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INVITED LECTURES

IJAE
Vol. 122, n. 1 (Supplement): 5, 2017
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Understanding muscle stem cell regenerative decline with aging

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Skeletal muscle has a remarkable capacity to regenerate by virtue of its resident Pax7-expressing stem cells (satellite cells), which are normally quiescent in the adult. Upon injury, quiescent satellite cells activate and proliferate, to subsequently differentiate and form new myofibers or self-renew to restore the quiescent satellite cell pool. Through a combination of global gene expression/bioinformatics and molecular/cellular assays, we found that resting satellite cells have basal autophagy activity, which is required to maintain the quiescent state. We will discuss the consequences of the autophagy failure in the regenerative potential of muscle stem cells, particularly in the context of aging.

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The good and bad of ERBB receptors in breast - *quanno viniti mi s'allarga lu cori, ma quanno vinni iti puru*

Carlo Tacchetti

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The mammary gland is a dynamic organ displaying structural changes throughout the female reproductive cycle. The gland differentiation follows defined stages (embryonic, prepubertal and pubertal stages, pregnancy, lactation and involution) connected to sexual development and reproduction. Complex two-way interactions between mammary epithelial cells and the surrounding stroma direct proliferation, duct formation, branching and terminal differentiation during these stages. The members of the ERBB family of receptor tyrosine kinases (RTK) are involved in each of these processes and play distinct and complementary roles.

Altered ERBB signaling, mostly due to over-expression and/or, to a minor extent, mutation of one or more of these receptors, results in aberrant cellular responses leading to breast cancers. Thus, the phenotype induced by altered ERBB modulation in breast cancer may highlight relevant aspects of the molecular mechanisms underlying normal breast development.

In the last 15 years, in collaboration with other groups, we have studied the molecular basis of RTK modulation, and contributed to the definition of relevant molecular events and organelle interactions underlying ERBB1 (EGFR) and ERBB2 internalization and trafficking (1-9). These studies brought us to approach the role of these events (10-18) in cancer pathogenesis and progression, and led to the identification of a key druggable molecular target to revert the resistance to Trastuzumab (Herceptin®), a humanized antibody to ERBB2, representing the front line treatment in ERBB2 over-expressing breast cancer (19).

In this lecture I will review the current knowledge on the role of ERBB receptors in normal breast development, their role in breast cancer onset and progression, and our recent results in the field.

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ABSTRACTS

IJAE
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Low oxygen availability and malignant evolution of non-invasive breast tumors: potential protective role of all-trans retinoic acid (ATRA)

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Reduced oxygen availability plays a crucial role in malignancy of solid tumors, including breast cancer. Even if the presence of hypoxic areas is common in all breast lesions, no data clearly correlate low oxygenation with the acquisition of malignant features by non-invasive cells, particularly by cells from ductal carcinoma *in situ* (DCIS), the most frequently diagnosed tumor in women in industrialized countries [1]. We demonstrated that 96 hours of culture under moderate hypoxia is sufficient to induce in DCIS-derived cells motility and epithelial-to-mesenchymal transition (EMT) and to enlarge the number of cells expressing the stem cell marker CD133, indicative of their increased malignant potential.

Administration of all-trans retinoic acid (ATRA), a well-known anti-leukemic drug with an anti-tumor role in invasive breast tumor cells [2], supports the epithelial phenotype of DCIS-derived cells cultured under hypoxia and reduces the number of CD133 positive cells, abrogating almost completely the effects of poor oxygenation. In DCIS-derived cells, as in leukemic cells, ATRA up-regulates the β2 isoform of PI-PLC (PLC-β2), ectopically expressed in invasive breast tumors in which counteracts the effects of hypoxia on both EMT and CD133 levels [3]. This suggests that the mechanisms triggered by ATRA in non-invasive breast tumor cells cultured under hypoxia may, at least in part, depend on PLC-β2 activity.

Overall, we assigned to hypoxia a role in increasing the malignant potential of DCIS-derived cells and identified in ATRA, currently used in treating acute promyelocytic leukemia (APL), an agonist potentially useful in preventing malignant progression of non-invasive breast lesions with hypoxic areas.

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Keywords
Ductal carcinoma in situ (DCIS), hypoxia, ATRA, PLC-β2

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Differentiation of Human iPSCs Into Telencephalic Neurons Using 3D Organoids and Monolayer Culture

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Human induced pluripotent stem cells (hiPSCs) are emerging as a useful tool for modelling *in vitro* early brain development and neurological disorders. Molecular mechanisms and cell interactions that regulate the neurodevelopment at early stages remain unclear because of human brain's complexity and limitations of functional studies. Two major culture methodologies are used to differentiate *in vitro* hiPSCs into neurons: monolayer (2D) and organoid (3D) cultures. Here we investigate the effect of cell dissociation and the loss of 3D organization during the early differentiation process of neuronal progenitors. Using the same culture media, we first differentiated hiPSCs into neural progenitor cells (NPCs) and then induced their differentiation into neurons in 3 different modalities: 3D undissociated organoids, dissociated NPCs followed by immediate re-aggregation into an organoid, and dissociated NPCs cultured as monolayer. We assessed neuronal differentiation efficiency of each method by immunocytochemistry, qPCR, western blot, and RNA-Seq analysis over a time course. Our data revealed substantial differences in gene and protein expression among the three systems, including genes of the Notch pathway (e.g. NEUROD1, NEUROG2), earliest determinants of cortical region differentiation (e.g. SOX1, FEZF1) as well as later transcriptional regulators that specify cortical neuron subtypes (e.g. TBR1, CTIP2), which were all downregulated in monolayer. Moreover, we found that genes and pathways mediating cell-to-cell interactions (e.g. CNTNs, CAMs) were mostly upregulated in the 3D culture systems, whereas cell-extracellular matrix interaction molecules (e.g. ITG, LAM) were mostly upregulated in 2D, indicating that cell surface molecules may be involved in specification of neuronal cell types. Our results address the methodological question of the appropriateness of a differentiation method for a particular experimental goal, and, beyond that, reveal important early determinants that exert a decisive influence on neuronal differentiation and regional specification of human neural stem cells.

Keywords
hiPSCs, organoid, monolayer, cell dissociation, cortical development, RNA-seq

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Morphology on the cloud - Virtual Campus, an integrated didactic platform for biomedical studies

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The current Core Curricula of Degree courses in Biomedical areas has enormously compressed the hours dedicated to the student for self-learning in morphological subjects. The result is a reduced student attitude to integrate the information received by attending lectures and practical sessions, with the indispensable consultation of texts dealing with morphological and 'functional' subjects, a key experience to autonomously logically identify the rational of the morphology/function relationship in the human body, at the macroscopic and microscopic level.

These changes are occurring at a time when new medical imaging technologies become more and more informative in both morphological and functional areas.

As a consequence, we are modifying our way of organize lessons compared to the generations of colleagues who have preceded us. More and more frontal lessons are organized with a logical morpho-functional approach. For example, the reference to the anatomy of the living, displayed through invasive or not invasive imaging, is added to the necessary and traditional anatomy of the cadaver. The reference to the pathology helps to define how the alteration of morphological integrity is reflected on function, both at the macro and microscopic level, and so on.

However, there are no organized easy-to-use guided tours for the student to allow, in the shortest possible time, to 'rationally see' what he has studied, in the various imaging contexts available at the macro- and microscopic level. At the same time, there are no 'data bank' of resources for the preparation of the lessons.

That is why we have imagined 'virtual campus' an integrated digital learning platform for self-learning. The platform has been thought and realized thanks to a group of teachers of 'morphologic' and 'functional' biomedical subjects and computer engineers belonging to a publishing house.

The presentation will explain the rationale behind the platform, its structure and the educational opportunities offered.

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Role of Stearoyl-CoA Desaturase 1 and 5 in breast cancer cell migration and survival

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We previously reported that a major component of breast tumor stroma, the "cancer-associated fibroblasts" (CAFs), induced epithelial-mesenchymal transition and an increase in cell membrane fluidity as well as in migration speed and directness in poorly (MCF-7) and highly invasive (MDA-MB-231) breast cancer cells. We next investigated the mechanisms responsible for the CAF-promoted tumor cell migration demonstrating the crucial role of Stearoyl-CoA desaturase 1 (SCD1), one of the main enzyme regulating membrane fluidity. We found SCD1 to be upregulated in tumor cells co-cultured with CAFs and that its inhibition (pharmacological or siRNA-based) impaired both intrinsic and CAF-driven tumor cell migration. In the present study, we deepen the understanding of the mechanisms involved in the SCD1-based modulation of tumor cell migration, as well as the possible role of the other human SCD isoform, SCD5. Thus, in the two above mentioned cell lines we studied whether the inhibitory effect produced on cell migration by SCD1 depletion was due to the deficiency of oleic acid (OA), the main SCD1 enzymatic product. By a wound healing assay, we found that the addition of OA nullified the inhibitory effects produced on tumor cell migration by the SCD1 inhibition in both the cell lines while SCD5 appeared not to be involved in the regulation of their motility but it was upregulated in MCF-7 cells co-cultured with CAFs. Because of the high number of detached MCF-7 cells silenced for SCD5, we investigated the role of the desaturase on tumor cell survival and an induction of necrosis was found. Consistently with the promotion of tumor cell migration, CAFs have also been found to induce the activated form of the hepatocyte growth factor receptor, p-MET, in the two cell lines.

These results provide further insights in understanding the role of SCD1 in both intrinsic and CAF-stimulated mammary tumor cell migration. Moreover, our data seem to suggest the ability of CAFs to promote the maintenance of tumor cell survival by the induction of SCD5 levels.

Keywords

Breast cancer cells, cancer-associated fibroblasts, SCD1, SCD5, cell migration, cell survival

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Generation of Induced Pluripotent Stem Cells from Patients with Duchenne Muscular Dystrophy and their induction to Neurons

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Duchenne muscular dystrophy (DMD) is an X-linked recessive disease characterized by deficient expression of the cytoskeletal protein dystrophin. DMD has been associated with intellectual disability and mental retardation (MR) and is present in about a third of all patients. Loss of Dp71, the major dystrophin-gene product in brain, and the dystrophin associated proteins (DAPs) are thought to contribute to severity of MR, but the specific function of the neural dystrophin proteins are poorly understood for a limited access to DMD patients brain tissue (1). Differentiation of induced Pluripotent Stem Cells (iPSCs) provides an opportunity to generate an unlimited supply of living neurons genetically identical to those present in patients.

In this study we obtained DMD-iPSCs from peripheral blood mononuclear cells of DMD patients with cognitive impairment and we performed morphological (fluorescence and electron microscopy), molecular (Western Blot and Real Time PCR) and functional (electrophysiology) characterization both of iPSC-derived Neural Stem Cells (NSCs) and the differentiated neurons. Preliminary data showed a reduction of Dp71 and DAPs proteins, including the AQP4, potassium channel Kir4.1, α- and β-dystroglycan (α/βDG) and α-syntrophin (αSyn), both at transcriptional and traductional level, coupled with membrane dys-arrangment in DMD-iPSCs compared with healthy iPSCs. Moreover, we demonstrated that the neurons obtained from the differentiation of iPSCs derived from DMD patient showed after confocal analysis, altered cytoskeleton and reduction in Dp71expression, and by single-cell imaging experiments and electrophysiology, altered intracellular calcium homeostasis, in analogy with what shown in the dystrophic mdx mouse neurons (2). Overall these results showed that the Dp71 and DAPs alterations affect also the neural precursor as well as the differentiated neurons in DMD patients, so suggesting a key role in the pathogenesis of neurocognitive deficits in DMD disease.

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Keywords
Duchenne Muscular Dystrophy, Induced Stem Cells

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An animal and cellular study on αB-crystallin activation in cardiac muscle by acute exercise

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Alpha-B-Crystallin (CRYAB), a Small Heat Shock Protein sensitive to oxidative stress, is expressed in many tissues and implicated in various biological processes. In cardiac muscle, CRYAB exerts a cardio protective role in ischemia-induced damage preventing apoptosis and necrosis.

In the present study we used forty young (7-weeks old) healthy male mice (BALB/c AnNHsd), which after 1 week of acclimatization to the new housing environment, runned 2 days per 10 minutes. The TR mice ran for 60 min at a speed of 5.5 m/min. Mice were sacrificed immediately after, 15 and 120 minutes after the end of the acute bout of endurance exercise (TR-0', TR-15' and TR-120', respectively). We prepared samples from the heart and from the group of posterior muscles study αB-crystallin' response at different time of recovery from an acute aerobic exercise (1 hour), correlating its modulation with oxidative stress level.

We found that a single bout exercise lead to a specific short-term increase of phospho-αB-crystallin level (pCRYAB), without changes of its total expression. Further, the level of 4-hydroxynonenal, a marker of lipidic peroxidation, has shown a similar trend of pCRYAB enhancement. This may indicate that CRYAB in cardiac muscle is activated and it has a putative role in oxidative stress during exercise. These results are supported by our previous data obtained in mouse skeletal tissues (i.e. gastrocnemius, soleus) and in H_2O_2-treated C2C12 myotubes. In particular we observed not only a fiber-dependent response of pCRYAB, but also its translocation into myofibrillar compartment.

Experiments are in progress to further investigate on CRYAB role during exercise and its interactions with cytoskeletal structures.

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Anatomical theatre place of Knowledge – the pivotal role of anatomist in its realization

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Human anatomy dissection represented a cornerstone in the evolution of medicine and modern scientific thought.

The anatomical theatres, some of which are considered true masterpieces of architecture, are the place where concretely men learned to know themselves with a scientific method.

Anatomists had a pivotal role in the buildings of anatomical theatres, using their knowledge in the research process of more functional architectures for demonstrative and experimental science.

Antonio Scarpa (1752-1832) is an emblematic figure in this scenario. He studied anatomy at the University of Padua in the oldest permanent anatomical theatre of the world, originate in 1594 from a joint project conceived by Paolo Sarpi, scientist and church reformer, and Hieronymus Fabricius Ab Aquapendente, anatomist.

In 1772, Scarpa became professor at the University of Modena.

Bearing witness to the architectural value of the theatre in Padua is the fact that in 1774 Scarpa, involved in the planning for an anatomical theatre in Modena, had the professor of surgery in Padua, Girolamo Vandelli, send him a wooden model of the theatre there. Another project, less expensive, was selected instead.

Later, in 1783, Scarpa was made professor of anatomy at the University of Pavia and promptly he promoted the building of an anatomical theatre there. The building was concluded in 1785, its semicircular layout is modeled on ancient theatres and the Palladian Olympic Theatre of Vicenza.

Today, most of the anatomical theatres are lost or forgotten. The Thesa project will provide a census of anatomical theatres, both survived and not, which will allow us to identify connections among them, among the anatomists who studied there and the mutual influences that characterize their form. We believe the achievement of these objectives defines the essential conditions necessary to regain full awareness of the value of anatomical theatres in both the academic and popular contexts, thus creating a fertile cultural basis for new initiatives that can continue the quest for knowledge undertaken in the past in these places. From an architectural and evocative perspective, they are and will remain places where man puts himself at the centre and at the same time observes himself.

Keywords

Anatomical theatres, Antonio Scarpa, history of anatomy, protecting cultural heritage, Thesa project

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The Mediterranean shipwreck of April 18 2015: challenges in the postmortem examination of the victims

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Background and Aims. On the 18th of April 2015, one of the largest shipwrecks in the Mediterranean sea occurred with around 800 gone missing. Among European indifference and inactivity, the Italian Government created a task force, through the will of the Commissioner's Office of Missing Persons, the Italian Navy, the Prefecture of Siracusa, the Police, the Military Red Cross and the University of Milano flanked by the Universities of Catania, Palermo and Messina and other 10 Universities for the recovery and the identification of these victims in a challenging scenario where collection of post-mortem and ante-mortem data is very difficult respectively because of the conditions of the bodies and the political situation of the countries of provenance of the victims as well as the dispersal of their relatives and loved ones all over the world. According to the DVI protocols, identification relies mainly upon primary (genetic, fingerprint, teeth) criteria, but previous experience on the Lampedusa disaster has proven that such criteria may not guarantee high success rates. Personal descriptors of faces (ante-mortem photographs) are becoming more and more important.

Materials and Methods. Since July 2015, 69 body bags bodies have been recovered around the wreck e and 458 body bags inside the boat; these were recovered by the Italian Navy and brought to a Naval area near Siracusa where a morgue was set up. Here PM examination on all bodies was performed and a biological profile was created through detailed pathological, anthropological odontological and radiological examination of the remains along with sampling for DNA analysis. 3D scans of the face or crania also were performed.

Results. Preservation of the bodies varied from decomposed bodies, partial skeletonization of the extremities to complete skeletonization (with lack of the skull). Over 550 bodies were recovered along with many commingled remains.

Preservation of bodies varied from partial skeletonization of the extremities (41%) to complete skeletonization (23%). All bodies so far belong to males.

Conclusions. The humanitarian disposition of countries, politicians and scientists is a fundamental prerequisite for identifying victims of these disasters. Because of the difference in type of AM data available in such cases, autopsy procotols and identification strategies may need to vary.

Keywords

Dead migrants, identification, biological profile, autopsy protocol.

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Spheroids from human primary skin myofibroblasts as experimental system for myofibroblast deactivation studies

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Myofibroblasts are activated fibroblasts, involved in tissue repair and cancer, characterized by *de novo* expression of alpha smooth muscle actin (α-SMA), increased secretion of growth factors and immunoregulatory phenotype [1].

At the end of wound healing myofibroblasts undergo apoptotic cell death, whereas *in vitro* they are also subjected to a programmed necrosis-like cell death, termed nemosis, associated with cyclooxygenase-2 (COX-2) expression induction and inflammatory response [1,2]. Moreover, fibroblasts form clusters during wound healing, fibrotic states and tumorigenesis.

In this investigation, we produced and analysed clusters such as spheroids from human primary cutaneous myofibroblasts to evaluate apoptotic or necrotic cell death, inflammation and activation markers during myofibroblasts clustering. The spheroids formation does not induce apoptosis, necrotic cell death and COX-2 protein induction. The significant decrease of α-SMA in protein extracts of spheroids, the anti- migratory effect of spheroid-conditioned medium on normal cell lines and the absence of proliferation marker Ki-67 after 72 h of three-dimensional culture indicated that myofibroblasts undergo a deactivation process within spheroids. The cells of spheroids, reverted to adhesion growth, preserve their proliferation capability and are able to reacquire a myofibroblastic phenotype. Furthermore, the spontaneous formation of clusters and spheroids on plastic and glass substrates suggests that aggregates formation could be a physiological feature of cutaneous myofibroblasts.

This study represents an experimental model to analyse myofibroblasts deactivation and indicates that fibroblast clusters could be a cell reservoir regulating tissue turnover.

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Keywords
Myofibroblasts, spheroids

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Synchrotron-based technique: a new high resolution imaging of nervous system

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X-ray phase contrast micro-tomography (μPCI-CT) is a high resolution technique that can be used to investigate vascular and neurodegenerative disorders overcoming the limitations of the conventional imaging modalities. In fact, currently available neuroimaging techniques are based on sample-invasive imaging protocols involving dissections, staining or labeling of nervous system structures. On the other hand, μPCI-CT permits to visualize the spinal cord micro-vasculature, to detect single neuronal cells in the vertebral column and even cells infiltrating the nervous system in pathological conditions. These properties make μPCI-CT a potential powerful instrument in the study of vascular and neurodegenerative disorders as well as in the patient evaluation during medical treatment. Moreover, it would be a powerful instrument to localize in preclinical model of immune mediated diseases ectopic cells infiltrating the nervous system in a multifocal and unpredictable way.

To optimize tissue fixation protocols for μPCI-CT analysis, several attempts were performed combining different protein and lipid fixation procedures and time points.

The high-resolution synchrotron μPCI-CT setup allowed recognition of full-organ spinal cord anatomy of healthy rats, including anterior/posterior gray horns, the dorsal/ventral roots and ganglions, the central canal and the meninges, was clearly depicted. Superficial and deep vessels were visualized without need of any contrast-agent. At the highest resolution used, single neuronal cells perfused by surrounding vasculature were recognized allowing the detection of specific structure such as bundles of nerve fibers, single motor neurons and neuro-glial cells, cell bodies and axons as well as intra-cellular structure (i.e. cell nuclei and nucleoli).

Moreover, in preclinical studies, the optimization of protocol for μPCI-CT allowed to localize ectopic infiltrating cells in nervous system organs in both mouse and rat models of Krabbe disease and Multiple Sclerosis which would allow a further accurate analysis of the areas and cell-parenchima fine interaction.

Keywords

Imaging, synchrotron, phase contrast micro-tomography, nervous system

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Scanning Electron Microscopy in forensic investigations: More views from more applications

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The purpose of this presentation is to expand and highlight the range of applications of scanning electron microscopy to forensic science, following the overview which was shown last year at the LXX SIAI Congress. All examples shown of forensic uses of SEM were carried out over the last few years at the Human Morphology Laboratory of the University of Insubria. These studies include:

- The identification and characterization of different toolmarks found on human bones. Some toolmarks have a distinctive morphology and allow a reliable identification of the weapon or instrument used. For this purpose, we will illustrate a few examples of dismemberment with different types of saw and will show the peculiar bone patterns left by different cutting edges (knives, axes, cutters ...);

- The examination of human tissues and of medical devices (catheters etc.) for the early detection and identification of slow-growing microorganisms (e.g. some fungi). The diagnosis of these microorganisms would have otherwise required molecular biology techniques, which are not only expensive but also not always available or applicable in the field of forensics (for instance, when the specimen is inadequate for external contaminations or is into a state of conservation far from optimal), or conventional cultures in vitro, which require much longer times and may be easily spoiled by inopportune drug administration;

- The use of scanning electron microscopy and of X-ray spectroscopy as auxiliary and "creative" tools to discover mystifications and frauds against insurance companies.

Keywords

Scanning Electron Microscopy, Forensic Science

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Ultrastructural modifications of human meniscus under different conditions

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Human meniscus presents two cell populations [1]. The main cell type present in its inner and middle part is the fibrochondrocyte, a round or oval shaped cell, while in outer zone fibroblast-like cells within a dense connective tissue [2] are mostly observable.

The aim of this work is to study a variety of pathological conditions. We have analized samples of meniscus obtained from 3 multiorgan donors (63 median age, years), 5 patients with traumatic meniscal tear (40 median age, years) and from 3 patients undergoing total knee replacement for osteoartritis (OA) (73 median age, years).

In elderly menisci we observed a progression of chromatin margination, and a partial cytoplasmic organelle conservation, but for the presence of occasional autophagic vacuoles. Both after trauma and in OA, an increasing chromatin condensation, organelle degeneration and cytoplasmic vacuolization appear.

In OA, similarly to elderly, autophagic vacuoles, which probably represent a cellular self-protection mechanism, appeared in the cytoplasm. The most evident ultrastructural changes have been observed when intervention takes place long time after trauma. In this case a high chromatin condensation, a large cytoplasmic vacuolization with degeneration of organelles and several necrotic cells appear.

Calcification areas occur in all conditions. In particular, specimens from traumatic menisci have a structure similar to OA ones, especially if trauma has not been surgically repaired

at appropriate times. In all there is disorganization of collagen fibers, and their replacement with proteoglycans.

We can conclude that trauma and OA induce an increasing meniscal degeneration, comparable to physiological aging. When surgery takes place long time after trauma we observed most evident menisci degeneration. In all pathological conditions apoptotic like features appeared [3].

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Keywords

Human Meniscus, Transmission Electron Microscopy, Ederly, Trauma, OA

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PI3Kα-selective inhibitor alpelisib (BYL719), may be effective as anticancer agents in Rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is a highly malignant and metastatic pediatric cancer that arises from the skeletal muscle. Recent studies have identified an important role of AKT signaling in RMS progression. This suggests targeting components of the PI3K/Akt pathway may be an effective therapeutic strategy. Here, we investigated the in vitro activity of the class I PI3K inhibitors [1] in human rhabdomyosarcoma cell lines (embryonal rhabdomyosarcoma RD and A204, alveolar rhabdomyosarcoma SJCRH30). We used a panel of four compounds which specifically target PI3K isoforms including the PI3Kα-selective (p110α) inhibitor alpelisib BYL719, currently in clinical development by Novartis Oncology, the p110β TGX-221 inhibitor, the p110γ CZC24832, the p110δ CAL-101inhibitor and the dual p110α/p110δ inhibitor AZD8835. The effects of single drugs and of several drug combinations were analyzed to assess cytotoxicity by MTT assays, cell cycle by flow cytometry , apoptosis by caspase 3/7 assay and Western blot, as well as the phosphorylation status of the pathway. BYL719 treatment resulted in G1 phase cell cycle arrest and apoptosis. BYL719 administered in combination with CAL-101, for 48 h and 72h, decreased cell viability and induced apoptosis in a marked synergistic manner. Taken together, our findings indicate that BYL719, either alone or in combination with p110δ CAL-101inhibitor, may be an efficient treatment for human rhabdomyosarcoma cells that have aberrant upregulation of the PI3K signaling pathway for their proliferation and survival.

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Keywords

Rhabdomyosarcoma, PI3K/Akt pathway, cancer

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Plasma redox response of Sicilian Opuntia Ficus Indica juice in young physically active women

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It is known amateur female athletes show an altered redox status [1] and the consumption of Opuntia Ficus Indica (OFI) decreases oxidative stress (OS) in healthy humans [2]. Therefore, the aim of this study was to investigate whether the supplementation with Sicilian OFI juice affected plasma redox balance following a maximal effort test in young physically active women . This study was randomized, double blind, placebo controlled and crossover design. Eight women (23.25±2.95 years old, weight of 54.13±9.05 kg, height of 157.75±0.66 cm and BMI of 21.69±0.66 kg/m^2) were randomly divided into 2 groups and each group was supplied with either 50 ml OFI, diluted to 170 ml with water, or 170 ml Placebo containing the same concentration of fruit juice ingredients except for Vitamin C and indicaxanthin. They consumed OFI or Placebo every day for 3 days before of effort test on cycle ergometer and continued to take it for 2 consecutive days after testing. Blood samples were taken before and after the effort test without supplementation (baseline), pre- and post-test, 24 h and 48 h post-test with OFI or Placebo supplementation. H2O2 levels and total antioxidant capacity (PAT) were measured with photometer and resonance Raman spectroscopy [1,2]. The differences within and between groups were calculated with ANOVA analysis and considered significant with $P<0.05$.

OFI group showed a significant lower quantity of H_2O_2 than Placebo group after the effort test. PAT levels of OFI group were significantly higher than pre/post those of baseline and 48 h post-test of Placebo group.

In conclusion, OFI supplementation might to be used to restore redox balance after intense exercises in moderately trained women.

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Keywords
Antioxidant supplementation, oxidative stress, redox balance, indicaxanthin

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Aerobic Fitness protects from Atherosclerotic Cardiovascular Risk Paralympic Athletes with a Locomotor Impairment

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Aim: This study, carried out on Paralympic athletes (PA) with a locomotor impairment (LI), was aimed at:

1. assessing the prevalence of atherosclerotic cardiovascular disease (ACVD) risk factors (RF) in PA with either a spinal cord injury (PA-SCI) or other (different from SCI) LI (PA-OLI);

2. evaluating the hypothesis that aerobic fitness (oxygen uptake peak - VO2peak) was inversely related to ACVD RF.

Methods: A total of 135 male PA (72 PA-SCI, 28 PA with lower limb amputation, 12 PA with a cerebral palsy/brain injury, 7 PA with poliomyelitis, 9 PA with other neurological disorders and 7 PA with other orthopedic disorders) were screened through anthropometric and blood pressure (BP) measurements, laboratory blood tests and graded cardiopulmonary maximal exercise test, to estimate both an ACVD-RF score and VO2peak. The ACVD-RF score was assessed summing 1 point for each of the following RF: obesity –OB- (BMI≥30 or waist circumference ≥102 cm), hypertension –HT- (systolic BP ≥ 140 mm Hg and diastolic BP ≥ 90 mm Hg), dyslipidemia -DL- (total Cholesterol -C- ≥200 mg·dl-1 or LDL-C ≥130 mg·dl-1 or HDL-C <40mg·dl-1), impaired fasting glucose -IG- (fasting plasma glucose ≥100 mg·dl-1) and subtracting 1 point when serum HDL-C was higher than 60 mg·dl-1.

Results: Prevalence of OB, HT, DL, IG and high HDL-C were equal to 5.9% and 3.2%, 13.9% and 14.3%, 58.3% and 49%, 29.2% and 34.9%, 27.8% and 17.4%, in PA-SCI and PA-OLI, respectively. Based on the ACVD RF, 3 groups were formed: group 1 (RF≤0, N=54), group 2 (RF=1, N=41), group 3 (RF≥2, N=40). VO2peak was equal to 37.9±14.71 ml·kg-1·min-1, 30.9±9.13 ml·kg-1·min-1 and 24.1±5.50 ml·kg-1·min-1 in the PA of groups 1, 2 and 3, respectively.

Conclusions: Being VO2peak inversely related to groups of ACDR RF, high aerobic fitness provides a protective effect on ACVD morbidity in PA.

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Clusterin enhances migration and invasion of prostate cancer cells through an isoform-specific Akt2/miR-190/PHLPP1 circuit

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During prostate cancer progression cancer cells undergo a variety of molecular alterations that lead to the acquisition of uncontrolled growth properties. One such set of molecular alterations is mediated by the PI3K/Akt signaling pathway. Here, we describe a regulatory system that modulates the phosphoinosited 3-kinase/Akt (PI3K/Akt) pathway downstream of secreted Clusterin (sCLU) in normal and cancer prostate cells. The overexpression of sCLU is very frequent in prostate cancer, and can lead to Akt-activation. This prompted us to investigate how sCLU overexpression influences PI3K/Akt signaling network in a study model represented by human epithelial prostate PNT1A cells stably transfected with sCLU or with empty vector alone. We found that CLU cells show a marked differential phosphorylation of several members of the PI3K/Akt cascade, and in particular of Akt2. Moreover, we found that the phosphatase PHLPP1, known to dephosphorylate Akt2 at S473, is severely downregulated in CLU compared to MOCK cells. We thus investigated whether sCLU alters PHLPP1 protein stability or expression. Our results indicate that sCLU indeed stimulates PHLPP1 degradation by β-TrCP. Interestingly, we further demonstrated that sCLU alters also PHLPP1 through the negative regulator miR-190. Next, because sCLU has been reported to inhibit or to stimulate the aggressive behavior of cancer cells depending on the cell model, we investigated the effects of CLU overexpression or addition of recombinant Clusterin to the medium on cell migration and invasion in PNT1A cell line, which is not expected to display an invasive phenotype, and in the cancer prostate epithelial cell lines LNCaP and PC3. The result was extremely clear: not only CLU overexpression gives PNT1A cells the same behavior of wild-type PC3 cells, but also increases the migration and invasion index of all the above cell models by two to four times, compared to controls. As a confirmation, in the same model silencing of Clusterin abrogates migration of CLU cells. Next, the effect of Akt single-isoform silencing on cell migration was explored. While silencing of Akt1 affected migration only slightly, silencing of Akt2 prevented migration of both MOCK and CLU cells completely. The same result was obtained by pharmacological inhibition of Akt2. All together our results, clearly demonstrate for the first time that Clusterin can switch the low migration phenotype of normal prostate cells towards a high migration phenotype through the modulation of the expression of the PHLPP1 and, in turn, the activity of Akt2.

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Keywords

Clusterin, PHLPP1, Prostate cancer, miR-190, Akt

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Osteocytes signaling events induced by intermittent *vs* continuous Teriparatide treatment affect *in vitro* osteoblast differentiation and mineralization

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PTH(1-34), also known as Teriparatide, is an active anabolic drug used in the treatment of some forms of osteoporosis and occasionally exploited to speed fracture healing. The effect of such therapies are dependent on the type of administration, in fact it has been largely demonstrated that a short administration of Teriparatide (also called *intermittent*) increases the bone mass, meanwhile a long administration of the same agent (known as *continuous*) leads to an increased resorption.

The molecular reason why the type of administration is so critical for the fate of the bone remodeling is still largely unknown but it is probably due to the fact that it affects several signaling pathways and alters the biological activity of a cohort of cells: osteoblasts, lining cells, osteoclasts, and osteocytes. In the present work, we firstly focused the attention on molecular events induced by intermittent vs continuous Teriparatide treatment in a well-known osteocytes *in vitro* model, the MLO-Y4 cells. By the use of a gene array platform, we found many molecules upregulated or downregulated depending on the the temporal administration modes, suggesting that the drug affects in diverse manner the osteocytes related signaling pathways. In particular, we paid attention to Wisp-2, a protein of the Wnt pathway that has been demonstrated to be able to interact and influence the differentiation of osteoblasts into osteocytes and their mineralization. Secondly, through the mineralization assay, we analyzed the functional effects, involving the differentiation of osteoblast IDG-SW3 cell line, upon the conditioning culture with MLO-Y4 medium, that were pre-treated with short and long time administration of Teriparatide. These findings, consistent with the crucial role performed by osteocytes on osteoblast differentiation, clarify the molecular events downstream the short treatment with Teriparatide, suggesting that the perturbation of certain signaling patwhays, such as the Wnt pathway, is crucial for the positive regulation of bone formation.

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Keywords

Osteocyte, Osteoblast, Teriparatide, Wisp-2, mineralization, gene array

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Converging orexinergic and reticular thalamic inputs on thalamic paraventricular neurons in normal conditions and experimental sleeping sickness

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A subset of excitatory neurons in the lateral hypothalamus, known to express the peptide orexin/hypocretin (Ox), play a key role in maintaining wakefulness. Projections from Ox neurons are widely distributed in the neuraxis but terminations are heavily concentrated in the thalamus along the midline, especially the paraventricular thalamic nucleus (PVT). The same areas receive afferents from inhibitory, GABAergic neurons expressing parvalbumin (Pv) in the thalamic reticular nucleus (Rt), which has long been considered essential for sleep regulation.

While the two circuitries have been regarded as distinct, we tested the hypothesis that PVT neurons represent a common target for both afferent systems by means of confocal microscopy of multiple immunofluorescence labeling in the mouse brain.

Calretinin (Cr) was used as marker of PVT neurons. Almost 90% of PVT perikarya were contacted by Pv+ terminals, confirming the prominent role of Rt in modulating PVT activity. Interestingly, about a third of these neurons were also reached by Ox+ terminals, suggesting a key role of the thalamic midline in integrating information pertaining vigilance state control. PVT cells receiving Ox+ but not Pv+ contacts were observed only rarely.

In mice infected with the parasite *Trypanosoma brucei brucei*, the causal agent of the neuroinflammatory disease "sleeping sickness", Pv+ afferents into PVT were largely preserved, while orexinergic fibers appeared fragmented and reduced in density. Importantly, the fraction of PVT perikarya receiving both Pv+ and Ox+ terminals was reduced by about 50%. The substantially decreased convergence of the two regulatory systems, in association with infection-induced disrupted sleep and sleep/wake cycles, further supports the hypothesis that PVT contributes to vigilance and arousal in physiological conditions.

Keywords

Neuroinflammation, diencephalon, African trypanosomiasis, immunofluorescence, confocal microscopy, orexin

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The cerebellum-periaqueductal gray connectivity: a constrained spherical deconvolution tractography study

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The periaqueductal gray (PAG) is a relevant neuronal station situated in the midbrain, which play a pivotal role in triggering behavioral responses to stressful stimuli, such as pain or threat. Current knowledge concerning PAG functions is based on several tract-tracing studies conducted on animals, which unveiled PAG connectivity to both cortical and subcortical areas [1]. Considering that descending projections to spinal cord reach the dorsal horn and connections to motor related cortical areas have never been described yet, the neural structure which best fits PAG modulation of motor behavior is the cerebellum. Direct connections between PAG and cerebellar cortex were firstly described in cats and neurophysiological studies conducted on animals, suggesting either direct or undirect PAG influence to cerebellar activity. In the last decades, the rise of diffusion weighted imaging and tractography have made possible to reliably reconstruct white matter pathways in the human brain. To the best of our knowledge, few tractography studies explored PAG connectivity in humans and the evidences concerning direct or undirect connections with the cerebellar cortex are still sparse. Aimed at investigating PAG connectivity with particular focus on PAG-cerebellum connections, we used high quality diffusion weighted imaging data of thirty healthy subjects from the Human Connectome Project. Fiber tracts have been reconstructed using Spherical Informed Filtering of Tractograms, a novel algorithm improving streamline reconstruction and selection [2]. Connectivity analysis revealed that the PAG is mainly connected with subcortical structures, such as the thalamus and the cerebellum. Taken together our results show a direct interplay between the PAG and the cerebellum, thus suggesting the cerebellum as a likely candidate to modulate complex features of motor behavior in stressful conditions, such as adaptation after social defeat and computing strategies to avoid threatening situations.

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Keywords

Periaqueductal gray, cerebellum, MRI, tractography, SIFT

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Platelet preparations in neuronal cell differentiation

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Concentrated Growth Factors (CGF) is a platelet rich preparation that has the important feature of a tight fibrin network and containing a large number of growth factors possessing great regenerative potentialities [1].

The regeneration of nervous system is one of the mail goal of regenerative medicine. The aim of this study is to test the *in vitro* CGF effects on both differentiated and undifferentiated SH-SY5Y cells, derived from human neuroblastoma.

To induce differentiation, SH-SY5Y cells have been treated with Retinoic Acid (RA) 10μM, in both basal and complete medium and in the presence and absence of CGF. After 72 hours, different parameters have been investigated: the morphological characteristics of the cells, the cell proliferation, the cellular vitality using the MTT test, the CGF and/or RA differentiation property and the immunocytochemical analysis of neuronal specific markers (NeuN, Sinaptophisine, β-III-tubulin, Nestin). Moreover the NGF (Nerve Growth Factor) and BDNF (Brain Derived Growth Factor) release have been assayed by ELISA test.

Our results obtained suggest that treatment with CGF, also used alone, positively affects cell differentiation and neuronal phenotype regulating the expression of the neuronal markers and improving the outgrowth of neurites.

Taken together these results seems to be promised into new approaches for neuronal regeneration using platelet preparations.

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Keywords

Concentrated growth factors, neuronal differentiation, SH-SY5Y

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The Organ Care System as a new promising tool for donor heart *ex vivo* preservation

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Heart transplantation remains the gold standard treatment for end-stage heart failure. To face actual donor shortage, heart warm perfusion with the Organ Care System (OCS) was introduced alternatively to usual cold ischemic storage [1]. Here, OCS-preserved hearts were matched against those subjected to cold ischemia in terms of (i) perioperative clinical parameters, (ii) histopathological, immunohistochemical, and ultrastructural features of pre- and post-implant left ventricle biopsies, and (iii) cardiomyocyte metabolism by NMR spectroscopy of blood samples. Concerning clinical outcomes and myocardium structural preservation, preliminary data seem to be encouraging. Namely, NMR spectra revealed OCS perfusion to reduce cardiomyocyte oxidative stress by lowering the lactate/glucose ratio. Ultrastructurally, cardiomyocytes from OCS-preserved hearts showed minor hypertrophy signs and few altered mitochondria. OCS preservation also seemed to mitigate reperfusion effects, decreasing the number of degenerating cardiomyocytes. Interestingly, disappearance of sarcomere banding in one heart undergone pre-explant arrest was found to be restored after OCS perfusion. In conclusion, these preliminary data suggest that the OCS can improve heart storage. Functional recovery of borderline hearts with actual broadening of the donor pool seems to represent additional advantages of OCS technology.

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Keywords

Organ Care System, heart transplant, cardiomyocyte metabolism, histopathology, ultrastructure

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Melatonin atheroprotective effects *in vivo*

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Chronic inflammatory fibro-proliferative changes leading to atherosclerotic plaques are considered hallmark of cardiovascular diseases [1]. Atherosclerosis pathogenesis is a complex entity, which has not been fully understood; however, many studies have demonstrated the role of oxidative stress and inflammation in its development. Melatonin effects on inflammation and oxidative stress process have been demonstrated in the last ten-year literature [2]. However, its role(s) and mechanism(s) of action as a therapeutic tool against atherosclerosis remain largely unexplored. Our aims were to assess the role of melatonin in the onset and developing of atherosclerotic plaques through radiologic and morphometrical tools in 20 apolipoprotein-E knockout (ApoE) mice fed with Western diet (42% calories from fat). 10/20 mice were treated with melatonin (10 mg/kg per os). ^{18}F-FDG PET-CT is a widely used tool to assess inflammatory changes, even before macroscopic changes have taken place. Glucose metabolism is known to be higher in areas of inflammation due to an increased expression of GLUT transporters on the cell membranes both in animals and humans. Using this feature PET/CT is able to determinate metabolic cellular changes and therefore it can be used as biomarker of atherosclerosis. All mice were scanned both before starting melatonin treatment and at the end of the study. After the last scan mice were sacrificed and vascular remodeling, oxidative stress and inflammation at aortic arch level were evaluated. CT-corrected PET datasets were used for computation of SUVmax.

Atherosclerotic vascular remodeling, oxidative stress and inflammation levels were significantly more conspicuous in the control cohort, compared to the treated mice ($p \leq 0.05$). ^{18}F-FDG PET/CT did not show significant difference in SUVmax. In summary, also in vivo, melatonin may have a protective effect in the atherosclerotic pathogenesis.

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Keywords
Atherosclerosis, inflammation, melatonin, oxidative stress, ^{18}F-FDG PET.

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Ischemic colitis following left antegrade sclerotherapy for idiopathic varicocele: the role of forensic clinical anatomy

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Introduction. The Tauber procedure, i.e. antegrade sclerotherapy for varicocele, can be complicated by ischemic colitis. Its possible pathogenesis is referred to the presence of an atypical kind of portal-systemic communication, which could represent an unfrequently reported anatomic variant. Aim of this study is to solve this anatomical controversy of utmost clinical usefulness.

Materials and methods. A computer-aided and hand-checked systematic review of the literature was implemented to identify relevant publications on the topic. Moreover, we reviewed the computed tomography CT-scan of a clinical case with medico-legal implications due to severe vascular complication following Tauber's procedure.

Results. Despite specific References were made on the issue in more dated hardbacks since the 19th century, only a few clinical cases reporting an ischemic colitis following the Tauber's procedure were found in contemporary literature. By reviewing the CT-scan images of a filed lawsuit we found traces suggestive for the presence of a significant communication between the internal spermatic and the left colic vein, as part of the portal-systemic anastomoses.

Conclusions. A significant anatomical finding identified in the past have been under-reported and subsequently underestimated in its clinical value. For the first time we demonstrated its pathophysiological role in a real clinical scenario, coupling the anatomical variation to the clinical complication hence stressing that its knowledge is of utmost importance to raise the scientific awareness and to prevent possible devastating complication in clinical daily practice. Progress in the medical field coupled with increased medical-legal awareness has supported the ripening of clinical anatomy and forensic clinical anatomy, whose multidisciplinary represents the best way to recover and hand down the medical knowledge at risk of being forgotten.

Keywords

Forensic clinical anatomy, antegrade sclerotherapy, internal spermatic vein, left colic vein, anatomical variation, ischemic colitis

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Effects of the endoplasmic reticulum signaling pathway on cadmium-induced impairment of the blood brain barrier

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Due to its high permeability to blood-brain barrier (BBB), cadmium (Cd) has been regarded as a possible etiological factor for human neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, and Huntington's disease [1]. However, the mechanism underlying cadmium-induced BBB permeability remains unclear. In this study, we investigated the effect of Cd in RBE4 cells (rat brain endothelial cells) and delineated the signaling pathway that, triggering endoplasmic reticulum (ER) stress and caspase 3 activation, leads to cytoskeleton disorganization and tight junctions disassembly.

Our results demonstrate a possible downstream pathway mediated through the Cd-dependent ER stress, assessed by the cytoplasmic expression of an ER protein GRP78, and the consequent caspase-3 activation that results in an extracellular ATP increase, which in turns induces a dislocation, evaluated by immunofluorescent staining, of Zonula Occludens-1 (ZO-1), a tight junction protein, and F-actin.

These findings, whereby Cd-induced permeabilization of BBB through a ER stress-dependent pathway on endothelial cells represent a possible novel mechanism of action for Cd that could explain, at least in part, the Cd-related central effects.

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Keywords

Cadmium neurotoxicity, blood brain barrier, ER stress, caspase-3 activation, tight junctions dislocation

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Zinc protection in cadmium-induced Blood Brain Barrier permeability: a metabolic and morphological in vitro study

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Cadmium (Cd) is a worldwide occupational and environmental pollutant [1]. Cd toxicity is widely known and studied in many tissues and organs such as testis, kidney, liver, lung and brain [2,3]. On the other hand, zinc (Zn) is a trace element known as coenzyme for many proteins such as methallothionein [4]. To date, very little is known about the role of Cd and Zn in BBB permeability.

To study their effects in BBB permeability in vitro, the RBE4 cell line was used and different concentrations of $CdCl_2$ and $ZnCl_2$ were tested. Metabolic activity (MTT assay) was performed to test the protective and preventive role of $ZnCl_2$ on $CdCl_2$ toxicity. Western blotting analysis was used to better investigate the molecular pathway involved in Cd-induced BBB permeability evaluating GRP78 (ER stress marker) and caspase-3 protein expression levels. Furthermore, ZO-1 and F-actin immunofluorescent staining was performed to better understand the morphological alterations and BBB permeability achieved by Cd treatment.

Our preliminary data highlight the role of Cd in evoking BBB permeability by F-actin and ZO1 dislocations, triggering the caspase-3 molecular pathway activation induced by GRP78-ER stress increase. Moreover, the data clearly show how Zn is able to counteract the metabolic impairment induced by Cd treatment.

Taken together these data point out the possible role of Zn in counteracting the Cd-induced BBB impairment.

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Keywords

Zinc, Cadmium, BBB permeability, morphology, in vitro study

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TLQP peptides in Amyotrophic Lateral Sclerosis

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TLQP peptides (TLQPp) act on synaptic strengthening or against neuronal apoptosis but their involvement in Amyotrophic Lateral Sclerosis (ALS) is not well known. We used an ALS animal model (SOD1-G93A) and human tissues (plasma and fibroblasts from both patients and controls), to address the TLQPp expression and their modulation in ALS mechanisms. Mouse motor neuronal cells (NSC-34), were used to study the neuroprotective role of the TLQP-21 against oxidative stress induced by Sodium Arsenite. TLQPp were immunoquantified by ELISA, their cell expression studied by immunofluorescence while cell viability was addressed by MTT assay. In the NSC-34 naïve cells, TLQPp were found within the cytoplasm, axons and growth cones. Upon stress, they appeared immunolocalized exclusively within a cytoplasmatic area, close to the nucleus, (probably the Golgi). Moreover, under stress, TLQPp levels were reduced (42%, $p=3.1x10^{-6}$), while the exogenous increase of TLQP-21 improved cell viability (13%, p= 0.018). In naïve fibroblasts, TLQPp were also similarly localized in an area, likely the Golgi, of patients and controls while their reduction (31%, p=0.03) was observed in cells with TARDBP-A382T mutation. Stress granules only appeared after SA treatment, and did not contain TLQPp. In plasma, a reduced release of TLQPp was associated with the beginning of the clinical motor symptoms in patients (12%, p=0.026), and with a pre-symptomatic stage in mutant mice (26.3%, p=0.048). Finally, in mouse spinal cord we identified by vescicular acetylcholine transporter (VAChT) antibody, a specific localization of the TLQPp in the motor neurons with a reduction in the pre-symptomatic stage of mutant mice. In conclusion, TLQPp are reduced in the stressed NSC-34 cells, mutant mouse motor neurons as well as in fibroblasts with TARDBP-A382T mutation that also induces production of reactive oxygen specie. Hence, we suggest that their reduction may occur in response to oxidative stress. They could be also considered as early biomarkers, while TLQP-21 acts as neuroprotective factor.

Keywords
ALS, VGF, TLQP peptides, motor neurons

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The internal thoracic vein for the breast and thoracic surgical reconstruction: anatomy of the valves

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The internal mammary veins (IMV) are suitable vessels for thoracic wall reconstruction thanks to their relatively predictable anatomy and because they are less affected by atherosclerosis, injury or scarring of previous surgery and radiotherapy. Their position near to the lateral border of the sternum allows easier access for surgeon and the possibility of placing the most vascularized part of a flap for breast reconstruction in the medial thoracic region [1]. As the complexity of reconstruction has increased, the use of the caudal portion of IMV has been reported as a convenient option for additional venous drainage. This procedure, requires retrograde blood flow that could be altered by the presence of efficient valves in the IMV.

In this study, we evaluated 32 IMVs dissected from 16 fresh cadaver thoracic walls. Retrograde blood flow and the presence of valves were investigated.

We observed an efficient flow in 18 IMVs, partial flow in 7 IMVs and no flow in 5 IMVs. In these last, single and/or multiple sacciform swelling and competent valves were macroscopically observed. Histomorphological analysis by Haematoxylin and Eosin and Masson-Goldner Trichrome staining confirmed their presence. In addition, some rudimental valves, not identified macroscopically, were found during histomorphological analysis.

Taking together, these data highlight the possible presence of complete or rudimental valves in IMV. This aspect should be considered when retrograde flow of IMV as a single venous drainage was performed during surgical breast and thoracic reconstruction.

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Keywords

IMV, valves, retrograde flow, free flap reconstruction

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An ultrastructural study of Sertoli cells inside alginate microcapsules

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Sertoli cells (SeC) are the main components of the blood-testis barrier, are essential for spermatogenesis and are long known for their ability to secrete trophic, anti-inflammatory and immunomodulatory factors [1]. For these reasons, SeC have been encapsulated in sodium alginate microcapsules and then used to create an ectopic immune-privileged environment to prolong survival of co-transplanted cells or modulate the immune responses [2]. Encapsulation has represented an improvement for the use of SeC. In fact, it has been reported that inside the microcapsules SeC (SeC-MC) act as a "micro-biofactory" and drug delivery system. By secreting immunomodulatory and trophic factors once injected into the peritoneal cavity of dystrophic mice [3], they can ameliorate muscle morphology and function. Since the manipulation of the microcapsules is rather complicated, we performed for the first time, an ultrastructure study on SeC-MC. The good cell morphology, along with viability of organellar compartment, was demonstrated.

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Keywords
Sertoli cells, encapsulation, ultrastructure, Duchenne muscular dystrophy

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The hidden geometry of the brain

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The human brain connectome is a topologically complex, spatially embedded network. One of the characteristic, basic, nonrandom rules on which brain topology relies on is the tendency of brain networks nodes to cluster into modules with high efficiency and short path length, thus reflecting an intrinsic small-world behavior, functionally segregated (local clustering) and integrated (global efficiency) [1]. Although network topology seems to be somehow connected to network geometry, one of the most challenging issues of the current network science is to infer the hidden geometry from the mere topology of a complex network. Here in, aiming at disclosing the latent geometry of the brain, we apply coalescent embedding – a novel advanced technique able to map a given network in the hyperbolic space inferring the node angular coordinates - on different structural brain networks [2]. Interestingly, we show that we can unsupervisedly reconstruct the intrinsic brain geometry with an incredible level of accuracy and that it strongly resembles the known brain anatomy. As a matter of fact, the first rule of organization of brain networks emerging in the hyperbolic space is their structural segregation into two distinct sections corresponding to the left and right hemispheres, which is a simple concept yet quite neglected in previous studies on brain connectomics. In addition, we demonstrate that the human structural brain networks exhibited a significant different geometry in two age range-specific groups. Finally, we show that the intrinsic geometry of Parkinson's Disease patients is significantly altered compared to the healthy subjects as revealed by two novel latent geometry markers. The present study may bridge the gap between brain networks topology and geometry and may open a completely new scenario towards the realization of latent geometry network markers for the evaluation of brain disorders.

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Keywords

Brain networks, connectome, latent geometry, topology, network neuroscience

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Does Pelvic Incidence Influence the Morphology of the Sacroiliac Joint?

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Pelvic Incidence is defined as the angle between the perpendicular line to the upper plate of S1 at its midpoint and the line between this point and the center of bicoxofemoral line, it describes the position of femural heads in relation to sacrum. Recently some authors described a direct correlation between high values of PI and large AP pelvic axis (horizontal pelvis) and a wide pelvic ring [1]. Also the acetabular orientation is influenced by PI ; high values of PI means a more vertical acetabulum. Having regard to the relationship between PI and the main structures involved in the load transfer, to date no studies that correlate the morphology of the Sacroiliac Joint (SiJ) and PI were performed. The aim of this study is to evaluate the different morphology of the auricular surface of the sacrum comparing two groups of healthy young people with low (<40°) and high (>40°) PI. We retrospectively analysed 51 consecutive young (between 20 and 35 y.o.) people. After the evaluation of PI the sample was divided into two groups: 31 people belong to the group A (PI < 40°) and 20 people belong the the group B (PI >40°). The following morphological parameters of the SiJ were analysed: length of long axis (LLA), length of short axis (LSA), length of oblique axis (LOA), ratio between long and short axis (RLSA), angle between axis (ABA) and surface; global shape of the joint was evaluated; two new parameters were introduced, SiJ Tilt (SiJT), defined as the angle between the vertical line and the long axis of the SiJ and SiJ Slope (SiJS), defined as the angle between the horizontal line and the short axis of the SiJ. We found a strong statistically significant correlations (p-value 0.05) between PI and RLSA, shape, ABA, SiJT and SiJS; a weaker correlations (p-value 0.10) between PI and LLA, LSA were observed; no statistically significant correlation between PI and LOA and surface were observed. The results underline that there is a strong correlation between pelvic morphology and SiJ anatomy. Further studies, about the different pattern of forces distribution among SiJ, will need to be performed to have a better knowledge that could help to understand the biomechanics and pathophysiology of normal and pathological SiJ.

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Keywords

Sacroiliac Joint, Pelvic Incidence, Biomechanics, Load Sharing

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Finite Element Analysis in different Subtype of Sagittal Alignment

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In humans, vertical posture acquisition caused several changes in bones and muscles which can be assumed as verticalization. It's widely known that six different morphological categories exist; each category differs from the others by pelvic parameters and vertebral column curvatures. Both values depend on the Pelvic Incidence, calculated as the angle between the axis passing through the rotation centre of the two femur heads and the vertical axis passing through the superior plate of the sacrum. The aim of this study is to evaluate the distribution of stress and the resulting strain along the axial skeleton using finite element analysis. The use of this computational method allows performing different analyses investigating how different bony geometries and skeletal structures can behavior under specific loading conditions. A CT of artificial bones, was used to obtain geometrical data of the model developed. Lines were imported into a commercial code in order to interpolate main surfaces and create the solid version of the model. Six different models were created according Roussouly's classification, by arranging geometrical position of the skeletal components. Loading conditions were obtained by applying muscular forces components to T1 to L5, and a fixed constrain was at the distal epiphisis of femurs. Materials were assumed as elastic; Elastic modulus of 15 GPa, a Shear Modulus of 7 GPa for bony parts; Elastic modulus of 6 MPa, a Shear Modulus of 3 MPa for cartilaginous parts [1]. Six different simulations have been carried out. Results confirm higher solicitations obtained varying configurations from case I to case VI. In particular way, first three cases seem to supply the different loading configurations spreading stresses in almost all the bony parts of the column, while the remaining others three cases produce a higher concentration of stress around the lower part of spine (L3, L4, L5). Results confirm a good agreement with those present in literature, an equivalent Von Mises average stress of 0,55 MPa was found on the intervertebral disks with the higher values reached on the lower part of the model. A comparison of results obtained for Case I with literature, shows a good agreement in terms of normal compressive force, while more evident differences can be found for shear force and sagittal moment. The results underline a relationship between PI increase, and accordingly of PT and LL, and the distribution of load forces. Load forces is exerted mainly on distal vertebrae, especially on L4 and L5.

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Keywords

FEM, finite element analysis, Sagittal Balance, Lumbar Spine, Biomechanics

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Cutaneous changes in varicose legs with normal skin appearance

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Background: In most of Western and European countries, reimbursement for the treatment of vein disorders depends upon the severity of the disease. This is currently evaluated according only to skin appearance (normal skin, edema, cutaneous changes and finally ulceration).

Aim: In this study, the occurrence of skin damaging was evaluated by ultrasonography in varicose legs with apparently normal skin (excluded from reimbursement in many countries).

Methods : Only legs with extensive varicose veins were considered (reflux originating from the saphenofemoral junction and descending along the great saphenous vein down to the lower leg). US findings fom the varicose leg were compared to those from the contra lateral healthy limb.

Results: In 13/18 varicose legs, skin changes related to inflammation were observed: dermal edema, subcutaneous edema, dermal or subcutaneous infiltration. In these legs, scoring for venous symptoms resulted higher than in the remaining 5 legs.

Discussion: Sonography demonstrated the occurrence of cutaneous and/or subcutaneous inflammation in varicose legs with apparently normal skin. These objective signs correlate to heavier symptoms and greater limitation in working, domestic and social activities.

Possible forensic and insurance implications are finally discussed.

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The role of virtual reality in improving gait abnormalities

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To regain walking after a neurological disorder is considered one of the primary goals of the rehabilitation process, given that gait abnormalities are often disabling, negatively impacting patients' quality of life. In the last years there has been an intense technological development of robotic devices to overcome such problems. The robotic rehabilitation tools are typically based on the so-called phenomenon of motor learning, resulting from intensive, repetitive, and task-oriented motor activities that require patient's effort and attention [1]. Such robotic devices can be classified into stationary and overground walking systems: stationary systems (treadmill gait trainers such as Lokomat, and programmable foot end-effector trainers including GeoSystem) and overground walking systems (e.g. Ekso-GT). Stationary devices and new treadmill and balance platforms, such as C-Mill and CAREN, may be equipped with virtual reality, to further improve functional outcomes. Virtual reality is conceived to put the patient in a situation to generate the augmented feedback towards his central nervous system (augmented feedback) through exercises performed in a virtual environment which help to develop knowledge of results of the movements (knowledge of results) and knowledge of the quality of the movements (knowledge of performance). Thanks to this, the central nervous system can activate a physiological key learning mechanism called "reinforcement learning" which implies an increase of the specific information of a movement to produce an effective improvement of performance quality.

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Keywords

CAREN, Lokomat, Virtual reality, Gait training

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In vivo Micro-computed tomography imaging of adipose tissue of mice fed Sicilian pistachio

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In obesity condition the adipose tissue undergoes molecular and cellular alterations affecting systemic metabolism via the release of different pro-inflammatory mediators. Nut-derived polyphenols and fatty acids have a documented role in the modulation of energy metabolism and antiobesity effect.

We investigate the effects of *Pistachia vera* nuts on body fat mass and its distribution in a mouse models of obesity by Micro-computed tomography (CT). For this purpose, three groups of C57BL/6J male mice were fed for 16 weeks with a standard chow, a high fat diet (HFD) or HFD supplemented with pistachio harvested in plantation of the valley of the Platani river (Sicily).

Fatty acids extraction from *Pistachia vera* nuts was carried out and analysed by a gas-chromatographic technique for contributing 20% of total diet energy. Total body fat mass was estimated and compared between groups by micro-CT (Quantum FX Micro CT scanner). Furthermore, the effects of pistachio consumption on total cholesterol, triglycerides, fat liver accumulation, adipose tissue inflammatory cytokines (TNFα and IL-1β) expression and serum ROS levels were also evaluated.

The micro-CT slices and three-dimensional image stacks of control and obese mice showed a reduced body fat deposit and less visceral fat in mice fed HFD supplemented with pistachios. Furthermore, cholesterol and triglycerides levels, hepatic lipid accumulation, pro-inflammatory cytokines and serum ROS levels were significantly reduced in mice fed HFD supplemented with pistachio compared with the HFD mice.

Our results suggest that pistachio consumption could have counteracting effects on metabolic dysfunctions in obesity through acting on the fat mass. In particular, it lower the accumulation of visceral and subcutaneous fat that could be responsible of lower level of circulating and hepatic lipids and of the decreased inflammatory condition.

Keywords

Micro-computed tomography, obesity, Pistachia vera, metabolic syndrome, adipose tissue

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Diffusion Tensor Imaging (DTI) in Head and Neck Oncology; a new method of Virtual Biopsy

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The objective of our study is to explore the feasibility and utility of Diffusion Tensor Imaging (DTI) in the non invasive evaluation of any lesion [1] in Head and Neck structure. We would reach a verified and certified step of Virtual biopsy. Ten patients with different histological lesions were recruited for this study. Morphological and diffusion weighted images were acquired with a 3T scanner during last 3 months. Probabilistic tensor-based tractography reconstruction of any lesions was performed and mean fractional anisotropy (FA) values for everyone were extracted [2]. The results showed that the Diffusion Tensor Imaging (DTI) was able to identify the lesion geometrical morphology and diffusion microstructural changes. The patient with a benign lesion reported a significant improvement in ADC mean values. We have evidence that pushes us to continue with this method. We are at the beginning of this work. We had to recruits more patients to get meaningfully significant data.

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Keywords

Diffusion tensor imaging, Tractography, Fractional Anisotropy

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LPS-stimulated human macrophages displayed sex differences in estrogen receptors α and β

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Macrophages play a key role in immunity, inflammation, and atherosclerosis. Moreover, several evidences demonstrate that 17-β-estradiol (E2) plays a key role in inflammation and atherosclerosis through estrogen receptors (ERs) ERα and ERβ, processes that display sex differences. It has been largely demonstrated that male tissues express active ERs, but there is still lack of knowledge on their role in inflammation in males. Macrophages, which have ERα and ERβ, are a good model to evaluate the role of ER levels and activation in inflammation. The aim of our work was to evaluate the ability of lipopolysaccharide (LPS) to modulate, in a sex-specific way, the expression and the activation status of ERα and ERβ in blood monocytes-derived macrophages (MDMs) from healthy men and women. MDMs were isolated from blood of 7 adult men and 7 adult and fertile women (aged 21 - 35 years), and cultured. After 10 days of culture, MDMs were incubated with 100 ng / ml LPS for 24 h and lysed for the analysis of ERα, ERβ, P- ERα, p38 and P-p38 expression by Western Blotting.

We found that in basal conditions, the expression of ERα and ERβ was significantly higher in female MDMs than in male ones. Importantly, LPS stimulation up-regulated ERα and ERα phosphorylation in both sexes, but this regulation was more pronounced in male MDMs. Moreover, LPS down-regulated ERβ level only in female MDMs. The expression of p38 and P-p38 proteins, used as marker of ERβ activity, did not display any sex differences. Finally, the ratios between ERα / ERβ and P-ERα / ERα were significantly higher in male than in female MDMs.

Our findings show, for the first time, that LPS can modulate the activation of ERα but not that of ERβ, identifying a critical role of the subtype ERα in inflammatory responses mediated by LPS, at least in human MDMs. These results represent a starting point in understanding the influence of sex in the relationship between LPS and ERα.

Keywords

sex differences, estrogen receptors, lipopolysaccharide, inflammation

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Histomorphometrical evaluation of cells and tissues in contact with a new anti-wear dental implant surface: Bioloy® coating

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Dental implants rehabilitation of edentulous patients is the current accepted treatment to increase prosthetic stabilization. Various implant surface modifications have been tried to enhance osseointegration and to reduce the spread of detrimental metallic ions toward host tissues [1].

The aim of this preliminary study was to investigate in vitro the viability, proliferation and adhesion of a Bioloy® (B®) coating compared to machined and sandblasted surfaces and to assess histologically in vivo the bone response to customized mini-implants coated with B® () placed in the mandible of patients. B® is a titanium niobium nitride coating applied on surface by physical vapor deposition (Permedica Spa). It is a thin ceramic monolayer, extremely hard and with high resistance against wear, scratches and corrosion [2]

Viability and adhesion was tested at 24, 48 and 72 hours after seeding of SAOS-2 on customised scaffold. Cell viability (2x104 cells) was evaluated by AlamarBlue® assay [3] and it resulted statistically higher on B® than in the other 2 groups (48 and 72 hours, p-value<0.05). No toxic response was observed. Adhesion (104 cells) was analysed by scanning electron microscope [3]. Cell morphology confirmed the healthy status. Cells adhered to the surface and proliferated, covering completely the surfaces of machined and B® at 72 hours.

Osseointegration was evaluated in 2 patients. After tooth extraction, 3 MIB® were placed. Three months later, during drilling process, the biopsies with MIB® were harvested for histological processing (Donath' protocol) [3]. In all sections MIB® resulted well osseointegrated and newly formed bone was highly mineralized and organized in lamellae (bone to implant contact 46.8%±9.15). The implant coils were filled with new bone for the 59.8% ± 4.23. Medullary spaces were rich in blood vessels without inflammatory infiltrate.

In conclusion, B® is a promising coating able to enhance the viability in vitro and to favor the osseointegration in vivo.

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Keywords

Titanium nitride, niobium, osseointegration, implant surface

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A Multi-dimensional Research Approach to Support Youth Taekwondo Athletes

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Youth sports encompasses a long-term developmental process, which varies in relation to the age and technical level of the athletes. At present, no single variable could be considered effective in providing information on relevant aspects related to the holistic development of talented athletes. This study aims to present a multi-dimensional research approach to taekwondo competitions, training, selection process and best practices in the combination of sport and academic commitments (e.g., dual career).

Findings from psycho-physiological and technical-tactical methods to investigate youth taekwondo athletes (age: 10-17 years) during competitions, training and a selection period will be presented, as well as European best practices of dual career policies for athletes.

In general, high psycho-physiological demands of official youth taekwondo competitions emerged. Conversely, children tended to perceive competition and training efforts as moderately high. In particular, qualitative psycho-physiological variables seem to be effective in discriminating talented athletes during intensive training periods. Thus, the assessment of the athlete's perceived efforts during training and competition could help coaches monitoring their training plans and effective recovery strategies. Although the European Parliament and Commission prioritize the holistic development of elite athletes, Member States present relevant differences to dual career policies, with Italy being characterized by laisser-faire/no formal structures. Therefore, a cooperation between Italian sport and educational institutions is deemed necessary to support the youth talented athletes in combining sport and education commitments.

Keywords

Rating of perceived efforts, match analysis, psycho-physiological responses, dual career

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KIR3DS1-mediated recognition of HLA-*B51: modulation of KIR3DS1 responsiveness by self HLA-B allotypes and effect on NK cell licensing

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Several studies described an association between killer-cell immunoglobulin-like receptor (KIR)/HLA gene combinations and clinical outcomes in various diseases [1-2]. Here, we show that KIR3DS1 mediates positive signals upon recognition of HLA-B*51 (Bw4-I80) surface molecules on target cells and that this activation occurs only in Bw4-I80neg individuals, including those carrying particular KIR/HLA combination settings. In addition, killing of HLA-B*51 transfected target cells mediated by KIR3DS1+/NKG2A+ NK cell clones from Bw4-I80neg donors could be partially inhibited by antibody-mediated masking of KIR3DS1. Interestingly, KIR3DS1-mediated recognition of HLA-B*51 could be better appreciated under experimental conditions in which the function of NKG2D was reduced by mAb-mediated blocking. This experimental approach may mimic the compromised function of NKG2D occurring in certain viral infections. We also show that, in KIR3DS1+/NKG2A+ NK cell clones derived from an HLA-B Bw4-T80 donor carrying 2 *KIR3DS1* gene copy numbers, the positive signal generated by the engagement of KIR3DS1 by HLA-B*51 resulted in a more efficient killing of HLA-B*51-transfected target cells. Finally, we analyzed KIR3DS1+/NKG2A+ NK cell clones from a HLA-B Bw4neg donor carrying cytoplasmic KIR3DL1. Although these clones expressed lower levels of surface KIR3DS1, they displayed responses comparable to those of NK cell clones derived from HLA-B Bw4neg donors that expressed surface KIR3DL1. Altogether these data suggest that, in particular KIR/HLA combinations, KIR3DS1 may play a role in the process of human NK cell education.

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Keywords
Human NK cells, activating KIRs, KIR3DS1, NK cell education, HLA-B alleles

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Hepatic Lysosomal Acid Lipase and lipophagy in the progression of NAFLD

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Lysosomal Acid Lipase (LAL) is an acidic enzyme that degrades cholesterol-ester and triglyceride inside lysosomes. Both genetic LAL deficiency and non-alcoholic fatty liver disease (NAFLD) are featured by lipid accumulation in hepatocyte leading to steatosis and eventually liver failure. Recently, a deficit in blood LAL activity was found in NAFLD patient (1). Lipophagy plays a pivotal role in degradation of lipids in the liver and consists in autophagic sequestration of lipid droplets and their degradation inside lysosomes by LAL (2). p62 serves as an autophagy/lipophagy receptor for selective autophagy and accumulates when the autophagy is blocked. We aimed to evaluate the hepatic expression of LAL in NAFLD patients and healthy subjects and to verify its association with histopathological features. Furthermore, we aimed to compare LAL levels with autophagic flux and lysosomal compartment status (LAMP1-positive vesicles). LAL expression was reduced in NAFLD patients with respect to healthy subjects ($p<0.001$), in patients with microvesicular steatosis ≥25% with respect to those with <25% ($p<0.01$), and inversely correlated with NAFLD activity ($r=-0.52$; $p<0.001$). p62 expression increased with the progression of NAFLD ($r=0.47$; $p<0.001$) and LAMP1-positive fatty vacuoles was inversely correlated with LAL expression ($r=-0.35$; $p=0.01$). Hyperdense vacuoles resembling typical lipolysosome and autophagosomes were observed with TEM analysis and accumulates in NAFLD patients. These results show that LAL expression is reduced in NAFLD patients and associated with features of genetic LAL deficiency and NAFLD activity. A lipophagy impairment, probably due to LAL dysfunction, could exert a pathogenetic role in NAFLD development.

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Keywords
Non-alcoholic fatty liver disease, lysosomal acid lipase, lipophagy

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Hepatic stem/progenitor cell activation differs between primary sclerosing and primary biliary cholangitis

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The activation of hepatic stem/progenitor cells (HPCs) is characterized by the appearance of ductular reaction (DR) in the liver parenchyma [1]. The aims of the present study were to evaluate the activation of HPCs in human cholangiopathies. Human liver tissue was obtained from liver donors (N=5), Primary Sclerosing Cholangitis (PSC; N=20), and Primary Biliary Cholangitis (PBC; N=20) patients. Ductular reaction extension was evaluated by Keratin(K) 7 immunoreactivity. HPC phenotype and signalling pathways were investigated by immunohistochemistry and immunofluorescence [2]. Ductular reaction in PBC is more extensive, appears earlier, and has a higher proliferation index compared to PSC. In PBC the extension of DR strongly correlates with clinical prognostic scores. A higher percentage of Sox9+ and K19+ cells characterized DR in PBC versus PSC. In cirrhotic-PSC, the HPC compartment showed signs of hepatocyte commitment. The study of the HPC niche indicated lower levels of laminin and NOTCH1 but higher expression of WNT pathway components in PSC compared to PBC. In conclusion, PSC and PBC are characterized by different patterns of HPC niche activation, reflecting the involvement of different portions of the biliary tree as primary target of damage. These aspects could have implications in the pathogenesis of cholangiopathies and could add prognostic value.

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Keywords
Stem cell, liver, biliary tree, cholangiopathies, regeneration, signalling

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Platelet gene expression profile in acute myocardial infarction

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Acute myocardial infarction is a sudden event that is fatal in around one-third of patients. It is primarily due to coronary atherosclerotic plaque rupture with subsequent platelet (PLT) activation/aggregation and thrombus formation. PLTs have a key role in the genesis and progression of atherosclerosis and in thrombus formation[1]. PLTs are anucleated cells which retain mRNA from their megakaryocyte precursor, therefore PLT mRNA is unique in representing a nearly fixed transcriptome[2].

We tested the hypothesis that platelet transcriptome acts as a fingerprint indicating the development of a future myocardial infarction, with the final goal of identifying a specific STEMI gene-signature, able to discriminate patients with acute event from healthy donors (HD) and from patients affected by stable coronary artery disease (sCAD), the phenotypically closest clinical condition to STEMI.

Peripheral blood samples (50mL) were collected in Na-citrate tubes from 20 myocardial infarction patients (MI), 20 sCAD and 20 HD. Highly purified platelets were obtained by leukocyte depletion as previously described[3]. Platelet RNA extraction were performed by TRIzolTM reagent according to the manufacturer's protocol. Gene expression profile was analyzed using an Affymetrix GeneChip system (Cancer Genomics and Bioinformatics Laboratory Facility, Kimmel Cancer Center, Jefferson University. Philadelphia, US).

The exploratory analysis of PLT transcriptome confirmed differences in gene expression between STEMI, sCAD and HD. Hence, the common differentially expressed genes (DEGs) derived from the STEMI vs sCAD and STEMI vs HD comparisons were obtained and tested by k-nearest neighbor classification and bootstrap. A set of 17 STEMI-related DEGs was identified, showing good sensitivity and specificity for the discrimination of STEMI patients.

Overall, we described a STEMI-specific gene expression patterns, suggesting that PLT transcriptome allows to characterize a powerful fingerprint of STEMI theoretically able to predict a future acute event.

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Keywords

Acute myocardial infarction, platelets, platelet transcriptome

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Immunoreactivity and expression of synucleins in the South African clawed frog *Xenopus laevis* peripheral nervous system

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Human synucleins (syns) genes coding for α-, β- and γ- isoforms are highly expressed in mammalian nervous system, in particular α-syn is implicated in several neurodegenerative diseases collectively named synucleinopathies, including Parkinson's disease, frequently associated with motor impairment. The precise functions of syns remain elusive, but there are evidence indicating their involvement in the regulation of vesicular trafficking, exocytosis and synaptic function.

Because of the high degree of conservation of syns among vertebrates, non-mammalian animal models may provide additional information on the evolution and the physiological role of these proteins [1,2]. Preliminary data are here reported on α- and β- syns expression and their morphological localization in different organs of adult specimens of the South African clawed frog *Xenopus laevis*, obtained by RT-qPCR, Western blot (WB) and immunohistochemistry (IHC). In WB and IHC experiments, two different commercial antibodies against mammalian α-, β- syns were used. Alpha- and β-syn immunoreactivities were differently distributed in the various tissues analyzed. Interestingly α -syn immunoreactivity was detected in both peripheral and autonomous nervous system respectively innervating skeletal muscles, cardiovascular system and gastrointestinal tract. Alpha-syn immunoreactive (IR) nerve fibers were found along skeletal muscle fibers, showing large varicosities typical of neuromuscular junctions. Moreover, both submucosal and myenteric plexuses of the gastrointestinal tract showed IR fibers. These preliminary observations suggest a conserved role for α-syn in synaptic vesicle trafficking in peripheral nerves and suggest that *Xenopus laevis* may be a promising model for the study of synucleinopathies.

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Keywords
Synucleins, peripheral nervous system, Xenopus laevis

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Identification of an HSP90 modulated multi-step process for ERBB2 degradation in breast cancer cells

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The receptor tyrosine kinase ERBB2 interacts with HSP90 and is overexpressed in aggressive breast cancers. Therapeutic HSP90 inhibitors, i.e. Geldanamycin (GA), target ERBB2 to degradation. We have previously shown that HSP90 is responsible for the missorting of recycling ERBB2 to degradation compartments. In this study, we used biochemical, immunofluorescence and electron microscopy techniques to demonstrate that in SKBR3 human breast cancer cells, GA strongly induces polyubiquitination and internalization of the full-length p185-ERBB2, and promotes its cleavage, with the formation of a p116-ERBB2 form in EEA1-positive endosomes (EE). p116-ERBB2 corresponds to a non-ubiquitinated, signaling-impaired, membrane-bound fragment, which is readily sorted to lysosomes and degraded. To define the sequence of events leading to p116-ERBB2 degradation, we first blocked the EE maturation/trafficking to late endosomes/lysosomes with wortmannin, and found an increase in GA-dependent formation of p116-ERBB2; we then inhibited the proteasome activity with MG-132 or lactacystin, and observed an efficient block of p185-ERBB2 cleavage, and its accumulation in EE, suggesting that p185-ERBB2 polyubiquitination is necessary for proteasome-dependent p116-ERBB2 generation occurring in EE. As polyubiquitination has also been implicated in autophagy-mediated degradation of ERBB2 under different experimental conditions, we exploited this possibility and demonstrate that GA strongly inhibits early autophagy, and reduces the levels of the autophagy markers atg5-12 and LC3-II, irrespective of GA-induced ERBB2 polyubiquitination, ruling out a GA-dependent autophagic degradation of ERBB2. In conclusion, we propose that HSP90 inhibition fosters ERBB2 polyubiquitination and proteasome-dependent generation of a non-ubiquitinated and inactive p116-ERBB2 form in EE, which is trafficked from altered EE to lysosomes.

Keywords
ERBB2, cleavage, geldanamycin (GA), polyubiquitin, proteasome

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A contribution to the gross anatomy of human extra-hepatic bile ducts with multi slice computed tomography

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Considering the high risk of biliary lesions during laparoscopic cholecystectomy the anatomy of the biliary tree has been the object of increasing interest. The aim of this study is mapping the extra hepatic biliary topography by 64-Multislice Computed Tomography, in patients who had undergone surgery for choledocolithiasis using T - Tube inside common bile duct.. This technique allows to remove virtually the liver parenchyma, and with the subsequent three-dimensional reconstruction of images, represents a good tool to visualise the biliary ducts. With 3 D reconstruction we are able to create the topography of extra hepatic ducts visible in several projections. We have selected 15 cases. Principal variants regard the morphology of cystic duct. This technique allows to map the biliary tract alternativamente to classic anatomical dissection, realizing a source of data directly from surgery. Furthermore it is possible to use the data in case of biliary iatrogenic lesion with legal sequences.

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Keywords

Extra hepatic biliary tree, 64 Multislice Computed tomography, map reconstruction

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"In vitro" osteogenic and angiogenic potential evaluation of a coculture of dental pulp stem and endothelial cells grown on the BisGMA/TEGDMA Chitlac coated thermosets

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Securing an adequate blood supply for survival of cell transplants is critical for a successful outcome in tissue engineering. Moreover during regeneration of weaken teeth, which is susceptible to reinfection, fracture and loss, the teeth apical canal is open and a limited blood supply is allowed. Thus the interactions between endothelial and dental pulp progenitor stem cells are important for vascularization of regenerating tissue cells. In particular, the interplay of dental pulp stem cells and endothelial cells can enhance "in vitro" osteo/odontogenic and angiogenic potential (Dissanayaka J Endod 2012, 38,454-463) and "in vivo" ensure angiogenesis and pulp regeneration (Dissanayaka Tissue Engin Part A 2015, 3-4, 550-563). Since dental pulp microenvironment supports HUVEC survival and capillary network formation in the absence of scaffolding material and external angiogenic stimulation , "in vitro" osteogenic and angiogenic potential of dental pulp stemcells cocultured with endothelial cells grown on BisGMA/TEGDMA Chitlac coated thermosets was evaluated.

Results: DPSCs were grown on BisGMA/TEGDMA Chitlac coated thermosets, a composite material used in dental restoration, in the presence of two different concentrations of endothelial cells (1:1 e 1:5) for 28 days and their metabolic activity and cytotoxic response were evaluated.

MTT analysis discloses that cell metabolic activity significantly increases in the presence of endothelial cells, mainly at 21 days of culture, along with cytotoxic response, while at 28 days of culture a light cytotoxic response occurs. An increasing ALP activity is evidenced in the coculture systems up to 28 days, both in the presence and in absence of Chitlac thermosets and this evidence is further supported by Alizarin red staining, which does not detect mineralization in the early stages of differentiation, but is significantly increased at 28 days of culture in both the conditions (1:1 e 1:5).

Even though the positive effect on DPSC differentiation, Chitlac thermosets could induce an inflammatory response in the system and thus an ELISA IL6 assay reveals an increased inflammatory response in 1:1 coculture system after 28 days of culture, furtherly increased in 1:5 coculture system.

In parallel an increased PGE2 release is evidenced in 1:1 coculture system in the presence of thermosets, reduced in 1:5 coculture system, suggesting the potential occurrence of neoangiogenesis, furtherly supported by a tubular network formation when DPSC are grown on matrigel.

These results evidencing that endothelial cells enhance "in vitro" osteo/odontogenic differentiation of DPSCs and angiogenesis, and that this response is revealed in the presence of Chitlac, usually used in dental restorative practice, indicate a coculture of DPSC and endothelial cells as a promising source for regenerative endodontics.

Keywords

Osteogenesis, angiogenesis, DPSC/endothelial cells coculture, BISGMA/TEGDMA Chitlac coated thermosets

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Colonization properties of different collagen sponges in skin repair

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Wound healing is a natural process that occurs in response to tissue injury [1]. In the skin, the entire process of wound healing is composed by different phases (haemostasis, inflammation, proliferation, and maturation) that could repair this complex organ consisting of the epidermis, dermis and appendages. Different collagenous sponges are used to help the regeneration of damaged skin [2]. These biomaterials are mainly formed by highly-organized natural collagen fibres, that are biocompatible, biodegradable, non-toxic and with high tensile strength [2]. In this study, we analysed the association of a keratinocyte cell line (HACAT cells) with collagenous biomaterials as an *in vitro* model for skin wound repair. In order to study the ability of this constructs to be considered an ideal regeneration template, HACAT cells were seeded on different collagenous scaffolds and cultivated for 7, 14 and 28 days in an "air-liquid interface" to promote keratinocyte differentiation. Gene expression analysis were performed with qRT-PCR on keratin genes. Moreover, histological analysis (H/E and MTT test) were performed to confirm the colonization and the proper distribution and proliferation of seeded cells. Results show that HACAT cells do proliferate and colonize in similar manner collagenous scaffolds and they differentiate to the horny layer more quickly than on plastic.

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Keywords

Wound healing, skin, collagenous sponges, HACAT cells, haematoxylin/eosin staining, gene expression analysis

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Safe use of human anatomical preparations in frontal and interactive teaching

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In the institute of Human Anatomy of Pavia, the use of cadaver dissection is not economically feasible. In order to improve students' preparation related to topography of the central nervous system, we decided to use formalin-fixed brains and cranial sections belonging to the collection of cadaveric specimens. These specimens, preserved in formalin, however cannot be manipulated as such by the students because formalin can cause headaches, burning sensation in the throat, difficult breathing and can trigger or aggravate asthma symptoms [1, 2]. Furthermore, formalin is known to be a human carcinogen [3]. In order to minimize toxic effects, whole brains were extensively washed in running water and then sliced according to different reference planes using a "home-made" device enabling cuts according to parallel planes. Finally, the resulting sections were inserted into transparent plastic envelopes, immerged in a solution composed by 0.5% agar and 1% sodium azide as preservative. Medical students can now use these human brain sections to test their own ability to recognize nervous system structures. This strategy optimize specimen's choice and focalize student's attention on peculiar, selected human samples in full compliance with current security laws.

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Keywords

Anatomical teaching, human specimens, security, central nervous system

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Post-traumatic taste disorders: presentation of three meaningful cases

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Since the late 1800s there are reports of post-traumatic anosmia [1], but few studies investigated post-traumatic gustatory deficit and adopted validated evaluation tests [2,3,4,5,6,7]. Post-traumatic ageusia is rare, occurring in ~ 0.5% of head traumas, while a gustatory deficit is more frequently referred (5-7%) when olfaction is impaired [2,4]. Unlike olfaction, gustatory sensation is mediated by several cranial nerves (VII, IX, X) and taste receptors are widely spread in the oral cavity, so that taste is considered a "robust" sense. Peripheral and/or central mechanisms may be involved in the genesis of post-traumatic gustatory dysfunction. Beyond a reduction/loss of gustatory function following a trauma, taste changes (dysgeusia) may occur, even if they are reported to be rare [7,8,9]. Gustatory disorders might not be immediately reported because patient often pays attention to other post-traumatic sequelae. Especially when persistent, taste deficits might be particularly relevant for patients' quality of life. Physicians are often not well-informed on the possible implications or treatment strategies.

Fifty-three consecutive patients with previous head trauma and chemosensory disorders were recruited by the olfactory and taste research group of the University of Verona. Every patient underwent a careful clinical examination, olfactory and gustatory testing by Sniffin'Sticks Extended test, Whole Mouth taste test and Taste Strips Test respectively (Burghart, Germany). Among them, we found 10 cases with hyposmia, 43 with functional anosmia, while 10 cases showed taste deficits (dysgeusia: n = 3, dysgeusia with hypogeusia: n = 1, hypogeusia: n = 5, ageusia: n = 1). Here we report anatomical, clinical correlations and detailed description of three cases representing central and peripheral injury patterns.

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Keywords

Trauma, taste disorders, anatomy of taste

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Relationship between wingate cycle test and 2000m rowing ergometer performance in youth athletes

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During 2000m indoor rowing performances, the estimated aerobic and anaerobic contribution are 65-75% and 25-35%, respectively2. In considering that anaerobic power could be an important predictor of performance1, the aim of this study was to analyse the relationship between the power outputs during a Wingate anaerobic test (WAnT) on a cycling ergometer and a 2000m rowing ergometer performance in young rowers.

In two separate days, 11 young (14.9±1.1yrs) male rowers performed a 2000m indoor rowing ergometer performance and a 30s WAnT on a cycling ergometer. WAnT peak power (PP) and mean power (MP), and 2000m time indoor rowing performance (t2000) were collected. Moreover, PP and MP were normalized with respect to body mass. Pearson correlation coefficients (r) were used to determine the association between t2000 and absolute and normalized PP and MP values.

Absolute PP and MP were 888.1±133.2W and 548.5±74.4W, respectively. The relative picture for normalized values was 13.4±1.5 W·kg-1 and 8.2±0.6 W·kg-1. High associations emerged between t2000 (431.5±19.5s) and absolute PP (r=-0.900, P=<0.05) and MP (r=-0.800, P>0.05) values, whereas no significant relationship was observed for normalized PP (r=-0.585, P=0.058) and AP (r=-0.561, P=0.072) values.

These findings indicate that PP and MP could be considered significant predictors of 2000m rowing ergometer performances, substantiating also the relevance of the anaerobic energy pathways to the 2,000m rowing performance.

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Keywords
Anaerobic power, Rowing ergometer, Youth athletes, Wingate test

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Rectus femoris proximal insertion anatomy - analysis of the clinically relevant anatomy and variations

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Rectus femoris muscle injury is one of the most frequent match-missing cause in sports requiring repetitive kicking and sprinting [1]. Although the injury pattern of the muscle belly and distal tendon is well documented, less is known about the specific lesions of the proximal tendons.

Its proximal insertion is a complex structure consisting of a tendinous direct head, an indirect head and an inconstant third head[2].

In this paper we systematically reviewed the current literature about the anatomy of the proximal insertion of rectus femoris in order to better understand the different sites where lesion can occur and clinical and prognostic implications.

We found that lesion of the indirect tendon is an under-recognized site of lesion and that no clinical data is present about the third head.

In clinical practice, knowledge of the possible structural variations in proximal insertion anatomy of the rectus femoris muscle might be useful to identify an higher risk of tendon or even muscle lesions and to define the best specific rehabilitation program for every athlete.

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Keywords

Rectus femoris, proximal insertion, diagnostic imaging, anatomy, structural variability, muscular-tendinous junction.

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Scleral ossicles as natural biomaterials on which vascular-like network is promoted from Mouse Aortic Endothelial cells (MAECs): preliminary results

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When a severe fracture is difficult to self-recovered, it is defined as "critical-size" bone defect. Till now, many efforts have been made by the tissue engineering (TE) to generate scaffolds suitable for recovering of this type of fracture, but the main obstacle remains the lack of an appropriate vascularization of the scaffolds. In the field of the regenerative medicine, the TE has developed many different biomaterials, with various features and peculiar functions, to be used in combination with cells and growth factors, in the generation of specialized constructs. Our proposal of natural scaffolds useful to obtain complex constructs concerns peculiar bony chips extracted from the eye bulb of adult chickens: the scleral ossicles (SOs). This proposed model is interesting because once SOs reach the definitive size in the adult animal, they are devoted only to mechanical stereotyped stress for their lifetime so that the activation of the bone remodelling should be avoided and, to do this, the osteocytes undergo massive apoptosis, making the ossicles like decellularized bones [1]. The novelty of our proposal is that the scaffolds do not require surface treatment (like further matrix deposition on the SO surface) since they are characterized, like all bones, by the well-known organic components such as type I-collagen fibres, proteoglycans and glycoproteins. The latter, for example, play the role of adhesion proteins and therefore can mediate the adhesion of the endothelial cells that should develop the vascular network. Our final goal is to obtain an *in vitro* 3D-vascularized natural constructs, from scaffolds easily available in nature to use *in vivo* for the healing of "critical-size" bone defeats. Previously [2] we identified the best preparation methods to obtain suitable SO surface for cell culture. Recently, we have performed a series of *in vitro* experiments to test the biocompatibility properties of the support; then, cell adhesion tests, viability and proliferation assay were carried out. Further, we tried to induce a vascular-like network organization of Mouse Aortic Endothelial Cells (MAECs) directly on the SOs surface, stimulating the cells with a known angiogenic factor, the Vascular Endothelial Growth Factor (VEGF), getting encouraging preliminary results.

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Keywords

Scleral ossicles, mouse aortic endothelial cells, vascularization, critical-size bone defects, bone scaffolds

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Judo training for older individuals with control group: An anthropometric evaluation

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The aim of this study was to investigate the effects of a 4-month judo training (1-hr training session, twice a week) on anthropometrical variables in older individuals (age: 60-76 yrs). The experimental group (JG) included 19 (F=9, M=10; 68.9±3.7yrs) participants to a 4-month judo programme, whereas the control group (CG) encompassed 14 (F=7, M=7; CG: 69.9±4.9 yrs) sedentary controls (CG). The considered anthropometric variables were: weight (Wt), height (Ht), body mass index (BMI), waist circumference (WC) and hip circumference (HC). A 2 (gender) x 2 (group) x 2 (intervention) ANOVA for repeated measures was applied to ascertain differences between groups ($p<0.05$).

A main effect emerged for gender ($p=0.017$) and intervention ($p=0.001$), whilst significant interactions group x gender ($p=0.007$) and intervention x group ($p=0.032$) were revealed. Regarding intervention effect, significant differences ($p<0.001$) emerged for weight (pre: 74.2±12 kg; post: 72.8±12.8 kg) and BMI (pre: 27.16±3.1 $kg \cdot m^{-2}$; post: 26.66±3.13 $kg \cdot m^{-2}$). For the intervention x group interaction, a significant difference ($p=0.01$) was confirmed only for hip circumference in the experimental group (pre: 101±5.4 cm; post: 99.9±4.9 cm).

In considering that individuals older than 55 years tend to adopt sedentary lifestyles [2] and to increase anthropometric variables related to health risks [3], intervention approaches are needed to increase the level of physical activity in older adults [1]. The present findings indicate that practising judo in older ages allows to control the anthropometric variables, thus opening a new research line on this discipline.

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Keywords:
Anthropometry, older persons, judoka, physical activity, physical health

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The pink adipocytes

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Most of white and brown adipocytes, in spite of their well-known different functions: i.e. storing energy (white) and thermogenesis (brown), are contained together in visceral and subcutaneous depots (adipose organ) in all mammals including humans (1, 2). A growing body of evidence suggests that the reason for this anatomical mixture could reside in the fact that adipocytes have peculiar plastic properties allowing them to convert directly each other under appropriate stimuli (3). Under chronic cold exposure white convert into brown to support the need for thermogenesis and under obesogenic diet brown convert into white to satisfy the need of energy storing. Adipocyte population in the mammary gland offers another striking example of adipocyte plasticity: during pregnancy and lactation adipocytes transdifferentiate into milk-producing epithelial cells (we propose to call them: pink adipocytes) and vice versa in the post-lactation period (4, 5, 6). The white into brown transdifferentiation is of great medical interest because the brown phenotype of the adipose organ is associated with obesity resistance and drugs inducing the brown phenotype curb obesity and related disorders (7).

We recently showed by transmission electron microscopy that in the post-lactating mammary gland interscapular multilocular adipocytes found close to the mammary alveoli contain milk protein granules. Lineage tracing system allowed showing that the involuting mammary gland of whey acidic protein-Cre/R26R mice, whose secretory alveolar cells express the lacZ gene during pregnancy, contains some X-Gal-stained and uncoupling protein 1 immunoreactive interscapular multilocular adipocytes. These data suggest that during mammary gland involution some milk-secreting epithelial cells in the anterior subcutaneous depot may transdifferentiate to brown adipocytes, highlighting a hitherto unappreciated feature of mouse adipose organ plasticity (8).

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Keywords

Adipose organ, plasticity, mammary gland, pink adipocytes, electron microscopy

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Sarcoglycan sub-complex in the adipose organ: a molecular and immunofluorescence study

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The sarcoglycan sub-complex (SGC) is made up of six glycoproteins which connect the cytoskeleton to the extracellular matrix in skeletal muscle. Recent data show that this complex is also expressed in epithelial tissue such as gingival, breast and prostatic ones [1]. The adipose organ is organized in multiple fat depots consisting of white and brown adipose cells. White adipocytes can store energy in triglycerides; brown adipocytes can dissipate energy for thermogenesis. It has been demonstrated that white adipocytes transdifferentiate to brown adipocytes after cold exposure [3].

In this study we examined the expression of sarcoglycans (SGs) in the adipose organ from two groups of mice: the first group was exposed at 25 C°, as control, and the second one was exposed at low temperature (4 C°) for 24 hours and 4 days. Fat depots from the visceral and interscapular region, but also from the mammary gland, have been examined by histological, immunofluorescence and molecular techniques.

Results have shown that SGC is expressed in the adipose organ, both in brown and white adipocytes of mice exposed at 25 C°. The main results is that the expression level of all sarcoglycans increase in cold exposure experiment.

For the first time the expression of all SGs in the adipose organ has been demonstrated, both at mRNA and protein levels. Since we found an increase in SGs expression after transdifferentiation from white to brown adipocytes cold exposure induced, we hypothesize that sarcoglycans could be associated with β3 adrenergic receptor; sarcoglycans associations with other receptors, as GABAr, has been already demonstrated in central nervous system. Although that, the function of these glycoproteins in the adipose organ remain still unclear and further investigation are required.

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Keywords
White adipocyte, brown adipocyte, sarcoglycans, mice, cold-exposure

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Treatment with r-irisin prevents and recovers disuse-induced bone loss and muscle atrophy

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Irisin is a hormone-like myokine secreted from skeletal muscle in response to exercise. We previously showed that treatment with recombinant Irisin (r-Irisin) in healthy mice improved cortical bone mass and geometry, supporting the idea that Irisin recapitulates some of the most important benefits of physical exercise on the skeleton and plays protective role on bone health (1). Here we show that treatment with r-Irisin prevented bone loss in hind-limb suspended mice when administered during suspension and induced recovery of bone mass when mice were injected after bone loss due to a suspension period of 4 weeks. MicroCT analysis of femurs showed that r-Irisin preserved both cortical and trabecular bone mineral density, and prevented the dramatic decrease of the trabecular bone volume fraction. Moreover, r-Irisin inhibited muscle mass decline during unloading, keeping proper fiber cross-sectional area. Notably, the decrease in myosin type II expression (MyHC II) in vastus lateralis of unloaded mice treated with r-Irisin was completely prevented. Our data reveal that r-Irisin treatment protects from disuse induced bone loss and muscle atrophy in mice. If these results will translate to humans, they may support a promising clinical strategy for the prevention and treatment of both osteoporosis and sarcopenia, particularly applicable to those patients who cannot perform physical activity, as occurs during aging, immobility and microgravity during space flight missions.

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Neuronal Antibodies and Brain alterations in APECED Patients

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APECED (Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Distrophy) is a rare autosomal recessive disorder. We previously found that sera samples from 9/14 patients revealed autoantibodies (Auto-Abs) reacting with cerebellum (GABAergic cells, n=5) and substantia nigra (SN; dopaminergic cells, n=5) [1]. Follow-up of the large majority of these patients was perfomed at least 10 years after the previous investigation. Indeed, on these patients, and on control age-matched subjects (n=14), we performed brain examinations using an MRI scanner. Obtained images were used to evaluate the volumes of white and gray matter (W.M and G.M., respectively) as well as the ventricles (III and IV). In addition, we used immunohistochemistry (IHC) on tissues from rat brain (after perfusion with 4% paraformaldehyde) in order to confirm the previous immunoreactivities or found new Auto-Abs cell targets. The brain MR revealed a reduction of G.M ($p = 0.042$) and cerebellum ($p = 0.0012$), and an increase of ventricles ($p = 0.0001$), compared to controls. Through IHC, after 10 years, we found 11/14 patients producing Auto-Abs against different brain neuronal cells. In detail, among the patients previously investigated and containing Auto-Abs against GABAergic perikarya in the cerebellum, 3 still contained the same immunoreactivity while 1 was unavailable, and 1 lost the reactivity. Instead, as to Auto-Abs against dopaminergic perikarya in the SN, 4 patients confirmed their previous reactivity, while 3 previously negative patients, revealed novel positivity (in total, n=7). A new immunoreactivity against the 5HT cells in the brainstem were also revealed in the same patients with Auto-Abs to SN (n=7). In conclusion, the co-presence of brain volume changes and neuronal Auto-Abs in APECED patients could suggest an autoimmune manifestation at the brain level that should be taken in consideration.

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Keywords

APECED, Autoantibodies, dopaminergic cells, GABAergic cells

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Morphological analysis of JAK1 intracellular pathway activation after pro-inflammatory psoriatic cytokines exposure: inside-out and outside-in the epidermis

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For their normal growth, cells depend on a continuous flow of signals from the environment. The Janus kinases (JAK) 1 transducers signalling pathway is a pleiotropic cascade used to transduce a multitude of signals among cells. A variety of ligands including cytokines, hormones, growth factors, and their receptors stimulate the JAK1 pathway. Cytokines, a large and very heterogeneous family of small and generally soluble glycoproteins, both control multiple biological processes as haematopoiesis, inflammation, and immunity playing a central role in cell-cell communication. Their action is mediated by the binding to specific receptors on the cell surface, thus transducing biological information to target cells [1]. Pro-inflammatory cytokines play a pivotal role in several inflammatory illnesses including psoriasis. Among them, interleukin (IL)-17, IL-22, IL-23 and tumor necrosis factor (TNF)-alpha play a central role. In the formation and progression of the psoriatic lesion a typical marker is keratin (K) 17 which is correlated with psoriasis severity. The aims of this study were to evaluate the early, direct, and specific effects of pro-inflammatory psoriatic cytokines i) on the activation of the intracellular pathway JAK1 and ii) on the correlation with the induction of K17 expression in a three-dimensional model (3D) of human skin (n=7) by immunofluorescence. Biopsies were cultured overnight epidermal side-up in a Transwell system and exposed to 50 ng/ml IL-17, or 100 ng/ml IL-22, or 50 ng/ml IL-23 or 100 ng/ml TNF-alpha. Samples were harvested 24 (T24), 48 (T48), and 72 (T72) hours after cytokine incubation.

In samples not exposed to cytokines, a JAK1 slight labelling was observed throughout the epidermis, decreasing at T72 in the lower layers. At T24, IL-17 and IL-22, but not IL-23 and TNF-alpha, induced an expression of JAK1 in the spinous layer. At T72, JAK1 immunostaining decreased in all samples, similarly to controls. K17 immunopositivity was induced and progressively increased with time in the suprabasal layers of epidermis in all experimental groups, with the exception of the TNF-alpha group. These results suggest that cytokines exert parallel effects on JAK1 pathway activation and K17 induction.

In conclusion, this 3D model, reproducing some features of psoriatic microenvironment, represents an useful experimental approach to dissect the specific role of each cytokine in the different steps of psoriatic lesion formation.

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Keywords

3D model, psoriasis, immunofluorescence, transmission electron microscopy, keratin 17

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The neuroprotective effect of estrogen pre-treatment in a model of neonatal hippocampal injury induced by trimethyltin

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Due to the relevance of hippocampal dysfunction in neurodevelopmental disorders, which affect memory, cognitive abilities and behaviour, developmental studies may represent an important tool for the understanding of cellular and molecular phenomena underlying early hippocampal damage, as well as to study possible therapeutic interventions, which may modify the progression of neuronal death. Since many findings support the neuroprotective effects of 17β-estradiol (E2) administration in different neurodevelopmental models of brain injury [1, 2], the present study investigates the effects of E2-pre-treatment in a model of neonatal hippocampal injury obtained by Trimethyltin (TMT) administration (6,5 mg/kg), characterized by neuronal loss in CA1 and CA3 subfields, associated with astroglial and microglial activation [3, 4]. At P5 and P6 animals received two E2 doses (0.2 mg/kg i.p.) or vehicle. At P7 they received a single dose of TMT (6,5 mg/kg i.p.) and were euthanised 7 days after treatment. Our data indicates that E2 administration significantly improves neuronal death in CA1, reduces the extent of microglial activation and restores TMT-induced reduction of both parvalbumin- and neuropeptide Y-expressing interneurons in the same hippocampal region. Our results add information on the role of in vivo E2 administration on mechanisms involved in neuroprotection and cellular plasticity in the developing brain.

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Keywords
Hippocampus, development, parvalbumin, neuropeptide y (NPY)

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The myotendinous junction plasticity following aerobic exercise

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The myotendinous junction (MTJ) is the site where muscle contractile force is transmitted from the myofibrils across the plasma membrane to the tendon extracellular matrix (ECM), therefore it is a key structure for the locomotor system [1]. In this work, we investigated the relationship between ultrastructural adaptations and the MTJ protein complex modulation after aerobic exercise. In particular, the answer of this anatomical interface to a month of moderate aerobic exercise has been analysed in Sprague-Dawley rats by means of confocal and transmission electron microscopy. Morphological observations confirm the exercise ability to increase the contact area between tissues, increasing the complexity of tendon finger-like processes, which penetrate into the muscle mass. Moreover, these observations suggest a possible MTJ protein complex adaptation after exercise. Confocal images, associated to an immunofluorescence quantification, confirm these ultrastructural observations. Taking together these data reveal that MTJ is a plastic interface. This plasticity can be induced by exercise, which is able to increase the contact area between tissues and to induce a protein synthesis at MTJ level.

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Keywords

Myotendinous junction, exercise, muscle, tendon, morphology, training

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Microenvironment regulation of the IL-23R/IL-23 axis overrides chronic lymphocytic leukemia indolence

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The development and progression of Chronic Lymphocytic Leukemia (CLL) require co-operation of both microenvironment and cytokines. Investigating the IL-23R/IL-23 axis we found that circulating cells of early-stage CLL patients with shorter time-to-treatment (but not of those with a more benign course) expressed a defective form of the IL-23R complex lacking the IL-12Rß1 chain. However, the cells from both patient groups expressed the com-plete IL-23R complex in tissue infiltrates and could be induced to express it when co-cultured with activated T cells or other CD40L-bearing cells. IL-23 production by CLL cells activated in vitro in this fashion and in lymphoid tissues was observed suggesting the exist-ence of an autocrine/paracrine loop causing CLL cell proliferation. Culture of CLL cells with stromal cells, nurse like cells and stimulation with anti IgM antibodies and IL-4 failed to activate this loop. Interference with the IL-23R/IL-23 axis using an anti-IL-23p19 anti-body proved effective in controlling disease onset/expansion in xenografted mice, suggesting potential therapeutic strategies.

Keywords
Chronic Lymphocytic Leukemia, IL23, microenvironment

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Cancer stem cells from peritumoral tissue of glioblastoma multiforme: the possible link between tumor development and progression

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In glioblastoma multiforme (GBM), cancer stem cells (CSCs) are thought to be responsible for gliomagenesis, resistance to treatment and recurrence. Unfortunately, the prognosis for GBM remains poor and recurrence frequently occurs in the peritumoral tissue within 2 cm from the tumor edge. In this area, a population of CSCs has been demonstrated which may recapitulate the tumor after surgical resection. In the present study, we aimed to characterize CSCs derived from both peritumoral tissue (PCSCs) and GBM (GCSCs) in order to deepen their significance in GBM development and progression. The stemness of PCSC/GCSC couples obtained from four human GBM surgical specimens was investigated by comparing the expression of specific stem cell markers such as Nestin, Musashi-1 and SOX2. In addition, the growth rate, the ultrastructural features and the expression of other molecules such as c-Met, pMet and MAP kinases, involved in cell migration/invasion, maintenance of tumor stemness and/or resistance to treatments, were evaluated. Since it has been recently demonstrated the involvement of the long non-coding RNAs (lncRNAs) in the progression of gliomas, the expression of H19 lncRNA, as well as of one of its two mature products miR-675-5p, was assessed in neurospheres. Our results show significant differences between GCSCs and PCSCs in terms of proliferation, ultrastructural peculiarities and, at a lower extent, stemness profile. These differences might be important in view of their potential role as a therapeutic target.

Keywords

Glioblastoma cancer stem cells, peritumoral cancer stem cells, stemness markers, proliferation markers, invasiveness markers, H19 lncRNA and miR-675-5p, ultrastructure

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Effect of NAP in diabetic retinopathy

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Diabetic retinopathy (DR) is a microvascular complication of diabetes leading to vision loss. Hypoxic/hyperglycaemic microenvironment is responsible of outer blood retinal barrier integrity impairment and uncontrolled vascularization typical of this pathology. Activation the hypoxia-inducible factors (HIFs) conduce to aberrant expression of some target genes such as vascular endothelial growth factor (VEGF). Many studies have showed the protective role of a small peptide, known as NAP, in counteract retinal damage induced by different insults (Gozes et al., 2004). In particular, we have previously demonstrated that a single intraocular dose of this peptide is able to protect retina from hyperglycaemic insult (Scuderi et al., 2014). However, the involvement of NAP in the modulation of HIFs and their downstream target genes has not been identified yet. In this work, we have instigated its effect on HIFs /VEGF system both *in vitro* and *in vivo* models of DR. Results have demonstrated that NAP treatment prevents outer BRB breakdown comprising human retinal pigmented epithelial cells (ARPE-19) grown in transwell supports and exposed to high glucose (HG) and low oxygen tension by adding desferoxamine mesylate salt (DFX). Peptide administration also reduced HIF1α /HIF2α, VEGF/VEGFRs and increased HIF3α expression in cells cultured in HG/DFX. Moreover, it reduced apoptotic cells rate by modulating BAX and Bcl2 expression, two genes involved in programmed death.

Furthermore, NAP intraocular administration in STZ-induced diabetic rat reduced retinal expression of HIF-1α, HIF-2α and VEGF by increasing HIF-3α levels. These data have been also confirmed by immunolocalization analysis detected through confocal microscopy showing the different distribution of these factors in retinal layers following hyperglycaemic insult.

Our data suggest that this small peptide may be efficacious in counteract retinal damages during DR.

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Keywords

NAP, Diabetic retinopathy, hypoxia, hyperglycaemia.

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Evidence for muscle synergies from virtual surgeries

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A fundamental challenge in neuroscience is understanding how the central nervous system (CNS) succeeds in controlling motor skills that require the coordination of many degrees-of-freedom. A long-standing hypothesis is that the CNS relies on muscle synergies, coordinated activations of groups of muscles, to simplify motor control. Evidence that the combinations of a small number of muscle synergies underlies the generation of muscle activation patterns has come from several studies performed in the last two decades with different species and experimental tasks. Muscle synergies, extracted from multi-muscle EMG recordings using multidimensional decomposition algorithms such as non-negative matrix factorization, capture regularities in the spatial, temporal, and spatiotemporal organization of the muscle patterns. However, whether muscle synergies are only a parsimonious description of the regularities of the motor commands rather than a key feature of their neural organization is still debated. Stronger evidence for a neural organization of muscle synergies would come from testing a prediction of how muscle synergies affect the difficulty in learning or adapting motor skills. An experiment with human subjects using myoelectric control to move a mass in a virtual environment has tested the prediction that it must be harder to adapt to perturbations that require new or modified synergies than to adapt to perturbations that can be compensated by recombining existing synergies. Novel perturbations were generated by altering the mapping between recorded EMG and simulated force applied on the mass, as in a complex surgical rearrangement of the tendons. After identifying muscle synergies, two types of virtual surgeries were performed. After compatible virtual surgeries, a full range of movements could still be achieved recombining the synergies, whereas after incompatible virtual surgeries new or modified synergies were required. In contrast, both types of surgeries could be compensated with similar changes in the recruitment of individual muscles. As predicted, adaptation after compatible surgeries was faster than after incompatible ones. These results suggest that muscle synergies are key elements organized by the CNS for controlling our complex musculoskeletal system by directly mapping task goals into a small number of synergy combination parameters.

Keywords

Motor coordination, motor adaptation, myoelectric control, dimensionality reduction, virtual reality

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Forces distribution during plantar stand among the myo-osteo-joint components of the foot. Simulations and analysis on a human anatomical network model

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The anatomical network analysis allows to explore the network of relationships among the anatomical parts of the human body. In our previous work the features of (2) a wide anatomical - biomechanical network were investigated.

The human foot represents a highly complex anatomical structure that carries motor-sensor functions and load distribution through a network of bones, muscles, joints and tendons. The main contacts with the ground during standing position match the metatarsal region for the forefoot and the calcaneus for the backfoot. Studies on the transmission of load in the forefoot area have shown that the latter cannot be considered as a metatarsal arch but rather as a continuous line in physiological condition of metatarsal motility (1, 3). Moreover, electromyographic studies (4, 5, 6) can only give information on the activation of the extrinsic muscles of the foot during walking, without providing any response about the distribution of the load, and the different role played by the numerous anatomical structures involved (7).

Here we present a weighted anatomical network of the foot, where every single node has a numerical value deriving from both the Young's modulus calculation and the number of connections with other nodes. The network consists of 116 nodes interconnected by 219 links and represents the biomechanical structure of the foot as activated by the plantar support.

By the collection of the data, the nodes cluster of the foot can be extrapolated and by detecting of the direct pressure on the plantar support, the virtual foot network can be reconstructed.

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Keywords

Anatomical network, foot, locomotor system

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The follicle-stimulating hormone receptor (FSHR) is expressed in human sperm and it may be considered as molecular marker of the detrimental effects related to the physiopathology of testicular varicocele

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Localization of the follicle-stimulating hormone receptor (FSHR), has been always closely related to the testis and ovary. FSH/FSHR role in Sertoli cell, has been known, however, the sites of FSH action within the male reproductive system are not resolved yet. Few studies have raised the intriguing possibility that germ cells may exhibit FSHR, all the reports point to Sertoli cells as the exclusive FSH target cells in testis. Besides, the attention has been always paid on the FSHR several polymorphisms which affect receptor sensitivity and expression. The presence of FSHR in germinal cells from spermatogonia to spermatocytes, including round spermatids is controversial or excluded. The mechanisms by which testicular varicocele affects fertility remain undetermined. Recently, our studies showed that the disease causes damage in sperm at the molecular level opening a new chapter in the already multifaceted physiopathology of varicocele. Samples used in this study were from normozoospermic and from diagnosed varicocele of grade III on the left testis patients. To date four FSHR isoforms were discovered, FSHR1, FSHR2, FSHR3 and FSHR4. The activity of FSHR1 is mediated by G proteins, which activate adenylate cyclase. FSHR2 and FSHR3 also bind FSH, but this does not result in activation of adenylate cyclase. FSHR4 does not bind FSH. By western blot analysis, we showed that healthy sperm express FSHR1, FSHR2 and FSHR3 while FSHR4 is almost absent. Varicocele does not express FSHR2. Immunofluorescence assay evidences FSHR localization prevalently at the midpiece level, which was strongly reduced in varicocele sperm. Responses to different FSH concentrations on motility and survival were significantly reduced in varicocele respect to the normal sperm, probably due to the lower FSHR1 expression and FSHR2 absence. The FSHR significance in human male gamete also emerged from the acrosome reaction histochemical studies, during FSH treatment which significantly induced the process. Our data showed for the first time that human sperm express the FSHR and constrain the need of further studies on the molecular anatomy of human male gamete both in healthy and in pathological conditions related to the male genital apparatus, considering the high couple infertility linked to the male. The translation of these new researches in the clinic surgery of testicular varicocele needs to be taken into account since molecular alterations in sperm imply a decline in the acquisition of fertilizing ability, and to date controversies exist on the opportunity to intervene surgically.

Keywords

FSHR, human sperm, testicular varicocele

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Insulin receptor-related receptor in the pancreas and in a β-cell line

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Insulin receptor-related receptor (IRR) belongs to the family of the insulin receptor (IR) along with the IR itself and the insulin-like growth factor receptor. Whereas the ligands of the latter receptors are known, identification of an IRR ligand has eluded investigations so that IRR has been considered an orphan receptor. A recent breakthrough in the understanding of IRR functional role came from the finding that IRR can be activated by mildly alkali media in absence of any protein agonist [1]. IRR shows highly specific tissue distribution, with highest concentration in kidney intercalated cells. However, significant amounts of the receptor are also found in the stomach and in α- and β-cells of the islets of Langerhans. Recent reports indicate that the pancreatic duct system is frequently associated with islet cells. Here, we show that those islet cells that are in contact with the excretory ducts are also IRR-expressing cells. Thus, when the exocrine pancreas is in an active state of secretion duct-associated islet cell behavior is potentially influenced by an IRR-mediated alkaline-induced signalling pathway. To explore this issue, we analyzed the effects of alkaline media on the pancreatic β-cell line MIN6. Activation of endogenous IRR was detected and could be inhibited with linsitinib, a synthetic inhibitor of the IR family of receptors. IRR autophosphorylation correlated with pH-dependent linsitinib-sensitive activation of IR substrate 1 (IRS-1). In contrast to insulin stimulation, no protein kinase B (Akt/PKB) phosphorylation was detected as a result of the alkali treatment. The alkaline medium but not insulin also triggered actin cytoskeleton remodeling in MIN6 cells that was blocked by pre-incubation with linsitinib. We propose that the activation of IRR by alkali is a component of a local loop of signaling between the exocrine and endocrine parts of the pancreas.

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Keywords

Insulin receptor-related receptor, MIN-6 cells, pancreas, insulin-secreting cells, insulin receptor substrate 1, immunofluorescence, western blotting

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Aerobic Training Improves Angiogenic Potential Independently of VEGF Modifications in Postmenopausal Woman

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Purpose: To evaluate the effect of walking-training on serum angiogenic potential in postmenopausal women.

Materials and Methods: Thirty-four postmenopausal women (56.18 ± 4.24 yr) participated in a 13 weeks program of walking-training. Anthropometric measures, VEGF, IL-1α, IL-1ß, IL-2, IL-8, IL-10, IL-12p70, TNF-α, C-reactive protein, insulin, IGF-1, cortisol, DHEA-S, leptin, visfatin, resistin and adiponectin were evaluated before and after training. Moreover, serum samples were tested for their ability to chemo – attract endothelial cells and to support the in vitro formation of capillary – like structures.

Results: After training, the levels of IL-8, TNF-α, leptin and resistin were significantly lower, levels of DHEA-S and adiponectin increased, serum angiogenic properties improved, whereas no changes in anthropometric parameters or VEGF were detected.

Conclusions: Walking training reduces inflammatory status and leads to a significant improvement in serum angiogenic properties in the absence of modifications in body composition and VEGF level.

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Aging of periosteal-derived stem cells during expansion: an alternative tool for a customized bone regenerative strategy

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Increased in life expectancy points out the necessity for tailored strategies to restore bone loss due to trauma and/or disease in elderly. Moreover, there is a compelling need for improved cell systems to test scaffolds interfacing with an "aging" tissue. For skeletal tissue regeneration, periosteal-derived stem cells (PDPCs) could represent an easily recruited source of Mesenchymal stromal cells (MSCs) [1,2]. This study investigated the effects of long-term in vitro expansion on the stability and function of PDPCs, since extensive culture expansion is usually performed to obtain clinically relevant cell numbers, but its impact on cell behaviour is still unclear. An integrated approach based on flow cytometry, ultrastructural and quantitative Real time PCR (qRT-PCR) analyses was adopted. Senescent cell data were compared with those of cells isolated from differently aged subjects. Both replicative-senescent PDPCs and cells isolated from old donors were permanently blocked in G1 phase of cell cycle, through a pathway that seemed to involve nitric oxide (NO) production and the expression of tumour suppressor proteins p16 or p53, respectively. Changes in the expression of MSC surface markers were detected in PDPCs during subculturing, whilst it was superimposable in young and aged PDPCs. Cytofluorimetric analysis of the physical parameters (i.e. FSC and SSC) showed a trend toward an increase in cell dimension and internal complexity in both populations analysed. This data was consistent with morphological observation that also evidenced similar alterations in mitochondrial shape. In addition, an intense autophagic activity in early passage PDPCs was observed, whilst in the late passages cells had a robust protein synthesis activity that could be related with "senescence-associated secretory phenotype" (SASP). In conclusion, the morphofunctional similarities detected in replicative-senescent and aged PDPCs suggest that their long-term expansion could be a reproducible and useful tool to mimic in vivo ageing.

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Keywords

Periosteal cells, replicative senescence, aging, NO, cytofluorimetric analysis

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SH3BGRL3 binds myosin 1c and is involved in MDA-MB-231 cell migration

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SH3BGRL3 is a gene belonging to SH3BGR family, it is ubiquitously expressed and encodes for a 93 AA thiorerodoxin-like protein evolutionarily conserved. A proteomic study reported that SH3BGRL3 binds the cytoplasmatic domain of ERBB2 receptor. On this basis we performed immuno-staining experiments in FLAG-SH3BGRL3 transfected SKBR3 cell line that showed SH3BGRL3 and ERBB2 co-localization. Nonetheless, co-immunoprecipitation (Co-IP) of ERBB2 using FLAG-SH3BGRL3 as bait and vice versa was not achievable. Therefore, to investigate SH3BGRL3 potential interactors we performed Co-IP experiments from SKBR3 lysates transfected with FLAG-SH3BGRL3 followed by mass spectrometry analysis. The results revealed myosin 1c (Myo1c) as a candidate interactor. Subsequent Co-IP experiments followed by WB analysis validated the interaction between the two proteins. To map the interaction site we performed Co-IP experiments using SKBR3 cells co-transfected with FLAG-SH3BGRL3 and HA tagged deletion mutants of Myo1c that showed SH3BGRL3 binding to the neck region of Myo1c. Since Myo1c neck region binds calmodulin in a Ca^{2+} dependent way, we assessed if the binding was Ca^{2+} dependent also for SH3BGRL3. The experiments showed that SH3BGRL3 binds the Myo1c neck in presence of Ca^{2+}, differently from calmodulin that binds it in absence of Ca^{2+}.

Myo1c is a motor protein involved, among its different functions, in cell membrane dynamics. Thus we investigated SH3BGRL3 involvement in cell migration using MDA-MB-231 cell line. We transfected MDA-MB-231 cells with FLAG-SH3BGRL3 and performed immuno-staining and Co-IP experiments that showed co-localization and interaction of Myo1c and SH3BGRL3. Accordingly, we performed migration assays using boyden chambers after silencing or not SH3BGRL3 expression by means siRNAs. The results showed a statistically significant decrease in migration capacity of silenced cells respect to controls. Our data show that SH3BGRL3 binds Myo1c neck region in a Ca^{2+} dependent way and that this interaction is involved in cell migration in our model.

Keywords
Protein interaction, cell migration

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Chitotriosidase expression in monocyte-derived dendritic cells

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Chitotriosidase (CHIT1) belongs to 18 glycosyl-hydrolase family, an ancient gene family that is widely expressed from prokaryotes to eukaryotes [1]. CHIT1 is a very critical enzyme to regulate the susceptibility to infection of organisms containing chitin as structural components. Conversely, during the development of acute/chronic inflammatory disorders, the enzymatic activity of CHIT1 increases significantly. The CHIT1 is expressed in activated macrophages as well in different lines monocyte-derived such as Kupffer cells and osteoclasts [2]. So far, it is unknown whether CHIT1 is expressed in other cells involved in the immune response such as monocyte-derived DCs. In this study we have investigated whether CHIT1 is produced in monocyte-derived DCs (moDCs) and the differential expression of CHIT1 during the different stage of moDCs differentiation. The presence of CHIT1 were examined by real time RT-PCR, Western Blot and Confocal Immunofluorescence, in Immature Dendritic cells (iDCs), generated from human monocytes by stimulation with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) and in mature Dendritic cells (mDCs), obtained by using lipopolysaccharide (LPS) and interferon-gamma (IFN-g). We observed that CHIT1 was expressed during the DCs differentiation and maturation process in time dependent manner. The maturation of DCs showed a significantly increased expression of CHIT1 mRNA and protein. Furthermore, the CHIT1 was evenly distributed in cytoplasm both in iDCs and in mDCs. The enzymatic activity confirmed that CHIT1 could play a role in moDCs function.

Taken together, our data confirm the crucial role of CHIT1 in primary immune responses and indicate that could be correlated with the immunogenicity of DCs.

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Keywords
Chitotriosidase, monocyte derived dendritic cells, CHIT1 activity

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Expression and localization of CHI3L1 in monocyte derived dendritic cells

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Chitinase-3-like-1 protein (CHI3L1) also called YKL-40, is a 40 kDa mammalian glycoprotein which is a heparin, chitin and collagen binding member of the mammalian chitinase-like proteins. Biological activities of CHI3L1 embrace regulation of cell proliferation, adhesion, angiogenesis, migration and activation. CHI3L1 is produced by variety of cells, including neutrophils, monocytes/macrophages, osteoclasts and Kupffer cells [1]. CHI3L1 secretion is induced by interferon (INF)-g and interleukin (IL)-6 and is an acute phase reactant associated with disease severity and mortality in a variety of infectious [2]. In this study, we have examined the expression and localization of CHI3L1 during the differentiation and maturation of monocyte derived dendritic cells by real time RT-PCR, Western Blot, Confocal Immunofluorescence, and Immunocytochemical assays. Potential nuclear localization signal (NLS) was determinated using the open source software cNLS Mapper and Chimera. Peripheral blood monocytes were differentiated toward immature DCs (iDC) and mature DCs (mDCs) through a combination of factors and cytokines. Our result showed, for the first time, that CHI3L1 is expressed during the process of differentiation and maturation of DCs in time dependent manner. Furthermore, CHI3L1 is evenly distributed in cytoplasm and in the nucleus of both the iDCs and mDCs.

In conclusion, the discovery of CHI3L1 expression in DCs has opened new dilemma for designing DC-based cancer immunotherapeutic. In fact, on the light of these results one can't exclude that as well as activated Tumor-associated macrophages (TAMs) also DCs infiltration could to be a significant unfavorable prognostic factor for cancer patients.

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Keywords
CHI3L1, YKL40, monocyte derived dendritic cells

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Effects of culture system and hypoxia on long-term expansion and differentiation of mesenchymal stem cells derived from periodontal ligament

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Periodontal ligament stem cells (PDLSCs), located in the perivascular space of the periodontium were able to differentiate into periodontal cell types in vitro [1]. In this study, we investigated the effect of three different culture media and of low oxygen tension (1%) on the immunophenotype, proliferation rate and osteogenic potential of PDLSCs. This study was the first report to compare the PDLSCs from the same person in different culture systems. PDLSCs were harvested from three healthy third molars and the single-cells suspensions were cultured in the culture media a-MEM, DMEM and a new medium formulation (Enriched Ham's F12 Medium, EHFM), respectively. PDLSCs were subcultured (4 x $10^3/cm^2$) until passage 7. The characterization of PDLSCs included FACS, immunofluorescence analysis and cell proliferation assay in both normoxia and hypoxia (1%). After culture in osteogenic medium for 7, 14 and 21 days, osteoblastic differentiation was evaluated by alkaline phosphatase activity, mineralization (alizarin red staining) and gene expression of osteogenic markers. Osteoblastic differentiation was also evaluated under hypoxic conditions. PDLSCs cultured in EHFM showed increased proliferation rate and CD73 overexpression compared to cells maintained in a-MEM and DMEM. On the other hand, PDLSCs grown in a-MEM and DMEM showed higher osteogenic differentiation potential compared to EHFM. Hypoxia affected both proliferation rate and osteogenic potential. On the basis of these results, we propose a two stages protocol for the osteogenic induction of PDLSCs, in which the early expansion stage could be performed in EHFM without loss of cell stemness. Furthermore, the results obtained in the different conditions (normoxia and hypoxia) suggest that oxygen tension plays a critical role in PDLSCs physiology.

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Keywords

Periodontal ligament stem cells (PDLSCs), Enriched Ham's F12 Medium (EHFM), hypoxia, CD73

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Engineered vesiscles from gingival stem cells: a new approach in 3D printed bone tissue regeneration

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In the bone regeneration field, properties of 3D scaffold could be improved using cellular and their released products. Even if previously documented, poly-(lactide) (PLA) scaffolds were not thoroughly evaluated for their design-related characteristics. The aim of the study was to investigate the properties of 3D printed PLA scaffolds for bone regeneration obtained through 3D printing, evaluating the differences in terms of structural properties, in vitro and in vivo cellular responses induced by different scaffold structures. Biofabrication is to generate a construct with biological function. In particular in our reserach we describe the fully process, including printing scaffold step, in vitro culture phase and subsequently in vivo transplantation.

Five porous scaffold designs (A-B-C-D-E) were fabricated from a poly-(lactide) (PLA) filament. Scaffold structural parameters, such as porosity and pore size, were measured using scanning electron microscopy, and micro-computed tomography. Nano-topographic surface features were investigated by means of atomic force microscopy.

Over a 112-day period, scaffolds were hydrolytically degraded and changes in weight, pH and mechanical properties were measured during degradation.

Osteogenic differentiation of hPDLSCs on different scaffold designs after 21 days of culture was measured by means of RT-PCR and Western Blot.

In vivo study was performed using C57BL/6 mice and was designed in 5 different groups:

- Group1: Scaffold loaded with hPDLSCs
- Group2: Scaffold loaded with conditioned medium (CM) derived from hPDLSCs
- Group3: Scaffold loaded with exosomes (Exo) purified from CM
- Group 4: Scaffold loaded with engineered exosomes (e-Exo), exosome treated with PEI (poly ether imide)
- Group5: Scaffold, used as control.

Histological analysis were performed after 60 days of in vivo transplantation and morphological evaluations revealed a high bone tissue formation and osteogenic cells commitment in group 3 and 4 when compared to other groups.

From these results, the cell-laden PCAMSC scaffold offers a significant advantage in the TM regeneration in a rat subacute TM perforation model. It may offer attractive opportunities in the conservative clinical treatment.

This study demonstrated that scaffold of group 3 and 4 significantly improved bone tissue regeneration in animal model and successfully showed new bone deposition in situ compared to the control scaffold (group 5) and group 1 and 2. Based on the results, we believe that the bioprinted scaffold may become a novel treatment for tissue regeneration approach therapy.

Keywords

Tissue engineering, mesenchymal stem cells, bone tissue regeneration, exosomes, conditioned medium

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3D morphometric evaluation of the face in Marfan syndrome: a better definition of dysmorphic features

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Marfan syndrome (MFS) is a rare genetic disorder of connective tissue caused by mutations in the *FBN1* gene, which result in alterations of fibrillin-1 and dysregulation of TGF-b bioavailability in the extracellular matrix. MFS is a clinical diagnosis. It is crucial to prevent acute aortic dissection; nevertheless it is difficult, due to the high degree of clinical variability of the disease. A quantitative definition of craniofacial abnormalities associated with MFS is not available and they are usually evaluated through a qualitative impression. The study aimed to better characterize the facial phenotype associated with MFS, identifying new morphometric features useful for the diagnosis of the disease. 3D facial images of 61 Italian subjects diagnosed with MFS, aged 16-64 years (21 males, mean ± SD age 38 ± 15 years; 40 females, mean ± SD age 41 ± 13 years), and divided in 6 non-overlapping age groups, were obtained by stereophotogrammetry [1]. From the coordinates of 50 soft-tissue facial landmarks, linear distances and angles were measured; z score values were calculated comparing patients with healthy Italian reference subjects (400 males, 379 females), matched for gender and age group. Statistical comparisons were performed by Student's t test. All subjects with MFS showed a greater facial divergence ($p<0.001$; mean z score = +1.9) and a reduced facial height index ($p<0.001$; mean z score = -1.9), being both the values mostly influenced by a shorter mandibular ramus ($p<0.001$; mean z score = -1.9) and an overall mild but significant increase of facial height ($p<0.001$; mean z score = +1.2). Gender differences or age-specific trends were not observed. Quantitative facial abnormalities pointed out in the current study enrich the information about the phenotypic expression of MFS and suggest their usefulness in the recognition of the disease.

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Keywords

Facial anthropometry, Marfan syndrome (MFS), stereophotogrammetry

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How to pinpoint the greater palatine foramen: a metrical analysis applied to a contemporary skeletal collection

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Anatomy of greater palatine foramen has acquired a growing relevance in the fields of dentistry, maxillofacial surgery and otorhinolaryngology [1,2]. Several publications are available concerning the collocation of greater palatine foramen; however available literature has so far focused on few metrical measurements and has not yet performed a complete analysis for the localization of the greater palatine foramen. This study provides a novel approach to the metrical assessment of the position of the greater palatine foramen on 100 skulls belonging to the Milanese contemporary collection, based on six linear measurements and two angles. Possible differences according to sex and side were assessed through two-way ANOVA test ($p<0.05$). Statistically significant differences according to sex were found for the distances of the greater palatine foramen from: the intermaxillary suture, the incisive foramen, the posterior palatal border, the most posterior point of the palate; palatal length; the position of the greater palatine foramen relative to palatal length ($p<0.05$). For what concerns side, only the distance from intermaxillary suture, from the most posterior point of the palate and the angle at the incisive foramen showed statistically significant differences ($p<0.05$). Results provide a complete metrical assessment of the localization of the greater palatine foramen and additional data for the assessment of differences according to sex and side.

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Keywords

Anatomy, greater palatine foramen, lesser palatine foramen, maxillary nerve block, maxillofacial surgery

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A proinflammatory microenvironment induces NFkB activation and beta-defensin expression through specific Toll Like Receptors in a 3D human skin model

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Psoriasis is an autoimmune skin disease characterized by the formation and the progression of silvery plaques on the extensory surfaces of our body. Proinflammatory cytokines as Tumor Necrosis Factor (TNF)-alpha, interleukin (IL)-17, IL-22 and IL-23 represent for the normal skin a psoriatic microenvironment. In the 3D human skin model standardized in our lab in the last decade, we were able to dissect the events in which each cytokine exerts a specific effect, e.g. keratinocyte proliferation, Langerhans cell activation, cytoskeleton arrangement, and, more recently, the epidermal expression of Toll like Receptors (TLRs) 2, 7, 9. Several experimental studies reported that in psoriasis TLRs are expressed and their activation triggers i) NFkB translocation from the cytoplasm to the nucleus and ii) the release of beta defensins (HBDs). The present study was aimed at investigating the intracellular NFkB activation and HBD1 and HBD2 expression induced by a cytokine mix (TNF-alpha, IL-17, IL-22, IL-23) by indirect immunofluorescence. Bioptic samples of normal human skin were obtained after aesthetic surgery of young healthy informed women (n=7). After overnight incubation to reduce mechanical and termical stress, skin fragments were incubated in a Transwell system for 5 (T5), 24 (T24), and 48 (T48) hours with the cytokine mix. Parallel control samples were carried out and each patient was represented at all time points. In controls at all time-points NFkB was localized only in the cytoplasm, while, starting from T5, scattered basal nuclei were observed in the cytokine-incubated samples. At later time points, in the upper spinous and granular layers, NFkB nuclear immunostaining was evident. HBD2 expression was affected after cytokine mix exposure, while HBD1 distribution was similar to controls.

Thanks to this simple but effective model, a deep knowledge of the early events occurring in the normal epidermis exposed to cytokines can be achieved, excluding the contribution of the blood and lymphatic vessels herein absent. This basic research can thus represent an important tool for targeting and counteracting single phenomenon leading to the formation/progression with the most innovative biological drugs.

Keywords

Proinflammatory cytokines, psoriasis, immunofluorescence

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Hippotherapy improves gait and balance in Down Syndrome

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Patients with Down syndrome (DS) present delays in motor development, showing a unique pattern of locomotion in clinical settings. Hippotherapy (HBR) is a field of rehabilitation therapy to achieve physical, social, and psychological well-being through therapeutic horse riding, providing a new stimulus related to gait and may helping balance and postural control [1], [2]. Herein, we have enrolled fifteen male individuals affected by DS, aged from 19 to 36 years old. All patients were vaccinated for tetanus and previously screened for any contraindications to practice HBR. The HBR protocol included a six-months period of horseback riding exercise, performed weekly. Before, during and after the study period, functional mobility, strength and performance in balance were assessed by Time Up and Go Test (TUG), 30s Chair-Stand-Test (30CST), MRC-scale and the Berg-Balance-Scale (BBS). Furthermore, the OPTO-Gait for dynamic analysis and the Diasu Ultrasensor systems for static analysis were applied at the same timepoints, in order to assess the HBR effects on movement reaction time, muscle activation, functional mobility, muscle strength and balance in DS. In conclusion, we provided objective clinical data on the role of HBR to determine a functional improvement on gait speed, rhythm, width, bilateral symmetry, gross motor function and balance in DS..

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Isolated quadriceps training restores whole body exercise capacity in CHF

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Patients with Chronic Heart Failure (CHF) are commonly characterized by exercise limitation. The benefit of isolated (i.e., small muscle mass) muscle training and its potential translation to whole body exercise in patients CHF has been recognized, however the mechanisms responsible for this positive outcome remain poorly understood. To study oxygen (O_2) transport and metabolism at maximal cycle (whole body) and knee extensor (KE, small muscle mass) exercise in this pathological condition, eight healthy controls and six patients with CHF with reduced ejection fraction commenced 8 weeks of KE training (both legs, separately). Before and after training, they underwent cycle and KE maximal exercise studies. Pre-training cycling and KE exercise peak leg O_2 uptake (VO_2) were 17% and 15% lower, respectively, in the patients compared to controls. Although KE training did not alter cardiac output at maximal KE or cycle exercise, it increased O_2 delivery (by 54%), arterial-venous O_2 difference (by 10%), and muscle O_2 conductance (by 39%) at maximal KE exercise, yielding an increase in peak single leg VO_2 of 53%, which exceeded untrained control subject values. Post-training, during maximal cycling, O_2 delivery (40%), arterial-venous O_2 difference (15%), and muscle O_2 conductance (DMO_2) (52%) all increased, yielding a 40% greater peak leg VO_2, matching that of the controls. Small muscle mass exercise training-induced improvements in both peripheral convective and diffusive O_2 transport and subsequent O_2 utilization were the main mechanisms responsible for the increased whole body exercise capacity in patients with CHF. Such clear improvements in these factors and exercise capacity support the efficacy of small muscle mass training as a powerful approach to promote a metabolic reserve and maintain physical function in the face of continuing central limitations associated with CHF.

Keywords

Adapted physical activity, training, exercise testing, exercise limitation

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Degeneration and regeneration of peripheral nerves: role of thrombin and its receptor PAR-1

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The peripheral nervous system has a striking regeneration potential and after damage extensive changes in the differentiation state both of the injured neurons and of the Schwann cells are observed. Schwann cells, in particular, undergo a large scale change in gene expression becoming able to support axonal regeneration. Nerve injury is generally associated to inflammation and activation of the coagulation cascade. Thrombin acts as a polyfunctional signalling molecule exerting its physiological function through soluble target proteins and G-protein-coupled receptors, the protease-activated receptors (PARs) [1]. Recently, we have demonstrated that the activation of the main thrombin receptor, PAR-1, in Schwann cells favours their regenerative potential determining the release of factors which promote axonal regrowth [2]. The pro-regenerative potential of thrombin seems to be exerted in a narrow range of concentrations (pM-nM range). In fact, our preliminary data indicate that high levels of thrombin in the micromolar range slow down Schwann cell proliferation and induce cell death. On the contrary, PAR-1 activating peptides mimic the pro-survival but not the pro-apoptotic effects of thrombin.

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

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

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Morphological and immunoistochemical evaluation of cadaveric gingival specimens to estimate the post-mortem Interval

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The estimation of the post-mortem interval (PMI) is a critical step in forensic medicine and remains one of the most challenging variables to determine. In general, the numerous methods currently used in estimating post-mortem interval yield to large post-mortem windows, are influenced by several factors and sometime contradict each other.

With the aim to obtain a much more accurate determination of the post-mortem interval we combined morphological ultrastructural and immunoistochemical analyses to reach a more detailed knowledge on tissue organization and degradation after death.

Samples of gingival tissues obtained from 20 cadavers at different post-mortem intervals were processed for transmission electron microscopy to evaluate ultrastructural modifications in the epithelium and connective tissue. Gingival cells and the extracellular matrix of gingival tissues have been observed by a transmission electron microscopy (TEM) in combination with the evaluation of potential post-mortem biochemical markers, with the final goal to find a tight correlation between the ultrastructural modifications, the biomarker expression and the time of death.

All the samples were also immunostained with anti-hypoxia-induced factor 1-α (HIF1-α) antibody, a transcription factor expressed in response to hypoxia, in order to evaluate the expression of HIF1-α, and to establish a correlation between the protein presence and the time of death.

Results showed nuclear chromatin changes and cytoplasmic vacuolization in both epithelial and connective tissues and a different pattern of expression of HIF1alpha protein that correlate to the time of death.

In conclusion, our preliminary findings suggest that ultrastructural investigations in combination with immunohistochemistry techniques in gingival specimens may represent a new tool to accurately and systematically estimate post-mortem interval.

Keywords

Post-mortem interval, hypoxia, HIF1alpha, ultrastructural morphological changes, gingiva

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Cancer stem cells and miRNA in the early diagnosis of colorectal carcinoma

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Colorectal cancer represents the third cause of death for malignant tumor by incidence and mortality.

Evidences suggest that tumor initiation, growth, invasion and cancer expansion are promoted by a small sub-population of tumor cells, called cancer stem cells (CSCs).

On the other hand, MicroRNAs constitute a recently discovered class of small non-coding RNAs (about 22 nucletides) found in plants, animals and some viruses, that play key roles in the regulation of gene expression. An increasing number of studies have identified miRNAs as potential biomarkers for human cancer diagnosis, prognosis and therapeutic targets. However, a real translation of miRNA significance into the clinical practice deserves and needs further investigation.

To this end, the aim of this work is to identify the expression of some specific microRNAs of this type of cancer, both in tissues and serum of cancer patients and in cancer stem cells, in order to allow early diagnosis. Therefore, some interesting microRNAs were chosen and their level was detected through amplification with real-time PCR method.

A preliminary analysis of results shows that in some patients microRNA 21, 221, 18a, 210, 34a, 10b, 16 are overexpressed, while in others they manifest a lower expression. Instead, the microRNA 31 is always overexpressed. We think that this result is related to the clinical stage of the tumor, because patients with similar clinical stage show the same expression.

Keywords

Colorectal cancer, miRNA, CSCs, biomarkers, tissue, blood, diagnosis

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Effects of melatonin oral treatment in pristane-induced lupus nephritis mice

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by lymphocytes hyperactivity and excessive production of pathogenic autoantibodies, pro-inflammatory cytokines and also reactive oxygen species [1]. One of the most severe manifestations of SLE is lupus nephritis (LN), which is characterized by glomerulonephritis and tubulointerstitial inflammation and oxidative stress with also immune-complexes depositing in the renal tissue [2]. Recent evidences suggest that the use of antioxidants in the treatment of LN have satisfactory outcomes. Melatonin is a small, highly conserved pineal indoleamine and due to its important and well known antioxidant and antinflammatory properties [3,4] may be an efficient tool against LN damages. In this study, pristane-induced LN mice were used to investigate the potential protective role of melatonin. At the end of the treatments, the LN animal model presented marked changes in the kidney cytoarchitecture (like glomerular sclerosis, marked tubular degeneration and matrix mesangial expansion) together with significative kidney inflammation, oxidative stress and fibrosis. Interestingly, pristane-mice treated orally with melatonin showed a significative reduction of inflammation, oxidative stress and fibrosis and also of morphological changes at both tubules and glomerular level. In summary, we thought that melatonin may be a potential tool in the management of SLE-glomerulonephritis.

Flamma S.p.A.-Italy (www.flammagroup.com) provided with melatonin. Thanks to Fondazione Cariplo e Regione Lombardia "New opportunities and ways towards ERC" (Project 2014-2256) for the grant support.

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Keywords

Lupus nephritis, fibrosis, kidney, inflammation, melatonin, oxidative stress

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Nutrient excess in a stabilized co-culture system of Caco2/HT-29 cells: an ultrastructural and functional time-course study

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The intestine represents one of the most important barriers of our body and interacts with several exogenous substances, among which nutrients. Today, the effects due to an excess of nutrients on intestinal morpho-functional changes, similar to the ones found in obesity, have been studied only in *in vivo* animal models. Many experimental difficulties hampered in establishing a physiological long-term experimental model starting from primary cultures of normal small intestinal and colon cells. For this reason, an intestinal Caco2/HT-29 (70/30) co-culture was set up in our lab starting from the differentiated parental cell populations to mimic the human intestinal epithelium. Co-culture was harvested at confluence (T0) and at 3, 7, and 15 days (T3, T7, and T15, respectively) post-confluence. Ultrastructural (TEM) and functional analysis (Alkaline Phosphatase, ALP; Aminopeptidase N, APN; Dipeptidyl Peptidase-IV , DPPIV; Transepithelial Electrical Resistance, TEER) were carried out. In the present study, two parallel experimental groups were cultured: the standard group and the excess group. In the standard group, the culture medium was changed every four days, whilst in the excess group on alternate days from T0. Transmission electron microscopy revealed that the excess of nutrients drives co-cultures towards a less differentiated absorptive phenotype. On the other hand, mucus granule presence was more and more evident from T3. The specific activity of ALP and APN, known markers of intestinal differentiation, and that of DPPIV, a specific marker of enterocyte differentiation, progressively increased. TEER, indicative of the barrier properties of the co-culture, increased at post confluence up to T15. In conclusion, data here presented show that the excess of nutrients can directly modify both morphology and function of the intestinal cells, opening the way to study at the effects due to specific nutrients on cell proliferation and differentiation involved in the acquisition of an obese human phenotype.

Keywords

Transmission electron microscopy, cell differentiation, hydrolase activity

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Interuniversity management support for learning verification based on multi-choice quiz-questions

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The assessment of learning both during the course and at the end represents a large and increasingly burdensome part of the overall teaching activity. A computerized evaluation system able to draw on a centralized archive of several thousands of multi-choice questions (MCQs), and equipped with programs for selecting, printing and correcting the tests, could offer a solution, providing rapid objective assessments. The concept has been widely explored in recent years both in Italy and abroad, but with differing modalities, and results were often disappointing. The need to use a truly multiple-choice format (with independent responses - all either True or False - and each with a statistical discriminative efficiency) was probably underestimated, as also the need for a huge archive of MCQs (several thousand, including questions on anatomical images) complete with the data-processing programs that only a centralized system can offer.

The central archive could be updated, revised, and implemented jointly by the peripheral user (compatibly with their experience) so becoming increasingly refined over time. In addition, a technical team could adjust the management programs and, eventually, correct the tests if there are difficulties locally. The system could best be used as a "high-pass filter" of the student's level of preparation, by which students can access a fast oral exam of the traditional type with the aim of improving their score obtained on the test. At the same time, the teacher would avoid being submerged by countless useless and frustrating exams with inevitably negative results.

The MCQs consist of a "text" sentence completed by 5 statements that can be individually and independently either true (T) or false (F), and which must be defined as such by the 5 relative responses. The presence of 5 independent statements (T or F) ensures a more complete evaluation of the topic proposed in each question and, in the test-trial as a whole, a well-balanced mix of true and false statements (and relative responses). For each multiple-choice quiz question used in the degree course, the percentage of correct answers by the students will be indicated, providing continuous feedback for updating the database. It is expected that the optimal size, by the end of the project, will be at least 10,000 MCQs, of varying levels of difficulty.

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Clinical Applications of Genome Anthropology

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Evolution is the only weapon that every living species has to survive to the changes of the surrounding environment. The clinical applications of genome anthropological studies has been studied. The goal is to understand with a global approach how certain body districts have changed during the evolution and how these changes have affected the contemporary man. All these researches investigated populations lived thousands of years ago and Tanaka craniofacial and cervical spine fresh cadaver dissective technic has enabled the creation of a comprehensive diagnostic protocol [1]. One of the most important tool of this diagnostic protocol is the use of all-skull CT cone beam which shows the anatomical structures in a real 3D perspective using a specific software. The use of this test has become a standard because the radiation dose administered is lower than the usual radiographs performed for the orthodontic diagnosis [2]. Among the innovative aspects of this protocol there is the evaluation of anatomical structures by means of cone-beam CT which allow us to create a specific therapy for each patient paying attention to gnathological and postural disorders. Patients are evaluated and monitored during the therapy using a clinical record which include a VAS test. VAS is a validated test and is used by patients to determine the effectiveness of the chosen therapy.

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Keywords

Anatomical diagnosis, evolution, posture, 3D, craniofacial dissections

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Constrained Spherical Deconvolution Tractography reveals a direct cerebello-ventro tegmental pathway in humans

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Introduction. In addition to its role in motor control, reflex adaption and motor learning in the past years numerous studies demonstrated the role of the cerebellum in non-motor functions. Furthermore, lesional animal and neuroimaging in vivo human studies demonstrated connections of the cerebellum with brain regions involved in cognitive, emotional, motivation linguistic processing [1, 2]. Although, studies suggest the role of the cerebellum in neuropsychiatric disorders of the mesocorticolimbic structure (i.e. schizophrenia), at the present time the existence cerebello-ventro tegmental pathway has been demonstrated in only in rodents and only hypothesized in humans.

Aim. The goal of this in vivo constrained spherical deconvolution (CSD) tractography study is the investigation on the presence of a direct cerebello-ventro tegmental pathway in the human brain.

Material and Methods. We recruited 15 human subjects with no previous history of neurological or psychiatric disorders. The entire study was performed using a 3T Achieva Philips scanner; a SENSE 8 channels head coil, acquiring T1 weighted 3D TFE, DTI sequence; data were analyzed by using constrained spherical deconvolution techniques (CDS). *Results.* We demonstrated with CSD dentate-ventral midbrain connections. In particular, we found a direct route linking between the dentate nucleus and the ventro tegmental area.

Conclusions. This study provides for the first time the existence of a human dentate nucleus connections with the ventro tegmental area, moreover the existence of this cerebello-midbrain pathway suggest that the cerebellum may be involved in the modulation of the mesocorticolimbic system and in related neuropsychiatric disorders such as the schizophrenia.

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Keywords

Cerebellum, dentate nucleus, ventral tegmental area, midbrain, human, connectivity, tractography

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Characterization of an *in vitro* model to study the role of human Polyomavirus BK in prostate cancer

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Prostate cancer (PCa) is one of the most common male neoplasm in the western world, being the most commonly diagnosed non-skin cancer and the second leading cause of cancer death. Various potential risk factors exist for the initial triggering events, including exposure to infectious agents, such as the human Polyomavirus BK (BKV). BKV is a good candidate as risk factor of PCa because it naturally infects the human reno-urinary tract, it establishes latency, and encodes oncoproteins that interfere with tumor suppressors pathways, thus altering the normal progression of cell cycle.

Previous studies suggested a potential association between BKV and PCa, revealing that the prevalence of BKV was significantly higher in cancer than in control tissues, with a significant association between viral expression and cancer. However, this hypothesis is controversial because BKV is not restricted to tumor tissues but also infects healthy individuals in a high percentage. Moreover, an in vitro model of BKV infection in prostate cells is not available to understand the role for BKV in pathogenesis of PCa.

Our aims were to determine whether BKV a) could infect normal epithelial prostate cells, b) affects cell phenotype and c) affects the phenotype of human prostate tumor cell line PC3.

For this purpose normal epithelial prostate cell line RWPE-1 and prostate cancer cells PC3 were infected with BKV for 21 days. Cell proliferation, epithelial-to-mesenchymal markers (EMT) and invasion potential were analyzed by, respectively, MTT, immunofluorescence and SDS-zymography.

Our results show that cell proliferation was increased or decreased by BKV, respectively, in RWPE-1 and PC3 cells. BKV induced E-cadherin downregulation and vimentin expression in both control and BKV-infected cells RWPE-1, suggesting that uninfected cells underwent EMT. Matrix metalloproteinase-2 and 9 activity was increased in RWPE-1 cells after BKV infection. By contrast, BKV did not significantly modified the phenotype of PC3 cells.

These preliminary results suggest that normal epithelial prostate cells RWPE-1 and PC3 are susceptible and permissive to BKV infection. However, RWPE-1 cells exhibit some phenotype modifications related to EMT, possibly induced by the papilloma virus used to obtain their immortalization, thus suggesting that further experiments will be necessary to define if they represent a good experimental model to study prostate cancer.

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Keywords

Prostate cancer, polyomavirus BK, epithelial-to-mesenchymal transition

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Extracellular matrix components affect cell migration and invasive potential of cultured human pancreatic ductal adenocarcinoma cells

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The tumor microenvironment influences cancer cell behavior in relation to tumor progression, as well as cell proliferation and invasion. Pancreatic ductal adenocarcinoma (PDAC) is characterized by an intense desmoplastic reaction and extracellular matrix (ECM) components in the tumor microenvironment are involved in a crosstalk between tumor cells, stromal fibroblasts and ECM components, influencing tumor cell behavior.

We aimed at analyzing *in vitro* the effect of the crosstalk between PDAC cells and the ECM of the microenvironment by culturing PDAC cells on different ECM proteins used as a substrate, in order to better understand the relationship between cancer cell phenotype and the proteins occurring in the desmoplastic tissue. For this purpose, we analyzed some epithelial-to-mesenchymal transition (EMT) markers and the migration and invasive potential in human HPAF-II, HPAC and PL45 PDAC cells cultured on collagen type I (COL), laminin (LAM) and fibronectin (FN).

Interestingly, the expression of E-cadherin was not significantly affected, but some differences were revealed by the wound healing assay. In fact, migration of HPAF-II and PL45 cells was decreased on FN and LAM, and increased on COL, compared to control cells grown on plastic (NC). By contrast, HPAC was very rapid and unaffected by the substrate. SDS-zymography showed that COL induced a strong upregulation of MMP-2 activity in HPAF-II and HPAC cells, and of MMP-9 in HPAF-II and PL45 cells, compared to NC.

These preliminary results suggest that ECM components could differently affect PDAC migration and invasion, possibly depending on the differentiation grade.

The characterization of the mutual effects elicited by the tumor-stroma interplay on the cancer cell will contribute to better understand the influence of the stroma on PDAC cancer cell phenotype, in order to develop new therapeutic strategies.

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Keywords

Epithelial-to-mesenchymal transition, tumor microenvironment, pancreatic ductal adenocarcinoma, matrix metalloproteinases

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Effects of physical exercise on Body Mass Index in Binge Eating Disorder

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Binge Eating Disorder (BED) is characterized by recurrent episodes of eating large quantities of food in a brief period. Bouts of binge are commonly associated with a lack of control on stop eating or on what or how much one is eating. The binge eating is not associated with recurrent use of inappropriate compensatory behavior as bulimia nervosa and occurs, on average, at least once a week for 3 months [1]. BED is also associate with obesity and motor inactivity [2].

Aim of this study was to estimate the effectiveness of a structured physical activity program on the Body Mass Index (BMI) in BED patients.

19 BED women were recruited for this study. The subjects were randomly assigned to two groups: Intervention Group (IG, n=10) and Control Group (CG, n=9). All participants underwent the following measurements: height and weight, to calculate BMI (kg/m^2) and participated into the weekly multidisciplinary program, constituted by Cognitive Behavioral Therapy and diet. In addition, the IG carried out a structured physical activity program for 6 months. The exercise session consisted of aerobic, balance and strength activity performed in four weekly sessions of 90 minutes. At baseline and after 6 months BMI was assessed in both groups.

Both groups improved their BMI for the influence of diet and changes in body composition but the IG achieved greater results probably for the effects of structured physical activity program.

The combination of traditional BED treatment and physical activity seem to improve the quality of life, to increase habitual activity level and to reduce Body Mass Index in BED patients.

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Keywords
Binge eating disorder, psychiatric subjects, body mass index, physical activity, women.

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"Muoversi in equilibrio" project: effects on balance capacity in Binge Eating Disorder

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Patients with Binge Eating Disorder (BED) are characterized by the consumption of a large amount of food in a short period of time, accompanied by a perceived sense of loss of control over the eating episode. Although obesity is not a DSM-V criterion for BED, there is a strong association between BED, obesity and physical inactivity [1].

In this study we evaluated if a structured physical activity program influences static and dynamic balance in BED patients.

For the study we recruited 18 BED patients, 15 females and 3 males, aged 53.1 ± 14.9 (mean ± SD). Subjects physically/pathologically unable in doing physical activity were excluded. The participants, in addition to medical treatment, performed a physical activity program for 6 months. The exercise session consists on aerobic, balance and strength activity performed in four weekly sessions of 90 minutes. In basal conditions and after 6 months were assessed: One Legged Stance Test to evaluate the static balance and Star Excursion Balance Test for the dynamic balance.

The comparison between baseline and after 6 months results showed a significant improvement for either motor skills tests: OLST dx-sx (t Student $p<.001$) and SEBT dx-sx (t Student $p<.01$).

The addition of exercise training in the traditional treatment for BED patients constitutes a novel potential therapeutic approach in eating disorder.

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Keywords

Binge eating disorder, psychiatric subjects, balance, OLST, SEBT, physical activity.

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Human Cardiopoietic Amniotic Fluid cell population: characterization and terminal differentiation

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Rationale. Human amniotic fluid-derived (hAF) stem cells are considered a novel class of multipotent stem cells, sharing characteristics of both embryonic and adult stem cells. In fact, they proliferate rapidly, are able to differentiate into cells of all the embryonic germ layers, but do not form teratoma. It has been already reported that the embryoid bodies (EBs) obtained from hAFs have a cardiac potential, but it has not been described a functional terminal differentiation in cardiomyocytes (CMs) yet.

Objective. Aim of this study was to foster the cardiomyogenic potential of hAFs in order to obtain a cellular population with morphological and functional features of CMs.

Methods and Results. AFCs were exposed sequentially to inducing factors (Ascorbic Acid, 5-Azacytidine, BMP4, ActivinA, VEGF) up to 15 days and differentiation was monitored. Only the hAF samples expressing the multipotency markers SSEA4, OCT4 and CD90 (CardiopoieticAF) responded to the differentiation process loosing their stemness and increasing the cardiac nuclear factors NKX2.5 and GATA4. After the differentiation cells expressed high levels of the sarcomeric proteins (cTnT, αMHC and αSA), the gap junction marker Connexin43 and both atrial and ventricular markers; moreover, up to 90% of the cells was positive for CACNA1C and SERCA2a, cardiac calcium pumps involved in the excitation/contraction coupling, and about 30% of the CardiopoieticAF-derived cells presented spontaneous intracellular Ca2+ waves and Ca2+ fluctuation in response to caffeine or adrenergic stimulation. Some spontaneous beating foci were also observed.

Conclusion. Our results demonstrate that CardiopoietichAFs can fully differentiate into a homogenous population of CM-like cells, characterized by cardiac-specific molecular, structural, and functional properties. Thus, CardiopoietichAFs can hold great promise for the development of in vitro models of cardiac genetic disorders, for drug discovery and testing, and for the emerging field of cardiovascular regenerative medicine.

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A novel approach to the assessment of anatomical uniqueness of ears: application of 3D-3D surface registration

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Uniqueness of body structures can be defined as the anatomical property consisting in the development of a unique and individual shape. From this point of view, the ear has always represented a field of particular interest for anatomy, especially for its individuality and the large variability in size and shape: however, its uniqueness has been assessed so far only through morphological methods, with limitations in quantifying the probability of finding the same shape within the sample [1,2].

The introduction of modern devices for 3D image acquisition and 3D models elaboration may provide additional data, especially through the 3D-3D registration of surfaces and calculation of respective distances.

Ten adults were recruited for the study. The right and left ear from each individual was acquired twice by stereophotogrammetry at the distance of few seconds. The ear surface obtained from the first acquisition was then superimposed onto the same structure derived from the second acquisition of the same subject (group of matches) and onto the ear surface from the second acquisition of all the other subjects taking part to the project (group of mismatches). In all the cases registration was reached according to the least point-to-point distance between the two 3D models. Point-to-point RMS (root mean square) distance was then calculated between the two surfaces. Possible statistically significant differences according to side and group were assessed by two-way ANOVA test ($p<0.01$).

A total 200 superimpositions were performed. On average point-to-point RMS distance in cases of matches was 0.31 mm (SD: 0.13 mm), in cases of mismatches 1.43 mm (SD: 0.31 mm): differences were statistically significant ($p<0.01$). No statistically significant differences were found according to side ($p>0.01$).

Results provided a novel contribution to the assessment of anatomical uniqueness of ear morphology, with a quantification of differences based on anatomy of auricles.

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Keywords

Facial anatomy, ear, stereophotogrammetry

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Assessing the precision of posttraumatic orbital reconstruction through 3D-3D "mirror" orbital superimposition: 3D surface point-to-point distance versus volume

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Posttraumatic orbital fractures are mainly reconstructed through orbital meshes. The most frequently used method to assess the success of orbital reconstruction is based on the comparison of orbital volumes [1]. However, the modern techniques of 3D elaboration enable to perform innovative procedures of comparison, such as the 3D-3D registration and quantification of point-to-point distances.

Ten patients who underwent to orbital reconstruction through titanium meshes in the Department of Maxillofacial Surgery, University of Milano-Bicocca, were recruited (patients group), together with a ten patient control group. Volumes of orbits were segmented on CT scans and automatically calculated. The 3D model from the unaffected orbit was then flipped according to the sagittal plane in order to obtain a "mirror" image of the contralateral orbit, and automatically registered on the reconstructed one. Point-to-point RMS (Root Mean Square) distance between the 3D models was then calculated. The same procedure was applied also to the CT-scans from unaffected patients. Possible statistically significant differences in volume and surface RMS distance were assessed through Student's t-test ($p<0.05$).

On average difference in volume between the unaffected and reconstructed orbit in patients was not discordant from the difference between the right and the left side in the control group ($p>0.05$); on the other side, mean RMS value was significantly higher in the former group than in the latter one (0.78 mm versus 0.59 mm, $p<0.05$). Discordant areas are more frequently observed on the floor of the orbital cavity where the titanium mesh is applied.

The present study highlights the use of 3D surfaces point-to-point distance as a parameter for assessing anatomical success of orbital reconstruction: the next studies will verify the relation of this parameter with clinical signs reported by patients.

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Keywords

Orbital reconstruction, volume segmentation, CT-scan, mirroring

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Does the experience of anatomical dissections change the mind of students? The opinion of Milanese undergraduate students towards the donation of bodies for didactic purposes

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Anatomical dissections have always represented an important cornerstone of medical education, especially in anatomy [1,2]. However, in several universitary context their practice has been abandoned or even not yet introduced. In the University of Milan a limited program of body donation has existed since 2014 (PANDORA, Programma Anatomico di Donazione di Cadaveri a Scopo di Ricerca Antropologica e Biomedica) and therefore only in the last four years medical students have been able to take advantage from this crucial experience.

This study aims at exploring the willingness of Italian undergraduate students towards whole body donation in order to ascertain the role of dissection in modifying the opinion: two groups of students belonging to the degree course in medicine and surgery were recruited. The first group included 43 students who were informed concerning the importance of dissection in anatomy through a specific course. The second group included 29 students admitted to a didactic autopsy. Students belonging to the two groups were then requested to specify if they would give their consent to the donation of their bodies, and why.

Results showed that students who attended the dissection were more likely to show willingness towards body donation (60.7% versus 39.5%). Among positive opinions towards donation the percentage of persons who found the experience useful increased from 47.1% in the first group to 80% in the second group. On the other hand, among negative opinions, percentages of persons reporting bad feeling towards the dissection increased from 16.7% in the first group to 27.2% in the second group.

This study shows that experience of dissection is a crucial step for emotional and professional improvement of medical students and contributes in a more detailed definition of their own opinion concerning body donation.

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Keywords

Body donation, anatomical dissections, questionnaire

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Stem cell differentiation for muscle regeneration

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Physical activity has a positive role on muscle remodelling and vascularization, involv-ing stem cells differentiation processes. Indeed, the skeletal muscle homeostasis and repair are maintained by a subset of muscle stem/progenitor cells called Satellite Cells (SCs), while for heart repair and remodelling the cardiac potential of progenitor cells is otherwise expressed by different stem cell types: bone marrow hematopoietic stem cells (BMHSC), bone marrow mesenchymal stem cells (BMMSC), cardiac stem cells and embryonic stem cells.

The ε isoform of the PKC family (PKCε) is a serine-threonine kinase that is expressed in muscle and in a variety of other tissues, regulating their homeostasis acting on cell death and differentiation.

We focused on the role of PKCε in skeletal, cardiac and smooth muscle differentiation of adult stem cells. We found that inhibition of PKCε prevents myogenic differentiation of the myoblast cell line C2C12 and of primary SCs. In vivo PKCε inhibition resulted in impaired muscle regeneration, as well [1]. On the contrary, in cardiac and smooth muscle differentia-tion of stem cells we observed a negative role of PKCε both in vitro and in vivo [2,3]. In fact, it impaired cardiac markers expression like NKX2.5 and GATA4 but also vascular differ-entiation markers like SMA and PECAM. PKCε should therefore be considered as a finely tuned modulator of muscle cell differentiation.

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Keywords

Stem cells, satellite cells, PKCε, muscle cell differentiation.

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Sternal foramina : anatomy and clinical significance

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The presence of one or more sternal holes is a congenital developmental anomaly that needs to be recognized and diagnosed to prevent accidental puncture of vital organs during procedures such as sternum biopsy or acupuncture. We present two cases of this bone anomaly characterized by the presence of sternal foramina that were found during tutorials for medical students in the anatomical museum" Leonetto Comparini "of University of Siena . Measurements of the foramina were carried out using a digital caliper and was subsequently made a photographic documentation.The first case shows a sternum with multiple oval foramina: one at level of body sterni with the larger diameter of mm 4,78 and two at xiphoid process with larger diameter of 8,83 and 7,44 mm respectively.The second case is a sternum with a single oval foramen at level of the lower part of body with a diameter of 12,8mm. In the fetus sternum cartilage is formed by two bars which merge with each other towards the eighth week of gestation forming the manubrium and the body of the sternum(1). At the tenth week of gestation .the subsequent ossification of the sternum takes place from six centers of ossification. The last part of the sternum to ossify in adulthood is the xyphoid process . A partial defect in the melting of cartilage bars can cause holes to form in the sternum. The incidence is between 3.1 to 27.4% in dried sterna (2). Sternal holes are observed in the manubrium, body, and in the xiphoid process, also if a highest incidence is verifyed in the xiphoid process. The presence of sternal holes may cause during sternal puncture the accidental punture of organs retrosternal as the heart and lungs with possible tamponade or pneumothorax .Moreover knowledge of this anomaly may be important in forensic medicine. The presence of a sternum holes may be mistakenly interpreted as penetrating traumatic injuries or bullet penetration. In conclusion, the recognition of this not uncommon anatomical abnormality is important for radiologists and in clinical and forensic medicine.

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Keywords

Foramina, congenital, sternum

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VEGF-induced intracellular Ca^2+ oscillations are weaker and do not stimulate proliferation in tumor-derived endothelial colony forming cells

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Endothelial colony forming cells (ECFCs) represent a population of truly endothelial precursors that may be mobilized from their vascular stem cell niches to promote the angiogenic switch in a growing number of solid malignancies, including breast cancer (BC). While normal ECFCs require VEGF to proliferate, tumor-associated ECFCs are seemingly insensitive to this growth factor. This phenomenon could contribute to the relative failure of anti-VEGF therapies in cancer patients. Recent work showed that the intracellular Ca^2+ toolkit, which is a crucial determinant of ECFC fate and controls the pro-angiogenic program triggered by VEGF, is remodelled in tumor-associated ECFCs. Herein, we adopted an array of techniques, including Ca^2+ imaging, electron microscopy, flow cytometry, real-time polymerase chain reaction, western blot analysis and functional assay to investigate whether and how VEGF uses Ca^2+ signalling to control proliferation in BC-derived ECFCs (BC-ECFCs). Our results finally demonstrate for the first time that BC-ECFCs are insensitive to VEGF, which might explain at cellular and molecular level the failure of anti-VEGF therapies in BC patients, and hint at SOCE as a novel molecular target for this disease.

Keywords

VEGF, breast cancer, endothelial colony forming cells, intracellular Ca2+ oscillations, proliferation

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Acetylcholine induces intracellular Ca^2+ oscillations and nitric oxide release in mouse brain endothelial cells

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Basal forebrain neurons increase cortical blood flow by releasing acetylcholine (Ach), which stimulates endothelial cells (ECs) to produce the vasodilating gasotransmitter, nitric oxide (NO). Surprisingly, the mechanism whereby Ach induces NO synthesis in brain microvascular ECs is unknown. An increase in intracellular Ca^2 + concentration recruits a multitude of endothelial Ca^2 +-dependent pathways, such as Ca^2 +/calmodulin endothelial NO synthase (eNOS). The present investigation sought to investigate the role of intracellular Ca^2 + signaling in Ach-induced NO production in bEnd5 cells, an established model of mouse brain microvascular ECs, by conventional imaging of cells loaded with the Ca^2+-sensitive dye, Fura-2/AM, and the NO-sensitive fluorophore, DAF-DM diacetate. Overall, our data shed novel light on the molecular mechanisms whereby neuronally-released Ach controls neurovascular coupling in blood microvessels.

Keywords

Mouse brain microvascular endothelial cells, bEND5 cells, acetylcholine, nitric oxide, Ca2+ signaling, intracellular Ca2+ oscillations, inositol-1,4,5-trisphosphate receptors, store-operated Ca2+ entry, Orai2

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A reversible carnitine palmitoyltransferase I (CPT1) inhibitor offsets chronic lymphocytic leukemia cell proliferation

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Crucial for CLL development and progression are iterative cycles of cell re-activation and proliferation in lymphoid tissues. Since cellular fatty acid (FA) import and oxidation (FAO) were recently reported to be upregulated in CLL, we explored in-vitro effects of ST1326, a reversible inhibitor of carnitine-palmitoyl transferase 1A (CPT1A), on leukemic cells subject to activating microenvironment-mimicking stimuli.

ST1326 induced dose-dependent mitochondrial dysfunction and cell death, which were remarkably higher in activated/proliferating than quiescent CLL cells. Drug sensitivity was observed irrespective of the presence of *TP53* alterations or chromosomal abnormalities.

ST1326 cytotoxicity was associated with decreased levels of intracellular acetyl-CoA and phosphorylated STAT3, known to favor CLL cell proliferation and upregulate antiapoptotic Bcl-2 family members. Indeed, rising of Mcl-1 and Bcl-xl expression in response to microenvironment stimulation was impaired by ST1326. Drug combination experiments with the BH3-mimetic ABT-199/Venetoclax, whose effects are counteracted by Mcl-1/Bcl-xl and cell proliferation, showed strong ST1326-mediated potentiation of ABT-199 cytotoxicity in activated/proliferating CLL cells.

The data indicate that CLL cells turning to an activated/proliferating state become more dependent on FAO and more sensitive to FAO-antagonists, and pave the way for ST1326 as an adjuvant tool in anti-CLL drug-combination regimens with drugs that loose efficacy on proliferating leukemic cells.

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Unexpected effects of biphosphonates in *in vitro* models of activated CLL cells

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Recent studies suggest that the commonly prescribed anti-osteoporosis drugs bisphosphonates (BPs) might also exhibit antitumor activity. We investigated a possible anticancer effect of BPs on B-chronic lymphocytic leukemia (CLL) cells obtained from peripheral blood of 26 CLL patients.

Zoledronate, etidronate and clodronate were administered in vitro simultaneously to following activation stimuli: i) CD40L-expressing fibroblasts, ii) soluble recombinant CD40L produced in our laboratory +IL-4, iii) CpG ODN 2006+IL-15 with or without bone marrow stromal cells (BMSC). CLL cell viability, activation/proliferation were monitored by flow cytometry.

We unexpectedly observed that BPs generated a protective effect from spontaneous apoptosis in 11/26 (42%) patients (viability + 18%-392%) and an augmentation in CLL cell activation/proliferation in 61% of the samples (S+G2M phase: +100%±25). Interestingly, protection from spontaneous apoptosis or increment of cell activation, required the presence of either fibroblasts, BMSC or autologous Nurse Like Cells (NLC).

We thus hypothesized that supportive cells are involved in the BPs effects either through cell-cell interactions with leukemic cells or T cells, or through soluble factors release in the medium. Functional experiments with transwells suggest that stromal cells, in presence of Clodronate, release soluble factors in the medium that may probably concur to the unexpected Clodronate-mediated enhancement of CLL cell activation/proliferation.

This work is in progress and several critical questions on the mechanisms are still unanswered. Nevertheless, the phenomenological data argue that caution should be taken when administering BPs against osteoporosis in elderly persons, who could have Monoclonal B Lymphocytosis or CLL.

Keywords

Bisphosphonates, pro-proliferative effect, stromal cells

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Primary vaginal leiomyosarcoma, a rare tumour: case report and review

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Primary vaginal leiomyosarcomas (pvLMS) are rare, recurrent tumours accounting for ca. 2% of all vaginal cancers. The etiology is still unknown, the prognosis is poor and there is no consensus guideline on its management. Diagnosis is usually made during the 5th decade due to the presence of a vaginal mass or nodule [1-2]. Current medical literature reports about 200 cases (PubMed®); only 3 studies have considered the ultrastructure [2-4]. Herein a pvLMS is presented and discussed. A nodular, 25 x 23 x 28 mm-mass, infiltrating the urethra but not the rectovaginal septum, was widely excised from the superior vaginal wall of a 58-year-old previously hysterectomized woman. Macroscopic images and MRI were performed. Iliac lymph nodes and HMB-45 were negative. The sample was fixed and prepared for light microscopy, transmission (TEM) and scanning (SEM) electron microscopy. Semithin sections showed a storiform pattern of spindle shaped cells with blunt-ended nuclei. Cells arranged in interwoven fascicles within a dense and richly vascularised stroma (neoangiogenesis). Some atypic mitotic figures and focal necrosis were seen. SEM evidenced a dense collagenous stroma with numerous microvessels. TEM showed neoplastic and pleomorphic cells with complex cytoplasm projections containing paranuclear crowds of dilated mitochondria, free ribosomes and a well-developed rough endoplasmic reticulum. Nuclei were large, mostly hyperchromatic, usually indented, with prominent nucleoli and nucleolonema. The dense intercellular space contained dense bundles of collagen fibers. A high and reactive endothelium lined blood vessels. After 4 follow-ups, the patient is fine and without recurrence. Best outcomes occur when the tumour is small, localized, and can be removed surgically with wide, clear margins, as it was for this case. As there are different kinds of LMS, biopsy followed by immunohistochemistry and electron microscopy still represents a good diagnostic choice.

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Keywords

Leiomyosarcoma, vagina, electron microscopy, light microscopy, cancer, clinical anatomy

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Training the "clinical eye". Rubens' Three Graces: how many pathologies?

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Art can serve as a powerful resource for medical students to both train the so called "clinical eye" and to better understand disease [1]. Herein a paleopathological analysis is performed on one of Ruben's final artworks, "The Three Graces" (1630-1635; oil on oak panel; 220.5 x 182 cm; Museo del Prado, Madrid). Rubens depicts the three Graces beside a fountain, under a garland of flowers in a landscape. The circular rhythm and elegant undulation are based on classical sculpture. Painted shortly after his marriage, it bears witness to the happiness of the artist's life. The figure on the left is directly inspired by his second wife, Hélène Fourment (23 years old); the central and right Graces probably illustrate Rubens' sisters-in-law. Besides overweight, scoliosis, and hyperlordosis observed in all three Graces, the left Grace evidences flat feet; hyperextension of the right metacarpal joints; signs of rheumatoid arthritis (even fibromyalgia has been proposed); lateral deviation of the nipple (Mondor's disease?); varicose thighs, and right hallux vagus. The central Grace (Clara Fourment?), in turn, shows cellulite and, interestingly, positive Trendelenburg sign. Finally, the Grace on the right -Susanna Fourment- has been subject of a long debate on signs of a locally advanced breast cancer in the left external upper quadrant. In fact, several specialists agree in the observation of signs of an open ulcer; redness of the surrounding skin (an inflammatory sign); nipple retraction; reduction of the left breast volume, and enlarged axillary lymph nodes [2-3]. Rubens was one of main Baroque and realist painters, i.e. he painted whatever his eyes captured. If the Graces were sisters, then they are likely to share genetic traits. The latter, together with all the other signs described, favour the working diagnosis of familial benign hypermobility syndrome. Observation has a key role in clinical medicine; the paleopathological observation in art show us how artists could record abnormalities long before doctors did [2]. Therefore, artworks still represent useful teaching tools for refining visual skills in traditional and innovative medical education.

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Keywords

Medical education, visual arts, innovative teaching, clinical anatomy, paleopathology

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Transmural remodelling of colonic wall following dopaminergic nigrostriatal neurodegeneration

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Background and Aim. Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor and non-motor clinical signs, among which gastrointestinal disturbances represent relevant manifestations [1]. Nevertheless, the morphological alterations associated with intestinal dysfunctions in PD have been barely investigated [2]. The present study was aimed at investigating the remodelling of colonic wall in a rat model of PD with central dopaminergic denervation by intra-nigral injection of the neuro-toxin 6-hydroxydopamine (6-OHDA).

Methods. Histopathological analysis of the whole colonic wall was performed 4 and 8 weeks after central 6-OHDA injection. Inflammatory infiltrates, collagen deposition as well as the remodelling of intestinal epithelial barrier and *tunica muscularis* were examined by microscopic techniques (histochemistry/immunohistochemistry/confocal immunofluorescence).

Results. Colonic tissue from 8-week 6-OHDA rats were significantly altered, as compared with controls. The *tunica mucosa* showed: eosinophil infiltration; altered lining epithelium (reduced claudin-1 and transmembrane 16A protein expression) and goblet cells (increased mucus expression); enhanced glial fibrillar acid protein-positive cells and vimentin-positive fibroblast-like cells. Along with transmural collagen deposition, significant changes were observed also in the *tunica muscularis*: reduced expression of alpha-smooth muscle actin/desmin and increased proliferation index in smooth muscle cells; increased vimentin expression and proliferative phenotype in myenteric ganglia.

Conclusions. A full-thickness structural remodelling occurs in the colon of PD rats 8 weeks after central dopaminergic denervation; the main changes include an alteration of the colonic epithelial barrier along with the activation of the mucosal defence and fibrotic switch of the colonic wall. Overall, these findings suggest that: a) early histological modifications occur in the colon of rats with experimental PD at both mucosal and muscular level; b) these changes and the fibrotic alterations might contribute to bowel motor dysfunctions associated with PD.

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Keywords

Parkinson's disease, rat model, intestinal dysfunctions, colon, morphological remodelling, histochemistry/immunohistochemistry/immunofluorescence

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Histomorphological analysis of the colonic barrier in a mouse model of obesity

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Background and Aim. Obesity is a metabolic disorder with an increasing incidence in Western countries and childhood. It is characterized by low-grade systemic inflammation and several comorbidities, including alterations of gastrointestinal (GI) functions, which impact negatively on patients' quality of life. There is currently limited information on the morpho-functional features of the GI tract in obese subjects. Of note, the intestinal barrier function has been found to be altered in obese subjects, even before the occurrence of body weight increase [1]. In this light, the present study was carried out to assess, in a mouse model of diet-induced obesity, whether high fat diet (HFD) is associated with morphological alterations of the colonic mucosal barrier.

Methods. C57BL/6 mice (n=5/group) were fed with standard diet (SD, 18% calories from fat) or HFD (60% calories from fat). After 8 weeks, body weight, and levels of blood cholesterol, triglycerides and glucose were evaluated. Malondialdehyde (MDA, colorimetric assay), IL-1β and IL-6 levels (ELISA assays) were examined in colonic tissues. Morphological features of colonic mucosal structures (lining epithelial cells, goblet cells, inflammatory infiltrates and enteric glia) were examined by histochemistry and immunohistochemistry.

Results. HFD mice displayed significant differences at both molecular and histomorphological level, as compared with SD animals: increased body weight and blood metabolic indexes; increased MDA, IL-1β and IL-6 levels in colonic tissues; altered pattern of claudin-1 expression along with upregulation of transmembrane 16A protein and induced nitric oxide synthase in the enteric epithelium facing the lumen; increased proliferation rate of crypts; altered composition of goblet cell mucous; mucosal gliosis and infiltrates with mixed inflammatory cells.

Conclusions. After 8 weeks, HFD intake led to significant alterations of systemic metabolic indexes, colonic tissue inflammation, and colonic mucosal barrier in obese mice, as compared with controls. Morphological studies can be useful to allow the characterization of histopathological patterns of colonic wall remodelling and inflammation underlying bowel motor dysfunctions associated with obesity.

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Keywords

Diet-induced obesity, mouse model, intestinal mucosal barrier, morphological remodelling

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Histomorphometrical evaluation of the effects of Aminogam® gel in oral healing process of post-surgical soft tissue

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Wound healing is a dynamic process that involves a complex interaction of inflammatory cells, cytokines and mediators of extracellular matrix [1]. One of the processes that occur during tissue regeneration is angiogenesis and it is considered to have a pivotal role in wound repair. Previous studies have shown that a topical application of proteins and sodium hyaluronate to wounds can expedite the repair of damaged tissue [2].

The aim of this preliminary study is to evaluate the efficacy of Aminogam® gel (A®) (ErreKappa Euroterapici SpA, Milano), a topical medication which contains 4 amino acids (glycine, leucine, proline, lysine) and sodium hyaluronate, used to improve and accelerate gingival flap healing following molar extraction by analyzing collagen fibers amount, orientation and microvascular distribution (MVD).

Ten patients (mean age 49ys) were included in the study. Two teeth (38 and 48) were extracted at an interval of 30 days. The "test" site (AM) was treated with A® while the "control" site (no AM) was not. Dental extraction was performed and the flaps were sutured with a consequent excess of tissue for histological processing (T0). A® had been applied only at the AM site for 10 days post-extraction. At suture removal, a gingivoplasty was performed and the exceeding tissue was histologically analysed (T1).

Paraffin blocks were cut and slides were stained with haematoxylin-eosin and Sirius Red. No signs of inflammatory infiltrate or necrosis were observed. Sirius Red staining highlighted a lower degree of organized collagen fibers at T1 vs T0. At T0 the fibers were organized in closely packed and well-oriented bundles. At T1-no AM fibers were thin and formed a disorganized grid. At T1-AM fibers appeared thicker and the tissue appeared more mature compared to T1-no AM.

Immunohistochemistry against CD31 was performed to mark endothelial cells and to calculate MVD by stereological method [3]. MVD resulted highest at T1-AM. The T1 data normalized on T0 presented a statistically significant difference ($p=0.012$) between AM and no AM group.

In conclusion, A® gel seems to increase new blood vessels formation and to promote collagen deposition and organization.

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Keywords

Wound healing, angiogenesis, collagen matrix

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From nucleus pulposus mesenchymal stem cells towards neural differentiation: an interesting prospect

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Regenerative medicine arouses great interest for the treatment of many neurological diseases. Since nucleus pulposus of the invertebral discs is a postembryonic vestige of the notochord, it has been hypothesized that mesenchymal stem cells (MSCs) isolated from nucleus pulposus (NP-MSCs) can more easily differentiate into neurons. In this study, MSCs from nucleus pulposus were successfully isolated and characterized. Then, neural differentiation was induced by using a medium consisted of DMEM/F12 supplemented with B27 and the growth factors FGF and EGF for 10 days. Immunocytochemistry, molecular studies, SEM and TEM microscopy analyses were performed.

NP-MSCs exhibited the typical features of MSCs, revealing spindle-shape morphology, specific immunophenotype attributable to MSCs and the ability to differentiate in osteogenic and chondrogenic lineages. After neurodifferentiation induction, compared to NP-MSCs in only DMEM/F12, proliferation rate decreased and cells changed morphology acquiring an increased number of the so-called neural-like extensions. Neural progenitor marker NESTIN and mature neuronal marker ENOLASE-2 were up-regulated, while GFAP was not detected. Moreover, cells after differentiation were small rounded and fusiform, with tendency to organize in clumps; they had elongated extrusions containing oriented cytoskeletal elements, classifiable as microtubules and intermediate filaments, as visualized by SEM and TEM microscopy. Dense vesicles similar to lipid droplet were also observed. NP-MSCs in differentiation medium were able to form neurospheres. In conclusion, even if more analysis have to be done and the way to treat neurodegenerative disease with regenerative medicine is still long, NP-MSCs represent a promising resource.

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Keywords

Mesenchymal stem cells from nucleus pulposus, neuronal differentiation, regenerative medicine

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The novel organelle autophagoproteasome is recruited to limit methamphetamine toxicity

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Protein clearing pathways represent a powerful physiological mechanism to control homeostasis within eukaryotic cells, while their dysfunction is a key factor in the molecular mechanisms underlying neuronal degeneration. These clearing pathways remove misfolded proteins and altered organelles. Two main pathways named autophagy pathway and ubiquitin proteasome are considered to be prominent in eukaryotic cells. These pathways are commonly viewed as distinct biochemical cascades occurring within specific cytosolic compartments where pathway-specific enzymatic activity is believed to take place. The classic view considers these clearing pathways as distinct depending on various items: different compartmentalization, different substrates, different enzymatic activities and different roles in cell homeostasis. We just described the morphological convergency of autophagy (ATG) and ubiquitin proteasome (UP) pathway to form a novel organelle named autophagoproteasome [1]. This is shown by confocal microscopy and immune-electron microscopy. Both ATG [2] and UP [3, 4] are recruited robustly during methamphetamine exposure playing a pivotal role in methamphetamine toxicity. Methamphetamine dramatically alters autophagoproteasomes which play a critical role in counteracting methamphetamine toxicity. Despite being segregated within a single organelle ATG and UP components undergo a slight different pharmacological regulation. Both pathways are up-regulated along with autophagoproteasome following methamphetamine administration, but UP prevails for low doses while ATG takes over for higher doses of methamphetamine, which demonstrates a common, dopamine-dependent regulation with slight differences for these clearing pathways within a single organelle. ATG and UP component appear to be molecularly bound within autophagoproteasome depending on specific pharmacological stimulation as shown by western blotting of immunoprecipitates. The structure and function of the autophagoproteasome critically relies on mTOR activity for all its components. The fine tuning of mTOR activity is likely to impact significantly methamphetamine toxicity as well as dopamine-dependent pathological conditions.

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Keywords
Autophagy, Ubiquitin Proteasome System, Methamphetamine, mTOR

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Multi-layer spiral CT with 2D, 3D and 4D volume rendered electronic reconstructions of wax models and natural bone made by Giuseppe Astorri kept at "Luigi Cattaneo" Museum in Bologna

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The Museum's collection of normal and pathological wax anatomical models provides a clear understanding of the developments in medical knowledge that took place during the 18th and 19th centuries. In this period the interest of the anatomists began to move from normal anatomy to pathological anatomy. The wax modelers made both types of wax anatomical models: normal and pathological. Our study investigates through the works of Giuseppe Astorri the differences between these two types of models, revealing hidden structures and materials used in a completely non-invasive way. The Computer Tomography (CT) analysis was carried out using an experimental CT system specifically designed for the analysis of Cultural Heritage materials, developed by the X-ray imaging research group at the Physics and Astronomy Department of the University of Bologna. The results of this project can also be shown through a dynamic 3D (i.e. 4D) virtual projection using a device capable of emulating a holographic representation.

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Keywords

Wax models, holographic representation, computer tomography, anatomical representation

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Human trophoblast differentiation: possible role for trophoblast cell surface antigen 2

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Human trophoblast cell surface antigen 2 (Trop2) is a 40-kDa transmembrane glycoprotein, encoded by TACSTD2 gene and identified for the first time in human trophoblast and choriocarcinoma cell lines. Trop2 has a short intracytoplasmic tail essential for the control of several pathways that regulate cellular functions such as cell- cell adhesion, cell proliferation and mobility [1]. We analysed the expression of Trop2 in human normal placentas during gestation and in placentas complicated by preeclampsia (PE). Trop2 protein expression and miR125b1 were analysed by morphological and bio-molecular techniques. Trop2 increased during gestation, i.e. from first to third trimester of gestation while it was low expressed in placental tissues collected from patients with PE. Since PE is a pathology associated with placental hypoxia, we demonstrated that Trop2 is downregulated in hypoxic conditions by in vitro model. Our study suggests a possible involvement of Trop2 in maintaining trophoblast morphology and function during placental development in normal and PE conditions.

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Keywords

Placenta, development, immunohistochemistry, preeclampsia

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P53 and VEGF expression in human temporomandibular joint discs with internal derangement correlate with degeneration

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Aim: Temporomandibular joint (TMJ) disorders are one of the most relevant causes of chronic facial pain and disability. During histopathological conditions biomolecular mechanisms occur inducing histologic changes of the tissue itself. Human Tumor Protein p53 and Vascular Endothelial Growth Factor are related with cell-cycle control, angiogenesis and both play a central role during inflammation. The purpose of the present research was to investigate the immunoexpression and immunolocalization of Human Tumor Protein p53 and Vascular Endothelial Growth Factor in temporomandibular joint discs of individuals with internal derangement with anterior disc displacement in order to gain insights into the apoptotic and angiogenetic processes in the three bands of articular discs with or without reduction and compare them to the histological degeneration score.

Methods: Paraffin samples of eighteen displaced temporomandibular joint and four control discs were analyzed by immunohistochemistry for the above evaluations.

Results: Data showed a strong Human Tumor Protein p53 and Vascular Endothelial Growth Factor immunoexpression in the anterior and intermediate disc areas and a weak immunoexpression in posterior area of anterior disc displacement with reduction patients while anterior disc displacement without reduction patients demonstrated a weak Human Tumor Protein p53 and Vascular Endothelial Growth Factor immunolabelling in the anterior and intermediate areas and a strong immunoexpression in posterior band. These immunoexpressions correlated with histological degeneration score.

Conclusions: According to our results it can be assumed in that when the more histopathological changes in the disc are releaved, major levels of p53 and VEGF are produced.

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Keywords

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Temporomandibular joint discs, internal derangement, p53, VEGF, immunohistochemistry

Effects of treatment with anti-HIV drugs on ovarian cell line

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Anti-retroviral drugs used for the treatment of Human Immunodeficiency Virus (HIV) have proven to be effective even against cancer. Drawing from this background the aim of our research project was to evaluate the effects of anti-HIV drugs that belong to the Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (NRTI, abacavir and tenofovir), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI, efavirenz and etravirine) and Protease Inhibitors (PI, darunavir) on ovarian adenocarcinoma cell line (Skov-3). We observed by FACS analysis that the treatment with NRTI and NNRTI showed a block in G0/G1 phase. In particular etravirine (ETR) displayed a relevant block in the progression of the G0/G1 phase of the cell cycle compared with the other examined drugs and it was also able to induce differentiation of SKOV-3 cells. In contrast, FACS analysis demonstrated that, abacavir (ABC) and PI inhibitor darunavir (DRV) showed no effect on proliferation cancer cells. DAPI (4',6-diamidino-2-phenylindole) staining demonstrated that cells treated with the NNRTI (efavirenz and etravirine) presented more DNA damages compared with other treatments. Immunoblotting analysis demonstrated tenofovir (TDF), efavirenz (EFV) and etravirine to be able to obtain a reduction in the expression of cyclin D1, Rb hypophosphorylation and increase of p21 concentration. Finally we also observed that etravirine also induced differentiation as demonstrated by Western Blot with high levels of E-cadherin expression. Therefore our study provides additional evidence supporting the in vitro cytotoxic effects of etravirine and efavirenz; furthermore it promotes the hypothesis for their potential use as therapeutic agents in ovarian cancer.

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Effects of running technique training on game-related sprint tests in 10-year soccer players.

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The present study aimed at comparing the effects of a 12-week soccer training on sprint ability in 16 pre-pubescent (9.5±0.3 yrs) soccer players. Twice a week the participants were administered the same technical and tactical training and friendly matches, whereas twice a week group 1 was trained exclusively on running techniques, and group 2 was administered multilateral workouts. Before (PRE) and after (POST) the experimental period, sprint ability was ascertained ("Chrono Time" photoelectric cell system; Globus; Codognè, Treviso, Italy) by means of four 20-m sprint tests performed twice with the best trial used for statistical analysis: linear sprint (L), linear sprint with ball possession (LB), sprint with change of direction (CoD), sprint with change of direction and with ball possession (CoDB). A Mann-Whitney U was applied to evaluate differences ($P \leq 0.05$) between groups and PRE-POST conditions. Whilst group 1 showed improvements in all sprint performances (L=3.5%, LB=5.4%, CoD=5.0%, CoDB=6.2%), group 2 showed progresses only in LB=5.8%, CoD=2.9%, CoDB=1.7%. However, differences between conditions ($p=0.046$) emerged only in group 1 for CoDB. Between groups, differences emerged for CoDB ($p=0.015$) in PRE (group 1: 7.46±0.37, group 2: 7.84±0.42; $p=0.046$) and POST (group 1: 7.00±0.47, group 2: 7.71; $p=0.078$) conditions. With respect to multilateral workouts, the present findings indicate that training focused on running techniques seems to be more effective for improving pre-pubescent soccer players' sprint performances, especially for change of direction with ball possession, which is the most game-related sprint activity. However, further studies need to clarify the optimal balance between multilateral and specialized training for youth soccer players [1].

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Keywords

Running technique, team sport, pre-pubescence, testing

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Post mortem computed tomography of heart

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Post mortem Computed tomography (CT) has been increasingly used in routine forensic practice and research. While radiological examination is generally considered to be a good complement for conventional autopsy, it was thought to have limited application in cardiovascular pathology. The aim of the present study is to show our experience of radiological analysis of the heart as single organ, as an integrative tool for research and forensic applications. The anatomo-radiologic study for forensic purpose was performed on 10 hearts sampled at autopsy (8M, 2 F, mean age 45 years old). The specimens underwent CT examinations. In 5 out of 10 of cadavers, a myocardial infarction was found at macroscopic and microscopic analysis. In these same cases, the CT examination showed the presence and the localization of calcifications, corresponding to the infarct area. In 90% of cases the presence of calcifications allowed the visualization of the coronary arteries and their branches. Basing on our experience, isolated single-organ CT could be considered a useful integrative tool in addition to traditional autopsy investigation (macroscopic sections and histology) in identifying the cause of death by recognizing the presence and degree of coronary artery pathology.

Keywords

Computed tomography, Coronary artery, Forensic clinical anatomy

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An anatomo-radiological study of the infrapatellar fat pad

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The infrapatellar fat pad (IFP) is an intracapsular, but extrasynovial structure, located between the patellar tendon, femoral condyles and tibial plateau. We have recently described the microscopic orgnisation of the IFP, which consists of white adipose tissue (fibroadipose, lobular type), organised in lobuli delimited by thin connective septa. The aim of the study is to describe the sonoanatomical features of IFP in subjects without knee pathology during flexo-extension movements. Twentyfour volunteers subjects with no history of knee diseases (5M, 19F, mean age: 45yo)were analysed. Examinations was performed using high-resolution grey-scale ultrasound. The mean area of the deepest recognisable adipose chamber in extension were 0,12 and in flexion 0,19 mm^2, and the circumference were in extension 1,36 and in flexion 1,19 mm. The area of the closest adipose chamber to the patellar tendon were in extension 0,29 and flexion 0,12 mm^2($p<0.01$), whereas the circumference were in extension 2,67 and in flexion 1,56 mm($p<0.01$). Our study demonstrated that the normal IFP is constituted by largest lobuli in the superficial part($p<0.01$) that become flattened in flexion movement. The deep lobuli are smaller and do not change their morphology. Our study demonstrated that ultrasound is useful to analyse the dynamic changes of the IFP.

Keywords

Infrapatellar fat pad, ultrasound

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Student learning performance in human anatomy using a virtual dissection table

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In medical training it is fundamental to have a 3D understanding of human anatomy [1]. Body dissection is considered mandatory in most of the bio-medical schools. Medical schools around the world, constantly face the problem of availability of the cadavers. Apart from the classic methods (lectures, podcasts, atlas or photographs, models) technology advances made available new instruments to learn/teach 3D anatomy, which allow cadaverless dissection with the help of simulator software or virtual cadavers. The Anatomage® and Sectra® tables are advanced anatomy visualization systems, adopted by many of the world's leading medical schools and institutions [2,3].

Here we report our experience with Anatomage® during the Academic Year 2016-17, in the Postgraduate Courses of Medical and Surgical Specialization, Master degrees in Medicine, Dental Medicine, Biology of Health and Nutrition, as well as Basic Degrees in Nursing and Biotechnology, of the University of L'Aquila. We enrolled 30 MD postgraduate students, and 440 undergraduate students. Both postgraduate and undergraduate medical students were allowed to handle the table. The other students assisted to class table demonstrations. An evaluation test was administered to all students at the end of the Courses.

Our preliminary observations suggest that the use of virtual dissection table into the anatomical curriculum improves the learning student performance. Each student have a different set of needs, and the base line skills may be different. So, the teacher should take in consideration the variable scope of practice of the specific health professions. We are currently evaluating the efficacy of this technology at the end of the examinations. In the present preliminary report, we account with our results that are indicative of a positive impact on both basic and advanced anatomical learning.

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Keywords
Virtual dissection table, teaching methods, gross anatomy, clinical anatomy

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Understanding the endocrine crosstalk between bone and muscle: molecular investigation of the impact of myokines on osteogenesis using C2C12 myoblast and 2T3 osteoblast cell lines

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Bones and skeletal muscles interact mechanically to allow motor activity in vertebrates and even invertebrates. Until the last decade of research, bone-muscle interactions had been gathered under the umbrella of the "mechanical coupling" theory, where muscles are the load suppliers and bones provide the attachment sites [1]. However, bones and skeletal muscles have recently been identified as endocrine organs, that secrete cytokines and chemokines, through which they interact to promote the motor activity. This molecular and biochemical interplay has been named "bone-muscle crosstalk". The bi-directional flow of signals between bone and muscle has been investigated experimentally by differentiating bone or skeletal muscle progenitor cells in a medium conditioned by myotubes or osteocytes respectively [2][3]. These studies have demonstrated that osteocyte and myotube secreted factors (osteokines and myokines, respectively) have a reciprocal inhibitory influence on myogenesis and osteogenesis, since they reduce the majority of the mRNA levels of genes associated with differentiation. We propose to study the effects of myokines on osteogenesis by differentiating 2T3 osteoblastic cells in a medium conditioned by either early (3-5 days) or late (7-10 days) myotubes. The study includes: *i)* analysis of mRNA and protein levels of marker genes of differentiation, to establish the effect of early and late patterns of myokines; *ii)* characterization of the differentiation process from the functional viewpoint by studying alkaline phosphatase activity and the deposition of mineralized matrix. As expected results, early and late myotube-conditioned media should affect differently the osteoblast lineage in the course of differentiation. The study includes also the successive identification of the metabolomic profile of the conditioned medium, to identify the cytokines most abundantly expressed. This first set of results will pave the way for further experiments of myoblast/osteoblast co-cultures aimed at a real-time tracking of the bi-directional signaling between bone and muscle tissues and its impact on all stages of differentiation. The results of this study will deepen the understanding of how the muscle secretome protects osteocytes and preserves their function and *vice versa* how bone factors maintain muscle function. Such knowledge will help to identify potential new target therapies for bone and muscle diseases, especially when they co-exist, as is the case of the twin syndrome of osteoporosis and sarcopenia.

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Keywords
Bone-muscle crosstalk, osteokines/myokines, osteogenesis, myotube, conditioned medium

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Analysis of isometric strength and force-velocity relationship after 7 weeks of stable and unstable training on partial push up

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Training with instability device seems to have useful adaptaments, but not all the autor confirm it. Instability training shows increase muscle activation due to the needed for stabilization. The increased stress associated with instability training has been postulated to promote greater neuromuscular adaptations, such as decreased co-contractions, improved intra and inter-coordination and set a lower stress on joint and muscle that can beneficial for musculoskeletal healt and rehabilitation. The aim of the research was to find the difference related in strength gain between an exercise under stable and unstable condition. Two groups of healty-fitness people follow a 7 weeks of stable and unstable training on partial push-up. The control group (CG) ($n = 4$, one female and three males, 25.0 ± 3.9 y) performed the push-up with hands on the floor, while the sperimental group (SG) ($n = 7$, two females and five males, 24.6 ± 2.3 y) performed push-up with hands on a Swissball. The execution time, the total volume and the articular ROM were standardized. The tests were: (1) a standardized isometric chest press and (2) force-velocity relationship of the chest muscle. For statistical analysis has been used the Wilcoxon matched-pairs signed rank test. The isometric strength has a positive correlation with the instability training ($p < 0.05$) while the force-velocity relationship hasn't got ($p > 0.05$). Instability training seems to show best adaptations on isometric strength, probably due to neural adaptations, while it seems that it doesn't happen in force-velocity relationship, probably due to the standardized time of execution.

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Keywords

Isometric strength, force-velocity relationship, instability training, neuromuscular adaptations

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Rigosertib as a radio-sensitizer for concurrent chemo-radiation treatment of cholangiocarcinoma (CCA): a comparative study *in vitro*

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Cholangiocarcinoma (CCA) remains a therapeutic challenge. The small-molecule Rigosertib can selectively synchronize cancer cells to G2/M phase improving the efficacy of radiation. In our study, we evaluated *in vitro* Rigosertib (gifted by Onconova Therapeutics Inc) effects on two human CCA cell lines: EGI-1 and TFK-1. Rigosertib was compared with Gemcitabine (GEM) and 5-Fluorouracil (5-FU), two antineoplastic and radio-sensitizer agents used in the treatment of CCA.

Rigosertib impaired cell viability (evaluated by Tripan-blue vital count) in both cell lines in a dose- and time-dependent manner (IC_{50} of 100nM at 24h). GEM and 5-FU had a IC_{50} of 30μM and 7μM after 24h, respectively. Cell migration and invasion tests was performed by scratch wound healing and Boyden chamber assay respectively. Rigosertib caused a 50% inhibition of the EGI-1 cell migration (10μM) and invasion (100nM), while the inhibitory effects on TFK-1 cells were observed with doses of 100μM and 10μM, respectively. GEM 30μM and 5-FU 7μM had no effect on cell migration and invasion. Evaluation of cell cycle by FACS cytometry showed a G2/M arrest in both cell lines after Rigosertib 100nM for 24h. Radio-sensitizing test was performed by clonogenic survival assay after irradiation. 24h Rigosertib pre-treatment (100nM for EG-1 and 1 μM for TFK-1) when followed by 2, 4 or 6 Gy irradiation, reduced survival in both CAA cell lines when compared with radiation alone. The Rigosertib radio-sensitizer effect was similar to that seen after GEM or 5-FU 24 pre-treatment both plus irradiation. However, 48h Rigosertib pre-treatment was more effective than radiation alone as well as GEM for 48h.

Our study highlights the preliminary but promising preclinical activity of Rigosertib both as antitumoral and as a radio-sensitizer agent in CCA and provides a background for further investigations.

Keywords

Cholangiocarcinoma, Rigosertib, radio-sensitizer, antitumoral effects

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Follicle-stimulating hormone receptor (FSHR) a promising novel target for cancer diagnosis in seminoma and embryonal carcinoma

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Adult testicular germ cell tumors (TGCTs) are the most frequent malignant tumors in male patients aged 15–45 years, their incidence is increasing in recent years. There are two main subclasses of TGCTs: seminomas (SE) and non-seminomatous germ cell tumors (NSGCTs). SE have histological features of primordial germ cells, whereas NSGCTs have varying degrees of differentiation (i.e. embryonal carcinoma, EC), they present distinctive clinical features and differ for therapy and prognosis. NSGCTs tend to be metastatic at presentation, and have a worse prognosis than seminomas at an equivalent stage of disease. Despite general advances in the management of TGCTs, the molecular bases underlying their progression remain almost unknown. The effects of the Follicle-stimulating hormone (FSH), central hormone in mammalian reproductive biology, are mediated by FSHR, which was believed to be expressed primarily in ovary and testis. Recently, FSHR expression has been shown in the blood vessels of different solid tumors, including prostate, urothelial and breast carcinomas, suggesting a role in neoangiogenesis. The expression of FSHR at the periphery of tumors, also suggests that FSHR may be of relevance to the metastatic process. In normal human testis, estrogen physiological actions are mediated by estrogen receptor (ER) β and highly variable ERβ expression has been reported in the different TGCTs. ERβ loss is associated with advanced tumor stage in several cancers and previously, we showed a higher expression of ERβ1 in SE with respect EC. In this study, we evaluated the expression of FSHR in normal and neoplastic human testis tissues. Further, we compared FSHR expression with that of ERβ1 in the same samples. In normal testes, immunohistochemical studies showed the presence of FSHR prevalently in somatic testicular cells, while ERβ1 is expressed both in somatic and germinal testicular cells. Intriguingly, we discovered that FSHR was strongly expressed in EC and absent in SE. Conversely, immunostaining for ERβ1 revealed higher intensity in SE as compared to EC. These data suggest distinct physiopathological roles for the two receptors in TGCTs progression, being ERβ1 protective and FSHR harmful. Our data report for the first time the expression of FSHR in TGCTs, suggesting its possible involvement in testicular carcinogenesis. FSHR may be considered an useful molecular marker to distinguish seminoma from embryonal carcinoma, the most common TGCTs subtypes, and this could be informative in clinical decision making and patient counseling.

Keywords
FSHR, TGCTs, seminoma, embryonal carcinoma

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Role of CX_3CR1^+ cell in the protection of the intestinal mucosa

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During infection intestinal $CX3CR1^+$ cells can either extend transepithelial cellular processes to sample luminal bacteria or, very early after infection migrate into the intestinal lumen to capture bacteria. However, up to date, the biological relevance of the intraluminal migration of $CX3CR1^+$ cells remained to be determined. We addressed this by using a combination of mouse strains differing in their ability to carry out CX3CR1-mediated sampling and intraluminal migration. We observed that, the number of S. Typhimurium traversing the epithelium did not differ between sampling-competent/migration-competent C57BL/6 and sampling-deficient/migration-competent Balb/c mice. By contrast, in sampling-deficient/migration-deficient $CX3CR1^{-/-}$ mice the numbers of S. Typhimurium penetrating the epithelium were significantly higher. However, in these mice the number of invading S. Typhimurium was significantly reduced after the adoptive transfer of $CX3CR1^+$ cells directly into the intestinal lumen, consistent with intraluminal $CX3CR1^+$ cells preventing S. Typhimurium from infecting the host. This interpretation was also supported by a higher bacterial faecal load in $CX3CR1^{+/gfp}$ compared to $CX3CR1^{gfp/gfp}$ mice following oral infection. Furthermore, by using real time in vivo imaging we observed that $CX3CR1^+$ cells migrated into the lumen moving through paracellular channels within the epithelium. Also, we reported that the absence of CX3CR1-mediated sampling did not affect antibody responses to a non-invasive S. Typhimurium strain that specifically targeted the CX3CR1-mediated entry route. These data showed that the rapidly deployed $CX3CR1^+$ cell-based mechanism of immune-exclusion is a defence mechanism against pathogens that complements the mucous and secretory (s)IgA antibody-mediated system in the protection of intestinal mucosal surface.

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β-caryophyllene and low-doses of doxorubicin against liver cancer cells: a "metronomic chemotherapy"

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Cholangiocarcinoma and hepatocellular carcinoma are primary liver cancers, both representing a growing challenge due to their increasing morbidity and mortality. A "metronomic chemotherapy", consisting of the repeated administration of low and/or continuous doses of anti-neoplastic drugs, represents an alternative approach to the standard chemotherapy [1]. Numerous natural substances exhibited *in vitro* chemosensitizing features: in particular, the natural sesquiterpene β-caryophyllene (CRY) has been proved to increase the cytotoxicity of doxorubicin (DOXO) in leukemic cells [2]. Hence, our aim has been to evaluate the ability of CRY to enhance the efficacy of low-dose DOXO in human liver cancer cells, by applying a metronomic protocol. To this end, human liver HepG2 and CCA cells have been used as models of hepatocellular carcinoma and cholangiocarcinoma. The metronomic protocol was based on a 2h low-time exposition to the test substances, followed by 72h incubation for restoring. This scheduling has been applied 3 times and cytotoxicity was measured by MTT assay. Both the substances alone (CRY 1-100 μg/ml; DOXO 1-500 μg/ml) and the combination of DOXO with a nontoxic concentration of CRY were assessed. We found that the repeated treatments with low concentrations produced a significant potentiation (about 30 %) of DOXO cytotoxicity in HepG2. The combination with CRY increased the DOXO activity, reaching a 70 % inhibition of cell viability at 50 μg/ml after 2 repeated treatments. Similar effects were found in CCA, although repeated treatments induced no additional potentiation. These results highlight a possible role of CRY as a chemosensitizing agent for DOXO-based chemotherapy of liver cancer.

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Keywords
Cholangiocarcinoma, hepatocellular carcinoma, CRY, doxorubicin

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Knockdown of hepatic GnRH reduces liver fibrosis in a mouse model of PSC

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Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by progressive inflammation and fibrosis of intrahepatic and extrahepatic bile ducts. [1]. Cholangiocyte proliferation occurs in all pathologic conditions of liver injury where it is associated with inflammation and regeneration. During these processes, biliary cells start to secrete different cytokines, growth factors, neuropeptides and hormones which represent potential mechanisms for cross talk with other liver cells [2].Gonadotropin-releasing hormone (GnRH) is a trophic peptide hormone synthesized by hypothalamic neurons and biliary epithelium and exerts its biological effects on cholangiocytes by interaction with the receptor subtype, GnRHR1, expressed by both cholangiocytes and HSCs[3]. Studies have shown that microRNA-200b is associated with the progression of hepatic fibrosis. However, the role of GnRH/GnRHR1/miR-200b axis in the progression of hepatic fibrosis in PSC is unknown. Using a mouse model of PSC (Mdr2-/-), we found that hepatic knockdown of GnRH decreased IBDM and liver fibrosis. In vivo, treatment with GnRH increases the expression of miR-200b and markers of fibrosis with an upregulation of the GnRH/GnRHR1 axis and miR-200b in human PSC samples. *In vitro*, inhibition of miR-200b decreases fibrotic gene expression in cultured murine cholangiocyte and HSC lines. In conclusion, these findings provides novel insights that the modulation of the GnRH/GnRHR1/miR-200b axis may regulates the progression of liver fibrosis in PSC.

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Keywords
Cholangiocytes, fibrosis, GnRH, PSC

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Pb effects on an experimental model of porcine prepubertal Sertoli cells

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The environmental pollution is one of the main factors implicated in the world's fertility decline. Lead (Pb) is one of the major heavy metal contaminants that impairs several organs but preferentially accumulates in male reproductive organs and alters *in vivo* and *in vitro* sperm quality [1]. Nowadays, the underlying mechanisms remain unclear. Sertoli cells (SC) provides structural and metabolic support to the spermatogenic cells within the seminiferous tubules, therefore, metabolic and structural changes in SC affect the developing germ cells and consequently alter spermatogenesis. This study aimed to assess whether exposure to subtoxic doses of Pb would adversely affect superior mammalian SC function. Highly purified and functional porcine pre-pubertal SC were isolated [2] and treated with three different Pb acetate concentrations. Parameters of SC functionality, such as inhibin B and anti-Müllerian hormone (AMH) mRNAs and proteins were decreased by Pb exposure respect to the control, such as the FSH-r integrity in terms of 17-β-estradiol production, under FSH stimulation. In addition, we observed an increase of AKT and mTOR mRNAs, p38 phosphorylation ratio and Akt phosphorylation ratio in all experimental conditions, respect to the control. In conclusion, the Pb-related toxicity on SC, even at low concentrations, is expected to alter spermatogenesis.

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Keywords
Lead, Sertoli cells, function

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Assessment of the aesthetic component of female classical ballet dancers performance

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Since the aesthetic component of dancers' performance is heavily related to their fitness level, the assessment of its qualitative aspect is highly needed (Angioi et al., 2009). This study aimed to assess the qualitative performance of classical ballet dancers by means of an adapted version of the "Performance Competence Evaluation Measure (PCEM)", developed and validated by Krasnow et al.(2009).

Eight female classical ballet dancers (age: 19,3±3,37 years; height: 165±4,65 cm; weight: 52,6±2,26 kg; total years of dance training: 10,5±2,26) were recruited to perform a movement sequence ("adagio") lasting 80 seconds and specifically choreographed for this study. All participants were free of injury and not involved in any supplementary fitness training or other sport activity. Each performance was video recorded, randomly ordered in an edited video and handed together with assessment guidelines to three judges (two very experienced and one professional). Then, the judges' scores for each one of the four parameters [1) Full Body Involvement (FBI); 2) Body Integration and Connectedness (BIC); 3) Articulation of Body Segments (ABS); 4) Movement Skills (MS)] were collected based on a Likert scale ranging from 1 to 5, in order to assess the inter-rater and intra-test-retest (one week after the first) reliability of their decisions. With regard to inter-rater reliability, the Kappa values were ranging from moderate (between 0.4 and 0.6) and very good (over 0.8) for FBI and MS and from good (between 0.6 and 0.8) and very good for BIC, while the professional judge differed from the others for ABS. With regard to intra-test-retest reliability there was mostly a very good agreement between all judges for all parameters. The reliability of the measures suggest that the PCEM can be serve as a useful tool to assess the aesthetic component of the performance of classical ballet dancers.

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Keywords
PCEM, ballet performance

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The open body: a "new" book

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At the beginning of the '300, Mondino de' Liuzzi, a physician from Bologna, was the first anatomist who started again the dissection of human body neglected from the III century. He hinted at the existence of the conflict between book and body, between "auctoritas" and the direct observation of the human body . The Mondino's masterwork "Anothomia" remained the key book up to the middle of the sixth century, when Andrea Vesalio wrote "De Umani Corporis Fabrica," in which the body (cadaver) eventually became the main player of the book .

During the years, the technologic evolution led to the wrong conviction that dissection could be dismissed, albeit, still in our day, doctors in training feel the need to associate the direct experience on the cadaver with the very valuable digital means and the modern imaging technologies even in 3D.

Thinking to Anatomy as an already fully well known discipline is a mistake. The most advanced methodologies for surgical access, namely the minimally invasive surgery, require the evolution of the traditional anatomical knowledge.

The Human Anatomy Institute of the University of Bologna, among the first in Italy, has recognized this need. Thanks to the generosity of the people enrolled in the Body donation programme for research and teaching, our Institute allows medical students to practice dissection on cadavers, beginning as Freshman, then Sophomore, Junior and Senior.

The sharing of Bologna's experience could be the chance to think about the perspectives offered by the dissection of the corpse: a wide range of possibilities spanning from research projects to advanced training courses in collaboration with clinicians and surgeons belonging to different branches. Moreover the practice of corpse dissection is extremely important for the recruitment of young graduates in Medicine which, by means of the experience vested acting as "tutor of anatomy", acquire interest in the field of research of morphological sciences, spanning from macroscopic up to the cellular and molecular level.

Hic mors gaudet succurrere vitae: the motto, reported in dissection room of most of the Italian anatomical institutes, represents the synthesis of the experience of an ancient discipline which, nowadays , has the chance to rewrite a new chapter dedicated to modern frontiers of scientific research and medical education.

Keywords

Dissection, anatomy, tutor, medical education.

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Proteomic insights in extracellular microvesicles from multiple sclerosis patients

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To date the most important biomarkers for Multiple Sclerosis (MuS) diagnosis are the oligoclonal bands (OCBs) in CSF and Link Index. CSF is the body fluid that might better provide information about the pathological processes occurring in the CNS, because of its proximity. Anyway, it is obtained through an invasive procedure, thus tears, may represent an useful alternative source of biomarkers. Emerging evidences showed that distinct types of brain cells release high number of Extracellular Vesicles (EVs), that play important roles in the CNS, and represent a relevant source of biomarkers, relative free from confounding factors. In the present study, we analysed EVs from MuS patients obtained from tears and CSF samples. In details, 50µl of CSF or 50 µl of tears/sample were processed by a common flow cytometry no-lyse and no-wash method, in order to identify EVs. Exosomes and microvesicles (MVs) were sorted (70 µm nozzle, FACSAria III cell sorter, BD) from pooled CSF samples on the basis of their positivity to specific tetraspainins (for exosomes) or markers identifying each MV subset. Fractions were analysed by electron microscopy and Dynamic Light Scattering. Purified MV fractions undergone to FASP tryptic digestion and nanoLC-ESI-QTOF-MS/MS based shotgun proteomic approach. Identified MVs proteins were processed by Ingenuity Pathway Analysis (IPA) and PANTHER - Gene List Analysis.

Our data shows the presence of subpopulations of extracellular MVs of neuronal and microglia origins in tears , indicating a cross talk between the two compartment. We also identified 55 proteins (FDR<2.38) for the MVs fraction. To uncover the molecular events underlying these proteins profiles, we studied the Gene Ontology (GO) information in terms of biological process and molecular function by using PANTHER software and we observed that about 70% of the identified proteins resulted were involved in binding processes, while 40% of them were related to cell communication. Ingenuity Pathway Analysis (IPA) of the identified MVs proteins revealed that the top network associated to them are "Cellular Movement, Hematological Disease, Immunological Disease", well matching with MS. Among the upstream regulators, the most significant one is PRDM with a p-value of 6.68E-07. The remaining upstream regulators, including APOE (the most relevant lipid carrier protein in the brain involved in brain development and repair), well related to neurological disease. These data underlined that MVs form neuronal and microcglial origin are detectable not only in the CSF, but also in tears from MuS patients. Of note, MVs of CSF origin carry relevant targets involved in immune responses in MuS patients, therefore they might be proposed as useful tools in MuS diagnosis and characterization.

Keywords

Proteomic, Extracellular vesicles, Flow cytometry, Biomarker

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Synaptic stripping and MHC class I expression in the facial motor nucleus of ALS mice

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Pathogenetic mechanisms involved in the fatal, still incurable neurodegenerative disease amyotrophic lateral sclerosis (ALS), characterized by progressive motoneuron death, await full clarification, important for the development of new therapeutic approaches. In the ALS murine model provided by mutant SOD1(G93A) mice, we here investigated the presynaptic wiring of facial motoneurons in basal conditions and after facial nerve transection (a classical paradigm to examine the retrograde motoneuron response to injury), and major histocompatibility (MHC) class I antigen expression after axotomy. The study was based on fluorescent retrograde labeling of motoneurons, synaptophysin and MHC class I antigen immunostaining, electron microscopy. A significant decrease of excitatory axosomatic boutons was found in presymptomatic ALS mice compared to the wild-type (Wt) counterpart, indicating the occurrence of excitatory synapse detachment (presynaptic stripping) in mutant motoneurons. Synaptic stripping, which seems to represent a protective mechanism preserving the inhibitory input, became more marked in facial motoneurons of symptomatic ALS mice. After axotomy, synaptic stripping was consistently enhanced in ALS mice. In the axotomized facial motoneurons of Wt mice synaptic stripping was accompanied by induction of MHC class I antigens, immune molecules implicated in activity-dependent changes in synaptic connectivity and regeneration after injury. MHC class I antigen induction was instead decreased in the axotomized facial nucleus of presymptomatic ALS mice, and was very low, occurring only in glial cells, in symptomatic ALS mice. The findings demonstrate enhanced loss of excitatory presynaptic terminals, as well as a dissociation between this process and MHC class I antigen expression after injury, in motoneurons which carry a mutation committing them to death. The findings also implicate MHC class I antigen induction in glial cells surrounding ALS motoneurons in this intercellular crosstalk.

Keywords

Synaptic plasticity, neurodegeneration, motoneurons, amyotrophic lateral sclerosis, neural-immune interactions, major histocompatibility complex antigens

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Effects induced by particles derived from two anthropogenic sources on respiratory, cardiovascular and central nervous systems

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Air pollution represents a well-known environmental problem related to public health. Particulate matter (PM) is a heterogeneous mixture of chemicals, metals and soils. Its adverse effects have been correlated with particles size, being smaller particles more likely to cause a worst damage, so their study deserves more attention. Ultrafine particles (UFPs, $d_{ae} < 100$ nm) are short-lived particles dispersed in the environment. In Lombardy, diesel combustion and solid biomass burning are the most relevant contributors to primary UFPs emissions (15-30 nm in diameter). Toxicological studies, mainly *in vitro*, indicate specific effects for particles of different origin but comparative *in vivo* studies are scarce. PM exposure has been primarily associated to pulmonary and cardiovascular diseases through oxidative stress and inflammatory response, but recently it has been postulated that PM exposure could also be an important risk factor for neurotoxicity and could have a role in neurodegenerative diseases.

In this study we analysed in BALB/c mice the effect of single and repeated intratracheal instillation of diesel (DEP) and biomass (BC) particles on respiratory, cardiovascular and central nervous systems, comparing the two different UFPs sources. The study was performed at biochemical and histopathological level. Different pro-inflammatory, cytotoxic, pro-coagulant and oxidative stress markers were measured. For the histopathological evaluation, sections of lung, heart and different parts of the central nervous system (CNS) were examined at light microscope, using standard staining tecniques and immunohistochemical methods. Inflammation was also monitored in living mice following BC or DEP intratracheal repeated administration using the FMT 1500 fluorescence tomography imaging system and the MMPSense 750 Fast probe.

Our results indicate that even a single instillation of both the sources of UFPs induces a wide range of biochemical changes in the respiratory and cardiovascular systems, then confirmed by repeated instillation. In the CNS similar modifications were observed, although these were much more evident after repeated instillations. Histological examination demonstrated the presence of macrophages containing particles in the lungs after UFPs single and, more abundantly, repeated administration. However, significant changes were not observed in sections of heart and CNS.

DEP was more effective in inducing oxidative stress and inflammation compared to BC.

Keywords

Air pollution, particulate matter, imaging, inflammation, oxidative stress

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A multidisciplinary approach to study the brain injury in Diet-Induced Obesity (DIO) rats

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Obesity represents an independent risk factor for the development of cerebrovascular disease and cognitive impairment. The systemic effects, such as increased fat mass, hypertension, insulin resistance and general metabolic dysfunction, have been identified as factors that may lead to impaired cognitive function.

To clarify the possible relationships between obesity and nervous system changes, high-caloric Diet-Induced Obesity (DIO) rats 7 weeks-old, were studied after 17 weeks of hypercaloric diet compared to control rats with not fat diet (Chow) or to rats not developing obesity (DIO-resistant DR). Food consumption, fat mass content, blood pressure and blood parameters were assessed. Different behavioural tests were used to estimate cognitive performance. RT-qPCR, immunochemical and immunohistochemical analysis were performed to evaluate neuronal, glial and vascular markers.

The obese phenotype developd after 5 weeks of high fat diet exposure and body weight values remained higher in DIO rats compared to the control group and DR rats during the treatment. Systolic blood pressure, glycaemia and insulin were higher in DIO rats only after 17 weeks. No differences in values of total cholesterol and triglycerides were observed. Furthermore increase of thiobarbituric reactive substances and increase of oxidated proteins, was observed in the serum of DIO rats compared to Chow rats. The open-field test revealed, in the older DIO rats, a decrease of cumulative distance travelled and in the number of rearings and an increase of total immobility time. Older DIO rats only, showed a reduction of retention latency time in the passive avoidance test.

RT-qPCR, immunochemical and immunohistochemical analysis showed an increased expression of the glial-fibrillary acid protein in the frontal cortex and hippocampus of older DIO rats compared to age-matched Chow and DR rats. A decrease of neurofilament expression was found in the hippocampus of older DIO rats without changes in the number of neurons. A modulation in the Transient Receptor Potential (TRP) channels and synaptic components was highlighted in cerebral areas.

These results indicate that obesity in rats, in addition to the development of correlate cerebrovascular risk factors, causes brain injury characterized by astrogliosis, neurodegeneration and impaired learning and memory tasks. The identification of neurodegenerative changes in DIO rats may represent the first step to better characterize the neuronal modifications occurring in the obesity and propose pharmacological treatments or food strategies to counter them.

Keywords
Obesity, hypertension, brain injury, Diet-induced obesity (DIO) rats

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Pattern and distribution of extracellular matrix proteins in human reparative dentin by an immunohistochemical approach

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Dentin is a large and complex component of the tooth synthesized by odontoblasts during the process of dentinogenesis. Dentin formed, before the completion of root formation, is define primary dentin (PD), while dentin formed after and associated with the normal aging process is designated secondary dentin (SD). Tertiary dentin (TD) is produced in reaction to external noxious stimulus/injury, such as attrition or dental caries, adjacent to the preexisting dentin layer and further classified reparative dentin (RD) (1, 2).

Aim this study was to compare pattern and distribution of extracellular matrix proteins, produced by odontoblast cells during dentin mineralization and during reparative process, in response to stimulus in human sound dentin *vs* human reparative dentin matrix.

Sixteen sound carious human molars were selected, demineralized, fixed in paraformaldehyde and then processed for immunohistochemical approach to detect extracellular matrix proteins. In particular specimens were submitted to an immunolabeling technique by using primary antibodies anti dentin matrix protein 1 (DMP1), dentin

sialophosphoprotein (DSPP), bone sialoprotein (BSP), osteoponti (OPN). Results indicate that the region of the exposed pulp, formed a layer of reparative dentin bridge sealing the communication between the cavity and pulp chamber. In addition results indicate that in RD is present a lower levels of DMP1 and DSP than PD layer, while BSP and OPN are present in RD but absent in PD layer. The expression of BSP and OPN in RD indicates that the odontoblast-like cells were attempting to produce a hard tissue at a very rapid process. In according with previous scientific literature, our results suggested that the deposition of OPN and BSP at the calcification front is essential for the type I collagen secretion by newly differentiated odontoblast-like cells to form reparative dentin during pulpal healing following cavity preparation.

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Keywords

Human reparative dentin, proteoglycans, immunohistochemical technique, scanning electron microscopy

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Retinoic Acid-induced differentiation sensitizes myeloid progenitors cells to ER stress

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The clonal expansion of hematopoietic myeloid precursors blocked at different stages of differentiation characterizes the acute myeloid leukemia (AML) phenotype. A subtype of AML, acute promyelocytic leukemia (APL), characterized by the chimeric protein PML-RARα is considered a paradigm of differentiation therapy. In this leukemia subtype the all-*trans*-retinoic acid (RA)-based treatments are able to induce PML-RARα degradation and leukemic blast terminal differentiation [1-2]. Granulocytic differentiation of APL cells driven by RA triggers a physiological Unfolded Protein Response (UPR), a series of pathways emanating from the ER in case of ER stress, which ensues when higher protein folding activity is required as during differentiation. We show here that, although mild, the ER stress induced by RA is sufficient to render human APL cell lines and primary blasts very sensitive to low doses of Tunicamycin (Tm), an ER stress inducing drug, at doses that are not toxic in the absence of RA. Importantly only human progenitors cells derived from APL patients resulted sensitive to the combined treatment with RA and Tm whereas those obtained from healthy donors were not affected. We also show that the UPR pathway downstream of PERK plays a major protective role against ER stress in differentiating cells and, by using a specific PERK inhibitor, we potentiated the toxic effect of the combination of RA and Tm. In conclusion, our findings identify the ER stress-related pathways as potential targets in the search for novel therapeutic strategies in AML.

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Keywords
Retinoic Acid, Myeloid differentiation, UPR, ER stress

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Role of nicotine during diabetic macular edema development

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Diabetic macular edema (DME) represents the major cause of visual loss in diabetes patients. It is characterized by retinal thickening in the macular area due to breakdown of the blood-retinal barrier (BRB) [1]. By altering blood vessels supplying retina, hyperglycemia triggers tissue hypoxia. The primary response to latter is mediated by hypoxia-inducible factors (HIFs) which in turn promote vascular endothelial growth factor (VEGF) expression. The most important psychoactive compound in cigarette smoke, nicotine (NT), binds nicotinic cholinergic receptors (nAchRs) which are widely distributed in several human tissues, including retinal pigmented epithelium (RPE) [2]. Until now, little is known about risk factors linked to cigarette smoke inducing DME development. In the present study, we have evaluated NT effect in an *in vitro* model of outer BRB following exposure to hyperglycemic/hypoxic insult mimicking DME microenvironment. Our results have suggested that NT deeply impacts on outer BRB integrity by increasing its permeability. To investigate the molecular mechanisms involved in negative effect of this compound, we have analyzed HIF/VEGF system in cells exposed to hyperglycemic/hypoxic damage. NT treatment induced upregulation of HIF-1α/HIF-2α, VEGF mediated through activation of MAPK/ERK1/2 pathway. In conclusion, all this data have suggested a unfavorable role of this psychoactive agent in smokers DME affected.

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Keywords

Diabetic macular edema, hyperglycemia, hypoxia, nicotine, hypoxia-inducible factors, vascular endothelial growth factor

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Morphologic, immunophenotypic, and ultrastructural characterization of telocytes in pterygium

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Telocytes (TCs) are a novel and peculiar interstitial cell type already described in many tissues and organs. Their name derives from their typical extremely long, thin, tortuous, and overlapping processes called telopodes (Tps), forming a stromal three-dimensional network. TCs occupy a strategic position in relation to stem cell niches, blood capillaries, and nerve bundles, then contributing to maintain tissues homeostasis. However, TCs involvement in the pathophysiology of several disorders is being increasingly investigated because of their role as "connecting cells", mostly oriented to intercellular signalling. Previous study provided evidences for TCs involvement in neoangiogenesis (2), and we recently demonstrated their presence also in pterygium, a common degenerative and hyperplastic disorder of bulbar conjunctiva, characterized by an intense process of neovascularization. TCs and TPs were detected both in the subepithelial layer and in the connectival stroma of pterygium, especially in close relationship to the newly formed vessels. Since it is well established that TCs share the same ultrastructural features but display totally different morphology and immunophenotype based on their organ and tissue localization, the purpose of the study was to perform in pterygium a morphological and immunohistochemical analysis by light microscopy of thin and semithin sections and an ultrastructural study by transmission electron microscopy (TEM). The results will be discussed.

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Keywords

Telocytes, pterygium, immunophenotype, morphology, Transmission Electron Microscopy (TEM)

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An *in vitro* study of the mTORC1/2 inhibitor PP242 in glioblastoma multiforme

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mTOR is a kinase complex involved in cell growth, proliferation, survival, metabolism and migration. The aberrant activation of mTOR has been previously demonstrated in glioblastoma multiforme (GBM), making it an interesting target for therapeutic approaches [1]. Unfortunately, the attempts to block mTOR activity made so far had disappointing clinical efficacy, as the mTOR inhibitor Rapamycin and analogs only target mTOR complex 1 (mTORC1) while mTOR actually exists in two distinct complexes, namely mTORC1 and mTOR complex 2 (mTORC2) that differ in terms of both regulation mechanisms and functions [2,3]. mTORC1 is inhibited by Rapamycin and acts as a downstream effector of the PTEN/PI3K/Akt pathway, linking growth factors, amino acids, ATP and O_2 signals to protein translation, cell growth, proliferation and survival. Differently, mTORC2 is insensible to Rapamycin and acts as an upstream activator of Akt via phosphorylation of serine 473 [3]. To analyze the contribution of mTORC1/2 to GBM biology, we studied the *in vitro* effect of PP242, a novel mTORC1/2 inhibitor, on glioma cell lines of different malignancy degree, and compared it to the effect of Rapamycin and of the irreversible PI3K inhibitor, Wortmannin.

Our results suggest that the inhibition of both mTOR complexes with PP242 induces sustained levels of autophagy that causes G0/G1 cell cycle arrest and a significantly reduction of cell viability, proliferation and migration. Additionally, we observed that administration of PP242 in U87MG cell line prevents stem cell growth, which results in the inhibition of neurospheres formation. This data confirms the pivotal role of mTOR in glioblastoma cells biology and expand upon this evidence suggesting a prominent role of the mTOR complex 2 in glioblastoma cell growth, migration and survival, and indicate that the mTORC2 might represent clinically valuable target in GMB.

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Pentose phosphate pathway inhibition induce Endoplasmic Reticulum stress and autophagy

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Pentose phosphate pathway (PPP) is a major glucose catabolism pathway that supplies the cell with a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and ribose-5-phosphate. NADPH is necessary for the detoxification of reactive oxygen species (ROS) and reductive biosynthesis. A key player in this pathway is the enzyme glucose-6-phosphate dehydrogenase (G6PD) that reduces NADP+ to NADPH, oxidizes glucose-6-phosphate and prevents ROS accumulation. Here, we show that the natural molecule 3,4',5-trihydroxystilbene-3-β-d-glucoside (Polydatin) inhibits glucose-6-phosphate dehydrogenase (G6PD). As expected, G6PD inhibition causes an imbalance in NADP+/NADPH ratio, leading to a redox imbalance, followed by Endoplasmic Reticulum (ER) stress, autophagy, cell cycle block and apoptosis. we have demonstrated a link between G6PD inhibition and ER stress, showing that Unfolded Protein Response mediator such as PERK and IRE-1 have a key role in inducing autophagy and apoptosis after PPP block. Moreover, combination of PPP inhibition with autophagy inhibitors, such as chloroquine, strongly potentiate cytotoxicity on cancer cells, evidencing the role of autophagy as an escaping mechanism. This results shows that double inhibition of PPP and autophagy may be an affective therapeutic strategy against cancer.

Keywords

Pentose Phosphate Pathway, PERK, IRE-1, Autophagy, Everolimus, Chloroquine

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Flavocoxid mitigates cadmium-induced toxicity: structural, immunohistochemical and molecular analysis in mice kidney

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Background: Cadmium (Cd), a diffused environmental pollutant, has adverse effects on urinary apparatus [1]. The role of flavocoxid, a flavonoid with antioxidant activity [2], on the morphological and biochemical changes *induced in vivo* by Cd in mice kidneys was evaluated.

Methods: C57 BL/6J mice received 0.9% NaCl alone, flavocoxid (20 mg/kg/day i.p.) alone, Cd chloride (CdCl2) (2 mg/kg i.p.) alone, or CdCl2 plus Flavocoxid (2 mg/kg i.p. plus 20 mg/kg/day i.p.) for 14 days. At the end of experiment, the mice were killed with an overdose of ketamine and xylazine and the kidneys were collected and processed for structural, immunohistochemical and biochemical analysis.

Results: Cd treatment alone significantly increased iNOS, TNF-α and MMP-9 expression, induced structural damages in the glomeruli and in the proximal tubule epithelium, and reduced claudin-11, occludin and N-cadherin immunoreactivity. Flavocoxid administration reduced iNOS, TNF-α and MMP-9 expression, ameliorated glomerular and tubular changes and enhanced claudin-11, occludin and N-cadherin immunoreactivity.

Conclusions: We demonstrated for the first time that flavocoxid has a protective role against Cd-induced damages in mice kidney. Therefore, flavocoxid may have a promising role against environmental Cd, in particular against its harmful effects on glomerular and tubular lesions.

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Keywords

Cadmium, flavocoxid, kidney, glomeruli, tubular epithelium, light microscopy, immunohistochemistry, Western blot analysis

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Stem Cells as a source to produce Red Blood Cells in vitro for Transfusion

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Blood transfusions have become indispensable to treat the anemia associated with a variety of medical conditions ranging from genetic disorders and cancer to extensive surgical procedures. In developed countries, the blood supply is generally adequate. However, the projected decline in blood donor availability due to population ageing and the difficulty in finding rare blood types for alloimmunized patients indicate a need for alternative red blood cell (RBC) transfusion products.

Increasing knowledge of processes that govern erythropoiesis has been translated into efficient procedures to produce cultured RBC (cRBC) using primary hematopoietic stem cells, embryonic stem cells, or induced pluripotent stem cells. In addition, proof-of-principle studies in lethally bled animal models suggest that these cRBC may represent alternative transfusion products. Compared to other cell therapies, however, transfusion poses the unique challenge of requiring great cell doses (2.5×10^{12} vs 10^{7} cells). Although production of such cell numbers is theoretically possible, current technologies generate cRBC in numbers sufficient only for quality control and safety studies. Since cRBCs have entered clinical evaluation, several issues related to their production are under intense scrutiny. Examples of issues that will be addressed in the future are the identification of stem cell sources more suitable for cRBC generation, the translation of cRBC culture methods into clinical grade production processes, and the development of protocols to achieve optimal cRBC quality, quantity, and maturation. We will discuss data on size, hemoglobin, blood group antigen expression and phosphoproteomic profiling obtained on cRBCs expanded ex vivo from a limited number of regular blood donors, including a donor with a rare blood phenotype, as examples of the type of measurements that are being generated as part of the quality control assessment of the suitability of cRBCs for transfusion. It is conceived that by the time all these quality studies will be completed, technical barriers to mass cell production will have been eliminated making transfusion with cRBCs a reality.

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Effects of in-vitro application of pentoxifylline on the morphology of human spermatozoa after vitrification in asthenozoospermic patients

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Cryopreservation of human spermatozoa is widely used in many assisted reproduction units to preserve male fertility [1]. Vitrification is based on the ultrarapid freezing and is routinely assayed for cryopreservation in assisted reproductive technology. Mohamed [2] showed that cryopreservation significantly affects progressive motility, viability and mitochondrial membrane potential of spermatozoa. Pentoxifylline (PX) is a phosphodiesterase considered to be a sperm movement enhancer, hyperactivation agent, inhibitor of reactive oxygen species and acrosome reaction-improving agent. The aim of our study was to evaluate the effect of in-vitro application of PX on sperm parameters and ultrastructure after vitrification. A total of 30 asthenozoospermic semen samples were selected and divided into two groups after vitrification: control (without PX) and experimental (with PX). A significant decrease in sperm motility, morphology and viability was observed post vitrification, but sperm motility was increased significantly following application of PX. On the other hand, PX did not exert any significant effect on the ultrastructure of the acrosome, plasma membrane and tail of vitrified spermatozoa.

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Keywords

Spermatozoa, pentoxyfilline, vitrification, human, ultrastructure

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In-vivo anatomical reconstruction of the optic radiations in the human brain

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The optic radiations are major white matter pathways funneling visual information from the lateral geniculate nuclei to the visual cortex in the occipital lobe. Given their relevance in visual processing and in several brain disorders, the optic radiations have been extensively investigated by using magnetic resonance imaging and diffusion tensor imaging tractography [1]. Herein, we use a powerful diffusion signal modeling, namely Constrained Spherical Deconvolution, in order to provide an exhaustive connectivity profile of the connections between the lateral geniculate nucleus and the visual cortex in the healthy brain, as well as pulvinar connectivity with visual-related structures. In addition, taking into account that visual deficits may precede motor symptoms' onset in Parkinson's Disease [2], we assessed whether the intracranial visual system can be involved at the early stage of the disease. Our connectivity analysis revealed that the optic radiations are mainly distributed in V1 and V2. Furthermore, we found significant alterations of optic radiations connectivity distribution in Parkinson's Disease patients, with decreased lateral geniculate nucleus-V2 density as well as significant increase of optic radiations' mean diffusivity. Voxel Based Morphometry analysis also showed significant reduction of visual cortical volumes and of the optic radiation in the patients group. In conclusion, our findings provide a reliable connectivity profile of the optic radiations, suggesting extrastriate-lateral geniculate nucleus connections in human brain. Finally, we showed that visual system alterations can be detected at early stages of Parkinson's Disease.

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Keywords
Optic radiations, tractography, MRI, Parkinson's Disease, brain connectivity

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Mesenchymal stem cells protect sensory neurons, but not cortical neurons, from the chemotherapeutics-induced neurotoxicity

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Mesenchymal stem cells (MSCs) have been often proposed for the therapy of several neurological diseases, due to their manifold peculiar properties. In particular, since it has been previously demonstrated that these cells are able to increase the survival of untreated sensory neurons [1], in this work we evaluated their possible protective effect on sensory neurons previously exposed to toxic agents. This could be particularly relevant to design a supportive therapy to counteract the peripheral neuropathy, a very common side effect of several chemotherapeutic agents, such as platinum and taxanes compounds, which often represents their dose limiting factor [2]. Several strategies have been suggested to reduce drug neurotoxicity without affecting the antineoplastic potential, but up to now results were not encouraging [3]. Here we demonstrated that Cisplatin (CDDP) and Paclitaxel-treated sensory neurons are protected by the co-culture with MSCs, but in two different manners: through a direct contact able to block apoptosis for CDDP-treated neurons, and by the release of trophic factors (including glutathione) for Paclitaxel-treated ones. In addition, the MSCs' effectiveness was also verified on cortical neurons, since the recent advances in targeted drug delivery allowed to drive chemotherapeutic drugs also to the central nervous system. We verified that cortical neurons are more vulnerable to the toxic action of the drugs, and overall that MSCs fail at all to protect them. All these data demonstrated that MSCs are potentially useful to limit the peripheral neuropathy onset for their protective effect on injured-sensory neurons, but they also identified for the first time a different susceptibility of cortical and sensory neurons to MSC action.

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Keywords

Chemotherapeutic induced peripheral neuropathy, mesenchymal stem cells, sensory neurons, cortical neurons, neuroprotection

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Effects of a physical activity program on functional fitness, oxidative stress and salivary cortisol levels in older adults

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Quality of life into later life is influenced by multiple factors. The physical ability to perform common everyday activities represents a key factor to maintain a healthy and independent lifestyle. Moreover, aging is a process characterized by physiological alterations resulting in a progressive decline in biological functions, decreased resistance to stress, and increased susceptibility to diseases. Especially in elderly people, alterations such as imbalance between pro and antioxidant activity and/or hormonal dysregulation negatively affect the physical capacity, the emotional status and the overall general health and quality of life [1]. On the other hand, regular physical activity is considered an effective strategy for older adults to prevent and reduce the risk of developing those negative conditions arising from aging. A 24-week regular physical activity program (twice weekly, 1 hour per session) focused on functional fitness exercises was performed by 20 older adults (aged 55 years or more). A set of anthropometric (height, weight, BMI and body fat percentage) and physical measurements (grip strength, chair sit to stand, sit and reach and back scratch) assessing the functional fitness performance [2] were evaluated. Moreover, biochemical markers (d-ROMs and BAP tests as assessment of oxidative stress and antioxidant potential; salivary cortisol levels) were measured before and after the intervention program. The results confirm the benefits of a regular physical activity in older adults resulting in improved physical strength and flexibility in the functional fitness parameters, and in regulating pro and antioxidant activity and cortisol levels.

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Keywords
Functional fitness, oxidative stress, cortisol

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Metabolic effects of Tart Cherries supplementation in an animal model of obesity

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Fruits and vegetables contain non-nutritive phytochemicals that may contribute to their health-promoting effects. Anthocyanins are phytochemical flavonoids principally found in fruits and vegetables. Several studies have suggested that anthocyanin-rich plant extracts can modify lipid metabolism in vitro and can reduce hyperlipidemia in vivo. Tart cherries (*Prunus cerasus L.)* are a rich source of anthocyanins.

This study was designed to evaluate the effects of anthocyanin-rich tart cherries extract and seeds powder on Diet-Induced Obesity (DIO) rats, that provide a useful animal model sharing several common features with human obesity.

DIO rats were studied for 17 weeks of hypercaloric diet with the supplementation of 0,1 mg/kg of tart cherries seeds powder (DS) and seeds powder plus tart cherries extract, containing 1mg of anthocyanins (DES). DIO rats were compared to the control rats with not fat diet (Chow).To determine the systemic effects of caloric dense expousure we examined food consumption, fat mass content and fasting glycemia, insulin levels, cholesterol and triglycerides.

Ultrasonographic (US) and computed tomography (CT) evaluations were performed to detect adipose tissue deposition. In CT, also fat infarction of the liver was investigated followed by histochemical analysis

17 weeks of fat diet, rats increased significantly their body weight in comparison to the control group. Glycaemia and insulin levels were higher in DIO rats. No difference in body weight was found in DS and DES rats compared to age-matched DIO rats. Supplementation of tart cherries in DS and DES induce a decrease of the blood pressure and the glycemia. Furthermore, decreased the serum levels of thiobarbituric reactive substances.

The US and CT analysis indicated an increase of deposition of visceral adipose tissue and evidenced a decrease of hepatic attenuation in DIO rats, suggests a moderate hepatic steatosis prevented by tart cherries supplementation in DS and DES rats. The evidence of the CT was confirmed by histological analysis. DIO rats present a distinctive pattern of steatosis with hepatocytic ballooning degeneration at the perivenular areas. The steatosis elements decrease in DS and DES rats.

Tart cherries supplementation, although did not reduce the body weight in DIO rats, prevent the development of related risk factors. Further studies are needed to better clarify the benefits of tart cherry supplementation on health and disease prevention.

Keywords:
Obesity, Diet-Induced Obesity rats, tart cherries, liver, steatosis

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The functional neuroanatomy resource (FNAR) at Weill Cornell Medicine

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INTRODUCTION. Computer assisted instruction has long proven useful in teaching of neuroanatomy, particularly when accompanied by traditional lecture presentations that present image and text data to students. The work describes the functional neuroanatomy resource (FNAR) innovation created at Weill Cornell Medicine – the first homegrown functional neuroanatomy teaching application developed for iPads by a medical school, including the learning options actively utilized by students and plans for continued development of the app. RESOURCES. Previously the teaching of functional neuroanatomy has relied heavily on gross brain and histological material created at the medical college and presented through computer technology initially server-based and then web-based. When the institution decided to move students to mobile devices, all students were provided with iPads. The functional neuroanatomy faculty and educational computing team accepted the challenge to make the FNAR content available through an iPad app. DESCRIPTION. This first local FNAR app integrates and indexes an image database along with various text resources. The app utilizes mouse-over and overlay technology, allowing users to easily highlight and select different areas of the brain and spinal cord and their related structures; it allows students to access the self-assessment tools onto the image overlays so that students can test their knowledge as they progress. CONCLUSIONS. A recent student evaluation reflects students rating the overall quality and usefulness of the FNAR as "excellent" (3.85 on a 4-point scale). Future plans include incorporating radiographic images and an "on-the-fly image set" technology, allowing students to query the database specifically designed to answer their questions.

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Rest-Activity circadian Rhythms and Body Mass Index In women with metabolic syndrome

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The metabolic syndrome is a complex of interrelated risk factors such as abdominal obesity, high blood pressure, dyslipidemia and high fasting glycemia. These risk factors expose the subject to cardiovascular diseases and type 2 diabetes mellitus. Furthermore, it has been shown that there is a correlation between circadian rhythms and metabolic syndrome. The circadian rhythms produce 24-hour oscillations of several physiological variables and any irregularity of these rhythms exposes the subject to an increased risk of metabolic syndrome [1]. Aim of the study was to investigate a possible direct correlation between Rest-Activity circadian Rhythms (RARs) and Body Mass Index (BMI) in subjects with metabolic syndrome. We recruited 52 adult women with metabolic syndrome in care at Fondazione IRCCS, Istituto Nazionale Tumori. All participants underwent a continuous 7-day actigraphic monitoring to detect the RARs. Subsequently, they were subdivided into 3 groups referring to their BMI: group 1, with BMI between 25 and 30 (n=18), group 2, with BMI between 30 and 35 (n=27), and group 3 with BMI >35 (n=8). All data were analyzed by single cosinor method to obtain MESOR (M), amplitude (A) and acrophase (Ø) for each subject. Then, on these values we applied the mean cosinor method to evaluate the parameters M, A and Ø for each group. We found statistically significant differences for MESOR (M group 1: 269.8 vs M group 2: 226.9; $p < .05$) and amplitude (A group 1: 212.1 vs A group 2: 171.8; $p < .05$) between group 1 and 2 by Hotelling test. These results show a trend to have an inverse correlation between BMI and MESOR, and BMI and amplitude.

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Keywords

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

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Motor recovery after stroke: the role of overground exoskeletons in shaping brain plasticity

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The use of neurorobotic devices into gait rehabilitative programs, including Ekso, is reported to increase the engagement and motivation of the patients while actively performing a task, and to shape the sensory-motor plasticity (SMP) and its balance between the primary motor areas (M1), and the fronto-parietal network (FPN) connectivity, thus contributing to successful gait rehabilitation [1]. Aim of our study was to assess whether Ekso would foster the recovery of deteriorated FPN connectivity and SMP patterns involved in limb coordination during walking [2] in a sample of patients with hemiparesis due to stroke. To this end, we enrolled ten patients who underwent Ekso training (24 sessions) and were evaluated about gait performance, FPN connectivity, and SMP pattern. Esko significantly increased gait performance index as revealed by surface EMG ($p=0.01$) and the deterioration of prefrontal-SMA and SMA-centroparietal connectivity (both $p=0.02$), and rebalanced the equilibrium between the SMP patterns of the two M1-leg areas ($p=0.03$). Moreover, the baseline plasticity and FPN connectivity were the most important factors in using Ekso fruitfully ($r=0.9$, $p=0.03$). Even though our findings need to be confirmed by future research further addressing the safety and effectively use of Ekso, our small cohort study provides new cues supporting the role of powered exoskeletons in rehabilitation protocols for persons with stroke.

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Keywords

Ekso, stroke, motor recovery, functional connectivity, frontoparietal networks

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BAG3 localizes in axonal structures during neuronal differentiation and is expressed in cellular processes of migrating cells in mouse cerebral cortex

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BAG3 protein belongs to the family of co-chaperones involved in protein quality control and in the clearance of misfolded proteins [1]. Few studies have addressed BAG3 distribution and function in the central nervous system (CNS) and little is known about the cellular localization of BAG3 during neuronal differentiation in vitro and migration in vivo. Therefore we analysed by immunofluorescence microscopy the cellular distribution of BAG3 in the PC12 cell model treated or not with NGF and in developing and adult cortex of mice brain. Our results shows that BAG3 localizes mainly in vesicle structures of the neuritic domain during cell differentiation, while in undifferentiated cells it appears confined to the cytoplasm near the nuclear membrane. These observations were corroborated by transmission electron microscopy (TEM) which revealed that in NGF-differentiated PC12 cells, BAG3 localizes into electron-dense vesicles clustered along the axon and showing the typical aspect of the large dense core vesicles (LDCVs). Interestingly, the change of BAG3 localization during neuronal differentiation was associated only to a slight increase in the total BAG3 immunoreactivity as shown by western blot analysis. In order to provide further insights on the role of BAG3 in neuronal differentiation and migration, we also analysed BAG3 localization in mice developing and adult cerebral cortex. In mouse developing cortex, BAG3 appeared to be intensely expressed in cellular processes of migrating cells, while in adult brain a low expression was detected in neuronal cell bodies and glial cells. In conclusion, our findings suggest that the presence and differential expression of BAG3 might be required for the correct development of the nervous system as well as for the maintenance of protein homeostasis.

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Keywords

BAG3, PC12 cells, neuronal differentiation, mouse cerebral cortex, confocal microscopy, transmission electron microscopy

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Physical activity and sport performance: adiponectin in relation to different physio-pathological status

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Adiponectin (Acrp30), and in particular its High Molecular Weight (HMW) oligomers, contributes to enhance insulin sensitivity and to reduce inflammation levels. Physical exercise improves body's biochemical balance and metabolism resulting effective in the prevention and therapy of metabolic diseases. Whether improvement of metabolic features mediated by physical exercise is associated with changes in Acrp30 serum composition is not yet clarified.

In the present study, we investigated total Acrp30 expression and its oligomeric status in two different metabolic status: professional Water Polo (WP) Players and adult patients affected by Cystic Fibrosis (CF) that performed regular physical exercise. CF is an inherited metabolic disease characterized by alterations in lipid and glucidic metabolism. Our results demonstrated significant elevated BMI, AST and LDH levels and, conversely, significantly lower concentrations of total cholesterol and VLD were present in WP players. No significant difference was found in total Acrp30 and/or HMW oligomers. Interestingly, in WP players, a direct relationship between total Acrp30 and monocytes as well as an inverse relationship between total Acrp30 and AST levels were found. ACDC molecular screening revealed previously described SNPs.

In CF patients, physical exercise has significant effects on lipid and glycemic metabolism. Indeed, patients that performed exercise are characterized by significant decrease of either VLDL, cholesterol and triglycerides, border-line significant decrease of either total cholesterol/HDL and non-HDL cholesterol/HDL ratio and by trend decrease of total, LDL and non-HDL cholesterol, although not significant. It's to highlight that physical exercise significantly reduces glycemia and HOMA-IR and increases serum albumin. However, physical exercise does not modify Acrp30 concentrations that, on the other hand, result significantly higher in all CF patients compared to controls. In conclusion, even if peripheral muscle abnormalities and respiratory factors limit exercise in patients with CF, our study indicated that physical activity has beneficial effects on lipid and glycemic metabolism in these patients not associated with Acrp30.

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Astrocyte clasmatodendrosis in a transgenic mouse model of Alzheimer's Disease

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Aging is frequently accompanied by a low-grade inflammation (inflammaging); on the other hand, inflammation is considered a prodrome of Alzheimer Disease (AD). Indeed, a distinctive event of both aging and AD is the deposition of beta amyloid (Aß) fibrils within the central nervous system, a condition that has been associated to cognitive decline. In a previous research we demonstrated that, in the hippocampus of aged rats, the fragmentation of astrocyte processes (clasmatodendrosis) is associated with a decrease of their activity in terms of Aß-fibril clearance, thus promoting neuron to neuron propagation of Aß-fibrils and therefore their prion like spread [1]. In this study we show preliminary data on the role of clasmatodendrosis in a double transgenic TgCRND8 mouse model, which overexpresses both Swedish and Indiana mutations in the human amyloid precursor protein, and displays early cognitive decline also in young animals [2]. We performed a 3D confocal analysis on optical volumes acquired in the CA1 hippocampal region of young (3m.)- and middle aged (7m)- TgCRND8 mice. We found that young TgCRND8 mice show Aß-amyloid deposition, astrocyte clasmatodendrosis and a decrease of the astrocyte cytoskeletal marker GFAP. In middle aged animals significantly higher levels of GFAP expression, indicating astrogliosis, were in concomitance with both Aß-amyloid deposition. These data appear to link the onset of early cognitive decline in TgCRND8 mice with astrocyte clasmatodendrosis and provide new perspectives on the role of astrocytes in Aß-amyloid deposition and spreading.

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Keywords

Clasmatodendosis, Alzheimer's Disease, Aß-fibril, Astrocytes, TgCRND8 mice

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Breast Implant Associated-ALCL: a possible role for inflammation and Mesenchymal Stem Cells

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In the last years, the use of breast implants has been cyclically associated with an enhanced risk of Anaplastic Large Cell Lymphoma (ALCL) onset (Breast Implant Associated-ALCL, BIA-ALCL). For the development of other different tumors, the involvement of Inflammation has been suggested. The use of breast implants often breaks out to inflammation, as proved by the formation of the periprosthetic capsule. Tumors take advantage of inflammation to influence and interfere with the host immune response by secreting multiple factors, and their onset and survival is in turn affected by the he paracrine effects from mesenchymal stem cells (MSCs). Our previous work [1] revealed the MSCs derived from inflamed capsules are different from those derived from control tissues. Here we deepen this dysregulation and test, by the criteria evinced from Elinav [2], if MSCs from inflamed tissues may exert a different paracrine effect (immunobiology) that in turn increases the risk of ALCL development.

MSCs derived from both inflamed (I-MScs) and control (C-MSCs) tissues were isolated and co-cultured with an ALCL cell line. Proliferation rate and the expression of selected cytokines related to inflammation were tested.

Our results show that I-MSCs secrete higher levels of cytokine related to chronic inflammation than C-MSCs. After co-cultures with KI-JK cells, C- and I-MSCs show the same variation in the cytokines expression, with an increase of IL2, IL4, IL5, IL10, IL13, TNF-α, TGF-β and G-CSF. Proliferation of ALCL cells was not influenced by co-cultures.

In conclusion our results state: i) inflamed microenvironment affects the immunobiology of MSCs modifying the expression of cytokines related to inflammation; ii) the paracrine effects exerted by MSCs on ALCL cells is not influenced by inflammation. ALCL cells are able to manipulate the MSCs immunoregulatory properties to evade the host immune control but this ability is not associated with inflammation and the question about BIA-ALCL is not proved by our experiments.

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Keywords

Inflammation, mesenchymal stem cells, breast implants, ALCL

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Rowing technique determinants: a comparison between international and national level rowers

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Rowing is a motor skill that requires high levels of consistency, coherence, accuracy and continuity, particularly at an elite level [1]. The rowing stroke consists of two phases: the drive, during which force is applied to the blade in order to move the boat relative to the water, and the recover, during which the rowers return to their former position [2]. The goal of the current study was to investigate the kinematic differences between rowers of International Level (IL) and National level (NL), in order to identify parameters that have the potential to characterise the best ergometer rowing technique. With this objective, we analysed three IL (age: 18.3 (0.5) [years]; height: 183.0 (8.8) [cm]; weight: 76.3 (8.9) [kg]), and thirteen NL rowers (22.2 (1.9) [years]; 182.5 (4.7) [cm]; 77.1 (7.4) [kg]), using a motion capture system (BTS SpA, Italy). Duration of the stroke phases, Range of Motion (RoM) of the knee 3D trajectories, length of the body Centre of Mass trajectory (COMd) and the curvature of the wrist path during the stroke (Index of Curvature, IC) were compared using the Mann-Whitney U test. The findings showed that the IL rowers presented a non-significant decrease of the drive phase duration (IL: 0.94 (0.04) [s]; NL: 1.05 (0.08) [s]; p= 0.122), higher Knee Rom (IL: 0.68 (0.04) [m]; NL: 0.51 (0.02) [m]; p= 0.018) in the Anterior-Posterior direction, a higher COMd (IL: 2.62 (0.12) [m]; NL: 1.74 (0.15) [m]; p=0.026) and a higher IC (IL 0.984(0.003); NL: 0.980(0.003); p=0.040). In conclusion, the stroke duration, the amplitude of the movement in the Anterior-Posterior direction and the control of the wrist path seem to be associated with high-level performances; the findings suggest that IL rowers present a better command of technique, timing and power, compared to NL rowers.

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Keywords

Ergometer rowing, elite athletes, sport kinematics, sport biomechanics

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Human adipose stem cell differentiation is highly affected by cancer cells both in vitro and in vivo: implication for autologous fat grafting

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Recent studies showed that mesenchymal stem cells derived from adipose tissue can promote tumour progression, raising some concerns regarding their use in regenerative medicine. In this context, we co-cultured either SAOS2 osteosarcoma or MCF7 breast cancer cells with human adipose stem cells (hASCs), in order to evaluate potential effects of cancer cells on hASCs differentiation, in vitro and in vivo. In this study we observed that both SAOS2 and MCF7 cell lines induced an increase in hASCs proliferation, compared to hASCs alone, but, surprisingly, neither changes in the expression of CD90, CD29, CD324 and vimentin, nor variations in the Twist and Slug mRNAs were detectable. Noteworthy, SAOS2 and MCF7 cells induced in hASCs an upregulation of CD34 expression and Stemness genes, including OCT3/4, Nanog, Sox2 and leptin, and a decrease in angiogenic factors, including CD31, PDGFα, PDGFRα, PDGFRβ and VEGF. SMAD and pSMAD2/3 increased only in hASCs alone. After 21 days of co-culture, hASCs differentiated both in adipocytes and endothelial cells. Moreover, co-injection of MCF7 cells with hASCs led to the formation of a highly vascularized tumour. Taken together our findings suggest that mesenchymal stem cells, under tumour cell induction, do not differentiate in vitro or facilitate the angiogenesis of the tumour in vivo, thus opening interesting new scenarios in the relationship between cancer and stem cells. These findings may also lead to greater caution, when managing autologous fat grafts in cancer patients.

Keywords

Adipose stem cells, cancer cells, breast cancer, co-culture, pSMAD2/3

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Naringenin as a novel inhibitor of Two-Pore Channel 2 controlling the angiogenic process *in vitro* and *in vivo*

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Two Pore Channels (TPCs) are an emerging family of intracellular channels, expressed on acidic compartments, which mediate calcium signaling evoked by NAADP. In particular, we demonstrated that TPC2 isoform has a main role in angiogenesis (Favia et al. PNAS 2014 Nov 4;111(44):E4706-15). TPC2 inhibition is emerging as a key therapeutic step in a range of important pathological conditions including the progression and metastatic potential of cancer, Parkinson's disease, and Ebola virus infection. We introduce naringenin, a natural flavonoid, as a novel TPC2 inhibitor as shown by electrophysiological evidence in a heterologous system, i.e. Arabidopsis vacuoles lacking endogenous TPCs. In view of the control exerted by TPC2 on intracellular calcium signaling and angiogenesis, we demonstrate that naringenin dampens intracellular calcium responses of human endothelial cells stimulated with VEGF, histamine or NAADP-AM, but not with ATP or Angiopoietin-1. The ability of naringenin to impair TPC2-dependent biological activities was further explored in an established *in vivo* model in which VEGF-containing matrigel plugs implanted in mice failed to be vascularized in the presence of naringenin. Our present data suggest that naringenin inhibition of TPC2 activity and the observed inhibition of angiogenic response to VEGF are linked by impaired intracellular calcium signaling. The relationship we describe here between naringenin and TPC2 is therefore likely to have wider implications in systems other than the vascular system, thus representing a novel tool for experimental, and possibly even clinical, research purposes.

Keywords

Calcium signalling, acidic stores, flavanones

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Ultrastructural analysis reveals differences in the secretory activity among four regions of amniotic membrane

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Human Amniotic Epithelial Cells (hAEC) from term placenta are a promising source of stem cells for regenerative medicine. In a previous study we observed histological heterogeneity, together with different expression of pluripotency markers and content in lipid granules among four regions of amniotic membrane (AM). To better investigate cell heterogeneity among different cell populations, we performed an ultrastructural study with Transmission Electron Microscopy. Term placentae from healthy women were collected after caesarean section and AM samples were freshly isolated from four regions: R1 (close to the umbilical cord); R2 (intermediate); R3 (peripheral to the placental disc); R4 (reflected amnion). Ultrastructural analysis revealed an epithelium of variable thickness, cellular shape, amount and type of vesicles in the four regions. The epithelium showed columnar hAEC with increased height in R1 and R3 and a multi-layered organization in R3, whereas it was a monolayer in the other regions. The highest amount of granules and vesicles was observed in R3, although R4 showed granules with a different density. Furthermore, in R1, R3 and R4 we noticed several vesicles of 100-150 nm in diameter, probably exosome-like structures, suggesting a consistent secretory activity. All along its length the epithelium was rich in microvilli both on the side facing the amniotic fluid and in lateral contacts (narrow desmosomal junctions) between cells. This *in situ* investigation shows for the first time differences in secretory activity and granules appearance along the AM as a proof of its heterogeneity. This could be relevant in clinical applications as the choice of the area could improve the effectiveness of AM/hAEC transplantation.

Keywords

Term placenta, amniotic membrane, ultrastructural analysis, secretory activity

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Effects of physical activity on postural balance in children with juvenile idiopathic arthritis: results from a pilot study

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Background: The juvenile idiopathic arthritis (JIA) is the main rheumatic disease in pediatric age. The rheumatic diseases are main causes of physical disability and have high economic costs for society. The aim of this study was to evaluate if the physical activity can prevent the decline in balance related diseases in children with previous diagnosis of JIA.

Materials and Methods: Fifty-six subjects were enrolled in this study. Thirty-nine healthy subjects were included in the control group (CG) and seventeen in juvenile idiopathic arthritis group (JIAG). Subsequently, the JIAG was stratified in two ones, respectively: JIAG active (JIAG-ACT) and JIAG sedentary (JIAG-SED). The analysis was measured through the FreeMed posturography system (by Sensor Medica). STATISTICA software was adopted to perform an unpaired t test. A P value lower than 0.05 was considered to be statistically relevant.

Results: Significant differences were identified in JIAG-SED vs CG in many parameters considered (Length of sway path of the CoP, $P< 0,0001$; Ellipse surface, $p<0,05$; Y mean, $p<0,05$). Against, except Length of sway path ($p<0,05$), the JIAG-ACT showed a similar trend respect to CG.

Conclusion: This pilot confirms the benefits for children with JIA to perform a training program due to prevent future diseases and increase the balance levels. Clearly, the sample is not adequate to make conclusions. More data coming from larger sample size studies are necessary to confirm these results.

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Investigation of human cadavers: one year of anatomic variants and their clinical correlation

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During the last year we found a plethora of variants while performing anatomical dissection on human cadavers. In the present report we describe the previously quoted variation morphologies focusing on their clinical relevance.

The first variant observed was a left vertebral artery (VA) arising from the aortic arch, it is present in the 5% to the 8 % of the individuals. The left VA of aortic origin showed a remarkably higher incidence of arterial dissection than left VA of a left subclavian artery origin. Also, the present pattern has to be taken into consideration before any intervention in the local region as to avoid unexpected events in relation to the aberrant vertebral artery.

A second dissection study led to the finding of the right posterior communicating artery of the Circle of Willis absence. This morphology may be a relevant risk factor for ischemic cerebral infarction if the patient suffers of internal carotid artery occlusion or severe stenosis. The same cadaver presented another variant: anterior inferior cerebellar artery bilateral absence.

While dissecting the vein of abdomen and thorax, we discover other two variants: bilateral iliolumbar veins draining into the testicular veins and three pulmonary veins entering into the right side of the left atrium. Cognition of the first variation it is critical during the anterior approach for spinal procedures, it will help surgeons to anticipate and to avoid potentially catastrophic complication such massive haemorrhages due to an avulsion of an unexpected extra vein; whether the knowledge of the pulmonary veins variant, compatible with R3a Marom's classification pattern, is essential for guidance during paroxysmal atrial fibrillation ablation procedures and preclude perioperative bleeding in video-assisted thoracoscopic surgery (VATS).

Anatomical variant findings enlarge the current knowledge of anatomists, but also the cognition of surgeons and physicians that need to be aware and always up to date on the incidence level of the variable course of arteries, veins, and nerves especially while performing clinical tests and surgical operations.

Keywords

Anatomic variation, clinical correlation, human cadavers, surgery, surgical complications

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Role of Associated Adherent-Invasive Escherichia Coli in Crohn's disease

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Several lines of evidence suggest that adherent-invasive Escherichia coli (AIEC) strains play an important role in Crohn's disease (CD). The objective of this study was to investigate the pathogenic role of two AIEC strains, LF82 and O83:H1, in CD patients. Organ cultures of colonic biopsies from patients were set up to assess the effects of LF82 and O83:H1 on the expression of CEACAM6, LAMP1, HLA-DR, ICAM1 by immunohistochemistry and of IL-8, IFNγ, and TNF-α genes by RT-PCR. Moreover, on Caco2 cells, we analyzed the cell cycle, the expression of MGMT and DNMT1 genes, and DNA damage induced by LF82 and O83:H1, by FACS, RT-PCR, and DAPI staining, respectively. Epithelial and lamina propria mononuclear cells (LPMNC) expression of CEACAM6 and LAMP1 were higher in biopsies cultured in the presence of both O83:H1 and LF82 than in biopsies cultured with non-pathogenic E. coli. Both AIEC strains induced increased expression of ICAM-1 on blood vessels and HLA-DR on LPMNC. We observed higher levels of TNF-α, IFN-γ, and IL-8 transcripts in biopsies cultured with both AIEC strains than in those cultured with NP. Both LF82 and O83:H1, block the cell cycle into S phase, inducing DNA damage, and modulate the expression of DNMT1 and MGMT genes. Our data suggest that LF82 and 083:H1 strains of E. coli are able to increase in CD colonic biopsies the expression of all the pro-inflammatory cytokines and all the mucosal immune markers investigated.

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The innate immune cross talk between NK cells and eosinophils is regulated by the interaction of Natural Cytotoxicity Receptors with eosinophil surface ligands

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Previous studies suggested that the cross talk between NK cells and other cell types is crucial for the regulation of both innate and adaptive immune responses [1,2]. In the present study, we analyzed the phenotypic and functional outcome of the interaction between resting or cytokine-activated NK cells and eosinophils derived from non-atopic donors. Our results provide the first evidence that an NCR/NCR ligand-dependent cross talk between NK cells and eosinophils may be important to up regulate the activation state and the effector function of cytokine-primed NK cells. This interaction also promotes the NK-mediated editing process of DCs that influences the process of Th1 polarization. In turn, this cross talk also resulted in eosinophil activation and acquisition of the characteristic features of antigen presenting cells. At higher NK/eosinophil ratios, cytokine-primed NK cells were found to kill eosinophils via NKp46 and NKp30, thus suggesting a potential immunoregulatory role for NK cells in dampening inflammatory responses involving eosinophils.

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Keywords

NK cells, eosinophils, dendritic cells, Natural Cytotoxicity Receptor (NCR), cross talk, cytotoxicity

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Morningness-Eveningness preferences and academic results: correlation between practical and theoretic discipline

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Human beings organize most of their biological and behavioural activities according to a 24h period. The biological rhythms show differences between individuals and this variability is known as *Circadian Typology* (CT). Morning-types (M-types), are active early in the morning and soon reach their peak in mental and physical performance but tire early in the evening. Evening-types (E-types) find difficult to get up in the morning and require more time to reach their optimal status. Neither-types (N-types) show intermediate characteristics. Many studies indicate that age and sex may influence: morningness preference increases with age in adults, and women show a stronger trend toward morningness than men [1].

Student chronotype can represent one of the factors that may affect academic achievement. This study investigates whether the CT of the students is related to the final exam grades of two different disciplines, theoretic (Anatomy) and practical (Athletics). Anatomy and Athletics grades are good indicators of the overall academic performance of the undergraduates. The aim of this study was to evaluate whether the performance in Anatomy is correlated with Athletics for the three chronotypes.

Participants were recruited among students of the School of Sport Science, University of Milan. They were 427 (294 males; 133 females). They completed the Morningness-Eveningness Questionnaire (MEQ): 44 students were classified as M-types, 280 as N-types and 103 as E-types. Individual performance in the final exams of Anatomy and Athletic were collected among them. M-type students achieved better results on final exams in Anatomy and Athletic than either E-type or N-type students. Moreover for M-types ($R^{2=}$ 0.187), it was observed a higher correlation concerning the results of the two disciplines than E-types ($R^{2=}$0.0727) and N-types ($R^{2=}$0.0236).

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Keywords

Chronotype, MEQ, academic performance

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The student academic performance in Anatomy is related to Circadian Typology?

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In human species, circadian rhythmic expression differs among individuals and may be classified with the concept of Circadian Typology (CT), which consists of three chronotypes: i) Morning-type (M-types), subjects that go to bed early and wake up early and achieve their peak of mental and physical performance in the early part of the day; ii) Evening-type (E-types), subjects that go to bed and wake up late, and perform at their best toward the end of the day, during evening hours; iii) Neither-type (N-types), subjects that show intermediate characteristics between the previous samples.

Circadian preferences may change during the life span and can influence academic and sport performance and job activities [1].

We collected data considering 427 students, 294 males and 133 females (age 18-25 years), attending the School of Sport Science, University of Milan. All participants compiled the Morningness-Eveningness Questionnaire (MEQ) for the assessment of chronotype; subsequently they have been evaluated taking into consideration their anatomy test marks. The chronotype distribution of the students was: 44 M-types, 280 N-types and 103 E-types. For M-types, the result in Anatomy exam was significantly higher compared to Evening-types ($p < .01$). Even the comparison between M-types and N-types showed a significant difference ($p < .01$). Instead, the performance for E- and N-types was similar.

The present results provide a clear indication of a better academic performance for M-types students compared to E-types referring to Anatomy exam. In this way, the italian academic organization seems to be less favorable for E-types.

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Keywords

Chronotype, MEQ, academic performance, anatomy test marks, university students

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An anatomo-radiological study of the renal segments

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An increasing number of observations call the general scheme of five renal segments into question, with anatomists, radiologists and surgeons that have reported discrepancies between Graves's scheme and morphological observations. The aims of the present study was to analyse the arterial vascular anatomy with reference to the renal segments. 15 kidneys were injected with acrylic resins to obtain vascular corrosions casts that were analyzed also with computed tomography. A mean number of 6,1 (range 4-8) avascular fissures were found, determining the presence of a mean number of 7,1 segments (range 5-9). The apical and posterior segments were in all the cases single. In the superior and middle territory there was a single segment in 6 cases (40%) and two segments in 9 cases (60%). In the inferior territory there was a single segment in 1 case (6,7%), two segments in 12 cases (80%), and three segments in 2 cases (13,3%). The renal arterial vasculature cannot be schematized according to the classical Graves classification because the majority of the evaluated cases showed a different number of segments. The presence of the fissures in the virtual vascular casts is a useful tool to identify the boundary between the vascular territories.

Keywords

Renal segments, Arterial vascular anatomy, Vascular corrosion casts, Computed tomography

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Morphological study of a mummified heart dated back to 1829: preliminary results

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In May 2016, a cylindrical lead container was found in the chapel of the Seminary of Sassari. Based on the archive documents, the cylinder should have contained the heart of Tommaso Arnosio, Archbishop of Torres and Primate of Sardinia and Corsica from 1822 to 1829, who died in 1829 at 54 years, in Turin, his home city.

The Archbishop died *in the midst of the most atrocious sorrows caused by a large purulent pocket near the heart*. After the autopsy and according to his will, the heart was shipped to Sassari, where the heart was buried in the chapel of the Seminary and forgotten for about 186 years. After the discovery, the container was brought for study to our CSAPS laboratory of the Department of Biomedical Sciences.

A multidisciplinary team of experts hypothesized as cause of death a suppurative pleuritis or a para-cardiac lung abscess. To verify this hypothesis the cylinder was opened in a laminar flow hood, avoiding any contamination, and a mummified organ immersed in cotton wadding was found.

The organ, typically heart shaped, appeared 13 x 9 cm sized, with a thickness of 1.5 cm, a weight of 80.5 g, a dark brown colour and a hard consistency. Few fragments of tissue were collected for analysis; then the organ, closed in the lead container, was returned to the chapel of the seminary.

Histological analysis required a rehydration using the Sandison Solution (1); the samples were treated for light microscopy and stained with HE, PAS and Gram's method. The first results have shown a morphology of cardiac and pericardial tissues partially preserved, and the banding and the intercalated disks appeared just in small portions. The Gram's method was negative till now. Further histological, immunohistochemical, ultrastructural and, molecular investigations are in progress.

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Keywords

Mummified tissue, heart, histology, microbiology, archive documents

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Three dimensional sphere culture system enhances neural crest-related properties of a sub-population of human dental pulp stem cells expressing STRO-1, c-Kit and CD34 markers

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Human dental pulp, a soft connective tissue contained within the pulp chamber of the tooth, is considered an interesting source of adult stem cells, due to the low-invasive procedures required for cell isolation, high content of stem cells and its peculiar embryological origin from neural crest [1-2]. Based on previous findings from our group, a dental pulp stem cells (hDPSCs) population sorted for the expression of STRO-1, c-Kit and CD34 showed a higher commitment towards neurogenic and glial lineages. Moreover, in standard culture conditions STRO-1+/c-Kit+/CD34+ hDPSCS, at late passages, underwent an arrest in cell proliferation and senescence occurred. To this regard, the aim of the present study was to evaluate the ability of three dimensional sphere structures to preserve the biological and stemness properties of this sub-population. In addition, the ability to differentiate towards neurogenic lineage as well as the expression of Fas ligand were investigated. Our data demonstrated that hDPSCs-derived spheres were able to maintain their fibroblast-like morphology and preserved the expression of the stemness markers and their proliferative capability. At late passages, only few cells derived from spheres were positive for β-Galactosidase activity. Interestingly, the expression of neural crest markers was maintained along the whole culture time and the neurogenic commitment was successfully achieved, as confirmed by confocal immunofluorescence and electrophysiological analyses. The expression of FasL, a key molecule for the modulation of immune response, was observed in undifferentiated hDPSCs derived from sphere culture and, surprisingly, it was maintained even after the neurogenic differentiation was reached, whereas after the induction towards osteogenic and myogenic lineages the expression of FasL significantly decreased ($P<0.05$). These data demonstrated that 3D spheres obatained from STRO-1+/c-Kit+/CD34+ hDPSCs represent a suitable culture system to preserve the stemness properties and provide a favorable micro-environment for neural crest derived hDPSCs.

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Keywords
hDPSCs, Neural crest, CD34, FasL

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Forensic Clinical Anatomy of Spine in Child Abuse

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Forensic Clinical Anatomy of Child Abuse includes studies of Functional and/or Biomechanical Anatomy which are performed on cadavers to verify compatibility of lesions with accidental dynamics. Moreover, some kinds of damages following Child Abuse are strictly anatomical in nature and require morphological/morphometric methods of investigation for adequate assessment. Problems of differential diagnosis between anatomical structures (normal or variant) and pathological findings also frequently arise **[1]**. In the present work, we focused on anatomical bases of spinal lesions in two autoptical cases of abusive head trauma, with particular reference to methodological issues. Both cases presented brain subdural haemorrhage and multiple bilateral retinal haemorrhages. In both cases, the spinal cord was sampled in continuity with the dura mater and it was subjected to complete sectioning. Spinal subdural haemorrhages were found along all the spinal levels. The histopathological characteristics of these haemorrhages also permitted to reveal different chronologies of the lesions, with consequent forensic implications. Hypoxic-ischaemic damages coexisted, mainly at the level of cervical and lumbar spinal cord, together with gliomesodermic response. On the basis of in vivo imaging suggesting cervical sub-dislocations, portions of the vertebral column were also sampled and subjected to postmortem imaging before further hystopathological sampling. In one case, postmortem imaging permitted to confirm anterolisthesis of the second vertebral body over the third one. Histopathological analysis also showed the presence of haemorrhagic infiltrations of the epidural adipose tissue at the level of the atlanto-axial joints. A consistent methodology of analysis of the spinal structures should involve integration of postmortem imaging with detailed and exhaustive histopathological study.

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Keywords

Forensic Clinical Anatomy, Abusive Head Trauma, subdural haemorrhage, postmortem imaging, spine, spinal cord

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In vitro and *in vivo* study of a novel biodegradable synthetic conduit for injured peripheral nerves

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In case of peripheral nerve injury (PNI) with wide substance-loss, surgical reconstruction is still a challenge. Bridging the gap by autologous sensory nerves as grafts is the current standard; nevertheless, the related issues have prompted the research towards the development of effective artificial synthetic/biological nerve conduits (NCs). Here, we manufactured a novel NC using oxidized polyvinyl alcohol (OxPVA) that is a biodegradable cryogel recently patented by our group [1]. Thus, its characteristics were compared with neat polyvinyl alcohol (PVA) and silk-fibroin (SF) NCs through in vitro/in vivo analysis. Considering in vitro studies, a morphological characterization was performed by Scanning Electron Microscopy (SEM). Thereafter, cell adhesion and proliferation of a Schwann-cell line (SH-SY5Y) were evaluated by SEM and MTT assay. Regarding in vivo tests, the NCs were implanted into the surgical injured sciatic nerve (gap: 5 mm) of Sprague-Dawley rats, and the functional recovery was assessed after 12-weeks. The NCs were then processed for histological, immunohistochemical (anti-CD3; -β-tubulin; -S100) and Transmission Electron Microscopy (TEM) analyses. In particular, morphometric analyses (section area, total number and density of nerve fibers) were performed at the level of proximal, central and distal portions with respect to NC. In vitro results by SEM showed that PVA and SF supports have a smoother surface than OxPVA scaffolds. Moreover, unlike SF scaffolds, PVA-based ones do not support SH-SY5Y adhesion and proliferation. Regarding the in vivo study, all animals showed a functional recovery with normal walk, even though only animals implanted with PVA and SF NCs sometimes showed spasms while walking. On the contrary, animals implanted with OxPVA NCs exhibited a normal movement. Anti-CD3 immunohistochemistry assessed the absence of severe inflammatory reactions in all the grafts. A strong positive immunoreaction for β-tubulin and S100 demonstrated the good regeneration of nervous fibers. TEM highlighted regeneration of myelinated/un-myelinated axons and Schwann cells in all the grafts. However, morphometric analysis demonstrated that OxPVA assure a better outcome in nerve regeneration in terms of total number of nerve fibers. Our results sustain the potential of OxPVA for the development of NCs useful for PNI with substance loss with the advantage of biodegradation.

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Keywords

Peripheral nerve injury, substance loss, nerve conduit, oxidized polyvinyl alcohol, peripheral nerve regeneration

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3D stereophotogrammetric facial analysis of SMAII patients

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Spinal muscular atrophy (SMA) is a rare neurodegenerative disease, due to autosomal recessive mutations on SMN1 gene. It is clinically classified into 4 phenotypes (SMAI-SMAIV) and it is characterized by muscular weakness and atrophy of the voluntary muscles of the legs, arms and trunk [1]. No information is available about soft tissue facial characteristics in these patients. To better define their facial phenotype and to evidence possible modifications, the 3D facial reconstructions of 12 male SMAII patients (3-8 years) were evaluated. All of them were able to sit, but not to walk independently and had respiratory problems, chewing and swallowing difficulties. The facial reconstructions were obtained through a stereophotogrammetric system, after the non-invasive identification of 50 facial reference landmarks, whose 3D coordinates were used to calculate a series of linear measurements. Data were compared with those of healthy controls, matched for age and sex, through the calculation of z-score values [2]. Results show that patients have larger skull base, mandibular and facial widths (z-score = 1.5, 2 and 1.8 respectively), together with an increased height of the nose (z-score = 3) and mandibular body length (z-score = 2.1). The mandibular ramus length is reduced (z-score = -2.6). Results are of interest to define the facial anatomy of these patients, since a detailed knowledge of their facial features can be useful to create ergonomic devices, as respiratory masks, that these patients must daily use.

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Keywords
Spinal muscular atrophy, stereophotogrammetry, face.

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Stereophotogrammetric assessment of the smiling capability after facial reanimation surgery

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Facial palsy causes functional and aesthetic problems; among those, the reduction of facial mimicry and smiling difficulties, require surgical treatment and rehabilitative procedures [1]. To quantitatively evaluate the recovery of the smiling capabilities after reanimation surgery (double cross-face, masseteric-facial nerve neurorraphy, hypoglossus-facial nerve neurorraphy), 11 patients (4 females, 7 males, mean age 59.6, SD 10.4 years) affected by acute unilateral facial palsy were acquired with a 3D stereophotogrammetric instrument. Each patient was acquired in neutral facial position and performing 4 different types of smile, executed taking advantage of the aforementioned surgical stimuli, both separately and together. The smiling facial images were divided in two hemifaces, successively registered on the corresponding neutral one. Root Mean Square (RMS) distances between neutral face and smiling hemifaces were automatically calculated by the software of the stereophotogrametric system. Inter and intra-operator repeatability in performing this procedure were assessed. A two-way ANOVA for repeated measurements was performed in order to verify the differences among the smiles and the facial sides. Results showed good intra and inter operator repeatability of the procedures (R^2 0.6 and 0.9, respectively). Statistical significant differences were found among the different smiles and the facial sides ($p < 0.01$ in both cases) and for the side x smile interaction ($p < 0.05$). For the affected facial side, post hoc tests revealed statistical significant differences ($p < 0.05$) between the smiles performed using the double cross-face (mean RMS 0.5 $\pm$ 0.2 mm) and masseteric-facial nerve neurorraphy, with this last being more powerful (RMS 0.9 $\pm$ 0.5 mm). The results offer the possibility to objectively quantify the recovery of the smiling capability, usually qualitatively evaluated, through subjective grading systems.

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Keywords

Facial palsy, stereopgotogrammetry, smile, Root Mean Square

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Endothelial cells are key-players in pilocarpine-induced epileptogenesis

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In recent years, the concept of the **neurovascular unit** (**NVU**) has emerged as a new paradigm for investigating both physiology and pathology in the CNS. This concept proposes that a purely *neurocentric* focus is not sufficient, and emphasizes that all cell types in the brain including **neuronal**, **glial** and **vascular components** (endothelial cells, blood cells, including immunity cells) must be examined in an integrated context. Cell–cell signaling and coupling between these different compartments thus form the basis for normal function (Lok et al. 2007). We tested the hypothesis that disordered signaling and perturbed coupling of these different components can be the basis for epileptogenesis in the pilocarpine model of epilepsy. We thus determined that pilocarpine can act on endothelial cells via receptors, comparing the response of the same stimulation in neurons as well.

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Keywords

Seizures, epilepsy, Bend3, BMVECs, neurovascular unit, endothelial cells

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IPMK and β-catenin take part in PLC-β1-dependent signaling pathway during myogenic differentiation

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Phospholipase C (PLC)-β1 catalytic activity plays an essential role in the initiation of myogenic differentiation but the effectors involved in its signaling pathway are not well defined[1,2]. Here, we show that the overexpression of the Inositol Polyphosphate Multikinase (IPMK) promotes myogenic differentiation, and that IPMK targets the same cyclin D3 promoter region activated by PLC-β1. Moreover, cyclin D3 promoter activation relies upon c-jun binding to the promoter, both in response to PLC-β1 and to IPMK overexpression. Furthermore, both IPMK and PLC-β1 overexpression determines an increase in β-catenin translocation and accumulation to the nuclei of differentiating myoblasts resulting in higher MyoD activation. Therefore, our data show that PLC-β1, IPMK and β-catenin are mediators of the same signaling pathway that regulates cyclin D3 and myosin heavy chain (MYH) induction during myogenic differentiation.

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Keywords

Myogenic differentiation, phospholipase C-β1, IPMK, β-catenin, inositol phosphates

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The quest for the third dimension: from the Electron Microscope to the 3D printer

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Conventional light microscopy (LM) and transmission electron microscopy (TEM) are meant to image planar sections, i.e. bidimensional specimens, and are therefore constrained into a bidimensional world. In contrast, the scanning probe microscopy (SPM) and scanning electron microscopy (SEM) are able to image surfaces, i.e. three-dimensional subjects. Of these techniques, SPM has the additional advantage of directly obtaining three-dimensional datasets from three-dimensional specimens, although this ability is seldom exploited. The SEM is *per se* limited to 2D pictures of 3D subjects, but its flexibility and performance make possible to re-obtain the third dimension indirectly.

A first, simple, time-proven approach is stereophotography. This makes possible an immediate visual appreciation of depth and volume but does not allow quantitative measurements.

A subsequent approach is represented by shape-from-stereo reconstruction, which builds a quantitative computer model of the specimen. This is now a consolidated technique and several solutions, both hardware- and software-based, are readily available. Although limited to the development of 2 ½ dimensions, rather than real 3D, this technique is simple and effective and for several years the authors have used a proprietary package [1] featured in a number of published papers.

More recently a new generation of shape-from-motion or shape-from-video photogrammetric software [2] makes possible the full recovery of the third dimension, complete with undercuts and texture mapping.

All these techniques are now complemented and extended by the availability of inexpensive three-dimensional printers. Going beyond visual appreciation and beyond computer graphics, this technique makes possible to obtain a tangible, material model of the specimen. 3D printing is already in use for educational purposes but can be effectively deployed also in morphological research, making possible to obtain highly magnified, accurate copies of microscopic structures such as molecules, cells and interfaces, adding to the visual appreciation the immediacy of the tactile experience. A few examples are shown.

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Keywords

Microscopy, photogrammetry, 3D reconstruction, 3D printing

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Nuclear DGKα regulates cell cycle progression in K562 cells

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The existence of an independent nuclear inositide pathway distinct from the cytoplasmic one has been demonstrated in different physiological systems and in diseases (1). Phosphatidylinositols (PIs) play an important role in nuclear function regulation and behave differently from their counterparts in the cytoplasm. The autonomous nuclear PI cycle in eukaryotic cells is involved in different regulation processes, from cell proliferation to differentiation and many others (2). At nuclear level an array of kinases and phosphatases can modulate PIs. Among these, Diacylglycerol Kinases (DGKs) are a class of phosphotransferases that phosphorylate diacylglycerol (DAG) and induce the synthesis of phosphatidic acid. We Investigated DGKα localization and function in human erythroleukemia cell line K562. Synchronization experiments at different cell cycle checkpoints showed an important expression of DGKα in the nuclear fraction of this cell model, slightly peaking at G2/M. This suggested that DGKα might have a function in nuclear signaling. In particular, nuclear DGKα expression can modulate cell cycle progression, leading to changes in the phosphorylated status of the Retinoblastoma protein (pRb), thus, regulating G1/S transition: DGKα silencing or downregulation leads to impaired G1/S transition and its overexpression leads to S phase progression. The molecular mechanism by which nuclear DGKα controls pRb phosphorylation and therefore cell cycle regulation in K562 cell line are still unclear. Further studies are needed to better understand the role of DGKα in relation to other pivotal PIs involved in cell cycle regulation in the hematopoietic system.

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Keywords

Nuclear lipid signaling, cell cycle, DGK, DAG

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FE-SEM and VP-SEM imaging of human calcified aortic valves: conventional vs Ionic Liquid innovative techniques

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Conventional FE-SEM protocol for calcified aortic valves (CAVs) consist of following steps: glutaraldehyde fixation, OsO4 post-fixation, dehydration in alcohol series, critical point drying and finally sputter coating. CAVs can be observed in their native state (fixed in glutaraldehyde with and without post-fixation in OsO_4) by Variable Pressure-SEM (range 6- 650 Pa). Gas presence allows an inferior resolution (low signal to noise ratio), however there is the possibility to perform EDS elemental analysis without background noise due to sputter coating. Recently Ionic liquids (IL, salts in the liquid state at room temperature) were used as suppliers of electronic conductivity with insulating properties, so we have tested their ability to replace sputter coating on CAVs in high vacuum condition. Samples fixed in glutharaldehyde 2,5% in PBS with and without OsO_4 post-fixation treated with ionic liquid (Hitachi HILEM® IL 1000) were compared with samples treated with conventional FE-SEM procedures. Several IL concentration (range from 5% to 20%) were tested, different operating voltages (range from 3 to 20Kv) were used. This novel technology requires a high degree of customization for each sample type. In our opinion fixation in glutaraldehyde with OsO_4 post-fixation is recommended to preserve finest details, moreover residual liquid elimination is important to increase resolution and avoid beam interference as linear markings. Setting of a proper accelerating voltage allows to correctly visualize the surface topography. Processing CAVs with IL with respect to conventional FE-SEM is useful for several reasons. Mainly this method is time saving (and cost saving), secondary the same sample can be processed for transmission electron microscopy after SEM observations (allowing correlative microscopy), finally EDS can be performed without background noise due to sputter coating. Perhaps now this technique can not completely replaces the conventional SEM in terms of resolution but in our opinion rapid technical improvement can further reduce this gap.

Keywords

FE-SEM, VP-SEM, EDS analysis, ionic liquid, aortic valve

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An 8-week rehabilitation training using the HBP exoskeleton improves cognitive brain functions in multiple sclerosis patients

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It has been showed that a single application of the exoskeleton (HBP) in multiple sclerosis patients is able to improve mobility and ambulation. These effects have been associated with brain changes in high-level executive functions decisive for improving patients' motor control [1].

We applied an 8-weeks rehabilitation protocol in 12 MS patients, half of them randomly assigned to a standard protocol (control group, CG) and the other half to a protocol based on the HBP use (experimental group, EG). Patients were evaluated before and after rehabilitation training using multiple neurological, physiotherapeutic and cognitive testing. During the cognitive task, high-resolution EEG was also recorded for ERP analysis. Results showed that both groups improved their performance in the Barthel, Rivermead, 2-WT, 25-FWT, Tinetti and BBS tests. Only in the EG, other positive treatment effects were observed as measured by the EDSS disability scale and the FSS. Accordingly, in cognitive testing, only the EG showed significant benefits in response time (RT) and accuracy. At brain level the EG showed enhancement in task-related preparatory activity in frontal and prefrontal cortices and stronger post-stimulus activity in the anterior Insula, whose activity is related to more efficient decision making. The CG didn't show enhanced performance in the cognitive task but only large activity in visual areas, as observed in EG. Concluding, both rehabilitation protocols brought substantial neurophysiological benefits to MS patients, however, the HBP protocol was particularly effective, boosting cognitive functions in prefrontal and frontal brain areas, it allowed improvements in RT and accuracy. The integration of HBP with standard rehabilitation procedure may considerably reduce disability in MS patients.

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Keywords:
Exoskeleton, electroencephalography, prefrontal cortex. multiple sclerosis

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The evolution of Clemente Susini's anatomical iconography from his beginnings at La Specola waxwork to his artistic maturity, as seen in the collection of Cagliari

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Museum of Clemente Susini's Anatomical Waxes, Department of Biomedical Sciences, University of Cagliari.

In 1772, Clemente Susini (1754-1814) freshly graduated at the Florentine *Accademia di Belle Arti* was hired, as assistant of the sculptor Giuseppe Ferrini and dissector aid, by Felice Fontana (1730-1805) physicist of the Grand-ducal court. The latter was then setting up the ceroplastic workshop of the *Regio e Imperiale Museo di Fisica e Storia Naturale* (called La Specola) funded by the Grand-Duke Peter Leopold. Ten years later, he was appointed first modeller, a job that he carried on until the end of his life. In forty years of work Susini realized, or oversaw, the production of over 2000 wax models most of which for the great collection of La Specola in Florence and that of the Josephinum in Vienna completed in 1780-1786 [1]. Aside from both the former, made under Fontana's directorship, Susini produced other models commissioned to the Museum from Italy and abroad. Noteworthy, among these is the collection for Cagliari made in collaboration with the Sardinian anatomist Francesco Antonio Boi (1767-1850) in 1803-1805. At the time, Fontana was no more interested in wax modelling and Susini was free, at last, to fully express himself. Cagliari's waxes are more realistic, there are no posing figures, and the models do not exhibit the "rosy skin" of those of La Specola and Vienna. Most of the 23 cases bear the date and Susini's signature, a seal of authorship lacking in the other collections of Florentine waxes. The target of Susini and Boi appears to be quite different from that seen in the earlier collections. The References to clinical and functional anatomy seem purposely pointed out in order to give students of surgery and medicine relevant information on their professional training. There is no attempt to make anatomy more attractive for a general public or to educate citizens according to the project of "popularizing" anatomy so dear to Fontana and Peter Leopold. Moreover, it seems that Susini and Boi have reached a degree of "cooperation of art and science" that anticipate the current trend of anatomical illustration [2].

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Keywords

Anatomical Waxes, Clemente Susini, Francesco Antonio Boi, Cagliari, La Specola

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Immunohistochemical characterization of axon terminals of the adult rat cerebellar cortex

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Aim of the present study was to immunohystochemically characterize the glutamatergic terminals in the cerebellar cortex. The study was carried out on adult rat, using immunohistochemistry for the vesicular transporters of glutamate, VGLUT1 e VGLUT2, and synaptophysin.

Results. Terminals positive for VGLUT1 e VGLUT2 were observed in the molecular layer (ML) and granular layer (GL). ML. VGLUT1-positive terminals appeared very numerous, homogeneously distributed throughout the layer with a distribution pattern resembling that of the parallel fiber terminals. VGLUT2-positive terminals appeared displaced along distinct spiral lines extending from the deeper part of the layer up to the its superficial part, likely corresponding to synapses between climbing fibers and Purkinje neuron dendrites. No colocalization of VGLUT1 and VGLUT2 was observed in ML. Double labelling for VGLUT1 and synaptophysin and, respectively, VGLUT2 and synaptophysin revealed colocalization, suggesting the axon terminal nature of the immunolabelled elements. Interestingly, some elements appeared synaptophysin positive, but negative for VGLUT1 and VGLUT2.

In GL, VGLUT1 and VGLUT2 positive terminals displayed similar distribution patterns, They appeared clustered in restricted regions of the layer, scattered within granule neurons, at the level of synaptic glomeruli. Most of these terminals showed a colocalization of VGLUT1 and VGLUT2. However a part of them shows positivity for VGLUT2 and negativity for VGLUT1. All the VGLUT1 and VGLUT2 positive elements also displayed positivity for synaptophysin. Finally, like in ML, some elements appeared synaptophysin positive, but negative for VGLUT1 and VGLUT2.

Conclusion. The results indicate that the glutamatergic terminals in the cerebellar cortex may be differentiated combining Immunohistochemistry for VGLUT1 and VGLUT2. Moreover, the results identify subpopulations within terminals of the parallel, climbing and mossy fibers. In particular, a subpopulation of mossy fiber terminals, displaying positivity for VGLUT2 and negativity for VGLUT1, are different from the vast majority of mossy fiber terminals, which display for both VGLUT1 and VGLUT2, but similar to climbing fiber terminals. It is intriguing to hypothesize that these mossy fibers may constitute a contingent of mossy fiber originated in the inferior olivary nuclear complex.

Keywords

Rat cerebellar cortex, climbing fibers, mossy fibers, vesicular glutamate transporters

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Optimization Of Human Heart Decellularization Method For Cardiac Regenerative Medicine

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Extracellular matrix (ECM) is an intricate mesh of collagenous and non-collagenous proteins, whose presence and amount vary according to type of tissue. ECM drew the attention of regenerative medicine scientists as natural scaffold suitable for stem cell delivery into damaged tissues. Although a multitude of protocols and combinations of chemical agents and physical methods have been tested and proved effective in the decellularization of human heart, none of the ones tried in our setting fulfilled the goal of obtaining a structurally preserved cardiac decellularized ECM (d-ECM). While testing already described procedures, we made several adjustments that led to the development of a novel, simpler and robust protocol to decellularize adult human heart. Specifically, we decellularized cardiac samples of the free wall of both ventricles of adult human hearts scaled down to fit into embedding cassettes used to avoid stirring stress and preserve structure. To shorten the procedure, a combination of SDS, Triton X-100 and antibiotics was used in simple and fast two-step protocol. After decellularization, d-ECM was fixed and processed for histological study or snap-frozen for molecular biology analysis or cytocompatibility test in vitro. Histochemistry and immunoistochemistry confirmed the absence of nuclei and the preservation of architecture and composition of d-ECM. Further, while DNA content in d-ECM was well below accepted standards, sGAG, elastin and growth factors were retained and d-ECM scaffolds supported cardiac primitive cell engraftment and survival in vitro. Hence, according to our evidence, our protocol is simple, fast, effective and is worth improving for clinical translation.

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Muscle hypertrophy and vascularization induction using human recombinant proteins

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Met-Activating Genetically Improved Chimeric Factor-1 (Magic-F1) is an engineered protein that contains two human Met-binding domains. Previous experiments in both homozygous and hemizygous transgenic mice demonstrated that the skeletal muscle specific expression of Magic-F1 can induce a constitutive muscular hypertrophy, increasing the vessel number in fast twitch fibers, also improving running performance and accelerating muscle regeneration after injury [1]. We also found that Magic-F1 could be responsible of muscular hypertrophy inteacting with Pax3 signal pathway in skeletal muscle precursor cells [2]. In order to evaluate the therapeutic potential of Magic-F1, we tested its effect on multipotent and pluripotent stem cells [3]. Murine mesoangioblasts (adult vessel-associated stem cells) expressing Magic-F1 were able to differentiate spontaneously forming myotubes. In addition, in Magic-F1 inducible murine embryonic stem cells subjected to myogenic differentiation, the presence of recombinant protein resulted in improved myogenic commitment. Finally, the microarray analysis of Magic-F1+/+ satellite cells evidenced transcriptomic changes in genes involved in the control of muscle growth, development and vascularisation [4]. Taken together our results candidate Magic-F1 as a potent myogenic inducer, able to affect positively the vascular network, increasing vessel number in fast twitch fibers and modulating the gene expression profile in myogenic progenitors.

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Keywords

Embryonic stem cells, Magic-F1, mesoangioblast, myogenic differentiation, recombinant protein.

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Transcriptome analyses unveils the unique requirement for human lipoproteins for optimal ex-vivo expansion of cultured red blood cells for transfusion.

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Cultured red blood cells (cRBCs) generated from discarded stem cell sources are being considered as alternative transfusion products. We developed a humanized media (HEMA^{def}) composed of clinical grade components and dexamethasone (Dex) that sustain amplifications of cRBCs from stem cells discarded from regular blood donations in numbers sufficient for transfusion [1]. These cells, however, have an altered morphology of their plasma membrane which suggests that may reduce their survival in vivo. Since ~50% of the cRBC mass is constituted by lipid, we hypothesized that the plasma membrane abnormalities were caused by insufficient lipid supply. cRBCs produce their plasma membranes starting from lipids supplemented by the media and through a dedicated biosynthetic pathway, well known to be affected by Dex. Comparison of the expression profiling of cRBCs generated with/without Dex [2] identified remarkable similarities in gene expression. The majority of the differences were however detected in genes involved in lipid metabolism. In particular genes involved in lipid synthesis (GPAM and PRKACB) and efflux/degradation (HMGCL and ABCA1) were respectively down and up-regulated in cRBCs obtained with Dex, suggesting that in cultures with Dex cRBCs are exquisitely dependent on exogenous lipids for their membrane biosynthesis. This hypothesis led us to optimize the lipid formulation of HEMA^{def} by replacing the synthetic liposomes with lipoproteins purified from human plasma which represents the natural carriers for delivering lipids to the cells. These experiments compared the levels of amplification/maturation of cRBCs in HEMA^{def} supplemented with either synthetic liposomes or the total lipoproteins contained in human plasma (TLP) or its high density (HDL), low density (LDL) and very low density (VLDL) lipoprotein fraction. Addition of LDL and VLDL both increased by 3-2-fold the numbers of cRBCs generated in HEMA^{def}. TPLF has modest effects on the number of cRBCs generated in HEMA^{def} but drastically reduced the frequency of cRBCs with membrane abnormalities. More importantly, addition of TLP increased both the number (by 2-3-fold) and the membrane quality of cRBCs generated in HEMA^{def}. These results confirm the importance of an appropriate lipid supply for correct generation of RBC and identify culture conditions which assure maximal expansion of morphologically normal cRBCs for transfusion.

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Keywords

Human plasma, lipoproteins, membrane biogenesis, human erythroid cells, blood farming

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Posture as a model of mechanosensitivity: the "Biotensegrity"

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The *Human body* must be considered as a complex motor biosystem where homeostasis and regional state or segmental state functions are inseparable from general ones and the control circuit is not linear-causality type, ie stimulus and reaction, but it is an interactive system made up of a large number of interconnected circuits.

Recent research paves the way for a new interpretation of connective tissue functions, understood as a true "communicative network" within the model of mechanical sensitivity that sees cells as a kind of "metal mesh" where elastic fibers, from cytoplasmic space, reach internal structures such as chromatin, allowing the cell to respond directly and immediately to the forces applied to the cell membrane. Moreover, we know today that, through specific membrane proteins (integrins), the connective system is able to interact with cellular mechanisms. Unlike the nervous system, the endocrine and the immune system, the myofascial apparatus presents a more archaic but not least important method of communication: the mechanical one. It "simply" pulls and pushes communicating thus from fiber to fiber, from cell to cell and from internal and external environment through mechanical signal transduction systems. We can, therefore, speak of biotensegrity bone-muscle-fascial system, that is the faculty of a system to stabilize mechanically through a game of tension and decompression forces that are divided and equilibrated.

The alterations of these forces determine pathological conditions that may affect the various sensory, central, motor, soft tissues subsystems with a progressive deterioration of the delicate compensatory mechanisms, causing the onset of postural disorder that is exacerbated by tissue suffering and results also in a morfological damage.

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Keywords

Posture, tensegrity, bone-muscle-fascial system, mechanical sensitivity

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Was it a fatal whiplash injury or not? Clinical forensic anatomy: a key to shed light on a case

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Autopsy is the oldest method of medical investigation. Many studies underscore the need for autopsies also in the era of technical progress emphasizing the continuing discrepancies between ante-mortem and post-mortem diagnoses. The forensic pathologist (and anatomist, too) has to know in depth the anatomy and how to study it using the dissection techniques with the help of new pre and post autoptical technologies.

Forensic radiology must integrate the expertise of forensic pathologist, the challenge is to unite all disciplines by direct and intense communication. Furthermore, histology plays a fundamental role in the final diagnosis and the collection of the samples requires the correct visualization and isolation of all the supposed organ lesions.

We present a case report with a multidisciplinary method to the cadaver, about a presumed "road murder", in which the forensic clinical anatomical approach was directed to the cause and means of death.

A case of a 79 years old man victim of a frontal crash is presented. At the scene, the driver was found comatose (GCS 3) and carried to the Emergency Department. At the ED, the patient was subjected to CT scan of brain and angio CT scan, directed, in particular, to epiaortic vessels. CT scan showed a widespread ischemia of cortical and subcortical areas of pariar, occipital and cerebellar lobes; angio CT scan revealed the complete occlusion of the lumen of both vertebral arteries, at the level of the third cervical vertebra. The man died about 4 days after his admittance to the hospital. Was it a death after a whiplash injury or not? Before performing autopsy, a head and neck CT scan was carried out. Autopsy was performed 6 days later, and was carried according to a protocol for the examination of the V3 – V4 segments of the vertebral artery. Imaging first, and then autopsy, revealed completely different findings from those shown in ante mortem CT scan, that revealed the true cause of death.

Keywords

Postmortem imaging, autopsy, forensic pathology, virtopsy

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Morpho-functional approaches to highlight skeletal muscle response to cell death inducers

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Cell death has been long described, with continuously growing interest, in a variety of tissues and models. Its presence in muscle disorders stimulated us to study it in striated muscle tissue, in particular, in " in vitro" differentiated myotubes. Apoptosis is a regulated mechanism of cell death which occurs in the absence of plasmalemma disruption, but with cell and organellar component swelling. It plays a crucial role in skeletal muscle pathology, in denervation and disuse [1]. Recently, "autophagy", an intriguing phenomenon characterized by progressive deletion of cell components, could have a role in skeletal muscle death progression, also acting as a survival mechanism. Here, in vitro C2C12 skeletal muscle cells were exposed to etoposide, H_2O_2 or staurosporine and cell response has been investigated by means of morpho-functional approaches. Myotubes appeared more resistant than myoblasts to apoptotic induction. In particular, etoposide- or H_2O_2-treated myoblasts showed characteristic apoptotic features, visualized also in etoposide-treated myotubes characterized by a diffuse DNA cleavage presence. After H_2O_2 exposure, necrotic cells could be observed and myotubes exposed to staurosporine, evidenced late apoptotic features and secondary necrosis. The coexistence of normal and apoptotic nuclei within the same fiber has been demonstrated, in particular in the case of etoposide and staurosporine treatments. The deletion of a single nucleus can occur without the death of the entire myotube, evidencing that a multinucleated cell dies 'more slowly'. After the majority of stimuli, autophagic vacuoles could be diffusely revealed in myotube cytoplasm. They could preserve muscle cell integrity counteracting chemical treatments, or could activate death pathways. It is the case of etoposide drug, which induced skeletal muscle apoptosis in the presence of an autophagic flux impairment. These findings reveal that apoptosis, necrosis and autophagy coexist in muscle biology and, ultrastructural analyses appear a useful approach for highlighting and describing these processes.

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Keywords

Myotubes, chemical triggers, apoptosis, autophagy

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E-cigarettes fluids trigger molecular and morphological response in oral fibroblasts

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Electronic-cigarettes (e-cigarettes) have been recently advertised as a safe alternative to the traditional ones and a possible smoking cessation tool. This electronic device was designed to transform a solution of variable compounds (some of them approved as food additives), in an inhalable aerosol. However, their safety is still not fully know (Lerner et al. 2016). The cytotoxicity of the fluids on human gingival fibroblasts (HGFs) was demonstrated on a previous study by Sancilio et al. (2016) where the occurrence of oxidative stress and apoptosis was found following the exposure to nicotine containing fluids. The aim of this study was to investigate the HGF biological response to e-cigarettes liquids (with and without nicotine) and to clarify the molecular mechanisms driving the cytotoxicity exerted by fluids themselves. To this purpose, cells were treated with e-cigarette fluids containing nicotine (final concentration 1mg/mL) and the equivalent volume of a fluid without nicotine, for times up to 48 h. Lactate Dehydrogenase Assay (LDH), electronic microscopy analysis, collagen I production, flow cytometry lysosome compartment evaluation and western blotting LC3 (microtubule-associated protein 1A/1B-light chain 3) expression were performed.

Fluids containing nicotine exerted cytotoxicity as demonstrated by the increased levels of LDH, in parallel to the formation of numerous vacuoles in the cytoplasm, as well as a decrease in collagen I production and an augmented LC3 II expression which characterized autophagy occurrence In conclusion E-cigarette fluids (with and without nicotine) trigger modification ultrastructure, collagen production and lysosomal compartment in HGFs, suggesting an involvement in the pathogenesis of oral diseases.

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Cytotoxicity and apoptosis induction by e-cigarette fluids in human gingival fibroblasts. Clin Oral Investig 2016;2: 477-483

Keywords
Fibroblast, nicotine, smoking, collagen, cell biology

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The endocannabinoid anandamide inhibits colon cancer cell growth by modulating different survival and proliferating pathways

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The Endocannabinoid System (ECS) comprising the CB1 and CB2 receptors and their endogenous ligands is a central signalling system regulating food intake and energy balance. It is also present in peripheral tissues where it is involved in cell proliferation and survival. It has been shown that in colon cancer cells, the CB1 receptor antagonist SR171416 reduces colon cancer cell growth by acting as an inverse agonist rather than an antagonist [1]. Starting from this observation and from evidence indicating that some biological responses to cannabinoids depend on estrogen levels and some selective estrogen receptor modulators can bind the CB1 receptor [2], we aimed to study the effects of the CB1 receptor ligand anandamide (AEA) on colon cancer cell proliferation and its ability to modulate some survival and proliferating pathways including Akt, MAPK/ERK and estrogen receptor (ER) b signalling which is the predominant ER pathway in colonic epithelium. We used an AEA-analogue and a selective inhibitor of fatty acid amide hydrolase (FAAH) that enhances intracellular levels of AEA and studied proliferation and cell cycle progression on human adenocarcinoma cells DLD1 and SW620. Our results showed that increased levels of AEA significantly reduced cell proliferation in both cell lines at 24 and 48 h also inducing an S phase cell cycle accumulation. The AEA-induced inhibition of cell growth was mediated by a reduced expression of phoshoAkt and phosphoERK and, at the same time, by an induction of ERβ expression. These data suggest that AEA can reduces colon cancer cell proliferation by interfering with different signalling pathways.

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Keywords

Endocannabinoid system, anandamide, colon cancer cells, Akt and MAPK/ERK pathways, oestrogen receptor β signaling

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Nerve growth factor-promotes primary cilium assembly in cholinergic neurons from the human basal forebrain

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The primary cilium is a non-motile sensory antenna protruding from the surface of nearly all cells of the body, able to mediate the cellular response to extracellular signals. Although many of its functions remain to be clarified, it has been recently shown a role in neurogenesis [1]. In this study we evaluated the presence of the primary cilium in neurons isolated from the human fetal nucleus basalis of Meynert (*hf*nbM), a basal forebrain region crucially involved in the cholinergic transmission required for learning and memory. The *hf*nbM cells are characterized by the expression of cholinergic markers, such as choline acetyl transferase (ChAT) and also express the primary cilium, which, in basal conditions, was detected in the 17% of cells. It is known that nerve growth factor (NGF) supports survival, maintenance, connectivity and function of the brain cholinergic neurons. Indeed, we demonstrated that *hf*nbM cells respond to NGF in terms of proliferation, neurite formation and ChAT expression. Interestingly, NGF exposure significantly increased the percentage of ciliated cells (34.9%±1.8%). Given the known adverse effect of systemic chronic inflammation in the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease, characterized by the loss of nbM neurons, we exposed our cells to tumor necrosis factor-α (TNF-α). We observed that TNF-α significantly reduced the number of ciliated cells (4.3%±2%). Our results strongly suggest for the first time that primary cilia may be involved in the NGF-driven maturation of human nbM cholinergic neurons and suggest that the deleterious effects of neuroinflammation may be linked to an altered formation of the primary cilium.

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Keywords

Nucleus basalis of Meynert, NGF, TNF-alpha, Alzheimer's disease

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Physical activity modify skeletal muscle fiber types in an animal model of metabolic syndrome

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Metabolic Syndrome (MetS) is a cluster of clinical conditions, associated to an increased cardiovascular risk, as well as to hypogonadism in males. Lifestyle modification (including physical exercise, PhyEx) may be beneficial for the condition. Skeletal muscles (SkM) are some of the most highly plastic tissues, able of remodeling in response to use, disuse and disease. In particular, transformations of fiber type may occur in response to physiological milieu to induce functional adaptations. This study is aimed at investigating in experimental MetS, high fat diet-induced in male rabbits [1], the effect of PhyEx on hormonal and metabolic parameters, as well as on SkM composition. Control and MetS rabbits were exercise-trained to run on a treadmill for 12 weeks. Quadriceps femoris samples were collected for histomorphological and gene expression analyses. We found that exercise resistance was significantly reduced in MetS rabbits, as demonstrated by the significant reduction of both running time and distance, compared to control group. MetS rabbits also exhibited the lowest quadriceps mass. Fiber typing by PAS-staining showed a pronounced shift from slower type I to faster type II fibers in MetS group in response to PhysEx, suggesting that MetS condition addressed SkM function towards anaerobic metabolism. Accordingly, extracellular lactate levels were significantly increased and mitochondrial respiration-related genes reduced in SkM of MetS rabbits respect to controls. Interestingly, PhyEx significantly counteracted MetS-related testosterone deficiency and hypercholesterolemia. In conclusion, our results indicate that dysmetabolic milieu induces a reduced proportion of fatigue-resistant type I fibers in response to PhysEx, which however resulted beneficial for MetS condition.

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Keywords:
Exercise resistance, metabolic dysfunction, testosterone

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Sarcopenia and muscle functions: the impact of aging and disuse

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Aging is typically associated with inactivity that can quickly result in deconditioning. This often ultimately leads to debilitating immobility. the rapid progression of several degenerative diseases, and the ballooning health care needs. The changes in muscle structure and function strongly affected the capacity of movement playing a central role in the dramatic spontaneous reduction of it. The impact of low grade of every day mobility in the elderly during this downward spiral of events is not well understood. In fact, our understanding has been clouded by what appear to be quite different findings in the old and the very old. From a health system point of view it is imperative that easily achievable and effective countermeasures be determined to better protect the elderly and the increasing number of them actually overcoming the threshold of 80/90 years old.. Therefore, this presentation will focus upon exercise efficiency in the old, and the very old, providing in vivo and in vitro data on how the lack of even a minimal usual movement can lead to a impressive modifications in muscle structure and function. Finally, a muscle-specific countermeasure that can target both the decreased exercise efficiency and greater fall prevalence associated with aging will be discussed.

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Keywords

Ageing, physical activity, muscle structure and function, sarcopenia, disuse

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Levetiracetam enhances the Temozolomide effect on glioblastoma stem cell proliferation

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Glioblastoma Multiforme (GBM) is a highly aggressive brain tumor in which cancer cells with stem cell-like features, called Cancer Stem Cells (CSCs), were identified. CSCs show a high capacity to resist to standard therapies, finally leading to a poor prognosis. Thus, the development of efficient strategies targeting these cells are urgently needed. We have previously demonstrated the presence of two CSC populations in GBM, one derived from the GBM area called enhanced lesion (GCSC) and the other one from the brain area adjacent to the tumor margin (PCSC), that greatly differ in their growth properties and tumor-initiating ability. Tumor recurrence occurs in tissue neighboring GBM suggesting a growing relevance for this area in translational research. To date the most effective chemotherapies to treat GBM are alkylating agents such as temozolomide (TMZ). Epigenetic mechanisms are increasingly recognized as a major factor contributing to pathogenesis of cancer including glioblastoma. Histone deacetylase (HDAC) inhibitors can interfere with TMZ activity by modulating methylguanine methyltransferase (MGMT) expression, resulting in increased TMZ efficacy. Levetiracetam (LEV), an antiepileptic drug, is known to modulate the transcription of HDAC, ultimately silencing MGMT.Since TMZ is the chemotherapeutic agent most widely used in newly diagnosed adult glioblastoma patients, we evaluated its effects on the proliferation rate of both GCSC and PCSC deriving from five patients, in comparison with the effects of other drugs such as etoposide, irinotecan and car-boplatin. Our results demonstrated that TMZ was the less efficient agent, hence we verified the pos-sibility to increase the effect of TMZ by combining it with LEV. Here we show that LEV signifi-cantly enhances the inhibitory effect of TMZ on the proliferation of the GCSC deriving from four patients and of the PCSC deriving from two patients. This effect seems to be mediated by HDAC6 since its expression is up-regulated in the TMZ resistant cells and correlates with MGMT expression. Taken together our results suggest that GCSC and PCSC differ in their ability to respond to the combined chemotherapeutic treatment we used and that the manipulation of HDAC6 expression might be a potential strategy for treating glioblastoma and overcoming resistance to TMZ.

Keywords

Glioblastoma, Cancer Stem Cells, Chemotherapeutic drugs, Temozolomide, Epigenetic modifications, Levetiracetam

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Comparative transcriptome and gene regulation in human iPSC-derived organoids and donor-identical brain tissue

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Modeling human brain development *in vitro* is critically important to understand the pathophysiology of neuropsychiatric disorders. As part of the PsychENCODE project, we generated human induced pluripotent stem cells (hiPSCs) from skin fibroblasts of three human specimens at 15, 16 and 17 postconceptional weeks. These hiPSC were differentiated into telencephalic organoids to study early genetic programs in forebrain development. By using RNA-seq and histone chromatin immunoprecipitation (ChIP-seq), we compared transcriptomes and epigenomes of hiPSCs-derived organoids to donor-identical cortical brain tissue. Immunocytochemical characterization of the organoids over a time course (TD0, TD11 and TD30) showed expression of radial glial markers and mature cortical neurons confirming telencephalic fate. Hierarchical clustering of the organoids' transcriptomes demonstrated stage-specific patterns of gene expression during *in vitro* development. Mapping organoids' transcriptomes against the BrainSpan dataset suggested highest correlations with neocortex and showed their correspondence to post-conceptional weeks 8-16 of human fetal development. We then inferred transcriptional alterations, by differential gene expression, between organoids and the two brain regions analyzed. We found ~5000 of differentially expressed genes (DEG) between TD0 and fetal cortex and a decreasing number of DEG at TD11 and TD30 suggesting a stronger, albeit incomplete similarity of the organoids to the cortex at later time points. ChIP-seq experiments identified H3K27ac and H3K4me3 peaks (putative promoters and enhancers) differentially active at different organoids developmental stages and between organoids and fetal brain. Overall, however, hierarchical clustering of H3K27ac and H3K4me3 peaks demonstrated clustering of organoids with human fetal brain samples from various databases, whereas neonatal and adult brain samples formed separate clusters. These data suggest that organoids recapitulate in part transcriptome and epigenome features of fetal human brain.

Keywords
Cortical development, human iPSCs, organoids, fetal brain

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BDNF, trkB and PSA-NCAM in the hippocampus of Roman rats after forced swimming

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The selective breeding of Roman High- (RHA) and Low-Avoidance (RLA) rats are considered as a genetic model of resilience to stress-induced depression and of vulnerability to that trait, respectively1. There is evidence that alterations in neuronal plasticity in the hippocampus and other brain areas are critically involved in the pathophysiology of mood disorders. Here, we investigated on immunochemical occurrence of Brain-derived neurotrophic factor (BDNF), tyrosine-kinase receptor trkB and polysialilated form of the neural cell adhesion molecule (PSANCAM) in the hippocampus of the Roman rat lines under baseline conditions and after acute forced swimming (FS). Western blot (WB) analyses showed that, in basal conditions, the relative levels of BDNF, trkB and PSA-NCAM markedly differed, appearing lower by 48%, 25% and 65%, respectively, in RLA vs RHA rats. WB analyses carried out after FST showed no differences between baseline and FST rats. In tissue sections, BDNF-, trkB- and PSA-NCAM-like immunoreactivity (LI) showed a distinctive labelling, mainly localized to proximal neuronal processes and nerve fibers distributed in the Ammon's horn and dentate gyrus (DG). A number of PSA-NCAM-positive neurons in the subgranular layer of dentate gyrus also occurred. Densitometric analysis further showed differences in the hippocampal subregions. Thus, upon FST, BDNF-LI was less abundant in the CA3 sector of the Ammon's horn of FST vs control RLA rats (-24%), whereas PSA-NCAM-LI was more abundant in the DG of RHA than RLA rats (+26%). Our findings suggest that an altered neuronal availability of and/or responsiveness to BDNF and inadequate dynamic events related to neuroplasticity may contribute to outline the molecular and morphological basis for the distinct vulnerability to stress-induced depression in the two rat lines.

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Keywords
Depression, BDNF, hippocampus, western blot, immunohistochemistry

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Osteological Markers of Malaria

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Malaria is an acute and chronic disease caused by a parasitic protozoan, the *Plasmodium*. Five species infect humans and one of them, the *Plasmodium falciparum*, is the most attested in the past by biomolecular research (1). Recently the connection between malaria and various skeletal and dental lesions like *Cribra Orbitalia* (2, 3), *Cribra Femuri* and Hypoplasia (4) was supposed, already related with nutritional deficiency during development. The aim of this study is to verify this connection comparing osteological and biomolecular data. Samples from Nord-West of Sardinia were examined: four necropolis ranging from the Prenuragic period (3000 BC) to Middle Age (1400 AD). The necropolis underwent analysis using standard anthropological methods. To verify the presence of *Plasmodium*, samples from each necropolis were analyzed using an immune-chromatographic approach; only the fragment from Nuragic period showed a positive signal to *Plasmodium falciparum*. *Cribra* were evaluated according to a scale present in literature for Orbitalia (5) and Femuri (6); to better evaluate them, each pathological sample underwent radiographic and TC analysis. Crossing malaria and osteological data we can see that hypoplasia seems not to be related to malaria because it is absent when there is the *Plasmodium falciparum*; on the contrary, Cribra seems to be related to *Plasmodium falciparum*, especially *Cribra Orbitalia* were the most severe and the most common. Thanks to our data, we can say that osteological diseases like *Cribra* can be used to diagnose ancient cases of malaria.

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Keywords
Malaria, Sardinia, Cribra, Paleopathology

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New pathogenetic perspectives in Pelvic Organ Prolapse (POP): the possible role of the cross-talk between AGEs, MAPK and Smads

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Collagen and MMPs play a pivotal role in the pathophysiology of the Pelvic Organ Prolapse (1). In POP samples a switch between type I and type III collagen together with a simultaneous activation of MMPs have been observed and the main consequence of these changes is the loss of mechanical support in the vaginal wall (2). Aim of this study was to prove that AGEs induces the activation of MMPs through ERK1/2 and synchronically stimulates changes in collagen composition directly through Smads. The case group consisted of 20 patients suffering from stage III genital prolapse undergoing colpohysterectomy and anterior and posterior plastic vaginal surgery and 10 control patients treated with laparohysterectomy for uterine fibromatosis. Histological and Immunohistochemical analysis using AGE, RAGE, ERK 1/2, Smads 2-3, Smad 7, MMP-3 and collagen I-III were performed. AGE and ERK 1/2 were also evaluated using Western-Blot analysis. POP samples from anterior vaginal wall showed disorganization and a distortion of the normal muscularis architecture. In POP samples AGE, ERK 1/2, Smad 2-3, MMP-3 and collagen III were upregulated in muscularis whereas in controls Smad 7 and collagen I were increased in the same layer. RAGE was mild or absent both in controls and prolapse. In summary we suggest the possible role of these new markers in the pathogenesis of POP but further studies are required to elucidate if the change of these molecules is the reason or the result of POP disease.

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Keywords
Pelvic organ prolapse, AGEs, RAGE, MAPK, Smads

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Inorganic nanoparticles interactions with dendritic cells

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Stimulation of the immune system may be of help for several diseases including cancer, for which the proposed vaccination strategies include the use of nanomaterials and dendritic cells (DCs) as adjuvants. Silica and gold nanoparticles (SiO_2NPs and AuNPs) are easy to produce and are endowed with high biocompatibility, tunable physicochemical properties and high adsorption power, which can lead to the formation of a protein corona. We have evaluated the interactions between human DCs and these types of NPs either alone or covered with a corona from cancer cell lysates.

AuNPs and SiO_2NPs were prepared [1-2] and exposed to lysates from two different cancer cell lines. Some SiO_2NPs were made fluorescent with rhodamine. The NPs and the protein corona were characterized by physico-chemical methods. DCs were generated in vitro from human monocytes [3], incubated up to 48 h with NPs at different concentrations and analyzed by phase contrast, fluorescence and electron microscopy, flow cytometry and mixed lymphocyte reaction.

When incubated with immature DCs, pure NPs were internalized and localized within vesicles and lysosomes. They did not cause cytotoxic nor stimulatory effects. The amount absorbed depended on NP concentration and did not increase appreciably between 4 and 24 h of incubation. Silica and gold NPs bound different pools of biomolecules from the same lysates. All lysate coated NPs promoted DC-mediated CD4+ cell proliferation. Lysate coated AuNPs also promoted DC maturation and DC-mediated CD8+ cell proliferation.

The results indicate that NPs are well tolerated by DCs and can represent a simple, cost-effective and versatile method to deliver antigens to DCs in view of cancer immunotherapy.

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Keywords
Silica, colloidal gold, protein corona, in vitro culture, microscopy, mixed lymphocyte reaction

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Musculocutaneous nerve variations. Meta-analysis of proportions and proposal for categorization

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The musculocutaneous nerve (MCN) is one of the main terminal branches of the brachial plexus. It provides motor innervation to coracobrachialis, biceps brachii and brachialis muscles and sensory innervation to the skin of lateral side of the forearm. In the normal anatomical description, the MCN arises from lateral cord and don't have communication with other terminal branches of brachial plexus. All motor branches arises from MCN, directly.[1] Despite these considerations, several variations of MCN have been reported. The most common are anomalous communications between MCN and median nerve. These communications could be relevant in clinical practice and could have several practical considerations that should be evaluated in different medical area, such as orthopedic surgery, traumatology or neurophysiology. Several classifications have been proposed but none of these is able to cover all aspects of this variation. Therefore, the aim of the present study are a systematic review of the available literature about MCN variations and a meta-analytic approach to define their prevalence.[2] At the same time, a new model of categorization with practical effects on clinical reasoning has been proposed. Several electronic databases have been searched. Articles have been screened and papers with anatomical description of MCN variations have been included. 43 out of 661 articles fulfilled inclusion criteria, with a description of 4695 brachial plexuses dissections. The random pooled prevalence of MCN variations is 18% (95%CI: 15-21%). The new categorization proposal is based on a 3 areas model: Area 1 (1A: absence of musculocutaneous nerve, 1B: variations before the division of the musculocutaneous nerve from lateral cord); Area 2: variations between origin of MCN from lateral cord and point of in coracobrachialis muscle (or same level if MCN does not pierce the muscle); Area 3: variations distal to point of entry in coracobrachialis muscle; Mixed areas: variations reported in more than a single area described above. Applying this model, the random pooled prevalence of reported variations is: Area 1A: 19% (95%CI: 11-28%), Area 1B: 26% (95%CI: 14-39%), Area 2: 46% (95%CI: 33- 59%), Area 3: 55% (95%CI: 40-70%), Mixed areas: 16 (95%CI: 8-25%). Therefore, MCN variations have a high prevalence. Among them, the most frequent are localized distal to coracobrachialis muscle. These results could be useful in clinical practice to point the attention at this anatomical region where variations in MCN are very common.

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Keywords

Musculocutaneous nerve, median nerve, anatomical variation

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Popliteal Artery Entrapment Syndrome (PAES) and Ankle-Brachial Index (ABI), any association?

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Popliteal Artery Entrapment Syndrome (PAES) is an uncommon pathology. Often, it affects young athletic males, with symptoms like calf claudication, weakness, numbness, pain, coldness of the foot, cramps, foot drop and paresthesia. The etiology of this syndrome is related to anatomical variations determining an altered relationship between popliteal artery and the surrounding structures. Based on described anatomical variations, PAES has been classified in several types (type I to V). In other cases, the compression of the popliteal artery may be caused by the hypertrophic gastrocnemius muscle, without anatomical variations, defining a "functional popliteal entrapment" (type VI). [1].

The Ankle-Brachial Index (ABI) is the ratio of the systolic blood pressure measured at the ankle to that measured at the brachial artery. It's a non-invasive measure of peripheral artery disease and can serve as a prognostic marker for cardiovascular events and functional impairment [2].

The ABI score is often pathological in patients affected by PAES. Nevertheless, to define diagnosis and type of PAES are often needed imaging studies and invasive procedures.

The aim of the present study is to systematically review the available literature to define if ABI score could be useful to predict a specific type of PAES.

Electronic databases have been searched using specific Keywords. Articles have been screened and full-texts of relevant papers have been retrieved. Case reports and case series with indications of symptoms, type of PAES, ABI score have been included. Results about ABI score (dependent variable) have been pooled and compared among types of PAES (independent variable). No statistical significance has been noted (ANOVA: $F=1.9$, $p=0.09$).

The use of ABI is insufficient to predict the type of PAES and its prognosis. Nevertheless, this non-invasive method could be useful to suspect PAES and as a tool in follow up in these patients.

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Keywords

PAES, ABI, popliteal artery, blood pressure

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Effects of acetylsalicylic acid on adiposity in a mouse model of diet-induced obesity

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Obesity is a growing public health problem and its prevalence has reached epidemic proportions in recent decades [1]. Several studies have demonstrated that obesity modifies the metabolic and endocrine functions of adipose tissue and is closely associated with chronic, low grade inflammation [2]. Because inflammation was proposed to be involved in the pathophysiology of obesity [1,2], we decided to evaluate the effects of the antinflammatory drug acetylsalicylic acid (ASA) in a mouse model of diet-induced obesity. We performed the experiments using C57BL/6J female mice fed for three months with Standard Diet (SD) or with High Fat Diet (HFD). At the end of three months, mice fed with HFD were separated in four groups and fed for other two months as follows: one group continued with HFD, one group returned to SD, one group continued with HFD with the addition of 30mg/kg of ASA and, finally, the last group returned to SD with the addition of 30mg/kg of ASA. ASA was administered in the drinking water. The metabolic and inflammatory status was evaluated by histological, molecular and biochemical analysis in all mice. As expected, HFD induced an increase in body weight and insulin resistance with a consequent reduction of glucose tolerance. Measurement of adipocyte size revealed that ASA significantly reduced HFD-induced adipocyte hypertrophy and it was able to revert insulin resistance with amelioration of glucose tolerance. Moreover, gene expression profiles of pro- and anti-inflammatory cytokines as well as the expression of macrophage and lymphocyte markers showed that HFD led to a significant increase in macrophages accumulation and an increase of inflammatory cytokines. However, we observed a significant trend for reduction of these molecules after treatment with ASA. The level of the anti-inflammatory molecules were also significantly increased after ASA administration. In conclusion, our results suggest that ASA can be proposed as pharmacologic option for reducing adipose tissue inflammation associated with obesity.

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Keywords

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High fat diet, Adipose tissue, Inflammation, Acetylsalicylic acid

Symmetry of zygomatic bone through 3D segmentation on CT-scan and "mirroring" procedure: a novel approach for reconstructive maxillofacial surgery

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Zygomatic bones are among those most frequently fractured facial bones [1]: symmetry is the golden standard for a correct restoration of zygomatic shape, but literature is divided about the best method for its quantification. Also, no information about the actual 3D symmetry of this bone in healthy subjects is available. This study aims at exposing an innovative approach for the assessment of zygomatic symmetry through 3D surface analysis.

One hundred patients (50 males and 50 females) were selected from the CT-scans database from a Northern Italy hospital. Zygomatic bones from each patient were segmented, the left bone was automatically mirrored according to the sagittal plane and registered on the right one according to the least point-to-point distance between the two surfaces. Mean and RMS (root mean square) distance between the two models was then calculated. Possible statistically significant differences according to sex and age groups were assessed respectively through two-way ANOVA test ($p<0.05$).

Repeatability of RMS measurements was 79%, with a technical error of 4.3%.Overall mean and RMS point-to-point distances were respectively 0.01 mm and 0.84 mm, without statistically significant differences according to sex or age group ($p>0.05$).

This study first provides an overall assessment of symmetry of zygomatic bone, based on surface analysis: results may provide a useful indication for the reconstruction of zygomatic bones in maxillofacial surgery.

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Keywords

Anatomy, zygomatic bone, radiology, maxillofacial surgery, CT scan

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Morphological characterization of a dietary challenged Sirtuin 1 heterozygous mice

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Sirtuin 1 (SIRT1), a member of the silencing information regulator 2 enzymes called sirtuins, is emerging as a master-regulator of metabolic functions like energy balance, mitochondrial health, browning of white adipose tissue, lipolysis. To best characterize its role in obesity, we analysed metabolic and morphological changes induced in SIRT1 heterozygous mice (HET) [1] by a high fat diet (5.4 Kcal/g from fatlard TD03584-Envigo) in comparison with C57BL6/J mice. Male C57BL6/J (WT) and HET mice received a standard maintenance diet (SD) (2.9 Kcal/g) or a high fat (HF) diet for 16 weeks from 12 to 28 weeks of age. Hepatic and epididymal white adipose depot (eWAT) reactions to the obesogenic diet were focused on hypoxia, inflammation, and endoplasmic reticulum stress. At euthanasia, blood was collected and the liver and eWAT removed for morphological analysis. WT HF and HET HF groups positively correlated with glucose intolerance, hepatomegaly and adipogenesis when compared with SD groups. Remarkably hepatosteatosis, fibrosis and inflammation were exacerbated in HET HF. Oxidative damage and abnormal lipogenesis were confirmed by elevated 4HNE and SREBP1 expressions. Hepatic mitochondria revealed myelinic figures and abnormal ER-mitochondria juxtapositions in HET HF [2]. eWAT adipocytes showed reduced perilipin but strong TNF-alpha and GRP78 signals in crown-like structures. In conclusion, HET HF mice might represent an intriguing animal model to best understand the complex pathogenesis of obesity and related disorders.

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Keywords

Sirtuin 1, perilipin, lipid droplets, ER stress, mitochondria, TEM

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Fasciacytes: specialized fibroblast-like cells that secrete the hyaluronan-rich matrix in fascial tissue

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The presence of hyaluronan (HA) in samples of fascia lata were determined in eight volunteers undergoing elective surgical procedures at the Orthopedic Clinic of Padova University. The methods used were: Alcian Blue staining with differential concentrations of the electrolyte MgCl2, immunohistochemistry with anti-HABP (HA-binding protein) and transmission electron microscopy (TEM). In additional biochemical studies, we extracted and quantified HA in fascial tissue using an HA assay (Biocolor). The results demonstrated the rich presence of HA within fascia. HA forms thin layers throughout the various fascial fibrous layers. The quantification assay documented a mean value of 40 μg HA/g in fascia. Histological and TEM analyses demonstrated the presence of two different types of stromal cells within fasciae: apparent fibroblasts and some modified fibroblast-like cells with specialized functions of HA synthesis and secretion. We termed these cells "fasciacytes". They may represent a new class of cells not previously recognized. This study confirmed that copious levels of HA occur within fascia, and provide quantification for the first time. In future studies, it will be important to compare these results in tissue from patients with myofascial pain and with rheumatic diseases.

Keywords

Fascia, connective tissue, hyaluronan, myofascial pain, fasciacytes

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Short Bowel Syndrome and Tissue Engineering: a preliminary study towards the development of a new regenerative approach in paediatric patients

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Pediatric Short Bowel Syndrome (SBS) is a malabsorption state following massive surgical resections of the small intestine. The current therapeutic options issues (i.e. parental nutrition, surgical lengthening, transplantation) have prompt the research in Tissue Engineering. Thus, our aim was to preliminary investigate in vitro/in vivo two composite scaffolds for engineering the small intestine without resorting to autologous intestinal epithelial organoid units which, to date, are the cell source mainly considered for this purpose. In particular, we developed composite supports consisting of a novel biocompatible/resorbable cryogel that is oxidized polyvinyl alcohol (OxPVA) [1] crosslinked with intestinal mucosa whole (wIM/OxPVA) or homogenized (hIM/OxPVA). After evaluating the scaffolds by histology and Scanning Electron Microscopy (SEM), we assessed their interaction with adipose mesenchymal stem cells. Thereafter, the in vivo behavior of scaffolds was studied implanting them in the omentum of Sprague Dawley rats. At 4 weeks, explants were processed by histology and immunohistochemistry (CD3; F4/80; Ki-67; desmin; α-SMA; MNF116). Considering the in vitro evidence, both histological and SEM results proved the effectiveness of the decellularization, and allowed to appreciate the preservation of intestinal villi of the wIM as well as the characteristic features of the hIM. At 7 days from cell seeding, MTT assay showed that hIM/OxPVA scaffolds could support cell adhesion/proliferation even if the wIM/OxPVA ones seem to significantly increase cell growth ($p < 0.01$). Considering in vivo data, around the cryogels was recognizable a continuous and relatively organized tissue wall; its thickness was greater in wIM/OxPVA scaffolds than in wIM/OxPVA and OxPVA (control) ones. The presence of Ki-67$^+$ elements, proving cell proliferation, was mainly ascribable to lymphocyte-macrophage populations and in minority to connective and myofibroblastic ones; primarily on the outer sides, CD3$^+$ and F4/80$^+$ cells were found. Moreover, the outer layer of the tissue wall showed a connective appearance partially immunoreactive for both anti-Desmin and -αSMA, which are related to myofibroblastic features and smooth muscle cells. In the parietal thickness, vascular structures with organized endothelium were found. Towards the polymer, cubic/cylindrical cells partially positive for anti-MNF116 were recognizable and they were ascribable to epithelial cells. Both scaffolds, albeit with some difference, are promising, nevertheless further analysis will be necessary.

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Keywords

Peripheral nerve injury, substance loss, nerve conduit, oxidized polyvinyl alcohol, peripheral nerve regeneration

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Specific ablation of phospholipase Cγ1 in forebrain causes manic-like behavior

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It is well known that manic episodes are one of the major diagnostic symptoms in a spectrum of neuropsychiatric disorders that include schizophrenia, obsessive-compulsive disorder and bipolar disorder (BD). Despite a possible association between BD and the gene encoding phospholipase Cγ1 (PLCG1), its etiological basis remains unclear. Here, we report that mice lacking phospholipase Cγ1 (PLCγ1) in the forebrain (Plcg1f/f; CaMKII) exhibit hyperactivity, decreased anxiety-like behavior, reduced depressive-related behavior, hyperhedonia, hyperphagia, impaired learning and memory and exaggerated startle responses. Inhibitory transmission in hippocampal pyramidal neurons and striatal dopamine receptor D1-expressing neurons of Plcg1-deficient mice was significantly reduced. The decrease in inhibitory transmission is likely due to a reduced number of γ-aminobutyric acid (GABA)-ergic boutons, which may result from impaired localization and/or stabilization of postsynaptic CaMKII (Ca2+/calmodulin-dependent protein kinase II) at inhibitory synapses. Moreover, mutant mice display impaired brain-derived neurotrophic factor-tropomyosin receptor kinase B-dependent synaptic plasticity in the hippocampus, which could account for deficits of spatial memory. Lithium and valproate, the drugs presently used to treat mania associated with BD, rescued the hyperactive phenotypes of Plcg1f/f; CaMKII mice. These findings provide evidence that PLCγ1 is critical for synaptic function and plasticity and that the loss of PLCγ1 from the forebrain results in manic-like behavior.

Keywords
Forebrain, Bipolar Disorders, Phospholipase Cγ1, Signalling

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Exercise and Nutrition effects on cartilage degenerative disorders

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Healthy lifestyle based on appropriate diet and not sedentary habits became of fundamental importance for the healthy aging and prevention of several diseases. The beneficial effects of Extra Virgin Olive Oil (EVOO), the main source of culinary and dressing fat of Mediterranean diet, have been, and still are, widely studied thanks to its anti-inflammatory and antioxidant properties. Lubricin is a chondroprotective glycoprotein, serving as a critical boundary lubricant between opposing cartilage surfaces. A joint injury causes an increased cytokine expression, which is associated with decreased lubricin production and predisposes to cartilage degeneration, leading to osteoarthritis. The aim of this study was to evaluate the beneficial role of EVOO-enriched diet and physical activity on cartilage tissue through the expression of lubricin in knee joints of rats after injury represented by anterior cruciate ligament transection (ACLT). To this purpose, we performed histomorphometric, histological, immunocytochemical, immunohistochemical, western blot and biochemical analysis for lubricin and interleukin-1 evaluations in articular cartilage and synovial fluid of rats. The results showed the beneficial effect of physical activity (treadmill training) and EVOO supplementation on the rat articular cartilage. ACLT determined an increase in interleukin-1 expression and a significant decrease in the lubricin expression, while physical activity and EVOO supplemented diet, determined that the values returned to a normal level when compared to the control group. In conclusion, the results showed a beneficial effect of the conjunction of EVOO-based diet, corresponding to the Mediterranean diet, and physical activity on the preservation of articular cartilage tissue.

Keywords

Extra Virgin Olive Oil, Physical Activity, Cartilage, Inflammation, Lubricin, Osteoarthritis

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Mesenchymal stem cell-based tissue engineering strategy for cartilage regeneration: A morphomolecular study

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Articular cartilage is an avascular and aneural tissue with poor self-repair capacity. Pathological conditions leading to the cartilage degeneration, such as osteoarthritis (OA), have prompted the development of strategies aimed to its regeneration, such as mesenchymal stem cells (MSCs)-based tissue engineering approach. The aim of this study was to investigate if chondrocytes, differentiated from rat adipose tissue derived-MSCs (AMSCs) and seeded on Collagen Cell Carrier (CCC) scaffolds, are able to constitute a morphologically and biochemically healthy hyaline cartilage. To this purpose the AMSCs were primarily differentiated in chondrocytes through chondrogenic medium and subsequently cultured for 6 weeks on CCC scaffolds. The expression of osteoblast (Runt-related transcription factor 2 (RUNX2) and osteocalcin), chondrocyte (collagen I, II and lubricin) and apoptosis (caspase-3) biomarkers were evaluated in undifferentiated AMSCs, AMSCs-derived chondrocytes cultured in monolayer and AMSCs-derived chondrocytes seeded on CCC scaffolds, by different techniques such as immunohistochemistry, ELISA, Western blot and gene expression analyses. AMSCs-derived chondrocytes cultured on CCC scaffolds showed the increased expression of collagen II and lubricin, whereas the expression of collagen I, RUNX2, osteocalcin and caspase-3 resulted decreased when compared to the other groups. In conclusion, the results of this study suggest a possible role of AMSCs and the use of CCC scaffolds for therapeutic strategies aimed to the articular cartilage regeneration.

Keywords

Mesenchymal Stem Cells, Collagen Cell Carrier, Cartilage, Apoptosis, Lubricin, RUNX2

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Possible Autophagy induction in Vernal Keratoconjunctivitis via Tumor Necrosis Factor Alpha Stimulation

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Tumor necrosis factor alpha (TNFα) is one of the main mediators of inflammatory response in many pathological diseases, involved in a widespread biological functions, including autophagy. Previous data obtained in our laboratory demonstrated that TNFα and some autophagy markers (which markers please indicate) are overexpressed in a severe inflammatory disease such as vernal keratoconjunctivitis (VKC).

In the present study we explored the role of TNFα in the induction of autophagy in VKC, using an *in vitro* model.

Primary conjunctival cell cultures were treated with TNFa and analysed by qPCR and western blotting for expression of some autophagy and lysosomial markers at 4, 10 and 24 hours after exposure. qPCR results demonstrated that LC3B, Beclin-1, LAMP1 and p62 strongly increased from 4 to 24 hours, whereas the expression of Catepsin D, a protein implicated in lysosomial apototic pathway, was comparable to that of untreated control. Western blotting analysis revealed lipidation of LC3B quantified as an increased LC3BII/LC3BI ratio. Moreover, double immunofluorescence for Cathepsin D and LAMP1 showed that Cathepsin D was localized within the lysosomes at 4, 10, 24 hours after cell exposure to inflammatory stimuli.

In conclusion, our data demonstrated that TNFα significantly induce in VKC LC3B lipidation, LC3BII/LC3BI ratio and p62 (qPCR) in the cells exposed to inflammatory stimuli which shows possible activation of autophagy pathway.

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Vascularized head and neck tumors and growth factors: immunohistochemical and rt-pcr profile in pediatric age

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Brain tumors account approximately for 20% of all childhood cancers and are characterized by a large diversity of morphological entities. The formation of abnormal, dysfunctional tumor vasculature and glioblastoma stem-like cells (GSCs) are believed to be the major components of the difficulty to treat these tumors effectively. Massive formation of blood vessels is one of the most important histological elements to determine the progression and histological grading of tumors. We hypothesized that an increased expression of TGF-β1 in tumor cells stimulates tumor neo-vascularization by mediating the secretion of relevant angiogenic factors via an autocrine mechanism. Expression of TGF-β in relation to VEGF and VEGF-receptors involved in angiogenesis and inflammation pathways was evaluated in pediatric patients with brain tumors and compared with normal tissues. Our results demonstrated that TGF-β1, VEGF-A and VEGF-RII were significantly related to the development and to the growth of glioblastoma. We can speculate that TGF-β1 and VEGF are involved in the cascade of the malignant progression of glioblastoma. These factors promote tumorigenesis and malignant progression of glioblastoma by a mechanism determining anti-apoptotic, angiogenetic and invasive behaviour of the tumor cells. Basing on our experimental data, we propose that VEGF may be the double promoter responsible not only for the tumor vessels, but also for the tumor stem cells [1]. Our data demonstrate that GSCs in association with high levels of VEGF-A and TGF-β play a key role in the development of the tumor vascularization acting on endothelial cells differentiation.

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Keywords
Childhood Cancers, Angiogenesis, Growth factors, IHC, RT-PCR

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Effects of acetylcholine precursors on inflammation markers in rat brain

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Choline is involved in important neurochemical processes. It is a precursor and metabolite of acetylcholine and plays a pivotal role in single-carbon metabolism and it is a fundamental constituent of membrane phospholipids such as phosphatidylcholine.

The role of choline and its precursors (e.g. choline alphoscerate, GPC) was recently investigated in experimental endotoxic shock. The obtained results suggest that these molecules may be useful in the treatment of endotoxemia/sepsis. On the other hand, a neuroprotective effect of choline precursors is well-documented and these actions may be related to their activity on inflammatory processes.

Based on these findings, the present study was designed to evaluate the effects of choline and GPC in inflammatory processes modulation in the rat brain.

Male Wistar rats were treated orally with choline, and GPC at choline-equivalent doses for 2 weeks or were left untreated. After this period, the brains were processed for Western blot analysis and immunohistochemistry. Inflammatory cytokines (IL1□, IL6, and TNF□) and endothelial inflammatory markers (ICAM-1, and VCAM-1) were studied in different cerebral areas (frontal cortex, hippocampus and cerebellum).

Treatment with choline or GPC has not influenced the expression of the inflammatory markers investigated in the brain areas examined. Hence, in this non-pathologic model, GPC, in spite of its neuroprotective effects [1,2], probably does not change or modulate brain inflammatory processes.

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Keywords
Choline, choline precursors, immunochemistry, immunohistochemistry, brain areas, inflammation

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Cobalt chloride supplementation differently affects human mesenchymal stem cells isolated from dental pulp, umbilical cord and adipose tissues in their chondrogenic potential

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Articular cartilage is an avascular tissue without innervations, characterized by low cell density and abundant extracellular matrix (ECM). These characteristics leave articular cartilage with very limited capacity of repair and regeneration.

Multipotent stem/stromal cells (MSC) are considered promising for cartilage tissue engineering. Stem cells are resided in a special microenvironment known as the stem-cell niche, characterized by the presence of low oxygen concentration.

Previous studies have reported that hypoxic conditions could enhance the chondrogenic differentiation of mesenchymal stem cells in the presence of an inductive medium. Cobalt chloride ($CoCl_2$) imitates hypoxia in vitro by preventing hypoxia-inducible factor-alpha (HIF-a) from being destroyed by oxygen. However, the long-term hypoxic culture of stem cells is difficult and requires special attention to avoid cell death due to cobalt treatment.

In this study we investigated if $CoCl_2$ affected MSCs isolated from dental pulp, umbilical cord and adipose tissue in their potential to differentiate toward the chondrogenic phenotype.

Cells were treated with concentrations of $CoCl_2$ ranging from 50 to 400 uM. Cell proliferation, mRNA expression of stem-cell marker and chondrogenic associated genes were analyzed by RT-PCR and Real-time PCR.

The results showed that the $CoCl_2$ supplementation had no effect on the proliferation of all the three type of cells analyzed, while the up-regulation of chondrogenic markers such as aggrecan, sox9, and type II collagen, was dependent on the cellular source.

This study shows that hypoxia induced by $CoCl_2$ treatment can differently influence the behavior of MSCs of different sources in their chondrogenic potential. These findings should be taken into consideration in the treatment of cartilage repair and regeneration based on stem cell therapies.

Keywords

Cobalt chloride, hypoxia, chondrogenic differentiation, mesenchymal stem cells, dental pulp, umbilical cord, adipose tissue

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The relation between moderate hearing loss with balance and postural control

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Background. Balance is a complex process that involves multiple sensory integrations. The auditory, visual and vestibular systems are the main contributors. Hearing loss or hearing impairment may induce inappropriate postural strategies that could affect the spine and consequently balance. The aim of this study was to understand if hearing loss could influence balance and posture

Methods. 13 patients (61±13 year; 161.8±11.0 cm; 70.5±15.9 Kg) with moderate hearing loss (Right ear -60±21 dB; Left ear -61±24 dB) underwent: 1) an audiometric examination 2) a postural examination (with open and closed eyes) through a stabilometric platform, and 3) a sternocleidomastoid EMG examination.

Results. No differences were found between right and left hemibody between the audiometric, posturographic, and the EMG amplitude. EMG parameters have shown no association with hearing loss, for both right or left head rotation. Multiple regression analysis has shown a negative regression coefficient (R2 -0.69) between hearing loss and the posturographic parameters.

Conclusions. Hearing loss is associated to increased posturographic measures (CoP, ellipse X and Y deviations) underlining a reduced postural control in people with hearing impairment. No association has been found between neck activation and hearing loss.

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Contribution of miR-145-5p/Ago2 complex to the regulation of epithelial-mesenchymal transition

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The epithelial-mesenchymal transition (EMT) is essential for cell fate determination during development but it is involved in pathological processes like cancer as well, being one of the first steps in the mechanisms leading to metastasis. miR-145-5p is one of the most widely recognized tumor-suppressor miRNAs, able to regulate cell migration and EMT through the contribution of the RISC complex in which Argonaute (Ago) proteins are required for target recognition and gene silencing [1]. Ago2 is an important member of the Ago family and its overexpression correlates with a transformed phenotype in breast cancer cells [2]. With the aim to unravel miR-145-5p/Ago2 contribution to the suppression of cancer progression in epithelial tumors, here we show that: i) miR-145-5p and Ago2 are down-regulated in breast tumor vs normal tissues; ii) the restored expression of miR-145-5p in breast cancer cell lines results in the reduction of tumor phenotype; iii) Ago2 expression is positively and specifically regulated by miR-145-5p; iv) miR-145-5p-dependent Ago2 induction is necessary for the inhibition of cell migration; v) when Ago2 is depleted, the formation of an alternative miR-145-5p/Ago1 active complex redirects miR-145-5p tumor suppressor function and correlates with a more invasive phenotype in breast cancer cells. These results open to the identification of miR-145-5p/Ago2-dependent molecular networks involved in the maintenance and progression of cancer phenotype.

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Keywords
EMT, miR145-5p, Ago2 protein

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A preliminary report on the characterization of epiretinal membranes excised from patients affected by macular pucker

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Among the various pathologies of the vitreoretinal interface, idiopathic macular pucker (MP) is one of the most puzzling. MPs are characterized by the formation of an epiretinal membrane (ERM) that grows in front of the fovea and results in a major impairment of vision. MPs are also frequently complicated by the deformation of the regular macular anatomy, with stretching and deformation of all retinal layers and loss of the foveal pit. This result is usually referred to the presence of myofibroblast-like cells on the retinal surface that alter macular anatomy possibly exerting a significant traction on ERMs. In order to shed light on the physiopathological events that lead to the development of idiopathic MPs, we carried out an immunofluorescence study by confocal microscopy on ERMs excised from 32 eyes with diagnosis of MP using a panel of antibodies including anti-collagen I, anti-collagen IV, anti-collagen VI, anti-smooth muscle actin (SMA), anti-glial fibrillary acidic protein (GFAP) and anti-vimentin antibodies. Some samples were also challenged with anti-heat shock protein (HSP) 47, anti-HSP 90 and anti-receptor II of the transforming growth factor (TGFβRII) antibodies. ERMs broadly varied in thickness. Mostly, they were formed by a layer of collagen 1 adjacent the internal limiting membrane, collagen IV and a layer of vimentin+ cells. Cells also co-expressed SMA or GFAP. Collagen VI, in contrast, was almost always scanty, frequently within the intracytoplasmic vesicles. Some membranes showed a very high content of collagen IV, so abundant to resemble the distribution of interstitial collagens. Cells were almost always restricted to the vitreal side of the membrane; only rarely they could be seen embedded between layers of extracellular matrix. They were frequently HSP90+ and sometimes they contained collagen-immunoreactive materials in their cytoplasm. They also showed TGFβRII within intracytoplasmic vesicles. All in all, ERMs have many features resembling those characterizing fibrotic processes.

Keywords

Macular pucker, retina, pancreas, epiretinal membrane, immunofluorescence, confocal microscopy

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Adaptive changes of telocytes in the urinary bladder of patients affected by neurogenic detrusor overactivity

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Urinary bladder activity involves central and autonomic nervous systems and bladder wall. Studies on the pathogenesis of voiding disorders such as the neurogenic detrusor overactivity (NDO) due to supra-sacral spinal cord lesions have emphasized the importance of an abnormal handling of the afferent signals from urothelium and lamina propria (LP). In the LP (and detrusor) three types of telocytes (TC) are present and form a 3D-network. TC are stromal cells able to form the scaffold that contains and organizes the connective components, to serve as guide for tissue (re)-modeling, to produce trophic and/or regulatory molecules, to share privileged contacts with the immune cells.

Specimens of full thickness bladder wall from NDO patients were collected with the aim to investigate possible changes of the three TC types by using histology, immunohistochemistry and transmission electron microscopy.

The results show that NDO causes several morphological TC changes without cell loss or network interruption. With the exception of those underlying the urothelium, all the TC display signs of activation (increase in Caveolin1 and caveolae, □SMA and thin filaments, Calreticulin and amount of cisternae of the rough endoplasmic reticulum, CD34, euchromatic nuclei and large nucleoli). In all the specimens a cell infiltrate, mainly consisting in plasma cells located in the vicinity or taking contacts with the TC, is present.

In conclusion, our findings show that NDO causes significant changes of all the TC. Notably, these changes can be interpreted as TC adaptability to the pathological condition likely preserving each of their peculiar functions.

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Distribution of synuclein immunoreactivity in the central nervous system of the South African clawed frog *Xenopus laevis*

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Alpha, β and γ synucleins (syns) have been identified in the nervous system of mammals and biochemical evidence suggest a crucial role for α-syn in the pathogenesis of several human neurodegenerative diseases. Our research is focused on the molecular expression and morphological localization of syns in the nervous system of representative species with the aim of understanding the evolutionary history of these proteins in vertebrates [1, 2]. Current model for our comparative analysis is the adult stage of the South African clawed frog *Xenopus laevis*. On the basis of previous studies on gene and protein expression of α- and β-syn in frog tissues, we have selected two antibodies immunoreactive for α- and β-syn for the immunohistochemical localization of *Xenopus* syns in the CNS. Double-immunohistostainings for ChAT , TH or serotonin were performed in order to analyze the distribution of syn immunoreactivity in cholinergic, catecholaminergic and serotoninergic areas. Both α- and β-syn were localized in the frog retina and in several brain regions with different patterns of distribution. Syn proteins are expressed in the retina of a wide range of vertebrates, including humans, and this suggests that retinal neurodegenerative diseases may be mediated by synucleinopathies. Strong α-syn immunoreactivity was also found in the visual projections and in the interpeduncolar nucleus, interspersed with cholinergic fibers, whereas β-syn immunoreactive axons formed a dense network in the ventral and dorsal striatum within the catecholaminergic plexus that plays a key role in the movement control in amphibians as in mammals. Present data are the background for further studies on physiological roles of syns during the vertebrate evolution.

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Keywords
Synuclein, central nervous system, eye, Xenopus levis

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Gross anatomy study on isolated formalin-fixed anatomical preparations

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The anatomic dissection has always had a crucial role in teaching gross Anatomy in medical academic courses. Nowadays the easy availability of several programs of virtual dissection together with the difficulties in finding useful human bodies as well as the complicated procedures of preparation, make anatomic dissection practice barely available in our Universities. The use of dissected preparations obtained from isolated organs or well-defined anatomical regions rather than the use of whole cadavers may be useful in many instances to overcome the difficulties described above. In particular, the long-established use of formalin-fixed specimens may still represent an easy and useful support to teach gross anatomy. However, due to its toxicity and cancerogenic activity, formalin use as a fixative has been recently strongly restricted, making both dissection as well as prosection of formalin-fixed specimens difficult to be performed. The E. Morelli's Museum, located in the C. Forlanini Hospital of Rome, contain a collection of anatomical preparations dating back to the half of past century, which represents not only a historical but also a scientific and didactic patrimony. This collection includes over one thousand of anatomical preparations, the majority of which are fixed and stored in unsealed glass vase. Furthermore, at present most preparations, many of which are of admirable workmanship, are abandoned. Therefore, on behalf of Sapienza University of Rome, during the last months we are engaged in the recovery and safeguard of these specimens. The procedures we are using include systematic cataloguing of all specimens, adding with photograph and comments as well as several recovery treatments, tailored to the singular specimen condition (change or recovery of split vases, complete or partial change of fixative fluid, improvement of the presentation of anatomical preparation, etc.). All vases were finally filled up with fixative and sealed with a synthetic resin, which polymerizes under water. This procedure of airtight closure of vases prevents any dispersion of formalin gas in the air, making anatomical preparations available for educational as well as museum purposes. Our final goal is the recovery, restoration and cataloguing of these preparations in order to reevaluate the Anatomic Museum center as the foundation of Medical school training.

Keywords

Gross anatomy, Anatomical Museum, dissection, didactic center

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Spinal cord pathology during chronic exposure to MPTP in mice

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MPTP (1-metyl 4-phenyl 1,2,3,6-tetrahydro-piridin) treatment is a validated toxic model to produce experimental parkinsonism. Chronic exposure to little amounts of MPTP has been reported to reproduce neuropathological features characterizing dopaminergic neurons in Parkinson disease (1). Recent studies by our group have found that MPTP, besides dopaminergic neurons, affects also spinal motor neurons (2), thus reproducing also the neuropathology of spinal cord in Parkinson's disease.

The aim of the present study is to investigate, through immune-histochemistry, the involvement of spinal motor neurons after chronic administration of MPTP.

Chronic MPTP treatment is able to induce motor neurons loss in the lumbar spinal cord. Increased immune-reactivity of the autophagy protein MAP LC3β is detectable in motor neurons which indicates the activation of autophagy. Immune-staining for alpha-synuclein, the hallmark protein for Parkinson's disease, was altered in spinal motor neurons of MPTP treated mice, as detected by two novel homemade monoclonal antibodies. Furthermore, immunohistochemical alterations have been detected in the Renshaw cells and in tyrosine-hydroxylase-containing axons in line with the alterations of noradrenergic projections in Parkinsons's disease (3).

Our results suggest that spinal cord may contribute to motor symptoms occurring in Parkinson's disease.

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Keywords
Alpha-synuclein, spinal cord, Parkinson's disease

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Modulation of MMP-2 function in bone marrow mesenchymal stromal cells requires sphingosine 1-phopsphate receptor 1 mediated signaling: implications for cytoskeletal assembly and proliferation

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Bone-marrow-derived mesenchymal stromal cells (BM-MSCs)–based therapy represents a promising option in the field of regenerative medicine. Their therapeutic potential is mainly dependent on paracrine secretion, proliferation and ECM remodeling abilities whose modulation involves Matrix Metalloproteinase (MMP)-2 functionality. Thus, the identification of paracrine/autocrine factors regulating MMP-2 expression/activity may be of great biological relevance for potentiating BM-MSC therapeutic efficacy.

Our research group has demonstrated that BM-MSCs release the bioactive lipid sphingosine-1-phosphate (S1P). Here we demonstrated : i) the requirement for BM-MSC of S1P production to synthesize functional gelatinases; ii) an impairment of gelatinolytic activity and MMP-2 expression/release when the S1P receptor subtype 1 (S1PR1) is blocked. Notably, in these experimental conditions BM-MSCs did not exhibit the formation of plasmamembrane-associated F-actin structures (lamellipodia, filopodia, microspikes) and, in turn, showed a reduction of the proliferation rate. Moreover, S1P1-mediated signaling is required for HIF-1alpha expression and MMP-2 expression/activity, reduction of vinculin expression and stress fiber formation and proliferation in hypoxia, an experimental condition mimicking the injured/regenerating tissue microenvironment.

In conclusion, our findings, demonstrating the trophic role exerted by the autocrine S1P/S1PR1 signaling in maintaining BM-MSC ability to modulate MMP-2 function, required for ECM remodeling, cytoskeleton assembly and cell proliferation may provide perspectives for considering S1P/S1PR1 as a pharmacological target to preserve BM-MSCs properties and improve their efficacy in tissue repair.

Keywords

Bone marrow-derived mesenchymal stromal cells (BM-MSCs), sphingosine 1-phosphate (S1P) receptor 1, metalloproteinases, cell proliferation, cytoskeleton remodeling, regenerative medicine

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The Master athlete: An extraordinary physiological model of aging study, a delicate issue for cardiologists and sports physicians

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The prolongation of average life in the industrialized countries and the definitive demonstration of preventive and therapeutic role of regular physical exercise and sport, have greatly increased the number of middle-aged and older subjects engaged in the regular practice of sports activities, not only for fun or healthy purposes, but also at competitive level. The creation by sports federations of age categories (five years in five years) has strengthened the agonistic nature of the activity. Master athletes compete not only against adversaries of the same age group but even against themselves and the Time flowing inexorably. At the scientific and clinical level, two are the fundamental implications of this phenomenon. The first is the positive effect of a regular and intense performance training, both anaerobic and aerobic power. In the latter, regular and intense training is able to slow down significantly (even 50%) the natural, progressive decline of cardiorespiratory functions observed in healthy sedentary subjects of the same age. The second, the reverse of the medal, is the difficulty encountered by sports physician and cardiologist to correctly interpret the clinical/instrumental features of the Master athlete who undergoes pre-participation screening for competitive sports. It is not always easy to differentiate the physiological, adaptive, changes of a middle-aged and older athlete from the pathological ones, related to cardiovascular disease, typical of aging, such as ischemic heart disease, arrhythmias, hypertension, valvular diseases. These difficulties can only be solved by having an adequate knowledge of the clinical and instrumental manifestations of the Master Athlete's Heart and individual cardiopathies, and with the careful use of all modern cardiological instrumental investigations. In addition to echocardiography and maximal ECG stress-test (preferably cardio-pulmonary test), the magnetic resonance imaging with Gadolinium, and coronary tomography (TC) are playing a decisive role. [1]

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Keywords

Master Athlete, anaerobic and aerobic power, aging, cardiovascular diseases

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The thrombopoietin/MPL axis is activated in the *Gata1low* mouse model of myelofibrosis and is associated with a defective RPS14 signature

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Myelofibrosis is characterized by hyperactivation of thrombopoietin signaling which induces a RPS14 deficiency that de-regulates GATA1 in megakaryocytes by hampering its mRNA translation. Since mice carrying the hypomorphic Gata1low mutation, which reduces the levels of Gata1 mRNA in megakaryocytes, develop myelofibrosis[1] (Zingariello M. et al. 2015), we investigated whether the thrombopoietin axis is hyperactive in this model. Gata1low mice contained 2-times more Tpo mRNA in liver and TPO in plasma than wild-type littermates. Furthermore, Gata1low LSKs expressed levels of Mpl mRNA (5-times greater than normal) and protein (2-times lower than normal) similar to those expressed by LSKs from TPO-treated wild-type mice. Gata1low marrow and spleen contained more JAK2/STAT5 than wild-type tissues, an indication that these organs were reach of TPO-responsive cells. Moreover, treatment of Gata1low mice with the JAK inhibitor ruxolitinib reduced their splenomegaly. Also in Gata1low mice activation of the thrombopoietin/MPL axis was associated with a RSP14 deficiency and a discordant microarray ribosome signature (reduced RPS24, RPS26 and SBDS expression). Finally electron microscopy revealed that Gata1low megakaryocytes contained poorly developed endoplasmic reticulum with rare polysomes. In summary, Gata1low mice are a bona-fide model of myelofibrosis which recapitulates the hyperactivation of the TPO/MPL/JAK2 axis observed in megakaryocytes from myelofibrotic patients.

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Keywords

MPL, Thrombopoietin (TPO), JAK2, GATA1, megakaryocytes

VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI SIAI, 2016

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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 Polo Universitario "Giovanni XXIII" della Facoltà di Medicina e Chirurgia "A. Gemelli", Università Cattolica del Sacro Cuore in Roma

In data 16 Settembre 2016, 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 Polo Universitario "Giovanni XXIII" della Facoltà di Medicina e Chirurgia "A. Gemelli", Università Cattolica del Sacro Cuore di Roma, in occasione del 70° Congresso Nazionale SIAI, con il seguente Ordine del Giorno:

1. Comunicazioni del Presidente.
2. Approvazione del verbale della seduta precedente.
3. Commemorazione dei Soci scomparsi.
4. Relazione del Tesoriere sul rendiconto finanziario del 2015 e sulla previsione finanziaria per il 2017. Relazione dei Revisori dei Conti.
5. Attività dei Collegi dei Docenti di Anatomia e di Istologia ed Embriologia: Relazione dei Presidenti o loro Delegati.
6. Assegnazione Premio Ricercatore under 40.
7. Assegnazione Premio alla Carriera.
8. Assegnazione Premi Poster.
9. Assegnazione Premio Migliore Comunicazione Orale.
10. Prossimi Congressi nazionali della SIAI e Congressi nazionali ed internazionali previsti per l'anno 2017; proposte di temi di relazione.
11. Problemi relativi all'Italian Journal of Anatomy and Embryology: relazione dell'Editor in Chief, Prof. Paolo Romagnoli.
12. Proposta di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.
13. Varie ed eventuali.

Presiede la riunione il Prof. Eugenio Gaudio, Presidente della SIAI; funge da Segretario Verbalizzante la Prof. Gigliola Sica, Segretario della SIAI.1. Comunicazioni del Presidente.

Il Presidente della SIAI, Prof. Eugenio Gaudio, apre i lavori e, a nome di tutti, ringrazia calorosamente i Presidenti del Congresso, Proff. Fabrizio Michetti e Gigliola Sica, nonché i loro Collaboratori, per l'impegno profuso nell'organizzazione del 70° Congresso Nazionale SIAI.

Il Prof. Gaudio ricorda che in data 7 Giugno 2016 è stato emanato il Decreto N.120 (G.U. n.155 del 5/07/2016) relativo al Regolamento recante criteri e parametri per la valutazione dei candidati ai fini dell'attribuzione dell'Abilitazione Scientifica Nazionale per l'accesso alla prima e alla seconda fascia dei Professori Universitari, nonché le modalità di accertamento della qualificazione dei Commissari. Questa problematica è stata affrontata nella riunione del Consiglio Direttivo del 15 Luglio 2016 e in tale

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occasione, dopo un serrato dibattito, è stata espressa una sostanziale adesione ai rilievi fatti, in data 7 Luglio 2016, dal CUN al Ministro dell'Istruzione, dell'Università e della Ricerca, Stefania Giannini, e si è ipotizzato di poter stilare un documento in cui esporre le riserve della SIAI ed i suggerimenti scaturiti dal dibattito, a supporto del CUN.

Successivamente, in data 26 Luglio 2016, il CUN, esaminando la proposta dell'ANVUR concernente la determinazione dei valori soglia degli indicatori da utilizzare, ha espresso un parere di non condivisione relativo al metodo e ai criteri con i quali sono stati identificati tali valori, poiché, specie in riferimento ai candidati, l'ASN viene trasformata in una procedura di natura intrinsecamente comparativa. Il CUN non condivide la scelta di fondare l'individuazione delle soglie esclusivamente su basi statistiche e di effettuare una selezione degli aspiranti commissari e degli abilitandi sulla base di una percentuale che, in generale, supera di poco la metà della platea dei potenziali candidati e, nel caso dell'abilitazione alla seconda fascia, rimane al di sotto della metà. Il CUN ha ribadito che i valori soglia dovrebbero essere fissati sulla base di pareri informati e motivati, fondati su principi di ragionevolezza e significatività e su criteri di adeguatezza, rilevando i numerosi aspetti critici presenti nella proposta dell'ANVUR.

Il Prof. Gaudio conclude affermando che la procedura per l'ASN influenzerà in maniera significativa il destino delle nostre Università e che sarà importante essere presenti in maniera intelligente nelle varie Sedi.

2. Approvazione del verbale della seduta precedente.

Il Presidente propone all'Assemblea l'approvazione del verbale della seduta precedente e l'Assemblea approva all'unanimità.

3. Commemorazione dei Soci scomparsi.

Il Prof. Lorenzo Fumagalli commemora il **Prof. Carlo Cavallotti**, scomparso nel mese di Marzo 2016. I Proff. Lucio Cocco e Nadir Mario Maraldi commemorano il **Prof. Francesco Antonio Manzoli**, scomparso nel mese di Aprile 2016. La Prof. Chiarella Sforza commemora la **Prof. Magda Gioia**, scomparsa nel mese di Aprile 2016. Il Prof. Sergio Castorina commemora il **Prof. Pietro Petriglieri**, scomparso nel mese di Settembre 2016.

4. Relazione del Tesoriere sul rendiconto finanziario del 2014 e sulla previsione finanziaria per il 2016. Relazione dei Revisori dei Conti.

Il Presidente legge il verbale stilato nella riunione dei Revisori dei Conti, Prof. Silvano Capitani, Prof. Andrea Porzionato e Prof. Elio Ziparo.

"Il giorno 16 Settembre 2016 si è riunita la Commissione dei Revisori dei Conti designata in seno alla Società Italiana di Anatomia e Istologia e costituita dai Proff.: Silvano Capitani, Elio Ziparo e Andrea Porzionato.

Dopo aver valutato attentamente il conto Consuntivo relativo all'anno 2015 e il conto di previsione relativo all'anno 2017 presentati dal Tesoriere, Prof. Amelio Dolfi, la suddetta Commissione approva all'unanimità le risultanze dei conti esaminati."

Il Presidente dà la parola al Prof. Amelio Dolfi, che illustra il rendiconto finanziario del 2015, qui di seguito riportato assieme alla relazione di accompagno.

Bilancio consuntivo anno 2014

Causale delle entrate	Entrate Euro	Causale delle uscite	Uscite Euro
Quote sociali incassate nel corso dell'anno 2015 (n°320) 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 (n°2)	**10.334,80**		
		Elenco spese per attività statutarie	
		Contributo I.J.A.E., anno 2014	**4.000,00**
		Quote di Iscrizione al Congresso SIAI 2015 di due Soci vincitori dei premi poster, anno 2014	**680,00**
		Contributo per l'organizzazione del 69° Congresso SIAI, anno 2015	**3.000,00**
		Contributo per l'organizzazione del Convegno G.I.S.N., anno 2015	**500,00**
		Premio alla Carriera, anno 2015	**585,60**
		Spese varie (mantenimento conto corrente postale e bancario, spese bollo e commissioni bancarie ecc., anno 2015)	**1.084,81**
		Rimborso spese per partecipazione alla riunione EFEM, anno 2015	**839,73**
		Spese impreviste: storno somme erroneamente versate alla SIAI, anno 2015	**74,80**
		Premio Giovane Ricercatore, anno 2015	**2.000,00**
		Iscrizione della SIAI a EFEM, anno 2015	**520,00**
		Premio alla migliore comunicazione orale presentata al Congresso Nazionale SIAI, anno 2015	**1.000,00**
		Elenco spese di funzionamento	
		Compenso per Consulenza Commercialista relativa alla stesura del Bilancio Consuntivo, anno 2014 e Bilancio Previsionale, anno 2016	**1.500,60**
		Versamento deleghe fiscali per compensi Commercialista, anno 2014	**280,80**
		Spese relative all'utilizzo del server UNIFI per il sito web SIAI, anno 2014	**272,06**
		Spese per il funzionamento del Consiglio Direttivo, anno 2015	**825,56**

Totale entrate	**10.334,80**	**Totale uscite**	**17.163,96**
Avanzo d' esercizio finanziario 2015	**- 6.829,16**		
Saldo Conto corrente Bancario al 31/12/2014	**17.800,70**		
Saldo Conto corrente postale al 31/12/2014	**5.045,23**		
Totale saldo finanziario al 31/12/2014	**22.845,93**		
Disavanzo dell'esercizio finanziario 2015	**6.829,16**		
Saldo finanziario al 31/12/2015	**16.016,77**		

Stanziamenti impegnati al 31/12/2015	**Euro**	**Euro**
Accantonamento premi poster e comunicazione, anno 2015 (rimane da erogare premio migliori poster, anno 2015)		**1.000,00**
Contributo all'It. J. Anat. Embryol., anno 2015		**4.000,00**
Spese per il sito web della Società, anno 2015		**400,00**
Spese per ECM, anno 2015		**500,00**
Spese per il funzionamento della Presidenza, anno 2015		**1.000,00**
Spese per il funzionamento della Segreteria, anno 2015		**1.000,00**
Spese per il funzionamento della Tesoreria, anno 2015		**1.000,00**
Compenso per Consulenza Commercialista relativa alla stesura del bilancio consuntivo anno 2015 e bilancio previsionale, anno 2017		**1.780,00**
Totale impegno di spesa		**10.680,00**
Saldo disponibile	**5.336,77**	

*Relazione di accompagnamento al rendiconto economico e finanziario per l'anno **2015***

Come risulta dal bilancio consuntivo il saldo finanziario al 31/12/ 2015 è pari ad **€ 16.016,77** ed è costituito dal saldo finanziario al 31/12/2014 pari a **€ 22.845,93** detratto del disavanzo dell'esercizio 2015 pari a **€ 6.829,16**.

A tale importo devono essere sottratti **€ 10.680,00** impegnati nel Bilancio Previsionale del 2015, ma non ancora effettivamente utilizzati alla data del 31/12/2015, per le seguenti voci di spesa:

- **Accantonamento premi poster e comunicazione orale, anno 2015: € 1.000,00;**
  Per questa voce risultano stanziati, nel previsionale del 2015, **€ 2.000,00** che in parte (**€ 1.000,00**) sono stati utilizzati nel corso del 69° Congresso Nazionale del-

la Società del 2015 per il premio alla migliore comunicazione orale e nella parte rimanente (**€ 1.000,00**) saranno utilizzati per il pagamento delle quote di iscrizione al 70° Congresso Nazionale della Società del 2016 di due Soci risultati vincitori dei premi poster nel Congresso societario del 2015;

- **Contributo all'It. J. Anat. Embryol.,** anno 2015: **€ 4.000,00;**
- **Spese per il sito web della Società,** anno 2015: **€ 400,00;**
- **Spese per ECM,** anno 2015: **€ 500,00;**
- **Spese per il funzionamento della Presidenza,** anno 2015: **€ 1.000,00;**
- **Spese per il funzionamento della Segreteria,** anno 2015: **€ 1.000,00;**
- **Spese per il funzionamento della Tesoreria,** anno 2015: **€ 1.000,00;**
- **Consulenza Commercialista,** anno 2015: **€ 1.780,00.**

Pertanto l'anno 2015 si chiude con un saldo disponibile di **€ 5.336,77**.

Durante il 2015 le quote associative incassate sono state 172 comprese alcune quote arretrate ed integrazioni di versamenti di quote non corretti, per un totale di **€ 10.334,80** che sommate al saldo finanziario al 31/12/2014 (**€ 22.845,93**), hanno dato la disponibilità di **€ 33.180,73**.

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, etc.**).

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 appena sufficiente, di conseguenza, al 31 dicembre 2015, rimane ancora un numero significativo di Soci che debbono regolarizzare la loro posizione; da questo fatto deriva la impossibilità di effettuare previsioni fondate. Il Tesoriere sottolinea che l'eventuale recupero delle quote arretrate consentirebbe alla SIAI di effettuare una adeguata programmazione delle attività statutarie e di intraprendere nuove iniziative.

Il Presidente, nel ringraziare il Prof. Dolfi per l'accuratezza del rendiconto pone in votazione il Bilancio Consuntivo 2015.

L'Assemblea approva all'unanimità.

Il Presidente dà quindi la parola al Tesoriere per illustrare la Previsione Finanziaria per il 2017, qui di seguito riportata assieme alla relazione di accompagnamento.

SOCI NEL 2015:	**526**
SOCI NEL 2016:	**534**
SOCI ORDINARI 2016:	**504**
SOCI DIMISSIONARI 2016:	**7**

ENTRATE

Quote sociali anno 2016 **(n. 497)**	€	29.820,00
Quote sociali arretrate 2007 - 2015	€	6.000,00
Totale entrate	**€**	**35.820,00**

USCITE

Contributo al Convegno Nazionale 2017, atti di convegni, altri contributi a convegni, partecipazione a convegni, organizzazione eventi scientifici, borse di studio, etc.	€	12.000,00
Accantonamento per premi poster dell'anno 2016 e per premio comunicazione assegnato nell'anno 2017	€	2.000,00
Accantonamento per premi SIAI (Premio alla Carriera e Premio Ricercatore under 40), anno 2017	€	4.000,00
Contributo all'Italian Journal of Anatomy and Embryology, anno 2017	€	4.000,00
Spese per sito web della Società, anno 2017	€	300,00
Spese per la partecipazione Meeting Comitato Internazionale per la Terminologia Anatomica e Istologica, FICAT, anno 2017	€	3.500,00
Quota adesione all'European Federation for Experimental Morphology, EFEM, anno 2017	€	540,00
Spese varie (bancarie, postali, necrologi, etc.), anno 2017	€	1.200,00
Spese impreviste, anno 2017	€	1.000,00
Totale spese per attività statutarie	***€***	***29.040,00***
Spese per il funzionamento della Presidenza, anno 2017	€	1.000,00
Spese per il funzionamento della Segreteria, anno 2017	€	1.000,00
Spese per il funzionamento della Tesoreria, anno 2017	€	1.000,00
Spese per il funzionamento del Consiglio Direttivo, anno 2017	€	2.000,00
Spese per consulenza Commercialista, anno 2017	€	1.780,00
Totale spese di funzionamento	***€***	***6.780,00***
Totale uscite	**€**	**35.820,00**

Relazione di accompagnamento alla previsione finanziaria per l'anno 2016

La chiusura del bilancio consuntivo del 2015 con un saldo disponibile di **€ 5.336,77** ha permesso al Tesoriere di sostenere alcune spese indicate nella previsione finanziaria del 2016.Il Tesoriere, nel corso di questo anno, oltre a cercare di riscuotere le quote associative del 2016, ha continuato l'azione di recupero di quelle arretrate. Al 31 agosto '16, sono state incassate 306 quote sociali comprensive di quelle relative all'anno 2016 e arretrate. E' probabile che in questo periodo altri Soci abbiano provveduto al pagamento, ma al momento non siano stati considerati in questo resoconto.

Il totale delle entrate è attualmente pari a **€ 18.401,00** e comprende le quote riscosse finora.

Comunque il piano previsionale del 2016 prevedeva entrate pari a **€ 35.640,00** dovute alla riscossione delle quote dell'anno in corso, più una cifra forfettaria concernente il recupero delle quote arretrate. In particolare, in tale previsione, come in

quelle degli anni precedenti, è stata indicata questa cifra forfettaria sulla base dell'esperienza relativa alle difficoltà di ottenere il pagamento degli arretrati da tutti i Soci non in regola.

La Società conta attualmente **534** Soci, di cui **504** Soci ordinari e **30** Soci Emeriti o Onorari (esonerati dal pagamento della quota sociale). Nel corso del 2016 **7** Soci ordinari hanno espresso la volontà di rassegnare le dimissioni dalla Società.

Allo stato attuale, dei 497 Soci che sono tenuti a pagare la quota associativa:

- 1 Socio è in regola fino al 2018
- 2 Soci sono in regola fino al 2017
- 133 Soci sono in regola fino al 2016
- 88 Soci in pari con il 2015 devono la quota 2016
- 64 Soci in pari con il 2014 devono le quote del 2015 e del 2016
- 25 Soci in pari con il 2013 devono le quote del 2014, 2015 e 2016
- 28 Soci in pari con il 2012 devono le quote del 2013, 2014, 2015 e 2016
- 40 Soci in pari con il 2011 devono le quote del 2012, 2013, 2014, 2015 e 2016
- 15 Soci in pari con il 2010 devono le quote del 2011, 2012, 2013, 2014, 2015 e 2016
- 31 Soci in pari con il 2009 devono le quote del 2010, 2011, 2012, 2013, 2014, 2015 e 2016
- 26 Soci in pari con il 2008 devono le quote del 2009, 2010, 2011, 2012, 2013, 2014, 2015 e 2016
- 25 Soci in pari con il 2007 devono le quote del 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015 e 2016
- 19 Soci in pari con il 2006 devono le quote del 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015 e 2016

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 2016 un certo numero di soci ha risposto positivamente all'azione di richiamo per il recupero delle quote arretrate. Rimane ancora un discreto numero di Soci che non hanno adeguatamente risposto ai solleciti di pagamento e, in base a quanto stabilito nello statuto e al parere in merito espresso dal Direttivo SIAI, è in atto una revisione dell'elenco dei soci.

Il tesoriere ricorda che gli scopi istituzionali della Società Italiana di Anatomia e Istologia sono essenzialmente la promozione della ricerca e della didattica nel campo delle discipline anatomiche e istologiche, pertanto l'incasso puntuale delle quote annuali e il recupero delle quote arretrate permetterebbero alla SIAI di raggiungere al meglio questi scopi.

Il Presidente ringrazia il Prof. Dolfi per la precisione nella rendicontazione dei documenti, per l'impegno che ha dimostrato nell'assolvimento della sua impegnativa carica di Tesoriere e pone in votazione la Previsione Finanziaria per il 2017.

L'Assemblea approva all'unanimità.

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

Il Prof. De Caro, Presidente del Collegio dei Docenti di Anatomia Umana, presenta una breve relazione che prende in esame le riunioni che si sono tenute, le principali comunicazioni rivolte ai Soci, i più importanti documenti prodotti ed il calendario dei prossimi incontri.

Riferisce che, in data 15 Settembre 2016, si è svolta l'Assemblea ristretta dei Professori Ordinari del Collegio dei Docenti di Anatomia Umana, in cui è stato presentato il documento elaborato dal Gruppo di Lavoro composto dai Proff. Giuseppe Anastasi, Raffaele De Caro, Eugenio Gaudio, Fabrizio Michetti e Stefania Montagnani, delegato dall'Assemblea ristretta dei Professori Ordinari del Collegio di Anatomia Umana nella seduta del 25.06.2016 al seguente compito: valutazione dei titoli dal 2 all'11 (all. A del DM n. 120 del 07.06.2016) al fine di proporre all'istituenda Commissione almeno 6 titoli fra i quali gli aspiranti candidati devono possederne almeno 3 (oltre i previsti valori soglia) per essere ammessi all'Abilitazione Scientifica Nazionale di I o di II fascia.

Il Gruppo di Lavoro ha preso visione del DM n. 120 del 07.06.2016, del Decreto Direttoriale n. 1532 del 29.07.2016, recante le procedure da seguire per la partecipazione all'Abilitazione Scientifica Nazionale, e delle considerazioni del CUN e ritiene importante sottolineare che l'obiettivo principale del DM n. 120 è quello di ampliare sino al 60% il numero dei potenziali aspiranti. Il Gruppo di Lavoro ha rilevato che limitare solo a 6 dei 10 possibili titoli, fra i quali ciascun candidato deve possederne almeno 3, determinerebbe una riduzione del potenziale numero dei candidati e, pertanto, suggerisce al Collegio dei Docenti di proporre che i candidati all'Abilitazione Scientifica Nazionale di I e di II fascia debbano possedere almeno 3 titoli tra i 10 richiesti per poter accedere all'Abilitazione Scientifica Nazionale.

In altri termini, il Gruppo di Lavoro propone al Collegio dei Docenti di Anatomia Umana che le Commissioni considerino tutti e 10 i titoli di cui ai punti 2-11 dell'Allegato A.

Il Gruppo di Lavoro ritiene, inoltre, che trattandosi di una Abilitazione Scientifica Nazionale, e non di una valutazione comparativa, non debba essere attribuita a ciascuno dei titoli una valutazione prioritaria.

Il Gruppo di Lavoro, prima di chiudere i lavori, ha suggerito che, oltre ai criteri per la valutazione delle pubblicazioni scientifiche, riportati all'art. 4 del DM n. 120 del 07.06.2016, sia da raccomandare alla Commissione di valutare la coerenza del percorso complessivo di maturazione scientifica del candidato.

La Prof. Sica, Presidente del Collegio dei Docenti di Istologia ed Embriologia, riferisce che, oltre a portare avanti la gestione di tale Collegio dal punto di vista amministrativo insieme al Tesoriere, Prof. Gianpaolo Papaccio, ha provveduto ad organizzare le necessarie riunioni della Giunta e l'Assemblea annuale. Tale Assemblea si è tenuta a Roma il giorno 11 Marzo 2016. In realtà essa era stata programmata nel mese di Dicembre 2015, ma successivamente annullata poiché il numero legale per la validità dell'Assemblea stessa non sarebbe stato raggiunto, a causa delle numerose comunicazioni di assenze ricevute.

Il Collegio ha una storia di grande partecipazione, ma recentemente, forse anche a causa dei risultati dell'ASN, si sono manifestati segnali di disinteresse e di scarsa risposta ad alcune sue iniziative che l'hanno portata a compiere una profonda riflessione sulla esistenza stessa del Collegio e sulla sua adeguatezza a presiederlo. La Prof. Sica rimane tuttavia convinta dell'importanza dello stesso, il cui scopo è quello di tutelare gli Associati facendosi carico di tutte le questioni relative al Settore Scientifico Disciplinare BIO/17.

La Prof. Sica ricorda che ha provveduto ad inoltrare a tutti i Soci del Collegio i documenti utili, con particolare riferimento ad un costante aggiornamento sull'ASN

e alla sua partecipazione alla Conferenza Permanente dei Collegi di Area Medica (Intercollegio). Relativamente all'ASN ha lavorato sempre in sintonia con la SIAI e con il Collegio dei Docenti di Anatomia Umana, come dimostrato dal fatto che già nel mese di Novembre 2015 era stato inviato al Prof. Lenzi, Presidente del CUN, e al Prof. Marco Mancini, Capo Dipartimento per la Formazione Superiore e per la Ricerca del MIUR, un documento congiunto frutto del lavoro della SIAI e dei Collegi dei Docenti di Anatomia Umana e di Istologia ed Embriologia, in merito alla determinazione dei valori soglia per le procedure di ASN. In tale documento, firmato dalla stessa Prof. Gigliola Sica, in qualità di Segretario della SIAI e Presidente del Collegio dei Docenti di Istologia ed Embriologia, e dal Prof. Raffaele De Caro, in qualità di Presidente del Collegio dei Docenti di Anatomia Umana, si manifestava apprezzamento per il tentativo fatto di superare nel nuovo Regolamento alcune delle notevoli criticità emerse durante le precedenti tornate dell'ASN, ma si sottolineava, in pieno accordo con il CUN, come la procedura delineata mantenesse alcune scelte di fondo della disciplina vigente ed un'impostazione numerologica. Il documento proponeva tutta una serie di osservazioni e di proposte.

In relazione a quanto riferito dal Prof. De Caro, la Prof. Sica ritiene condivisibile quanto deciso nell'ambito del Collegio dei Docenti di Anatomia Umana.

6. Assegnazione premio ricercatore under 40.

Il Presidente riferisce che il Consiglio Direttivo della SIAI, su indicazione della Commissione formata dai Proff. E. Gaudio, S. Montagnani e S. Adamo, ha attribuito il premio **al Dott. Vincenzo Desiderio**, Ricercatore a Tempo Determinato, presso il Dipartimento di Medicina Sperimentale della Seconda Università degli Studi di Napoli (Indice H: 16). La Commissione ha espresso inoltre un sentito apprezzamento per la qualità della produzione scientifica della **Dott.ssa Gaia Favero**, candidata allo stesso premio.

Il Presidente consegna il premio al Dott. Vincenzo Desiderio.

7. Assegnazione premio alla carriera.

Il Presidente comunica che il Consiglio Direttivo della SIAI, sulla base delle proposte pervenute, ha all'unanimità deliberato l'attribuzione del premio alla carriera al **Prof. Nadir Mario Maraldi**, di cui traccia un breve profilo.

Il Prof. Maraldi, nella sua ultraquarantennale carriera ha dato lustro alla Morfologia Italiana a livello nazionale, ma anche e soprattutto a livello internazionale. Tutti ricordano l'eccezionale lettura magistrale, tenuta al Congresso di Ancona, in cui il Prof. Maraldi ha dimostrato come la Ricerca di Base, senza tradire la propria vocazione, sia stata in grado di determinare avanzamenti della Clinica. I suoi allievi, nelle varie sedi italiane, sono testimonianza del valore di quanto da lui seminato.

Pertanto, a nome di tutta la SIAI, Il Presidente consegna una Targa d'argento ed una pergamena al Prof. Maraldi che, con un breve discorso, ringrazia per l'onore riservatogli.

8. Assegnazione premi poster.

Il Presidente riferisce che la Commissione per l'attribuzione dei Premi Poster della SIAI e di due Buoni Premio offerti dalla Casa Editrice Ambrosiana (nel catalogo Zanichelli), formata dai Proff. L. Fumagalli, S. Zecchi e P. Onori, nominata dal Consiglio

Direttivo, nel congratularsi per l'elevato livello scientifico raggiunto dai vari gruppi di ricerca, dopo un'attenta valutazione, ha deciso di attribuire i Premi ai seguenti Poster:

- *The amniotic membrane from the human placenta contains different subregions with different morphofunctional features.*
  Francesca Passaretta, Luca Centurione, Maria Antonietta Centurione, Silvia De Munari, Elsa Vertua, Antonietta Silini, Ornella Parolini, Roberta Di Pietro.
- *Histopathological rearrangements of the colonic wall following dopaminergic nigrostriatal neurodegeneration.*
  Chiara Ippolito, Cristina Segnani, Carolina Pellegrini, Matteo Fornai, Luca Antonioli, Rocchina Colucci, Saro Dini, Mariella Errede, Daniela Virgintino, Amelio Dolfi, Nunzia Bernardini.

La Commissione ha deciso di attribuire i due Buoni Premio offerti dalla Casa Editrice Ambrosiana ai seguenti poster:

- *Preliminary observations on scleral ossicles in performing functionalized 3D vascularized scaffolds for "critical-size" bone defect healing.*
  Marta Checchi, Alberto Smargiassi, Marzia Ferretti, Paola Sena, Marta Benincasa, Francesco Cavani, Marco Sola, Antonio Ranieri, Stefania Mitola, Carla Palumbo. (Buono premio da Euro 300,00).
- *An intriguing relation between periodontal and cardiovascular diseases.*
  Francesca Diomede, Jacopo Pizzicannella, Ilaria Merciaro, Simone Guarnieri, Oriana Trubiani. (Buono premio da Euro 200,00).

Il Presidente comunica che la premiazione dei vincitori dei Premi Poster SIAI e dei due Buoni Premio offerti dalla Casa Editrice Ambrosiana avverrà alla fine del Congresso e il verbale della Commissione che ha valutato i Poster verrà spedito a tutti i Soci SIAI.

9. Assegnazione premio migliore comunicazione orale.

Il Presidente riferisce che la Commissione, nominata dal Direttivo della Società nelle figure dei Proff. L. Cocco, C. Tacchetti e E. Ziparo, pur esprimendo un giudizio estremamente lusinghiero sulla qualità scientifica di tutte le presentazioni, ha unanimemente deciso di attribuire il premio di Euro 1.000,00 alla comunicazione della Dott.ssa Emanuela Marcenaro intitolata: "**Identification of a subset of human Natural Killer cells expressing high levels of Programmed Death 1: A phenotypic and functional characterization**".

Il Presidente consegna il Premio alla Dott.ssa Marcenaro.

Il verbale della Commissione che ha valutato le comunicazioni orali verrà spedito a tutti i Soci SIAI.

10. Prossimi Congressi nazionali della SIAI e Congressi nazionali ed internazionali previsti per l'anno 2017; proposte di temi di relazione.

Il Presidente ringrazia ancora una volta i Proff. Michetti e Sica che hanno dato la loro disponibilità ad organizzare per il 2016 il 70° Congresso Nazionale della SIAI nella sede di Roma e ricorda che il 71° Congresso Nazionale della SIAI si terrà a Taormina. I temi delle relazioni saranno oggetto di definizione nel corso dei prossimi Consigli Direttivi.

Il Presidente, annuncia due importanti Meeting Internazionali:

- 44th SCUR (Society for Cutaneous Ultrastructure Reseach) Annual Meeting, che si terrà dall'8 al 10 Giugno 2017 a Milano (Prof. Elena Donetti);
- 25th International Symposium on Morphological Sciences, che si terrà dal 27 al 30 Luglio 2017, a Xi'an, China (Prof. Guido Macchiarelli).

11. Problemi relativi all'Italian Journal of Anatomy and Embryology: relazione dell'Editor in Chief, Prof. Paolo Romagnoli.

Il Prof. Gaudio dà la parola al Prof. Romagnoli, il quale riferisce che "L'Italian Journal of Anatomy and Embryology riceve numerosi manoscritti da autori di vari Paesi di tutti i continenti. Poco più della metà dei manoscritti sono accettati, di solito previa revisione sia sostanziale sia formale e linguistica, altri sono respinti, altri ancora non sono più rinviati dagli autori a seguito della richiesta di modifiche in base al referaggio.

Presso SCImago

(http://www.scimagojr.com/journalsearch.php?q=9500154001&tip=sid&clean=0)

l'Italian Journal of Anatomy and Embryology risulta avere un SJR (corrispondente all'IF per quella banca dati) di 0.32. Per confronto, presso la stessa banca dati, Anatomical Record ha un SJR di 0,74, Nature di 21,94.

Sotto il profilo finanziario la Rivista è solida; grazie al contributo della Società Italiana di Anatomia e Istologia e al pagamento delle tariffe da parte degli autori le spese sono tutte coperte. Con i fondi della Società è possibile aiutare alcuni autori di Paesi emergenti coprendo le spese per una parte delle pagine pubblicate e garantire la pubblicazione su supplementi della Rivista degli Atti dei congressi della Società. La pubblicazione come supplementi della Rivista reca lustro a quest'ultima, ma è un bene anche per la Società:

presso la FUP il supplemento ha la stessa dignità dei fascicoli ordinari, è inserito stabilmente in rete in libero accesso e ne è garantita la definitiva documentazione anche dopo esaurite le scorte stampate.

La Rivista sta soffrendo della mancata indicizzazione su PubMed, ferma al fascicolo 1 del 2015. Ciò è dipeso dal fallimento dell'intermediario che curava questa indicizzazione e dal tempo richiesto per studiare, avviare e compiere le procedure per riprenderla; questa operazione è ancora in corso, a carico della Firenze University Press (FUP) che cura la pubblicazione della Rivista a stampa e on line.

Appena risolta la questione

dell'indicizzazione in PubMed, insieme ai collaboratori della FUP, si cercherà di risolvere altri problemi aperti: il referaggio è lento, in media richiede tre mesi e potrà avvantaggiarsi di una procedura computerizzata in corso di studio; la pubblicazione non è del tutto tempestiva nonostante l'impegno della FUP, anche per le difficoltà ad ottenere il pagamento della tariffa da parte di autori di alcuni paesi stranieri; anche per questa difficoltà si cercheranno strategie idonee a evitare i ritardi".

12. Proposta di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.

Sono pervenute 13 domande di ammissione a Socio SIAI da parte di:

Aquila Saveria

Bernardi Sara

Carmagnola Daniela
Centurione Lucia
D'amico Agata Grazia
Miglietta Selenia
Murtas Daniela
Palmerini Maria Grazia
Pellegrini Gaia
Santoro Antonietta
Quondamatteo Fabio
Toesca Amelia
Ratti Stefano

Come previsto dallo Statuto, tutte le domande sono corredate dalla firma di presentazione da parte di due Soci.

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

Nulla al punto 13.

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. Eugenio Gaudio	*Prof. Gigliola Sica*	*Prof. Amelio Dolfi*

INDEX OF AUTHORS

Paolo Romagnoli, Direttore responsabile
Registrato presso il Tribunale di Firenze con decreto n. 850 del 12 marzo 1954

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Errata corrige

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Aging of periosteal-derived stem cells during expansion: an alternative tool for a customized bone regenerative strategy

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Rigosertib as a radio-sensitizer for concurrent chemo-radiation treatment of cholangiocarcinoma (CCA): a comparative study in vitro

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The neglected non-traditional large neuron types in the granular layer of the cerebellar cortex: Morphofunctional and Neurochemical data

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Five classical corticocerebellar neurons are commonly involved in the circuitry of the cerebellar cortex: stellate, basket, Purkinje, granule and Golgi neurons. Numerous morphofunctional studies demonstrate the presence of different large neuron types in the granular layer of the cerebellar cortex of mammals: candelabrum neuron, neuron of Lugaro, unipolar brush neuron, globular neuron, synarmotic neuron and perivascular neuron [1-7] distributed in three different zones of the granular [1,4]. Although, studies demonstrate that this large neuron types play a not negligible role in the microcircuitry of the cerebellum, they continue to be neglected and still now called 'non-traditional neurons' [2]. Finally, these data open a new scenario: in the cerebellar cortex of mammals at least 11 different neuron types must be considered, which may play a considerable role in the motor and non-motor functions of the cerebellum and in its disorders.

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Key words

Cerebellar cortex, non-traditional neuron types, neuron of Lugaro; candelabrum neuron; unipolar brush neuron; globular neuron; synarmotic neuron; perivascular neuron, immunohistochemistry

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