PACAP and its related receptors has been largely demonstrated in human gliomas, where the peptide has shown to interfere with the hypoxic microenvironment through the modulation of hypoxia-inducible factors via PI3K/AKT and MAPK/ERK pathways inhibition. Considering that hypoxic tumor mi- croenvironment is strictly linked to EMT, in the present study we have investigated for the first time the ability of PACAP to regulate this event. Our results showed that PACAP and its receptor are expressed either in epithelial as well as mesenchymal cells of human GBM. Moreover, the peptide interferes with EMT process in GMB cells exposed to deferoxamine mesylate, a well-known hypoxia mimetic agent, by reducing the expression of mesenchymal markers and affecting cell migratory ca- pacity. The complex regulating mechanism carried out by PACAP might open new insight in the therapeutic approach to this fatal malignancy.

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Key words

Glioblastoma, hypoxia, PACAP, EMT.

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Novel regulation of astrocyte response during parkinson's disease

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Astrocytes are key players in Parkinson's disease where they can contribute to α-Synuclein pathology but also to neuroprotection via α-Synuclein clearance. The acquisition of inflammatory profile in astrocytes is due to a different gene expression program which potentially exacerbates the damage. Recent evidence suggests an important role for post- transcriptional gene regulation during neurodegeneration. Here, we unravelled a novel function for the RNA Binding Protein Quaking (Qki) in astrocytes during Parkinson's disease. We used primary cortical astrocytes cultures derived from C57BL/6J mice treated for 18 hours with human a- Synuclein pre-formed fibrils (PFFs) as an *in-vitro* model of Parkinson's Disease. We performed super-resolution microscopy analysis of PFF- treated astrocytes and we found an alteration in Qki distribution and a strong inflammatory response in astrocytes.

In healthy conditions Qki recognizes its mRNA targets through Quaking Responsive Element (QRE) consensus sequence leading to mRNA degradation and inhibition of gene expression. To identify Qki mRNA targets expressed in astrocytes and altered in Parkinson's disease, we crossed different datasets present in literature and performed computational analysis using Galaxy web-based platform. We found that key astrocytic mRNA altered in Parkinson's disease had QRE and therefore could be potential directly repressed by Qki.

To test whether Qki mRNA regulatory activity is altered in Parkinson's disease, we tested its function in PFF-treated astrocytes. Our results showed that QRE-containing mRNAs had an altered expression following PFFs exposure thus suggesting that Qki is linked to pathological astrocytes activation. Overall, these findings reveal for the first time the role of Qki in astrocytes post-transcriptional regulation and the importance of its activity in modulating homeostatic functions. Ongoing experiments are addressing the expression of QKI in human astrocytes of Braak stage 6 brains to uncover its involvement in Parkinson's disease patients.

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Keywords

Qki, RNA Binding Protein, Astrocytes, Inflammation, α-Synuclein, Parkinson's disease.

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The expression of raf kinase protein inhibitor (rkip) on different histological types of leiomyoma

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Uterine leiomyomas are smooth muscle tumors of the uterus and represent the most common benign tumors of female reproductive tract [1]. A classification can be made according to histological characteristics of leiomyomas. We can distinguish into usual leiomyoma the most commons, atypical also called bizarre leiomyoma, that is characterized by polymorphic and bizarre nuclei; leiomyoma with high mitotic activity, cellular leiomyoma, lipoleiomyoma and leiomyosarcomas which are the rare malignant counterparts [2]. In addition, in 2014 the World Health Organization (WHO) introduced the so-called category of STUMP (smooth muscle tumors of uncertain malignant potential) which includes any lesion that is difficult to characterize. In particular, the bizarre leiomyoma is described as a benign histology but, since it has intermediate characteristics, it is often difficult to distinguish with certainty whether it is benign or malignant. For this reason, bizarre leiomyoma is often classified as a tissue belonging to the STUMP category. Raf Kinase Protein Inhibitor (RKIP) is considered primarily as a suppressor of the metastasis gene.

We evaluated the expression of RKIP in the different histotypes of leiomyoma, it is being reduces in high proliferating variety (such as in cellular leiomyoma and leiomyoma mitotically active or apoplectic) and in leiomyosarcoma by immunohistochemistry. RKIP expression is different among leiomyoma histotypes, and in metastatic tissues, such as leiomyosarcoma [3]. RKIP is present in smooth muscle cells and it is highly expressed in endothelial cells of the usual leiomyoma. In lipoleiomyoma, it has high expression in adipose cells with inflammation, this particular has been confirmed with immunohistochemistry for the CD68 which shows the presence of "crown like structures", typical structures of adipose cells with inflammation [4]. In cellular leiomyoma, the expression of RKIP is low and confirmed to endothelial cells. Interestingly it has a high expression in the bizarre cells of the bizarre leiomyoma, while it is completely absent in leiomyosarcoma. These results suggest that RKIP could be used as a marker that could aid in the distinction between bizarre leiomyoma that has a high expression of RKIP and leiomyosarcoma in which RKIP is absent. Therefore, RKIP could be used to evaluate and distinguish STUMP and help to discriminate between benign and malignant lesions.

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Characterization of α-synuclein (α-syn) in normal human jejunum and its correlations with the neuroendocrine system

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Alpha-synuclein (α-syn) is a presynaptic neuronal protein and its structural alterations play an important role in the pathogenesis of neurodegenerative diseases, such as Parkinson's disease (PD) (1). It has been originally described in the brain and aggregated α-syn has also been found in the peripheral nerves including the enteric nervous system (ENS) of PD patients (2). ENS is a network of neurons and glia found in the gut wall which controls gastrointestinal function independently from the central nervous system. Moreover, two types of epithelial cells are crucial in the creation of an interface between the lumen and the ENS: they are the tuft cells and the enterochromaffin cells (EECs) (3-4). In addition, the abundant enteric glial cells (EGCs) in the intestinal mucosa play a key role in controlling the intestinal epithelial barrier (5). Our aim has been to localize and characterize the presence of α-syn in the normal human jejunum wall. Surgical specimens of proximal jejunum wall were collected from patients submitted to pancreaticoduodenectomy and intestinal sections underwent immunohistochemical procedure using monoclonal antibody for α-syn. α-Syn has been found both at the level of ENS and the epithelial cells. To characterize α-syn immunoreactive epithelial cells we used markers as choline acetyltransferase (ChAT), useful to the identification of tuft cells (3). Then, we evaluated the co-presence of α-syn with 5-hydroxytryptamine (5-HT), expressed in EECs (4). Finally, we used the low-affinity nerve growth factor receptor (p75NTR), to detect peripheral EGCs. The presence of α-syn has been demonstrated in EECs but not in the tuft cells. Additionally, p75NTR has been highlighted in EGCs of the mucosal layer, and co-localized with α-syn in EECs but not in ChAT-positive cells. These findings suggest that α-syn could play a crucial role in synaptic transmission of the ENS and may contribute to maintain the integrity of the epithelial barrier of the small intestine through EECs.

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Key words

α-syn: alpha-synuclein, p75NTR: nerve growth factor receptor, ChAT: choline acetyltransferase, EECs: enterochromaffin cells, Tuft cells.

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Posture and gait in the early course of schizophrenia

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While correlations between postural stability deficits and schizophrenia are well documented, information on dynamic motor alterations in schizophrenia are still scarce, and no data on their onset are available yet. Therefore, the aim of this study was i) extensively investigate gait and posture parameters in patients with schizophrenia and, in a secondary analysis, ii) to look across patients to capture a possible motor profile for the early detection of the disorder. Body composition, posture and gait parameters were assessed in a group of 30 patients with schizophrenia and compared to 25 healthy subjects. Based on time from first hospitalization, the schizophrenia group (SG) was subdivided in three subgroups: ≤5 years (early-term disease, ETD), 6–14 years (middle-term disease, MTD), ≥15 years (long-term disease, LTD) from the first hospitalization.

Sway area was significantly higher in the schizophrenia group compared to controls regardless of whether the participants were in eyes open or eyes closed condition. Gait cadence and speed were significantly lower in patients with schizophrenia. Similarly, in the early stages of disease sway area was significantly higher and gait cadence and speed were reduced in the ETD group.

Overall, we show that: i) sway area is increased, while gait cadence and speed are reduced in schizophrenia individuals; ii) the visual component of postural control is less relevant in patients with schizophrenia, as their balance–which is impaired–does not significantly suffer from eye closure; iii) increase of sway area and decrease of gait cadence and speed are the earliest detectable events in terms of motor alterations.

We concluded that the combination of an increased sway area (independent from eye closure) and a gait cadence reduction—in the presence of normal gait speed and stride length—might be considered peculiar postural and gait profile characteristic of early schizophrenia. The results of the present study highlight the importance of motor dimension as a core feature in schizophrenia. The recognition of specific motor markers might represent a valid tool for the early detection of the disorder and a possible treatment target.

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Astrocytes expressing Vitamin D-activating enzyme identify Parkinson's disease

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Astrocytes are important players in Parkinson's disease (PD), a neurodegenerative disorder characterized by α-Synuclein aggregates as the main pathological hallmark. It has been demonstrated in cellular and mouse models that astrocytes can internalize α-Synuclein aggregates thus protecting neurons. Conversely, the acquisition of an inflammatory profile in astrocytes can amplify the tissue damage.

Different studies linked Vitamin D to neuroprotection. Vitamin D3 (25- hydroxyvitamin D [25(OH)D]) is present in human serum and is activated by 1α-hydroxylase (CYP27B1) in kidney and also brain. In fact, CYP27B1 and 1,25-dihydroxyvitamin D receptor (VDR) are expressed in neurons and glial cells of *post-mortem* human brain. Despite their wide expression, CYP27B1 and VDR role in PD pathology still needs to be investigated.

Our aim was to analyse Vitamin D activating and deactivating (CYP24A1) enzyme and VDR in *post-mortem* human brain PD (n = 9, Braak stage 6) compared to control samples (n = 4).

To investigate if there were differences in Vitamin D pathway components, we analysed the distribution of VDR and CYP24A1 in the *substantia nigra* and we found similar expression in PD patients and control subjects. On the contrary, CYP27B1 was increased in a particular subpopulation of astrocytes not only in *substantia nigra* but also in dorsal motor nucleus of vagus and frontal cortex of PD patients. To characterize CYP27B1 astrocytes, we performed super-resolution microscopy analysis and quantified the expression of proinflammatory marker (i.e. complement component C3) and α-Synuclein aggregates (fibrils and oligomers). We found that only a small fraction of CYP27B1 astrocytes were C3 positive and contacted dopaminergic neurons lacking Lewy bodies in the *substantia nigra* of PD. Furthermore, CYP27B1 positive astrocytes contained α-Synuclein oligomers detected by proximity ligation assay, thus indicating that these astrocytes contribute to α-Synuclein oligomers clearance in human brain.

Finally, we analysed whether CYP27B1 positive astrocytes are related to neuropathological features, like white matter alteration and clinical aspects of PD (i.e dementia). Interestingly, we observed a decrease in CYP27B1 positive astrocytes in the frontal cortex of PD patients with dementia.

Taken together, the presence of CYP27B1 positive astrocytes distinguishes PD patients and suggests their potential role in neuroprotection.

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Key words

Astrocytes, α-Synuclein oligomers, CYP27B1, Vitamin D, Parkinson's disease.

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Restoration of ER proteostasis augments the autophagic flux and mitigates remote degeneration after spinal cord injury

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The pathogenic mechanisms underlying the progression of remote degeneration after axonal damage are not fully understood. In this study, we aimed to assess the relationship between endoplasmic reticulum (ER) stress and autophagy in remote degeneration after axonal damage due to spinal cord injury (SCI).

Starting from 1 day after SCI, significant increases in markers of ER stress (activating transcription factor 4 (ATF4), glucose-regulated protein 78 (GRP78/BiP) and C/EBP homologous protein (CHOP) were found in remote axotomized neurons compared with sham-lesioned animals. In SCI animals' ER stress-mediated signaling was parallel to the blockade of the autophagic flux assessed by increase in protein levels of SQSTM1/p62, microtubule-associated protein 2 light chain 3 (LC3-II), and decrease of the lysosomal marker LAMP1, suggesting an accumulation of dysfunctional autophagosomes in axotomized neurons. Pharmacological modulation of ER stress by Guanabenz significantly modulated ER stress response and elicits autophagy machinery homeostasis in remote axotomized neurons, consistent with the detrimental effects of persistent ER stress on the autophagic flux and, consequently, on remote degeneration. These effects correlate with an increased activation of TFEB —the master regulator of autophagy/lysosomes biogenesis– and the halt of neuronal cell death as well as with an improved functional recovery.

Thus, our results suggest that therapies aimed to restore ER proteostasis might attenuate the progression of remote degeneration after spinal cord injury.

Key words

Xonal damage, autophagy, ER stress, apoptosis, lysosome, proteostasis.

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Altered Sexual Behavior in Dopamine Transporter (DAT) Knockout Male Rats: A Behavioral, Neurochemical and Intracerebral Microdialysis Study

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Dopamine-Transporter knockout (DAT KO) rats exhibit several behavioral dysfunctions related to increased extracellular dopamine levels and altered dopamine turnover due to DAT gene silencing. Central dopamine plays a key role in sexual behavior. This prompted us to characterize several aspects of the sexual behavior of the DAT KO rats compared to their heterozygote (HET) and wild type (WT) counterparts. Extracellular dopamine and glutamic acid concentrations were also measured by intracerebral microdialysis from the nucleus accumbens (Acb) shell of sexually experienced DAT KO, HET and WT rats when put in the presence of a sexually receptive female rat. Expression of markers of neural activation (Δ-FosB), neurotrophism (BDNF, trkB, Arc) and synaptic plasticity (synaptophysin, syntaxin-3, PSD-95) was also evaluated in the ventral tegmental area (VTA), Acb and medial prefrontal cortex (mPFC) by Western Blot assays. The results obtained indicate that sexual behavior of DAT KO rats shows peculiar differences compared to their HET and WT counterparts, as for instance a more rapid acquisition of sexual experience and higher levels of sexual motivation and activity. These differences were observed along with differential changes in dopamine and glutamic acid concentrations in dialisates from the Acb during sexual behavior, with lower increases of dopamine and glutamic acid in DAT KO versus WT and HET rats, and a lower expression of the markers investigated, mainly in the mPFC, in DAT KO versus WT rats. Together these findings confirm a key role of dopamine in sexual behavior and provide evidence that the permanently high levels of dopamine triggered by DAT gene silencing cause alterations both in the frontocortical glutamatergic neurons projecting to the Acb and VTA and in the mesolimbic dopaminergic neurons, and lead to specific brain regional changes in neurotrophism and neuroplasticity, which may have a role in the sexual behavior alterations (i.e., hypersexuality) found in the KO genotype.

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Key words

Arc, BDNF/trkB, Δ-FosB, DAT knockout rats, dopamine, glutamic acid, sexual behavior, synaptic proteins.

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Effect of acute stress on the expression of BDNF and trkB in the mesocorticolimbic system of the Roman rats

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The Roman High-Avoidance (RHA) and the Roman Low-Avoidance (RLA) rats, represent two psychogenetically-selected lines that are, respectively, resistant and prone to displaying depression-like behavior, induced by stressors. Evidence that the aberrant reward processing and responses represent a common symptom in patients with depression has drawn attention to the role of the mesocorticolimbic circuitry. Most of the experimental studies regarding the reward circuitry refer to the dopaminergic mesocorticolimbic system as the pathways originating in the ventral tegmental area (VTA) and projecting to the Nucleus Accumbens (Acb) and to the medial prefrontal cortex (mPFC), but involving also other brain regions (1-3). Given the key role played by the neurotrophic factors and neuronal plasticity in the pathophysiology of depression, here we extend previous studies on the hippocampus of the Roman rats (4) and aimed at assessing the effects of acute stress, i.e., forced swimming (FS), on the expression of brain-derived neurotrophic factor (BDNF) and its trkB receptor in regions of the mesocorticolimbic system, such as the VTA, the Acb core and shell, and the anterior cingulate (AC) and prelimbic/infralimbic (PL/IL) areas of the mPFC, of the RHA and the RLA rats, by means of western blot and immunohistochemistry.

A 15 min session of FS elicited different adaptations in the expression of BDNF and trkB in the Roman rat selected brain areas by generating line-dependent changes and line x FS interaction. After FS i) in the RLA rats levels of both markers are lower than those in the RHA rats in the Acb core, whereas in the Acb shell BDNF shows no change and an effect of line is present for trkB; ii) in the VTA, BDNF and trkB levels are higher in RHA than in RLA rats, where the two proteins appear not to change; c) in the AC cortex, a significant higher expression of BDNF, but non trkB, occurs in RLA rats; d) in the PL/IL cortex, BDNF is lower in FS than in control RHA rats, while both markers showed higher expression levels in FS vs control RLA rats.

The results are consistent with the hypothesis that the differences in the BDNF/trkB signaling and neuroplastic mechanisms are involved in the activation of the mesolimbic DA system as a site of molecular neuroadaptations that appear to be uniquely associated to the susceptibility of RLA rats and resistance of RHA rats to stress-induced depression.

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Effect of conjugated linoleic acid (CLA) on activated microglia

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Microglia represent the resident immune cells of the brain and have phenotypic and functional features similar to tissue macrophages of peripheral organs. Like other macrophages, activated microglia can synthesize and secrete a great variety of soluble factors, including growth factors, cytokines, chemokines, complement factors, fatty acid metabolites, proteolytic enzymes and free radicals. A number of these factors exercise trophic functions, but most of them are proinflammatory factors. For these reasons, it has been suggested that activated microglia may play a pivotal role in the pathogenesis of several neurodegenerative diseases.

Inflammation is modulated by several fatty acid derived molecules. In particular, those derived from arachidonic acid able to influence the expression of inflammatory molecules. However, it is not clear whether a decrease of arachidonic acid is able to influence inflammatory markers expression. Conjugated linoleic acid (CLA) consists of a group of linoleic acid isomers that are naturally present in food, such as meat and dairy products. Numerous biological activities have been attributed to CLA related to inflammatory processes, diabetes, obesity, cancer. We previously demonstrated that c9,t11 and t10,c12 CLA isomers easily pass the BBB in rats and in humans and downregulate inflammatory markers in human cultured astrocytes (Fa et al. 2005: Saba et al., 2019).

In this study, we aimed to evaluate whether CLA isomers are able to modulate in vitro the expression of pro-inflammatory molecules in mouse murine BV2 microglia under inflammatory conditions induced by poly I:C or LPS.

CLA isomers (c9,t11 and t10,c12) were able to downregulate inflammatory markers expression (IL-1β, IL-6, RANTES. iNOS) as observed by ELISA, qPCR and western blot in activated BV2 cells. Moreover, we observed an increase in PPAR-alpha expression, suggesting the involvement of this receptor in the mechanism of action of CLA.

Our data demonstrate that CLA can reduce neuroinflammation by decreasing the inflammatory properties of activated microglia, suggesting a nutritional role of CLA in the modulation of neuroinflammation.

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Brain innate immune system and extracellular matrix are differentially modulated during glioblastoma progression in the GL261-C57/Bl6J syngeneic mouse model

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Glioblastoma multiforme(GBM) is the most common primary brain tumor, associated with a poor prognosis. The brain's innate immune system comprehends microglia, macrophages, and astrocytes which move in the extracellular matrix net. Recent studies showed their involvement in nurturing tumor cells and grant GBM invasiveness. In the present study, we aim to characterize the time-related changes in the GBM landscape, using a syngeneic mouse model of primary GBM.

GL261 glioma cells were injected in the right striatum of immuno-competent C57Bl6J mice and animals were sacrificed after 7, 14, and 21 days (7D, 14D, 21D). The tumor development was assessed through 3D tomographic imaging. Brain tissues were processed using immunohistochemistry, immunofluorescence, and western blotting.

Our results showed how tumor progression changes the morphology and the molecular profile of astrocytes and microglia/macrophages. In the early stages, the proliferating tumor (Ki67+) appeared with a speckled distribution. The proliferating elements triggered astrocyte activation (GFAP+, glial fibrillary acidic protein) surrounding the GBM cells. The phagocytic cells, microglia, and macrophages (Iba1+, Ionized calcium-binding adaptor molecule 1), on the other hand, were scarcely represented and the brain architecture was conserved.

A dense multilayer of reactive astrocytes with a morphological gradient of activation is evident at 14D towards the well-defined tumor bulk. At the same time point, we observed the increase of the phagocytic elements in the peritumoral area. The cellular changes may prompt the release of extracellular matrix (ECM) proteins and proteases to favor the GBM invasiveness across the contralateral hemisphere. Metalloproteinase 9, fibulin, and tenascin C expression were affected at this stage.

Accordingly, CD133+ glioma stem cells migrated out of the bulk at 21D, while the necrotic primary lesion was infiltrated by macrophages. Protein expression of the specific microglial marker TMEM-119 (transmembrane protein 119) was downregulated, suggesting diminished activity of tumor-associated-resident microglia and increased contribution of circulating invading macrophages.

The present study emphasizes the role of functional changes in the microenvironment during the GBM progression, fostering the studies on novel multi-targeted, time-dependent therapies in an experimental model similar to the human disease.

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Regional development of high-grade gliomas: the anatomical conundrum of cancer biology

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High-grade glioma (HGG) still represents a challenge for the medical community worldwide. The new WHO classification tried to explain the biological behavior of these tumors, analyzing their metabolic and genetic pathways[1].

We hypothesize that anatomical factors (regionalized glial cells, extracellular composition, committed gene-expression) could account for the biological behavior and invasiveness routes of gliomas.

Astrocytes showed, particularly in mammalian CNS, both inter-regional and intra-regional distinct features both during development and adulthood, structuring astrocytic domains, with local regulation of synaptic plasticity[2].

Neurons, on the other hand, induce astrocyte's maturation via cell-cell contact and the exchange of growth factors[3]. Neurons in brain tissues adjacent to tumors express PD-L1, which induces caspase-dependent apoptosis in GBM cells by activating an unknown receptor.

Moreover, gliomas release in their defense excessive amounts of glutamate, inducing glutamate excitotoxic neuronal cell death in the peritumoral brain parenchyma, which in turn stimulate glial tumor cells by activating glutamate receptors in a paracrine and autocrine manner. Therefore, up-regulation of the GLT-1 protects neurons and glia in peritumoral areas[4].

Tumor patterns of migration could follow ECM organization. A non-radial invasion pattern was shown in murine models using specific cellular markers. In the same study, clinical verification was performed, and most malignant temporal lobe gliomas were located lateral to the collateral sulcus. Despite widespread pathological fluid-attenuated inversion recovery (FLAIR) signal in the temporal lobe, 74% of the "lateral tumors" did not show signs of involvement of the amygdala-hippocampal complex[5].

The lateralization of the GBM also seems important as a malignancy hallmark. A pivotal study characterized human cortical asymmetry[6]. The authors identified and verified 27 differentially expressed genes, which suggests that human cortical asymmetry is accompanied by early, marked transcriptional asymmetries. LMO4 is consistently more highly expressed in the right perisylvian human cerebral cortex than in the left.

The anatomical factors such as neuronal and glial subtypes, lateralized gene-expression, spatiotemporal committed stem cells, and extracellular composition could explain the biological diversity of gliomas and predict their invasiveness.

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Role of contactin-1 axonal glycoprotein and polyphenol in a mice model of friedreich ataxia

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Friedreich ataxia (FA) is an autosomal-recessive genetic disase, mainly characterized by cerebellar ataxia and cardiomyopathy. FA is caused by the expansion of a GAA triplet repeat in the first intron of the Frataxin (FXN) gene, which encode for Frataxin, a mitochondrial protein involved in the iron-sulfur cluster biogenesis in turn responsible for mitochondrial ATP production.

In this study we used the Fxntm1MknTg(FXN–)YG8Pook/J line a FRDA transgenic mouse models which implied the carrying the human Frataxin (Pook) gene in emizygosis and the occurrence of a variable number of supernumerary GAA triplets.

In this study a phenotypes of 6 month-old mutant mice and of their wild type littermates were compared. We submitted to immunohistochemical procedures cerebellar and spinal cord cryostat sections, and immunostained them, with antisera against the neuronal marker β-tubulin and the glial marker, glial fibrillar acid protein (GFAP) as well as with a specific antiserum to Contactin 1 axonal glycoprotein.

Moreover, we analysed in the same observed phenotype the administration effects of polyphenol epigallocatechin gallate (EGCG).

In the mice mutants line the neuronal phenotype was significantly counteracted while a glial upregulation was rather observed and, as well as for Contactin 1 a reduced expression was detected, potentially indicating the involvement of the underlying gene in FA pathogenesis. Finally, EGCG administration counteracted the observed phenotype, indicating protective effects of antioxidant administration on the evolution of the disease.

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Cellule staminali, istogenesi e differenziamento

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Fire effects on bone tissue

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Introduction. Bones are an important resource in physical anthropology. Many occurrences can burn bones, and this can have consequences on tissue. Colour modification (Krap et al. 2019), size of fragments (Thompson 2005), shrinkage (Goncavales et al. 2011) and microscopic structure (Absolonova et al. 2012) can be analyzed to evaluate the damage of the fire.

Materials and methods. Starting from standard temperature-colour classification (Shipman et al 1984; Mays 1998), different heat-induced modifications were analyzed.

Samples come from 12 cremated burials of a Sardinian necropolis of the imperial roman age. Macroscopic changes were measured (Hubelaker 2009) and osteon structure was analyzed with SEM (Holden et al.1995), comparing fragments of different colours and the step-by-step modification from the outer to the inner edge of the bone.

Results. Most calcined districts were in the central part of the body; in fact, large parts of bones were white, or grey-white, except for the skull, generally brown-black. More variability was present in the size and weight of fragments; the same for the pattern of fracture suggesting that they may not be related to the temperature of cremation.

The destruction of bone organization appears to be associated with fire temperature; this was nearly survived in brown fragments and the inner edge, mostly destroyed in white or blue-white fragments and the outer edge of the bone.

Conclusion. Macroscopic and microscopic modifications of bones seem to be related to the temperature of the fire in cremated remains. It would be interesting to investigate the survival of aDNA in this kind of sample.

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Epigenetic features of human perinatal stem cells unveil a novel stemness capacity

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Human perinatal stem cells can be isolated from fetal annexes without ethical or safety limitations. Despite they are generally considered multipotent, their biological characteristics are still not fully understood. The aim of this study was to investigate the pluripotency potential of human perinatal SCs as compared to human induced pluripotent stem cells (hiPSCs). Despite the low expression of the pluripotent factors NANOG, OCT4, SOX2, and C-KIT in perinatal stem cells, we observed minor differences in the promoters DNA-methylation profile of these genes with respect to hiPSCs; we also demonstrated that in perinatal stem cells miR-145-5p had an inverse trend in comparison to these stemness markers, suggesting that NANOG, OCT4, and SOX2 were regulated at the post-transcriptional level. The reduced expression of stemness markers was also associated with shorter telomere lengths and shift of the oxidative metabolism between hiPSCs and fetal annex-derived cells. Our findings indicate the differentiation ability of perinatal stem cells might not be restricted to the mesenchymal lineage due to an epigenetic barrier, but other regulatory mechanisms such as telomere shortening or metabolic changes might impair their differentiation potential and challenge their clinical application.

Key words

DNA methylation, NANOG, OCT4, SOX2, amniotic epithelial cells, amniotic fluid stem cells, fetal membrane mesenchymal stromal cells, miRNAs expression, perinatal stem cells, telomere length.

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Human Mesenchymal Stromal Cells isolated from the amniochorionic membrane disclose an unexpected differentiation potential toward the dopaminergic neuronal lineage

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Parkinson's disease is one of the most targeted neurodegenerative diseases in clinical research, as its incidence is increasing worldwide. It is characterized by the degeneration of the dopaminergic neurons of the substantia nigra pars compacta. The replacement of dead cells with new healthy ones may represent an appealing therapeutic approach to this pathology, but currently, only pluripotent stem cells can generate dopaminergic neurons with high efficiency. However, the use of these cells arises safety and/or ethical issues. Human mesenchymal stromal cells (hFM-MSCs) are perinatal stem cells that can be easily isolated from the amniochorionic membrane after delivery. Generally considered multipotent, their real differentiative potential is not completely elucidated. The aim of this study was to analyze their stemness characteristics and to evaluate whether they may overcome their mesenchymal fate, generating dopaminergic neurons. We demonstrated that hFM-MSCs expressed embryonal genes OCT4, NANOG, SOX2, KLF4, OVOL1, and ESG1, suggesting they have some features of pluripotency. Moreover, hFM-MSCs that underwent a dopaminergic differentiation protocol gradually increased the transcription of dopaminergic markers LMX1b, NURR1, PITX3, and DAT. We finally obtained a homogeneous population of cells resembling the morphology of primary midbrain dopaminergic neurons that expressed the functional dopaminergic markers TH, DAT, and Nurr1. In conclusion, our results suggested that hFM-MSCs retain the expression of pluripotency genes and are able to differentiate not only into mesodermal cells, but also into neuroectodermal dopaminergic neuron-like cells.

Key words

Human mesenchymal stromal cells, perinatal stem cells, hFM-MSCs, pluripotency, neural differentiation, dopaminergic neurons, DAT, TH, PITX3, Parkinson's disease.

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CEA Cell Adhesion Molecule 6 (CEACAM6), Collagen Type I Alpha 2 Chain (COL1A2), Galectin 4 (LGALS4) and Tetraspanin 8 (TSPAN8) mRNAs as blood biomarkers for colorectal cancer

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Colorectal cancer (CRC) develops over several years, thus, early diagnosis and prevention are fundamental to reduce CRC burden. A new non-invasive blood test based on 4 mRNAs encompassing adhesion molecules such as *CEACAM6*, as well as a member of the collagen I superfamily, namely *COL1A2*, combined with *LGALS4* and *TSPAN8*, was recently discovered and referred to as CELTIC panel [1]. The expression of the CELTiC panel was measured by quantitative PCR. The panel of putative biomarkers was subsequently evaluated in 101 subjects resulted positive to fecal immunochemical screening test (FIT) highlighting the potential to distinguish colonoscopy negative-FIT positive patients (NFIT, n=36), patients with low risk lesions (LR, n=36) and with high risk lesions or CRC (HR/CRC, n=92) [2]we searched for mRNA blood markers (CELTiC panel. In this study 174 healthy FIT negative subjects (FITN) were analysed [3] and compared to previous groups, evaluating also the influence of age and sex. Within the FITN group, *CEACAM6* and *COL1A2* display significantly lower expression in female than in male, confirming this sex difference of *CEACAM6*, a differentiation marker in normal colonocytes, also in the older groups (60–70 y.o.) and supporting current data on the importance of gender- and age-specific reference intervals for the early diagnosis of CRC [4,5]. The four genes showed significantly lower expression in FITN than in HR/CRC. Interestingly, *TSPAN8* and *COL1A2* were significantly lower expressed in FITN also than in NFIT and LR patients. *TSPAN8*, an integral membrane protein which upregulation promotes metastasis [6], was confirmed by logistic model as able to discriminate FITN from NFIT, LR and HR/CRC. Interestingly, also *LGALS4*, involved in cell-cell interaction and studied in CRC patients [7,8], was able to differentiate FITN from NFIT (false FIT positive). *TSPAN8* and galectins have been described also in exosomes [9] suggesting the origin of the mRNAs of the CELTiC panel. Finally, the CELTIC panel showed good sensitivity (84-90%), specificity (76-81%) and AUC (0.87-0.89) to discriminate FITN from the other groups. The CELTiC panel was confirmed as a useful tool to diagnose CRC highlighting the importance of sex and age. A multicenter cross-sectional study will enroll 800 FIT positive subject at the Universities of Amsterdam and Bologna to better estimates the performances of the CELTiC panel and to further validate its robustness and usefulness in the early diagnosis of CRC and to exclude false FIT positive subjects.

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Key words

Colorectal cancer, blood biomarkers, CELTiC panel, CEACAM6, LGALS4, TSPAN8, COL1A2.

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Midkine is responsible of drug resistance in melanoma patients after treatment with MEK and ERK inhibitors

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Malignant melanoma is the deadliest type of skin cancer, with an incidence increasing yearly worldwide (1-3). Targeted therapy to key mutated or altered signaling cascades (i.e in the MAPK pathway) are the golden standard in clinical setting as they are highly efficient. Despite a sudden recovery observed in most of the patients, many relapse after a short period with more aggressive and drug resistant tumors. Therefore, understanding the mechanisms underlying targeted therapy resistance is paramount to design more effective therapies (4). To this aim, we have recently identified the growth factor MIDKINE (MDK) as a new driver of melanoma progression. MDK is overexpressed in aggressive melanoma cells and is involved in neo-lymphangiogenesys and metastatic process (5). A pharmacological screen showed that drugs targeting MAPK pathway elements such as BRAF, MEK or ERK induced a strong production and release of MDK suggesting its involvement in drug resistance mechanism. We observed that different melanoma cell lines with different mutations, after treatments with MEK and ERK inhibitors, show an increase in both expression and secretion of MDK. To evaluate the effect of MDK on cell survival and resistance, we selected few cell lines with low MDK expression and secretion, and treated them with both MEK/ERK inhibitors and exogenous MDK. As expected, treatments with MEK/ERK inhibitors lead to cell death in different cell lines, while exogenous MDK was able to completely rescue the cells. These results were validated in an *in vivo* model of melanoma xenograft treated with Trametinib (MEKi). MDK overexpressing cell lines showed a significative increase of tumor volume compared to control. These results strongly point out that MDK has a fundamental role in protecting melanoma cells MEK/ERK induced cytotoxicity, and promoting drug resistance. Moreover, MDK promote tumor proliferation and consequently the increase in tumor volume.

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Key words

Melanoma, targeted therapy, midkine.

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Exosomes derived from human amniotic fluid mesenchymal stem cells modulate microglia and neuron cells exposed to Aβ

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Neuroinflammation is involved in brain aging and neuronal cell death in neurodegenerative diseases such as Alzheimer's disease (AD). The pathological progress of AD can derive from imbalanced homeostasis of amyloid beta (Aβ) in the brain. In such cases, microglia plays important roles in regulating the brain Aβ levels. Human amniotic fluid stem cells (hAFSC) may be a potential therapeutic candidate to treat this pathology given their immunomodulatory properties. Evidence suggests that the mechanism of action of hAFSC is expressed through their secretome, which includes extracellular vesicles such as exosomes (exo). Some studies have been done to evaluate whether hAFSC-exo exert protective effects on neurons, however, their therapeutic role has not been fully elucidated with respect to human microglia.

In this study, we examined the effect of hAFSC-exo on BV2 microglia cells that had been activated by lipopolysaccharide (LPS) as neuroinflammation model. Here we found that exposure to hAFSC-exo prevented the overproduction of NO by inducible nitric oxide synthase (iNOS), a marker of activated microglia. Moreover, the expression of proinflammatory proteins was inhibited, while markers of anti-inflammatory macrophage phenotype were not affected by hAFSC-exo.

Then, conditioned medium (CM) obtained from activated BV2, in presence or absence of exo, was added to SH-SY5Y neuroblastoma cells exposed to Aβ, as in vitro model of AD. Interestingly, hAFSC-exo pretreatment significantly increased SH-SY5Y cell viability by inhibiting the apoptosis normally induced by microglia-CM and Aβ.

Collectively, our results indicate that hAFSC-exo effectively mitigate inflammatory injury caused by activated microglia, and significantly recover the neurotoxicity from Aβ + LPS-induced microglial conditioned media. These findings provided evidence that hAFSC-exo confer neuroprotection against Aβ- induced microglial toxicity associated with the production of pro-inflammatory mediators and may be a potential therapeutic agent for inflammation-related neurological conditions including Alzheimer's disease.

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Regulation of the EGFR endocytic route by an Endoplasmic Reticulum-related Ca^{2+} binding protein

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EGFR is a receptor-type tyrosine kinase exposed on the surface of epithelial cells, mediating responses to EGF family ligands via a complex signaling pathway. The binding of EGF ligand to EGFR induces its activation and internalization, stimulating multiple intracellular signaling cascades including the Ras/MAP kinase, phospholipase Cγ/protein kinase C, phosphatidylinositol 3-kinase (PI3K)/Akt, Jak/STAT, and Src family kinases. Since all these pathways are involved in cell proliferation, differentiation, migration, and survival, EGFR plays an important role at both physiological and pathological levels. Overexpression and/or genetic alteration of the EGFR gene are indeed strictly associated with carcinogenesis[1,2].

The endocytic trafficking of EGFR is regulated by two mechanisms, i.e. clathrin-mediated endocytosis (CME) and non-clathrin-mediated endocytosis pathways (NCE), that are responsible for EGFR recycling or intracellular degradation. Many studies have reported that at low doses of EGF ligand, EGFR is recycled back to the plasma membrane, while at high concentration of EGF is carried to the lysosomes for degradation. In this context, the release of intracellular calcium from the Endoplasmic Reticulum (ER) to the cytosol, upon EGF-EGFR binding, plays a critical role in EGFR recycling and degradation[3].

In our study, we investigated the role of a SERCA-regulating calcium-binding protein in the EGFR recycling in Non-Small Lung Cancer cells.

We evidenced an accumulation of EGFR protein in Golgi apparatus, a downregulation of AKT pathway, and a reduction in cellular migration capability, upon silencing of this SERCA-regulating calcium-binding protein. Moreover, the silencing of this protein cooperates with an EGFR inhibitor in reducing the migratory capability of these cells. Altogether these data highlight the relevance of the ER-related calcium-binding proteins in the regulation of EGFR recycling and EGFR downstream pathways. In conclusion, we suggest a possible novel functional approach to modulate the EGFR signaling pathway.

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HtrA1 modulation in Preeclampsia

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The High temperature requirement A 1 (HtrA1), a member of the HtrA family, is a multi-domain secretory protein with serine-protease activity involved in different cellular processes. HtrA1 is more expressed in human first trimester placenta suggesting that this serine protease is tightly associated with early phases of human placenta development [1] In addition, HtrA1 expression was altered in both placental tissue and maternal plasma of pregnancies complicated by Preeclampsia (PE), a gestational syndrome that affects the 3-5% of the pregnancy worldwide characterized by new onset of maternal hypertension and proteinuria suggesting that HtrA1 could be considered a key molecule in the development of PE [2, 3]. Although PE symptoms appear after 20th week of gestation, the pathology begins to develop before this period. An early identification and treatment of pregnant woman that will develop PE, before 12 weeks of gestation, would make possible to treat these women before the appearance of symptoms preventing damage to the placenta and consequently to the fetus.

In this study we found increased HtrA1 plasma levels in first trimester pregnancy which will later develop PE. Moreover, we found HtrA1 expressed in the cytoplasm of four placental cell lines (HTR8/SVneo, JEG3, BeWo and HUVEC) normally used as *in vitro* models to study human placental physiology. In addition, we found that HtrA1 expression was decreased when HTR8/SVneo cell line was exposed to oxidative stress (by using H_2O_2), a feature of PE. Interestingly, we proved that pre-treatment of HTR8/SVneo with heparin, a drug normally used to treat and prevent PE, was able to restore HtrA1 normal expression.

Our data showed that HtrA1 could be considered a useful early marker of PE onset and pre-treatment of patients at risk to develop PE during first trimester of gestation with heparin may contribute to restore HtrA1 expression improving or avoiding PE onset.

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ATG7 immunohistochemical expression in Asbestiform fibers-Induced Malignant Pleural Mesothelioma. A preliminary report

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Literature evidence has demonstrated a high incidence of asbestos-related malignant pleural mesothelioma (MPM) in a Sicilian town (Biancavilla, Italy), where fluoro-edenite (FE) fibers have been discovered some decades ago (Delgermaa et al., 2011; Filetti et al., 2020a). As ATG7 immunohistochemical analysis has been ascribed as a prognostic tool of improved survival, we decided to investigate, in MPM patients, exposed to FE fibers, the immunohistochemical expression of this autophagy-related protein named ATG7 (Fimia et al., 2010; Dyczynski et al., 2018). We analyzed the correlation between ATG7 immunohistochemical level and clinicopathological parameters. Ten MPM tissue samples, from patients with available clinical and follow-up data, were included in paraffin and processed for immunohistochemistry. The immunohistochemical results confirmed activation of the autophagic process in MPM. Densitometric and morphometric expressions of ATG7 were significantly increased in MPMs when compared to the control tissues. An association of a high level of ATG7 with increased survival was demonstrated, with a mean overall survival (OS) of 23 months for patients with high expression *vs*. a mean OS of 13 months for patients with low ATG7 expression. In addition, a significant correlation between ATG7 expression and the age of MPM patients was observed. This study represents a starting point to hypothesize the prognostic role of ATG7 which could be a reliable prognostic indicator in MPM.

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Key words

ATG7, malignant pleural mesothelioma, immunohistochemistry.

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CAM assay: a versatile low-cost experimental method offering a 3D environment to test new polymeric biomaterials

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Pathologies, debilitating conditions, organ and tissue decay are the most critical factors within an aging population. Regenerative medicine and tissue engineering are trying to address such impelling demands by developing new materials, devices and therapeutic treatments. Before moving on to clinical application, every new proposed strategy must get through a series of safety and efficacy tests including, firstly, *in vitro* assays and, secondly, experiments on animal models. The majority of these potential and promising treatments raises ethical concerns, especially involving *in vivo* experimentation. For this reason, animal testing requires alternative models to speed up and facilitate the screening of a large number of samples with relative low cost.

The Chorio-Allantoic Membrane (CAM) assay is a simple, rapid and low-cost model, which has already been used in the past to evaluate the angiogenic potential of tissue grafts, including metastasis, the activity of pro- and anti-angiogenic molecules and drugs, but also for biocompatibility tests, toxicological analysis and organotypic cultures.

This is due to the fact that this *in ovo* model has numerous advantages. First of all, the CAM assay does not require the approval of ethical committee for animal experimentation since the chick embryo is not considered as living being until the 17th day of development in most countries. Secondly, it provides rapid outcomes while maintaining the complexity of an *in vivo* system. Moreover, since CAM is naturally immunodeficient, it may receive transplantations from different tissues and species without adverse immune responses.

In the present work, we have repurposed, renewed and standardized the use of this powerful method as a platform for testing the biocompatibility, the toxicity and the angiogenic potential of new polymeric formulations. In particular, we tested Ethylene-Vinyl Acetate (EVA) and poly-3-hydroxybutyrate-CO-3-hydroxyhexanoate (PHBH) added with different percentages of Cellulose Nano Crystals (CNC), in particular 5%, 10%, 15% and 20%. The CAM assay allowed us to select the most suitable biomaterial, obtained by 3D printing technology, for future applications in the field of bone regeneration. The quantification of angiogenesis and the morphological aspect of the graft, evaluated with the histological analysis, suggested PHBH with 10% and 15% of CNC and EVA as the most promising materials for 3D printing techniques. This study proposed by our research group is only an example among multiple possible applications of the CAM model.

Key words

CAM assay, polymeric biomaterials, biocompatibility, bone regeneration.

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Preliminary structure-activity relationships and *in vitro* cytotoxicity profile of the marine bisindole alkaloid 2,2-bis(6-bromo-1*H*-indol-3-yl)ethanamine

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3,3'-diindolylmethane scaffold (DIM) is known for its apoptotic activity, but its use is limited due to the lipophilic nature and the gastric chemical instability. However, it seems that the presence of the alkylamino side chain improves its chemical and therapeutic activities. Therefore, the compounds which contain both DIM and alkylamino side chain could be considered pharmacologically active and applicable in the field of pharmaceutical chemistry. Among these molecules, a marine sponge alkaloid 2,2-bis(6-bromo-1H-indol-3-yl)ethanamine, has shown a variety of biological activities such as anti-inflammatory, antibacterial and anticancer properties (Salucci et al., 2018). Therefore, modifying its structure had proven to be useful in the search of new therapeutic agents. For that in this study the potential antiapoptotic activity of three alkaloid derivatives has been investigated in a tumor cell line by a morpho-functional analysis and the results compared with those obtained treating hemopoietic cells with the original compound. Data showed that the methylation of indole NH (compound 1) does not alter the alkaloid apoptotic activity which decreases when bromide units (compound 2) were deleted or substituted with the fluorine atoms (compound 3). Human tumor cells exposed to compound 1 showed the same ultrastructural apoptotic features (Salucci et al., 2020) observed treating the cell line with the original bisindole alkaloid, and as this latter, the derivative 1 led to peroxidation events suggesting the mitochondrial pathway involvement in inducing cell death.

In conclusion, this study highlights the potential role of a marine sponge alkaloid derivative as anti-apoptotic agent and demonstrates that the presence of the bisindole scaffold with the bromide atoms appears an essential condition for maintaining the anticancer activity of this bisindole series.

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Key words
Bisindole alkaloid derivatives, tumor cell line, apoptotic cell death.

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Eco-friendly biodegradable materials as new promising 3D-printed scaffold for eco-sustainable regenerative medicine

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In the last years, additive manufacturing technologies have led regenerative medicine to a higher level: the possibility of printing patient-customized scaffolds together with human cells and tunable biomaterials has been opening brand new frontiers in tailor-made medicine. To date, many materials designed in different geometries have been tested, but these approaches have not taken place on a large scale yet, due to the high manufacturing costs and the difficulty to obtain regulatory approval. Thermoplastics, such as poly(lactic acid) (PLA) and polycaprolactone (PLC), have been studied more extensively and are currently in use in the biomedical field, structured in 3D geometries with a Fused Filament Fabrication (FFF) technique. The FFF technology is an extrusion-based method that has the great advantage of not making use of any toxic solvent for printing 3D structures. Despite this, the impelling environmental issues are moving all the industrial sectors towards a sustainable development, avoiding the use of non-renewable resources such as petroleum-based plastics. For this reason, the very first aim of this study was to find eco-friendly materials that may become a valid alternative for traditional thermoplastics. We focused on a polyhydroxyalkanoate copolymer, i.e., poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBH)[1], a biodegradable aliphatic polyester which is synthesized by microorganisms under conditions of oxygen and nitrogen imbalance. Since PHBH is an eco-friendly material with good biocompatibility and biodegradability, but poor thermomechanical proprieties, it has been reinforced with Cellulose Nano Crystals (CNCs). CNCs can significantly increase not only the degree of disintegration of PHBH, under simulated composting condition, but also its thermal stability, allowing the 3D printing of complex architectures by means of FFF technique. By exploiting the outstanding proprieties of PHBH-nanocellulose composites[1], our first objective was to 3D-print a suitable geometry for cell culture, testing scaffold colonization and cytotoxicity both *in vitro* and *in ovo* (CAM assay). The second aim was to find a suitable formulation for future applications in bone tissue engineering, enabling scaffold colonization and vascularization that are mandatory for bone regeneration. Finally, we encourage the use of eco-friendly biodegradable bioplastics to reduce the environmental impact.

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Key words

3D scaffolds, Fused Filament Fabrication (FFF), regenerative medicine, biodegradable plastics, PHBH-Cellulose Nano Crystals composites.

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Reproducing the bone marrow niche on scleral ossicles: a peculiar 3D scaffold for studying leukemic lymphoblasts/bone marrow stroma crosstalk *in vitro*

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It is widely acknowledged that stromal cells play a fundamental role preserving the physiological homeostasis of the bone marrow niche (BMN) and supporting haematopoiesis. However, in the niche also the leukaemia initiating cells (LICs) can benefit from the paracrine signalling with the stroma, which promotes aberrant proliferation of immature blood elements, ultimately leading to repression of normal haematopoiesis and onset of leukaemia. To date, a large body of data showed that the interactions between the stroma and the LICs overrule the success of drug treatment[1]. An *in vitro* study model able to mimic the BMN is therefore mandatory to get conclusive knowledge of the leukaemia-stroma crosstalk and establish effective therapies[2]. Hitherto, however, because of the complexity of the 3D microenvironment of the BMN, most studies have been carried out with user-friendly and economical bidimensional models, which lack cell-to-matrix interactions, typical of the 3D organization of tissues and organs, and consequently often failed to translate into a clinical setting. In a previous work, we demonstrated that scleral ossicles (SOs) are small, biocompatible and spontaneously decellularized bony plates extracted from the eye-bulge of adult chickens[3]. Since it has been shown that an optimized decellularization protocol can preserve with striking detail the 3D microenvironment of natural scaffolds[4], here we propose SOs, recently patented by our Labs for bone regeneration, as a peculiar platform also for recreating the bone marrow niche. To exploit SOs properties, human stromal cells expressing GFP (GFP-HS-5) were seeded either on the SOs or on plastic and allowed to grow up to 2 weeks. Next, the suitability of SOs to support cell adhesion and growth was assessed by both fluorescence microscopy and SEM analysis, while cell proliferation and viability were monitored by trypan blue dye exclusion cell counting. The proliferation rate of GFP-HS-5 cells on SOs was significantly higher than that of their counterpart seeded on plastic. Thus, we aim to take advantage of this 3D model for co-culturing GFP-HS-5 with human primary blasts from acute leukaemia to investigate the leukaemia-stroma crosstalk *in vitro*. In summary, our work indicates that the above *in vitro* SOs-stroma 3D model might represent a powerful tool to improve the efforts in translating into a clinical setting many findings obtained from *in vitro* bidimensional models and may contribute to develop more effective therapies for hematologic malignancies.

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Key words

Bone marrow niche, Scleral Ossicles (SOs), biomaterials, co-culture on 3D scaffolds, leukaemia-stroma crosstalk.

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Enhancing osteogenic differentiation by means of Scleral Ossicles and Pulsed Electromagnetic Fields (PEMFs): a low-cost and effective combination to promote bone regeneration

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Bone lesions, secondary to different pathologic conditions, are not able to heal spontaneously in about 10% of cases. Thus, when large bone losses occur (*i.e.*, the so called critical-sized bone lesions), it is necessary to resort to alternative strategies. The current standard treatments, commonly used in orthopedic surgery, including allograft and autograft, present some limitations such as the limited amount of tissue harvestable, rejection problems, and pathogenic transmission. An approach that allows overcoming such problems is the Bone Tissue Engineering that uses scaffolds together with cells and chemical/physical stimuli, with the aim to reproduce the interactions between cells and extracellular matrix during the osteogenesis processes. It is known that not all scaffolds reported in literature have physical-chemical characteristics suitable for use in regenerative medicine. The improvement of cell adhesion, proliferation and differentiation is considered the key point for the best success in the use of scaffolds. One of the most interesting alternatives could be to combine the presence of biocompatible scaffolds with the physical stimulation, such as Pulsed Electromagnetic Fields (PEMFs) application.

As an innovative proposal, the scleral ossicles (SOs), recently patented by our Labs, have been suggested as natural, biocompatible, and spontaneously decellularized scaffolds for bone repair. SOs are peculiar bony plates forming a ring at the scleral-corneal border of the eyeball of lower vertebrates[1]. Concerning the physical stimulations, PEMF application plays an important role in bone cell differentiating processes. In fact, with appropriate intensity and frequency, they can activate different cellular pathways, induce pre-osteoblast proliferation, promote osteogenesis and decrease bone resorption; for all these reasons, they are largely used for bone fractures treatment. Among their advantages, it is to be mentioned that they represent a simple and not-invasive therapy, also remembering that the PEMFs used for the treatment of bone lesions have low energy and frequency and, for this reason, to date no negative effects have been reported on human health. In this research work, human osteoblasts were differentiated in presence of SOs (used as biochemical stimulus)[2] and/or stimulated with PEMFs, in order to assay if the combination of the two stimuli could enhance the differentiation process compared to the single contribute.

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Beneficial effects of horsetail (*Equisetum arvense*) in *in vitro* models of sarcopenia and osteoporosis

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Sarcopenia, defined as loss of skeletal muscle mass (muscle atrophy) and strength with aging, is strictly connected to the decrease of bone mass (osteoporosis), predisposing to frailty, bone fractures and loss of personal independence in over-60s [1,2]. Due to the growing life expectancy, sarcopenia and osteoporosis represent primary social and financial problem for western countries [2,3]. The imbalance between myofibrillary protein breakdown (especially, type II myosin heavy chain, MyHC-II) and synthesis, and the excessive presence of osteoclasts, responsible of bone resorption [1-3], are recognized causes of the loss of muscle and bone tissues. Low-grade chronic inflammation and excessive treatment with glucocorticoids (GCs) are common processes underlying the pathogenesis of sarcopenia and osteoporosis [2,3].

Horsetail (*Equisetum arvense*) is traditionally recommended for the maintenance of bone homeostasis [4]. We tested a standardized dry extract of horsetail on well-characterized *in vitro* experimental models mimicking i) muscle atrophy, i.e., C2C12 myotubes treated with proinflammatory cytokines (TNFα/IFNγ) or excess GCs (dexamethasone, Dex) [1]; or ii) osteoclastogenesis, i.e., RAW 264.7 cells treated with RANKL [5].

We found that horsetail extract, not toxic for myotubes up to 600 μg/mL, protects myotubes against MyHC-II degradation under atrophying stimuli, as indicated by the analysis of myotube diameters and MyHC-II expression. Horsetail reduces the activity of the catabolic pathways, p38 MAPK and STAT3 in the presence of TNFα/IFNγ. Moreover, it blunts the activation of FoxO3a-dependent ubiquitin-proteasome system and the reduction of anabolic Akt-mTOR pathway in the presence of Dex. Interestingly, horsetail rescues MyHC-II expression and Akt activity in myotubes derived from muscle biopsies of sarcopenic subjects.

When tested on RAW 264.7 cells, horsetail shows marked inhibition of RANKL-dependent osteoclast formation, as demonstrated by dose-dependent reduction of TRAP-positive cells and TRAP enzymatic activity, and by down-regulation of osteoclastogenic markers.

Based on our results, horsetail is a promising source of active compounds to sustain muscle functionality and balanced bone remodelling in aged people and in diffuse atrophying conditions, improving the quality of life and reducing health-care costs.

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Human resident cardiac progenitor cells remodel dermal matrix scaffold developed for cardiac tissue regeneration

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Cardiac Tissue Engineering (CTE) brought the extracellular matrix (ECM) into the spotlight. Notoriously, the ECM is the naturally occurring tissue scaffold that conveys to cells biochemical and mechanical signals that control parenchymal and supporting cell behavior. Therefore, the decellularized ECM (d-ECM) is an attractive biomaterial for CTE applications. We have recently described the decellularized human skin (d-HuSk) as a suitable scaffold material for CTE. However, despite the similarity reported between dermal and cardiac matrix, any scaffold should be intended as a temporary implant that should comply with resident cell requirements in terms of composition and signaling. Therefore, the scope of the present study is to evaluate whether d-HuSk can be remodeled and converted from a cardiac-like to a cardiac matrix by resident human cardiac progenitor cells (hCPCs) and differentiating cells in vitro.

To this aim, we decellularized human abdominal skin to prepare three-dimensional scaffolds of d-HuSk that were then recellularized with hCPCs isolated from explanted human hearts and cultured for two weeks. Afterwards, hCPCs seeded on d-HuSk scaffolds were induced to myogenic differentiation and cultured for two additional weeks. d-HuSk scaffolds not recellularized were used as control to evaluate by immunohistochemistry, SEM analysis and quantitative assays the effects of hCPCs and differentiating cells on the composition and organization of d-HuSk.

The histological analysis by immunofluorescence revealed that repopulating d-HuSk with hCPCs and differentiating cardiac cells induces a reorganization of the dermal matrix involving mostly fibronectin, tenascin and laminin. Moreover, the amount of ECM components, like collagen, elastin and GAGs, resulted affected by seeded cells as well, as shown by quantitative dye binding assays. From SEM observation ultrastructural changes are evident both at matrix and cellular level suggesting a possible active remodeling of extracellular matrix caused by the cell attachment.

The collected data support the hypothesis that d-HuSk could represent the ideal biomaterial for cardiac tissue engineering applications. Particularly, the capability of cells to attach and establish a dynamic equilibrium with it and its autologous origin validate the possibility to use d-Husk as a platform to boost cardiac regeneration by resident hCPCs.

Key words

Decellularized extracellular matrix, tissue engineering, cardiac repair, regenerative medicine, stem cells.

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A new device enriched with functionalized nanofibers to regenerate the oral mucosa: an in vitro study

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AIM: The loss of the interdental papilla (IDPL) can be caused by various factors such as periodontal disease and side-effects of orthodontic treatments and can lead to a hard and soft tissues defect whose restoration is particularly challenging and is still an unsolved issue. The aim of the study was to test polycaprolactone (PCL) nanofibers enriched with hyaluronic acid (HA) and antioxidant vitamin E on human mesenchymal stromal cells isolated from gingival interdental papilla (GinPa-MSCs), as such a construct might be useful in the development of devices able to enhance gingival regeneration.

METHODS: Nanofibers were produced from a solution of 24% PCL (NF) or PCL enriched with 1,5 % of Hyaluronic Acid (HA) and 1 ml EREVIT 300 (Vit E) (NFE) by electrospinning technology (Nanospider, Elmarco, Czech Republic) in order to obtain fibers similar to those characterizing the extracellular matrix (ECM). NF and NFE were mechanically, chemically and morphologically characterized. They were further cultured to evaluate cell adhesion and viability. The cells were obtained from a biopsy of interdental papilla (GINPA), phenotypically characterized, and tested with NF and NFE to evaluate the metabolism of the cells by means of Alamar Blue assay at 24,48 and 72 hours.

RESULTS: FTIR analysis demonstrated the presence of Vit E and HA embedded in NFE. NF and NFE showed different mechanical characteristics, with a higher modulus of elasticity for NF compared to NFE (39.262±7.127 vs. 34.406±3.850) and, on the contrary, a tensile strength higher for NFE compared to NF (4.86±0,202 vs 5.226±0,335). At SEM analysis the diameter distribution of the fibers ranged between 0.10 and 2.98 μm for NFE, higher values compared to NF (0.05 to 2.16 μm), suggesting the presence of encapsulated substances in the nanofibers. At 24 hours GinPa-MSCs adhered on NF and NFE and showed a preserved fibroblast-like morphology with extending cytoplasmatic protrusions on the fibers. After a slight reduction of viability observed after 24 hours from seeding, at 72 hours GinPa-MSCs proliferated more than plastic in both groups.

CONCLUSION: These preliminary in vitro findings suggest that both NF and NFE might be tested in scaffold devices aiming at creating an ECM-like network that could be useful for gingival regeneration.

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Key words

Interdental papilla, mesenchimal stem cells, nanofibers, tissue regeneration.

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In vitro assessment of alendronate effects on periodontal ligament stem cells

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Alendronate (ALN) is a second-generation bisphosphonate widely used for cancer indications and osteoporosis [1]. Many studies confirmed that ALN was a contributing factor to the developing of osteonecrosis of the jaw (ONJ), however the exact pathogenesis has not yet been clarified [2]. Reduced bone turnover and inflammation observed in ONJ have been associated to the action of ALN on the different cell types harbored in oral microenvironment, such as osteoclasts, endothelial cells and periodontal ligament stem cells (PDLSCs). PDLSCs play a dual role as both osteoprogenitor cells and immune-modulatory cells in periodontal tissue homeostasis. For this reason, we investigated the effects of ALN at different concentrations (2 μM, 5 μM, 10 μM, 25 μM, 50 μM) on the phenotypic and functional properties of PDLSCs.

PDLSCs showed a decrease in cell viability (MTT assay) only when treated with a concentration of 25 μM or larger of ALN for 48h and 72h. Cell cycle analysis revealed a moderate increase in number of cells in the S phase after exposure to ALN at low concentration (2-25 μM), an effect that was reverted after exposure to 50-100 μM ALN. Accordingly, apoptotic cells were evidenced via Annexin V/PI assay at very high concentration of ALN (50 μM) after 72h of treatment. Next, we explored whether the apparent opposite effects exerted by ALN at low (2-25 μM) and high (50-100 μM) concentrations on PDLSCs growth and survival can be mediated by its ability to modulate oxidative stress. To this, we quantified the intracellular ROS amount and lipid peroxidation by using DCF probe and Bodipy staining, respectively. Flow cytometry analysis showed that ALN induced a dose-dependent reduction of intracellular oxidative stress and lipid peroxidation upon treatment with low concentrations at both 48h and 72h. Notably, once exceed the 50 μM concentration, this effect was broken-down. ALN action on immune-regulatory properties of PDLSCs appeared dose- and time-dependent, resulting in altered production of specific interleukins (IL-1a, IL-1b, IL-6, IL-8), MCP-1 and VEGF.

Our preliminary results confirm that ALN can interfere with the normal behavior of PDLSCs. The evidence that drug exposure accounted for impaired cell survival only at very high concentration of ALN, while ALN-mediated changes in cytokines production by cells occurred at low concentration suggest that PDLSCs could contribute to ONJ development through changes in the production of inflammatory cytokines.

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Key words
Periodontal ligament stem cells, alendronate, oxidative stress.

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Cellular senescence in vascular mesenchymal stromal cells and their potential contribution in the development of abdominal aorta aneurysm

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Abdominal aortic aneurysm (AAA) is an age-related disease characterized by chronic inflammation, increased local activation of matrix-degrading proteinases, and weakening of the vascular wall. The main complication is the rupture of the abdominal aortic wall which leads to death. Nowadays, no pharmacological therapies are available and the understanding of the molecular mechanisms that lead to AAA onset and its development are poorly defined.

Reparative abilities of vascular mesenchymal stromal cells (MSCs) have a key role in vascular remodeling. However, unbalanced activity of vascular MSCs support AAA pathogenesis.

Cellular senescence is considered one of the main hallmarks of aging. It is classified as an irreversible growth arrest of the cell cycle which has beneficial roles in physiological conditions. However, the accumulation of senescent cells during aging has adverse consequences and it is believed to have a key role in the onset and development of several age-related diseases.

The aim of this study was to demonstrate the presence of cellular senescence in vascular MSCs isolated from AAA segments (AAA – MSCs), responsible of an impaired vascular remodeling process. AAA-MSCs were assayed for their proliferative ability, cellular senescence markers, autophagy and in vitro vascular differentiation. The results were compared to MSCs isolated from healthy segment of the abdominal aorta of the same patients (h – MSCs).

The results from AAA-MSCs clearly demonstrated a reduced proliferation ability, an increase of ROS levels, the positive expression of the senescent markers $p21^{CIP1}$ and $p16^{INK4a}$, a dysregulated autophagy and a strongly impaired ability in differentiating toward an endothelial phenotype.

All these results indicate the presence of senescent vascular MSCs in the wall of AAA and strongly support the hypothesis that an accumulation of senescent vascular MSCs could have a pivotal role in the onset and development of AAA.

Keywords

Vascular MSCs, cellular senescence, vascular differentiation, abdominal aorta aneurysm.

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Impaired TFEB-dependent autophagy leads to higher anti-apoptotic c-Flip protein levels in cancer cells

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The role of autophagy in cancer cells is largely debated. Experimental data suggest an impairment of autophagy during the initial steps of cancer development while an increase of autophagy in established tumors. In the present work we analyzed autophagy involvement in a human cholangiocarcinoma cell line (HuCCT1) *vs* a normal cholangiocyte cell line (H69). By comparing these experimental models we highlighted a strong difference in the expression level and subcellular localization of Transcription Factor EB (TFEB), previously shown as a master protein in driving autophagy (E Zhao and MJ Czaja, 2012) as well as in inducing liver cell commitment toward cholangiocyte lineage (Pastore N et al. 2020). More in detail we found higher TFEB nuclear localization in healthy H69 than in HuCCT1, correlating with the higher level of autophagy flux observed in H69 cells, evaluated through LC3II and p62 analyses. We therefore hypothesized that TFEB trafficking in the nucleus might be pivotal to control cholangiocyte cell differentiation status and autophagy, thus representing a possible player in counteracting cholangiocarcinoma growth. We also analyzed GSK-3beta and c-Flip proteins expression since GSK-3beta inhibition has been previously shown to promote degradation of the anti-apoptotic protein c-Flip (Na Zhang et al., 2018) *via* autophagy. Remarkably we found higher levels of c-Flip and higher activity of GSK-3beta in HuCCT1 as compared to H69 cells. We thus speculate that a fine autophagy control via TFEB may be involved in apoptosis modulation through c-Flip degradation in cancer cells.

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Keywords

C-Flip, apoptosis, autophagic flux, differentiation.

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Cancer-nerve crosstalk in human cholangiocarcinoma

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Advancement in cancer research has shed light on the importance of the tumor microenvironment, which is made of extracellular matrix (ECM) and various cell types that interact with cancer cells autocrinally and paracrinally. Nerves take part in the tumor microenvironment and increasing evidence show that they have a key role in tumor growth, invasion, metastasis and survival [1]. In particular, Schwann cells (SC) are now being investigated as main players of the nerve boost for the tumor progression, since their well-known regenerative potential can be exploited by cancer cells to thrive [2]. While some data about these phenomena are available for pancreatic, lung, and some head and neck cancers, data about cholangiocarcinoma are lacking. The aims of the project are to investigate whether SC are involved in cholangiocarcinoma (CCA) progression and to describe the putative underlying cellular and molecular mechanisms. To do so, we set up a 3D SC migration assay where we placed nerve explants from a mouse pup into an ECM scaffold, and seeded some CCA cells next to it. We observed the system by phase contrast for 7 days, then fixed it and stained it by immunofluorescence for activated SC marker GFAP. Our preliminary data show migration of activated SC from the nerve towards CCA cells (carcinotropism) starting at day 4-5. We also performed neuritogenesis assays using PC12, a well characterized cell line widely utilized in literature to study neuritogenesis. PC12 differentiate in a neurite expressing phenotype if placed in culture with conditioned media containing neuritogenic factors (i.e. neurotrophins). We tested the conditioned media from HuCCT-1 (human CCA) and found some neuritogenic activity in the media compared to the positive control (NGF). We then performed Western Blot analysis on untreated HuCCT-1 cells investigating autophagy (TFEB, c-FLIP, LC3B, p62), cell death (cleaved Caspase-3) and proliferation (PCNA) markers to compare them to those of HuCCT-1 cells treated with conditioned media from unactivated and activated human primary SC in order to study the modulation of cancer cell behavior by activated SC. Our data show that CCA cells do have neuritogenic activity suggesting they can lure new nerve fibers towards them to establish a contact; also, activated SC from nerve explants leave the nerve and reach CCA cells in our 3D ECM system, indicating a "priming" of SC by HuCCT-1 cells which may benefit from factors released by activated GFAP+ SC.

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Keywords

Cancer biology, Schwann cells, carcinotropism.

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Biological effects of Hyaluronic Acid Nanoparticles: how the molecular weight influences the cellular uptake of human mesenchymal stem cells

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Nanomedicine is the medical application of nanotechnology, which makes use of nanoparticles (NPs) for the selective delivery of drugs [1]. To fully exploit the properties of NPs, it is essential to study the specific biological mechanisms by which they are incorporated into cells and try to avoid intracellular routes able to discard the formulation [2]. Since their discovery, mesenchymal stem cells (MSCs) have gained significant attention in biomedical field [3,4]. Among their extraordinary properties, MSCs overexpress the CD44 marker, a receptor known also to be upregulated in some cancer cells and involved in inflammatory diseases [5]. Therefore, MSCs are flawless model cells to accurately reproduce pathological conditions. Furthermore, CD44 is the main receptor of Hyaluronic Acid (HA), a natural polymer widely used in nanomedicine [6].

The aim of this work is to investigate the biological effects of three distinct nanosized formulations with similar physical features, each composed of a precise HA Molecular weight (Mw), in human bone marrow MSC CD44+.

Selected HA Mw (280, 540, 820 kDa) were used to obtain three nano-formulations by microfluidic technique [7]. Cytocompatibility and cell proliferation were assessed by MTT assay and Bromodeoxyuridine assay. Cellular uptake experiments were performed using pharmacological and physical inhibitors of the major endocytic pathways: Chlorpromazine and Genistein to inhibit clathrin and caveolin mediated endocytosis; CytochalasinA and Nocodazole to suppress the polymerization of actin and cytoskeletal microtubules, respectively. ATP-dependent mechanisms were blocked pre-incubating cells at 4°C; CD44 receptor was blocked with anti-CD44 antibody.

Despite the different HA Mw, microfluidic technology allowed to obtain HA NPs with the same characteristics; all NPs tested were biocompatible and did not affect cell proliferation activity. Cellular uptake results showed that all formulations were selective for CD44 receptor and the three different HA Mw swayed the NPs endocytosis: NPs made of HA 280 kDa were exclusively internalized by clathrin-mediated endocytosis, while NPs composed of 820 kDa exploited the caveola-mediated endocytosis, macropinocytosis and other clathrin-independent pathways. NPs made of HA 540 kDa were able to across the cell membrane using all the mechanisms aforementioned.

In conclusion, HA Mw clearly influences NPs uptake in human MSCs thereby regulating the subcellular drug delivery and fate.

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Key words

Mesenchymal stem cells, endocytosis, CD44 receptor, hyaluronic acid, nanoparticles.

Anatomia clinica e forense
e anatomia per immagini

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Shape and size of foramen ovale: differences according to sex and side

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Shape and size of foramen ovale (FO) is crucial for several surgical procedures. For its position and the specific structures passing in, FO is the target of a surgical transforaminal route which is routinely applied to the treatment of trigeminal neuralgia, (through ablation, radiofrequency rhizotomy, glycerol injection or balloon compression), as well as for the insertion of opiates into the trigeminal cistern for pain treatment in case of cancer[1]. In literature very few data are available on sexual dimorphism of morphological characteristics of FO, and none of them has taken into consideration the possible influence of cranial size.

One hundred CT-scans of adult patients, equally divided between males and females, were retrospectively assessed. Shape of FO was classified in circular, oval, almond-shaped, irregular. In addition, length (antero-posterior diameter) and width (diameter perpendicular to the length) were manually measured. The surface area was measured on 3D model of FO segmented through ITK-SNAP open source software. In addition, distance between anterior and posterior nasal spine, upper facial height and bizygomatic breadth were measured.

Statistically significant differences in prevalence of different shapes according to side and sex were assessed through Chi-squared test ($p<0.05$). Statistically significant differences in length, width and surface area of FO according to side and sex were assessed through two-way ANCOVA test, using cranial measurements as covariates ($p<0.05$).

Results showed that almond type was prevalent without statistically significant differences according to sex ($p>0.05$). Length, width and surface area were significantly higher in males than in females; moreover, length was higher on the right than on the left side ($p<0.05$).

The study provided novel data for the analysis of sexual dimorphism of FO, useful for the management of transforaminal route in surgical context.

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Key words

Anatomy, foramen ovale, sexual dimorphism, trigeminal neuralgia.

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Facial dysmorphism in Prader-Willi syndrome: a stereophotogrammetric analysis

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Prader-Willi syndrome (PWS) is a rare neurodevelopmental imprinting disorder caused by the lack of expression of a paternally derived region of chromosome 15. In addition to hypotonia, cognitive impairment, hypothalamic dysfunction with pituitary hormone deficiencies and hyperphagia, patients may show a distinctive facial appearance. Despite it has been known for a long time, it almost always refers to a qualitative evaluation of the face and minor anomalies could have escaped from a mere visual inspection. The study aimed to better characterize the facial phenotype of patients with PWS through a noninvasive quantitative approach [1].

Facial images of 15 Italian adult subjects with genetically confirmed diagnosis of PWS (6 males, mean±SD age 41±5 years; 9 females, 37±8 years), without previous history of craniofacial traumas or surgery, were acquired through stereophotogrammetry. From the 3D coordinates of 50 facial landmarks, 42 linear distances, 30 angles and 9 ratios were calculated and expressed as z-score values by referring to 403 healthy subjects matched for age and sex.

Concerning the face as a whole, patients with PWS showed decreased middle and lower facial depths (mean z-score±SD -1.0±0.9 and -1.2±1.0, respectively). With reference to parts of the face, patients showed decreased mandibular ramus length (-1.0±1.0) with smaller gonial angles (-1.9±1.2), short palpebral fissures (-1.2±0.8), lesser inclination of the ears (-1.2±1.1), and decreased height of both upper and lower vermilion (-1.2±1.0 and -1.1±1.3, respectively).

The current study provided a quantitative description of facial phenotype of PWS, detailing information about known distinctive features and pointing out facial abnormalities that had never been reported, such as a thin lower vermilion. Albeit slight, it could represent a phenotypic trait of PWS; nevertheless it needs to be confirmed on more patients. Facial morphometric analysis proved once again to be useful in detecting even subtle anomalies not always appreciable on clinical inspection.

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Key words

Facial features, Prader-Willi syndrome, stereophotogrammetry.

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What is fredet's fascia? A systematic review of definitions and use of the term fredet's fascia in the era of complete mesocolic excision

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Background: Fredet's fascia represents a crucial mesocolic plane, especially in laparoscopic complete mesocolic excision (CME) for right-sided colon adenocarcinoma. Fredet's fascia allows, in fact, the access to the gastrocolic trunk of Henle (GCTH), the most critical step of both open and minimally invasive right-sided CME techniques. Despite this, a recent workshop of expert surgeons on the standardization of laparoscopic right hemicolectomy with CME did not recognize or include the term of *Fredet's fascia or area*.

Methods: A systematic review of MEDLINE (PubMed), WOS, SCOPUS, Google Scholar databases, and grey literature was performed to identify records that used the term of "Fredet's fascia or area", or synonyms thereof, with special emphasis on the mentioned Fredet's fascia terminology and definition, article type, study setting and surgical relevance of Fredet's fascia.

Results: The inclusion criteria were met by 26 study, of which 15 (57.7%) were from Italy. The terminology and definition of the Fredet's fascia varied (widely) in the literature. Moreover, the eponym "*Fredet*" was poorly used in world medical literature and not at all in the French and British ones.

Conclusions: Despite the knowledge of Fredet's fascia surgical anatomy is essential for colorectal surgeons the fusion Fredet's fascia still remains neglected embryological structure also due to a lack of standardization of its terminology. To clarify this fascia for future use and avoid confusion, we propose moving beyond the use of the eponymous term by using a "descriptive term" instead, based on the fascia's anatomic structure in accordance with its embryonic development such as "*inframesocolic pre-duodenopancreatic fascia*".

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Key words

Mesocolic anatomy, Fredet's fascia, fusion fasciae, right hemicolectomy, CME, laparoscopy, anatomical termin.

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Coexistence of linguo-facial and occipito-auricular trunks: uncommon branching pattern of the external carotid artery

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Knowledge of the anatomical variations of the external carotid artery (ECA) is important in head and neck surgery, and for radiologists in the image interpretation of these regions (1).

About that, in this case report, we described an uncommon dual variation in the branching pattern of the left ECA observed during the dissection of head and neck in a 65-year-old Caucasian female cadaver at the Anatomical Training Center of the University of Brescia. We found two common trunks: the first between the lingual and the facial artery and the second between the occipital and the posterior auricular artery. Linguo-facial common trunk (2.14 mm diameter) originated from the anterior part of the ECA, ran for 13,92 mm and then gave the lingual (1.42 mm diameter) and facial (1.89 mm diameter) branches; accessory facial artery (1,21 mm diameter) was also observed originating from the facial artery below the inferior mandibular edge. Additionally, an occipito-auricular common trunk (1.57 mm) originated from the posterior part of the ECA was noted; it ran for 15.38 mm before splitting into the occipital artery (1.48 mm diameter) and the posterior auricular artery (0.81 mm diameter). These anomalies were unilateral and normal pattern was observed on the right side.

This work gives an additional contribution to the whole detailed anatomical description of the different ECA branching patterns, clinically important for several interventional procedures.

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Key words

External carotid artery, linguo-facial trunk, occipito-auricular trunk, anatomical variations.

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Anatomical and pathological variants of neck structures in a forensic case: comparison between anatomage and autopsy and radiological data

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We present the case of a 60-years-old man, with a long-lasting tracheostomy, who died due to a haemorrhage from the brachiocephalic trunk caused by a tissue wear following a "cannula-tracheal conflict". In this case, the man had been hospitalized in the Intensive Care Unit at the end of March 2021, due to the onset of acute respiratory failure in bilateral interstitial pneumonia from SARS-CoV-2. He was subjected to orotracheal intubation and invasive ventilation, with improvement of respiratory function. At the end of April, due to the prolonged weaning from mechanical ventilation, he underwent an effective percutaneous tracheostomy, performed according to the Ciaglia-Blue-Rhino method. During the subsequent hospitalization, the anatomical conformation of the man, which was obese and endomorphic, with an enlarged thyroid gland, complicated the management of the tracheostomy, with the appearance of several episodes of oxygen desaturation. At the end of May 2021, the health workers reported blood leaking from the tracheal stoma, so they performed a CT scan of the neck, showing that the superior profile of the brachiocephalic artery was in close contact with the tracheostomy tube. The man underwent a cervical-mediastinal surgical revision, during which a laceration of the brachiocephalic artery wall was detected and sutured. However, a few days later, the subject died due to the presence of numerous blood clots, formed in the bronchial tree following the previous bleeding in the trachea. The cadaveric section made it possible to detail the position of the tracheostomy, located between the VII and IX rings of the tracheal wall, and the peculiar anatomical characteristics of the cervical structures and the upper thorax.

The described case was investigated using the Anatomage Table. The anatomical characteristics of the subject (in particular the distances between trachea, thyroid, brachiocephalic artery) were compared with those of four subjects (two Asian and two Caucasian) loaded on the Anatomage Table software.

The CT scan of the patient has been studied in detail, also using the same Anatomage Table, and compared with the autoptic results.

The integration between the Anatomage data, the radiological images and the cadaveric section offer useful information, allowing students and professionals to come into contact with different anatomical and pathological variants, influencing, on a daily basis, the clinicians' and forensic pathologists' activity.

Key words

Anatomage, brachiocephalic artery, anatomical variants.

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Clinical anatomy of the patent foramen ovale: implications for sport medicine

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Patent foramen ovale (PFO) is a common congenital atrial septal defect with an incidence of 15-35% in the adult population. The development of the interatrial septum begins in the fourth gestational week and is completed only after birth. During intrauterine life, the foramen ovale allows the passage of highly oxygenated blood from the right to the left atrium and into the systemic arteries, thus bypassing the pulmonary circulation. In 75% of the general population, the foramen ovale closes after birth, and only an oval depression, called fossa ovalis, remains on the right side of the interatrial septum. Patent foramen ovale can be associated with various clinically important conditions, including migraine and stroke, or decompression illness in divers. The aim of this study is to analyse the PFO developmental and anatomical features and to discuss the clinical risks associated with this atrial septal defect in adults.

With the advent of new diagnostic imaging techniques, such as transthoracic echocardiography with bubble test, transesophageal echocardiography, and transcranial Doppler ultrasound, the confirmation of the PFO has become clinically attainable. The transthoracic echocardiography with bubble test is a noninvasive exam that detects PFO with 99% specificity and 46% sensitivity. Unlike traditional echocardiography, this test is performed after injecting an agitated saline contrast into the patient's peripheral vein. The presence of PFO is suggested by the observation of saline microbubbles shunting to the left atrium in at least three cardiac cycles.

Most people with PFO remain asymptomatic throughout life, but life-threatening events can develop in association with certain pathologies or activities, including cryptogenic stroke, migraine with aura, or decompression sickness in divers. The interest in the close relationship between PFO and diving has grown rapidly, as it has been observed that the PFO can cause paradoxical embolization of nitrogen bubbles that form during diver ascent. This cardiac malformation is to be considered a risk factor for the onset of decompression sickness, one of the most feared complications of diving.

In concusion, PFO is present in up to 35% of adults. Often clinically silent and of little hemodynamic significance, it still needs to be considered in patients with migraine or stroke or individuals involved in certain hemodynamically challenging activities, such as diving.

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Morphological alterations of human gustatory and non-gustatory lingual papillae associated with SARS-CoV-2: a histological and molecular study

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Introduction. COVID-19, a recently emerged disease caused by SARS-CoV-2 infection, can present a large variety of signs and symptoms with different degrees of severity, including olfactory and taste dysfunctions. Different mechanisms, at the central and peripheral level, have been suggested to be responsible for COVID-19 related dysgeusia. The aim of the present study was to i) evaluate the presence of SARS-CoV-2 in the lingual tissues ii) describe the possible modifications of the lingual papillae morphology in SARS-CoV-2 positive subjects.

Material and Methods. Lingual samples were collected from 16 cadavers at the Institute of Forensic Medicine of Milan: 8 were SARS-CoV-2 positive (+) and 8 negative (–). Circumvallate, foliate and filiform papillae were harvested from the tongue by means of a 5 mm diameter punch. The samples underwent (i) molecular examination by means of PCR, in order to investigate the presence of the virus in the tissues and (ii) processed for histological analysis with Haematoxylin and Eosin staining and Silver impregnation to evaluate their morphology by optic microscopy.

Results. All samples were adequate for analysis. PCR revealed the presence of SARS-CoV-2 in filiform, foliate and circumvallate papillae in 6 out of 8 COVID-19 + subjects while all COVID-19 – samples resulted negative.

In circumvallate and foliate papillae from COVID-19 + patients, the epithelium was generally thin due to a reduced number of layers. Some portions of the papillary wall showed no epithelium at all. The structure of the buds was damaged or completely destroyed in some areas. Circumvallate and foliate papillae presented signs of inflammation. All papillae, including the filiform ones, presented signs of fibrosis in COVID-19 + samples. Circumvallate, foliate and filiform papillae from COVID-19 – samples were morphologically preserved with no signs of alterations. Silver impregnation staining for nerve fibres evaluation did not evidence any sign of alteration in any + or - sample.

Conclusions. Our study highlighted that SARS-CoV-2 can be detected in the oral tissues and particularly in the gustatory and non-gustatory lingual papillae. The presence of the virus may trigger tissue inflammation and fibrosis that in turn might be responsible of the taste disfunction that affects a great proportion of COVID-19 + subjects.

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Key words

SARS-CoV-2, lingual papillae, taste buds, inflammation.

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Assessment of the human mesenchymal stem cells structural and functional characteristics associated to a prolonged exposure of morphine

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The discovery of opioid receptors expression in skin and their role in orchestrating the tissue repairing process, gave rise to questions regarding the potential effects of clinical morphine treatment in wound healing. Although short term treatment was reported to improve wounds regeneration, in vivo chronic administration was associated to immune cell recruitment delay and abnormal extracellular matrix deposition, impairing the physiological tissue healing progression and causing systemic fibrosis.

Human mesenchymal stem cells (hMSCs) are a versatile class of multipotent adult stem cells. Their self-renew and multidirectional differentiation ability, combined with the release of immunoregulatory molecules, make them fundamental in restoring the damaged tissues. In this regard, opioid receptors expression was recently observed even in hMSCs[1], for which acute morphine exposition induced a significant functional characteristics decline[2]. However still little is known about its long-term effects.

To determine how a prolonged treatment could impair their regenerative potential, we exposed hMSCs to increasing morphine concentrations respectively for nine and eighteen days, evaluating in particular the fibrogenic potential.

Our results showed a time dependent cell viability decline and conditions compatible with a cellular senescent state. Ultrastructural analysis combined with beclin-1 (BECN1) and microtubule-associated proteins 1A/1B light chain 3B (LC3) expression, after eighteen days of exposition, were indicative of increased autophagy, suggesting a correlation to an augmented detoxification activity. In addition, the enhanced transcription of type I collagen (*COL1A1*) together with the related genes involved in its regulation, namely: metalloproteinase-1 and -2 (*MMP1*, *MMP2*), transforming growth factor-beta1 (*TGFB1*) and tumour necrosis factor-alpha (*TNFA*), suggested the possibility that a prolonged morphine treatment might exert its fibrotic potential risk even involving the hMSCs.

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Key words

hMSCs, stem cells, fibrosis, senescence, morphine.

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An anatomical variant of *palmaris longus* muscle: from the historical preparation to the present day

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Palmaris longus (PL) muscle is a superficial flexor of the forearm with restricted functions (its harvest or absence does not result in any functional disorder) but great clinical importance. It is used as landmark to identify median nerve, it is the first choice as tendon graft in reconstructive plastic, orthopedic, ophthalmologic and oto- laryngologic surgery and its variation can lead to some compression syndromes. It is phylogenetically classified as a retrogressive muscle and one of the most variable muscles of the human body. Agenesis is the most common anatomical variation while anomalies in insertion are quite rare, having an overall incidence of less than 1% [1]. During routine dissections of 32 forearms (from 16 embalmed cadavers) in the dissecting room of the University of Bologna, a female Caucasian cadaver aged 78 years was found to present a variation of insertion of the right PL tendon. The flattened tendon, measuring 10 cm in length and 0.6 cm in width, presented a cleft about 3.5 cm from the palmar aponeurosis. The lateral division was slender, located more superficially and fanned out distally into the palmar aponeurosis. The medial division, sharing a common sheet with the lateral one, run deeper and inserted at the ipothenar eminence. Median nerve crossed the lateral division. Such a V- shaped distal insertion is known as the PL *bicaudatus* of Gruber [2]. We conducted a detailed review of the literature and surprisingly we discovered that *Luigi Cattaneo* Anatomical Wax Collection of *Alma Mater Studiorum*, which displays a rich collec- tion of anatomical preparations dating back to the 19th century, hosted a dry speci- men of a left forearm. This preparation, displayed on a wooden platform, exhibited a case of *bicaudatus* PL tendon, described by professor Calori in 1868 [3] and likely prepared in the Anatomical Cabinet to faithfully display and reproduce the variation observed during autopsies/dissections. Even more fascinatingly, a hint to PL varia- bility was already found in Vesalius' masterpiece *De Humani Corporis Fabrica* [4]. The iconic second book tables in fact seemed to depict a *bicaudatus* insertion of PL at the right wrist.

Though it is a vestigial muscle, the PL continues to arouse interest because of its great variability. Understanding anatomical variants is certainly a fundamental skill of anatomists as well as of clinicians involved in diagnostic and surgical techniques.

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Key words

Palmaris longus muscle, tendon, variability, historical preparation.

Stili di vita e prevenzione:
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Low back pain prevalence and risk factors in Italian adolescent male soccer players: results from an online survey

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Background. Low back pain (LBP) is a widespread musculoskeletal complaint reported by athletes, often from an early age. The purpose of this retrospective survey was to analyse the prevalence of LBP among Italian adolescent soccer players, and to identify potential risk factors.

Methods. Participants were requested to answer an online survey based on the standardized Nordic questionnaires for musculoskeletal symptoms.

Results. Data were obtained from 204 male soccer players aged 14-17 years competing at the national and regional level. More than half of the players had experienced LBP in their lives. One-way ANOVA revealed that the players with LBP were taller, heavier and with a higher BMI (all p values<0.00001). When considering the playing position, ANOVA revealed that 14-15 years-old strikers displayed higher LBP scores than all other roles (all p values<0.05). Accordingly, strikers were exposed to a higher risk of LBP than midfielders (RR=1.48; 95%CI:1.10-2.01; p=0.01) and goalkeepers (RR=1.48; 95%CI:1.02-2.971; p=0.04), but not defenders (RR=1.23; 95%CI:0.93-1.63; p=0.15). Within the 14-15 age-class, strikers were, again, those most exposed to LBP risk (all p values<0.05).

Conclusions. Anthropometric and soccer-related features should be monitored to ensure early identification of potential risk factors for LBP. This information should be considered along with the specific playing position as strikers emerged as the roles most exposed to LBP

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Cardiac changes in Sirtuin 1 heterozygous mice on lard diet plus melatonin

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Sirtuin 1 (SIRT1), a conserved member of the family of the silencing information regulator 2 enzymes called sirtuins, is a NAD+ dependent deacetylase, and a key mediator of lifespan, and metabolism [1]. Melatonin, the pineal indolamine, is a potent SIRT1 activator which alleviates the cardiometabolic syndrome in genetically obese leptin-deficient mice [2]. Here, to best characterize its role in dietary obesity, we analysed morphological changes induced in whole body SIRT1 heterozygous mice (HET) on a high fat diet (58.4% E from fat-lard TD03584-Envigo) vs mice on standard maintenance rodent diet (8.4% E from fat). Male wildtype C57BL6/J mice (WT) were placed on above regimens for comparison. HET and WT mice received a standard diet (STD) or a high fat (HFD) diet for 16 weeks and melatonin, in drinking water at 10 mg/kg/day. Cardiac reaction to the hypercaloric lard diet was characterized analysing inter-myofibrillary mitochondria, fibrosis, lipid peroxidation, inflammation, and endoplasmic reticulum stress. Remarkably, round mitochondria with abnormal cristae, fibrosis and inflammation were exacerbated in HET HFD despite melatonin. Oxidative damage, endoplasmic reticulum stress and inflammation have been validated by strong 4HNE, GRP78, CHOP and NLRP3 immunostaining. Unfortunately, melatonin failed to improve cardiac alterations in HET HFD group. These data highlight the strict interdependence between melatonin and SIRT1 in the treatment of cardiac complications in obesity.

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Key words

Sirtuin1, obesity, heart, mitochondria, ultrastructure.

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Combined effects of physical activity and sleep on fatigue in haematological cancer patients

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Reduced physical activity (PA) and increased sleep deficiency are two of the several symptoms reported by haematological cancer patients (HCP). Differently from solid cancers, in which sleep deficiency and PA's beneficial roles have been intensely studied, less attention has been paid to these topics in HCP. HCP's sleep deficiency is usually linked to higher levels of cancer-related fatigue (C-RF), preventing HCP from being physically active. By acting in a vicious circle with C-RF, the reduced PA could negatively interact with the side effect of the disease's treatment. Moreover, the frame times during or after chemotherapy are usually the most studied in HCP. The present study assesses the differences in sleep by comparing PA and fatigue among HCP at the onset of chemotherapy. Furthermore, it investigates the relationship between sleep, PA and fatigue.

Fifty-eight newly diagnosed HCP (47.1 ± 15.4 yrs; 51.7% males) were anthropometrically evaluated and filled in five questionnaires within two weeks from starting treatment to assess sleep (PSQI), PA (visual analogue scale), fatigue (MFI-20), anxiety and depression (HADS), and quality of life (EORTC QLQ-C30). SPSS software was used to analyze the comparison between good and bad sleepers (ANCOVA analysis) and describe the relation between sleep, PA and fatigue (Mediation analysis). The HCP classified as *good sleepers* were less represented than those classified as *bad sleepers* (25% and 75%, respectively). The two groups showed no differences in body mass and BMI values. *Bad sleepers* displayed less frequent PA ($p < .04$), higher fatigue ($p < .032$), anxiety ($p < .003$), depression ($p < .011$) and pain ($p < .011$). The mediation analysis disclosed PA as a mediating factor between sleep and fatigue; in other words, it revealed significant indirect effects of sleep on fatigue through PA.

Sleep deficiency characterizes haematological cancer patients even at the onset of high dose chemotherapy. Impaired sleep quality is associated with less frequent PA and higher fatigue. In addition, the results highlight the combined action of sleep and PA on fatigue: fatigue could be improved by an increment in PA frequency, which, in turn, could be enhanced by sleep. In this view, PA and sleep could represent meaningful intervention targets to improve a patient's status before and at the onset of chemotherapy.

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Can habitual physical activity improve anthropometric and metabolic parameters in *BRCA 1/2* women?

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The *BRCA 1/2* mutations are the most known typologies of hereditary breast cancer (BC). Studies among women carrying these mutations have showed preliminary evidence of a protective role of physical activity (PA) against BC, particularly during adolescence or early adulthood. Previous data confirmed a significantly lower BC prevalence in women reporting higher PA in their adolescence [1, 2]. In addition, on healthy pre-menopausal women at high risk of BC, the practice of PA showed that it raised adiponectin and lowered leptin, controlling for a change in body fat and suggesting the importance of adipokines in *BRCA* penetrance [3]. Aim of the current study was to assess the role of PA on BC risk factors in women carrying *BRCA 1/2* mutations.

Data analysis involved 63 women (47.6±12.4 years) with *BRCA 1/2* mutations, in care at Fondazione IRCCS Istituto Nazionale dei Tumori, Milan. The participants filled in Godin-Shepard Leisure-Time Physical Activity Questionnaire for the evaluation of the PA levels. Moreover, they underwent to anthropometric, metabolic, and blood sample assessments. Data were analyzed with SPSS version 27.

The women were classified as *inactive* (n=41) and *active* (n=22). Insulin levels were found significantly lower in active compared to the inactive group ($p<.05$); in addition, there were no differences for the other variables analyzed.

The correlation analysis on the total sample showed that higher PA levels were significantly correlated with lower weight ($r2$=-0.26, $p<.05$), BMI ($r2$=-0.30, $p<.05$), hip circumference ($r2$=-0.30, $p<.05$), fat mass both in % ($r2$=-0.31, $p<.05$) and in kg ($r2$=-0.28, $p<.05$), and triglycerides ($r2$=-0.28, $p<.05$).

These findings suggest that higher levels of PA can play an important and protective role against BC. Therefore, structured PA interventions could be a tool for modulate the penetrance of hereditary BC.

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Key words

Hereditary breast cancer, physical activity, anthropometry, quality of life.

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Is foot velocity during a soccer kick associated with kinematics of the swinging and support limbs?

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INTRODUCTION: Besides being accurate, successful kicks in soccer usually generate the highest ball velocity possible (often measured in laboratory as the foot velocity), giving the goalkeeper less time to react (1, 2). Also, a high level of intersegmental coordination is required to perform an effective kick (3). The swinging limb must coordinate to achieve maximal foot velocity at the moment of ball impact, while the support limb must position the body's center of mass in an optimal position. Although intersegmental coordination is fundamental for a successful kick, it is still unclear if there are direct associations between joint angles and foot velocity at the moment of impact. Therefore, we evaluated the associations between joint angles and foot velocity during different maximal instep kicks.

METHODS: 18 male soccer players (age: 21±2 yrs, BMI: 22.4±1.9 kg·m-2, exp: 14±2 yrs) participated in the study. Players performed three kicks with the ball stationary or rolling (~2 $m \cdot s^{-1}$) from four different directions (anterior, posterior, medial, or lateral) and with both feet. They approached the ball at a 45° angle and kicked it as fast as possible with the instep portion of the foot, hitting a target (1.2x1.2m) positioned 3m away. Swinging foot velocity, the distance between support and swinging feet, and angles in the sagittal plane for the hip, knee, and ankle joints at the moment of ball impact were measured with 3D motion capture (BTS, Italy). Correlations between kick performance (foot velocity) and joint angles and feet distance were assessed with Pearson's correlation (α<0.05).

RESULTS: Foot velocity was significantly correlated with all variables, except swinging knee flexion angle (p=0.118; r=-0.117). Higher foot velocities were correlated with increased swinging ankle plantarflexion (p<0.001; r=-0.436), swinging hip extension (p=0.003; r=-0.217), support hip (p=0.008; r=-0.198) and knee (p<0.001; r=-0.331) extension, support ankle plantarflexion (p<0.001; r=-0.288) and feet distance (p=0.001; r=0.241).

CONCLUSION: Foot speed velocity was associated with joint angles in both limbs, with the highest correlations associated with increased swinging ankle plantarflexion and support knee flexion. Furthermore, the distance between the support and swinging feet at the moment of ball impact also seems to be relevant factor. Coaches and athletes should consider the hip, knee and ankle angles of both limbs and the distance between feet to improve kicking performance.

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Key words

Kicking, movement analysis, soccer, performance, coordination.

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How does leg dominance and ball condition affect lower limb muscle activation during soccer kicking?

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INTRODUCTION: To score in soccer, players need to kick the ball with a high velocity, giving the goalkeeper less time to react. In a match, players often need to kick a moving ball and, to be more successful, need to be able to kick the ball with both feet proficiently. Although muscle activation during the kicking task has been studied [1,2], it is still unclear how limb dominance and ball condition affect lower limb muscles activation during an instep kick.

METHODS: Ten experienced male soccer players (age: 21.9±1.9 yrs; BMI: 21.9±2.9 $kg \cdot m^2$; experience: 13.5±3.3 yrs) participated in the study. Eight surface EMG probes (FreeEMG300, BTS, Italy) were positioned on the muscle belly of the vastus medialis (VM), biceps femoris (BF), gastrocnemius medialis (GM) and tibialis anterior (TA) of both limbs. Subjects were asked to kick the ball inside a 1.5 X 1.5m target 3m away as fast as possible in four conditions: stationary ball and rolling ball with both the dominant and non-dominant feet (the ball rolled from behind the subjects at 2 $m \cdot s^{-1}$). Normalized peak activation was obtained for each kick. A repeated measures two-way ANOVA was used to identify differences between conditions and feet and partial eta-squared ($\eta p2$) was used to measure effect size.

RESULTS: There were no significant differences between positions or limbs. However, there were large partial eta-squared values for some comparisons: the swinging limb BF activation was higher when kicking with the dominant foot ($\eta p2 = 0.19$) and when kicking a stationary ball ($\eta p2 = 0.25$); the GM and the TA activation from the supporting limb were higher when kicking a moving ball ($\eta p2 = 0.35$ and $\eta p2 = 0.18$, respectively).

CONCLUSIONS: Although we found no significant differences in this pilot study, the large effect sizes in some comparisons suggest that there could be a trend toward different muscle activations in these comparisons. The larger swinging BF activation in the stationary and dominant conditions suggest that there is greater co-contraction when performing these kicks; the larger support GM and TA activations when kicking a rolling ball suggest that muscles that act on the ankle joint could be more active to stabilize the joint during this task. Future studies with a greater sample size can clarify how lower limb muscle activation function in different kicking conditions and possibly help to direct training and consequently improve player's performance.

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Key words

EMG, kicking, movement analysis, soccer, performance.

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Covid-19 pandemic: lifestyle differences in university students attending the course of Anatomy at the University of Milan

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The lockdown, along with other restrictions related to the COVID-19 pandemic, has negatively impacted our daily lives, exposing people to adopt sedentary and unhealthy behaviors, and increasing the risk to develop diseases [1].

The present study aimed to investigate physical activity, nutrition, and sleep habits in two different population of university students.

533 university students attending the course of Human Anatomy at the University of Milan (Italy) were recruited. Students filled out a questionnaire to evaluate their anthropometric characteristics (weight, height), age and lifestyle habits. Specifically, physical activity was evaluated by the Godin- Shephard Leisure Time Physical Activity Questionnaire, nutrition was investigated by the Mediterranean Diet Quality Index for children and adolescents, and sleep was evaluated by the Pittsburgh Sleep Quality Index proposed for both weekday (WD) and weekend (WE). Moreover, we investigated the consumption of cigarettes, wine and alcohol. The students were divided into two groups according to the degree course: Nursing (N) (258 students, 50 males and 208 females) and Sport Sciences (S) (275 students, 148 males and 127 females).

The comparison between N and S students showed statistically significant differences in weight ($p<0.001$) and height ($p<0.001$), but not in age and Body Mass Index. N showed lower levels than S in physical activity ($p<0.001$) and for adherence to the Mediterranean diet ($p<0.001$). Moreover, N were classified as bad sleepers and slept worse compared to S during WD ($p<0.001$). During the WE both N and S were good sleepers, but S slept better compared to N students ($p<0.002$). The smokers were significantly higher in N (28%) compared to S (15%) ($p<0.001$), while no differences were found for the consumption of wine (N=35% and S= 31%) and alcohol (N= 25% and S= 26%).

Data showed that, even during the COVID-19 lockdown, Sport Sciences university students, usually more active compared to the other students, maintain healthy lifestyle habits. These findings suggest the importance to promote in young people an active lifestyle, particularly during in this lockdown situation.

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Key words

Covid-19 pandemic, lifestyle, quality of life, physical activity, nutrition, sleep, university students.

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Melatonin Protects Corneal Epithelial Cells from Dry Eye Disease Injury

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Dry eye disease (DED) is one the most common disorder of the ocular surface that result in symptoms of eye dryness, light sensitivity, itching, irritation, and visual disturbance so compromising the quality of life of patients. Two common mechanisms contributing to the pathogenesis of ocular surface injury in DED are tear hyperosmotic stress and ocular surface inflammation [1]. The increased osmolarity of the damaged tear film stimulates inflammatory response and induces reactive oxygen species overproduction, which trigger apoptosis of corneal and conjunctival epithelial cells [2]. Pharmacotherapies to treat DED are actually limited and they only provide temporary relief and do not affect DED chronic course. Thus, molecules that suppress inflammation and oxidative damage would be strong potential candidates against DED. Melatonin, endogenous indoleamine synthetized by the pineal gland and locally by perhaps in all organs and cells, is an attractive candidate that not only meets, but also exceeds these requirements [3-5].

Therefore, to explore the potential effect of melatonin against the DED pathogenesis, we investigated its beneficial role and mechanism(s) of action using an *in vitro* hyperosmotic stress-induced DED model. Hyperosmolarity significantly altered human corneal epithelial cells (HCECs) morphology, increased the expression of pro-inflammatory cytokines, pro-apoptotic markers and metalloproteinases and induced reactive oxygen species overproduction, underlying mechanism of many ocular surface diseases, including DED. Interestingly, melatonin protected HCECs, in a dose-dependent manner, from morphological alteration and inflammatory injury, reduced oxidative damage, restoring the balance between oxidative damage and anti-oxidative protection, and prevented HCECs apoptosis. At the aim to sought to determine the underlining mechanism(s) by which melatonin protects HCECs from DED injury, we observed that melatonin acting on oxidative stress and inflammation can become a promising and safety therapeutic tools against ocular surface diseases.

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Effect of the new transdermal route of melatonin administration by cryopass laser in prostate cancer

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Melatonin is an indoleamine secreted by the pineal gland in response to darkness which controls various physiologic processes. Moreover, this molecule is known for its anti-oxidant, anti-inflammatory and anti-tumor activities [1]. Among the anti-tumor effect the best dose, the correct route of administration and the mechanism of action are not yet fully known. In recent years alternative delivery route based on transdermal administration of melatonin by cryopass-laser treatment shown the effect of this new technique in reducing the progression of prostate tumor cells (LNCaP) xenografted into nude mice [2].

The aim of the present work was to evaluate the impact of this administration route of melatonin on tumor morphology, microenvironment, and immune response.

For this aim we used three groups of nude mice xenografted with LNCaP cells: 1) control group 2) laser (treated only with cryopass-laser) and 3) laser plus melatonin. The animals of the last group were treated with melatonin at the dose of 4 mg/kg/treatment delivered by cryopass-laser for 6 weeks (3 treatments/week). At the end of the treatment the tumors were collected and were morphologically analyzed. The microenvironment and inflammation were studied by immunohistochemistry for lymphocytes $CD4^+$, $CD8^+$ and FOXP3 (forkhead box P3), a protein involved in immune system responses.

Our results showed that this technique of melatonin delivery significantly impaired the tumor microenvironment, increased the collagen deposition around the tumor and triggered an immunological response with increased recruitment of CD4+, CD8+ and FOXP3.

We can conclude that transdermal application of melatonin influencing the tumor growth and could be an effective and safe route of administration also in humans.

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Key words
Melatonin, prostate Cancer, cryopass-laser, immune system.

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Heart alterations in spontaneously hypertensive rats: immunochemical and immunohistochemical assessment of the activity of dextrorotatory form of thioctic acid

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Hypertension represents a risk factor for cardiovascular diseases [1]. Increasing evidence attributed the main role of oxidative stress in cardiovascular damage, promoting endothelial dysfunction, vascular remodeling, and inflammation. Excess bioavailability of reactive oxygen species (ROS), often is accompanied by structural mitochondria abnormalities in the cardiomyocyte [2,3]. The most effective treatment in the management of hypertension seems to be the administration of anti-hypertensive drugs with antioxidant properties [4].

Thioctic acid (TIO) is an antioxidant existing in nature and expressed in two optical isomers. The dextrorotatory form is the naturally occurring eutomer, whereas the most used formulation of the compound in clinical practice is the mixture of (+) and (-) enantiomers. Previously, we demonstrated that TIO-(+) is one of the more appropriate antioxidant molecules to prevent cardiac and renal alterations associated with hypertension [5].

The present study was designed to investigate the effect of 4 weeks of treatment with TIO-(+) on the heart of spontaneously hypertensive rats (SHR), using immunochemical and immunohistochemical techniques. 125 mmol/Kg/day of TIO-(+) was administered intraperitoneally in 24-weeks-old SHR. Hypertensive rats were compared to age-matched normotensive Wistar Kyoto (WKY) rats.

Blood pressure values were significantly decreased in treated SHR compared to the control one. This is probably related to the effects at the levels of the endothelial vessels that determine vasodilation. Left ventricular cardiomyocytes' hypertrophy deposition of reticulin, collagen fibers, proteins, and nucleic acid oxidation accompanied by an increased expression of interleukins, such as IL-1 beta, IL-6 and tumor necrosis factor-alpha were found in SHR. These alterations were reduced in TIO-(+) treated animals. The effects observed after treatment with TIO-(+) nominate this molecule as one of the more appropriate antioxidants to prevent heart injury associated with hypertension, opening the opportunity to further evaluations in clinical trials.

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Effects of tart cherry supplementation on brown adipose tissue of high-fat diet obese rats

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This study is a part of a project aimed to assess the effects of tart cherries on a commonly used rat model of diet-induced obesity (DIO). To date, a preventive effect of tart cherry against the onset of obesity-related risk factors and its protective role against oxidative stress and liver steatosis has been demonstrated [1]. In addition, a decrease of inflammation in the visceral fat has proven to be associated with tart cherry supplementation [2].

Here, we have investigated the impact of a high-fat diet (HFD) on interscapular brown adipose tissue (iBAT) in DIO rats and the possible beneficial effects of tart cherries in addition to the high caloric diet. Rats were divided into 3 groups fed an HFD (DIO), an HFD added with seed powder (DS), and an HFD added with seed powder plus juice (DJS) of *Prunus cerasus* L. The animals were monitored for 17 weeks and compared to control fed with standard diet (CHOW).

Histologic observations revealed that HFD induces iBAT dysfunction in DIO rats, characterized by expansion of white adipose tissue versus iBAT, mainly through the conversion of brown adipocytes to white-like unilocular cells. Although with individual differences, tart cherry supplementation resulted in a reduction of obesity-induced whitening of iBAT both in DS and in DJS, compared to DIO rats. No differences in tissue weight were found among the obese groups.

A lower intensity of the Uncoupling Protein 1 (UCP1) staining in brown adipocytes and a higher amount of weakly or negative white-like brown adipocytes in both supplemented groups were found. These results were also confirmed by the WB results. As expected, based on the large brown to white conversion in obesity, the gene expression analysis showed a decreased UCP1 gene and related peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PCG-1a) expression in DIO rats respect to the controls. Instead, in the obese DS rats, the upregulation of the thermogenic genes not only concerning DIO rats but also control might suggest the activation of a tissue remodeling program, distinct from that induced in DIO rats.

Finally, additional immunochemical investigations indicated that tart cherry supplementation would attenuate the obesity-induced profile of both inflammatory markers and Glucose-Regulated Protein 94 (GRP94) protein, suggesting the possible effectiveness of both juice and seeds against the pro-inflammatory environment and the ability to improve the recovery of the ER function.

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Effect of physical exercise on Telocyte population in the skeletal muscle tissue of rats

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Skeletal muscle atrophy refers to biochemical, morphological, and functional changes in skeletal muscle that may result from states of hypokinesis or immobilization, and represents a major topic in the fields of regenerative and rehabilitation medicine. On the contrary, both resistance and aerobic training induce metabolic changes within the muscle by altering protein synthesis, muscle proteolysis, therefore inducing molecular and cellular adaptations that regulate homeostasis and hypertrophy. A large variety of stromal cells are involved during physiological processes following physical exercise, in order to sustain remodeling and regeneration [1]. Telocytes represent a recently discovered population of stromal cells, which has been increasingly identified in several human organs and appears to participate in sustaining cross-talk, promoting regenerative mechanisms and supporting differentiation of local stem cell niche [2-4]. The aim of this morphologic study was to investigate the presence/absence of Telocytes in tibialis anterior muscle of healthy rats who underwent a protocol of endurance training for either 4 weeks or 16 weeks in comparison to sedentary rats who were inactive, i.e., not engaging in any physical exercise, throughout the duration of the experiment. Histomorphometric analysis of muscle fibers diameter revealed muscle atrophy in sedentary rats. Telocytes were identified by double-positive immunofluorescence staining for CD34/CD117 and CD34/vimentin. The results showed that Telocytes were significantly reduced in sedentary rats at 16 weeks, while rats subjected to regular exercise maintained a stable Telocytes population after 16 weeks. Finally, immunohistochemistry showed CD34+ cells, within the interstitium between muscle fibers, with characteristic features of TCs and telopodes, i.e., spindle nuclei and multiple long cytoplasmic processes. These findings herein are intended to encourage knowledge about Telocytes population and their role in the stem cells niche of skeletal muscles. The understanding of the mechanisms between the cells involved in the tissue remodeling process and the role of physical activity could offer new chances in regenerative tissue strategies and insights about finding possible triggers for Telocytes in sarcopenia and other musculoskeletal disorders.

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Ferroptosis-resistance supports cellular senescence in the development of liver injury in an experimental mouse model of Nonalcoholic fatty liver disease/ Nonalcoholic steatohepatitis (NAFLD/NASH) induced by "Wester-style" diet assumption

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NAFLD/NASH is a worldwide and unresolved health problem. Several factors concur to its onset and among them, a crucial cause is represented by unbalanced alimentary habits (1). One of the worst regimens for the liver fitness is the so called "Western-style diet" (WSD), characterized by an excessive assumption of fats and sugars. This dietary regimen induces severe alterations in liver parenchyma ranging from simple steatosis to fibrosis and hepatocarcinoma (2,3). Many different mechanisms have been considered as key players of the liver damage, including most recently senescence and ferroptosis. Indeed, several studies revealed that an alteration of these processes is involved in the exacerbation of many diseases, among which nonalcoholic livers alterations. However, despite senescence and ferroptosis share several molecular players and are triggered by the reactive oxygen species (ROS), limited studies focused their attention in the possible direct connection between senescence and ferroptosis in the progression of NAFLD/NASH. On these bases, we analyzed the molecular pathways of the senescence and ferroptosis in livers of a mouse model of WSD. In particular, we used an Amylin-modified liver NASH mouse model fed for 23 weeks with an unbalanced diet (fat 40%, sucrose 20%, cholesterol 2% Kcal). The main features of liver injury such as hepatomegaly, hepatic lipidic accumulation, elevated plasmatic ALT, inflammatory infiltration was confirmed, as well as an increase of apoptosis rate, Ki67 and p53 expression. On the senescence side, the number of β -galactosidase (β-gal) positive cells increased, as well as expression of secretory associated senescent phenotype (SASP) members interleukin-(Il)-6 and matrix metalloprotease (MMP)-1. On the ferroptosis side, iron overload and increase of DNA damage revealed by positiveness for phospo-histone H2 (p-H2AX) were confirmed. The expression of glutathione peroxidase-4, gpx-4, counteracting iron-mediated ferroptotic cell dead, was also increased. Notably, a high number of gpx-4 and β-gal positive cells were noted in WSD fed mice compared to control. In conclusion, our data suggest that a mechanism of ferroptosis resistance mediated by gpx4 occurs in senescent cells, fostering the deterioration of the liver fitness.

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Keywords

NAFLD/NASH, senescence, ferroptosis, beta-galactosidase, gpx-4.

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Effects of contact sports on temporomandibular disorders: preliminary results

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Temporomandibular disorders (TMD) are a group of craniofacial disorders involving the temporomandibular joint (TMJ), the masticatory muscles and the musculoskeletal structures of head and neck. TMD are characterized by orofacial pain, limited or asymmetric mandibular movements and TMJ sounds. Tinnitus, neck pain and headache may also occur. Contact sports are an etiological factor in the development of TMD (1). They are associated with a higher risk of injury of the orofacial district due to intentional or accidental physical contact among players (2). One hundred male patients were recruited and equally divided into five study groups (20 patients for each group), based on the sport practiced: soccer, rugby, American Football, boxing group and basket. A control group (CG) of 20 non-athletes was matched by sex and age with the study groups. TMD signs and symptoms were valued following the standardized Diagnostic Criteria for Temporomandibular Disorders. Symptoms and signs of the study groups were compared to those found in the CG. Neck pain, arthralgia, temporal headache and joint noises are more present in the study groups.

On the contrary, myofascial pain evoked during palpation was significantly greater in the CG, except for the anterior temporal in soccer and American football. About the alterations of the oral opening, the mandibular deviation was statistically significant in soccer, rugby and American football. There were no statistically significant differences in mandibular kinematics in boxing and basket. In American football and rugby a limited laterotrusion was found, whereas in soccer a reduced opening. In the literature, few studies investigate the correlation between TMD and contact sports. Our preliminary data indicate that the sports investigated seem to play an important role in the onset of TMD, both in myofascial syndromes and joint alterations.

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Key words

Temporomandibular disorders, competitive sports, contact sports.

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Low levels of lamin A and mislocalization of LINC complex proteins are linked to metastatic potential in Ewing sarcoma

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Nuclear morphology abnormalities, loss of nuclear lamina integrity and changes in its constitution are known cancer hallmarks [1]. Alterations in nuclear architecture are mediated by nuclear proteins, such as lamin A/C, the main constituent of nuclear lamina, or LINC complex proteins SUN1, SUN2 and nesprins, which mediate a mechanical link between nucleus and cytoskeleton, determining the overall nuclear structural properties [1]. Lamin alterations may decrease nuclear rigidity, promoting invasiveness or increase protection against mechanical forces, such as interstitial pressure within the tumor [2, 3]. It has been observed that the expression of lamin A can be increased, reduced or absent, depending on the type of cancer. However, changes in lamin A levels are often associated with poor prognosis in multiple human cancers [4].

Here, we reported that low *LMNA* levels are associated with a worse 5-year OS in Ewing Sarcoma (EWS) patients and correlated with a more metastatic phenotype. To gain mechanistic insights into lamin A-dependent pathways in EWS, a pediatric tumor characterized by a high degree of aggressiveness, we focused on the LINC complex proteins, the major lamin A partners in mechanosignaling transduction [5]. We found that SUN1, SUN2, and nesprin 2 were severely mislocalized, due to a lamin A-dependent mechanism, as forced lamin A expression rescued their nuclear localization and SUN1 expression involved in cytoskeleton remodelling, and directly correlated to EWS invasiveness [6]. While in naïve EWS cells, YAP was recruited to the nucleus, lamin A overexpression reduced its nuclear retention, inhibiting its activity. Lamin A rescue of SUN1 levels/localization was associated with significantly lower ROCK2 levels and reduced motility, in agreement with the critical role played by Rho/ROCK2 pathway in EWS migration [7].

Interestingly, we obtained the same effects treating EWS cells with mevinolin, a statin already employed in clinical practice. Mevinolin treatment caused prelamin A accumulation as expected, but also significantly increased *LMNA* gene and protein expression, rescuing LINC complex localization and pushing the cells in a more differentiated status.

Thus, our findings recognize mevinolin, as a tool capable of reducing invasiveness, while triggering neural differentiation, paving the way to new therapeutic options for this very aggressive cancer.

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Key words

Lamin A, LINC complex, mechanosignaling, Ewing sarcoma, tumor invasiveness.

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Cellular and metabolic changes in the liver tissue of patients with Mitochondrial Neurogastrointestinal Encephalomyopathy

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Objective: Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an extremely rare disease caused by a genetically-driven thymidine phosphorylase (TP) defect. TP converts the nucleosides thymidine and deoxyuridine into respective nucleotides. In MNGIE, these nucleosides accumulate promoting tissue damage and mtDNA abnormalities responsible for neurological impairment and severe gastrointestinal dysmotility. The resulting clinical phenotype has a tremendous impact on patients' poor quality of life and fatal outcome.1;2 One possible treatment strategy to MNGIE patients is the stable reduction of circulating toxic nucleosides via liver transplantation. This approach has been successfully achieved in MNGIE patients in Italy and in many Western Countries.3 Although the liver has never been described as a target organ of the disease, explanted livers of MNGIE patients show clear macroscopic changes.

Aim: The objective of our study was to provide an accurate morphologic and metabolic analysis of MNGIE liver tissue *vs.* controls (CTR).

Methods: Liver tissue biopsies (1x1 cm each sample) were collected from n=7 MNGIE (2F; 20-38 yrs) and n=7 CTR (2F; 22-67 yrs) subjects. Formalin fixed- paraffin embedded tissue sections were processed for: 1) Hematoxylin and Eosin staining to evaluate architectural changes; 2) Sirius Red/Fast Green collagen assay to localize and quantify fibrosis; 3) Orcein, vascular endothelial cells growth factor and CD31 immunostaining to assess vascular changes; 4) hypoxia-inducible-factor- 1α (HIF-1α) immunostaining to test hypoxia. Part of the liver tissue biopsies (0.5x0.5 cm each sample) were collected and snap frozen from n=3 MNGIE and n=3 CTR for metabolomic NMR assay.

Results: MNGIE liver was characterized by intense steatosis. Fibrosis increases (P=0.0025) with patient age leading to the formation of porto-portal and porto-central collagen septa. The vascular and biliary branches were altered and these findings correlated with the increased hypoxia in hepatocytes, as indicated by HIF-1α positive staining. Preliminary analysis performed with NMR indicated a rearrangement characterized by triglyceride production and a significant decline in aerobic metabolism.

Conclusions: Our data, showing marked morphological and metabolic changes, indicate that the liver is a target organ of MNGIE. Liver alterations should be carefully taken into account as a compromised liver may interfere with the clinical response to targeted treatments.

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Key words

Fibrosis, hypoxia, liver abnormalities, mitochondrial neurogastrointestinal encephalomyopathy.

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The intriguing role of astrocytes in autosomal-dominant leukodystrophy (ADLD)

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Autosomal Dominant Leukodystrophy (ADLD) is a rare and fatal adult-onset neurodegenerative disorder that affects the central nervous system. It is characterized by Lamin B1 (LMNB1) gene duplication or deletion upstream the gene[1], but the molecular mechanisms responsible for driving its onset and development are not clear yet. Considering the pivotal role that glial cells as oligodendrocytes and astrocytes and Leukemia Inhibitory Factor (LIF)-activated pathways have in the myelination process, this work aims to analyze the specific alterations in different cell populations. Specific morpho-functional aspects of primary cells deriving from ADLD patients and engineered cellular models overexpressing Lamin B1 have been analyzed. Result demonstrated that cells from ADLD patients and astrocytes overexpressing LMNB1 show nuclear alterations, not present in oligodendrocytes. Moreover, Lamin B1 accumulation in astrocytes induces a reduction in LIF and LIF-R levels with a consequential decrease in LIF secretion, leading to Jak/Stat3 and PI3K/Akt axes downregulation. Administrating exogenous LIF, the toxic effects, induced by LMNB1 accumulation, may be partially reverted with differences between astrocytes and oligodendrocytes[2]. In addition, Lamin B1 overexpression induces alterations in cell survival pathways with GSK3β inactivation, but not the upregulation of β-catenin targets resulting in a reduction of astrocyte survival. Moreover, Lamin B1 build up affects proliferation and cell cycle progression with PPARγ and p27 increase and Cyclin D1 decrease. These events are associated to cell viability reduction and apoptosis, suggesting a great sufferance of this specific cell population. Interestingly, astrocytes overexpressing Lamin B1 show increased immunoreactivity for both GFAP and vimentin together with NF-kB nuclear translocation and c-Fos increase, suggesting astrocytes reactivity and substantial cellular activation. Moreover, it has been shown the activation of proinflammatory mechanisms and the increase of reactive oxygen species (ROS) in ADLD patients' cells. These data demonstrate that Lamin B1 accumulation is correlated to cell sufferance, probably related to the induction of reactive astrocytes phenotype that could be associated to ADLD pathological mechanisms. Our studies, for the first time, hint at a pivotal role of astrocytes in the development of the disease.

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Key words

LaminB1, cellular signaling, ADLD, astrocyte, LIF, apoptosis.

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Potential role of Phospholipase C β1 in high-grade gliomas: a new prognostic biomarker

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Gliomas are primary brain tumors originated from the supporting neuroglial cells of the Central Nervous System (CNS). Gliomas can be classified into different histopathologic grades according to World Health Organization (WHO) grading system and the grade of gliomas is highly correlated with the type of prognosis. Gliomas can, therefore, be classified into high-grade gliomas (HGG), including Glioblastoma Multiforme (GBM) as the most common malignant brain tumor in adults, and low-grade gliomas (LGG). Today the main therapies for this type of cancers are based on neurosurgical, chemotherapeutical and radiotherapeutical procedures that are highly invasive for all gliomas and cannot cure definitively the tumor in high-grade patients. For this reason, the management of gliomas represents today a great challenge and the understanding of the molecular mechanisms related to tumor transformation could help to find a successful targeted therapeutic strategy. Several studies have shown the key role of phosphoinositides (PIs), and in particular of phospholipase C β1 (PLCβ1) in the regulation of many mechanisms within the CNS1 such as cell adhesion, migration and cell cycle that, interestingly, are altered in GBM2. In silico studies have already evidenced that PLCβ1 gene expression is inversely correlated with the gliomas' pathological grade, suggesting PLCβ1 as a potential prognostic factor and novel signature gene in the molecular classification of high-grade gliomas3. This study aims to determine the pathological impact of PLCβ1 in GBM patients' samples, in engineered cell lines and primary astrocytes. PLCβ1 gene expression was analyzed through qPCR. Mesenchymal markers and survival pathway targets were evaluated by Western Blot and Immunofluorescence. Migration and invasion were carried out through transwell and wound healing assays. This study confirmed that PLCβ1 gene expression is lower in 50 GBM samples compared to 20 healthy controls. Moreover, PLCβ1 silencing in U87-MG, U-251 MG cell lines and HA primary astrocytes, leads to a more aggressive cellular phenotype by increasing cell migration, invasion, proliferation and by the activation of the main survival pathways, such as β-catenin, Stat3 and ERK1/2 pathways. These data confirm that PLCβ1 is involved in GBM pathogenesis and a complete understanding of its role may be strategical from both pathological and clinical point of view.

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Nuclear localization of PI-PLCbeta1 in Myelodysplastic Syndromes (MDS) during Iron-Induced Oxidative Stress and Cyclin D3/PKCalpha regulation

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Alterations of inositide signalling regulated by PI-PLCbeta1/Cyclin D3/PKCalpha or Akt/mTOR pathways are associated with MDS[1].

Most MDS patients may need transfusions of RBCs to correct anemia, but iron overload and increased oxidative stress are common collateral effects of this approach[2], that is why they are treated with Deferasirox (DFX) as iron chelator[3].

Here, we further analyzed the molecular effect of iron stress and DFX therapy on inositide signalling, using hematopoietic cell lines and MDS samples, with the aim to identify new molecular markers of DFX response.

The study included BM samples obtained from 6 transfusion-dependent MDS patients treated with DFX from 2006 to 2019, and 6 BM samples from healthy subjects who had given informed consent according to the Declaration of Helsinki. 3 patients were responders, and 3 were non responders. All samples came from the Institute of Hematology "L e A Seràgnoli", Bologna, Italy, where also the clinical data were collected.

Gene/protein expression were evaluated on mononuclear cells at diagnosis and during the therapy, by Real- Time PCR and immunocytochemistry. Cell lines were treated with 150 μM FeCl for 48h and DFX 50 μM for 6h, used alone or in combination to imitate in vitro the patients' dose. Cell cycle and apoptosis were examined by flow cytometry, along with the analysis of ROS production. We also studied the expression of enzymes related to inositide signalling, cytokine secretion and Akt/ mTOR activation.

Responder patients showed higher baseline levels of PI-PLCbeta1/Cyclin D3/PKCalpha expression, as compared both with non-responders and healthy subjects. During DFX therapy, ROS production and inositide signalling increased only in non responder patients, while in responder patients, as well as in K562 cells treated with FeCl +DFX, gene and protein expression remained almost constant. On the contrary, THP-1 cells were not affected.

All in all, our results show for the first time that the expression of PI-PLCbeta1/Cyclin D3/PKCalpha pathway is directly associated with iron-induced oxidative stress and ROS production in MDS and hematopoietic cells. The apparent nuclear localization of inositides during treatment could hint at a specific involvement of this signalling pathway during oxidative stress, that could be linked to a specific regulation of hematopoietic differentiation and needs further investigation, first in a larger number of patients' cells, then in other in vitro experimental models.

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Sertoli cells have promyogenic and antifibrotic effects, and induce utrophin expression in human DMD myotubes with different mutations in a heregulin β1/ErbB2/ERK1/2-dependent manner

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Sertoli cells (SeC) are the major component of the seminiferous tubules, where they secrete a plethora of trophic and immunomodulatory factors necessary to development of germ cells [1]. For these characteristics, SeC have been widely used as a therapeutic approach in many pre-clinical studies, including Duchenne muscular dystrophy (DMD) [2,3]. DMD is a lethal, rare X-linked disease caused by mutation in the dystrophin gene (*DMD*). The absence of dystrophin compromises the integrity of the dystrophin glycoprotein complex (DGC) increasing susceptibility of sarcolemma to rupture during contraction, with consequent necrosis of myofibers and muscle chronic inflammation. In DMD patients, the contractile component of skeletal muscles is progressively replaced by adipose and fibrous tissues, compromising muscle functionality and leading to premature death [4]. Despite huge effort to find a cure, glucocorticoids remain the standard therapy for DMD patients, despite their limited efficacy and undesired side effects. We reported that a single intraperitoneal injection of microencapsulated porcine SeC translates into recovery of muscle morphology and performance in dystrophic mice. This was linked to the ability of SeC to restrain inflammation and upregulate the dystrophin paralogue, utrophin at the sarcolemma through the release of heregulin β1 [5,6]. Here, we report the direct effect of SeC on myoblasts/myotubes of murine origin or derived from healthy subjects and DMD patients with different mutations. We observed that SeC-secreted factors i) stimulate cell proliferation in the early phase of differentiation in C2C12, and human healthy and DMD myoblasts; ii) delay the expression of differentiation markers in the early phase, nevertheless stimulating terminal differentiation in DMD myoblasts; iii) restrain the fibrogenic phenotype in human fibroblasts, and inhibit myoblast-myofibroblast transdifferentiation in C2C12 cells treated with TGF-β, and in DMD myoblasts; iv) induce the upregulation of utrophin at the sarcolemma in DMD myotubes regardless of the mutation in a heregulin β1/ErbB2/ERK1/2-dependent manner, recruiting components of the DGC thus providing functional replacement of dystrophin. Altogether, these results further support the use of SeC as a potential treatment of DMD patients, and suggest that SeC might improve muscle regeneration, especially in the early phase when myoblasts have to proliferate to efficiently replace damaged myofibers.

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Key words

Duchenne muscular dystrophy, Sertoli cell, muscle differentiation, fibrosis, heregulin β1, utrophin.

Tessuto muscolare e connettivo

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Givinostat as metabolic enhancer reverting mitochondrial biogenesis deficit in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a rare disorder characterized by progressive muscle wasting, weakness, and premature death. Remarkable progress has been made in genetic approaches, restoring dystrophin, or its function. However, the targeting of secondary pathological mechanisms, such as increasing muscle blood flow or stopping fibrosis, remains important to improve the therapeutic benefits, that depend on tackling both the genetic disease and the downstream consequences. Mitochondrial dysfunctions are one of the earliest deficits in DMD, arise from multiple cellular stressors and result in less than 50% of ATP content in dystrophic muscles. Here we establish that there are two temporally distinct phases of mitochondrial damage with depletion of mitochondrial mass at early stages and an accumulation of dysfunctional mitochondria at later stages, leading to a different oxidative fibers pattern, in young and adult mdx mice. We also observe a progressive mitochondrial biogenesis impairment associated with increased deacetylation of peroxisome proliferator-activated receptor- gamma coactivator 1 α (PGC-1α) promoter. Such histone deacetylation is inhibited by givinostat that positively modifies the epigenetic profile of PGC-1α promoter, sustaining mitochondrial biogenesis and oxidative fiber type switch. We, therefore, demonstrate that givinostat exerts relevant effects at mitochondrial level, acting as a metabolic remodeling agent capable of efficiently promoting mitochondrial biogenesis in dystrophic muscle.

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Giorgia Catarinella, Cecilia Barbieri, Lucia Latella.

Key words

Skeletal muscle, Duchenne Muscular dystrophy, epigenetic, mitochondria.

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The role of ECM protein crosstalk in the maintenance of a correct bone architecture

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During life, bone extracellular matrix (ECM) undergoes a continuous remodeling process that determines the proper preservation of bone mass and architecture. An increase in bone remodeling level, as well as the imbalance in the osteoblasts- osteoclasts coupling, leads to an altered bone tissue microarchitecture, enhancing bone fragility and fracture risk, features of osteoporosis (OP) [1,2]. Dysregulation in bone ECM proteins is considered a key marker of OP [3,4] and an accurate analysis of their distribution, expression and mutual relationship in the onset or maintenance of OP is far from being elucidated.

In the present study we correlated the microarchitectural changes observed in osteoporotic femoral heads with the expression of Collagen Type I (COL1A1, COL1A2) and non-collagenous proteins (NCPs) like Transforming Growth Factor beta (TGF-beta), Decorin, Osteopontin, Bone Sialoprotein 2 (BSP-2), Osteonectin and Osteocalcin. Sirius Red staining, immunohistochemistry, Western Blotting analysis, and histomorphometry were used.

Our analyses confirmed the alterations of bone area fraction, cortical and trabecular thickness in OP bone tissue. Moreover, we detected opposing correlation between the changes in Type I Collagen distribution and the expression of Decorin, Osteocalcin, Osteopontin, and BSP-2. These NCPs exert a central role in bone ECM structure and function (e.g., cell-matrix interaction, OB and OC recruitment and maturation, and HA nucleation) with agonist or antagonist effect in tissue maturity, bone formation and resorption and mineralization. An unbalance in their crosstalk supports the increase of tissue immaturity, also possibly caused by a high resorption rate, observed in OP tissue. These heterogeneous ECM network- dependent effects need to be taken as a whole for targeted therapeutic strategies aimed at preserving bone mass.

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Key words

Bone ECM, osteoporosis, type I collagen, Decorin, SIBLING proteins, osteocalcin.

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Platelet rich plasma prevents voltage-dependent gap junction currents and Connexin 43 expression in TGFβ1-induced myofibroblasts *in vitro* through Vascular endothelial growth factor (VEGF)-A Mediated Signaling

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Although increasing evidence supports the antifibrotic potential of Platelet rich plasma (PRP), currently there is not a clear consensus and the cellular and molecular mechanisms, underpinning PRP action, need to be clearly identified. In this context, we recently demonstrated the capability of PRP to prevent the *in vitro* differentiation of fibroblasts towards myofibroblasts, the key cell effectors of fibrosis induced by the pro-fibrotic agent transforming growth factor (TGF)-β1. Moreover, PRP reduced the expression of α-smooth muscle actin (sma), a well-known myofibroblastic marker, engaging the vascular endothelial growth factor (VEGF)-A/ VEGF Receptor-1-mediated signaling [1]. In addition, we demonstrated the capability of PRP to abolish the TGF-β1-induced voltage-dependent gap junction (GJ) current onset, while preventing the expression of connexin (Cx) 43, the typical Cx isoform forming voltage-dependent connexons [2]. Here we extended our research assessing the involvement of VEGF-A/ VEGF receptor signaling in mediating the in vitro PRP-induced response of fibroblasts cultured in the presence of TGF-β1 in terms of GJ currents and Cx43 expression, by combining morphological and electrophysiological analyses. First, we confirmed the ability of PRP to prevent the acquisition of myofibroblastic phenotype by TGF-β1-treated fibroblasts as judged by cell shape and surface area analyses, confocal immunofluorescence analysis of α-sma and vinculin expression and electrophysiological recordings by whole-cell patch-clamp of the resting membrane potential. Furthermore, we found that VEGF-A neutralization by blocking antibodies or the VEGFR inhibition by KRN633 in TGF-β1-treated fibroblasts prevented the PRP-induced down-regulation on the GJ currents and Cx43 expression. The role of VEGF-A signaling in preventing voltage dependent GJ currents occurrence and CX43 expression upregulation was confirmed by the treatment of fibroblasts with soluble VEGF-A. This study contributes to provide new insights into the molecular mechanisms underpinning PRP action on counteracting fibroblast to myofibroblast differentiation, recognizing GJs and Cx43 crucial targets of the VEGF-A mediated pathway.

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Key words

Connexin 43, fibrosis, gap junctions, myofibroblasts, platelet rich plasma, VEGF.

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Characterization of collagen content in skeletal muscles in a mouse model of Duchenne muscular dystrophy during ageing

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Skeletal muscles of patients affected from Duchenne muscular dystrophy (DMD) exhibit progressive skeletal muscle degeneration and weakness, since muscle fibers are gradually replaced by deposition of collagen, leading to muscle fibrosis [1,2]. The extracellular matrix (ECM) in muscles, mainly composed of collagen type I and III, is located in the endomysium, perimysium and epimysium, that play a key role in organizing the hierarchical structure of muscles [3,4].

Since muscular fibrosis is a striking characteristic of DMD, this study was aimed at evaluating muscle fibrosis in skeletal muscles of healthy and MDX mice (the mouse model of DMD), by analyzing collagen content by morphological methods. For this purpose, young and adult mice were studied in order to better understand whether different muscles are differently involved and whether aging influences the extent of fibrosis.

Skeletal muscles were obtained from young (1 month old) and adult (5 months old) healthy (CT) and dystrophic mice (MDX), fixed and paraffin embedded. Sections of quadriceps (QD), tibialis anterior (TA), gastrocnemius (GC) and diaphragm (DF) were stained with Sirius red. Collagen content was expressed as fibrosis index, that is the ratio between the collagen relative to the whole section analyzed expressed as a %.

Our preliminary results show that collagen content is similar in all the considered muscles of CT young mice, whilst it significantly increases in adult CT mice in QD ($p<0.01$) and DF ($p<0.05$) compared to TA and GC. The comparison between young and adult CT and MDX mice showed that there is a significant increase of collagen in QD of young MDX mice ($p<0.001$ vs CT) and adult mice ($p<0.001$ vs CT and young MDX). In TA, collagen increased in adult MDX ($p<0.05$ vs CT) but not in young MDX. In GC from MDX mice the fibrosis index is higher in adult compared to young MDX ($p<0.05$) and in adult MDX compared to CT ($p<0.05$). In DF there is a significant increase in young MDX compared to CT ($p<0.005$) and in adult MDX ($p<0.001$ vs CT and young MDX).

These findings show that collagen content increases at a different extent in the different skeletal muscles in CT. Moreover, fibrosis is higher in MDX mice in QD and DF, suggesting that these are the muscles mainly affected. Indeed, DF, playing a crucial role in breathing, becomes highly fibrotic also in humans. Overall, our results could contribute to the characterization of the progression of fibrosis in DMD.

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3D Bioprinting of different hydrogels and scaffolds: rational design strategies in skeletal muscle tissue engineering

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Skeletal muscle tissue engineering aims at repairing defective skeletal muscles. Among different strategies, 3D bioprinting emerges as an innovative strategy to provide the unprecedented capacity of depositing various types of biomaterials, cells, and biomolecules in a layer-by-layer fashion, with precisely controlled spatial distribution [1]. In this study, we demonstrated an application of 3D Bioprinting using different commercially available hydrogels with the aim to identify the most suitable biomaterial for the proliferation and differentiation of murine myoblast cells (C2C12). We studied, at different time points, the proliferation and differentiation capabilities of C2C12 cells in different commercial bioinks and encapsulated in a fibrinogen based-bioink co-printed on a PCL scaffold, using cells seeded on plastic as controls [2]. Moreover, the expression patterns of master regulatory myogenic genes and cell viability have been analyzed in order to evaluate the capabilities of the scaffolds to induce myoblast elongation, alignment and differentiation [3]. Commercially available hydrogels were provided by CELLINK (CELLINK® FIBRIN, CELLINK® GelMA A, CELLINK® GelXA, Cellink AB, Gothenburg, Sweden) and applied following the manufacturer protocol. C2C12 myoblast cells laden into the hydrogels were printed using CELLINK INKREDIBLE+, a pneumatic extrusion-based 3D bio-plotter. After 24h, 7, 14, 21 and 28 days of cell culture on a hierarchical scaffold, viability and gene expression of C2C12 cells were evaluated. Live/Dead assay showed that the average viability of cells in almost all structures was 90% within 24h after printing which significantly increased in both fibrinogen-based hydrogel, fibrinogen hydrogel and PCL in the other time points. Molecular biology results indicated that, at 7 and 14 days of culture, muscle genes are more expressed in control cells with respect to cells growth in the bioinks and in the bioink co-printed with PCL. At 21 and 28 days C2C12 cells bioprinted in fibrinogen-based hydrogel expressed in a statistically significance myogenic genes, indicating the beginning of the differentiation process. Hence, we have demonstrated a positive effect of PCL and Fibrin hydrogel co-printing in mimicking the structural properties of muscular tissue, in particular topographical cues to guide cell orientation. Further studies will be necessary to ameliorate the geometry of the PCL and to design a device capable to promote myotube formation.

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Development and characterization of a tumor stromal microenvironment and analysis of its interaction with breast cancer cells: an in vitro model to study breast cancer associated fibroblasts inactivation

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Breast cancer associated fibroblasts (BCAFs) represent the most abundant cell type among non-cancer stromal cells of the breast tumor microenvironment (TME). It is known that BCAFs, by interacting with cancer cells, contribute dramatically to breast cancer growth and dissemination. This cell type, as well as myofibroblasts, displays up-regulation of activation and inflammation markers represented by α-smooth muscle actin (α-SMA) and cyclooxygenase 2 (COX-2). Moreover, BCAF aggregates have been detected in the peripheral blood of patients with metastatic breast cancer. Therefore, we have developed and characterized an in vitro stromal model constituted by BCAF monolayers, BCAF aggregates, such as spheroids collected after 72 or 216 h of three dimensional (3 D) culture, and BCAFs reverted to adhesion growth after 216 h of 3 D culture (reverted-BCAFs). BCAF spheroids collected at 216 h display an inactivated phenotype as indicated by a significant reduction of α- SMA and COX-2 proteins, compared with control BCAF monolayers. The deactivated phenotype of BCAF spheroids collected at 216 h is confirmed by the cytostatic effect of their conditioned medium on MCF-7 cells. It is noteworthy that also reverted-BCAFs exhibit an inactivated phenotype linked to significant α-SMA protein level reduction compared with BCAF monolayers. Of note, the conditioned medium of reverted-BCAFs decreases significantly the migratory capability of MCF- 7 cells compared with the conditioned medium of BCAF monolayers. The study of this in vitro experimental model suggests that the activated phenotype of BCAFs is reversible and it could be a starting point to develop new therapeutic strategies targeting BCAFs.

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The role of hypusinated eIF5A in leiomyoma and leiomyosarcoma: a possible novel therapeutic target

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Myometrium (MM) can transform itself into leiomyoma (LM), the most common benign tumor of the uterus or into the leiomyosarcoma (LMS), a very rare malign tumor. LM etiology is not known. It is defined as typical fibrotic tissue. In fact, it is characterized by a large amount of extracellular matrix (ECM). Furthermore, ECM is a reservoir of cytokines, chemokines, angiogenic and inflammatory response mediators and growth factors that represent key molecules for the development and the growth of LM [1]. Moreover, ECM is tightly interconnected with cell membrane and inner cellular environment. In addition to this, we have recently demonstrated that omega-3 fatty acids can down-regulate the expression of genes involved in the mechanical signaling process in LM [2]. Baring in mind all this, in this study, we analyzed the role of the translation factor eukaryotic initiation factor 5A (eIF5A) in LMS, LM and MM. eIF5A is the only protein containing the hypusine residue and by means of the hypusine, obtained after a post-translational modification called hypusination, it plays an important role in tumors and in cell proliferation [3], it modulates macrophage activation [4] and so, it could have a key role in inflammation and fibrosis. First of all, the differences of hypusinated eIF5A quantity in LMS, LM and MM tissues and cell lines were assayed respectively by immunohistochemistry (IHC) and western blotting (WB) using anti-hypusine antibody. IHC revealed that LMS showed the highest level of hypusinated eIF5A, MM showed the lowest level of hypusinated eIF5A and LM showed an intermediate level of hypusinated eIF5A. The SK-LMS-1 LMS cell line, the A006-8 LM cell line and the A005-7 MM cell line were used for WB analysis. The WB confirmed the same pattern detected in the correspondent tissues by IHC. Besides this, since the N1-guanyl-1,7-diamineheptane (GC-7) is known as the specific inhibitor of the hypusination, SK-LMS-1 LMS, A006-8 LM and A005-7 MM cell lines were treated with 100 nM GC-7. The WB using anti-hypusine antibody showed that GC-7 impaired the levels of hypusinated eIF5A in every cell line. Subsequently, the proliferation of SK-LMS-1 LMS, A006-8 LM and A005-7 MM cell lines after the 100 nM GC- 7 treatment was assayed by MTT. The GC-7 reduced proliferation in every cell line. This study highlights that hypusinated eIF5A may play an important role in LM and LMS pathologies and it suggests hypusinated eIF5A as a possible therapeutic target for LM and LMS.

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Key words

Myometrium, leiomyoma, leiomyosarcoma, ECM, eIF5A, hypusine, hypusination, GC-7.

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Mitochondrial shape and function in C2C12 myotubes

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It is well known that mitochondria play an essential role in cell energetic homeostasis. Many studies have been carried out on mitochondrial cristae morphology, as well. Nonetheless, studies on the combination of these two features (cristae morphology and their functionality) have not been deeply investigated.

The aim of the present study is to correlate mitochondrial bioenergetics with cristae appearance.

To this end we used mouse myoblasts cell cultures (C2C12) that were differentiated into myotubes for 4-5 days. Mitochondrial oxidative phosphorylation (OXPHOS) has been assessed by Clark-type electrode on permeabilized myotubes tested with complex I and II substrates. On differentiated myotubes, treated with or without permeabilizing agents, mitochondrial cristae morphology has been evaluated by the osmium maceration technique, that allows three-dimensional observation of cristae at high resolution scanning electron microscopy (HRSEM).

As regards mitochondrial bioenergetics, we tested several concentrations of digitonin and chaconine (a new kind of cell permeabilizing agent) and the best concentrations were 0.003% for digitonin and 10μM for chaconine. Myotubes displayed a regular OXPHOS.

The basic mitochondrial morphology revealed pleomorphic mitochondria that were thin and elongated or short and roundish, with the shape of mitochondrial cristae that could be lamellar, tubular or both.

In summary, C2C12 myotubes revealed rather various mitochondrial shapes, that were not discernible by transmission electron microscopy, and seem to have a normal OXPHOS. This is a preliminary study on basic mitochondrial bioenergetics and morphology of this cell line that will be followed by an analogous study on the loss of function of specific mitochondrial proteins that regulate cristae formation.

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Mitochondria OXPHOS after acute hypoxia in trained rats

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The human body adjusts to low oxygen concentrations that are found at high altitudes, basically by increasing the autonomic nervous system tone and red blood cells production, through eritropoietin release. Recently, the use of devices that provide low oxygen air is spreading among athletes, because it is believed to improve their performances. The physiologic and metabolic events that occur at high altitudes have been studied deeply when chronic, but we ignore whether acute hypoxia in athletes is harmful.

The aim of the present work was to investigate on cardiac and brain mitochondrial bioenergetics after exercising in acute hypoxia and normoxia.

We used male Wistar rats that had been trained for 5 weeks, 1h/day, on a treadmill set at 35 cm/s. The day of the experiment they were allowed to run on the treadmill for 30 minutes in hypoxia (the same oxygen concentration as at 4000 mt.) or in normoxia. After euthanasia, we removed the brain and the heart and we isolated brain mitochondria, subsarcolemmal (SSM) and interfibrillar (IFM) heart mitochondria. Mitochondrial bioenergetics was assessed by Clark-type electrode, and we tested complex I (glutamate plus malate), complex II (succinate), complex III (durohydroquinone, DHQ), complex IV (tetramethyl-p-phenylenediamine, TMPD, plus ascorbate), lipid substrates for oxidative phosphorylation (OXPHOS) and dinitrophenol (DNP) to test uncoupled respiration with these substrates.

Brain mitochondria showed an increase of uncoupled respiration with the complex II substrate after acute hypoxia. Between heart mitochondria IFM only showed an impairment at the complex I after acute hypoxia, as ADP/O decreased compared with controls, whereas the respiratory control ratio increased in the same complex. These wo data suggest a mild uncoupling of hypoxic IFM at complex I.

Therefore, athletic training in acute hypoxia affects both brain and heart mitochondria bioenergetics at different complexes. In brain complex II is involved and in heart that impairment is restricted to IFM at complex I. Future studies will verify whether hypoxia is accompanied by ROS production and whether it affects mitochondria morphology, too.

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Biological effects of-cigarette fluids on human gingival fibroblasts (HGFs)

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In the last years, electronic cigarette (e-cigarette) use is becoming more prevalent and is particularly popular among adolescents and conventional smokers as a safer and cheaper alternative to traditional cigarettes. Indeed, e-cigarettes are promoted as a smoking cessation device and less dangerous way to self-administer nicotine than conventional cigarettes[1]. However, although all ingredients contained in e-cigarette liquid are approved as food additives and no combustion occurs, the impact of e-cigarettes on oral health is still unknown[2]. The goal of this work was to investigate the effects of e-cigarette liquids on primary human gingival fibroblasts (HGFs) and compare the effects of nicotine-containing fluid with the fluid itself. HGFs were treated with different concentrations (0–5 mg/mL) of fluids of e-cigarettes for different time intervals (0–72 h). Fluids were administered also after being vaped (e.g., warmed into the cartomizer). Cytotoxicity was analyzed with MTT assay. Apoptosis occurrence and Bax expression were evaluated with flow cytometry; ROS production was analyzed with fluorescence microscopy. Both nicotine-containing and nicotine-free fluids induced an increased ROS production after 24 h, along with an increased Bax expression, followed by apoptosis occurrence after 48 h of exposure. Thus, the cytotoxicity exerted on HGFs by e-cigarettes fluids was not entirely ascribable to nicotine. Since the e-cigarettes are advertised as a safer alternative to traditional ones, especially for the possibility of smoking nicotine-free fluids, further studies are necessary to clarify the effects of such compounds at cellular level.

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The hidden interface of the articular cartilage

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The articular cartilage is a distinctive feature of all mobile joints and has been for a long time the subject of extensive research because of its clinical relevance. From a structural standpoint it can be divided into three or four ill-defined regions, and the most superficial layer has already been shown to differ from the intermediate ones in both its collagen fibrils and its glycoconjugates (1,2). In the deepest portion the chondrocytes become hypertrophic, begin to express collagen type X and the matrix undergoes mineralization by replacing part of its water with carbonated hydroxyapatite.

The calcified cartilage is an essential connection of the articular cartilage with the underlying bone, not unlike the structure of the entheses; its surface (the mineralization front) is located in the depth of the tissue and is only revealed by a thin line (the *tidemark*). This surface is usually imaged only by histological cross-sections, which are an inadequate approach to the study of a surface and have an inherently limited resolution.

The present research was carried out by HRSEM and 3D reconstruction by a proprietary symmetrical neural network (3) and showed that

- chondrocytes become extremely hypertrophic and keep dividing until the matrix hardens and prevents further growth;
- at higher resolution the mineralization front looks much more rough and irregular than revealed by LM, resulting in a much better mechanical coupling;
- as suggested by a previous research (4) the mineralization takes place by formation of fractal-like spheroidal clusters approx. 2 μm wide that gradually coalesce, in a pattern different from any other calcified tissue.

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Key words

Cartilage, bone, extracellular matrix, SEM.

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Autophagy in chronic obstructive pulmonary disease: a modelling study

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Chronic obstructive pulmonary disease (COPD) is a severe inflammatory disease characterised by persistent airflow limitation. The most common risk factor for COPD is cigarette smoke with pulmonary emphysema involving epithelial cell and fibroblast alterations caused by smoking.

We explored the role of autophagy in COPD using bronchial epithelial cells (16HBE) and primary bronchial fibroblast cultures treated with the inflammatory media of activated U937 monocytes (CM) and analysed by qPCR and western blotting for the expression of the autophagy markers, at 4,

10 and 24 hours after exposure. Moreover, we assessed autophagy markers by immunohistochemistry (IHC) in bronchial biopsies of nonsmokers (CNS), smokers with normal lung function (CS), patients with mild-moderate and severe COPD and by ELISA test of protein extracts in lung specimens of CNS, CS and mild-moderate COPD.

In vitro exposure of bronchial cell cultures to CM of activated U937 revealed that LC3B expression was increased at both gene and protein level in HBE cultures and only at gene level in fibroblast cultures. Moreover, the expression of p62 was enhanced in bronchial fibroblast cultures CM treated, and in protein extracts of COPD patients compared to CS and CNS.

LC3B expression was also increased in bronchial biopsies of severe/very severe COPD compared to mild to moderate diseased patients, and in comparison with control non-smokers.

In conclusion, our *ex vivo* data demonstrated that autophagy markers are mainly increased in the more severe disease, and they are also altered in their function in milder COPD (p62 increase). *In vitro* data support the fact that macrophage-mediated inflammation playa role in the increase of autophagy markers.

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The expression of raf kinase protein inhibitor (rkip) on different histological types of leiomyoma

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Uterine leiomyomas are smooth muscle tumors of the uterus and represent the most common benign tumors of female reproductive tract [1]. A classification can be made according to histological characteristics of leiomyomas. We can distinguish into usual leiomyoma the most commons, atypical also called bizarre leiomyoma, that is characterized by polymorphic and bizarre nuclei; leiomyoma with high mitotic activity, cellular leiomyoma, lipoleiomyoma and leiomyosarcomas which are the rare malignant counterparts [2]. In addition, in 2014 the World Health Organization (WHO) introduced the so-called category of STUMP (smooth muscle tumors of uncertain malignant potential) which includes any lesion that is difficult to characterize. In particular, the bizarre leiomyoma is described as a benign histology but, since it has intermediate characteristics, it is often difficult to distinguish with certainty whether it is benign or malignant. For this reason, bizarre leiomyoma is often classified as a tissue belonging to the STUMP category. Raf Kinase Protein Inhibitor (RKIP) is considered primarily as a suppressor of the metastasis gene.

We evaluated the expression of RKIP in the different histotypes of leiomyoma, it is being reduces in high proliferating variety (such as in cellular leiomyoma and leiomyoma mitotically active or apoplectic) and in leiomyosarcoma by immunohistochemistry. RKIP expression is different among leiomyoma histotypes, and in metastatic tissues, such as leiomyosarcoma [3]. RKIP is present in smooth muscle cells and it is highly expressed in endothelial cells of the usual leiomyoma. In lipoleiomyoma, it has high expression in adipose cells with inflammation, this particular has been confirmed with immunohistochemistry for the CD68 which shows the presence of "crown like structures", typical structures of adipose cells with inflammation [4]. In cellular leiomyoma, the expression of RKIP is low and confirmed to endothelial cells. Interestingly it has a high expression in the bizarre cells of the bizarre leiomyoma, while it is completely absent in leiomyosarcoma. These results suggest that RKIP could be used as a marker that could aid in the distinction between bizarre leiomyoma that has a high expression of RKIP and leiomyosarcoma in which RKIP is absent. Therefore, RKIP could be used to evaluate and distinguish STUMP and help to discriminate between benign and malignant lesions.

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VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA DEI SOCI, 2019

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Verbale della seduta amministrativa e dell'assemblea generale dei soci della Società Italiana di Anatomia e Istologia (SIAI) tenutasi presso il centro congressi "Federico II" di Napoli.

In data 23 Settembre 2019, alle ore 17:30, in seconda convocazione, si è svolta l'Assemblea Generale dei Soci della Società Italiana di Anatomia e Istologia (SIAI), presso il Centro Congressi Federico Il di Napoli, in occasione del 73° Congresso Nazionale SIAI, con il seguente Ordine del Giorno:

1. Comunicazioni del Presidente.
2. Relazione del Tesoriere sul rendiconto finanziario del 2018 e sulla previsione finanziaria per il 2020. Relazione dei Revisori dei Conti.
3. Attività dei Collegi dei Docenti di Anatomia e di Istologia ed Embriologia: Relazione dei Presidenti o loro Delegati.
4. Aggiornamento sull'Italian Journal of Anatomy and Embryology.
5. Aggiornamento e presentazione del Sito web della SIAI.
6. Assegnazione Premio alla Carriera.
7. Assegnazione Premi Ricercatori under 40.
8. Assegnazione Premio Miglior Comunicazione Orale.
9. Prossimi Congressi Nazionali della SIAI e Congressi nazionali ed internazionali previsti per l'anno 2020; proposte di temi di relazione.
10. Proposta di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.
11. Proposta di istituzione di un Premio alla Ricerca.
12. Commemorazione dei Soci scomparsi.
13. Varie ed eventuali.

Presiede la riunione il Prof. Giuseppe Anastasi, Presidente della SIAI; funge da Segretario Verbalizzante la Prof. Gigliola Sica, Membro del Consiglio Direttivo della SIAI.

1. Comunicazioni del Presidente.

Il Presidente della SIAI, Prof. Giuseppe Anastasi, apre i lavori e porge il benvenuto ai Soci presenti in Assemblea e li ringrazia per essere intervenuti così numerosi. A nome di tutti ringrazia calorosamente i Presidenti del Congresso, Proff. Stefania Montagnani, Michele Papa e Gianpaolo Papaccio per la loro ospitalità e si complimenta per la splendida organizzazione dell'evento congressuale dichiarandosi sicuro che questo Congresso rimarrà a lungo nella mente di tutti i Congressisti come un evento davvero bellissimo. Successivamente, il Presidente, manifestando soddisfazione, comunica ai Soci che da oggi è visibile in rete il nuovo Sito della Società Italiana di Anatomia e Istologia e, per questo, ringrazia in particolare la Prof. Gigliola Sica,

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la Prof. Sandra Zecchi Orlandini e il Prof. Carlo Tacchetti per essersi impegnati nel lavoro di elaborazione del nuovo Sito. Come sarà ampiamente presentato al punto specifico all'OdG, il Presidente ricorda che sarà chiesta a ciascuna Sede la nomina di un Redattore di Sede, che avrà il compito di fornire tutti i dati relativi all'Anatomia e all'Istologia, in modo da poter costruire una mappa aggiornata dei Componenti della Società e delle loro peculiarità scientifiche e didattiche. Il Presidente comunica inoltre ai Soci che ha ripreso la pubblicazione dell'Italian Journal of Anatomy and Embryology con i numeri del 2019 e riferisce che sono in corso le trattative con la Firenze University Press per il rilancio della storica rivista che rappresenta l'organo ufficiale della SIAI e la preparazione di un nuovo formato di stampa consono alle esigenze delle banche dati internazionali. A tale proposito il Presidente precisa che questo aspetto sarà approfondito nello specifico punto all'OdG. Esprime inoltre, a nome di tutto il Consiglio Direttivo, che si è impegnato in quest'opera di rilancio, orgoglio ed i più sentiti ringraziamenti al Prof. Domenico Ribatti, Direttore Scientifico, e al Prof. Ferdinando Paternostro, Direttore Esecutivo della rivista. Il Presidente infine, prima di passare la parola al Tesoriere, Prof. Gianpaolo Papaccio, è lieto di comunicare all'Assemblea che sono pervenute durante l'anno numerosissime richieste di iscrizione alla SIAI.

2. Relazione del Tesoriere sul rendiconto finanziario del 2018 e sulla previsione finanziaria del 2020. Relazione dei Revisori dei Conti.

Il Presidente legge il verbale stilato nella riunione dei Revisori dei Conti, Proff. Monica Mattioli Belmonte, Domenico Ribatti e Luca Tamagnone, tenutasi il 23 Settembre 2019.

"La Commissione dei Revisori dei Conti della Società Italiana di Anatomia e Istologia, nelle persone dei Proff. Monica Mattioli Belmonte, Domenico Ribatti e Luca Tamagnone, ha esaminato il Bilancio Consuntivo per l'anno 2018 e quello Preventivo per l'anno 2020 e le rispettive relazioni di accompagnamento.

La Commissione ne approva integralmente il contenuto."

Il Presidente dà la parola al Tesoriere, Prof. Gianpaolo Papaccio, che illustra il rendiconto finanziario del 2018 qui di seguito riportato insieme alla relazione di accompagno.

Bilancio consuntivo anno 2018

Causale delle entrate	Entrate	Causale delle uscite	Uscite
Quote sociali incassate nel corso dell'anno 2018 (n° 282) 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	**€ 16.920,00**		
		Elenco spese per attività statutarie	
		Quote di Iscrizione al Congresso SIAI 2018 di due Soci vincitori dei premi poster anno 2017	**€ 561,00**

		Contributo per l'organizzazione del Convegno G.I.S.N. anno 2018	**€ 500,00**
		Premi alla Carriera 2018 (targa) e pergamena premio giovane ricercatore 2018	**€ 400,00**
		Spese varie (mantenimento conto corrente postale e bancario, spese bollo e commissioni bancarie ecc.) anno 2018	**€ 597,54**
		Rimborso spese per partecipazione alla riunione EFEM e alla riunione per la Terminologia Anatomica anno 2018	**€ 682,00**
		Premio "Giovane Ricercatore" anno 2018"	**€ 2.000,00**
		Iscrizione della SIAI a IFAA anno 2018	**€ 120,00**
		Iscrizione della SW a EFEM anno 2018	**€ 375,00**
		Elenco spese di funzionamento	
		Compenso per Consulenza Commercialista relativa alla stesura del bilancio consuntivo anno 2017 e bilancio previsionale anno 2019	**€ 1.500,60**
		Spese relative all'utilizzo del server UNIFI per il sito web SIAI anno 2017	**€ 407,00**
		Spese per il funzionamento del Consiglio Direttivo, anno 2018	**€ 1.154,00**
Totale entrate	**€ 16.920,00**	**Totale uscite**	**€ 8.297,14**
Avanzo d' esercizio finanziario 2018	**€ 8.622,86**		
Saldo Conto corrente Bancario al 31/12/2017	**€ 14.719,38**		
Saldo Conto corrente postale al 31/12/2017	**€ 5278,69**		
Totale saldo finanziario al 31/12/2017	**€ 19.998,07**		
Avanzo dell'esercizio finanziario 2018	**€ 8.622,86**		
Saldo finanziario al 31/12/2018	**€ 28.620,93**		

Stanziamenti impegnati al 31/12/2018	**Euro**	**Euro**
Accantonamento premi poster e comunicazione anno 2018		**€ 2.000,00**
Spese per sito WEB SIAI anno 2018		**€ 300,00**

Contributo all'It.J. Anat. Embryol. anno 2018		**€ 4.000,00**
Spese per ECM anno 2018		**€ 500,00**
Spese per il funzionamento della Presidenza anno 2018		**€ 1.000,00**
Spese per il funzionamento della segreteria anno 2018		**€ 1.000,00**
Spese per il funzionamento della Tesoreria 2018		**€ 1.000,00**
Compenso per Consulenza Commercialista relativa alla stesura del bilancio consuntivo anno 2018 e bilancio previsionale anno 2020		**€ 1.780,00**
Totale impegno di spesa		**€ 11.580,20**
Saldo disponibile	**€ 17.040,93**	

Relazione di accompagnamento al rendiconto economico e finanziario per l'anno 2018

Come risulta dal bilancio consuntivo, il saldo finanziario al 31/1212018 è pari ad € 28.620,93

A tale importo devono essere sottratti € 11.580,00 impegnati nel bilancio previsionale del 2018, ma non ancora effettivamente utilizzati alla data del 31/12/2018 per le seguenti voci di spesa:

- **Accantonamento premi poster e comunicazione anno 2018: € 2.000,00;**

Per questa voce risultano stanziati, nel previsionale del 2018, € 2.000,00 che per € 1.000,00 sono stati resi disponibili ma non incassati nel corso del 72° Congresso Nazionale della Società del 2018 per il premio alla migliore comunicazione e, nella parte rimanente (€ **1.000,00),** saranno utilizzati per il pagamento delle quote di iscrizione al 73° Congresso Nazionale della Società del 2019 di due Soci risultati vincitori dei premi poster nel Congresso societario del 2018; inoltre, a tale impegno, vanno sommati € 500,00 relativi all'impegno dell'anno precedente (2016) per la stessa voce e non utilizzati perché un premio poster era stato congelato con parere favorevole del Direttivo SIAI, motivato dalla impossibilità di partecipazione del vincitore al congresso 2018.

- **Contributo all'lt.J. Anat. Embryol.** per l'anno 2018: € **4.000,00;**
- **Spese per il sito web della Società** anno 2018 € **300,00** ;
- **Spese per ECM** anno 2018 € **500,00;**
- **Spese per il funzionamento della Presidenza € 1.000,00;**
- **Spese per il funzionamento della Segreteria € 1.000,00;**
- **Spese per il funzionamento della Tesoreria € 1.000,00;**
- **Consulenza Commercialista** anno 2018: € **1.780,00.**

Pertanto l'anno 2018 si chiude con un **saldo disponibile** di € **17.040,93.**

Durante il 2018 le quote associative incassate sono state **282** comprese alcune quote arretrate ed integrazioni di versamenti di quote non corretti, per un totale di € **16.920,00,** che sommate al saldo finanziario al 31/12/2017 pari ad € **19.998,07** hanno dato la disponibilità di € **36.918,07**

Le voci relative alle competenze di liquidazione del conto Bancoposta e del conto corrente Unicredit sono risultate negative e sono considerate nel totale della voce spese varie (mantenimento conto corrente postale e bancario, ecc.)

Le entrate hanno permesso di coprire le spese previste e non previste, includendo i fondi impegnati e non erogati.

La rispondenza dei Soci ai solleciti da parte del Tesoriere in merito alla regolarizzazione dei pagamenti delle quote associative si è rivelata buona; comunque, al 31 dicembre 2018, rimane ancora un certo numero di Soci che debbono regolarizzare la loro posizione. Il Tesoriere sottolinea che l'eventuale recupero delle quote arretrate consentirebbe alla SIAI di effettuare una adeguata programmazione delle attività statutarie e di intraprendere nuove iniziative

Il Prof. Papaccio procede ad illustrare il Bilancio Preventivo del 2020 qui di seguito riportato insieme alla relazione di accompagno.

Previsione finanziaria 2020

SOCI NEL 2018:	**392**
SOCI NEL 2019:	**391**
SOCI ORDINARI 2019*:	**368**
SOCI DIMISSIONARI/ CANCELLATI/DECEDUTI 2019:	**43**
SOCI EMERITI/ONORARI:	**23**

*compresi i nuovi soci in numero di 41 ammessi sino al Direttivo di Giugno 2019

ENTRATE

Quote sociali anno 2020 **(n. 368)**	€	22.080,00
Quote sociali arretrate 2015 - 2019	€	9.400,00
Totale entrate	**€**	**31.480,00**

USCITE

Contributo al Convegno Nazionale 2020, atti di convegni, altri contributi a convegni, partecipazione a convegni, organizzazione eventi scientifici, borse di studio, etc.	€	10.000,00
Accantonamento per premi poster dell'anno 2019 e per premio comunicazione assegnato nell'anno 2018	€	2.000,00
Accantonamento per premi SIAI (Premio alla Carriera e Premio Ricercatore under 40), anno 2020	€	4.000,00
Contributo all' Italian Journal of Anatomy and Embryology, anno 2020	€	4.000,00

Spese per sito web della Società, anno 2020	€	500,00
Spese per la partecipazione Meeting Comitato Internazionale per la Terminologia Anatomica e Istologica, FICAT, anno 2020	€	2.000,00
Quota adesione all'European Federation for Experimental Morphology, EFEM, anno 2020	€	400,00
Quota adesione all'International Federation of Anatomical Associations, IFAA, anno 2020	€	200,00
Spese varie (bancarie, postali, necrologi, etc.), anno 2020	€	800,00
Spese impreviste, anno 2018	€	1.000,00
Totale spese per attività statutarie	**€**	***24.900,00***
Spese per il funzionamento (Segreteria, Tesoreria, Presidena e Direttivo)	€	4.800,00
Spese per consulenza Commercialista, anno 2019	€	1.780,00
Totale spese di funzionamento	**€**	***6.580,00***
Totale uscite	**€**	**31.480,00**

Relazione di accompagnamento alla previsione finanziaria per l'Anno 2020

La chiusura del bilancio consuntivo del 2018 con un saldo disponibile di € **17.040,93** ha permesso al Tesoriere di sostenere alcune spese indicate nella Previsione Finanziaria del 2019.

Il Tesoriere , nel corso di questo anno, oltre a cercare di riscuotere le quote associative del 2019, ha continuato l'azione di recupero di quelle arretrate. Al 31 agosto 2019, sono state incassate 428 quote sociali comprensive di quelle relative all'anno in corso e arretrate. È probabile che nella prima metà di Settembre altri Soci abbiano provveduto al pagamento e che non siano stati considerati in questo resoconto.

Il totale delle entrate è attualmente pari a € **27.552,57** e comprende le quote riscosse finora.

Comunque il piano previsionale del 2019 prevedeva entrate pari a € **35.280,00,** 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 ed evidentemente si ipotizzava che i Soci che si erano dichiarati dimissionari fossero disponibili a regolarizzare la loro posizione.

La Società conta attualmente 391 Soci, di cui 368 Soci Ordinari e 23 Soci Emeriti o Onorari (esonerati dal pagamento della quota Sociale). Nel corso del 2019, 11 Soci Ordinari hanno espresso la volontà di rassegnare le dimissioni dalla Società; 32 Soci Ordinari sono stati cancellati poiché o in debito da più di 8 anni o deceduti.

Su tale punto occorre assolutamente che i soci non in regola e morosi dal 2015 ad oggi siano chiamati singolarmente onde definire la loro situazione anche perché non è corretto che si giunga ad un aumento della quota sociale in presenza di un discreto numero di soci non in regola da molti anni.

Allo stato attuale, dei 368 Soci Ordinari che sono tenuti a pagare la quota associativa:

- 65 Soci sono in regola fino al 2019;
- 26 nuovi Soci dal 2019 che devono ancora versare la quota sociale dell'anno in corso; 155 Soci sono in regola fino al 2018 devono la quota 2019;
- 71 Soci in pari con il 2017 devono la quota 2018 e la quota 2019;
- 41 Soci in pari con il 2016 devono le quote del 2017, 2018 e la quota 2019;
- 10 Soci in pari con il 2015 devono le quote del 2016, 2017, 2018 e la quota 2019.

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 2019 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 e al parere in merito espresso dal 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.

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 dalla Giunta tutta.

3. Attività dei Collegi dei Docenti di Anatomia e di Istologia ed Embriologia: Relazione dei Presidenti o loro Delegati.

Il Prof. Raffaele De Caro, Presidente del Collegio dei Docenti di Anatomia, presenta la relazione sull'attività svolta dal Collegio nell'anno accademico in corso. Le riunioni si sono svolte il 20 Febbraio 2019 in seduta plenaria a Roma, il 30 Aprile e 1° Maggio in forma di Consiglio Direttivo per via telematica, il 17 Giugno 2019 in occasione dell'evento organizzato su iniziativa della Senatrice Paola Binetti intitolato *"Donazione Del Corpo Post Mortem a Fini Didattici e di Ricerca"* , svolto nella sala Zuccari del Senato a Roma. Tale evento è stato introdotto dall'Onorevole Pierpaolo Sileri, Presidente della Commissione Igiene e Sanità Pubblica del Senato della Repubblica, e il DDL intitolato "Norme in materia di disposizione del proprio corpo e dei tessuti post-mortem a fini di studio, formazione e di ricerca scientifica" è stato presentato dal Senatore Giuseppe Pisani, relatore del provvedimento. Sono intervenuti i seguenti membri del Collegio dei Docenti di Anatomia: i Proff. Giuseppe Pio Anastasi, Raffaele De Caro, Giuseppe Familiari, Lucia Manzoli e Sergio Morini. Successivamente si è svolta una tavola rotonda con i contributi dei Proff. Rocco Bellantone, Marco Caricato, Fabrizio Consorti, Giuseppe Petrella e Massimo Rossi.

Le principali comunicazioni date nel corso delle riunioni sono state: a. Relazioni sulla partecipazione all'attività della Conferenza dei Presidenti dei Collegi dell'Area Medica; b. incontro con il Prof. Paolo Pedone, candidato per l'Area 05 alle prossime elezioni CUN.

I principali documenti prodotti sono stati:

a. Parere del Collegio dei Docenti di Anatomia Umana sul Disegno di Legge "Norme in materia di disposizione del proprio corpo e dei tessuti post-mortem a fini di studio, formazione e di ricerca scientifica" approvato dal Senato della Repubblica.

La prossima riunione è programmata per Febbraio 2020.

Il Presidente della SIAI, Prof. Anastasi, invita il Prof. Papaccio, nella sua qualità di Presidente del Collegio dei Docenti di Istologia ed Embriologia a prendere la parola.

Il Prof. Papaccio ringrazia ed illustra brevemente all'Assemblea l'attività del Collegio durante quest'anno (da Settembre 2018 a Settembre 2019). Egli riferisce che la Giunta si è riunita per sei volte e che l'Assemblea annuale si è tenuta nel Febbraio 2019.

La prima riunione della Giunta è stata allargata a tutti i Professori Ordinari (PO) ed è stata effettuata al solo fine di voler chiarire a tutti i PO sorteggiabili per l'ASN quali fossero i punti critici, nel pieno rispetto dell'autonomia della Commissione, specie riguardo ai titoli.

L'Assemblea si è svolta alla presenza di numerosissimi soci.

È stato presente il Prof. Pedone, candidato al CUN area 05, che Istologi ed Anatomici hanno appoggiato.

Il Prof. Anastasi lo ebbe ad accompagnare. È seguita l'Assemblea che si è occupata di numerosissime questioni e nel pomeriggio si è svolta la Seduta Scientifica.

Le altre riunioni della Giunta sono state volte a:

- produrre documenti contro le voci e le iniziative dirette all'eliminazione del numero programmato a Medicina;
- produrre documenti contro iniziative di alcune sedi che volevano sperimentare impattando sulla autonomia dell'Istologia;
- completare il core curriculum della disciplina;
- produrre un documento sulla presenza della Istologia nelle Scuole di Specializzazione;
- stimolare le varie sedi prive di PO a voler porre in essere tutte le iniziative utili al fine di chiamare le prime fasce.

Circa quest'ultimo punto il Prof. Papaccio fa presente che in questo lasso di tempo hanno preso servizio ben 5 PO in Istologia. Questo numero ha solo parzialmente coperto i pensionamenti. In corso vi sono due procedure ed una è in avvio. Con esse si copriranno le 3 quiescenze che si avranno entro novembre 2020, per cui l'aumento del numero degli Ordinari non si è potuto realizzare, pur se ben 11 nuovi PO sono entrati in servizio negli ultimi 2 anni, il che ci fa ben sperare e ci rallegra per il lavoro svolto, che resta ancora da portare avanti specie in talune specifiche sedi.

Il Prof. Papaccio infine puntualizza che numerosi Istologi (pur se anche alcuni Anatomici) non sono soci della SIAI, per cui si impegnerà a chiedere a tutti di voler meditare bene se intendano o meno che "questa" sia per tutti la casa comune.

4. Aggiornamento sull'Italian Journal of Anatomy and Embryology.

Il Presidente riferisce, così come anticipato nelle Comunicazioni, che il Consiglio Direttivo ha nominato Direttore Scientifico dell'Italian Journal of Anatomy and Embryology il Prof. Domenico Ribatti e Direttore Esecutivo il Prof. Ferdinando

Paternostro e li ringrazia per aver accettato la nomina e per l'impegno e la passione dedicati per far ripartire la pubblicazione della rivista. Il Presidente infatti comunica che proprio in questi giorni uscirà il nuovo numero dell'Italian Journal of Anatomy and Embryology con oltre venti contributi scientifici e che successivamente verranno pubblicati un altro o altri due numeri al fine di dare esito positivo ai lavori accettati ma non pubblicati. Per quanto riguarda il futuro della rivista, è confermata la volontà del Consiglio Direttivo a proseguire nell'operazione di rilancio della stessa e il Presidente riferisce che, insieme al Prof. Ribatti e al Prof. Paternostro ed ai vertici e tecnici della Firenze University Press, si sta valutando la possibilità di dare vita ad una nuova edizione dell'Italian Journal che poggi su una moderna piattaforma informatica, capace di garantire la tracciabilità dei processi revisionali dei contributi scientifici e di riversare i dati sulle più importanti banch.e dati al pari delle altre più prestigiose riviste scientifiche internazionali. A questo proposito, il Prof. Anastasi ringrazia il Magnifico Rettore dell'Università di Firenze per la grande disponibilità dimostrata. Tuttavia, il Presidente afferma che, se tale accordo non si dovesse concretizzare, si valuterà la possibilità, in accordo con l'Università di Firenze, di affidare la pubblicazione della rivista ad altro publisher e pertanto ha chiesto al Prof. Amelio Dolfi di esplorare la possibilità di stipulare un accordo con la Pisa University Press; su questo punto si sono espressi favorevolmente il Prof. Ribatti e il Prof. Paternostro.

5. Aggiornamento e presentazione del Sito web della SIAI.

Il Presidente Comunica che nel Consiglio Direttivo della SIAI, tenutosi in data 26 Gennaio 2019, è stato deliberato un rinnovamento del Sito web, anche in seguito alla impossibilità di proseguire con il vecchio Sito e con l'hosting che verranno prossimamente dismessi d'ufficio. A questo scopo sono stati richiesti dei preventivi (Allegati 1, 2 e 3) e, in base alla migliore offerta, è stata selezionata la Ditta PHOOPS di Firenze. In primo luogo, a seguito di incontri sia in presenza che a distanza del Presidente e della Prof. Zecchi con gli esponenti della Phoops è stata definita l'organizzazione gerarchica del Sito, con la descrizione dei ruoli e degli accessi alla gestione del sito stesso. Successivamente, la Prof. Zecchi, con la collaborazione dei Proff. Sica e Tacchetti, che il Presidente ringrazia, ha definito quale dovesse essere il layout con la richiesta di immagini per le diverse finestre ed è stato chiesto a tutti i Soci di inviare i dati relativi alla propria Sede secondo un formato diffuso dal Segretario, Prof. Roberto Di Primio.

Il Presidente dà la parola alla Prof. Zecchi che procede alla presentazione della versione provvisoria del Sito con proiezione delle immagini relative. Allo stato il Sito, che presenta uno sfondo di microscopia confocale del sistema nervoso, vede una breve storia della SIA! con Statuto e Regolamento ed indicazioni delle Quote di Iscrizione e delle Modalità di Pagamento; la Composizione del Consiglio Direttivo; l'Elenco dei Soci; il Bilancio; una sezione dedicata all'Italian Journal of Anatomy and Embryology; la Struttura, la Sede, i Servizi ai Soci, lo Statuto dei Collegi degli Anatomici e dei Docenti di Istologia ed Embriologia (per quest'ultimo è stato inserito uno sfondo ad hoc); l'indicazione degli Eventi e dei Congressi, le Notizie sulle Sedi (che potrebbero includere l'attività didattica e scientifica); le Notizie Scientifiche dai Soci, le Notizie in evidenza ed ovviamente l'indirizzo del sito: https://siai.phoops.it/.

6. Assegnazione Premio alla Carriera.

Il Presidente comunica che il Consiglio Direttivo della SIA!, sulla base delle proposte pervenute, ha all'unanimità deliberato l'attribuzione del Premio alla Carriera al Prof. Saverio Cinti dell'Università Politecnica delle Marche. Il Prof. Cinti ha sempre mantenuto centrale l'aspetto morfologico ed ultrastrutturale confermando ed implementando le sue osservazioni con tutte le più recenti tecniche bio-funzionali ed elevando il ruolo dell'osservazione morfologica a strumento essenziale nelle attività di ricerca e di diagnostica. Il Prof. Cinti è autore di numerosi testi a carattere didattico-scientifico, di oltre 300 lavori originali pubblicati su riviste internazionali nonché detentore di brevetti nazionali ed internazionali.

Il Presidente consegna il Premio al Prof. Cinti, che ringrazia sentitamente la Società anche per avergli dato per 4 volte la possibilità di tenere una plenary lecture.

7. Assegnazione Premi Ricercatori under 40.

Il Presidente comunica che nel Direttivo del Giugno 2019 sono stati designati i Commissari per la valutazione dei curricula nelle persone dei Proff. Antonio Filippini, Stefania Montagnani e Carlo Tacchetti. Il Presidente comunica che sono pervenute 9 domande che sono state affidate alla Commissione ed invita il Prof. Tacchetti a dare lettura dei risultati e delle motivazioni. Il Prof. Tacchetti comunica che sono state selezionate le domande dei Dott. Alberto Cacciala e Saverio Marchi. Il Dott. Cacciala dell'Università di Messina, tramite l'utilizzo di imaging avanzato di risonanza magnetica, combinato con sofisticate metodiche di modeling del segnale e di ricostruzione trattografica, ha identificato nuovi pattern di connettività strutturale tra i nuclei della base, la corteccia cerebrale ed il cervelletto , caratterizzandone l'organizzazione topografica e analizzandone le implicazioni fisiopatologiche nella pratica clinica. Il Dott. Marchi, dell'Università Politecnica delle Marche, si è interessato dei meccanismi molecolari coinvolti nel trasporto del calcio a livello mitocondriale e del loro coinvolgimento nel controllo della proliferazione cellulare in modelli normali e tumorali. Il Presidente consegna i Premi.

8. Assegnazione Premio Migliore Comunicazione Orale.

La Commissione per l'Attribuzione del Premio per la Migliore Comunicazione Orale, nominata dal Presidente della SIAI e composta dai Proff. G.C. Panzica, G. Cavaletti ed F. Michetti (assenti giustificati i Proff. Fabene, Papa e Rezzani) hanno ripercorso le note prese durante le comunicazioni orali ed hanno selezionato le due presentazioni migliori del mattino e del pomeriggio. Per la sessione mattutina si è scelta la comunicazione Di F. Girolamo e coli. soprattutto per la iconografia estremamente chiara e curata. Per la sessione pomeridiana è stata selezionata la comunicazione di F. Casoni e coli. per l'argomento di attualità e per le modalità di presentazione dei risultati ottenuti. Non potendo assegnare un premio ex-aequo (come nella prima intenzione), i Commissari hanno ritenuto la presentazione di F. Casoni et al. più meritevole. Pertanto la vittoria è stata attribuita alla comunicazione di Filippo Casoni, Laura Croci, Francesca Vincenti, Ottavio Cremona, Giacomo Consalez, dal titolo: "Severe malformation of the fourth ventricle coroid plexus in Zfp423 mutants".

Il Presidente consegna il premio.

9. Prossimi Congressi Nazionali della SIAI e Congressi Nazionali ed internazionali previsti per l'anno 2020. Proposte di temi di relazione.

Il Presidente comunica che il prossimo Congresso Nazionale SIAI si svolgerà a Bologna e sarà organizzato dai Proff. Lucio Cocco e Lucia Manzoli e sollecita le candidature per il Congresso del 2021.

Comunica altresì che dal 15 al 16 Novembre 2019 si svolgerà a Bari il XXIX Convegno organizzato dal GISN.

10. Proposta di ammissione nuovi Soci e proposte per Soci Emeriti e Onorari.

Il Presidente comunica che sono pervenute 35 domande di ammissione a Socio SIAI da parte di:

1. Barbiera Alessandra
2. Belviso Immacolata
3. Calì Corrado
4. Camaioni Antonella
5. Cappelletti Graziella
6. Caruso Bavisotto
7. Celeste Cavedon
8. Valentina Ciccocioppo
9. Fausta Cirillo Giovanni
10. Diomede Francesca
11. Fucarino Alberto
12. Germano Giovanni
13. Giovarelli Matteo
14. Guarnieri Giulia
15. Lombardo Claudia
16. Lucariello Angela
17. Marchi Saverio
18. Marmiroli Sandra
19. Maugeri Grazia
20. Noli Barbara
21. Pergolizi Simona
22. Perna Angelica
23. Pierdomenico Laura
24. Pitruzzella Alessandro
25. Ricci Giulia
26. Romano Veronica
27. Sacco Annamaria
28. Sapte Elena
29. Scalia Federica
30. Schellino Roberta
31. Sirico Felice
32. Sorrentino Silvia
33. Spinoso Giulio
34. Tamagnone Luca
35. Viscomi Maria Teresa

Come previsto dallo Statuto, tutte le domande presentano la firma di presentazione da parte di due Soci.

L'Assemblea approva all'unanimità tutte le richieste sopra riportate.

11. Proposta di istituzione di un Premio alla Ricerca.

Il Presidente comunica che il Consiglio Direttivo della SIAI ha deliberato di istituire un Premio di eccellenza da conferire ad un Ricercatore la cui attività scientifica sia di elevata qualità. Il Premio potrà essere attribuito sia ai Soci che ai non Soci.

L'Assemblea approva all'unanimità.

12. Commemorazione dei Soci Scomparsi.

Il Prof. Amelio Dolfi commemora il Prof. Mario Lupetti, scomparso nel Marzo 2019. La Prof. Zecchi Orlandini commemora il Prof. Paolo Pacini, scomparso nel Luglio 2019, leggendo un ricordo scritto dal Prof. Giovanni Orlandini.

L'Assemblea osserva un minuto di raccoglimento.

13. Varie ed eventuali.

Il Presidente comunica che le Commissioni deputate all'attribuzione dei due Premi per i migliori Poster non hanno ancora terminato il loro lavoro, ragion per cui i risultati saranno comunicati successivamente.

Il presente verbale viene approvato seduta stante dall'Assemblea.

Il Presidente ringrazia i presenti anche a nome del Consiglio Direttivo e, alle ore 19:00, dichiara conclusi i lavori dell'Assemblea.

Il Presidente	Il Segretario	Il Tesoriere
Prof. Giuseppe Anastasi	*Prof. Gigliola Sica*	*Prof. Gianpaolo Papaccio*

ALLEGATO N.1

K.Group
orange company

Offerta commerciale

Redesign sito web della Società Italiana di Anatomia e Istologia completo di Content Management System e di un'area privata dedicata alla condivisione di file e informazioni tra gli utenti registrati.

K.Group
orange company

Tabella dei Contenuti

K.Group

Executive summary

La business proposal in oggetto riporta indicazioni circa il redesign del sito della Società Italiana di Anatomia e Istologia (d'ora in poi SIAI), del *Content Management System* (CMS) e della *private area* del sito che consente agli utenti abilitati di condividere documenti, contatti, note e informazioni generiche con tutti gli utenti registrati del sito.

L'obiettivo del progetto è quello di ridisegnare il sito web della SIAI secondo gli attuali standard web e secondo i criteri di accessibilità definiti dal W3C in conformità con la normativa vigente.
I contenuti attualmente presenti sul sito Web SIAI verranno integrati nella nuova versione e saranno consultabili da qualunque tipo di dispositivo che sia dotato di un moderno browser web grazie ad un design *responsive*.
Il CMS fornito consentirà agli utenti abilitati di creare pagine, modificare contenuti, pubblicare immagini e contenuti multimediali.
Ogni utente registrato sarà dotato di un'area privata tramite la quale potrà condividere con altri utenti registrati documenti e informazioni.

Le sezioni seguenti del presente report riportano la proposta tecnica (sezione 1) e la proposta commerciale (Sezione 2).

Proposta tecnica

La sezione riporta le specifiche tecniche riguardanti il sito web SIAI completo di area privata di condivisione e collaborazione.

Lo strumento di pubblicazione utilizzato nel progetto sarà il CMS open Source Drupal
Il design del sito seguirà i principali trend attuali (responsive design, accessibilità, mobile first).
IL CMS consentirà all'utente amministratore di gestire tutti gli aspetti del sito (organizzazione dei contenuti, pagine pubblicate e voci presenti nel menù di navigazione).

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L'utente amministratore avrà la possibilità di definire altri utenti abilitati che, seppur non avendo i suddetti privilegi, potranno produrre e pubblicare dei contenuti specifici del sito a seconda del ruolo a loro assegnato.
Il progetto consentirà lo sviluppo di una area privata integrata al sito web SIAI che potrà essere gestita dall'utente amministratore che avrà la facoltà di creare degli *workplaces* organizzati gerarchicamente.
A discrezione dell'amministratore, l'accesso a queste aree potrà essere consentito anche ad altri utenti o gruppi di utenti. Nelle suddette aree sarà possibile scambiare contenuti multimediali di vario genere e definire le attività che vengono assegnate a specifici utenti.
L'area privata consente inoltre di pubblicare delle news di carattere generale che restano visibili solo agli utenti registrati e che potranno essere commentate tramite appositi forum.

Proposta commerciale

Sulla base delle attività previste e stimando le risorse necessarie, la miglior offerta formulabile per la realizzazione del sito web completo di CMS e di area privata con spazi di condivisione dedicati agli utenti registrati è pari a euro **9000 (novemila/00).**

I termini dell'offerta prevedono che tutti i prezzi siano da intendersi **IVA esclusa.** Le relative condizioni di pagamento risultano momentaneamente ancora da definire.

La presente offerta è da ritenersi **valida per un periodo di 60 giorni** dalla data di sua emissione.

Letto, approvato e sottoscritto

Perugia. 05.10.2018

K.Group srl

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ALLEGATO N.2

OPENBOX

Offerta Commerciale

Redesign sito web della Società Italiana di Anatomia e Istologia completo di Content Management System

Area riservata del sito dedicata alla condivisione di file e informazioni tra gli utenti

OPENBOX
Via Sesta Strada Poggiolupi 343 – 52028 Terranuova Bracciolini (AR) – Italia
P.IVA 01952550513 – Tel. 055 91 98 312 – Fax 055 97 30 870
email: info@open-box.it – www.open-box.it

OPENBOX

1. Summary dei contenuti

La presente Business Proposal dettaglia le caratteristiche relative al redesign del sito web della *Società Italiana di Anatomia e Istologia (SIAI)*, del *Content Management System* (CMS) finalizzato alla gestione del sito e dell'area riservata del sito stesso. Tramite tale area l'utente registrato potrà condividere documenti, note, contatti e generiche informazioni con altri utenti registrati sul sito.
Il processo di re-design del sito web SIAI si atterrà agli attuali standard web e ad i criteri di accessibilità definiti dalle norme che attualmente regolano la materia.
Ii contenuti della versione attuale del sito verranno integrati nella nuova versione e verranno gestiti tramite un *CMS* che consentirà agli utenti di creare pagine, modificare e pubblicare contenuti multimediali (come video e immagini) ed indicare altri utenti abilitati alla pubblicazione. Il sito verrà fornito di un'area riservata, dedicata ai soli utenti registrati, che consentirà la creazione di *workplaces* per la condivisione di documenti informazioni e attività con altri utenti registrati.

2. Proposta tecnica

Le successive sezioni presentano in maggiore dettaglio le specifiche tecniche riguardanti sia il *redesign* del sito web SIAI (Sezione 2.1), sia la creazione dell'area riservata di condivisione (Sezione 2.2).

2.1. Sito web SIAI

Per pubblicare e gestire i contenuti del sito verranno impiegati il CMS open source OpenCms, basato sulla tecnologia Java, completo delle approriate estensioni e plugin. Il processo di redesign del sito sarà conforme ai moderni standard web (responsive design, accessibilità e mobile first).
Grazie all'utilizzo del CMS l'utente definito come 'amministratore' potrà gestire qualsivoglia aspetto del sito, comprensivamente delle pagine pubblicate, dell'organizzazione dei contenuti e delle voci presenti nei vari livelli del menu di navigazione.

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L'utente amministratore sarà anche in grado di definire altri utenti che, pur non definiti come amministratori, avranno la possibilità di pubblicare contenuti specifici all'interno del sito a seconda del ruolo assegnato.

2.2 Area riservata di collaborazione e condivisione

Per l'implementazione dell'area privata verrà utilizzato il software open source *Alfresco*, nella versione Community Edition. Alfresco consente di gestire ogni tipo di contenuto, consentendo agli utenti registrati di condividere documenti, collaborare alla loro modifica, scambiare informazioni.
Tutti gli utenti che abilitati dall'amministratore del sito disporranno di un'area privata nella quale potranno creare aree di lavoro (*workplaces)* organizzate gerarchicamente. A queste aree potranno accedere sia utenti singoli che gruppi di utenti. Gli *workplaces* consentiranno lo scambio di messaggi, note o contatti oltre a consentire la condivisione di file e definire le attività che potranno essere assegnate ad altri utenti specifici.
L'area riservata consentirà inoltre la pubblicazione di news di interesse generale che saranno visibili solo agli utenti registrati e potranno essere commentate attraverso message boards dedicate.

3. Proposta commerciale

L'offerta viene formulata seguendo le due attività principali del progetto, tenendo in considerazione che il redesign del sito web SIAI e la creazione del CMS sono propedeutici alla realizzazione della piattaforma di condivisione e collaborazione.

Viste le attività dettagliate alla sezione 2.1 e sulla stima delle risorse necessarie, l'offerta proposta è pari a euro **7.000,00 (settemila/00).**

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Viste le attività dettagliate alla sezione 2.2 e sulla stima delle risorse necessarie, l'offerta proposta è pari a euro **5.000 (cinquemila/00).**

Tutti i prezzi sono da intendersi **IVA esclusa.** Restano ancora da definire le condizioni generali di pagamento. La presente offerta è da ritenersi valida entro e non oltre i **30 giorni** successivi alla sua approvazione e sottoscrizione.

Terranuova B.ni, 05.10.2018

Open-Box s.r.l.

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ALLEGATO N.3

phoops

Business Proposal:

Redesign sito web della Società Italiana di Anatomia e Istologia completo di Content Management System.

Realizzazione di un'area privata del sito dedicata alla condivisione di file e informazioni tra gli utenti registrati.

Codice BP: 18G_SIAI_WEB_1.0

Autore: A. Meiattini

Cliente: Società Italiana di Anatomia e Istologia

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Certificate No.: 226196-2017-AQ-ITA-ACCREDIA
Valid: 29.06.17-15.09.18

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Premessa

Il presente documento descrive le caratteristiche del redesign del sito web della **Società Italiana di Anatomia e Istologia** (SIAI), del *Content Management System* (CMS) per la gestione del sito e dell'area privata del sito stesso, tramite la quale gli utenti registrati potranno condividere documenti, note, contatti e generiche informazioni con altri utenti registrati al sito.

Panoramica

Il sito web della Società Italiana di Anatomia e Istologia verrà ridisegnato tenendo conto degli attuali standard web e dei criteri di accessibilità definiti dal *W3C* e recepiti dalle norme attualmente in vigore.
I contenuti del sito saranno consultabili da qualsiasi tipo di dispositivo dotato di un moderno browser web, grazie ad un design *responsive*.
Tutti i contenuti presenti nella versione attuale del sito saranno integrati nella nuova versione.
Per la gestione dei contenuti del sito sarà fornito un *CMS* grazie al quale gli utenti abilitati potranno creare pagine, modificare contenuti, pubblicare immagini e contenuti multimediali e definire altri utenti abilitati alla pubblicazione.
Al sito web sarà associata un'area privata, dedicata agli utenti registrati, nella quale ogni utente potrà creare *workspaces* per condividere con altri utenti registrati documenti, informazioni e attività.

Proposta tecnica

Nelle sezioni successive vengono descritte in maggiore dettaglio le caratteristiche delle varie sezioni del sito web e dell'area privata di condivisione.

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Sito web SIAI

Come strumento per la pubblicazione dei contenuti e la gestione del sito verrà utilizzato il CMS open source WordPress, completo delle opportune estensioni e plugin.
Il design del sito verrà definito seguendo i moderni standard web (*responsive design*, *mobile first*, *accessibilità*) e tenendo conto della attuale organizzazione dei contenuti.
Attraverso il CMS, l'utente amministratore potrà gestire ogni aspetto del sito, inclusa la organizzazione dei contenuti, le pagine pubblicate, le voci presenti nei vari livelli del menu di navigazione. L'amministratore potrà anche definire degli utenti che pur non avendo i suddetti privilegi potranno produrre e pubblicare contenuti specifici all'interno del sito secondo il ruolo assegnato.

Area privata di collaborazione e condivisione

Per gli utenti abilitati, a discrezione dell'amministratore, sarà implementata un'area privata nella quale ciascun utente potrà creare aree di lavoro (*workspaces*) secondo una struttura gerarchica. L'accesso a queste aree di lavoro potrà essere fornito ad altri utenti o gruppi di utenti. Nelle aree di lavoro sarà possibile scambiare messaggi, note o contatti, condividere file, definire attività che potranno essere assegnate ad utenti specifici.
Attraverso l'area privata sarà anche possibile pubblicare news di interesse generale che resteranno visibili solo agli utenti registrati e potranno essere commentare attraverso *message board* dedicate.

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Proposta commerciale

Riteniamo opportuno definire offerte separate per le attività di realizzazione del sito web completo di CMS e di realizzazione dell'area privata di condivisione e collaborazione per gli utenti registrati, tenendo presente che la seconda attività non potrà essere completata in assenza della prima.

Sulla base delle attività previste nelle sezioni precedenti del presente documento e sulla stima delle risorse necessarie, la nostra migliore offerta per la realizzazione del sito web completo di CMS risulta pari a euro **4.000,00 (quattromila/00)**.

La nostra migliore offerta per la realizzazione dell'area privata e degli spazi di condivisione dedicati agli utenti registrati al sito risulta pari a euro **3.500,00 (tremilacinquecento/00)**.

Termini dell'offerta

Tutti i prezzi si intendono IVA esclusa.
Condizioni di pagamento: da definire.
Validità dell'offerta: 30 giorni.

Letto, approvato e sottoscritto.
Firenze, 19/09/2018

per phoops srl
L'Amministratore unico
Filippo Severi

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