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INVITED LECTURES

IJAE
Vol. 121, n. 1 (Supplement): 5, 2016
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Novel views on brainstem structure and function and their implications in brain disorders

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The brainstem as the evolutionary oldest part of the brain plays a pivotal role in the regulation of cardiac and respiratory function. It regulates the central nervous system, and maintains consciousness and regulates the sleep cycle. Despite these fundamental roles of the brainstem in regulating vital functional abilities such as autonomic nervous system activity as well as regulating all higher cerebral functions via neurotransmitter projections systems originating in the brainstem, the role of the brainstem is mostly neglected in most neuropsychiatric disorders. Besides the dorsal and median raphe nuclei complex comprising mainly serotonin-producing neurons, the brainstem also contains noradrenalin, dopamine and histamine-producing nuclei, i.e. resp. the Locus Coeruleus, the Substantia Nigra and the Mamillary Bodies. Most of the focus on neurobiological questions on above mentioned disease are related to forebrain structures since they are often associated with cognitive dysfunction. As a consequence the brainstem has been highly neglected in neurodegenerative diseases, including Alzheimer's (AD) and Parkinson's (PD) disease and frontotemporal lobar degeneration. In the area of depression, several observations have been made in relation to changes in one particular brain structure: the Dorsal Raphe Nucleus (DRN). In addition dysfunction of the cerebellum is also observed in AD and associated with pulmonary deregulation. The DRN is also related in the circuit of stress regulated processes and cognitive events. The ascending projections and multitransmitter nature of the DRN in particular and the brainstem in general stress its role as a key target for AD/PD research and autonomic dysfunction. It also points towards the increased importance and focus of the brainstem as key area in various neurodevelopmental and age-related diseases. The current presentation aims to review the neuroanatomy of the brainstem as well as the current status on findings, derived from a wide range of studies using molecular, cellular and imaging technologies, of brainstem involvement in neurodevelopmental (i.e. autism, schizophrenia) and neurodegenerative disorders (Alzheimer's and Parkinson's disease).

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From fetal development and beyond: human term placenta as a source of stem cells for regenerative medicine

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Over the past decade, human term placenta has become more than a vital organ during pregnancy, but also a precious reservoir of cells. An increasing number of studies have shown that these cells harbor beneficial properties. In fact, we and others have reported the therapeutic effects of placental cells in preclinical models of lung and liver fibrosis, sepsis, inflammatory bowel disease, autoimmune encephalomyelitis, cardiac ischemia and hind limb ischemia. Remarkably, the diseases which were most attenuated by placental cell treatment were those with underlying altered immune reactions. We have significantly contributed to the understanding of the immune-modulatory properties of cells from the amniotic membrane in vitro, showing that they can reduce the proliferation of T cell subsets, down regulate Th1 and Th17 subsets, and increase T lymphocytes with regulatory functions (Treg). Contributing to their regenerative potential, placenta-derived cells have been reported to secrete a variety of growth factors that could act on progenitor and/or resident cells to favor tissue regeneration. For example, placental cells can release pro-angiogenic factors, such as hepatocyte growth factor, and mediators in extracellular matrix degradation, such as matrix metalloproteinases (MMPs). Moreover, the release of growth factors could stimulate resident stem cells to proliferate, altogether contributing to tissue regeneration. Interestingly, to further substantiate the observation that secreted factors are the main players in the therapeutic properties of placental cells, an increasing number of studies have shown that these beneficial effects are evident when conditioned medium obtained from cell culture is used or when cells are cultured in transwell systems.In conclusion, the placenta is a rich resource of therapeutic derivatives, such as cells, their secreted factors, and also others such as amniotic membrane patches, the latter of which have been successfully used in medicine for over a century. More recently, placental cells and their derivatives are being testing in clinical trials in patients with immune-dysregulated diseases.

This work was supported by Fondazione Poliambulanza, Fondazione Cariplo, Ministero dell'Istruzione, dell'Università e della Ricerca, and Ministero della Salute.

Keywords

Regenerative medicine; human term placenta; stem cells immunomodulation; paracrine effects.

ABSTRACTS

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Correlation between expression profile of Wilms tumor 1 gene isoforms and neuroblastoma grade malignancy

Velia D'Agata [1] - Grazia Maugeri [1] - Rita Reitano [1] - Salvatore Saccone [2] - Daniela Maria Rasà [1] - Agata Grazia D'Amico[1]

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Wilms tumor 1 gene (WT1) is expressed in neuroblastoma (NB) which represents the most aggressive extracranial pediatric tumor. This latter may transform into a more benign form such as ganglioneuroblastoma and ganglioneuroma or progress into a highly aggressive metastatic cancer with a poor survival rate. WT1 acts as tumor suppressor gene in NB by inducing the maturation in a less invasive mass. To date, it has been identified 13 mRNA WT1 variants encoding 13 proteins, however, most of the studies have focused their attention exclusively on isoform of ~49 kDa molecular weight (1, 2). In the present study, we have analyzed, the expression profile of WT1 isoforms, in undifferentiated and all-trans retinoic acid (RA) differentiated NB cells in order to evaluate their involvement in tumor malignancy. Results have shown that different isoforms are expressed both in untreated and RA treated NB cells. Their expression is significantly increased in RA treated cells, suggesting that WT1 isoforms are inversely related to NB malignancy grade. In accord to this hypothesis, WT1 isoforms and nestin expression are inversely related in undifferentiated and RA treated cells. Furthermore, the inhibition of the two signalling pathways specifically involved in differentiation of NB, PI3K/Akt and MAPK/ERK respectively, trigger an overexpression of all WT1 isoforms. In conclusion, these data suggest that overexpression of WT1 isoforms might promote trans-differentiation of NB into a more benign tumor such as ganglioneuroblastoma or ganglioneuroma.

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Keywords

Wilms tumor 1 gene; neuroblastoma; WT1 isoforms.

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PACAP and VIP counteract glioblastoma cells invasiveness

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Glioblastoma multiforme (GBM) is an aggressive brain tumor characterized by hypoxic areas. The low oxygen supply induces expression of hypoxia-inducible factors (HIFs) leading to overexpression of epidermal growth factor receptor (EGFR) [1]. It is well known that pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) are involved in human tumors, however, their role on GBM invasiveness has not been well elucidated [2]. To this end, we have investigated, for the first time, the anti-invasive effect of PACAP or VIP on GBM cells following exposure to hypoxia, by using desferrioxamine mesylate salt (DFX), an hypoxia-mimetic agent.The results have shown that under low oxygen tension, both PACAP and VIP reduce cells migration by modulating HIFs and EGFR expression. This effect is mediated through the inhibition of phosphoinositide 3 kinase (PI3K)/Akt and mammalian mitogen activated protein kinase/Erk kinase (MAPK/ERK) signaling cascades, which, as previously demonstrated, interfere with HIF-1 α and HIF-2α expression. In conclusion, our data suggest that PACAP and VIP might be good candidates to modulate GBM invasiveness exacerbated by hypoxic microenvironment.

This work was supported by grants from MIUR FIRB 2010 and MIUR PRIN-2009.

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Keywords

PACAP; VIP; glioblastoma multiforme invasiveness; hypoxia; HIFs; EGFR.

IJAE
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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Possible interactions between HDACs and TGF-beta/ Smads pathway in Glioblastoma

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Glioblastoma (GBM) is the most common and aggressive tumor of the Central Nervous System (CNS). Unfortunately, patients afflicted with this disease have a very poor prognosis, due to high level of invasiveness and resistance to standard therapies (1). Although the molecular profile of GBM has been extensively investigated, the events responsible for its pathogenesis and progression remain largely unknown. Reports have indicated that HDAC (Histone deacetylases) dependent epigenetic modifications (2) and the Tgfβ/Smad pathway (3) play roles in GBM tumorigenesis. The aim of this study was to evaluate the involvement and the possible interaction between these two molecular cascades in the pathogenesis, therapeutic responsiveness and prognosis of GBM. Immunohistochemestry (IHC) was performed on microdissected GBM samples, collected from 14 patients (n.8 men and n.6 women) ranging in age from 43 to 74 years. The patients were previously divided, on the basis of their overall survival (OS), into three groups: low, intermediate and high OS. Patients with poor prognosis showed hyperexpression of HDAC4 and HDAC6, an activation of the Tgfβ/Smad pathway, with high levels of IL-13, Smad2, PDGF and MMP3 expression, compared to the intermediate and high OS groups, whereas the expression of Smad7 was reduced. The high OS group also exhibits an increase in p21 immunostaining, which represents a common target of the two cascades. The IHC data was confirmed by Immunoblotting. Our results suggest that both HDAC4 and HDAC6 together with the Tgfβ/Smad pathway are involved in progression of GBM and could be a useful prognostic markers and may predict responsive to therapy.

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Keywords

GBM; HDAC; Tgfβ; Smad.

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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

The original slides of Camillo Golgi

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As it is well known, Camillo Golgi (1843-1926) reported in 1873 his discovery of the black reaction (reazione nera), based on nervous tissue hardening in potassium dichromate and impregnation with silver nitrate. This method first revealed neurons, including their processes, in their entirety, thus providing the tool for a breakthrough in the knowledge on the structure of the nervous system. Professor of Histology and of General Pathology, Camillo Golgi worked for decades at the University of Pavia, leading a very active laboratory. Most of the original histological preparations of Golgi's laboratory have unfortunately been lost. However, some slides are still kept at the Museum of the University of Pavia ("Sistema Museale di Ateneo") but they have not been examined in detail until now. This presentation will provide an account of Golgi's original slides available nowadays. Images from these preparations (e.g. from the hippocampus, cerebellum, and spinal cord), mostly based on Golgi impregnation, will be shown and compared with Golgi's drawings and his descriptions of neuronal wiring. The presentation is thus aimed at showing, for the first time, the images which have led to the pioneering observations made by Camillo Golgi, which have opened the field of neurohistology and neuroanatomy and have contributed to the foundations of modern neuroscience.

Keywords

History of neuroscience; Camillo Golgi; Golgi staining.

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Functional anatomy of cortical areas characterized by Von Economo neurons

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Von Economo's neurons (VENs) are large, bipolar or corkscrew-shaped neurons located in layers III and V of the frontoinsular and the anterior cingulate cortices. VENs are reported to be altered in pathologies such as frontotemporal dementia and autism, in which the individual's self control is seriously compromised. We have recently reviewed the evolutionary appearance of VENs and we are currently studying their distribution in different neurodegenerative diseases. To investigate the role of VENs in the active human brain, we have explored the functional connectivity of brain areas containing VENs by analyzing resting state functional connectivity (rsFC) in 20 healthy volunteers. Our results show that cortical areas containing VENs form a network of frontoparietal functional connectivity. With the use of fuzzy clustering techniques, we find that this network comprises four sub-networks: the first network cluster resembles a "saliency detection" attentional network, which includes superior frontal cortex (Brodmann's Area, BA 10), inferior parietal lobe, anterior insula, and dorsal anterior cingulate cortex; the second cluster, part of a "sensory-motor network", comprises the superior temporal, precentral and postcentral areas; the third cluster consists of frontal ventromedial and ventrodorsal areas constituted by parts of the "anterior default mode network"; and the fourth cluster encompasses dorsal anterior cingulate cortex, dorsomedial prefrontal, and superior frontal (BA 10) areas, resembling the anterior part of the "dorsal attentional network". Thus, the network that emerges from analyzing functional connectivity among areas that are known to contain VENs is primarily involved in functions of saliency detection and self-regulation. In addition, parts of this network constitute sub-networks that partially overlap with the default mode, the sensory-motor and the dorsal attentional networks.

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Keywords

Von Economo neurons; insula; frontotemporal cortex.

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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Painting a global picture of basal ganglia network: from past to present!

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Since the 70s it has been thought that basal ganglia integrated sensorimotor, associative and limbic inputs and then projected this information through the thalamus to the motor cortex, supplementary motor area and frontal cortex, thus playing a relevant role in planning movement. Recent literature on basal ganglia networks is going beyond the classical "dogma" of dorsal striatum as the main station for cortical inputs in basal ganglia loops and several neurophysiological studies have suggested a more segregated organization of these neural circuits. In the classical view, various tract-tracing methods combined with immunohistochemistry and in situ hybridization demonstrated that the cortical information flows through the basal ganglia via a dual-network model, based on the "direct" and "indirect" routes. However, in addition to these two major projection systems, a glutamatergic hyper-direct pathway between cerebral cortex and subthalamic nucleus has been demonstrated first in monkeys and then in humans. Furthermore, we have recently shown a i) cortico-pallidal connection; ii) a cerebello-pallidal connection; iii) a cerebello nigral connection [1, 2]. Herein, we extensively examined basal ganglia network of fifteen healthy subjects by using probabilistic constrained spherical deconvolution tractography on magnetic resonance diffusion weighted imaging data and we also performed weighted connectivity analysis for each of the subcortical nuclei. In addition, we demonstrated for the first time tractographic evidences of the existence of a direct cortico-nigral pathway in humans. We found that substantia nigra is connected with cerebral cortex as a whole, with the most representative connections involving prefrontal cortex, precentral and postcentral gyri and superior parietal lobule. These findings would strength the hypothesis that the cortico-basal ganglia network consists of several, parallel, segregated, and functionally distinct, but homologous loop, and may be relevant for the comprehension of the pathophysiology of several basal ganglia disorders.

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Keywords
Basal ganglia; MR; Network.

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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Topo-pathological re-wiring in brain structural connectomes of de novo Parkinson's Disease patients

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Although several studies in the last decades have challenged our understanding of Parkinson's Disease (PD) pathophysiology, an important gap at a network and system level still remains to be filled in order to understand the fundamental changes in high-order motor and non-motor circuits underlying PD symptoms. The wide spectrum of both motor and non-motor symptoms suggests that Parkinson's Disease may reflect extended alterations of the global brain network, thus justifying the onset of this heterogeneous symptomatology. Such hypothesis would be suitable with the idea of an "associationist" brain, which goes beyond the classic cortical "localizationist" theory. According to the former, the brain might consist of several, segregated and parallel distributed networks around critical and participating cortical epicenters. To the best of our knowledge, only few studies attempted to improve our understanding on structural MRI networks in PD. With the aim of detecting altered topological rewiring of brain networks in early stage de novo PD patients, we reconstructed tractography-based brain structural connectomes [1] in a pilot population of 10 PD patients and 13 controls. Topological features of structural connectomes were computed and compared between the healthy controls group and the group with PD at different level of cut-off. Significant group differences were showed at certain cut-off in the structural connectivity from the measurement of the Local Community Paradigm-correlation (LCPcorr), Characteristic Path Length, Betweenness Centrality and Edge Betweenness Centrality. Increased value of LCPcorr in the pathological group reflects a topological (and not spatial) network local community re-organization of structural interactions between common neighbors nodes [2]. As a result, the PD group has an increased correlation between the number of common neighbors and the number of their internal-interactions across all the structural local communities in the networks. On the other hand, decreased values in Characteristic Path Length, Betweenness Centrality and Edge Betweenness Centrality suggest also a global topological network re-wiring. Taken together these findings strongly indicate altered topological rewiring in de novo PD brain connectome and could shed new light on the pathophysiology of the disease and in the definition of network-based markers for a more quantitative and precise diagnosis.

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Keywords
Parkinson; tractography; topology.

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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Limbic-motor areas interactions as revealed by Constrained Spherical Deconvolution tractography: a mechanism to shape complex motor behaviors?

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Although there are several evidences in animal research on the emotional motor-limbic subcortical system including amygdala, hyppocampus, parahyppocampal cortex and nucleus accumbens, little is known about its connections to cortical motor-related areas. All these structures are in a position to influence behavior via cortical motor-related areas, which in turn have access, both directly and indirectly, to descending motor pathways. If on the one hand, many animal studies have investigated the neural connectivity of the motor-limbic system using electrophysiological and tracing techniques, on the other hand the use of these methods in the live human brain is limited and elusive due to their invasive nature [1]. By contrast, recent developments in diffusion magnetic resonance imaging and tractography have allowed for non-invasive and in vivo investigation of the human brain. Diffusion-based tractography is a method analyzing the preferential water diffusivity directionality along white matter bundles, thus calculating the highest mathematical probability that water diffuses in a given direction [2]. Using diffusion-weighted magnetic resonance imaging and Constrained Spherical Deconvolution tractography on a population of 15 healthy subjects, we provided tractographic evidence of a structural connection between the amygdala and motor-related areas in humans. These direct limbic-motor pathways may allow for the regulation and modulation of complex motor behaviors and subtle behaviors such as social interactions. The demonstration of these interactions might be fundamental for the comprehension of the pathophysiology of several limbic-sensorimotor diseases, such autism spectrum disorders and motor conversion disorders.

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Keywords
Amygdala; tractography; MRI.

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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Deconvolution increases the accuracy of measurements by image analysis in a model of trimethyltin-induced reactive gliosis of the rat entorhinal cortex

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Digital images were used in applications such as astronomy, medicine, physics and biology, to record and analyze results from experiments Due to the features of the imaging system, the recorded images can be degraded by blurring and noise. Image deconvolution, or image deblurring, is the process of reconstructing or estimating the true image from the degraded one [1]. In order to optimize morphometrical analysis of Glial Fibrillary Acidic protein (GFAp)-immunoreactive astrocyte of the whole rat entorhinal cortex of both trimethyltin hydrochloride- and saline treated rats, large images of it (about 30 000 x 20 000 pixels) were digitized by a microscope with a X – Y motorized computer-managed stage and an autofocusing system, using an objective 40x, a digital camera 2560x1920 RGB. Moreover it has been optimized a procedure of deconvolution and segmentation under the NIH ImageJ system. Such large images were first deblurred, by Modified Residual Norm Steepest Descent (MRNSD) and Wiener Filter Preconditioned Landweber (WPL) algoritms, and segmented, then analyzed, to measure the % of the area (in μm2) occupied by GFAp-immunoreactive cell bodies and processes, and classical morphometrical parameters. Statistical analysis was performed to describe obtained data and to point out differences between segmented only versus deblurred-segmented images. Our results can be summarized as follows. 1. Large images can be an useful tool to identify precisely the distribution of reactive astrocytes in the rat entorhinal cortex. 2. Deconvolution avoid an overestimation of the area of immunoreative astrocytes of about 10-15%. Segmentation allow a measurement with improved accuracy, precision and uncertainty. 3. This approach is time consuming and requires a multi-core hardware with a large amount of available RAM.

Thanks to Prof. G. Metafune and Dr. A. Falconieri for making possible the access to resources of the computing center of the Department of Mathematics and Physics of the University of Salento.

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Keywords

Deconvolution; rat; entorhinal cortex; glial fibrillary acidic protein.

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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Image analysis evaluation of astroglial and microglial markers distribution in the medial entorhinal cortex of trimethyltin hydrochloride treated rats

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Trimethyltin acute administration is a useful tool to investigate neurodegenerative processes (1). This research was carried out to characterize regions of the rat medial entorhinal cortex, where vimentin (VIM), glial fibrillary acidic protein (GFAp) immunoreactivity of reactive astroglia, and IBA1 immunoreactivity of microglia are related to neuronal loss after administration of trimetyltin hydrocloride. Male Sprague-Dawley rats, weighting 250 g were given a single intraperitoneal dose of 8 mg/kg of trimethyltin hydrochloride (TMT), or the vehicle only, and were sacrificed after 21 days. 10 μm horizontal serial sections of paraffin embedded brains of all specimens were stained with cresyl violet (CV) 10 μm horizontal serial sections of paraffin embedded brains of all specimens were stained with cresyl violet (CV) or immunocytochemically tested with anti-GFAp, anti-VIM or anti-IBA1 monoclonal antibodies. Each section was digitized using a 20x or 40x objective to get a 'mosaic' of all the entorhinal cortex. Such large images of three adjacent sections, the first VIM-, the second GFAp- and the third IBA1-immunotested (or CV-stained) were placed each one into a RGB stack and aligned showing VIM as red, GFAp as green and IBA1 or CV as blue false colors. In the medial entorhinal cortex of TMT-treated rats a few VIM-immunoreactive astrocytes were found mainly in the layer II and also, with less density, in the layer III, while GFAp-immunoreactive astrocytes appeared very numerous, increased in size and located in the layers I, II and III, and IBA1-immunoreactive microglial cells distributed at least partly similarly to GFAp-immunoreactive astrocytes. Such central core VIM - immunoreactive, surrounded by a halo GFAP – and IBA1 - immunoreactive, focused attention mainly on the layer II where was found a significant neuronal loss, and also, on the more lateral part of the layer III. In addition, some Iba1 - immunoreactive cells showed a morphology not type microglia, as they were similar to astrocytes or neurons, suggesting a possible neuroinflammatory-induced phenotypic plasticity process.

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Keywords

Image analysis; rat; medial entorhinal cortex; reactive gliosis.

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Gender differences in estrogenic compounds effect on human EPCs

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Endothelial dysfunction has been defined as an "integrated risk factor", and several gender difference have been reported in endothelial function. Several evidences suggest that endothelial progenitor cells (EPCs) are an important endogenous system that maintains integrity and vascular homeostasis. Their function is regulated by estrogens and estrogen receptors (ERs), but the effect of estrogenic compounds such as bisphenol A (BPA) and (R,R)-5,11-diethyl-5,6,11,12-tetrahydro-2,8-chrysenediol (THC) on human EPCs is unknown. BPA is used in plastic industry and BPA exposure is associated to abnormalities such as obesity, diabetes, disorders of the reproductive and immune systems, to endothelial dysfunction, and oxidative stress. This endocrine disruptor binds to ERα and ERβ with higher affinity for ERβ. THC is a specific agonist of ERα with a stronger ERβ antagonist activity. Therefore, the present work aimed to analyze if BPA and THC influence in a sex-specific manner the migration of human EPCs, an essential process in endothelial regeneration after vascular injury. EPCs were isolated from healthy adult men and women aged between 18 and 30 years, using a magnetic positive selection with the CD34 MicroBeads, a well-established marker of human progenitor cells. EPCs were also characterized for acetylated LDL Dil- (acLDL) and isothiocyanate (FITC)-conjugated with Ulex europaeus agglutinin I (lectin) uptake. The expression of ERα and ERβ was analysed by Western Blotting, while the migration assay was performed with the transwell chemotaxis assay. Male and female EPCs expressed both classical ERs: ERα was higher, but not significantly, in female cells, while ERβ was similarly expressed in both sexes. Male and female EPCs did not differ in basal migration. 17-β-estradiol (10^{-9} M e 10^{-10} M) significantly inhibited migration in female EPCs but not in male ones. Moreover, both 10^{-5} M THC and BPA (10^{-8} M) were able to bock migration only in female cells. Considering that BPA has a ERα and a prevalent ERβ agonist activity while THC has ERα agonistic activity and a prevalent ERβ antagonist activity, our data show that the effect on migration observed in female EPCs is mediated by ERα. Our data demonstrate that estrogenic compound have a sexual divergent effect on human EPCs, improving our knowledge on the gender differences observed in the pathophysiology of endothelial function.

Keywords

EPCs; gender; migration; estrogenic compounds.

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Direct effects of estrogens on cholinergic primary neurons from the human fetal nucleus basalis of Meynert

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Epidemiological studies have indicated that Alzheimer's disease (AD) is more common in females and that post-menopausal women are at increased risk than their male counterpart, thus suggesting that estrogens could play a protective role to counteract neurodegenerative processes (1). However, the mechanisms underlying this association remain to be clarified. Since the nucleus basalis of Meynert (nbM) is the major source of cholinergic innervation selectively vulnerable to degeneration in AD, our study is aimed at investigating the effects of estrogens on human cholinergic primary neurons (hfCNs) isolated from the nbM of 12-week old fetuses. The primary culture obtained was immunophenotyped with flow cytometry and resulted almost totally positive (97±2 %) for the neuronal marker MAP2 and for the choline acetyltransferase (ChAT). We demonstrated that hfCNs express receptors for hormones of the reproductive axis (ERs, LHR, GnRHR). In particular, besides to classical estrogen receptors (ERa and ERb), hfCNs express the transmembrane receptor GPR30, which is known to mediate rapid non-genomic estrogen actions. Increasing concentrations of 17-β estradiol (E2, 0.1-100 nM) determined a dose-dependent significant increase in cell number after 24h exposure, which was antagonized by tamoxifen treatment. In addition, E2 exposure determined a significant increase in ChAT expression, thus indicating a direct positive effect of E2 on cholinergic phenotype. Given that substantial evidence now indicates that estrogens exert an anti-inflammatory activity even in the central nervous system (2), we exposed hfCN cells to the proinflammatory cytokine TNFα. E2 treatment (1nM) was able to significantly counteract the TNFα-induced nuclear NF-kB p65 translocation. Interestingly, this effect was mimicked by G1, a GPR30 agonist, and abolished by pretreating cells with the GPR30 antagonist G15, but not by tamoxifen, which usually antagonizes classical ERs. Overall, our results indicate that estrogens exert direct neuroprotective mechanisms on hfCNs through the activation of either classical (trophic) and non-classical (anti-inflammatory) receptors.

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Keywords
Neuroinflammation; GPR30; Alzheimer's disease.

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Ameliorative effect of VIP family members on blood retinal barrier breakdown in diabetic macular edema

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Diabetic macular edema (DME) is one of the main complications of diabetic retinopathy [1]. This pathology is owed to impairment of the blood-retinal barrier (BRB) [2]. Many factors, such as hypoxia, contribute to barrier dysfunction and progression of the disease. Low oxygen tension is one of the main events involved in the formation of new blood vessels that characterize the typical uncontrolled angiogenesis in proliferative stage of diabetic retinopathy. In the last decades, various studies have focused their attention on the role of pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) in the pathophysiology of DME. However, the effects of these peptides in maintaining the integrity of the BRB exposed to hypoxia remains to be elucidated. In the present work we have studied, for the first time, the effect of these peptides on outer BRB integrity following hypoxic insult in an experimental model of DME. To this end, we have used the human retinal pigment epithelial cells (ARPE-19) to test the effect of both peptides on cellular permeability, transepithelial electrical resistance, tight junctions expression and hypoxia-induced apoptosis. Results have demonstrated that both PACAP and VIP are able to rescue the integrity of cell monolayer during the hypoxic event, minimizing apoptotic damages induced by low tissue oxygen tension through the activation of phosphoinositide 3 kinase /Akt and mammalian mitogen activated protein kinase/ Erk kinase signaling pathways. Furthermore, these peptides modulate the expression of vascular endothelial growth factor which is one of the downstream transcription factor activated during the hypoxic process. In conclusion, we have demonstrated that PACAP and VIP are able to counteract the damage induced by hypoxia on BRB through the modulation of hypoxia inducible factors expression.

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Keywords

PACAP; VIP; diabetic macular edema; blood-retinal barrier.

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Vasopressin induces cholangiocyte proliferation in experimental cholestasis and in Polycystic Liver Disease

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The hormone vasopressin (hereafter AVP) is a neuropeptide mainly synthesized in the brain's hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei, works by three distinct receptor subtypes: V1a, V1b, and V2 [1]. In liver, AVP is involved in glycogenolysis and neoglucogenesis and regenerative processes [2]. Cholangiocytes are the cells that line the biliary ducts and they are the target in a number of animal models of cholestasis including bile duct ligation (BDL) and in several human pathologies such as polycystic liver disease (PLD) characterized by the presence of numerous cysts within the liver that arise from biliary epithelium [3]. Since no data exist about the presence and the role of AVP and receptors in biliary epithelium, we aimed to evaluate the effects of AVP in experimental model of cholestasis and in course of PLD. In vivo, normal and BDL liver fragments from rats, normal and PLD from human patients were collected to evaluate: (i) intrahepatic bile duct mass (IBDM) by immunohistochemistry for citokeratin-19 (CK-19); and (ii) expression of V1a, V1b and V2 by immunohistochemistry, immunofluorescence and real time PCR. In vitro, small and large mouse cholangiocytes, H69 (non-malignant human cholangiocytes) and LCDE (human cholangiocytes from cystic epithelium) were stimulated with AVP in the absence/presence of antagonists such as OPC-31260 and Tolvaptan, before assessing cellular growth by MTT proliferation assay, cAMP levels by a RIA kit and the expression of some angiogenic factors, such as platelet-derived growth factor (PDGF) and Angiopoietins (Ang-1 and Ang-2). Cholangiocytes express V2 receptor that was upregulated following BDL and in course of polycystic disease. Treatment with AVP of cholangiocyte cultures increased proliferation, cAMP levels and expression of PDGF, Ang-1, Ang-2 in small cholangiocytes and LCDE cells. These increments were blocked by pre-incubation with the AVP antagonists. Our results showed that AVP play an important role in growth of the biliary epithelium during cholestasis and in cystic epithelium in course of PLD acting on the cAMP signalling pathway and increasing angiogenic factors. Additional studies are necessary, but these first results may be considered important in the regulation of the biliary growth/loss in course of cholangiopathies.

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Keywords

Biliary epithelium; vasopressin; cholestasis; polycystic liver disease.

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Identification of a subset of human Natural Killer cells expressing high levels of Programmed Death 1: A phenotypic and functional characterization

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Background: PD-1 is an immunological checkpoint that limits immune responses by delivering potent inhibitory signals to T cells upon interaction with specific ligands expressed on tumor/virus-infected cells, thus contributing to immune escape mechanisms (1). Therapeutic PD-1 blockade has been shown to mediate tumor eradication with impressive clinical results. Little is known on the expression/function of PD-1 on human NK cells (2). Objective: To clarify whether human NK cells may express PD-1 and analyze their phenotypic/functional features. Methods: Multiparametric cytofluorimetric analysis of PD-1+ NK cells and their functional characterization by degranulation, cytokine production and proliferation assays. Results: We provide unequivocal evidence that PD-1 is highly expressed (PD-1bright) on a NK cell subset detectable in the peripheral blood of approximately one fourth of healthy individuals. These donors are always serologically positive for HCMV. PD-1 is expressed by CD56dim but not by CD56bright NK cells and is confined to fully mature NK cells characterized by the NKG2A-KIR+CD57+ phenotype. The proportions of PD-1bright NK cells were higher in the ascites of a cohort of ovarian-carcinoma patients suggesting their possible induction/expansion in tumor environments. Functional analysis revealed a reduced proliferative capability in response to cytokines, low degranulation and impaired cytokine production upon interaction with tumor targets. Conclusions: We have identified and characterized a novel subpopulation of human NK cells expressing high levels of PD-1. These cells have the phenotypic characteristics of fully mature NK cells and are increased in ovarian-carcinoma patients. They display low proliferative responses and impaired anti-tumor activity that can be partially restored by antibody-mediated disruption of PD-1/PD-L interaction.

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Keywords

Natural Killer cells; programmed death receptor (PD-1); ovarian carcinoma; tumor escape; immune checkpoint; NK cell degranulation; NK cell proliferation; NK cell cytokine production; CD57+ NK cells; CMV.

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The isoprenoid end product N6-Isopentenyladenosine inhibits inflammation in bronchial epithelial cells through modulating the NFκB pathway

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N6-Isopentenyladenosine (iPA) is a cytokinin identified in plants but also present in a free form or as a modified nucleoside bound to selenocysteine tRNA in human cells. It is an adenosine modified by an isopentenyl chain which derives from dimethylallil pyrophosphate (DMAPP), an intermediate of the mevalonate pathway. iPA is required for efficient translational decoding of selenoproteins, and can modulate a variety of biological processes including cell cycle progression, DNA synthesis and apoptosis (1). Recently, it has been shown that iPA can exhibit immunomodulatory and anti-inflammatory properties by activating NK cells and modulating cytokine production in a way depending on the concentration used (2). In order to further investigate the anti-inflammatory properties of iPA and its possible mechanisms of action, we analyzed its ability to inhibit TNFα-induced inflammation, either in normal human bronchial epithelial cells or in a model of exacerbated inflammation represented by bronchial cells derived from a Cystic Fibrosis (CF) patient bearing the ΔF508 mutation. Results showed that iPA inhibited IL-8 and RANTES release in both type of cells in a different manner. The analysis of the key enzymes of the STAT3 and NF-κB signalling pathways showed that iPA decreased the phosphorylation of STAT3 enzyme and markedly increased the expression of the direct NF-κB inhibitor, IκBα. These results were corroborated analyzing directly the NF-κB activity in HEK 293/T cells transfected with a NF-κB reporter plasmid. In these cells, iPA was also able to decrease IκBα levels. Of interest, we found that iPA also increased the expression of the antioxidant selenoprotein glutathione peroxidase only in CF cells. Altogether these data suggest that iPA can negatively regulate inflammation with a general mechanism of action involving the inhibition of NF-κB pathway but also propose that, in the presence of an altered inflammatory response such as in CF disease, iPA might act by modulating expression and/or synthesis of glutathione peroxidase.

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Keywords

N6-isopentenyladenosine; inflammation; NF-κB.

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Langerhans cells and Toll Like Receptors: how do they act and react in an in vitro psoriatic microenvironment?

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Tumor Necrosis Factor (TNF)-α, interleukin (IL)-17, IL-22 and IL-23 are involved in the psoriasis pathogenesis and represent a strong proinflammatory stimulus. Both epidermal keratinocytes (KCs) and Langerhans cells (LCs) early respond promoting an early epidermal response [1, 2]. Human skin can count on the cellular response supported by LCs and on innate immunity through the expression of Toll-like Receptors (TLRs) [4]. We aimed at investigate whether the exposure of normal human skin to a combination of TNF-α, IL-17, IL-22, and IL-23 (cytokine mix) affected i) LCs immunophenotype, ii) expression of TLR2 and TLR9 and iii) KC proliferation. Human skin samples were obtained after plastic surgery (n = 5) and exposed to the cytokine mix in a Transwell system at air-liquid interface, with a parallel control group. Samples were harvested 24 and 48 hours after cytokine stimulation, processed in parallel for immunofluorescence or ultrastructural analysis. A decrease of cell proliferation was evident in samples exposed to cytokine mix for 24 hours and this phenomenon was more and more evident later. TLR2 immunopositivity progressively disappeared in the basal layer after cytokine mix exposure compared to the control group, while TLR9 expression was induced in scattered granular keratinocytes. By TEM, LCs showed an activated phenotype. In conclusion, these results suggest that, in a microenvironment mimicking the psoriatic plaque, epidermis early stimulates two important lines of defense, thus proposing that a therapeutic intervention in this direction can interfere with the formation/progression of the psoriatic plaque.

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Keywords

Human epidermis; transmission electron microscopy; cell proliferation; cell differentiation.

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Hsp60 and interleukins expression in the skeletal muscle and its implications in exercise and cachexia

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Heat shock protein 60 (Hsp60) is a chaperon localizing in skeletal muscle mitochondria, whose role is poorly understood. This chaperone has been found also in other cellular localizations. In the three years we studied the levels of Hsp60 in fibres of the entire posterior group of hindlimb muscles (gastrocnemius, soleus, and plantaris) in mice after completing a 6-week endurance training program. In this evaluation we correlated Hsp60 levels with the expression of four isoforms of the peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α). Moreover, the short-term overexpression of hsp60, achieved by in vitro plasmid transfection was performed to determine whether this chaperon could have a role in the activation of the expression levels of PGC-1α isoforms. The levels of Hsp60 protein were fibre-type specific in the posterior muscles and endurance training increased its content in type I muscle fibers. Concomitantly with the increased levels of Hsp60 released in the blood stream of trained mice, mitochondrial copy number and the expression of three isoforms of PGC-1α increased. Overexpressing hsp60 in cultured myoblasts induced only the expression of PGC-1 α1, suggesting a correlation between Hsp60 overexpression and PGC-1 α1 activation. We are now studying the expression of Hsp60 in the muscles of trained and untrained C26-bearing mice, to understand if Hsp60 over expression may improve muscle performance and reduce cachexia. Four different interleukins have been also studied in cachectic mice, to understand which can be the effect of them on Hsp60 expression both in the tumor mass and the trained muscle.

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Keywords

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

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Local expression of SOD1G93A mutant protein triggers neuromuscular junction dismantlement

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The alteration of Reactive Oxygen Species (ROS) homeostasis plays a causal role in several chronic pathology such as aging and neurodegenerative diseases like Amyotrophic Lateral Sclerosis (ALS). Although it is recognized that axon and synapses are first cellular sites of degeneration in ALS disease, controversy exists on whether pathological events initially begin at the NMJs and then, in a dying back phenomena, contribute to motor neuron degeneration. Moreover, the precise molecular mechanisms of pathology-associated deterioration in neuromuscular system have remained elusive (1). Here we provide evidences that muscle specific accumulation of SOD1G93A in the transgenic mice model MLC/SOD1G93A (2) induces mitochondria dysfunction and triggers NMJ dismantlement. Further, we demonstrate that treatment of MLC/SOD1G93A mice with Trolox, a potent antioxidant, is sufficient to rescue mitochondria and NMJ defects in the MLC/SOD1G93A mice, stabilizing muscle-nerve connection. The analysis of potential molecular mechanisms that mediate the toxic activity of SOD1 revealed the activation of specific Protein Kinase as a downstream player of NMJ dismantlement. Overall our data demonstrate that muscle specific expression of SOD1G93A mutation causes mitochondrial impairment and NMJ dismantlement, suggesting that muscle defects and NMJs alteration precede motor neuron degeneration rather than resulting from it.

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Keywords

Oxidative stress; NMJ; aging; ALS; muscle.

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Sarcoglycans and mucin in epithelial tissues of digestive and respiratory tracts: an immunofluorescence study

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Sarcoglycans are transmembrane glycoproteins which play a key role in maintaining sarcolemma stabilization during muscle contraction. Several studies have demonstrated that this complex is not muscle specific and that it is also expressed in epithelial tissues as gingival, breast and prostatic epithelia. In the present study we investigated sarcoglycans expression in the epithelia of digestive and respiratory tracts. We performed immunofluorescence reactions using antibody against a-, b-, g-, d-, e- and z-sarcoglycans and against mucin 4 and 16. Mucins are a superfamily of proteins which serve to protect the underlying epithelia against a wide range of injuries (bacteria, virus, parasites, toxins, pH). This protection leads to coordinate cell proliferation, differentiation and apoptosis among other cellular responses; in fact, mucins are promising biomarkers and therapeutic targets in cancer and inflammatory diseases. Our results show the expression of sarcoglycans in the basal, lateral, and apical epithelial cell's surface; moreover, sarcoglycans show to colocalize with mucins in the cell's apical surface of bronchi and bronchioles, stomach and intestine but no apical localization has been detected in the esophageal epithelium. These results support the role of sarcoglycans in cell-cell and cell-matrix interaction. Moreover, the colocalization between sarcoglycans and mucins at apical level of epithelia which have high mucosecretory activity suggest that sarcoglycans could interact with mucus, maybe involving in maintainig omeostasis of gastro enteric epithelia. It will be necessary to demonstrate the hypothetical correlation between sarcoglycans and the maintaining epithelial homeostasis.

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Keywords

Mucins; sarcoglycans; epithelia; immunofluorescence.

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Isolation and characterization of extracellular vesicles secreted by pre-pubertal Sertoli cells

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Recent studies have shown that extracellular vesicles (Ev) are an important mechanism of intercellular communication. In fact, Ev may contain proteins, DNA and mRNA. In particular, the latter play an important role in various biological processes including regulation and cell differentiation [1]. Sertoli cells (SC), previously considered as a mere "sustentacular cell", were re-evalued in their functions and elevated to the rank of a "sentinel" in spermatogenesis due to production of trophic, differentiation and immune-modulators factors. Porcine pre-pubertal SC, isolated by our method [2], upon 48 hours culture, SC were stimulated with recombinant a-follitropin (rFSH) or FSH + testosterone (T) to evaluate both the presence in the medium of SC-derived Ev (SC-Ev) and SC-Ev content, in terms of mRNA for Anti-Müllerian hormone (AMH), inhibin B, Androgen Binding Protein (ABP) and FSH-receptor (FSH-r), by RT-PCR. SEM analysis highlighted the presence of SC-Nv in culture medium with mean diameters < 149 nm. We have also demonstrated, within the SC-Ev, significant increase in mRNA for AMH, ABP and FSH-r after both FSH and FSH+T stimulation, as compared to unstimulated SC-Ev. Differently from unstimulated SC-Ev, mRNA inhibin B levels were unchanged in FSH-stimulated SC-Ev, and increased after FSH+T stimulation. Interestingly, an opposite trend was shown in mRNA secretion, in control SC monolayer where, we demonstrated a decrease of AMH and FSH-r mRNA (after both stimulations with FSH or FSH + T) and an increase of inhibin B mRNA. On the contrary, mRNA ABP levels, in SC monolayer, decreased after stimulation with FSH but were unchanged in the presence of FSH+T. For the first time in the Literature, our work has shown the presence of SC-Nv containing AMH, inhibin B, ABP and FSH-r mRNA regulated by FSH with or without T. This result may suggest that other testicular cells could produce factors that, until now, were considered an exclusive SC secretory product.

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Keywords

Sertoli cells; extracellular vesicles.

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The frontoethmoidal architecture: a developmental point of view

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The anatomy of frontal sinus drainage pathway (FSDP) and surrounding spaces is extremely complex and variable. Its anatomical variability can be simplified based on the knowledge of the developmental mechanism of the frontal recess. The frontal sinus develops from the 13th week of intrauterine life to the age of twenty through a number of well-known steps of progressive extension within the frontal bone. Its development results from an upward epithelial migration of the anterior ethmoidal cells that penetrate the inferior aspect of the frontal bone between its two diploic plates. Even though this developmental theory is almost universally accepted, only few Authors focused on the formation of FSDP prior to the extramural pneumatization (1-2). The results of the present study conducted on 14 human heads match with the developmental model proposed by Terracol and Ardouin (2), in fact a number of significant associations are conform to the process of growing of the frontal sinus from one out of the three primordial cells (i.e. orbital, nasal, or bullar cell). In this model, renewed in view of the observation of the present study, the hierarchical order of growing among primordial cells determines the final frontoethmoidal architecture.

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Keywords

FSDP; frontal sinus; frontoethmoidal architecture; development.

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Hypo and retrotympanum: the importance of anatomical variants

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The hypo- and retrotympanum host a variety of crucial anatomical structures[1], characterized by high variability, which are poorly been described. The aim of our study is to describe and classify the anatomical variants of the hypo- and retrotympanum by the means of transcanal endoscopy[2]. We hypothesize that the retro- and hypotympanum are subject to more anatomical variability than actually thought. Moreover, the configuration as bridge variants and variably shaped sinus interconnects the different subregions. A total of 125 middle ears (83 cadaveric dissections) were explored by the means of 3mm straight and angled scopes. The variants were documented photographically and tabularized. The bony crests ponticulus, subiculum and finiculus[1] were most frequently represented as ridges. The ponticulus showed the highest variability with 38% ridge, 35% bridge and 27% incomplete presentation. The subiculum was bridge - shaped only in 8% of the cases, while the finiculus in 17%. The sinus tympani had a normal shape in 66% of the cases. A subcochlear canaliculus was observed in 50%. The retro- and hypotympanum were classified respectively to the present bony crests and sinus in chambers type I to IV. In our opinion, the retro- and hypotympanum have to be considered as a tightly coherent region of the middle ear. For this purpose, we propose a straightforward classification, according to the presence of the different bony crests and sinus forming the different chambers of the retro- and hypotympanum. The introduced classification may also serve as intraoperative assessment, to be aware of the different anatomical subregions. The hidden areas of the retro- and hypotampanum are difficult to access and therefore represent a region of risk for residual cholesteatomatous disease after surgical treatment. The extension below a bridge bony crest or into a deep sinus demands thorough exploration; therefore, exact anatomical knowledge and an effective technique to visualize the whole middle ear are required.

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Keywords

Endoscopic ear surgery; middle ear anatomy; sinus tympani; retrotympanum; hypotympanum; ponticulus; subiculum; finiculus; subcochlear canaliculus.

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Morphometric evaluation of the pedicles of the lumbar spine according to L5 lateral tilt classification

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A classification of the lumbar spine according to the pedicle lateral tilt (PLT) of L5 pedicle was recently proposed [1]. In this work the sample was divided into three categories, the first or Wing Type (WT) includes people with a PLT >36° (41,8%), the second or V Type (VT) includes people with a PLT between 30° and 36° (48%) and the third or U Type (UT) includes people with a PLT <30 (10,2%). The aim of the study is to evaluate the bone morphometric values and the distance between the pedicles and the nervous structures. Similar works are present in literature [2], but some are lack or for the size of the sample or for the parameters analysed. In our work seven parameters were considered: Pedicle Width (PW), Pedicle Height, Interpedicular Distance (IPD), Pedicle-Inferior Root Distance (PIRD), Pedicle-Superior Root Distance (PSRD), Root Exit Angle (REA), Nerve Root Diameter (NRD). In this study 325 patients were evaluated, a CT and MRI scan were taken to analyse respectively bone morphometry (CT) and distance between nervous structures (MRI). Statistically significant results were observed in five out seven categories, at L5: PW has a mean value of 18,5 mm in WT, 17,2 mm in VT and 15,8 mm for UT; PH has a mean value of 13,4 mm in WT, 12,8 mm (VT) and 11,2 mm in UT; IPD has a mean value of 29,2 mm in WT, 27,3 mm in VT and 25,8 mm in UT; PSRD has a mean value of 4,9 mm in WT, 4,6 mm in VT and 4,4 mm in UT; PDSD has a mean value of 1,9 mm in WT, 1,5 mm in VT and 1,3 mm in UT; REA has a mean value of 43° in WT, 40,2° in VT and 37,8° in UT. No differences were observed for PIRD (with a mean value of 1,5 mm) and NRD (with a mean value of 4 mm). Similar results were also observed for the pedicles of L4, whereas for the proximal pedicles (L3, L2 and L1) were not observed statistically significant differences into the three categories. In conclusion, the results obtained in this paper confirms the need to adopt our proposed classification according to the anatomic differences observed into our sample; in particular VT pedicle of L5 and L4 could be considered as "complicated" in a pedicle screw fixation surgery, based on the reduced bone volume and the close distance to nervous structures.

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Keywords

Pedicle; nerve root; morphometry.

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Echographic study of the muscular fasciae

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Today there is a great interest about the muscular fasciae and their possible role in myofascialpain, but it is still unclear what are their main features in living. For example the thickness of the thoracolumbar fascia, that is probably the most studied fascia, varies from 0.37 mm [1] to 0.68 mm [2]. The lack of a standard value for the fascial thickness has a great clinical relevance, indeed it seems that their increased thickness could be related to myofascial pain or reduction of the range of motion. Therefore, the definition of standard values of fascial thickness is the first step to investigate fascial alterations that may play a role in myofascial pain. The fascial thickness was evaluated in 24 subjects with a mean age of 30.46 years (SD ± 9.241).The mean BMI was of 22.08 (SD ± 3.696),in particular women with an BMI of 20.30 and 25.08 for men. The measurement was performed with the portable ultrasound system of SonoSite®, linear probe of 15 Hz. For each subject 13 deep fasciae were analyzed, both in the trunk, superior and inferior limbs. The collected data showed that the average thickness of the fasciae ranges from 0.71 ± 0.15 mm (deep fascia of the anterior region of the arm) and 1.62 ± 0.39 mm (plantar fascia). The fasciae of the anterior compartments are thinner respect to the fasciae of the posterior ones (p value <0.001). There is also a variability among the different subjects, having a range from 0.76 ± 0.19 mm (25 year old woman) to 1.12 ± 0.43 mm (20 year old men). The fascial thickness shows also a significant difference among women (mean 0.99 mm ± 0.31 mm) and men (average 1.09 mm ± 0.32 mm) (p value <0.0001). we found also a moderate correlation (p value <0.05) between thickness and age, in particular comparing the subjects under 25 years old and over 35 years. Finally, there is a strong correlation between fascial thickness and BMI, above all if we compare the thickness of subjects with BMI<19 and BMI>25 (p value <0.001). Our study is the first to demonstrate a variability in the fascial thickness in living. These data have to be considered when the fasciae will be studies in patients.

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Keywords

Fascia; myofascial pain; ecography.

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Inter and intra-procedural hemodynamic variations in the orbit of children affected by intraocular retinoblastoma and treated with intraarterial chemotherapy

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It is always assumed that in the absence of vascular disease the blood within the ophthalmic artery flows from the orbital apex towards the anterior orbital opening. As a corollary, the flow should be directed from proximal to distal also in the ramifications of the ophthalmic artery. We report a study that we carried out on 99 children affected by intraocular retinoblastoma who underwent several sessions of intra-arterial chemotherapy and that unveiled some unexpected findings [1]. As in some cases the disease was bilateral the treated orbits were 108. In all orbits the ophthalmic artery was constantly present though not always visible by selective angiography of the internal carotid artery. The blood flow within the ophthalmic artery, in fact, did not always flow anterogradely. The orbits could be entirely supplied either by the internal carotid artery or by the external carotid artery. Between these two extreme situations (internal carotid artery or external carotid artery dominance), a variety of possible hemodynamically intermediate conditions (balanced hemodynamic) could be found with part of the orbit supplied by branches of the external carotid artery and part from the internal carotid artery. These three possibilities were not always stable conditions. When a series of angiographies was carried out monthly on the same child it was not unusual to find different hemodynamic outlines. It is evident that the extension of the territories supplied by the external carotid artery and internal carotid artery could change in a matter of days at least in children. These findings unveil that in children a subtle balance exists between external carotid artery and internal carotid artery, the two vessels competing for the orbital blood supply.

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Keywords

Ophthalmic artery; orbit; hemodynamic variations; external carotid artery; internal carotid artery; retinoblastoma; intraarterial chemotherapy.

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Vessels of the umbilical cord: an anatomo-microscopic study in normal and pathological newborns

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Intrauterine growth restriction (IUGR) due to placental insufficiency is associated with blood-flow redistribution. Placental insufficiency in late-onset IUGR often goes undetected by umbilical artery Doppler scan. Despite a broad general body of literature referencing placentas of IUGR pregnancies, there are no report on the structural characteristics of the vessels of the umbilical cord in IUGR. Thus, the aim of the present study was to compare the microscopic anatomy of the umbilical arteries and veins in normal, IUGR and small for gestational age (SGA) newborns. Twenty six umbilical cords were taken from 10 IUGR, 5 SGA, and 11 controls newborns. The histological and morphological examination was performed with EE, Azan–Mallory, Sirius Red stains and morphometric evaluation was performed through a computer image analysis approach. In the controls, the umbilical artery shows a muscular tunica, organized by two layers, an outer one with circularly arranged cells, and an inner one, with irregularly arranged cells. In the IUGR longitudinal muscular fibers are observable. In IUGR the percentage of the muscular fibers of the umbilical artery was greater with respect of SGA and controls. In IUGR and SGA the percentage of the muscular fibers was minor with respect to controls. In the umbilical artery in IUGR and SGA the elastic fibers and collagen I was major and collagen III was minor with respect to SGA. In the umbilical vein the collagen III was major in IUGR and SGA with respect to controls. These data agree with those of intrauterine life, in which a major thickness of the abdominal aortic wall was observed in fetuses with abnormalities of Doppler flussimetry. The rearrangement of umbilical artery may affect the mechanical properties of these vessels and disturb fetal blood circulation.

Keywords

Umbelical artery; umbelical vein; morphometry; IUGR.

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Brödel's line: an anatomo-radiological study of the avascular kidney's plane

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The division in anterior and posterior branches of the renal artery implies the existence of an avascular plane, the so call Brödel's line (1). This longitudinal zone is described along the convex renal border (2) or just posterior to the lateral aspect of the kidney (3). The aim of this study was to describe the extension of Brödel's line with reference to the renal segments. 12 kidneys were injected with acrylic resins to obtain vascular corrosions casts that were analyzed also with computed tomography. We observed the presence of a relative avascular plane in all vascular casts, located on the posterior surface, ascribable to the Brodel's line. In 33% of cases the line extended from the apical to the inferior segments, in the 33% of cases it extended from the superior to the inferior segments, in 33% of cases it is limited to the superior and middle segments. Since the Brödel's line corresponds with the plane of the anterior surface of the posterior hilar calyces, the knowledge of its extension is relevant from the surgical point of view: this area permits a relatively safe access route to the pelvicalyceal system for nephrostomy insertion and incision within this plane results in significantly less blood loss than outside this plane.

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Keywords

Brödel's line; kidney; anatomy; arteries; computed tomography.

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Peptidergic innervation of the olfactory bulb: a sleep/wake-regulatory route through the nose

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Olfactory epithelium receptor neurons in the nasal cavity, which are exposed to the external environment, reach the olfactory bulb (OB), representing a direct port of entry to the brain. Through retrograde axonal transport, pathogens, toxins and misfolded proteins can reach brain cell groups which innervate the OB and result in functional alterations. Indeed, influenza virus nasal instillation was found to target brainstem and hypothalamic cell groups and result in narcoleptic-like sleep/wake changes [1]. These cell groups included the wake-promoting orexin (OX)-containing neurons, and the sleep-promoting melanin-concentrating hormone (MCH)-containing neurons [1]. Orexinergic innervation of the OB has been reported, but OX and MCH neurons innervating the OB have never been visualized. OX immunoreactivity in the mouse olfactory receptor neurons has been ascribed to the olfactory mucosa. Sources of input to the OB have been studied [2] before the discovery of OX in 1998. Orexinergic innervation of the prefrontal cortex is instead well established. Aim of this study was to reveal OX- and MCH-containing neurons projecting to the OB. Unilateral injections of the retrograde fluorescent tracer Fluoro-Gold (FG) confined to the OB of adult mice were combined with immunophenotyping and quantitative analysis of retrogradely labeled neurons. The findings were compared with those obtained after FG injections in the prefrontal cortex. Following FG injections in the OB, labeled neurons were found in the ipsilateral lateral hypothalamus, and included intermingled OX-A- or MCH-immunoreactive cells. About 8% of orexinergic neurons were labeled when the tracer was confined to the OB. This proportion increased (13±2.49 %) in cases in which a faint halo of tracer diffusion to the lateral portion of the prefrontal cortex was observed. Preliminary data indicate retrograde labeling from the OB of almost 15% of MCH-containing neurons. The findings demonstrate that OX and MCH neurons reach the OB directly, thus providing to environmental agents a route to sleep/wake-regulatory nodes via the nasal cavity.

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Keywords

Orexin/hypocretin; MCH; connectomics; olfactory bulb; sleep.

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Neuroendocrine circuits controlling food intake: a target for endocrine disruptors

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Tributyltin (TBT), a pesticide used in antifouling paints, is toxic for aquatic invertebrates. In vertebrates, TBT may act in obesogen- inducing adipogenetic gene transcription for adipocyte differentiation (1). In a previous study, we demonstrated that acute administration of TBT induces c-fos expression in the arcuate nucleus (2). Therefore, in this study, we tested the hypothesis that adult exposure to TBT may alter a part of the nervous pathways controlling animal food intake (3). In particular, we investigated the expression of neuropeptide Y (NPY) immunoreactivity. This neuropeptide forms neural circuits dedicated to food assumption and its action is mediated by Y1 receptors that are widely expressed in the hypothalamic nuclei responsible for the regulation of food intake and energy homeostasis. To this purpose, TBT was orally administered at a dose of 0.025 mg/kg/day/body weight to adult animals [male and female C57BL/6 (Y1-LacZ transgenic mice] for 4 weeks. No differences were found in body weight and fat deposition, but we observed a significant increase in feed efficiency in TBT-treated male mice and a significant decrease in circulating leptin in both sexes. Computerized quantitative analysis of NPY immunoreactivity and Y1-related b-galactosidase activity demonstrated a statistically significant reduction in NPY and Y1 transgene expression in the hypothalamic circuit controlling food intake of treated male mice in comparison with controls. In conclusion, the present results indicate that adult exposure to TBT is profoundly interfering with the nervous circuits involved in the stimulation of food intake.

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Keywords

Neuropeptide Y; tributyltin; pesticides; hypothalamus; transgenic mice.

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Characterization of the autophagoproteasome a novel cell clearing organelle in eukaryotic cells

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The autophagy (ATG) and Ubiquitin-Proteasome (UP) pathways clear proteins and membranes from eukaryotic cells. Their dysfunction is associated with systemic diseases, and neuronal degeneration. These pathways are commonly viewed as independent biochemical steps owing pathway-specific enzymatic activity taking place within different, site specific cell domains. While ATG is placed within a double/multiple membrane structure named autophagosome, the UP pathway is viewed as a protein complex dispersed in the cytosol. Scattered recent data provided functional evidence suggesting an interplay between ATG and UP. We recently provided morphological and biochemical evidence suggesting the existence of a close relationship between ATG and UP which may converge to form a novel organelle named autophagoproteasome. In the present study we characterized the autophagoproteasome by using various experimental approaches in vitro and in vivo. We studied the autophagoproteasomes in baseline conditions, following mTOR inhibition, and during specific neurotoxic treatments. The quantitative evaluation of ATG and UP component within autophagoproteasomes was carried out by confocal microscopy and ultrastructural morphometry. The number of autophagoproteasomes increases following mTOR inhibition. Again, specific neurotoxins as well as endogenous neurotransmitters modulate the expression of autophagoproteasomes. Remarkably, within autophagoproteasomes the relative amount of ATG compared with UP components varies depending on experimental conditions. Despite its morphological novelty the autophagoproteasome appears to be the organelle where ATG and UP (originally regarded to be independent structures) co-exist and share the catalytic activity. In addition, ATG and UP co-immunoprecipitate, suggesting a reciprocal binding and functional interplay.

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Keywords

Autophagy; proteasome; ubiquitination; protein clearing pathways.

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Membrane protein remodeling in microglia exposed to amyloid peptides

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Infection, neurodegeneration, and other conditions associated with loss of brain homeostasis, induce changes in microglial morphology, gene expression and function, generally referred to as "activation". Alzheimer's disease (AD) is the most common dementia and is characterized by neuroinflammatory changes, including alterations in the morphology and distribution of microglia and astrocytes, and deposition of complement and other inflammatory mediators. Our previous observations show that microglial cells challenged in vitro with amyloid peptides clustered and rounded up, dramatically changing their morphology. Besides, in these cells we observed the early acetylation and then the phosphorylation of STAT3 which is required for the expression of the epsilon isoform of 14-3-3, a marker of Abeta-activated microglia (1, 2). We applied affinity partitioning approach combined with high throughput mass spectrometric analysis in order to identify variation of proteins on plasma membrane of BV2 immortalized microglia upon treatment with amyloid peptides. By this method several proteins up- or down-regulated by amyloid treatment were identified in microglial plasma membrane. Among them annexins (5 and 7), IFITM3 and MARK3. These data have been confirmed in primary microglial cultures.

In microglia, plasma membrane plays a relevant role in the cross-talking with the external neuronal environment and in the resulting trophic or inflammatory response of these sentinel cells. As such, knowledge of the microglia responsiveness to beta amyloids in term of changes in its plasma membrane proteome is imperative for unveiling the molecular landscape in which AD occurs.

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Keywords

Microglia; Alzheimer's disease; neurodegeneration.

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Cell-to-cell communication within the neurovascular unit (NVU) in a model of cerebral cortex demyelination

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The concept of neurovascular unit (NVU) emphasizes the critical role of cell-to-cell interaction and communication between glial, neuronal, and vascular cell components during blood-brain barrier (BBB) development, and in adult normal and pathological conditions. In this study we have analysed the involvement of the nerve glial antigen 2, NG2, a chondroitin sulphate proteoglycan, highly expressed in developing and adult CNS, in cell cross-talk within the NVU. During CNS development NG2 is expressed by activated pericyte and appears downregulated as these cells undergo terminal differentiation. NG2 has also been identified on the surface of oligodendrocyte precursor cells, OPCs, evenly distributed throughout the CNS already by the end of the first postnatal week in mice and throughout adulthood. In a previous study on cerebral cortex experimental autoimmune encephalomyelitis (EAE) in mice, we firstly observed and described the glia-limitans-like position of NG2-bearing OPCs that during neuroinflammation extend processes to the pial surface and acquire a perivascular arrangement, coming in contact with the wall of EAE cortex microvessels. With the aim of understanding if a subset of OPCs specifically contributes to the cell composition of the NVU during EAE, we have explored, by morphometric analyses applied to laser confocal microscopy, OPCs distribution and vascular relationships in the cerebral cortex of WT controls and naïve NG2KO and in EAE WT and EAE NG2KO mice, at both early (20 dpi) and late (40 dpi) disease stages. In EAE WT mice, juxtavascular (JV) and perivascular (PV) OPCs were identified in a higher number compared to healthy mice. On the contrary, absence of NG2 in EAE NG2 KO mice seemed to affect the proliferative response of OPCs, specifically inhibiting the emergence of the JV and PV OPC subsets. The results indicate that in WT mice during EAE, the NVU microenvironment, classically formed by perivascular astrocytes, receives the insertion of OPCs as a specific vascular subset and suggest NG2 as the molecule involved in the observed NVU damage.

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Cadmium-induced neurotoxicity: impairment of the blood brain barrier

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Cadmium (Cd), an ubiquitous heavy metal, known to be accumulated outside of the blood–brain barrier (1) and to cause neurotoxicity, has also been demonstrated to induce an increase in the blood–brain barrier (BBB) permeability (2). Key components of BBB integrity are primarily the tight junctions (TJs) between adjacent brain microvascular endothelial cells that confers low paracellular permeability, making the barrier to function (3). Cd-dependent BBB alterations are elicited by a caspase-3 activation-dependent pathway (4) that triggers the irreversible open of pannexin-1 (panx-1) (5), a large transmembrane channel that allows an ATP massive spillage (6), imparing the neurovascular unit (NVU) homeostasis (7). In this study, we investigated the Cd cytotoxicity in a rat brain endothelial cell line (RBE4). Results from the cell viability assay showed that Cd caused a remarkable decrease in cell viability in a dose-dependent manner. 10 μM Cd induced caspase 3 activation and an increment in extracellular ATP concentration, indicative for a panx-1 involvement. The increase of BBB permeability was evaluated analyzing zonula occludens-1 (ZO-1) expression levels and its subcellular dislocation. ZO-1 is a protein localized on the plasma membrane in areas of cell-cell contact that acts as a crucial central regulator of the structural organization of the TJs (8). The presence of Cd 10 μM caused a significative reduction of ZO-1 expression levels (as determined by western blot technique) and an altered distribution of this protein (analyzed by immunofluorescence) that appears patchy or faded away from membrane areas. Summarizing, these data offer an initial image of the NVU homeostasis impairment induced by Cd, suggesting Panx-1 as a novel target to counteract its neurotoxicity.

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Keywords

Cadmium; BBB permeability; pannexin-1.

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PAR1 activation induces the release by Schwann cells of factors promoting cell survival and neuritogenesis

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Schwann cells (SCs) regulate a wide variety of axonal functions in the peripheral nervous system, providing a supportive growth environment following nerve injury (1). Here we show that rat SCs express the protease-activated receptor-1 (PAR1) both in vivo and in vitro. PAR1 is a G-protein coupled receptor eliciting cellular responses to thrombin and other proteases (2). To investigate if PAR1 activation affects the neurotrophic properties of SCs, this receptor was activated by a specific agonist peptide (TFLLR) and the conditioned medium was transferred to PC12 pheocromocytoma cells for assessing cell survival and neurite outgrowth. Culture medium from SCs treated with 10 μM TFLLR reduced significantly the release of LDH and increased the viability of PC12 cells with respect to the medium of the untreated SCs. Furthermore, conditioned medium from TFLLR-treated SCs increased neurite outgrowth on PC12 cells respect to control medium from untreated cells. To identify putative neurotrophic candidates we performed proteomic analysis on SC secretoma and real time PCR experiments after PAR1 activation. Stimulation of SCs with TFLLR increased specifically the release of a subset of five proteins: Macrophage migration inhibitory factor (Mif), Aldose reductase (Akr1b1), Matrix metalloproteinase-2 (Mmp2), Syndecan-4 (Sdc) and Decorin (Dcn). At the same time there was a significant decrease in the level of three proteins: Complement C1r subcomponent (C1r), Complement component 1 Q subcomponent-binding protein (C1qbp) and Angiogenic factor with G patch and FHA domains 1 (Aggf1). These data indicate that PAR1 stimulation does induce the release by SCs of factors promoting cell survival and neuritogenesis. Among these proteins, Mif, Sdc, Dcn and Mmp2 are of particular interest.

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Keywords

Protease-activated receptor 1; thrombin; peripheral nerve; Schwann cells.

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Oxidative stress related to obesity: results in plasma and brain areas of obese Zucker rats

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Oxidative stress may be a consequence and/or cause of several pathologies, such as diabetes, metabolic syndrome (MetS) and cerebrovascular disease. Oxidative stress is also tightly bound to the physiological condition of obese individuals. The obese Zucker rats (OZRs), with a mutation in leptin receptors represent a model of type II diabetes mellitus, characterized by the simultaneous occurrence of obesity, hyperglycemia, hyperinsulinemia, hyperlipidemia and moderate hypertension similar to MetS. The present study has investigated oxidative stress alterations occurring in OZR compared to Lean Zucker Rats (LZRs) of 12, 16 and 20 weeks of age. Thiobarbituric acid reactive substance (TBARS), gluthatione-peroxidase (GPX) and superoxide dismutase (SOD) activity, oxidative state of protein and the expression of 8-hydroxy-2'deoxyguanosine (8-oxo-dG) as a nuclear marker, were evaluated in plasma and in various brain areas (frontal cortex and hippocampus) of rats at different age. OZRs were characterized by higher body weight, an increase of systolic pressure, glycemia, triglycerides and cholesterol values in comparison with age-matched LZRs. An age-dependent increase of these parameters was observed in OZRs. TBARS values were higher in plasma of OZRs at all ages. The SOD activity was decreased in plasma of OZRs whereas GPx activity did not show differences between lean and obese rats. Oxi-blot analysis showed an obvious increase of oxidative state of proteins on obese rats samples, particularly in the 20-weeks-old rats. In frontal cortex the TBARS values were higher in 20-weeks-old OZRs, but no difference was found in hippocampus. The enzyme activity decreased, whereas the oxidative status of protein increased in the brain of OZRs compared to age-matched LZRs. An increase of nuclear 8-oxo-dG immunofluorescence detection was revealed both in frontal cortex and hippocampus neurons of 20-weeks-old LZRs. These findings demonstrate an increase in lipid peroxidation, protein carbonylation and an alteration of enzyme activity, suggesting an increase of oxidative stress in plasma and in the brain of OZRs. These observations consistent with previous studies, confirm that the increase of oxidative stress is associated with metabolic complications in OZRs. In terms of practical consequences these data may help to better manage MetS progression and the correlations with neurodegenerative processes.

Keywords

Obesity; oxidative stress; brain; obese Zucker rats.

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Tubulin involvement in Bortezomib peripheral neurotoxicity

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Axonal transport of mitochondria (Mt) controlled by specialized motor and docking proteins that distribute Mt throughout the axon where they provide energy for metabolic and synaptic activity is a vulnerable target in neuronal pathology (1). Bortezomib (BZ) is a proteasome inhibitor active in multiple myeloma (2). One of its key toxicities is painful peripheral neuropathy (BIPN), which frequently requires treatment discontinuation (3). BIPN is dose-related and predominantly sensory, resulting from axonal degeneration. Recent results indicate that BZ modifies axonal tubulin dynamic and we hypothesize that BZ alters fast axonal transport. Here we studied using time-lapse imaging the effect of different BZ concentration on axonal Mt transport in isolated dorsal root ganglion (DRG) neurons from adult male mice. We used kymograph to quantify the total number of Mt and to discriminate antero and retrogradely moving Mt from stationary Mt. Twenty-four hours of BZ treatment (0.1 to 15 μM) induced a dose-dependent reduction in Mt trafficking. Moreover, BZ had no impact on MT motion directions, but it induced a progressive reduction of both anterograde and retrograde axonal transport velocities. These events were associated with increase in tubulin polymerization and of MAP2 expression, but they occurred only after 72h of chronic BZ treatment. We have developed an in vitro model of BIPN demonstrating that transport impairment is already present before evident tubulin polymerization, suggesting that transport deficit represents an early stage of axonal dysfunction. Perpetuated transport dysfunction could impair distal organelle supply and play a critical role in advanced stages of BIPN.

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Keywords

Bortezomib; peripheral neuropathy; mitochondrial trafficking; adult DRG sensory neurons; mice.

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Protective effect of selenium and zinc against cadmium toxicity in SHSY-5Y neurons

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Cadmium is a widespread heavy metal environmental toxicant and pollutant. Cadmium toxicity affects many tissues and organs including the central nervous system and cadmium exposure has been related to many neurodegenerative diseases (1). Cadmium-induced toxic effects include oxidative damage, apoptosis, interference with calcium/zinc-dependent mechanisms, inhibition of cellular respiratory processes and others, but the underlying mechanisms of cadmium neurotoxicity are not completely understood (2). On the other hand heavy metal toxicity can be counteracted by bioelements such as zinc, selenium and others mainly through the induction of metallothionein expression levels and other antioxidant pro-teins (3). Human neuroblastoma SHSY-5Y cell line, in both the undifferentiated and neuronal-like differentiated state, were used in this study to better elucidate the mechanisms un-derlying the protective effect of zinc and selenium against the cadmium neurotoxicity. Toxic effects of cadmium chloride (10 mM, 24h) observed by cell viability assay, western blot analysis of Bax and Gap-43, and immunostaining of b3 tubulin and cytochrome c proteins, were reverted to control values by a 24h-pretreatment with zinc chloride (50 mM) both in undiffer-entiated and differentiated neurons. Interestingly, the reverting effect of a 24h-pretreatment with sodium selenite (100 nM) against cadmium toxicity, was observed only in undifferentiat-ed neurons. In conclusion we can hypothesize that in undifferentiated and differentiated SHSY-5Y neurons, the protective effects of zinc and selenium compounds against cadmium toxicity depend on the activation of partially common signalling pathways. Moreover sodium selenite dont exert a significant protective effect in differentiated SHSY-5Y demonstrating that the differentiated and undifferentiated phenotypes show different responses.

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Keywords

Cadmium; SHSY-5Y cell line; zinc; selenium.

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Polydatin (3,4′,5-trihydroxystilbene-3-β-d-glucoside) is a new inhibitor of glucose-6-phosphate dehydrogenase affecting cancer metabolism and producing a strong cytotoxic and antimetastatic effect

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Polydatin (3,4′,5-trihydroxystilbene-3-β-d-glucoside) is a natural precursor of resveratrol already approved for commercialization as food supplement. It is present in many types of plants, including grape, peanut, and cassia seed where it acts as antimycotic agent. Polydatin has been proposed to have effect on cancer, including HNSCC and breast cancer, with promising results, but its mechanism of action seems to be different from resveratrol and is poorly understood. Glucose-6-phosphate dehydrogenase (G6PD) is the limiting enzyme of the pentose phosphate pathway which have been widely shown to be fundamental for tumor growth and metastasis formation. In this work our results show that polydatin inhibit G6PD in a dose dependent manner affecting cancer cell viability, causing a dose-dependent apoptosis and cell cycle arrest. Moreover, treated cells showed a strong increase of the Unfolded Protein Response (UPR), activated by a stress in the endoplasmic reticulum (ER), autophagy, reduced migration and invasion ability. Moreover, we developed a metastatic orthotopic HNSCC model in immunocompromised mice and showed that treated group had reduced tumor growth and reduce lymph nodes metastases. In conclusion here we show that Polydatin exerts a significant inhibitory effect on pentose phosphate pathway inhibiting HNSCC growth and metastases, pointing out that polydatin may be a reliable anticancer drug.

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Keywords

G6PD; pentose phosphate pathway; ER Stress; metastasis.

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Valproic acid and 5-azacytidine promote an increase of stemness phenotype in human osteosarcomas

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Osteosarcoma (OS) is the most common pediatric tumour of bone in the world. It leads to local invasion and early metastasis to lung. The prognosis is poor with the 5-year survival rate of 65% for patients with primary tumour and 20% for patients with metastasis. OS contain a small cell population with stem cell like properties, referred to as cancer stem cells characterized by CD133 expression [1,2]. Recent studies have implicated epigenetic alterations, including DNA methylation and histone modifications, as prominent factors that contribute to the phenotype of cancer progenitor or stem cells. Here, we examined the epigenetic effects of the HDAC inhibitor valproic acid (VPA) and demethylating agent, 5'azacytidine (DAC), on the stemness phenotype in osteosarcoma cell lines. Saos-2 and MG63 cell lines were treated with 0,5 mM VPA and 3μM DAC for 48 hours alone, and in combination. CD133 expression as well as stemness markers expression including OCT4, Sox2 and Nanog was analyzed by flow cytometry and real-time PCR. Vimentin and osteocalcin levels were also tested. Sarcospheres formation rate was assessed as spheres number/seed single cells number. Specific histone modifications including H3-trymethyl-k9, H3-acetyl-k9, H3-trymethil-k27 and global methylation were analysed by flow cytometry and immunofluorescence. Moreover, soft agar and invasion assays were performed. Our findings indicated that DAC or VPA and their combination induced an increase of stemness characteristics of OS cells in terms of high expression of CD133, OCT4, Sox2 and Nanog and high sarcospheres-forming efficiency. Interestingly, combined treatment with DAC and VPA induced an increase of CD133 expression in a synergistic manner in all cell lines. Vimentin resulted up-regulated after treatment, whereas, the level of osteocalcin remained similar before and after treatment. Furthermore, soft agar assay revealed major colony-forming efficiency in treated cells compared to untreated cells, whereas invasion potential decreased after drug treatment. Interestingly, histone modification analyses correlated with those typical of embryonic stemness with an increase of H3-acetyl-k9 and decrease of H3-trymethyl-k9, and H3-trymethil-k27 after drug treatment as well as global methylation decreased in treated cells. In conclusion, DAC and VPA induced an increase of stemness associated to an decrease of global methylation, increase of acetylation and decrease of H3-trymethyl-k9, and H3-trymethil-k27.

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Keywords

Cancer stem cells; osteosarcomas; sarcospheres; methylation; histone deacetilase inhibitor.

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Activation of anti-apoptotic machinery downstream to Fas/FasL pathway in primary mixed and pure mucin producing cholangiocarcinoma cells: key role of c-FLIP

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Cholangiocarcinoma (CCA) comprises a heterogeneous group of malignancies lacking of effective strategies for prevention and cure. Recently, we have established a protocol for the isolation of primary cells from mixed and mucin specimens of human CCA. To this regard, the aim of this study was to analyze the influence on proliferation and apoptosis as well as the related modifications of apoptotic machinery downstream to Fas/FasL pathway in primary cultures of human mixed and mucin-producing CCA after direct co-culture with peripheral blood mononuclear cells (PBMCs). Our findings show that both IH-CCA subtypes constitutively express high levels of Fas and FasL. Following direct co-culture with PBMCs, the expression of Fas and FasL significantly increased after 24, 48 and 72 hours of exposure ($p< 0.05$). At the same time, a significant increase of percentage of apoptotic CD4+ and CD8+ T-cells or Natural Killer CD56+ cells was observed along the co-cultures compared to PBMCs cultured alone ($p<0.05$). Conversely, both IH-CCA subtypes showed an augmentation of the proliferation rate after co-culture with PBMCs ($p<0.05$). WB analysis revealed a stable expression of FADD in IH-CCA primary cells either cultured alone or co-cultured with PBMCs. Interestingly, both IH-CCA subtypes showed an increased expression of c-FLIPS/L, namely, a 47±3% increase in mucin CCA co-cultured for 24 hours with PBMCs vs cells cultured alone ($p< 0.05$), and a 35±3% increase in mixed CCA co-cultured for 24 hours with PBMCs vs cells cultured alone ($p< 0.05$). IF analysis showed a strictly nuclear staining for c-FLIPS/L in both IH-CCA subtypes cultured alone, whereas, after co-culture with PBMCs, either a nuclear and a cytoplasmic staining for c-FLIPS/L were observed. Interestingly, a significant increase of the expression of pro-caspase 8 and Bcl-2 was detected. In conclusion, these data demonstrate that a direct co-culture with PBMCs induces an increased expression of Fas and FasL, followed by an increase of c-FLIPS/L in primary cultures of mixed and mucin IH-CCA, culminating in anti-apoptotic and proliferative effects in cancer cells. Moreover, as shown for other cancer cells, c-FLIPS/L proved to be the key molecule of cell proliferation and survival in the immune-escape in subtypes of IH-CCA and might represent a potential therapeutic target in deadly and drug refractory cancers.

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Epigenetic regulation of nuclear PLCbeta1 and Cyclin D3 during Azacitidine treatment

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The Myelodysplastic Syndromes (MDS) are a heterogeneous group of bone marrow disorders characterized by alterations of the hematopoietic stem cells that lead to anemia, neutropenia, bleeding problems and infections. The evidence of a clinical correlation between the presence of a monoallelic gene deletion of Phospholipase Cβ1 (PLCβ1) and the progression of MDS to Acute Myeloid Leukemia (AML) opened new perspectives of research and treatments. Patients affected by MDS with a higher risk of AML evolution have a reduction in the expression of the nuclear PLCβ1, which is also epigenetically relevant in MDS. This strengthens the importance of PLCβ1 localization. In fact, PLCβ1 is a molecular target for hypomethylating agents, such Azacitidine (AZA)(1). High-risk MDS patients that respond to the drug showed an increased expression of nuclear PLCβ1 and its downstream target Cyclin D3 (CCND3), an induction of normal myeloid differentiation, and a better prognosis. Stemming from these data, our goal was to analyze the correlation between CCND3, PLCβ1 and AZA treatment. Firstly, we treated two different cellular lines, AML HL60 and histiocytic lymphoma U937, with AZA 5μM (Ec50 for HL60 cells) for 24 hours. Then, we used Real-Time PCR and Western blot to quantify both gene and protein expression. Moreover, we showed that CCND3 promoter has one CpG island. For this reason, it is possible that AZA could directly affect both PLCβ1 and CCND3 promoters. Therefore, we studied PLCβ1 binding to CCND3 promoter by chromatin immunoprecipitation (CHIP), before and after AZA treatment. Our results evidenced that the recruitment of PLCβ1 to CCND3 promoter is specifically increased after AZA treatment, leading to suppose that PLCβ1 could have a pivotal role in MDS with either a direct or indirect effect on cell cycle, proliferation and differentiation. These complicate relations need future deepening in order to demonstrate how PLCβ1 binding actually regulates CCND3 expression and how much this expression depends on CCND3 direct promoter demethylation and PLCβ1 control.

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Keywords

Nucleus; phospholipase Cβ1; azacitidine; myelodysplastic syndromes; cyclin D3.

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Cyclin D1 silencing suppresses tumorigenicity, impairs DNA double strand break repair and thus radiosensitizes androgen-independent prostate cancer cells to DNA damage

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Patients with hormone-resistant prostate cancer (PCa) have higher biochemical failure rates following radiation therapy (RT). Cyclin D1 deregulated expression in PCa is associated with a more aggressive disease: however its role in radioresistance has not been determined. Cyclin D1 levels in the androgen-independent PC3 and 22Rv1 PCa cells were stably inhibited by infecting with cyclin D1-shRNA. Tumorigenicity and radiosensitivity were investigated using in vitro and in vivo experimental assays. Cyclin D1 silencing interfered with PCa oncogenic phenotype by inducing growth arrest in the G1 phase of cell cycle and reducing soft agar colony formation, migration, invasion in vitro and tumor formation and neo-angiogenesis in vivo. Depletion of cyclin D1 significantly radiosensitizes PCa cells by increasing the RT-induced DNA damages by affecting the NHEJ and HR pathways responsible of the DNA double-strand break repair. Following treatment of cells with RT the abundance of a biomarker of DNA damage, γ-H2AX, was dramatically increased in sh-cyclin D1 treated cells compared to shRNA control. Concordant with these observations DNA-PKcs-activation and RAD51-accumulation, part of the DNA double-strand break repair machinery, were reduced in shRNA-cyclin D1 treated cells compared to shRNA control. We further demonstrate the physical interaction between CCND1 with activated-ATM, -DNA-PKcs and RAD51 is enhanced by RT. Finally, siRNA-mediated silencing experiments indicated DNA-PKcs and RAD51 are downstream targets of CCND1-mediated PCa cells radioresistance. In summary, these observations suggest that CCND1 is a key mediator of PCa radioresistance and could represent a potential target for radioresistant hormone-resistant PCa.

Keywords

Prostate cancer; cyclin D1; radioresistance.

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Relevance of fatty acid metabolism in proliferating CLL cells

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Chronic lymphocytic leukemia (CLL) cells undergo, during their life, iterative cycles of re-activation and subsequent clonal expansion. We previously demonstrated that the antidiabetic drug metformin, known to also inhibit oxidative phosphorylation (OXPHOS), inhibits cell cycle entry of leukemic cells derived ex-vivo from the peripheral blood of CLL patients and stimulated in vitro by cell culture systems that recreate a microenvironment to drive their proliferation (Bruno et al, Oncotarget, 2015). However, overtly proliferating CLL cells were resistant to the cytostatic effects of metformin. Since metformin switched the energetic metabolism of activated, not yet proliferating, CLL cells from OXPHOS to accelerated glycolysis, in the present study we asked whether combining metformin with glycolysis impairment could inhibit also proliferating CLL cells. Still, CLL cells recovered from a transitory block and rescued in vitro proliferation. What kind of energetic reprogramming was involved in the resistance of proliferating CLL cells to glucose utilization? Recent studies highlight on the role of fatty acid utilization of CLL cells. We asked 1) whether inhibitors of lipid metabolism could impair proliferation of in vitro stimulated CLL cells; 2) whether impairing glucose energetic pathways could act synergistically with beta oxidation inhibitors.We found that inhibitors of critical steps of fatty acid metabolisms, such as carnitine-palmitoyl transferase 1A (CPT1A) -rate-limiting enzyme for fatty acid import into mitochondria- or Peroxisome Proliferator-Activated Receptor (PPAR)-alpha -regulator of beta-oxidation- administered at clinically achievable doses, were ineffective on quiescent CLL cells and on CLL cells stimulated by the microenvironment during the first stages of activation. Conversely, remarkable susceptibility to undergo apoptosis was observed at later stages of cell activation and during overt proliferation. Synergism with impairment of other energetic pathways occurred depending on the stage of activation of the in vitro stimulated CLL cells. The results suggest that energetic metabolic pathways could be relevant targets for CLL treatment, provided that the complex metabolic reprogramming network during the transition of leukemic cells from quiescence to proliferation, and back, are clearly elucidated.

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Keywords

Energetic metabolism; cell proliferation; chronic lymphocytic leukemia.

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Plantar support for a correct gait

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Postural adjustments to maintain static and dynamic balance depend on the relationship between sensory input organs and the environment. The study of foot posture assists in the evaluation of overall posture. The human foot is the basis of support and propulsion for gait and it provides support and flexibility for effective weight transfer (1). Foot posture involves the integration of sensory information from the periphery to the body, especially mechanoreceptors in the sole of the foot, related to gravitational acceleration, the environment and the position of the segments of the body. Numerous studies have investigated the effectiveness of orthotics that, by increasing the contact surface between the foot and the ground, were going to decrease the load associated with certain areas of plantar surface (2). The aim of our study was to evaluate the effects of innovative insoles, named Regular Gait (RG), on plantar pressures distribution during standing position and walking in healthy subjects; therefore, we investigated whether these effects are maintained after insole removing. 30 subjects were tested; these were free of foot diseases or damage to the anatomical structures involved in the processes above posture. These subjects underwent rating scales and static and dynamic baropodometric examination before and after using RG. The results obtained, subjected to statistical analysis for significance, show that the RG, for as we have designed, is able to restore a correct distribution of the parameters both in static and dynamic conditions. We have also shown that the best results were obtained only after a month of treatment with RG and that the results obtained persist even in the tests post-treatment without insoles. The fact we charged to the special geometry with which the insole is designed: its supports, that are located in specific regions of the plantar arch, go to stimulate the mechanoreceptors found there. In this way, through the streets proprioceptive, you can obtain a reorganization of the plantar stance even at the higher nervous centers level. This allowed the subjects treated to improve their posture both while walking and during the maintenance of the upright position. As far demonstrated , the RG seems to be a tool whose potential does not end in the modification of the plantar stance, but that influences a number of processes, by acting on the kinetic chains that originate from the foot.

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Keywords

Proprioception; arch support; deambulation.

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The role of ankle plantar flexion in the monitoring of diabetic patients at risk of foot ulcer

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Limited ankle joint mobility (AJM) is a major risk factor for ulcers and a useful parameter for monitoring the health of patients with diabetes. The aim of this study was to test the feasibility and usefulness of evaluating AJM for monitoring ulcerative risk. AJM in plantar (PF) and dorsiflexion (DF) was evaluated in 99 patients with diabetes, type1/type2: 50/49, (58/41;M/F), and 59 healthy controls (32:27/M:F). Patients and controls were divided into 6 groups by age and neuropathy: 32 young patients (group YD), mean age 12.4±2.0 yr, 29 young controls (group YC), mean age 11.4 ± 3.3 yr, 38 elderly patients without neuropathy (group ED), mean age 58.5 ±10.3 yr, 15 neuropathic patients without history of foot ulcer (group ND), mean age 62.1 ± 7.9 yr, 14 neuropathic patients with history of foot ulcer (group NUD), mean age 64 ± 8.4 yrs, and 30 elderly healthy controls (group EC), mean age 60.3 ± 6.4 yr. AJM was evaluated by an inclinometer with the patient lying supine, the subtalar joint in neutral position and with the ankle in the position freely taken at the beginning. The knee, corresponding to the evaluated ankle, was extended and put over a rigid 5-cm high support. Diabetes duration was respectively: group YD 5.5 ± 3.5 yr, ED 16.5 ± 10.6 yr; ND 18.2 ± 13.1 yr and NUD 13.7 ± 9.6 yr. The NUD group showed a more significant AJM reduction in DF and PF than all other groups ($p < 0.005$). The reduction was 40.1% compared to the EC group and 46.9% compared to the YC group (78.1 ± 18.4 vs 147.2 ± 19.1, 130.4 ± 15.1). Only the DF was significantly reduced in the NUD group compared to the ED group ($p < 0.001$). The YD had more reduced AJM in both movements compared to the young controls (YC) ($p < 0.001$) with PF more reduced than DF (30.9% vs 15.5%). Among patients and controls the elderly groups had significant reduction of only DF (EC vs YC $p < 0.001$; ED vs YD $p < 0.05$). As in previous studies, these results confirm that an AJM reduction of about 40% (28-32) in patients with diabetic neuropathy can be considered as a threshold for ulcer risk. The method used permits direct evaluation of AJM in plantar flexion that seems to show an early reduction in diabetic subjects, thereby providing useful information for patient monitoring.

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Keywords
Diabetic foot, diabetes, limited joint mobility.

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The impact of diabetes mellitus and sport practice on joint mobility, muscle strength and posture of young subjects

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It is known that diabetic disease may modify patients' joint mobility, muscle strength and posture. The aim of this study was to evaluate the presence of differences between young patients with T1DM and young sports subjects. In 23 patients with diabetes (13/11:m/f), mean age 10.7±1.2 yrs, duration of diabetes 6.1±3.0 yrs, mean HbA1c 7.6±0.9 %, (group D), and in 52 healthy control subjects (39/14:m/f), mean age 11,1±1,5 yrs (group C) were evaluated muscle strength (standing long jump, hand grip, key pinch test), ankle joint mobility (AJM) (inclinometer), flexibility (sit and reach test), foot plantar pressure distribution in quiet standing and posture (baropodometric analysis, images). Group C was composed by 2 teams of soccer players: 23 soccer players (23/0:m:f), mean age 12,0±0,3 yrs (group SP1); 15 young soccer players (15:0/m:f), mean age 9,0±1,3 yrs (group SP2); and a team of 14 volleyball players (0:14/:m:f), mean age 11,8±0,3 yrs (group VP). The group D showed a significant reduction of AJM in plantar flexion compared to VP group (31.2 ±5.5 vs 41.0 ±5.6; $p<0.001$). The SP1 group showed a significant lower AJM compared to the VP group (122.5 ±30.0 vs 149.3 ±13.7; $p<0.005$), and the ankle plantar flexion (28.4 ±7.5 vs 41.0 ±5.6; $p<0.001$) was more reduced compared to the dorsal one (108.3 ±10.2 vs 94.1±23.7; $p<0.05$). Considering all subjects investigated AJM was directly correlated with flexibility ($r=0.37$; $p<0.005$). Only in the group D, AJM in plantar flexion and flexibility had not a significant correlation. The strength tests did not show differences between the groups age-matched. Strength exerted in the hand grip test and in the key pinch test were correlated ($r=0:44$; $p<0.01$). On the sagittal plane the inclination of the axes that arise from the center of the lateral malleolus and passing through the center of the head of the fibula or the tragus of the ear, was directly correlated in control groups (control: $r=0.52$, $p<0.01$). The inclination of the axis passing through lateral malleolus and head of the fibula was significantly correlated with the forefoot and rear-foot plantar pressure distribution ($r=0.38$; $p<0.05$). The results of this pilot study show that diabetes and playing soccer may negatively affect ankle mobility and the posture on the sagittal plane of young subjects. The confirmation of these results in larger studies might justify appropriate preventive interventions.

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Keywords

Diabetic foot, diabetes, limited joint mobility.

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Moderate exercise improves cardiac hypertrophy in female aged mice

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Age-related diastolic dysfunction has a significant impact on the elderly health, in fact the left ventricular filling is impaired, limiting intense exercise tolerance and reducing the quality of life. Pathological hypertrophy is commonly associated with up-regulation of fetal genes, fibrosis, cardiac dysfunction, fat deposits and increased mortality. Regular and moderate physical activity improves cardiac performance in elderly people. Type 5 cyclic nucleotide phosphodiesterase (PDE5) regulates intracellular cGMP levels and its increased expression has an important role in the development of cardiac hypertrophy (1). Our hypothesis is to assess if moderate exercise modulates PDE5 expression and reduces cardiac hypertrophy in old mice. CD1 female mice were grouped in young (2 months) sedentary (YS), young trained (YE), old 20 months sedentary (OS) and old old trained (OE). Exercise was performed at moderate intensity (speed of 13 m/min, for 30 minutes) on tapis roulant for 5 days/week. Morphometric (left ventricular weight/tibial length ratio) and histological (cardiomyocyte size) analyses showed that cardiac hypertrophy is present in OS compared to YS and YE and significantly reduced in OE group compared to OS. Moderate exercise also attenuated cardiac fibrosis in OE group. Molecular analysis revealed that hypertrophic markers such as ANP, BNP, GATA 4 and NKX 2.5 were significantly down-regulated in OE group. SIRT1 and PPARα, two regulators of oxidative stress and fat metabolism, were up-regulated in aged trained mice. PDE5 expression is down-regulated after exercise in OE group. These results suggest that exercise leads to a beneficial effect in old mice. Interestingly PDE5 expression correlates with the anti-hypertrophic effect of training in old mice.

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One repetition maximum bench press performance: a new approach for its evaluation in inexperienced males and females. A pilot study

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The aim of this study was to evaluate a new method to perform the one repetition maximum (1RM) bench press test, by combining previously validated predictive and practical procedures. Eight young male and 7 female participants, with no previous experience of resistance training, performed a first set of repetitions to fatigue (RTF) with a workload corresponding to ⅓ of their body mass (BM) for a maximum of 25 repetitions. Following a 5-min recovery period, a second set of RTF was performed with a workload corresponding to ½ of participants' BM. The number of repetitions performed in this set was then used to predict the workload to be used for the 1RM bench press test using Mayhew's equation. Oxygen consumption, heart rate and blood lactate were monitored before, during and after each 1RM attempt. A significant effect of gender was found on the maximum number of repetitions achieved during the RTF set performed with ½ of participants' BM (males: 25.0 ± 6.3; females: 11.0x± 10.6; $t = 6.2$; $p < 0.001$). The 1RM attempt performed with the workload predicted by Mayhew's equation resulted in females performing 1.2 ± 0.7 repetitions, while males performed 4.8 ± 1.9 repetitions. All participants reached their 1RM performance within 3 attempts, thus resulting in a maximum of 5 sets required to successfully perform the 1RM bench press test. We conclude that, by combining previously validated predictive equations with practical procedures (i.e. using a fraction of participants' BM to determine the workload for an RTF set), the new method we tested appeared safe, accurate (particularly in females) and time-effective in the practical evaluation of 1RM performance in inexperienced individuals.

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Keywords

1RM bench press test; performance; predictive equations.

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Effects of a ludic-motor program on motor development and early literacy skills in preschool children

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There is insufficient evidence in the literature about many correlations between motor and cognitive skills in 3-5 year old children [1]. The aim of this study was to explore the relationship between the development of the gross motor skills and the prerequisites of reading/writing following a ludic-motor program (LMP) in pre-school children. This study has involved 189 children (age: 4.62 ± 0.97 years; height: 107.83 ± 7.82 cm, body weight: 19.84 ± 4.95 kg) attending 8 kindergartens in Palermo. The children were randomly divided in a control group (C, n= 29), a 1-intervention group (I-1, n= 120) and a 2-intervention group (I-2, n= 40). I-1 and I-2 respectively performed 4 and 10 hours/week of a 16-week LMP carried out by outside experts; while C children do not perform any LMP. This program was planned in 21 learning modules aimed to develop bodily schemes, basic motor skills, fine motor control and coordination abilities. Before and after the LMP, locomotor and object control skills were evaluated with the Test of Gross Motor Development, while early reading/writing skills with the PRCR-2 test. Analyses of covariance were performed to compare outcomes for I1, I2 and C groups at post-test and the covariate was the participants' measure of cognitive skills at pre-test. Statistical significance was defined at $p<0.05$. I-1 and I-2 groups showed a significant increase in both motor skills compared with C group after the LMP. A significant decrease in the number of errors concerning the serial work skills from left to right, the visual analysis and memory was found in I-1 and I-2 groups compared with C group following the LMP. Moreover, in I-2 group we observed a positive correlation between the pre-requisites of writing/reading and the Quotient of Gross-Motor Ability. The level of motor skills of children of 3-5 years is dependent on the amount of structured physical activity executed. More the level of motor skills reached is high more is correlates with a greater learning of cognitive skills. Therefore, this activity may be a tool to encourage academic achievement of all children and, even more, of those with specific learning disorders.

This work was supported by a grant from the Municipality of Palermo

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Keywords

Children; motor skills; cognition; motor program.

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Effects of mini screw placement in mandibular bone of rats treated with low dose zoledronate

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Bisphosphonates are a category of drugs that are commonly used in dentistry and orthopedics to treatment of several bone disorders. The two most potent and widely used nitrogen-containing BPs are zoledronate and alendronate, which inhibit the intracellular mevalonate pathway. In recent years it has been observed Biphosphonate-associated osteonecrosis of the jaws which is a real complication of intravenous biphosphonates therapy in patients with cancer or osteoporosis. In our previous studies, performed on rat model, we observed mandibular bone characteristics after long term of low dose zoledronate treatment without tooth extraction or trauma; results showed several areas of bone with empty osteocyte lacunae, absence of matrix and presence of unorganized fibrillar structures but no spontaneous bone exposure has been observed. In the present study we have treated 20 rats with intraperitoneal injections of zoledronate at the lower dose for three times a week. After 30 days of treatment we applied trauma on mandibular bone by application of screw. Specimens were analysed by histological staining, immunofluorescence techniques and scanning electron microscopy. Our results show that the bone area of screw application is characterized of empty osteocyte lacunae, empty Volkmann'and Havers'canals and some inflammatory cells. Although the presence of small necrotic areas no bone exposure has been observed after low dose zoledronate treatment and trauma application. These results suggest us that the bisphosphonates-associated osteonecrosis of jaw is strictly correlated to drug's dose. It will be necessary to perform the same study using the highest dose of drugs.

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Keywords
Biphosphonates, mandibular bone, BRONJ

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Sexual dimorphism of the first metatarsal bone: volumetric assessment for diagnosis of sex

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Sex assessment is one of the principal activities in the establishment of a biological profile in both forensic anthropology and osteoarchaeology; in addition, most of methods for diagnosis of sex are based upon anatomical findings, including morphological traits of cranium and pelvis [1]. As standard procedures based on cranial and pelvic morphology are not applicable in every context, literature is focusing on novel methods for assessing sex also from measurements of single bones. In the last years volumetric (CT, MRI) and surface (stereophotogrammetry, laser scanners) 3D acquisition systems enabled researchers to perform statistical evaluations not only of linear measurements, but also of surfaces and volumes. This article aims at assessing volume parameters of the first metatarsal bone through 3D acquisitions by laser scanning: 129 first metatarsal bones from 68 skeletons (35 men, 33 women) were acquired from the Milanese Skeletal Collection hosted by University of Milan (66 right and 63 left elements). A male-female cut-off value of 13,330 mm3 was found, with an overall accuracy of 82.4% ($p<0.05$). Area under the receiving operating characteristic curve (AUC) was computed to assess the ability of the cut-off value to distinguish between men and women with a global performance of 87%. A novel method for sex assessment from the volume of the first metatarsal bone was developed, which however seems less reliable than similar procedures based on linear measurements [2]. Further studies are needed to verify the real advantage for sex assessment provided by volume measurements and possible bias due to ethnic variables.

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Keywords

Anatomy; sex assessment; first metatarsal bone; laser scanner; bone volume.

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Variations of midfacial soft-tissue thickness between 6 and 18 years for the reconstruction of the profile: a help for facial reconstruction of children

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Facial approximation techniques used in forensic anthropology are based on soft-tissue thickness databases. A potentially relevant application concerns the facial reconstruction of children in cases where only the skull can be recovered. Although several databases concerning facial soft tissues thicknesses already exist [1-3], no study has so far taken into consideration the Italian population. This study aims at providing data concerning facial thickness on the midline in a population of Italian children. Diagnostic cephalometric X-ray films were obtained from 222 healthy Caucasoid children (91 boys and 131 girls), aged between 6 and 18 years. After setting the Frankfurt plane horizontal, 15 measurements were taken at the mid-facial landmarks: supraglabella, glabella, nasion, nasale, subnasale, nasal tip, superior labial sulcus, labrale superius, stomion, labrale inferius, inferior labial sulcus, suprapogonion, pogonion, gnathion, menton. Mean and standard deviation of soft-tissues thickness at each point were calculated. A two-way analysis of variance (ANOVA) was performed to test the modifications of facial parameters with age and sex ($p<0.01$). The results demonstrated that there is an increase in tissue thickness as individuals grow; in most occasions, males showed thicker soft tissues than females of the same age, especially after the adolescent growth spurt. Facial thicknesses at subnasale, nasal tip, superior labial sulcus, labrale superius, labrale inferius, inferior labial sulcus, suprapogonion, pogonion and gnathion significantly modified with age, whereas the same parameters at subnasale, superior labial sulcus, labrale superius, labrale inferius, stomion and suprapogonion were significantly sexually dimorphic. In addition, a database for soft-tissue thicknesses in children aged between 6 and 18 years was created, which may be of interest in cases of facial approximation of Italian minors.

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Keywords

Facial anatomy; facial approximation; George reconstruction; soft-tissue thickness.

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Collateral vascular network in a case of cardiac lymphoma: anatomo-clinical considerations

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Primary cardiac tumours have an incidence of 0.2%1. Lymphomas represent 1.3% of all malignant primary cardiac tumours2 and are highly aggressive neoplasms. We describe a case of a rare primary cardiac tumor and the establishment of a collateral circulation. A 62-year-old white man referred to our clinical center with sinus tachycardia, congestion in the face and neck, pathologic jugular turgor, and hypovolemia in the superior caval system. Laboratory investigations revealed no abnormalities, except for a slight anemia and a moderate increase in carcinoembryonic antigen and in the lactate dehydrogenase level. The Multislice Computed Tomography (MSCT) revealed a right atrial infiltrative mass and superior vena cava (SVC) syndrome, due to obstruction of SVC drainage. In one week, after admission and without any therapy, congestion and turgor progressively disappeared because venous collaterals were recruited and dilated, offering alternative pathways through the parietal and mediastinal veins, from the superior (brachiocephalic system) to the inferior caval system (countercurrent or retrograde flow). On the other hand, from the inferior vena cava (IVC), the venous blood followed the normal route to the right atrium. In other words, three compensatory venous circles, anterior (ventral), posterior (dorsal), and confluent posterior-anterior, were constituted. A transjugular endomyocardial biopsy revealed that the mass was a large B-cell non-Hodgkin lymphoma, and positron emission tomography was positive for a primitive location, without any evidence of extracardiac involvement. The patient underwent 6 cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy. After 8 months, CT venous phase venography with 64-detector CT angiography revealed that the superior caval-right atrium flow was restored, along with depletion of the anastomotic circles that had secured the return of retrograde flow through the IVC. After treatment, the patient experienced complete remission, and no recurrence has been observed to date. MSCT has proven to be very useful in the early detection of a cardiac mass and it allows for a noninvasive evaluation of the vascular network. In our case, no other older diagnostic tool would have been able to detect the reversal of venous blood flow from the cephalic district to the heart using the IVC. The images obtained by MSCT confirm that the vascular system responds to the functional needs of organs.

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Keywords
Cardiac lymphoma; collateral circulation; 64-MSCT.

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Forensic Clinical Anatomy and Medical Responsibility. Implications for methods of ascertainment and criteria of evaluation

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Forensic Clinical Anatomy may be defined as the practical application of Clinical Anatomy to the ascertainment and evaluation of medico-legal problems. Implications of Forensic Clinical Anatomy may invest various fields of Forensic Medicine but the field of Medical Responsibility and/or Liability represents the most intriguing one. European Guidelines have been recently released regarding methods of ascertainment and criteria of evaluation. In the present work, we analyse how individual anatomy may acquire specific significance in the application of the various steps of analysis in cases of Medical Responsibility and/or Liability: how relevant aspects of individual anatomy may arise from application of methods of ascertainment and how they may be furtherly ascertained through specifically anatomical methodology; how data about individual anatomy, once fully ascertained, may help in the correct application of the criteria of evaluation and in final judgment about identification of medical responsibilities. The main methods of ascertainment on living and/or dead persons have been itemized as follows: examination of clinical/documentary data; consultation with specialist; clinical examination; further instrumental diagnostic exams; pre-autopsy examination; autopsy; post-autopsy diagnostic procedures; clinical synthesis. Anatomical data of forensic interest may arise from the correct application of the above steps and anatomical methodologies are frequently required for a comprehensive analysis. In the analysis of medical liability cases, the phase of ascertainment is followed by assessment of a series of evaluation criteria which may be summarized as follows, with particular reference to cases with relevant anatomical aspects: Reconstruction of the Physio-Pathological Pathway; Identification-Evaluation of Errors; Discussion of Causal Value; Damage estimation. In some cases, the rigorous interpretation of the anatomical data, derived from ascertainment phase and analysed on the basis of pertinent literature, is pivotal for correct applying of each evaluation step. In literature about radiologic, clinical, and surgical anatomy, methods and findings are discussed with reference to clinical/surgical implications but forensic implications (although of potential interest) are frequently overlooked. A better awareness about the forensic relevance of some clinically-oriented anatomical data may also invest the research on radiologic/clinical/surgical anatomy.

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Keywords

Forensic clinical anatomy; medical liability; medical responsibility.

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Skin morphology as revealed by High Frequency Sonography. Findings from the skin of legs with venous and lymphatic insufficiency

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The structure of the skin was sonographically evaluated since the mid of '80 by Ultra High Frequency (UHFP) probes. These instruments were very expensive but allowed to evaluate only the cutaneous layers (epidermis and dermis). Recent improvements in ultrasound technology allowed to possibly evaluate the morphology of the cutaneous and subcutaneous layers by the same High Frequency Probes (HFP) used for routine vascular investigations. The epidermis appears as a thin hyperchoic band due to the echoes created between the gel and the skin surface. The papillary dermis (PD) appears as a thin and low-echogenic band parallel to the skin surface, immediately below the hyperechoic epidermis. The reticular dermis (RD) appears as a regular band, with homogenous thickness and echogenicity. The subcutaneous tissue consists of hypoechoic fat lobules separated by echolucent connective trabeculae. In legs with impairment of venous or lymphatic drainage, HFP sonography allows to better define the pathology of the skin changes observed during clinical examination. More importantly, HFP sonography may reveal skin changes not evidenced by clinical examination like dermal edema, dermal thinning or thickening, skin inflammatory infiltration, and other. In legs with venous or lymphatic insufficiency HFP sonography demonstrates different patterns of subcutaneous edema and may reveal skin changes that precede ulcer opening. Finally, HFP sonography may reveal skin lesions related to therapeutic procedures (open or endovascular surgery, sclerotherapy) without the need to perform skin biopsy.

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Forensic Autopsy versus Anatomic Dissection: Playing the same role in medical malpractice claims?

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Autopsy is a term derived from the ancient Greek "αὐτοψία"; compound word from 'autos' (αὐτός) literally "own" and 'opsis' (ὄψις), literally eyes. Therefore it means 'to see with its own eyes'. Autopsy has a long history that stems from mummification and human dissection in 3000 BC in ancient Greece, where Hirophilus discovered the duodenum by live human dissection, to Rokitansky (1804–1878), regarded as the father of the modern autopsy and who performed or supervised over 100,000 examinations. Despite that: (i) autopsy has to be considered the oldest method of medical investigation; (ii) many studies underscore the need for autopsies in the era of technical progress emphasizing the continuing discrepancies between ante-mortem and post-mortem diagnoses; and (iii) autopsies are considered valuable in medical education, e.g., delivering problem-based learning cases for students; there is a continuing decline in the number of autopsy worldwide. This occurred due to several reasons with complex interactions, and autopsy has been placed in a peculiar position over the last decades. Some regard it as an unnecessary procedure, one that has been superseded in importance by newer methods of study, including: biochemistry, cardiac catheterization, angiography and isotope scanning, virtopsy techniques and 'virtangio' (post-mortem virtual angiography). However, there is a general agreement that autopsies are important in quality management, teaching, training, tissue collection for research (when permitted), death statistics and education. In view of all these reasons, we are strongly convinced that medical mal practice autopsies are the best practice model to perform an autopsy that covers all these goals. When performing an autopsy for the evaluation of an alleged mal practice claim, one must take into consideration the fact that in most cases, the 'normal' anatomy would be altered due to pathological, traumatic, and iatrogenic factors. The pathologist (also forensic and/or anatomist) must have a sound knowledge of the human cadaver anatomy and how to examine it using the traditional dissection techniques and the new pre and post autoptical technologies. Histology plays a fundamental role in the final diagnosis, and the collection of the samples requires the correct visualisation and isolation of all the hypothetical organ lesions. In conclusion we strongly agree with Van den Tweel & Wittekind who state that "The decline of the autopsy rate is a reality, and with the limited number performed, it is increasingly difficult to acquire sufficient experience in performing, interpreting, and reporting autopsies. It is essential that pathologists who perform autopsies are enthusiastic, interested, and competent and respected for their knowledge in this field of our discipline. Only these qualities will make them appreciated partners of clinicians and good teachers of our residents. The only way to achieve this goal is subspecialization in clinical autopsy pathology, much like what has developed for forensic pathology". A personal selection of forensic clinical anatomy cases is presented.

Keywords

Autopsy; virtopsy; virtangio.

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Post mortem computed tomography and magnetic resonance imaging of single organs

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Computed tomography (CT) and magnetic resonance (MR) have been increasingly used in routine forensic practice and research, and, recently, also in cases of natural deaths. Post mortem CT and MR of single organs is currently applied only for investigation of cardiovascular pathologies. The aim of the present study was to show our experience of radiological analysis of single organs, as an integrative tool for research and forensic applications. The anatomo-radiologic study for forensic purpose was performed on single organs sampled at autopsy and on historical specimens. The specimen underwent CT and MR examinations. Basing on our experience, post-mortem CT and MR on single organ are very useful tool in detection of anatomical variations; diagnosis of cardiovascular pathologies in combination with macroscopic examination and histological evaluation; evaluation of findings shown at post-mortem CT examination of the body and not confirmed by macroscopic examination; analysis of historical anatomical specimens.

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Friar Leopoldo Mandic (1866–1942): the computed tomography of the body of a Saint

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Post-mortem Computed tomography (CT) is an imaging technique for documentation and analysis of consecutive autopsy findings, including fractures and gross tissue injuries. Various post-processing techniques can provide strong forensic evidence for use in legal proceedings. On the other hand, this technique is implied also in paleopathology, in particular in mummy studies, with the aim of providing a permanent record of the mummy's features, investigating the embalming procedure employed and analyzing the extent of the preservation in detail. For the Extraordinary Jubilee of Mercy, the mortal remains of Saint Leopoldo Mandic and Saint Pio da Pietrelcina, as examples of Mercy known by everyone, have been brought to Rome from 3 to 11 February. During the survey that preceded the preparation for transport to Rome, a whole-body CT was performed on the mummified corpse of Leopoldo Mandic, Capuchin Saint of Padova, Italy. The CT examination demonstrated the presence of arthritis at the level of the vertebral column, of the left knee and of the hand. Moreover, CT showed the preservation of skeleton, and partial preservation of the brain, ear ossicles, major arterial vessels (aorta and carotid arteries), pleurae, esophagus, heart, urinary bladder, nervous structures (plexuses and spinal nerves). Pseudopathologic changes, primarily postmortem skeletal dislocations were also present at the level of the hip joints. It is to emphasize the fact that San Leopoldo was not subjected before the CT to any conservative treatment, with the exception of a surface treatment with celluloid. CT demonstrated to be a non-destructive method to investigate Saint Leopoldo, in order to maintain the integrity of the body and to acquire data on his pathologies and on his preservation. CT allows not only the acquisition of sectional images but also, thanks to dedicated software, the post-processing and reconstruction of three-dimensional models, that can be used also for public displays.

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Different eyes, different views. Scanning Electron Microscopy applied to forensic investigations

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The Scanning Electron Microscope (SEM) is an extremely versatile instrument, essential in a wide array of applications in forensic analysis: for example, it is used to analyze gunshot residue, bullet fingerprints, bullet wipe or patterns around the bullet hole, to examine traces of foreign material embedded in or adhered to bullets (which can provides critical information in the trajectory reconstruction of spent bullets); to study environmental dusts, fibers (both natural and artificial) and to identify unknown small particles; to detect non visible blood stains; to analyze diatoms in drowning cases; and for ink and paper analysis. One central feature of SEM is its ability of providing both panoramic and highly magnified views of the same sample, giving an almost 3D view of the specimen. It is the ideal trait d'union between macroscopic information collected during autoptic or investigative activity and microscopic information obtained with the light microscope. Above all, SEM allows performing a progressive and targeted microdissection of the sample. In this presentation, a selected number of investigations are shown in order to illustrate through specific cases general purpose applications. An elderly man was killed with several blows of axe at the head. SEM investigation allowed us to reconstruct the sequence of the blows, to recognize the type of weapon, to determine how this latter was used and how sharp it was. These results allowed the police to reject the initial version of the suspected, which was eventually convicted of willful murder. A young man died with multiple traumatic and fulguration lesions. SEM analysis allowed us to perform a detailed study of the burnt tissue and to reconstruct the path of the electric discharge, concluding that the primary causa mortis was an accidental electrocution, which caused the subsequent trauma. A child died of a sudden, dramatic internal bleeding. The autopsy revealed that some time before she had swallowed a coin battery which had become lodged in the oesophagus. Here the decaying products of the battery caused an electro-chemical dissection of the oesophagus and, finally, of the descending aorta. The SEM analysis revealed the details of the progressive degeneration of the surrounding tissues.

Keywords

SEM; forensic.

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High-quality digital 3D reconstruction of the terminal pathway of a heart stab wound

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High-quality digital 3D reconstructions of microscopic findings have been involved in anatomical and histopathological research, but their potentialities in forensic pathology may also be of particular interest. We here present the use of such methods to furtherly analyse a heart stab wound in a suicidal case. External examination revealed multiple incised wounds on the wrists, three stab wounds at the neck and a single stab wound on the chest. At autopsy, injuries to the neck and wrists were superficial whereas the thoracic stab wound penetrated the chest wall and pericardium. Heart examination showed an 8-mm-long stab wound on the anterior surface of the left ventricle. Heart sectioning and inspection of the correspondent internal aspect of the left ventricle did not show a macroscopic injury, but the irregularity of the trabeculae carneae did not permit to exclude a microscopic pathway. Thus, the heart wall including the stab wound was paraffin-embedded and subjected to complete sectioning for microscopic analysis along all the wound extension. Every 10th section was stained with haematoxylin-eosin and was acquired by using a Leica DMR microscope and a high resolution digital camera. The three-dimensional aspect of the lesion was reconstructed with a software system for 3D computer graphics. Microscopic examination and 3D reconstruction demonstrated that the lesion extended to the internal surface of the ventricle wall, although for a very limited extension. 3D reconstruction also showed a certain curvilinear pattern of the lesion in the myocardium, consistent with myocardial contraction at the moment of injury and consequent vitality of the lesion. Moreover, 3D reconstruction permitted to obtain the dimensions of the intra-myocardial injury (corrected for shrinkage and evaluated in the context of muscle contraction), also permitting to furtherly confirm the identification of the knife involved. In conclusion, the present case is indicative of how complete microscopic sectioning and 3D reconstruction may add further information about characteristics of injuries of forensic interest.

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Medical imaging techniques as tools for the study of forensic clinical anatomy

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The last decades have been characterized by a growing interest in the field of imaging techniques for the study of the anatomy of human body. This is a fundamental concept since it is not only interesting to anatomical theory but also useful to clinical medicine, especially in the field of surgery. In this work, we demonstrate that advanced medical imaging techniques may play a major role in forensic clinical anatomy, especially for the evaluation of iatrogenic and non-iatrogenic lesions. Here in, we evaluate the usefulness of CT- and MR-based direct volume rendering reconstruction [1] and tractography [2] for the study of: i) medial rectus lesion; ii) oro-antral fistulas; iii) dislocation of dental implants; iv) brain injuries. Our results, which improve the conventional three-dimensional images, reveal a new avenue of research studies and clinical fields, mostly in anatomy. Computer-assisted, model-based procedures typically cover specific modifications of virtual anatomy as well as numeric simulations of associated phenomena, like mechanical loads or diffusion processes, in order to evaluate potential therapeutic and post-operative outcomes. In the future, a combined approach of these advanced tools with other radiological techniques may lead to an imaging data set with unsurpassable anatomical, physiological and pathological information, offering unique advantages in the forensic science field.

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Keywords

Direct volume rendering; MRI; CT.

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The brain tissue reaction to blunt trauma: a field of possible cooperation between neuroanatomists and forensic pathologists

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The goal of this presentation is to describe, through the histological examination, the evolution over time of the biological processes, at the cellular and molecular level, in the neurological tissue after a blunt trauma. The origin of the secondary ischemia, that often occurs after a brain trauma, leading to death the patient, is almost studied on animal models and is not well known yet. It is presumed that hemorrhages and contusions result in brain ischemia, and that also brain edema arises intra-cranial pressure producing ischemia. Forensic pathology deals everyday with cases of traumatic deaths, and is therefore able to study the inflammatory reaction to trauma in a human casuistry giving information to other disciplines like neuroanatomy. The time-dependent appearance of different leucocyte subtypes can contribute to a forensic wound age estimation but, in contrast to peripheral tissue, the cellular reaction in the CNS is characterized by a minimal neutrophil exsudation and a delayed increase in mononuclear cell numbers. 62 deaths due to head injury with a survival time from few minutes till 30 days were studied. Samples of brain tissue were stained with immunohistochemistry using selectin P and E, GFAP, HIF1-α, CD 117 (c-kit), LCA. The schematic information about chronology of head trauma are given as follows: survival of a few minutes, of 1 hour, of 2-4 hours, of 4-12 horus, of 12-24 hours, 24-48 hours, 2-6 days, 6-14 days, 15-30 days. The number of platelets microthrombi increases with TBI age up to 3 days, afterward leukocytes start to take their place. Platelets aggregates may impair cerebral circulation causing ischemia. Cerebral ischemia plays an important role in SBD. There is also an involvement of CD 117+ cells and HIF-1 α in the modulation and progression of the brain injury. After brain injury a cascade of events occurs leading sometime to a brain secondary ischemic injury. Thanks to the availability of injured human brain tissues, forensic histopathologists might work together with neuroanatomists in order to help in the identification of glial cells and leukocytes communication with endothelial cells, and on the post-traumatic ischemic process that causes the death in prolonged survival time after brain injury.

Keywords

Traumatic brain injury; cell movement; immunohistochemistry; secondary brain injury; biological processes.

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Anatomical bases of a case of retroperitoneal haemorrhage

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Forensic Clinical Anatomy includes the evaluation of the normal anatomical basis of pathological findings in forensic context; this is frequently pivotal to verify the anatomical plausibility of alleged iatrogenic injuries. The case is here reported of a 20-year-old woman who died for sudden haemorrhagic shock, due to retroperitoneal haemorrhage of unknown origin. About three months before she had undergone surgical decompressive fasciotomy for traumatic haemorrhage at the level of the right calf. About one months before death, another trauma had also occurred with hematoma of the posterior aspect of the right thigh. Four days before death, she had also undergone angiographic TC which showed arterio-venous malformation of the homolateral calf. Autopsy examination performed by another medico-legal consultant reported triple laceration of the right superficial femoral artery. The anatomical bases and plausibility of the various hypothetical anatomo-physio-pathological pathways (iatrogenic lesion of the superficial femoral artery during angio-TC? Delayed post-traumatic hematoma of the thigh or ileo-psoas? Spontaneous retroperitoneal haemorrhage? Artefactual autopsy lesions of the superficial femoral artery?) are discussed.

Keywords

Forensic clinical anatomy; vascular system.

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In vitro effects of Concentrated Growth Factors on BMP-2 synthesis by human osteoblasts

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Bone morphogenetic proteins, and especially BMP-2, play an important role in bone homeostasis and regeneration, stimulating osteoblasts differentiation. Concentrated Growth Factors (CGF) is an autologous platelet preparation, obtained from the patient's own blood, with a specific protocol of centrifugation and containing several different growth factors, including BMP-2, with an average release of 5-10 pg (1). So, in this study, we investigated the in vitro effect of CGF on BMP-2 synthesis by human osteoblasts (HOBs). Cells were cultured in presence of CGF for 9 days and then the cell number were determined by using an automated cell counter. To test the effect of CGF on BMP2 release from osteoblasts, the supernatants were collected, centrifuged at 1200 rpm for 10 min at room temperature and used to perform the BMP-2 ELISA assay. In addition, the expression of BMP-2 on fixed HOBs was also evaluated by performing an immunocytochemical analysis. Our results showed that CGF significantly enhances HOBs proliferation and increases BMP2 synthesis by HOBs that could act also in paracrine way, together with CGF derived BMP2, to better promote bone regrowth.

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Keywords

BMP-2; Concentrated Growth Factors; lithium chloride; human osteoblasts.

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Involvement of Notch signaling in the osteogenic differentiation of human mesenchymal stem cells stimulated by pulsed electromagnetic fields

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Biophysical stimulation with pulsed electromagnetic fields (PEMFs), used in clinics to promote bone repair, favour osteogenic differentiation in human mesenchymal stem cells (hMSCs), however their molecular mechanisms are not clarified. Notch is a pathway regulating cell fate decisions which play a role in skeletal development. Notch signaling is initiated by binding a Notch ligand to a cell surface Notch receptor, resulting in a cleavage of receptor and releasing Notch intracellular domain which translocates to the nucleus and activates transcription of nuclear Notch target genes, such as the Hes/Hey family.

The aim of this study is to establish if the known PEMF-induced osteogenic effects may occur through the modulation of Notch pathway. Bone marrow hMSCs cultured in basal condition (control) and in osteoinductive medium (OM) for 28 days were unexposed or continuously exposed to PEMFs (75 Hz, 1.5 mT) (Igea, Carpi, Italy). To block Notch pathway, the Notch inhibitor DAPT was used to treat a series of hMSCs cultured in OM. At different time points (day 1,3,7,14,21,28), osteogenic markers (alkaline phosphatase activity, osteocalcin and matrix mineralization), mRNA expression of osteogenic transcription factors (Runx2, Dlx5, Osterix) as well as of Notch receptors (Notch1-4), their ligands (Jagged1, Dll1 and Dll4) and nuclear target genes (Hey1, Hey2, Hes1, Hes5) were analysed.Our results showed that osteogenic markers and transcription factors increased in OM compared to control and they were further stimulated by PEMFs. Notably, PEMFs significantly increased the expression of Notch4, Dll4, Hey1, Hes1 and Hes5 in the middle phase of differentiation in OM compared to control. In the presence of DAPT, osteogenic markers as well Hes1 and Hes5 expression were significantly inhibited, in unexposed and PEMF-exposed hMSCs. Hey1 was not inhibited by DAPT suggesting a possible regulation by other signaling pathway.These new findings show that PEMFs favor osteogenic differentiation acting through Notch pathway, adding important knowledge concerning the molecular mechanisms by which PEMFs can modulate osteogenesis.

This work was supported by grants from IGEA, Carpi, Italy.

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Keywords

Osteogenic differentiation; electromagnetic fields; Notch.

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Characterization of novel autologous leukocyte fibrin platelet membranes for tissue engineering applications

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Autologous hemocomponents have recently emerged as potential biologic tools for regenerative purpose, consisting mainly of platelet concentrates which locally release growth factors (GFs) to enhance the tissue healing process. Despite two decades of clinical studies, the therapeutic efficacy of platelet concentrates is still controversial. This work represents a first characterization of a novel autologous leukocyte fibrin platelet membrane (LFPm), which is prepared by the Department of Immunohematology of Belluno Hospital according to a well standardized protocol. The quantification of their specific content showed that LFPms are enriched not only with platelets, but also with monocytes/macrophages, fibrinogen and CD34+ cells. Mechanical properties of LFPms were investigated by tensile tests, revealing that the specific elasticity of membranes was maintained over time. Furthermore, the release kinetics of Platelet Derived Growth Factor, Vascular Endothelial Growth Factor, Tumor Necrosis Factor alpha and Interleukin-10 was assessed by ELISA, demonstrating that LFPms act as GF delivery systems which sustain the local release of bioactive molecules. For in vitro biodegradation analysis, LFPm samples were incubated into PBS solution for 4, 7, 14, 21 days. SEM micrographs showed a progressive loss in cellular elements associated to a simultaneous exposure of the fibrin scaffold, also confirmed by histological and immunohistochemical investigations. In parallel, LFPm disks were implanted into a subcutaneous dorsal pouch of healthy nude rats and explanted after 4, 7, 14, 21 days for in vivo biodegradation study. SEM, histological and immunohistochemical analysis revealed that the typical LFPm fibrin structure was maintained until day 7, with a contemporary loss of cellular elements. From day 14, the morphology and texture of samples became less and less recognizable, confirming that a progressive biodegradation occurred. Overall, collected evidences could support the rationale for the clinical use of LFPms, shading some light on the regenerative effect they may exert after the autologous implant on a defect site.

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Keywords

Autologous hemocomponents; platelets; tissue healing.

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Hepatic progenitor cell activation is influenced by liver macrophages in the progression of non-alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is one of the most important causes of liver-related morbidity in children. In NAFLD, the activation of hepatic progenitor cells (HPC) is a central event in the progression of liver injury (1). The aim of the present study was to evaluate the cross-talk between HPC activation and polarization of liver macrophages in the progression of pediatric NAFLD. 32 children with biopsy-proven NAFLD were included. 20 out of 32 patients were treated with docosahexaenoic acid (DHA) for 18 months and biopsies at the baseline and after 18 months were included (2). HPC activation, macrophage subsets and Wnt/β-catenin pathway was evaluated by immunohistochemistry and immunofluorescence. Our results indicated that in pediatric NAFLD, pro-inflammatory macrophages were the predominant subset. Macrophage activation was correlated with NAFLD Activity Score, HPC activation, and portal fibrosis; DHA treatment determined a macrophage polarization towards an anti-inflammatory phenotype in correlation with the reduction of serum inflammatory cytokines and with the up-regulation of macrophage Wnt3a expression; macrophage Wnt3a expression was correlated with β-catenin phosphorylation in HPCs and signs of commitment towards hepatocyte fate. In conclusion, macrophage activation seems to have a key role in driving HPC response by Wnt3a production in the progression of pediatric NAFLD.

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Keywords
Non-alcoholic fatty liver disease; stem cells; liver; WNT.

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Key role of MEK/ERK pathway in sustaining tumorigenicity and in vitro radioresistance of embryonal rhabdomyosarcoma stem-like cell population

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The identification of signaling pathways that affect the cancer stem-like phenotype may provide insights into therapeutic targets for combating embryonal rhabdomyosarcoma. The aim of this study was to investigate the role of the MEK/ERK pathway in controlling the cancer stem-like phenotype using a model of rhabdospheres derived from the embryonal rhabdomyosarcoma cell line (RD). Rhabdospheres enriched in cancer stem like cells were obtained growing RD cells in non adherent condition in stem cell medium. Stem cell markers were evaluated by FACS analysis and immunoblotting. ERK1/2, myogenic markers, proteins of DNA repair and bone marrow X-linked kinase (BMX) expression were evaluated by immunoblotting analysis. Radiation was delivered using an x-6 MV photon linear accelerator. Xenografts were obtained in NOD/SCID mice by subcutaneously injection of rhabdosphere cells or cells pretreated with U0126 in stem cell medium. MEK/ERK inhibitor U0126 dramatically prevented rhabdosphere formation and down-regulated stem cell markers CD133, CXCR4 and Nanog expression, but enhanced ALDH, MAPK phospho-active p38 and differentiative myogenic markers. By contrast, MAPK p38 inhibition accelerated rhabdosphere formation and enhanced phospho-active ERK1/2 and Nanog expression. RD cells, chronically treated with U0126 and then xeno-transplanted in NOD/SCID mice, delayed tumor development and reduced tumor mass when compared with tumor induced by rhabdosphere cells. U0126 intraperitoneal administration to mice bearing rhabdosphere-derived tumors inhibited tumor growth . The MEK/ERK pathway role in rhabdosphere radiosensitivity was investigated in vitro. Disassembly of rhabdospheres was induced by both radiation or U0126, and further enhanced by combined treatment. In U0126-treated rhabdospheres, the expression of the stem cell markers CD133 and CXCR4 decreased and dropped even more markedly following combined treatment. The expression of BMX, a negative regulator of apoptosis, also decreased following combined treatment, which suggests an increase in radiosensitivity of rhabdosphere cells. Our results indicate that the MEK/ERK pathway plays a prominent role in maintaining the stem-like phenotype of RD cells, their survival and their innate radioresistance. Thus, therapeutic strategies that target cancer stem cells, which are resistant to traditional cancer therapies, may benefit from MEK/ERK inhibition combined with traditional radiotherapy, thereby providing a promising therapy for embryonal rhabdomyosarcoma.

Keywords

Rhabsomyosarcoma; MAPKs; stem cells.

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Mesenchymal stem cell extracellular vesicles: potential use in regenerative medicine

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Complex biological systems are composed of great amount of cells with sophisticated mechanisms for information exchange, involving the molecular as well as the cellular level. Vesicle release results in a process highly conserved in prokaryotes as well as in eukaryotes, therefore representing an evolutionary link and suggesting that such a dynamic extracellular vesicular compartment may play a key role in remote organ and tissue regulation. Microparticles serve as a vehicle to transfer proteins and messenger RNA and microRNA (miRNA) to distant cells, which alters the gene expression, proliferation, and differentiation of the recipient cells. Microparticles released from mesenchymal stem cells have the potential to be exploited in novel therapeutic approaches in regenerative medicine to repair damaged tissues, as an alternative to stem cell-based therapy.

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The analysis of the dermal collagen matrix in the absence of α11β1-integrins suggests a potential role for integrins α11β1 in the regulation of skin biomechanics

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Integrins α11β1 are major collagen receptors and are thought to play a central role in fibrillar collagen arrangement [1;2], but this has not been demonstrated in vivo. In order to answer this question, here, we analysed the overall organisation of the dermal collagen network fibril diameter in samples of back skin of α11β1-integrin-deficient mice (KO). Dermal collagen organisation was assessed for its complexity and its heterogeneity on paraffin sections after Sirius red staining (4 KO and 4 controls), by quantifying fractal dimension and lacunarity respectively. The results showed that fractal dimension was increased in KO mice (1,40±0,06 in α11β1 KO mice vs 1,24±0,05 of control mice, p=0,009), whereas Lacunarity was reduced (0,78±0,06 in α11β1 KO mice 0,97±0,02 of control mice p=0,002), indicating a re-organisation of the dermal collagen network in absence of integrins α11β1. Fibril diameter was studied in images taken at the Transmission Electron Microscope (5 KO and 5 controls). The total number of fibrils examined was 22,212 (for the 5 controls) and 28,446 (for the 5 KO). The analysis showed a proportional increase in smaller fibrils with a proportional decrease in larger fibrils in α11β1 KO mice, being these differences were most evident in fibrils with smallest (<40nm) and largest (>120nm) diameter. Chi squared test confirmed statistical significance of these changes (equivalent to p=0,001). Given the fundamental role of dermal collagen in skin stability, these changes in collagen organisation and fibril size also suggest a potential implication of α11β1 integrins in the control of skin biomechanics.

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Keywords

Transmission electron microscopy; stereological/morphometric analysis; a11b1 integrins; dermal collagen.

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Melatonin oral supplementation against fibromyalgia-related skeletal muscle alterations

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Fibromyalgia is a chronic idiopathic pain syndrome characterized by widespread musculoskeletal pain and a deep range of other symptoms including disordered sleep, paresthesia, depression and anxiety (1). To date, its aetiopathogenesis and pathophysiology are still not understood, but the musculoskeletal, neuroendocrine and central nervous systems appear to play major roles in the development and progression of fibromyalgia (2). Important factors involved in the pathogenic process of fibromyalgia are oxidative stress and inflammation suggesting that antioxidative supplementation might be important in the management and modulation of fibromyalgia. Recent evidences suggest that melatonin may be suitable for this purpose. Melatonin is a small, highly conserved pineal indoleamine and due to its important and well known antioxidant and antinflammatory properties, together with also its analgesic effects, our research group studied the beneficial effects of the melatonin oral supplementation against the pathogenetic process of fibromyalgia. In detail, Sprague Dawley rats were randomly treated with reserpine, to reproduce the pathogenic process of fibromyalgia (3), and/or with melatonin (MelapureTM by Flamma S.p.A.). At the end of the treatments, the animals treated with reserpine showed moderate alteration at hind limb skeletal muscle level with difficult in moving, together with a significant expression of several oxidative stress and inflammatory markers at the gastrocnemius muscle level. Interestingly, melatonin, dose and time dependently, reduced the difficulties in walking and the musculoskeletal oxidative stress and inflammatory processes. In summary, this pilot study suggested that melatonin could be an in vivo effective tool against muscoloskeletal morphofunctional damages and dysfunctions in the management of fibromyalgia-related complications.

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Keywords

Inflammation; melatonin; oxidative stress; skeletal muscle.

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Effects of recombinant Irisin on the musculoskeletal system of hind-limb suspended mice

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We previously showed that Irisin, a myokine released from skeletal muscle after physical exercise, plays a central role in the control of bone mass, driving positive effects on cortical mineral density and geometry in vivo (1). Here we demonstrated that r-Irisin treatment prevents bone loss in hind-limb suspended mice when administered during suspension and recovers bone mass when mice were injected after a suspension period (4 weeks) during which they developed bone loss. Micro computed tomography of femurs showed that r-Irisin treatment positively affected both cortical and trabecular bone. As expected, unloaded mice treated with vehicle displayed a remarkable decrease of cortical and trabecular bone mineral density (BMD), whereas in Irisin-treated unloaded mice no loss of BMD was observed with respect to control mice kept under normal loading. Likewise, by treating mice after they already developed disuse-induced bone loss, r-Irisin was able to restore the damaged mineral component. Furthermore, trabecular bone volume fraction (BV/TV), which dramatically decreased in unloaded mice, was prevented by r-Irisin therapy. In particular, r-Irisin treatment preserved the number of trabeculae (Tb.N) and the fractal dimension, an index of optimal micro-architectural complexity of trabecular bone.We also showed that r-Irisin treatment protects muscle mass suffering from atrophy during unloading. Thus, unloaded mice treated with vehicle displayed a severe loss of muscle mass, as confirmed by ~ 60% decline of vastus lateralis weight and ~33% decrease of fiber cross-sectional area. Conversely, Irisin-treated unloaded mice showed no loss of muscle weight and similar fiber cross-sectional area to control mice. Our data reveal for the first time that r-Irisin treatment prevents and retrieves disuse-induced bone loss and muscle atrophy. These findings may lead to develop an Irisin-based therapy for the prevention and treatment of osteoporosis and sarcopenia in all patients who cannot perform physical activity, as occurs during aging and immobility, and it could also represent a countermeasure for astronauts exposed to microgravity during space flight missions.

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Keywords

Osteoporosis; sarcopenia; mechanical loading; Irisin.

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Bone marrow-derived mesenchymal stromal cells and platelet rich plasma in skeletal muscle regeneration

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Adult skeletal muscle is able to undergo regeneration after damage, thanks to satellite cells. However, in case of severe damage, the efficiency of these cells cannot be sufficient to promote tissue repair [1]. Recent trends are attempting to identify strategies aimed to improve the endogenous muscle repair potential. The administration of bone marrow-derived mesenchymal stromal cells (BM-MSCs), thanks to their secretion of paracrine factors, displays promising clues in skeletal muscle healing [2]. However, some criticisms hamper their clinical application, namely scarce survival in the host tissue and the need to avoid animal serum contamination in manipulation. In this context, platelet rich plasma (PRP) offers several advantages. Indeed, as a source of multiple growth factors it could represent an optimal substitute for animal serum in vitro and could provide beneficial therapeutic effects in vivo in the tissue injury site. However, controversial clinical findings exist for its therapeutic application in skeletal muscle injuries [3]. Here we evaluated: i) the effect of PRP on both C2C12 myoblasts and BM-MSCs in term of viability, survival, proliferation and myogenic differentiation and ii) the effect of PRP in combination with BM-MSCs in sustaining and promoting myogenic differentiation. We found that PRP induced an increase of myoblast and BM-MSC survival, viability and proliferation as judged by MTS and EdU incorporation assays, Ki67 expression and Akt and Notch-1 signalling activation. PRP promoted also C2C12 myoblast differentiation as evaluated by the analysis of myoD, myogenin, and α-sarcomeric actin expression. Finally, the co-colture C2C12/BM-MSCs in the presence of PRP showed an increase of all parameters of survival, proliferation and differentiation as compared to PRP treatment alone. In conclusion, our data suggest that the combined use of PRP and BM-MSC can be considered as a valuable tool in the field of skeletal muscle regenerative medicine.

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Keywords
Platelet rich plasma; skeletal myoblasts; mesenchymal stromal cells.

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Colonic wall remodeling in patients with short- and long-lasting ulcerative colitis

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Inflammatory bowel disease (IBD) are chronic and progressive pathologies associated with invalidating abdominal symptoms and increasing incidence in Western countries. Although fibrostenosis is a rare event in ulcerative colitis [UC], it may evolve to fibrosis in the later stages (1, 2). In the present study we examined the histopathological remodeling of the colonic wall from short- and long-lasting (SL and LL) UC patients. Full-thickness left colonic surgical samples were obtained from non-stenotic SL (≤ 3 years) and LL (≥ 10 years) UC patients with a severe exacerbation of colitis and active disease, without clinical signs of fibrostenosis. Collagen and elastic fibres, vascular networks and parameters of fibrosis have been evaluated by histochemistry, immunohistochemistry, confocal immunofluorescence and/or western blot. For comparison, normal colonic control samples from subjects who underwent surgery for uncomplicated colon cancer and without IBD and diverticular disease, were considered.Both SL- and LL-UC showed a thickening in tunica muscularis and activation of transmural neovessels, with proliferating CD105-positive endothelial cells and activated nestin-positive pericytes, as compared with controls. The colonic wall of LL-UC displayed a significant increase in collagen deposition and fibrotic markers (type I and III collagen, fibronectin, vimentin, RhoA), an enhancement of proliferation (PCNA), a decrease in elastic fibres, together with a fibrotic rearrangement of the tunica muscularis smooth muscle cells. In conclusion, a significant full-thickness remodeling of the colonic wall has been documented in the colonic tissues from both SL-UC and LL-UC, as compared to controls: transmural fibrotic thickening and angiogenesis were found in both UC groups, together with a cellular fibrotic/proliferative switch in the tunica muscularis which occurs in the later stages. These histopathological remodeling may contribute to the reduced elastic properties and tissue stretch capability of the wall, thereby impairing the occurrence of a normal motor activity in these subjects.

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Keywords

Ulcerative colitis; active disease; colonic muscle remodeling; intestinal angiogenesis/fibrosis.

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Expression of the endocannabinoid receptors in human fascial tissue

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Endocannabinoids are endogenous lipid mediators with wide range of biological effects similar to those of marijuana. They exert their biological effects via two main G-protein-coupled cannabinoid receptors, the CB1 (cannabinoid receptor 1) and CB2 (cannabinoid receptor 2). Cannabinoid receptors have been localized in the central and peripheral nervous system as well as on cells of the immune system, but recent studies gave evidence for the presence of cannabinoid receptors in different types of tissues (1,2) Their presence was supposed in myofascial tissue, suggesting that the endocannabinoid system may help resolve myofascial trigger points, suppressing proinflammatory cytokines such as IL-1beta e TNF-alpha and increasing anti-inflammatory cytokines (3, 4). However, until now the expression of CB1 and CB2 in fasciae and in fascial fibroblasts has not yet been established. In this work small samples of fascia were collected from volunteers patients: for each sample were done a fibroblast cell isolation, immunohistochemical investigation (CB1 and CB2 antibodies) and real time RT-PCR to detect the expression of CB1 and CB2. The immunostaining results demonstrate the expression of CB2 and CB1 on fascial fibroblasts and fascial tissue. In the tissue not all the fibroblasts are positive, whereas the isolated and expanded cells are homogeneous. These results are confirmed by the real time PCR where the specificity of the reaction on fibroblasts and fascial tissue is the same, but the amount of expression in the tissue is lower, for both CB1 and CB2. This is the first demonstration that the fibroblasts of the muscular fasciae express CB1 and CB2. These results could represent a new target for drugs to care fascial fibrosis and inflammation. The presence of the endocannabinoid system in the fascial fibroblasts can also explain the efficacy of cannabis to care myofascial pain and the possible stimulation during manipulative treatments and exercises (5). More studies about the interactions between fibroblasts, extracellular matrix and CB1 and CB2 receptors could help to understand the role of these receptors on myofascial pain.

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The role of MICAL2 gene in myogenic differentiation

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The dystrophin-glycoprotein complex (DGC) is composed of several transmembrane and peripheral components localized in the sarcolemma of skeletal muscle. Mutations in genes that encode DGC components lead to the loss of either expression and/or function of the DGC in muscle. As DGC complex interacts with F-actin it is reasonable that the multidomain F-actin binding protein MICAL2 that transduces semaphorin/plexin external signaling into cytoskeletal modifications, might interact either directly or indirectly with the DGC complex. MICAL2 is indeed expressed in skeletal and cardiac muscles and drosophila Mical mutants reveal that the architecture of contractile muscle filaments is negatively affected. We focus here on the role of MICALs in myogenic differentiation. The rationale to investigate MICAL2 in muscle differentiation is also highlighted in a paper regarding a complex muscle genome-wide expression profiling during the disease evolution in mdx mice, a mouse model of Duchenne muscular dystrophy (1). In this study the authors found MICAL2 among a set of totally ten functionally linked genes involved in the decline of muscle necrosis in mdx mice. For this purpose MICAL2 gain and loss of function studies have been performed in myogenic cell line and compared to in vivo analysis of MICAL2 expressions in acute and chronic muscle degeneration. Recently we showed that that differential myogenic propensity influences the commitment of isogenic induce pluripotent stem cells and a specifically isolated pool of mesodermal iPSC-derived progenitors (MiPs) toward the striated muscle lineages (2). Analysis of MICAL2 expression in MiPs is currently under investigation. Taken together modulation of MICAL2 has an impact on skeletal muscle commitment and could be considered a potential therapeutic target for Duchenne patients.

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Keywords

Myogenic differentiation; MICAL2; stem cells; muscular dystrophies.

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Origin of fat cells

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Adipose tissue expansion involves the enlargement of existing adipocytes, the formation of new cells from committed preadipocytes, and the coordinated development of the tissue vascular network. Here we find that murine endothelial cells (ECs) of classic white and brown fat depots share ultrastructural characteristics with pericytes, which are pluripotent and can potentially give rise to preadipocytes. Lineage tracing experiments using the VE-cadherin promoter reveal localization of reporter genes in ECs and also in preadipocytes and adipocytes of white and brown fat depots. Furthermore, capillary sprouts from human adipose tissues, which have predominantly EC characteristics, are found to express Zfp423, a recently identified marker of preadipocyte determination. In response to PPARg activation, endothelial characteristics of sprouting cells are progressively lost, and cells form structurally and biochemically defined adipocytes. Together these data support an endothelial origin of murine and human adipocytes, suggesting a model for how adipogenesis and angiogenesis are coordinated during adipose tissue expansion.

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LIGHT/TNFSF14 affects basal bone remodeling

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LIGHT (TNFSF14), expressed by different cells of the immune system, binds two trans-membrane receptors: HVEM and LTβR. It is over-expressed in erosive rheumatoid arthritis and lytic myeloma-bone disease and controversial data have been published on its role in osteoclast (OC) formation in vitro. Here, we investigated the role of LIGHT on in vitro murine osteoclastogenesis model and bone phenotype in LIGHT-/- mice. Firstly, we showed that murine macrophages stimulated with LIGHT alone did not differentiate into OCs. Interestingly, the presence of LIGHT and sub-optimal RANKL concentration displayed synergic effects on OC formation through the early and sustained activation of Akt, NFκB and JNK pathways. Secondly, by microCT we found that the femurs of LIGHT-KO mice exhibited a 30% ($p<0.01$) decrease in trabecular BV/TV due to a significant reduction in trabecular thickness and number as well as the increase in trabecular spaces respect to wild-type (WT) mice. Furthermore, a five fold increase of OC number/bone surface was found in femora from KO mice compared to WT ($p<0.008$). To investigate the possible molecular mechanism/s responsible for this bone phenotype in LIGHT-/- mice we studied OPG levels in whole bone marrow (BM) extracts from the femurs of these mice and demonstrated a significant reduction in OPG mRNA transcript respect to WT. Further investigations showed that BM CD8+ T cells and B cell subpopulations from KO mice expressed lower levels of OPG compared to those from WT mice. Consistently, LIGHT treatment in a dose dependent manner increase OPG expression in BM CD8+ T cells and B-cells. In conclusion, our results identified LIGHT as a new important regulator of bone remodeling and highlighted a new modulator of OPG expression.

Keywords

LIGHT/TNFSF14; osteoclasts; bone remodeling.

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Hepatic matrix metalloproteinase-10 exerts a hepatoprotective role after acute liver injury

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After injuries that lead to a loss of liver tissue a regenerative and reparative response is performed in order to restore an adequate hepatic mass. The remodeling of the extracellular matrix, accompanies the liver regeneration and when the reparative reaction goes awry in the setting of chronic liver injury, could be involved in the carcinogenic process (1,2). Following the damage, a provisional matrix is deposed, intended to be successively replaced, which has the function of stabilizing the lesional area and constitutes a support for guiding regenerating cells. Matrix metalloproteinases are increasingly recognized as important modulators of the matrix remodeling process. Matrix metalloproteinase-10 (MMP-10) has been implicated in the reparative process in other organs and has effects on the plasminogen system, which plays a fundamental role in liver repair (3). The hepatic expression of MMP10 in animal models of acute liver injury was tested in order to investigate the role of MMP-10 in liver repair and regeneration. The liver regeneration after two thirds partial hepatectomy (PH) and bile duct ligation (BDL) models were examined. Hepatic MMP-10 expression, analyzed by immunohistochemistry, western blot and qPCR showed a rise early after injury. In the MMP10-deficient mice a diminished and delayed resolution of necrotic lesions, enhanced fibrogenesis and a fibrinogen/fibrin and fibronectin compromised turnover were observed. These findings showed that the MMP10 expression plays a role in the hepatic wound healing response probably through its profibrinolytic activity.

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Key words

Metalloproteinase-10; liver repair and regeneration; hepatocarcinogenesis.

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Looking is not seeing: visual art as a useful eLearning tool for teaching clinical anatomy

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An old Chinese proverb reads "A picture paints a thousand words…". Even though arts were used as a pedagogical tool as early as 1902, arts have not traditionally been part of medical education. Anatomy teaching is undergoing significant changes; long-distance education associated to web 2.0 tools have enlarged communication and interaction possibilities between users and virtual communities (1,2). We are developing a project consisting of a series of asynchronous (pre-registered) short video tutorials covering a wide range of clinical anatomy-related topics which can be posted on an internet/intranet site and then actively and autonomously be followed. Videos include short introductory remarks and legends which might be developed in different languages and which make them useful even to disable eLearners. The relationship between Art and: anatomy, thyroid gland, osteomuscular alterations, breast, ageing, artists' diseases, as well as the presence of anatomists and physicians in Art, are some of the topics developed. Each tutorial considers numerous paintings and some sculptures covering a long time span, from Prehistory to the Classical period, Late Middle Ages, the rich Renaissance period up until modern times. Artworks were chosen in order to be narrative in nature and rich in detail, thus stimulating reflection and self-discussion. These video tutorials could be a valuable teaching/learning complement to theoretical knowledge within medical students' education or even within a larger "art-loving" audience. eLearners are guided by the teacher's voice and then get immersed in an artwork, discovering it. Clear and/or hidden clinical anatomical features can be enhanced and made easier to assimilate. This nontraditional format brings a new lens through which students can learn valuable visual skills on human clinical anatomy. It may favor critical thinking, opening the mind to alternative ways of seeing, thus enhancing medical students' abilities to more deeply observe and to better understand real clinical situations.

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Keywords

eLearning; medical education; visual arts; video tutorials; innovative teaching.

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Corrado Tumiati, Luigi Castaldi and their reappraisal of the role of Clemente Susini (1754-1814) in the accomplishment of the anatomical wax models of La Specola and of those now in Cagliari

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Corrado Tumiati (1885-1967) the youngest among the four brothers to whom the city of Ferrara dedicated a memorial tablet on the front of their house, was a psychiatrist who, at the age of 47, for disagreement with the authorities (he had refused to become a member of the fascist party) left the medical profession to devote himself to literary activities. As stated by Giuseppina Bock–Berti (2005), there is much to be said of him as writer, poet, translator and editor of famous literary magazines. In between the two wars, his prestige was so high that, despite his political credo, he was given responsibility for the editorship of the literary page of the Corriere della Sera, a job he maintained till 1946. Just in this capacity, on 18/01/1939 he published the article: "Le cere della Specola" that he extended and repeated in two essays published in 1941 and in 1942, by which he vindicated to Susini the authorship of the waxes produced in La Specola in a time when, outside from Sardinia, they were known in all Europe under the name of Fontana, the founder of the museum. Tumiati, who gives a vivid description of the artistic value of the models, even challenges the reader to find the name of Clemente Susini in any encyclopedia worldwide. A few years later, possibly inspired by Tumiati, Luigi Castaldi (1890-1945) the then anatomist of Cagliari, as appear from the news published in L'Unione Sarda on 03/12/1942, started his seminal work on the collections of Cagliari and Florence. Moreover, he reports on F.A. Boi, the Sardinian anatomist who performed the dissection then reproduced by Susini (Riva and Conti, 2015). Boi was unknown to Tumiati who does not mention him. Castaldi's masterly essay: "Francesco Antonio Boi primo cattedratico di Anatomia a Cagliari e le cere fiorentine di Clemente Susini" (Olschki, Firenze), gives an unprecedented description of La Specola Museum and of the persons responsible for its establishment. The book published posthumously in Florence (1947) through the good offices of his friends, was instrumental in reinstating the fame of Clemente Susini.

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Keywords

Cere anatomiche; Susini; Tumiati; Castaldi; Boi.

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Realdo Colombo in the fifth centenary of his birth

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The date of birth of Realdo Colombo is still uncertain. However, 1516 is conventionally credited as the year where he was born in Cremona. Colombo's life can be divided into three periods, according to the cities where he worked: Padua, Pisa and Rome. A talented anatomist, in Padua Colombo became assistant of Andreas Vesalius in 1541. In 1545 he moved to Pisa at the behest of the Grand Duke Cosimo I de' Medici. Finally, he was invited in Rome by Pope Paul III and became the physician of many important patients, including Michelangelo Buonarroti. He also performed the autopsy on the body of Saint Ignatius of Loyola. In his unique masterpiece, De re anatomica, consisting of 15 books, Colombo reported original observations. He hoped to have a text illustrated by Michelangelo that would have competed with the fabrica of Vesalius, but that purpose did not realize. Indeed, the unique engraving of the volume, published posthumously in 1559, is the frontispiece. The most important anatomical discovery attributed to Colombo is the original description of the pulmonary circulation, based on hundreds of dissections and vivisections. The Galen's long-standing doctrine of the blood circulation from the right ventricle to the left ventricle through invisible pores of the interventricular septum was definitively rejected. Although two other figures had already described the pulmonary circulation – the thirteenth century Arabic physician Ibn al-Nafis, in the Commentary on Anatomy in Avicenna's Canon, and the Spanish philosopher Michael Servetus, in the theological book Christianismi restitutio – Colombo seems to have arrived at his conclusions independently. He also understood the function of the cardiac valves. Colombo's book had a profound effect on William Harvey, when he prepared his lectures on anatomy for the College of Physicians of London, and was determinant for the publication of his description of the blood circulation in De motu cordis (1628). Other anatomical observations are attributed to Colombo. He corrected previous misconceptions, demonstrating that the right kidney is lower than the left, and showing that the lens is in the anterior chamber of the eye. He recognized anatomical variants, such as the presence of palmaris longus muscle, and described congenital malformations, such as the horseshoe kidney. He also seems to have coined the term "placenta" and claimed to have been the first to describe the clitoris and its function.

Keywords

Michael Servetus; Ibn al-Nafis; pulmonary circulation.

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The role of the LISTANet Consortium in the European DEDIPAC-KH project

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Aim:To improve understanding of the determinants of dietary, physical activity (PA), and sedentary behaviours, the European multi-disciplinary consortium on "Determinants of Diet and Physical Activity Knowledge Hub" (DEDIPAC-KH) includes 46 consortia and organisations supported by joint programming grants from 12 countries across Europe (Lakerveld et al., 2014). Six Italian Universities (e.g., Cassino, Chieti-Pescara, Palermo, Roma Foro Italico, Roma Sapienza, and UCSC) participating in the LISTANet consortium supported by MIUR (B84G14000040008) contributed to the Thematic Area2 "Determinants of dietary, PA, and sedentary behaviours across the life course and in vulnerable groups". In particular, the coordinator of LISTANet Prof Capranica and Prof. MacDonncha from the Irish Physical Activity and Health Consortium act as Work Package (WP) Leaders of PA determinants (WP2.2). Methods: A mix of methods has been used in identifying PA determinants by developing PA taxonomy and a European framework (EU-PAD), seven umbrella systematic literature reviews (e.g., behavioural, biological, economic, physical, policy, psychological, and socio-cultural), and identifying ongoing/recently completed European-funded projects and data sets for secondary data analyses. Results: LISTANet participated in DEDIPAC-KH meetings/seminars/courses/conferences, and organized two workshops dedicated to the EU-PAD framework and umbrella SLRs. Outcomes included internal reports, presentations to international conferences, and scientific papers submitted for publications. Conclusions: The DEDIPAC-KH project represents an excellent start in setting up a complex, cross-country, organisational structure to: 1) guide a European strategic plan for novel and multi-disciplinary research addressing the complexity of determinants of PA behaviours across the life course; and 2) identify key aspects for potential strategies and intervention programmes to implement multi-sectoral European policies in PA. Finally, the cumulated experience of LISTANet could be valuable to fully exploit effective research and actions to increase PA levels of Italian citizens.

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The role of the WISE Consortium in the European DEDIPAC-KH project

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WISE (Wellness, nutrItion, Sport and Exercise prevention) is a research consortium including five Italian research teams (University of Turin, University of Milan, University of Trieste, Universiy of Rome "Foro Italico", University of Bari), operating within the broader framework of the DEDIPAC-KH joint action (Determinants of Diet and Physical Activity Knowledge Hub). Research actions within the WISE consortium, funded by the Italian Ministry of Higher Education & Research, are in line with the main objective of the DEDIPAC-KH of developing an international and interdisciplinary network of researchers on dietary, physical activity and sedentary behaviours, related determinant research and policy interventions. More specifically, the WISE consortium research aimed to contribute to the following task (1.2.4 - Task Leader: Prof. Alan Donnelly): perform SLRs to identify state-of the art methods for physical activity and sedentary behaviour measurements. The focus of task 1.2.4 was to examine the methodological effectiveness (validity, reliability and sensitivity/responsiveness) of measures of physical activity and sedentary behaviours. The approach taken with this task was to examine the methodological effectiveness of measures of physical activity and sedentary behaviours in two populations; i) child/adolescence and ii) adults. Findings on methodological effectiveness of measures of physical activity and sedentary behaviours constitute the basis for a variety of publication and reports, and conference communications. The DEDIPAC-KH project created an unique opportunity for developing a comprehensive analysis on the determinants of diet and physical activity in Italy, and fostered successful collaboration with leading international groups. The findings of the WISE project created valuable information for the implementation of successful policies in Italy.

This work was supported by MIUR.

Keywords

Physical activity; sedentary behavior; DEDIPAC.

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The role of the IRILD Consortium in the European DEDIPAC-KH project

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The IRILD Consortium (partner in the DEDIPAC-KH joint Action) has been working in a few activities of Thematic Area1 and Thematic Area2 aimed at:

-Developing a toolbox with state-of-the-art methods to assess dietary intake and their determinants based on their quality and suitability to be used in pan-European studies (task 1.1.1)

- Identifying existing pan-European datasets including dietary intake to do secondary analysis according to variation in dietary intake by demographic (task 1.1.2)

- Examining the validity and reliability of the measurement methods employed to assess physical activity and sedentary behaviours on children/adolescent and adults populations (task 1.2.4)

-Identifying national state-of-the-art surveillance systems and infrastructures in Europe (task 1.3.3).

-Mapping and defining life course determinants, correlates and key research challenges of dietary intake in old population (task 2.1.1)

-Mapping and defining life course determinants, correlates and key research challenges of physical activity behaviour (task 2.2.1)

Theoretical frameworks of determinants have been taken as the starting point for developing the frameworks and taxonomies. On-going/recently finished EU-funded projects on determinants has been consulted. Finally, SRLs have been conducted .

The IRILD (Infrastructure to support Research In promoting active Lifestyles and healthy Diet) Consortium has been contributing to obtain the following results:

Methodological assessment and measurements for evaluation of vitamin B12 and folate intake have been extracted. Information on the validity and reliability of assessment methods were collected and all essential data for a toolbox have been prepared (task 1.1.1);

A report on variation of food consumption throughout Europe, concerning a secondary analysis to estimate food habits variation by food groups in different European age-gender population groups, has been prepared (task 1.1.2);

Methodological effectiveness of measures of PA and SB on children/adolescent and adults populations have been examined (task 1.2.4);

Information on nutritional surveillance in Italy have been delivered for sub-task 1.3.3.1 (task 1.3.3);

Systematic Literature Review on determinants of dietary intake in community-dwelling older adults to get an overview is going to be concluded (task 2.1.1).

The IRILD consortium was financially supported by the Italian Ministry of Agriculture Food and Forestry Policies (DM.14474/7303/13).

Keywords

DEDIPAC-KH, IRILD Consortium; systematic literature reviews; data set; dietary intake determinants; physical activity determinants; methodological effectiveness.

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The role of the INTREPID Consortium in the European DEDIPAC-KH project

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INTREPID (INTegrated Research on Eating and Physical activity Interventions and Drivers) is a research consortium including four Italian research teams (University of Bologna, University of Verona, Sapienza University in Rome and Catholic University in Piacenza), operating within the broader framework of the DEDIPAC-KH joint action (Determinants of Diet and Physical Activity Knowledge Hub) [1]. The DEDIPAC joint action has produced a taxonomy and a framework on the determinants of diet. Drawing from these, the INTREPID consortium has produced several statistical analyses, including: modelling of the socio-economic determinants of diet in Italy for different demographic and socio-economic groups; evaluation of interventions to promote physical activity in children; choice experiments on the use of smartphones to promote healthy dietary behaviours in children; quasi-experimental methods to assess the impact of the economic crisis on food purchasing behaviours of the Italian households; policy evaluation methods to assess the effects of the 2012 French soda tax; demand modelling to explore trade-offs between nutritional choices and sustainability outcomes. Furthermore, data merging methods were used to combine data from different sources (household budget surveys, omnibus surveys) and develop a measure of dietary quality. A variety of data sources were exploited. Ad hoc Kantar home scan data were purchased from GfK Italy and analysed in conjunction with French Kantar data (provided by ALISS France) to explore the impact of the French soda tax. Other sources used for the research include: the omnibus (Multiscopo) survey on Italian individuals between 2003 and 2015, the Italian Household Budget Survey between 1997 and 2014; the UK Living Cost & Food Survey between 2001 and 2014. The preparation of this paper was supported by the DEterminants of DIet and Physical Activity (DEDIPAC) knowledge hub. This work is supported by the Joint Programming Initiative 'Healthy Diet for a Healthy Life', and funded by the Italian Ministry of Education, University and Research (MIUR).

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Keywords
Diet; physical activity; nutrition policy; evaluation.

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Compensatory component of PRP-technology and knee-joint osteoarthrosis of dogs

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Input: Osteoarthrosis belongs to heterogenous group of diseases with similar morphological and clinical implications that leads to the cartilage and subchondral bone lesion, as well as lesion of other joint components (synovium, ligaments, capsule and periarticular muscles) (patent RU 2117997, 2240602, 2240603, 22464304, 22464305, 2271139, 2271140, 2303436, 2323694, 2323695) . Aim of research – to develop treatment technology of animals with aseptic osteoarthrosis by applying thrombocyte rich plasma.

Objectives: The objects of the study were dogs with knee-joint osteoarthrosis of I-III level. Every animal was older than 6 years old, with a live weight ≥30 kg. Methods: clinical, X-ray research, endoscopy.

Results: A week later after the autoplasma re-introduction, positive dynamics in all the animals was observed. In particular, lameness decreased, volume of the affected limb muscles recovered a little. At the end of therapy lameness disappeared in all animals virtually, but in some animals it was appeared after loading. Regain mobility joint crepitus disappeared. Joint mobility was recovered, crepitation disappeared. At the end of the treatment, control radiography of affected limbs showed a positive dynamics, consist in unevenness reducing of the articular surface, size of osteophytes reducing, osteosclerosis percent reduction. In the time of re-arthroscopy at the end of treatment some positive dynamics had observed; chondromalacia of I stage was marked, edema and hyperemia of synovial villi reduction, no generalized synovitis, no pulping of partial anterior cruciate ligament in particular. Discussion: Leanness, joint rigidity reduction in all animals by the end of the treatment course proves the presence of anti-inflammatory, regenerative effect of thrombocyte-rich plasma. Chondroprotective effect of platelet-derived growth factor cause reduction of edema of the synovial membrane and cartilage chondromalacia reduction, and reduction in the size of the cartilage defects observed during arthroscopic visualization. Lack of side effects and complications indicate high availability and safety of the method, in comparison with the standard treatment methods of animal osteoarthritis.

Keywords

PRP-technology; dog; knee-joint osteoarthrosis.

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Hemo-biochemical component in dogs' pancreatitis dynamics

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Input: Pancreatitis – aseptic inflammation of pancreas of demarcation character, which is based on necrobiosis processes of pancreatocytes and fermental autoaggression followed by necrosis, glandular degeneration and secondary infection development (patent RU 2305844). Lipidemia – increased content of lipids in blood is one of the main causes of pancreatitis development. The aim of research became - evaluation of therapeutic efficacy of a medicine that based on beta-sitosterol and polyprenyl phosphates (BSPP). Objectives: Twenty sick dogs with diagnosis of pancreatitis and lipidemia served as a material of research. Dogs were divided into 2 groups of 10 animals in each according to the principle of analogues. Pancreatitis therapy of first animal group was carrying out by drugs of protease inhibitor group, H-2 histamine receptors blockers, and also antibioticotherapia. Methods: haematological, biochemical, statistical. Results: Before therapy beginning both groups had an increased hematocrit, number of erythrocytes and hemoglobin. Increased number of leucocytes was marked. After 14-th day therapy all hematological indicators returned to the reference values in both groups of animals. By biochemical research at the beginning of the treatment increased level of a-amylase, pancreatic lipase glucose, cholesterol and triglycerides had been marked. On completion of therapy (2 months) in both groups, indicators of α-amylase and glucose were within reference values. Level of cholesterol and triglycerides had returned to normal by that time in the second group of animals, which indicated the normalization of lipid metabolism. Whereas in the first group of animals these indicators remained high. Discussion: Inclusion in the scheme of therapy in acute pancreatitis protease inhibitors, H-2 histamine receptors blockers and antibiotics don't allow to achieve complete recovery as evidenced by the high content of cholesterol and triglycerides. This promotes further transition of process to a chronic form. Inclusion of BSPP in the therapy in pancreatitis of dogs achieves significant depression of cholesterol and triglycerides in the serum of blood, thereby prevent relapses of disease.

Keywords

Dog; lipidemia; beta-sitosterol and polyprenyl phosphates.

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Morphological aspect of the superior wall of the cavernous sinus

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The lateral wall of cavernous sinus (CS) is made of two distinct layers of dura: meningeal dura (the external one) and endosteal dura (internal one). On the other hand, some debates about the histomorphological aspects of the superior and medial wall of CS are present in literature (1-2). In this study we aimed to demonstrate the histomorphological features of the superior wall of CS during cadaver dissection studies. We considered only the sellar area, bounded anteriorly by the posterior margin of planum sphenoidale, antero-inferiorly by the antero-superior surface of sphenoid sinus, inferiorly by the superior surface of sphenoid sinus, posteriorly by dorsum sellae, laterally by the interclinoid ligament (preserving the medial wall of CS) and superiorly by diaphragma sellae. The samples were collected, decalcified, paraffin embedded and serial sections were finally processed for standard histomorphological staining. Sections of each sample were analyzed using optical light microscope equipped with a digital camera. Our results showed that the two layers separated themselves at the superior wall of CS: the meningeal layer continued as diaphragma sellae and the endosteal layer continued downward between the venous compartment and pituitary capsule. In addition, between these two layers it was possible to define an interdural pathway. These data should be considered in the evaluation of the rare, though possible, extension of pituitary adenomas in the interdural space, rather than into the cavernous sinus.

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Keywords

Cavernous sinus; dura mater; interdural space; endosteal dura; pituitary gland.

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A mouse model of alcoholic liver disease reveals protection by Lactobacillus fermentum

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The knowledge and treatment of alcoholic liver disease is still plagued with gaps mostly due to the inherent limitations of research with patients. We developed an animal model for studying liver histopathology, Hsp-chaperones involvement, and response to treatment. The system was standardized using mice to which ethanol was orally administered alone or in combination with Lactobacillus fermentum for 4, 8 and 12 weeks and applying a battery of techniques (histology, immunohistochemistry, Western blotting, real-time PCR, immunoprecipitation, 3-nitrotyrosine labeling) to assess liver pathology and Hsp60, iNOS gene expression and protein levels, and Hsp60 post-translational modifications. Steatosis score, iNOS levels, and nitrosylated proteins (e.g., Hsp60) decreased after probiotic intake reducing considerably ethanol-induced tissue damage. However, one may assume that the probiotic tested has a gut protective effect and, possibly, anti-steatotic and antioxidant effects in the liver. Our results provide novel insights that may be taken into account while devising new approaches for treating liver diseases associated with alcohol consumption (1).

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Keywords

Ethanol-induced liver pathology; steatosis; probiotics.

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Parkinson's disease and taste function: a prospective investigation at three, four and five years from the first evaluation

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It is well-known that Parkinson's disease is characterized by a variety of non-motor symptoms. A gustatory deficit is hypothesized to be one of them. Because the few previous works assessed taste in a case-control way, the aim of our study was to investigate taste function in Parkinson's disease patients in a longitudinal fashion, after three, four and five years from the first evaluation. A group of 26 patients was re-examined (16 men, 10 women; age range: 54-88 years; mean age: 70.9 ± 8.4 years). As previously, taste function was assessed by means of the Whole Mouth Test (WMT) and Taste Strips Test (TST). Olfaction was also evaluated with the Sniffin' Sticks Identification Test (SST). All patients were able to understand and complete the procedure. Both for smell (p=0.45, Mann-Whitney U-Test) and taste results (WMT: p=0.234, Mann-Whitney U-Test; TST: p=0.747, Mann-Whitney U-Test) even if there is a score decrease, no significative difference was found between first and second evaluation, so suggesting a quite steady condition of chemosensory impairment across time. This could be in support of the hypothesis reported by various studies that an important taste dysfunction can be linked to the advanced phases of the disease associated with cortical involvement. Considering the objective difficulty in finding Parkinson patients suitable for this kind of evaluation in time (e.g. comorbidities onset, cognitive impairment) future research designed on a multicentric recruitment is needed.

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Keywords
Parkinson's disease; non-motor symptoms; taste function assessment.

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Teaching and learning human Anatomy in the University of Pavia: from models and clinical specimens to prosection on 3D models from our museum collection

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Due to decline of resources and support for teaching human anatomy, in our Institute the use of cadaver dissection is not economically feasible. After a few years in which I was able to perform prosection on fixed organs belonging to the Institute collection, over the last years students learned topographical anatomy on commercial plastic models (bones, muscles, joints, trunks and brains). New perspectives recently occurred thanks to a collaboration with Prof. Auricchio's group, which is involved in the strategic plan "3DPRINTING" (http://www. unipv.eu/site/home/area-stampa/articolo12952.html). First, we have segmented DICOM images of Computed Tomography (CT) to reconstruct 3D models of all the feet's bones from a patient. Then, these 3D models have been post-processed to obtain suitable file for 3D printing. A 3DSYSTEMS ProJet 460 Plus, professional, full-color binder jetting printer (property of General Surgery2), has been used to create 3D models of feet's bone by chalk powder binding. Medical students will use these models to test their own ability to recognize feet's bones shape and to recompose them. Second, a plastic 3D anatomical model has been scanned by Artec Eva 3D Object Scanner to obtain a 3D virtual model of the physical one; this model has been modified to create a new modular model, printed with a process similar to one described above. Our Anatomy Institute is enriched by a Museum, established in the late eighteenth century by universally known anatomists (Rezia, Scarpa, Panizza, Zoja). This historical collection contains several sections (osteology, angiology, splanchnology, neurology and topographic anatomy). It is impossible to use these anatomical specimens of historical interest for prosection, but their life-size copies will constitute a cheap and effective method of learning. This strategy could not replace cadaver dissection experience but we hope that it could assist students in the comprehension of anatomical systems in a cost effective way within a systemic anatomy course. Besides, this method should optimize specimen's choice and focalize student's attention on peculiar, selected samples, preparing more appropriately medical students to their clinical practice.

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Keywords

Anatomical teaching; medical education; 3D printe; 3D scan.

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Anatomy of the superior cerebellar artery

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Currently the superior cerebellar artery (SCA) attracting the attention of neurosurgeons, radiologists and anatomist because of its variations. The aim of this study was to investigate the anatomy and variations of the SCA in the Macedonian population and to emphasize their clinical significance. We examined radiographs of 103 patients who had CT angiography undertaken for a variety of clinical reasons, performed as a part of their medical treatment at the University Clinic for Radiology in Skopje, R. Macedonia. The study population included 103 patients, 58 male and 45 females, age range from 25-82, mean age 58.4 years. In 96.14% of the patients SCA have origin from the distal portion of the basilar artery on both sides as a single vessel. The most common variations of the SCA was duplication (frequency 1.94%) and origin from PCA (frequency 1.94%).The diameter of SCA at its origin on the left side was in the range between 0.40 - 2.41 mm, mean 1.36 ± 0.47 mm, and on the right side from 0.44 - 2.40 mm, mean 1.32 ± 0.44 mm. The distance between SCA and PCA on the left side was 1.59 ± 0.41 mm, and on the right side was 1.61 ± 0.39 mm. Although anatomically interesting, an awareness of the SCA anatomy and variations is clinically important for save performance of diagnostic and interventional procedures in radiology and for surgeons during planning and accomplishing surgical interventions.

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Keywords

Superior cerebellar artery; anatomy; variations.

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Cell damage induced by asbestos similar particles

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The presence, in nature, of asbestos similar particles, highly toxic and potentially cancerogenic for human healthy is well known (1). Inhalation of the fibrous form of erionite, has been shown to cause effects compared to those observed with mineral fibers classified as ''asbestos,'' including malignant mesothelioma, a disease typically associated with occupational and environmental exposures to asbestos (2). In this work various zeolite materials have been considered because of their suspected carcinogenic activity and, the possible interactions occurring between asbestiform fibers and U937 cell, a human hemopoietic cell line, have been evaluated. Chemical and morpho-functional analyses have been carried out, both to characterize fiber structure and cell response. Cells showed the ability to internalize the minerals, as observed after TEM analyses. With zeolite exposure time increasing, a diffuse cell damage with features of apoptotic and necrotic death can be evidenced (3). These findings suggest that the fibrous form of scolecite or offretite too can be considered potentially toxic for cell culture in vitro.

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Keywords
Asbestiform fiber; U937 cells.

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Morphological study of cartilage cell death in patients affected by osteoarthritis and chondrocalcinosis

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The role of chondrocyte death in the pathogenesis of osteoarthritis (OA) has been largely discussed in literature, but its relative contribution is difficult to assess (1). Chondrocyte death, be it apoptotic, necrotic or chondroptotic, has been clearly documented in OA and a certain correlation between the degree of cartilage damage and chondrocyte apoptosis has been demonstrated (2;3). Conversely, the relationship between the different types of cell death and chondrocalcinosis (CC) is still little known, as well as the presence and role of chondroptotic cells. The aim of this research was to compare chondrocyte behavior in the cartilage of osteoarthritic and chondrocalcinotic knees, evaluating the different types of cell death by means of optical and electron microscopy. During total knee replacement surgeries, cartilage specimens of femoral condyle have been withdrawn and their transversal semithin sections, stained with toluidine blue and alizarin solutions, have been investigated by optical microscopy. From the same samples, thin sections were obtained for transmission electron microscopy to evaluate, at high magnification, the specific ultrastructural features of different types of cell death. Cartilage specimens from both conditions revealed a thickness reduction of superficial layer and a high number of empty lacunae in the middle layer. Calcium pyrophosphate crystals appeared in the samples of patients affected by CC. In osteoarthritic cartilage, numerous chondrocytes revealed necrotic features, whereas, in chondrocalcinotic tissue, the middle zone was characterized by morphological patterns suggestive of chondroptosis, such as chromatin condensation mostly localized at the nuclear periphery, mitochondria alterations, a marked increase in endoplasmic reticulum, the presence of a diffuse autophagic component and the extrusion of cellular material into the lacunae. In conclusion, a different distribution of cell death types seems to characterize the intermediate layers of cartilage specimens from patients affected by CC compared to OA.

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Keywords

Chondrocalcinosis; osteoarthritis; chondroptosis; necrosis.

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Ultrastructural detection of environmental nanoparticles in circulating blood

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The increase in the incidence of acute myeloid leukemia (AML) may suggest a possible environmental etiology. PM2.5 was declared by the International Agency for Research on Cancer (WHO organ) a Class I carcinogen. To date, no reports have focused on particulate environmental pollution together with AML. The study investigated the presence and composition of particulate matter in circulating blood with a Environmental Scanning Electron Microscope coupled with an Energy Dispersive Spectroscope for the elemental analysis of the samples. 38 peripheral blood samples, 19 AML cases and 19 healthy controls, were analysed. A significant overload of particulate matter-derived nanoparticles linked or aggregated to blood components was found in AML patients, while almost absent in matched healthy controls. Two tailed Student's t-test, MANOVA and Principal Component Analysis indicated that the total numbers of aggregates and particles were statistically different between cases and controls (MANOVA, $P<0.001$ and $P=0.009$ respectively). The particles detected showed to contain highly reactive, non-biocompatible and non-biodegradable metals; in particular, micro- and nano-sized particles grouped in organic/inorganic clusters, with statistically higher frequency of a subgroup of elements in AML samples. The demonstration, of an overload of nanoparticles linked to blood components in AML patients suggests a possible, additional, pathogenetic mechanism for AML development.

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Keywords

ESEM; environmental pollution; nanoparticles; circulating blood; acute myeloid leukemia.

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Rhinosinusal polyposis and metals: morphological aspects

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Metals have strong toxic effects in humans and can act as immunoregulatory factors. The purpose of our study was to determine whether the concentrations of metals are associated with the clinical course of nasal polyposis (NP). We measured the concentrations of 10 metals (Zn, Mn, Se, Fe, Cr, Ni, Pb, Al, Cd, and Cu) in 58 patients with NP, and 29 controls with a healthy nasal mucosa. We used electron microscopy to compare the ultrastructural features of the nasal mucosa between NP patients and healthy controls. Concentrations of metals in nasal polyps and healthy mucosa were determined by mass spectrometry. Transmission electron microscopic (TEM) and scanning electron microscopic (SEM) images of the nasal mucosa were obtained. The mean tissue concentrations of all 10 metals were significantly lower in NP patients than in healthy controls ($P < 0.001$). Tissue concentrations of each metal were lower in stages III and IV NP than in stages I and II NP, although the differences were not statistically significant. TEM and SEM revealed changes in the mucosal ultrastructure in NP with progression from isolated polyposis (stages I and II) to massive polyposis (stages III and IV) with progressive fibrosis, devascularisation, and inflammation. Tissue concentrations of metals were lower in NP patients than in healthy controls, and this was particularly evident in massive polyposis. Polyp structure could contribute to the lower concentrations of metals by exposing the tissue to increased oxidative stress.

Keywords

Metals; nasal polyposis; TEM; SEM.

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Body structure of the macedonian population

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Establishing the structural components of the body: bone, muscle and fat is of great significance in sport orientation and selection as in entire biophysical development in young people. Nowdays, when there is hypokinetics in the modern world, there is a greater disbalance between active (muscle) and inactive (fat) components, which enlarge the value of "ballast" tissue. The material comprised personal files of 200 Macedonian examinees 14 years old,100 females and 100 males. On the basis of manifested anthropometrical variables, structural components of the body are established, in Macedonian children at age 14 who live in Skopje area, living in approximately equal socio-economic conditions. For quantitative determination of the absolute values of the bone (0 kg), muscle (M kg) and fat tissue (D kg), the dynamic anthropometric method by J. Meteigka was applied. At the age of 14 years, the absolute values of the bone mass (0 kg) was 9.91 (17,04%) in the males and in the females it was 7,83 (14,63%). The muscle mass (M kg) was 31.52 (52,04%) in the males and 27.89 (51.49%) in the females. The fat structural component (D kg) was 7.18 (11.14 %) in males and 7.02 (12.32 %) in females. The difference among sexes in the Macedonian population at the age of 14 years is very significant for the bone mass. At this age in male examinees bone mass is in high correlation with the diameter of the knee joint and in females with the diameter of the elbow, so they can serve as most valid sign in maintaining the bone mass. Muscle mass at 14 year age is in correlation with the circumference of the upper arm in males and with total muscle mass in females. Fat structural component at this age is higher in females then in male examinees, and is in high correlation with the body mass.

Keywords

Physical anthropology; body structure; adolescent.

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A case of cilioretinal artery with hemiretinal distribution

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The retinal vasculature is usually supplied by the central retinal artery (CRA), This is the first branch of ophthalmic artery in 77.5% (1). Cilioretinal arteries are reported to be present in up to 50% of eyes, and are considered to be the most common retinal vascular anomaly. Cilioretinal arteries take rise from a posterior ciliary artey (2). They may vary in size, number, distribution and point of origin from the optic disc. In most of the cases they are small arterioles supplying a part of central retina from the fovea to the optic disc. Only in 0.6%, large cilioretinal arteries can supply more than a quarter of the retinal circulation (3). We present a rare case of an individual with asymmetrical cilioretinal artery that arise inferiorly and supply the entire inferior emiretina. D.N., 7 years old male, affected by hyperopic astigmatism in both eyes. Clinical exam revealed no diseases. Examination of the fundus oculi showed, in the right eye, the presence of a very common small cilioretinal artery running from the disc to the foveal avascular zone. In the left eye, the central retinal artery gave rise only to two superior branches; a large cilioretinal artery entered the disc area from its inferotemporal edge, and gave rise to two inferior arterial branches. Thus, in the left eye, the CRA feeds the superior half of the retina; the large cilioretinal artery supplies the inferior half of the retina. Retinography was performed using a Confocal Color Scanning Ohthalmoscope (Eidon). This type of uncommon variant of retinal vasculature prevents extended retinal ischemia due to CRA occlusion.

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Keywords

Cilioretinal artery; retinal arteries; central retinal artery.

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Morphological characteristics of the anterior cerebral artery

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Cerebral circulation, especially arterial, in recent decades has attracted the interest of anatomists and clinicians. The anterior cerebral artery (ACA) arises from the termination of the ipsilateral internal carotid artery, and supplies blood to the medial regions of the frontal and parietal cortex, corpus callosum and falx cerebri. The ACA is usually divided into 5 segments. The aim of this study was to determine the morphological and topographic characteristics of the A1 or precommunicating segment of the ACA. The investigations of anatomical characteristics of the A1 segment of ACA was made on 133 human brains without cerebrovascular pathology, from both sexes at age from 23 to 68. Brains were fixed in a 10% solution of formaldehyde, and the obtained material was analyzed using a stereoscopic light microscope. The length of the A1 segment of ACA was in range from 6.8 to 20.8 mm, with mean value of 13.9 mm on the left side and from 7.4 to 21.8 mm, with mean value of 14.6 mm on the right side. The diameter of A1 segment of ACA on the left side was in range from 1.1 to 3 mm, with a mean value of 2.2 mm. The diameter of A1 segment of ACA on the right side was in range from 0.6 to 3.1 mm, with a mean value of 2.0 mm. Hypoplastic caliber of A1 segment of the ACA was noticed in 8% and in 0.5% duplication of the A1 segment of the ACA was registered. Detailed anatomical knowledge of the A1 segment of ACA is important when considering vascular surgery in the area of the anterior portion of the circle of Willis, since is the most common site of intracranial aneurysm formation.

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Keywords

Anterior cerebral artery; anatomy; origin; diameter.

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The aortic arch branching pattern: a 14,632 cases review

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Variations of the aortic arch branching pattern have a direct repercussion and influence not only in vascular surgery, but also in otorhinolaryngological and endocrinological surgery of the neck, radiological interpretation and procedures, intensive care unit patients management, mostly those with long-term nasogastric tubes, ischemic brain injuries linked to vascular interventions and during trauma patients diagnosis and management. Most of these variations are asymptomatic and have been largely considered clinically benign. Recent studies have pointed some of these vascular patterns as potential anatomical markers for thoracic aortic disease. The main objective of this work is to determine the prevalence of the aortic branching pattern reviewing a high number of cases, 14,632 (the highest as far as we know) from 38 anatomical and radiological studies, seven cohort studies and one case-control study The second objective is to propose a novel classification which includes most of the aortic arch patterns described until now in literature, and that easily allows to add new patterns that might be described in the future. This classification was used to group the results, based primarily in the number of main arterial branches arising directly from the aortic arch, and subsequently sub-classified according to the arise of secondary arteries from the arch. Pattern IIIA of the proposed classification represents the normal anatomical disposition, and along with its subtypes, determined by secondary arteries arising directly from the arch, they represented 81.87% of the reviewed cases. Pattern II A, commonly known as "bovine arch", when grouped with its five subtypes, represented 10.87% of the total, becoming the second anatomical variation most frequently found. Frequency of pattern II B and its subtypes was 5.40% in our review. Among secondary arteries arising frequency, 589 cases or 4.03% had a left vertebral artery emerging directly from the arch. The hardest part was to merge the results from the different studies due to the inhomogeneity of their descriptions. We believe that one of the most remarkable aspects of this work is the new classification we have created to describe the results. Since it is based on the anatomical pattern rather than frequency, it easily allows to add new patterns.

Keywords

Aortic arch; branching pattern; classification.

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Hypotrophic pseudoarthrosis: methodological problems of the impact interpretation of compensatory effects

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Input: The humanitarian trauma use methods of mesenchymal stimulation (curettage, tunnelization) widely and bring positive results. On this basis, the aim of this study was: treatment technology of animal with hypotrophic pseudarthrosis development. To achieve this aim we have identified the following tasks: study histological changes in the hypotrophic pseudarthrosis area, develop the tunneling zone of pseudarthrosis technology, evaluate the effectiveness of tunnelization under hypotrophic pseudarthrosis on the basis of clinic-morphological and radiographic changes (patent RU 2117997, 2240602, 2240603, 22464304, 22464305, 2271139, 2271140, 2303436, 2323694, 2323695). Objectives: The objects of the study were dogs with emerging complications (hypotrophic pseudarthrosis) after osteosynthesis of 57 goals. Methods: clinical, biochemical , hematological, radiographic, histological. Results and discussion: On the basis of histological changes (absence of the periosteum, the abundance of coarse fiber connective tissue, the presence of hondroida, isolated fragments of a full cartilage resorption of trabecular bone) indicates the presence of hypotrophic pseudarthrosis. Using of the term "atrophic pseudarthrosis" is not entirely competent, as nutrition of the periosteum was carried out, but not properly. Accordingly, the term "hypotrophic pseudarthrosis" should be used when referring to this process. Treatment technology of patients with hypotrophic pseudoarthrosis proposes tunnelization of pseudoarthrosis zone that provokes vascularization of pseudoarthrosis area. Changes in hematological and main biochemical indicators of sick animals within the reference quantities shows, firstly, that the basis of hypotrophic pseudarthrosis is trophicity breaking of the bone fragments, secondly, tunnelization method is invasive and less traumatic. That indicates by reduction of C-reactive protein from 2,8±0,7 before operation to 1,9±0,3 30 days later, and to 0,4±0,05 on 120 day. Radiologically, hypotrophic pseudarthrosis characterized by thinning of cortical and erosion of cortical layers of proximal and distal bone fragments, optical density and diameter of the bone regenerate decrease, appearance in the zone of diastase radiographically weakly visible tissues (fibrous).

Keywords

Dog; hypotrophic pseudoarthrosis; compensatory effects.

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Variations of lobes and fissures in human fetal lung: a cadaveric study

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The human lungs are divided by fissures into lobes, which facilitate movements of lobes in relation to one another. Anatomical variations of lungs including number, fissures and lobes are at utmost important. The study was done to note the morphological variation of the fissures and lobes in fetal lungs. 62 human fetuses from 12 weeks to 40 weeks of gestational age were collected from the department of Obstetrics and Gynecology, University Clinic Hospital , after getting formal permission from the concern authority/ persons and the Institutional Ethics Committee. After fixation in 10% formalin, fetuses were dissected and both lungs were removed for examinations. On the right side, 8 specimens showed incomplete oblique fissure, 39 specimens showed incomplete horizontal fissure, 1 specimen showed absence of horizontal fissure and 9 specimens showed superior accessory fissure. On the left side, 5 specimens showed incomplete oblique fissure and the left minor fissure was seen in 8 specimens. Knowledge of lobes and fissures in a particular population might help the clinician during diagnosis and partial resection of lungs. This may reduce morbidity and mortality associated with lung disease.

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Keywords

Lungs, oblique fissure, horizontal fissure, fetuses.

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Protocol of ultrasound detection of multifidus in the lumbar spine tract

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A correct morphological analysis is strictly related to the modalities of acquisition. A protocol to standardize cross-sectional ultrasound scans of lumbar multifidus (LM) was set, aiming to correct and complete information (1). Three authors of the study took turns as subject and operator mutually, repeating the scans until definite, correct, and complete images were obtained for each of the lumbar levels investigated. The convex probe (5.0Mhz emission) of a LogiqE (Ge Medical Systems, Milwaukee, WI, USA) in B-mode Real Time modality was used. During each scan, the setting of the machine was fixed for all planes. The gain was set at 82, parameters of depth control at 7cm, focal frequency at 31Hz with the implementation of bifocal setting. Each scan conducted on the posterior aspect of the lumbar vertebra produced characteristic shapes that were indicative of the level at which the scan was performed. For a complete and correct definition of the area and section of interest of LM, the recognition of the lateral side of the osteofibrous space was particularly critical (2). Once the optimal plane to scan LM had been defined, three different morphologies, characteristic of the vertebral recesses corresponding to vertebrae L5-L4, L3, L2-L1, were identified (3). To guarantee uniformity in data collection, in the communication and individualization of the parameters characterizing the clinical pictures, the protocol of acquisition had to be repeatable, reliable, and it had to reduce the operator dependence (1,2,3). In conclusion, a correct and complete image is subject to qualitative and quantitative evaluation in the area of interest of LM. This undeniably favours communication between different diagnostic centres and the detection of morphological modifications that characterize both function and pathology.

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Keywords

Spine; ultrasound; lumbar multifidus; reliability; diagnostic imagine.

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Anthropometrical indexes as nutritional indicators in 5 years-old macedonian children

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Evaluation of sex-specific differences of anthropometrical indexes that were used as indicators for assessment of nutritional status in children aged 5. The study included 226 healthy children (113 boys, 113 girls) aged 5 from Macedonian nationality. With standard methodology (IBP) were taken following body measurements (body weight, height, mid upper arm circumferences-MUAC and skinfolds thickness triceps–SFTr and subscapular-SFSc), and according to standard formulas were calculated: weight-for-age (BW), height-for-age (BH), body mass index-for-age (BMI), mid upper arm circumferences-for age (MUAC), and skinfolds thicknes-for-age (SFTr and SFSc). Results showed sex-specific differences in a large number of the examined anthropometrical parameters (BW, BH, BMI) in favour of the boys. On the other hand, skinfolds thickness (SFTr and SFSc) were significantly higher in girls. Values of the 50th percentile in boys were as follows: 21 kg for BW, 115 cm for BH, 15.48 kg/m^2 for BMI, 15.5 cm for MUAC, 4.1 mm for SFSc, and 7 mm for SFTr. The values of these parameters in girls were: 20 kg for BW, 113.5 cm for BH, 15.01 kg/m^2 for BMI, 16cm for MUAC, 4.7mm for SFSc and 7.8 for SFTr.These results can be used as criteria for the assessment and detection of deviations in the nutritional status in children aged 5.

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Keywords

Children; anthropometry; nutritional status.

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Anatomical variations of coronary sinus ostium and Thebesian valve

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The coronary sinus (CS) is the main cardiac vein and it has become a clinically important structure especially through its role in providing access for different cardiac procedures. The study was carried out on 100 randomly selected adult human cadaver hearts fixed in 10% formalin. The transverse and craniocaudal diameters of the coronary sinus ostium (CSO) were directly measured. The presence of the Thebesian valve was noted and the anatomical details of the valve were documented in each case in terms of the shape and extent of coverage of the CSO. Considerable variations in the diameter of the CSO were observed. The mean craniocaudal diameter of the CSO was 8.1±1.51 mm, and the mean transverse diameter was 7.67±1.72 mm. Heart specimens without Thebesian valve tended to have larger ostia. The mean craniocaudal diameter and the mean transverse diameter of the CSO were statistically larger in the specimens without Thebesian valves ($p=0.000$ and $p=0.001$, respectively). The Thebesian valves were observed in 86 hearts, and a wide variety of their morphology was seen. The majority of the Thebesian valves were semilunar in shape (74.42%). The extent to which the valve covered the ostium was variable, including remnant valves that covered <15% of the CSO (35%), and valves that were large and covered at least 75% of the CSO (22.09%). In 3 specimens the valve completely occluded the ostium.

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Keywords

Thebesian valve; coronary sinus ostium; variations.

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Microscopic evaluation of tongue dorsum biofilm from halitosis patients: an ex vivo study using confocal laser scanning microscopy (CLSM)

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A category of oral biofilm which is still not well understood is the one coating the tongue, although various reports have associated its presence with halitosis in patients (1). The aim of the study was to visualize the three-dimensional bacteria distribution within the biofilm in order to better understand the ecological balance which regulates it. Tongue plaque samples from four halitosis-diagnosed patients and four healthy volunteers were analysed and compared. The biofilm was collected using a 0.1ml sterile inoculating loop. The visualization of the tongue dorsum biofilm was performed combining fluorescence in situ hybridization (FISH) and confocal laser scanning microscopy (CLSM) (2). Eubacteria, Streptococcus spp. and Fusobacterium nucleatum were stained using specific fluorescent genetic probes. Morphological analysis by CLSM illustrated the different distribution of the species which were tracked: Streptococcus spp. appeared immerged within the samples, while F. nucleatum was found in the peripheral areas of the samples. Furthermore, F. nucleatum appeared to exist without the presence of the Streptococcus spp. in the halitosis group. This study showed the architecture of tongue dorsum biofilm by means of imaging techniques, highlighting the distribution of the tracked bacterial species within the biofilm sample of the plaque.

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Keywords
Tongue dorsum biofilm; Confocal Laser Scanning Microscopy (CLSM); oral bacteria; halitosis.

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Orthodontic brackets removal: morphological in vitro evaluation

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Debonding procedure is a fundamental stage in the orthodontic treatment (1). Adhesive removal should lead to a complete polished enamel in order to prevent the plaque accumulation and enamel injures (2). Aim of this study is to evaluate in vitro by use of Scanning Electron Microscopy (SEM) the enamel surface after the bracket removal and the enamel polishing with four different techniques. Two groups of teeth, group A (n=12) and group B (n=12) were evaluated. The images were analyzed by the Image J software. After the debonding procedure the two groups were subcategorized in four groups (1A, 1B, 2A, 2B 3A, 3B and 4A, 4B, n.=3). The discriminant between the two main categories was the use of a magnification system (Group A) during the polishing stages. From the qualitative and quantitative images analysis the most conservative technique resulted to be the use of tungsten carbide bur, followed by the final polishing using the soft-polisher tip for composites. In group A, the percentage of the residual adhesive resulted 8% and the damages on the enamel surface showed to be 7%. In group B the percentage of the residual adhesive resulted 35% and the damages on the enamel surface showed to be 15%. This analysis showed how the use of a magnification system aids in significant way during the debonding procedures in the enamel surfaces' preservation.

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Keywords

Dental debonding; Scanning Electron Microscopy (SEM); enamel surface.

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Maxillary sinus lift with or without biomaterials in humans: radiographic and histomorphometric evaluation

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Biomaterials are frequently used for sinus lift procedures at the time of implant installation in the posterior upper jaw. Nevertheless, some authors have reported the possibility of leaving an empty space, in order to exploit the regenerative potential of the residual periosteal and bony walls following the Schneiderian membrane elevation. The aim of this study was to compare the radiographic changes and histological characteristics of newly formed bone following sinus lift without any biomaterials or Bio-Oss®. Fifteen patients with edentulousness of the maxillary molar-premolar area and a residual crest thickness < 6 mm, were randomly allocated to a test group (sinus lift without biomaterial) or a control group (with Bio-Oss®). Two submerged Astra Tech implants were placed in the most mesial and distal portion of the augmented area. Six months later, a bone biopsy was harvested in the area between the 2 previously placed fixtures, where a third central implant was placed. Standard radiographies were taken before sinus lift and 6 months later in order to measure and compare the vertical bone changes. The biopsies were processed for ground sectioning. All implants but one case were successful. Radiographically, the basic level of bone tissue in the test group (n=5) was 5 ± 0.86 mm and 3.5 ± 0.57 mm in the control group (n=10); in the test group the bone tissue was increased vertically of 10 ± 2.53 (range 7-13), in the control group 13 ± 1.41 (range 12-14). At histological observation, all samples showed new bone formation without signs of inflammation. Bone remodeling was observed in the apical portion of both groups. Residual particles of biomaterial were embedded in mineralized new bone. Histometric results for the control group were: LB 38.8% ± 8.1, WB 9.2% ± 2.4, BM 30.2% ± 7.5, BO 21.7% ± 8.9; for the test group: LB 54.5% ± 2.1, WB 115.5% ± 6.1 and BM 33.5% ± 6.4. A clinically significant bone increase was achieved both with and without the use of biomaterials. The implants showed similar performances when inserted at sites augmented with or without biomaterials. The success of such procedure might depend on the anatomical conformation of the crest and on the level of surgical experience of the surgeon.

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Keywords
Dental implants; sinus lift.

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Comparison of direct linear measurements on dental plaster cast and digital measurements obtained from laser scanner and Cone-Beam CT dental models

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Different dental imaging technologies are now daily used in clinical practice to evaluate oral anatomy. These new techniques allow to replace dental plaster casts with digital models that are easier to manage and store. Such models can be acquired with optical methods like laser scanner, stereophotogrammetry and intraoral scanner or reconstructed by 3D CT or CBCT images [1]. Since these digital casts are used in clinical routine, it is important to evaluate accuracy and reliability of measurements taken from them, in relation to traditional methods [2]. We wanted to compare linear measurements taken on digital models obtained from CBCT images and laser scanner surfaces, with direct measurements obtained with digital calliper on dental plaster casts. Data from 6 adult Caucasian subjects with full dentition, no history of implant surgery and without dental filling were obtained. The absence of implants and metal fillings was selected as inclusion criterion to reduce the presence of metal artefacts that can affect the measurement process. All patients were retrospectively selected from a clinical database and underwent CBCT examination for clinical reasons uncorrelated with this study. Six dental distances in the upper and six in the lower jaw were examined: the mesio-distal distance of teeth 21, 23, 24 and 26, the palatal-vestibular distance of teeth 24 and 26, and the corresponding distances on teeth 41, 43, 44 and 46. All measurements were performed using: 1) a digital calliper on dental plaster casts; 2) a virtual calliper on digital models obtained from CBCT images; and 3) a virtual calliper on laser scanner surfaces. Kruskal-Wallis test compared measurements performed with the 3 different techniques. There was no statistical significant difference among different techniques for all measurements ($p>0.05$) except for one distance, the mesio-distal distance of tooth 24 ($p<0.05$). Measurements on digital dental models seem as reliable as direct measurements performed on dental plaster casts. Results are promising, nevertheless further evaluation on a larger sample is advised.

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Keywords

Dental model; CBCT; laser scanner; calliper.

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The face in Marfan syndrome: a 3D morphometric study

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Marfan syndrome (MFS) is a rare congenital disorder of the connective tissue mainly caused by mutations in the FBN1 gene, resulting in an altered assembly of extracellular matrix microfibrils and TGF-beta signalling dysregulation. Major clinical manifestations of MFS involve the skeletal, ocular, and cardiovascular systems, with a high risk of life-threatening aortic dissection and rupture. An early recognition of the disorder is essential, but it could be difficult, due to the variable phenotypic expression of the disease and the current incomplete sensitivity of molecular genetic testing of FBN1. It has been suggested that craniofacial dysmorphism associated with MFS could facilitate obstructive sleep apnea, which in turn may promote aortic dilation. The study aimed to investigate the face in MFS through a 3D not invasive approach [1], identifying new morphometric features which could facilitate the early diagnosis of the disease. The 3D coordinates of 50 anatomical facial landmarks were obtained using a stereophotogrammetric system in 68 Italian subjects diagnosed with MFS, aged 4-64 years (27 males, mean ± SD age 29.6 ± 18.2 years; 41 females, mean ± SD age 37.2 ± 15.5 years). Subjects were divided in 11 non-overlapping age groups. Facial linear distances and angles were measured; z score values were calculated comparing patients with healthy Italian reference subjects (347 males, 388 females), matched for gender and age. Subjects with MFS showed a shorter mandibular ramus than controls (mean z score = -1.9), a greater facial divergence (mean z score = +2.0), a reduced ratio between posterior and anterior facial height (mean z score = -1.9), and a reduced ratio between facial width and facial height (mean z score = -1.5), together with an expected but overall mild increase of facial height (mean z score = +1.3). Noteworthy gender differences or age trends were not observed. Facial abnormalities pointed out in the current study could represent phenotypic traits of MFS; since they were observed also in young patients, their detection could facilitate the early recognition, management, and follow up of the disease. These promising findings need to be confirmed extending the study on more patients.

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Keywords

Anthropometry; Marfan syndrome (MFS); facial morphology; stereophotogrammetry.

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Nerve endings in rat periodontal ligament: an immunofluorescence study

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Periodontal ligament is a structure between tooth root and alveolar bone. A few morphological study on the sensory innervation of periodontal ligament are available. Although there has been controversy over the distribution and shape of the sensory nerve terminals, researchers largely agree on the presence of two types of nerve endings: free endings and organized structures as Ruffini-like and Meissner corpuscles. Ruffini-like are slowly adapting receptors and give informations about the intensity and duration of the mechanical stimulus applied to the periodontal ligament; Meissner's corpuscles are rapidly adapting receptors and give informations about direction of mechanical stimuli to the tissue. In the present study we investigated, by immunofluorescence for protein gene product PGP 9.5, the distribution of nerve endings among the different regions of periodontal ligament both of incisors and molars of rat. We found a variety of nerve endings morphology and several difference about their distribution between incisors and molars periodontal ligament. Moreover, we found that the morphology of nerve endings changes depending on the modification of the force applied to periodontal ligament, highlighting its importance in regulation of muscle activity.

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Keywords

Periodontal ligament; PGP 9.5; rat.

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Qualitative and quantitative analysis of gingival microvessels by capillaroscopy in healthy subjects

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Gingiva is composed by attached gingiva and free gingiva that are separated by free gingival line. Attached gingiva covers the alveolar bone and adheres to the bone and root surface by fibres. Free gingiva ends with the gingival margin and in clinical practice it can be displaced from the tooth surface to locate the prosthetic margin. Capillaroscopy allows to take microphotographs of the microvessels and to observe their abnormalities in autoimmune rheumatic diseases (1). Aim of this study was to analyse microvessels of the attached gingiva, free gingiva line and free gingiva by means of capillaroscopy. In correspondence of upper incisors of 12 young healthy volunteers, after placement of liquid vaseline, microphotographs (x200) were taken at level of the free gingiva and 2-3 mm more apically within the attached gingiva. Capillaries structure and organization were evaluated in the three areas of interest. In 10 randomly selected microphotographs of the attached gingiva, the amount and percentage of microvessels per mm2 were also calculated. For each subject, two analyses were performed at 3 weeks of distance for repeatability assessment. At the observation, in attached gingiva vessels appeared as tortuous capillary loops perpendicular to the epithelial surface. At level of free gingival line vessels get linear and parallel to the arch of gingival margin. In free gingiva capillaries run superficially and parallel to the epithelial surface, toward the margin and fell back with a loop on the tooth side. At the quantitative analysis, the method resulted repeatable (Wilcoxon signed-rank test, $p>0.05$). A mean of 49.8 ($\pm$ 9.5) microvessels for mm2 was found. Capillaries represented the 10.3% ($\pm$3.5) of the attached gingiva. Capillaroscopy is a non-invasive repeatable method to observe gingival capillaries. This method may be proposed in clinical practice to detect and monitor changes or abnormalities after placement of prosthetic margins.

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Keywords

Microvessels; capillaroscopy; gingival margin.

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The anastomotic network around the anterior superior alveolar nerve: an anatomical and radiological study

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Innervation of superior teeth is supplied by the posterior (PSAN), anterior (ASAN) and sometimes by middle superior alveolar nerve (MSAN). PSAN arises from the maxillary nerve and passes through the posterolateral maxillary wall towards the posterior teeth. ASAN arises from the anterior portion of the infraorbital nerve and courses within the infraorbital canal passing nearby the piriform aperture and premaxilla. When present, MSAN arises from the posterior portion of the infraorbital nerve and runs along the lateral maxillary wall. However, an additional nasopalatine or sublabial injection is frequently required to obtain a complete anesthesia of the maxillary teeth due to rich anastomotic network (1-2). With the aim to better describe the complexity of the superior alveolar nerve network, fifty-seven high-definition sinonasal cone-beam CT (CBCT) were analyzed. PSAN, ASAN and MSAN were detected by specific bony landmarks/canals and nervous anastomoses were accurately evaluated. In addition, medial anastomotic branches from the palatal and/or nasal nervous plexi were also considered. PSAN and ASAN were identified in 100% of cases whereas MSAN in 19.6% of cases. Anastomotic branch versus ASAN was identified in all cases from MSAN and in 60.3% from PSAN. Medial anastomotic branch was detected in 62.0% of cases from the nasal plexus and in 6.2% from the palatal plexus: the former passed through a bony defect in the floor of the piriform aperture or at the base of the nasal septum; the latter passed through a tiny canal in the interface between maxilla and premaxilla. These data confirm that maxillary teeth innervation, especially for incisor teeth, could be provided not only by alveolar nerves but also from palatal and nasal plexi via small branches running within maxillary bony canals. These results support the need of additional anesthetic injection to obtain adequate anesthesia of the maxillary teeth; moreover, the role of CBCT in the identification of the nervous pattern was underlined.

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Keywords

Superior alveolar nerve; anastomosis; CBCT.

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Assessment of facial asymmetry using stereophotogrammetry

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Asymmetry in the dimensions and spatial position of facial structures is a common finding in healthy individuals and in esthetically pleasing faces (1). Additionally, a variety of craniofacial anomalies are characterized by severe hard- and soft-tissue asymmetry (2). Facial asymmetry can impair the affected people from both aesthetical and functional points of view. Currently, facial asymmetry is mainly evaluated using the entire facial surface, thus providing measurements that give only general information about facial morphology. In contrast, several pathologies affecting facial appearance are localized in selected parts of the face, and a local assessment can provide helpful information for clinical decisions. For these reasons a detailed, focused and objective evaluation of facial asymmetry is advised, both for surgical planning and treatment evaluation. In this study we present a new quantitative method to assess symmetry in different facial thirds, objectively defined on the territories of distribution of trigeminal branches. Forty healthy young adults (21 women; 19 men; average age 39 $\pm$ 12 years) were acquired with a stereophotogrammetric system and the level of asymmetry of their hemi-facial thirds was evaluated, comparing the root mean square of the distances (RMSD) between their original and mirrored facial surfaces. The method resulted highly reproducible (Bland and Altman coefficient of reproducibility for area selection, 98.8%). In the upper facial third, median asymmetry was 0.726 mm (IQ range: 0.579-0.954 mm); in the middle facial third, median asymmetry was 0.739 mm (IQ range: 0.558-0.887 mm); in the lower facial third, median asymmetry was 0.679 mm (IQ range: 0.552-0.907 mm). No significant differences in RMSD values among the facial thirds were found (ANOVA, $p>0.05$). The presented method provides an accurate, reproducible and local facial symmetry analysis, that can be used for different conditions, especially when only part of the face is asymmetric.

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Keywords

Facial asymmetry; photogrammetry; 3D.

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New therapeutic applications of ultrasounds: a preliminary study

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Ultrasound is well known for its imaging role in medicine. Recently, a new role of non-thermal ultrasound has been investigated concerning the central nervous system. Effectively, ultrasound treatment is able to modulate brain functions in humans (1), as well as the opening of the blood brain barrier in primates and rats, with the absence of tissue damage (2). Furthermore, an in vitro study has demonstrated the role of focused ultrasound in regulating neuronal sprouting in human neurons (3). The aim of this study is to evaluate, for the very first time, the effects of diagnostic ultrasound (12 MHz, 1 cycle) on murine microglial cell line (BV-2). For this purpose, BV-2 cells were stimulated with ultrasound (12 MHz, 1 cycle) for 3 minutes. Cell viability assay and western blotting, morphological, and immunofluorescence analyses were performed. Our results show that ultrasound stimulation did not affect cell viability. Conversely, western blotting analysis, as well as immunofluorescence staining, revealed a decrease in B7-2 (CD86) expression, and this latter feature was confirmed by morphological analysis, highlighting an increase in resting-ramified cells, with the capability to survey the surrounding area. Taken together, these results demonstrate that ultrasound safely affect the central nervous system in normal (physiological) condition. Furthermore, ultrasound own the features of temporary change the morpho-functional state of microglial cells, reinforcing their new role in the field of neuroscience.

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Keywords
Ultrasound; microglia BV-2 cell line; B7-2 (CD86).

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The role of autophagy in vernal kerato-conjunctivitis

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Autophagy is involved in many biological aspects, including cell survival and death, innate and adaptive immunity and cancer. An involvement of autophagy is also reported in some inflammatory diseases such as asthma. In the present study we explored the role of autophagy in vernal keratoconjunctivitis (VKC), a severe inflammatory disease mainly found in children and adolescents. Autophagy and apoptosis markers (LC3A, LC3B, Beclin-1, cathepsin B, BCL-2, BAX, caspase 3) expression in conjunctival biopsies from 9 active VKC patients and 9 healthy age matched normal subjects were analyzed using immunohistochemistry and qPCR techniques. Conjunctival cells cultures were treated with inflammatory stimuli (IL-1b, histamine, IL-4, TNFa) and analysed by western blotting for autophagy markers expression. LC3B, Catepsin D and B and Beclin-1expression strongly increased in the stroma of VKC whereas the epithelium was consistently negative for all of the molecules studied but positive for Beclin-1 in VKC. qPCR analysis demonstrated a similar mRNAs expression in VKC and normal subjects. In "in vitro" experiments autophagy induction revealed that only LC3B expression was changed in conjunctival fibroblasts by inflammatory stimuli. In particular, both LC3BI, the LC3B free form, and LC3BII, the phosphatidyl-ethanolamine-conjugated form, involved in the autophagosome formation, were decreased in fibroblast cultures at 24h after TNFα stimulation. However, since LC3B-II is normally degraded by lysosomes and the total amount of LC3B-II depends on the balance between its formation and degradation, we analyzed the expression of LC3B-II in the presence and absence of chloroquine, an inhibitor of lysosomal degradation. We found a significant increased amount of LC3BII compared to the control, indicating an over-expression of this protein in stimulated fibroblasts that is quickly damped by its degradation. Since one of the key steps in autophagy is the conversion of LC3B from LC3B-I to LC3B-II, our results suggest that autophagy may be involved in the pathogenesis of VKC.

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Synergistic effects of selective inhibitors targeting the PI3K/AKT/mTOR pathway and NUP214-ABL1 fusion protein in human acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is a neoplasm of precursor cells committed to the B-cell and T-cell lineages involving bone marrow and blood, with a rapid onset and frequent chemotherapy resistance and refractory relapses (1). Philadelphia chromosome-positive (Ph+) ALL accounts for 25–30% of adult ALL and its incidence increases with age in adults >40 years old. Irrespective of age, the ABL1 fusion genes, among which BCR-ABL1 is the most commonly found, are markers of very poor prognosis. Amplification of the NUP214-ABL1 oncogene can be detected only in patients with T-ALL (2). The PI3K/Akt/mTOR signaling pathway is activated in many solid cancers and in leukemias and plays a crucial role in tumorigenesis. Furthermore, the presence of RTKs (Receptor Tyrosine Kinases) by ABL1 fusion proteins may result in activation of the PI3K/Akt/mTOR axis. T cell malignancies bearing the ABL1 fusion genes are sensitive to many cytotoxic agents, but up to date complete remissions have not been found. In this work we analyzed the effects of three BCR-ABL1 tyrosine kinase inhibitors (TKIs), alone and in combination with a panel of selective PI3K/Akt/mTOR inhibitors, on two NUP214-ABL1 positive T-ALL cell lines, ALL-SIL and PEER that also displayed Akt hyperactivation. Cells were sensitive to anti BCR-ABL1 TKIs Imatinib, Nilotinib and GZD824, that specifically targeted the ABL1 fusion protein, but not the PI3K/Akt/mTOR axis as deducted by a readout of drug efficacy, four drugs against the PI3K/Akt/mTOR cascade, GSK690693, NVP-BGT226 (BGT226), ZSTK474 and Torin-2, showed a relevant cytotoxic efficacy on T-leukemic cells, without affecting the NUP214-ABL1 kinase and related pathway. Dephosphorylation of pAkt and pS6 showed the cytotoxicity of the compounds. Either single or combined administration of drugs against the different targets displayed inhibition of cellular viability which was associated with a concentration-dependent induction of apoptosis, cell cycle arrest in G0/G1 phase and autophagy, having the combined treatments a significant synergistic cytotoxic effect. Co-targeting NUP214-ABL1 fusion gene and PI3K/Akt/mTOR signaling pathway could represent a new and effective pharmacological strategy to improve the outcome in NUP214-ABL1 positive T-ALL.

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Keywords

BCR-ABL1; PI3K/Akt/mTOR signalling; T-acute lymphoblastic leukemia; targeted therapies; autophagy.

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Raf Kinase Inhibitor Protein (RKIP) expression and function in human myometrium and leiomyoma

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Many growth factors been identified in human myometrium and leiomyoma and activate multiple signaling pathways in order to regulate major cellular processes, including proliferation and fibrosis which are linked to uterine leiomyoma development and growth. The Raf kinase inhibitor protein (RKIP) has emerging roles as regulator of multiple signaling networks including mitogen activated protein (MAP) kinase cascade, as well as interaction with glycogen synthase kinase 3 (GSK3). In our study, we aimed to investigate the presence of RKIP in human myometrium and leiomyoma as well as to determine the effect of locostatin (RKIP inhibitor) on extracellular matrix (ECM) production, proliferation and migration in human myometrial and leiomyoma cells. Myometrial and leiomyoma tissues were used to investigate the localization and the expression level of RKIP through immunohistochemistry and western blotting. Myometrial and leiomyoma cells were treated with locostatin to measure ECM expression by real time PCR, GSK3b expression by western blotting, cell migration by wound-healing assay and cell proliferation by MTT assay. We found that RKIP is expressed in human myometrial and leiomyoma tissue. Locostatin treatment resulted in the activation of the MAPK signal pathway (ERK phosphorylation), providing a powerful validation of our targeting protocol. Further, RKIP inhibition by locostatin reduces ECM components. Moreover, the inhibition of RKIP by locostatin impaired cell proliferation and migration in both leiomyoma and myometrial cells. Finally, locostatin treatment reduced GSK3β expression. Therefore, even if the activation of MAPK pathway should increase proliferation and migration, the destabilization of GSK3β leads to the reduction of proliferation and migration of myometrial and leiomyoma cells.

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Keywords

Myometrium; uterine leiomyoma; RKIP; locostatin; extracellular matrix; cell migration.

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Ultrastructural changes of the intestinal mucosa in Non-Celiac Gluten Sensitivity patients could represent an early indicator of cellular stress

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Non-Celiac Gluten Sensitivity (NCGS) is a wide-spreading syndrome and an emerging problem in clinical practice linked to the increase of gluten content in some populations diet. It is characterized by intestinal and/or extra intestinal symptoms that improve or disappear after removing gluten from diet in non-celiac or non-wheat allergic patients [1]. In order to perform and support a precise and early diagnosis, as others recently suggested [2], the aim of this work was to analyze in detail ultrastructural features of the duodenal mucosa. Biopsy specimens were obtained from 10 patients who underwent gastrointestinal endoscopy for a diagnostic check-up at the Department of Gastroenterology of the University of Chieti and prepared for electron microscopy. Semithin sections were blindly observed but only biopsies showing well-shaped intestinal villi were selected for the ultrastructural study, observed with a ZEISS EM109 equipped with a Gatan videocamera. We analyzed: 1) brush border, 2) epithelial cell cytoplasm, 3) cellular junctions and 4) the villus connective axis with respect to inflammatory cell number and vascular alterations, evaluating amount and localization of cellular damages. Interestingly, only 3 of these biopsies, obtained from subjects in which clinical history and diagnosis was uncertain, presented fine spot damage in the epithelium from intestinal villi with an apparently normal morphology. Some epithelial cells showed sever distress such as heterochromatic and nuclei not-round shaped, dilated endoplasmic reticulum, increased number of mitochondria and a messy brush border thinned and reduced in width. Numerous damaged cellular junctions and remarkable basal detachment of cell plasma membranes were observed. These findings pave the way to a deepen characterization of intestinal mucosa from NCGS patients to identify, by means of electron microscopy, potential morphological and functional markers of NCGS.

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Keywords

NCGS; duodenal villi; ultrastructural morphology.

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In vitro comparison of new bisphosphonic acids and zoledronate effects on human gingival fibroblasts viability, inflammation and matrix turnover

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Bisphosphonates (BPs) are well known clinically used drugs, commonly applied to treat osteoclast-mediated bone resorption. Some clinically used BPs were demonstrated to be able to inhibit the activity of matrix metalloproteinases (MMPs) (1), a protease family required to fully degrade all the components of the extracellular matrix during connective tissue remodelling (2). Combining the arylsulfonamide function with the bisphosphonic group, several compounds were synthesized to obtain selective inhibitors of MMPs. The aim of the present work is to compare the effects on cell adhesion, cytotoxicity, inflammatory response occurrence and matrix turnover process in an in vitro model of primary human gingival fibroblasts (HGFs) treated with newly synthesized sulfonamide BPs and with zoledronic acid (ZA), a clinically used drug. Western blot was used to measure Procollagen I, β1 integrin MMP-8 and MMP-9, phase contrast and MTT for cell viability, LDH was performed for toxicity evaluation, ELISA for Prostaglandin E2 (PGE2) secretion assessment. When compared with ZA, the treatment with the newly synthetized compounds shows increasing viability, Procollagen I expression and decreased expression of β1 integrin in HGFs. Higher levels of released LDH, PGE2 and MMP-9 expression are recorded in ZA-treated HGFs. Increased levels of MMP-8 are recorded in newly synthetized compounds-treated samples.These findings imply that new BPs could accelerate the physiological matrix turnover, they are more able to preserve the soft tissue surrounding bone as they have neither inflammatory effects nor toxicity, along with reduced effects on the cell viability, which are instead typical side effects of ZA administration. We can conclude that the newly synthesized compounds are better tolerated, leading to the hypothesis that their use leads to connective tissues side effects reduction compared to clinically used drugs, even though several studies are required to deeply investigate the signaling cascades involved in the mechanism of action of these new BPs.

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Keywords
Bisphosphonate; human gingival fibroblast; metalloproteinase; zoledronate.

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Doxorubicin anti-tumor mechanisms include Hsp60 post-translational modifications leading to the Hsp60/p53 complex dissociation and instauration of replicative senescence

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Hsp60 is a pro-carcinogenic chaperonin in certain tumor types by interfering with apoptosis and with tumor cell death. In these tumors, it is not known whether or not doxorubicin anti-tumor effects include a blockage of the pro-carcinogenic action of this protein. We used the human lung mucoepidermoid cell line NCI-H292 and different doses of doxorubicin to measure cell viability, cell cycle progression, cell senescence indicators, Hsp60 levels and its post-translational modifications as well as the release of the chaperonin into the extracellular environment. Cell viability was reduced in relation to doxorubicin dose and this was paralleled by the appearance of cell senescence markers. Concomitantly, intracellular Hsp60 levels decreased while its acetylation levels increased. The data suggest that Hsp60 acetylation may interfere with the formation of the Hsp60/p53 complex and/or promote its dissociation, both causing an increase in the levels of free p53, which can then activate cell senescence. On the other hand, acetylated Hsp60 is ubiquitinated and degraded and, thus, the anti-apoptotic effect of Hsp60 is impaired with subsequent tumor cell death.

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Keywords

Doxorubicin; Hsp60; p53; replicative senescence; post-translational modifications.

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Macrophage polarization by the microenvironment of atherosclerotic plaques

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Macrophages are key cellular mediators of innate immunity: they are positionally and transcriptionally programmed to respond to pathogens and environmental challenges. When activated by inflammatory signals in their microenvironment they develop into functionally and phenotypically distinct polarized subpopulations: classically activated macrophages, M1, characterized by cytotoxix/proinflammatory activity; alternatively activated macrophages, M2, characterized by anti-inflammatory/wound repair activity. M1 pro-inflammatory macrophages drive atherosclerotic plaques progression towards instability, cap fragilization and rupture. Our study provide new informations about the role exherted by IL-23 and its receptor in human carotid atherosclerotic plaque progression. We show the presence of IL-23 immunoreactivity, mRNA and protein in macrophages infiltrating human carotid atherosclerotic plaques. Our immunohistochemical analysis demonstrated a strong IL-23 immunoreactivity within the inflammatory infiltrate at the shoulder of the plaques, and at the level of cells lining the fibrous cap. FISH analysis confirmed the expression of IL-23 detected by immunohistochemistry. Immunofluorescence, followed by FISH analysis, showed that cells positive for IL-23 mRNA bind anti-CD68 mAb, thus indicating that these cells belong to the macrophage components of the inflammatory infiltrate. This result was further confirmed by double labelling experiments. IL-23 immunoreactivity was detected within the fibrous layer and co-localized with cells belonging to the monocyte-macrophage lineage as shown by their strong CD68- and CD14-related reaction. Clusters of double-positive cells were found at the border of the plaque, as well as in the subendothelial space. Immunohistochemistry and immunofluorescence showed a strong immunoreactivity for IL-23R at the level of inflammatory mononuclear cells accumulated within the plaque. In vitro, only M1 pro-inflammatory, but not M2 anti-inflammatory macrophages produced IL-23, upon stimulation with zymosan or bacterial lipopolysaccharide. Our results suggests that a hyperactive and highly pathogenic IL-23-IL-23R system drives chronic inflammation in atherosclerosis, while the presence of IL-23 proximal to the fibrous cap may contribute to the atherosclerotic plaque instability.

Keywords

IL-23; macrophages; human carotid atherosclerosis; immunohistochemistry.

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Effect of TNF-alpha and IL-17 on TLR expression and Langerhans cells phenotype in a three-dimensional model of normal human skin: a morphological study

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Toll-like receptors (TLRs) are essential for innate immunity and contribute to create the skin barrier. Their abnormal stimulation is involved in the development of several dermatological diseases, among which psoriasis. Tumor Necrosis Factor (TNF)-alpha and interleukin (IL)-17 play a pivotal role in the pathogenesis of psoriatic plaques and their proinflammatory activity can affect Langerhans cell (LC) phenotype. In a well characterized three-dimensional model of organotypic cultures of normal human skin [1-3] we evaluated the effect of TNF-alpha and IL-17 on the expression of TLR2 and 9 by immunofluorescence, on the ultrastructural morphology of keratinocytes and LCs by transmission electron microscopy (TEM). Human skin explants (n=7) were cultured at the air-liquid interface overnight in a Transwell system and exposed to 50 ng/ml IL-17 or 100 ng/ml TNF-alpha or a combination of both cytokines. Samples were harvested 24 (T24) and 48h (T48) after cytokines incubation. After incubation with IL-17 and IL-17+TNF-alpha, TLR2 immunostaining was not detectable in the basal layer, differently from controls and TNF-alpha-treated samples. Conversely, TLR9 expression was progressively induced in granular keratinocytes in all cytokine-exposed groups. By TEM, enlargements of intercellular spaces were evident especially and, after IL-17 treatment, LCs showed an activated phenotype. At T24 LCs number increased indicating that TNF-alpha and IL-17+TNF-alpha exert a chemoattractant activity, while at T48 only IL-17+TNF-alpha maintained this effect on trapping LCs in epidermis. TNF-alpha and IL-17 differently affect LCs behaviour and TLR expression, with a specific contribution to the inflammatory loop underlying the lesion formation. These results suggest that the simultaneous inhibition of the effect of different cytokines with a defined role in the pathogenesis of psoriasis could improve psoriasis treatment.

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Keywords

Transmission electron microscopy; proinflammatory cytokines.

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Characterization of pancreatic ductal adenocarcinoma cells in a 3D-cell culture model: focus on epithelial-to-mesenchymal transition

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Three-dimensional (3D) cell cultures provides a key to the information encoded in the tissue architecture, therefore mimicking the functions of living tissues [1]. Considered the key role of epithelial-to-mesenchymal transition (EMT) in carcinoma progression [2], we aimed at analyzing the effect of the 3D-arrangement on the expression of some key markers of EMT in pancreatic adenocarcinoma (PDAC) cells cultured in either 2D-monolayers or in 3D-spheroids by morphological and molecular methods. HPAF-II, HPAC, and PL45 cell ultrastructure was analyzed by transmission electron microscopy. The main EMT markers E-cadherin, β-catenin, N-cadherin, collagen type I (COL-I), vimentin, α-smooth muscle actin (αSMA), Snail, Slug, Twist, Zeb1 and Zeb2 were evaluated by confocal microscopy and molecular methods. Moreover, the expression of cytokeratins was characterized in PDAC cells grown in 2D-monolayers and 3D-spheroids to better understand PDAC cell behaviour. We show important differences in the phenotype of PDAC cells grown in 3D-spheroids or in 2D-monolayers, especially providing additional correlative evidence of EMT marker expression in PDAC cells and contributing to a clarification of the role of EMT in PDAC progression. Considered as a whole, our results suggest that a 3D cell culture model could provide deeper insight into the understanding of the biology of PDAC.

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Key words
Epithelial-to-mesenchymal transition; pancreatic adenocarcinoma; spheroids; E-cadherin.

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A novel role of c-FLIP protein in regulation of ER stress response

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Cellular-Flice-like Inhibitory Protein (c-FLIP) is an apoptosis modulator known to inhibit the extrinsic apoptotic pathway thus blocking Caspase-8 processing in the Death Inducing Signalling Complex (DISC). We previously demonstrated that c-FLIP localizes at the Endoplasmic Reticulum (ER) and that c-FLIP-deficient Mouse Embryonic Fibroblasts (MEFs) display an enlarged ER morphology. In the present study, we have addressed the consequences of c-FLIP ablation in the ER stress response by investigating the effects of pharmacologically-induced ER stress in Wild Type (WT) and c-FLIP-/- MEFs. Surprisingly, c-FLIP-/- MEFs were found to be strikingly more resistant than WT MEFs to ER stress-mediated apoptosis. Analysis of Unfolded Protein Response (UPR) pathways revealed that Pancreatic ER Kinase (PERK) and Inositol-Requiring Enzyme 1 (IRE1) branch signalling is compromised in c-FLIP-/- cells when compared with WT cells. We found that c-FLIP modulates the PERK pathway by interfering with the activity of the serine threonine kinase AKT. Indeed, c-FLIP-/- MEFs display higher levels of active AKT than WT MEFs upon ER stress, while treatment with a specific AKT inhibitor of c-FLIP-/- MEFs subjected to ER stress restores the PERK but not the IRE1 pathway. Importantly, the AKT inhibitor or dominant negative AKT transfection sensitizes c-FLIP-/- cells to ER stress-induced cell death while the expression of a constitutively active AKT reduces WT cells sensitivity to ER stress-induced death. Thus, our results demonstrate that c-FLIP modulation of AKT activity is crucial in controlling PERK signalling and sensitivity to ER stress, and highlight c-FLIP as a novel molecular player in PERK and IRE1-mediated ER stress response.

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The correlation between PLC-β2 and miR-146a in breast ductal carcinoma in situ (DCIS) defines its malignant potential

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Ductal carcinoma in situ (DCIS), which represents the most frequently diagnosed tumor in women in industrialized countries, may be a crucial step in the progression of breast lesions to invasive ductal carcinoma (IDC) (1). Among the signaling molecules deregulated in breast tumors, the beta2 isoform of the phosphoinositide-dependent phospholipase C (PLC-b2) strongly correlates with malignancy of invasive tumors and breast tumor-derived cells (2, 3). In breast tumor-derived cell lines cultured under hypoxia, PLC-b2 regulates the levels of cancer stem cell and epithelial-to-mesenchymal transition (EMT) markers (4), suggesting its involvement in breast cancer progression. By using archival FFPE breast tumor samples, we demonstrated that PLC-β2 is up-regulated in DCIS, in which it inversely correlates to the levels of miR-146a, known to act as a tumor suppressor in breast cancer (5). By using the MCF10DCIS cell line, a well-established model of DCIS-derived cells, we demonstrated that the de-regulation of miR-146a is sufficient to modulate the expression of PLC-β2, in turn able to affect the epithelial-to-mesenchymal shift as well as the number of cells expressing CD133. These data indicate that miR-146a and PLC-β2 are members of an intracellular network able to ensure the maintenance of the non-invasive phenotype of DCIS and suggest that alterations in their levels can determine the appearance of an invasive phenotype. The potential prognostic relevance of PLC-β2/miR-146a relationship was investigated in primary DCIS from patients who developed an invasive ductal carcinoma in the contralateral breast. The PLC-β2/miR-146a correlation was found negative in primary DCIS from patients who did not recur and strongly positive in DCIS from patients who developed a contralateral IDC. We propose that the assessment of the correlation between the levels of PLC-β2 and miR-146a in primary DCIS at diagnosis could be beneficial to identify patients with either low or high propensity to develop invasive recurrence. Since a major problem in the management of patients with DCIS is the lack of reliable prognostic markers, our results might be of value in selecting the most appropriate therapies for individual women with non-invasive breast neoplasia.

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Keywords

Ductal carcinoma in situ (DCIS); PLC-β2; miR-146a.

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Diabetes induces changes in salivary gland melatonin reactivity

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The fine localization of melatonin and its receptors in the human salivary glands were reported in our previous works revealing, by transmission electron microscopy (TEM), that serous cells are able to store melatonin and to secrete it by regulated pathways (1, 2). Moreover, changing in morphology during secretion was observed after melatonin treatment by high resolution scanning electron microscopy (3). As in saliva of patients suffering from type 2 diabetes melatonin was reduced, we focused our study on salivary glands removed from diabetic subjects, in order to add diabetic data to our survey on melatonin and salivary glands. Aim of this investigation was to establish if diabetic status may affect subcellular melatonin distribution and traffic. Bioptic samples of parotid and submandibular glands, removed from diabetic patients, were fixed, dehydrated, embedded in Epon Resin and processed to search for melatonin reactivity by the immunogold staining method. The labelling density (expressed as number of gold particles per μm2/granule) and the percentage of melatonin-positive granules were estimated in diabetic samples. The resulting values were compared with those of non-diabetic ones and the differences were statistically evaluated. In diabetic samples the pattern of melatonin staining was unchanged with respect to non-diabetic ones, as the gold particles were specifically localized within secretory granules and vesicles of serous cells. The quantitative evaluation of gold particles showed that the labeling density changed in parotid diabetic samples with respect to those measured in non-diabetics, as the percentage of melatonin positive granules showed a tendency to decrease in the diabetic status in both glands.

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Keywords

Melatonin; salivary gland; diabetes; immunogold method.

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Combination of genes rs2241423, rs12444979 and rs6732220 spreading analysis in patients with hyperplastic processes of the uterus

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The aim of the research was to study part of combination of genes rs2241423, rs12444979 and rs6732220 among Russia Central Chernozem region population. Research group was made of 1873 individuals: 908 women with uterus hyperplastic processes and 965 women of control group. In the sample of sick and control group were included Russian nationality women, which were born in Russia Central Chernozem region and the non-kinship between them. Venous blood in the volume of 6 ml taken from the cubital vein of a proband served as a material of research. Genomic DNA extraction was realized by phenolic-chloroform extraction method. SNP-research was realized by polymerase chain reaction method with associated primers and probes using on amplifier IQ-5.Three molecular genetic markers was genotyped: MAP2K5 (rs2241423), CNVs (rs12444979) и FSHR (rs6732220).A statistically significant difference in the concentrations of the combination of alleles G rs2241423, genotype CC rs12444979 and allele G rs6732220 was established among group of patients with hyperplastic processes of the uterus (28,55%) and control group (33.09%, p=0.01, P=0.05, OR=0.81, 95% CI 0.66-0.98). Combination of genetic markers G rs2241423, CC rs12444979 и G rs6732220 (OR=0.81) reduces the risk of uterus hyperplastic processes development in population of Central Chernozem region of Russia.

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The study of the role of genetical combination rs6729809 and rs10769908 in the formation of hyperplastic processes

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The aim of the research was to study the role of the combinations of the genes rs6729809 and rs10769908 in the formation of hyperplastic processes of uterus among the population of the Central Chernozem region of Russia. The group of the research was made of 1873 individuals: 908 patients with hyperplastic processes of uterus and 965 women of the control group. The sample of patients and controls were included women of Russian nationalities who are the natives of the Central Chernozem region and non-relative to each other. Material for the study is the venous blood in the amount of 6 ml taken from the cubital vein of a proband. The eduction of genomic DNA from peripheral blood was done by the method of phenol-chloroform extraction. Polymorphism study was carried out with the help of the method polymerase chain reaction with using appropriate primers and probes for thermocycler IQ5. Genotyping of the two molecular genetic markers was carried out: LHCGR483 (rs6729809) and STK33 (rs10769908). It was revealed that the combination of alleles C rs6729809 with C rs10769908 are recorded among the patients with hyperplastic processes of uterus (20.81%) was significantly less compared with the control group (27.78%, p=0.03, OR=0.68, 95%CI 0.55-0.85). Thus, it was found that among the women of the Central Chernozem region protective factor in the development of hyperplastic processes of the uterus is the combination of alleles C rs6729809 with C rs10769908 (OR=0.68).

Keywords

Hyperplastic processes of uterus; molecular genetic markers.

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Loss of nuclear BAP1 protein expression is a marker of poor prognosis in patients with clear cell renal cell carcinoma

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BAP1 is a gene situated on chromosome 3p in a region that is deleted in over 90% of Renal Cell Carcinomas (RCCs) (1,2). In the present study we studied BAP1 immunohistochemical expression in a large series of conventional clear cell RCCs (ccRCCs) treated with radical nephrectomy and we assessed the prognostic value of their expression in terms of patients survival at long-term follow-up. 154 consecutive patients with ccRCC were selected from a prospective database and considered for the study purpose; all patients were treated with radical nephrectomy and lymphadenectomy at our Institute of Urology between 1983 and 1985. The features considered in this study were tumor size, grade and stage, vascular and capsular invasion, incidence of metastasis and patient specific survival; all these parameters were correlated with immunohistochemical cytoplasmic and nuclear expression of BPA-1 in tumoral tissue. Median follow-up was 196.18 months (range 5 to 274); median survival was 125.34 months (range 5 to 274 months). We found that nuclear BAP1 expression showed a high frequency of loss in tumoral cells; nuclear BAP1 negative tumors had higher tumor size, higher Fuhrman grade, and higher stage, a greater amount of vascular and capsular invasion and a higher incidence of metastases. We have demonstrated that nuclear BAP1 expression is a marker of prognosis in ccRCC, having an impact on cancer-specific survival. The clinical importance for BAP1 will be realized with the identification and application of targeted therapies and with individualized approaches in the adjuvant and/or metastatic setting.

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Keywords

BAP1 protein; clear cell renal cell carcinoma; immunohistochemistry; cancer-specific survival.

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Role of the secretin/secretin receptor axis in the modulation of the liver fibrosis

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Proliferating cholangiocytes, the cells that line the biliary ducts, secrete and respond to neuroendocrine hormones, including secretin. Secretin stimulates biliary proliferation by downregulation of let-7a and subsequent upregulation of the growth-promoting factor NGF [1]. It is not known if the secretin/secretin receptor (SR) axis plays a role in subepithelial fibrosis observed during cholestasis [2]. Our aim was to determine the role of secretin/SR axis in the development of biliary fibrosis in animal models and human primary sclerosing cholangitis (PSC). Studies were performed in Wild-type (WT) mice with bile duct ligation (BDL), BDL SR-/-mice or Mdr2-/-mouse models of cholestatic liver injury. In selected studies, the SR antagonist (Sec 5-27) was used to block the secretin/SR axis. Biliary proliferation and fibrosis were evaluated as well as the secretion of secretin (by cholangiocytes), the expression of markers of fibrosis, TGF-β1, TGF-β1R, let-7a and downstream expression of NGF. Correlative studies were performed in human control and PSC liver tissue biopsies, serum and bile. SR antagonist reduced biliary proliferation and hepatic fibrosis in BDL WT and Mdr2-/- mice. We found a decreased expression of let-7a in BDL and Mdr2-/-cholangiocytes that was associated with increased NGF expression. Inhibition of let-7a increased liver fibrosis due to cholestasis. Moreover, we showed an increased expression of TGF-β1, TGF-β1R. Significantly higher expression of secretin, SR and TGF-β1 was observed in PSC patient liver samples compared to controls. In addition, there was higher expression of fibrosis genes and an important decreased expression of let-7a with an increased expression of NGF compared to the control. In conclusion, we found that in proliferating cholangiocytes during cholestasis there is an upregulation of the secretin/secretin receptor axis.

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Keywords

Biliary epithelium; secretin; cholestasis.

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Retinoic acid sensitizes acute myeloid leukemia cells to ER stress

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Acute myeloid leukemia (AML) is caused by the blockade of hematopoietic myeloid precursors at different stages of differentiation. A subtype of AML, acute promyelocytic leukemia (APL), is a paradigm of differentiation therapy since retinoic acid (RA) is able to induce leukemic blast terminal differentiation leading to cure rates exceeding 80% when administered in combination with chemotherapy. Although APL patients refractory to RA or who relapsed are very effectively treated with arsenic trioxide (ATO) in combination with RA, the elevated costs limit its use in developing countries and in first line therapy so that RA plus chemotherapy currently remain the standard of care (1, 2). Most importantly non-APL acute myeloid leukemia do not respond to RA indicating the need for novel strategies to sensitize AML cells to RA. Here we show that RA-triggered differentiation of APL cells induces endoplasmic reticulum (ER) stress slightly activating the unfolded protein response (UPR). This is sufficient to render leukemic cell lines and human primary blasts very sensitive to doses of ER stress inducing drugs, like tunicamycin (Tm), that are not toxic for the same cells in the absence of RA or for most cell types. Furthermore we observed that low doses of Tm, even in the absence of RA, are sufficient to strongly increase ATO toxicity. Indeed both RA-sensitive and RA-resistant APL cell lines resulted sensitive to Tm-ATO combined treatment at low doses of ATO that are ineffective in the absence of ER stress. The use of inhibitors targeting specific UPR branches indicate that the Protein Kinase RNA-like Endoplasmic Reticulum kinase (PERK) pathway protects differentiating APL cells from ER stress rendering it an interesting therapeutic molecular target. Finally, we extended our observations in a non-APL model, assessing that RA sensitize the non-APL cell line HL60 to ER stress. Altogether our data indicate ER stress as a possible target for designing novel combination therapeutic strategies in AML.

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Keywords

AML; ER stress; RA; ATO.

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Antitumoral effects of Hibiscus Sabdariffa on human breast cancer cells

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Hibiscus Sabdariffa (HS) is a plant commonly used in folk medicine (1). In recent years HS has gained great interest due to its important antioxidant, anti-inflammatory and antitumoral properties. In our work, we evaluated the in vitro anticancer effects of HS extract against two different human breast cancer cell lines: estrogen receptor (ER) positive MCF-7 cells and ER negative MDA-MB-231 cells. We tested both total extract (HSE) and one fraction obtained by ethyl acetate extraction (HSEC). MTT assay and Trypan Blue vital count showed a dose and time dependent reduction of the viability in both cell lines treated with different concentrations of HSE or HSEC compared to untreated control cells. A significantly marked reduction was observed in MCF-7 cells treated with HSEC. On the basis of our results we used the concentrations of 7.5mg/ml and 3.5mg/ml respectively for HSE and HSEC. In order to evaluate ER involvement in HS effect, we analyzed the cellular localization of the receptor (ERα isotype) by immunofluorescence experiments. Untreated MDA-MB-231 cells showed a low expression of the receptor mostly localized at the cytoplasmic level and treatment with HSE or HSEC didn't change this state. Untreated MCF-7 cells showed a greater expression of the receptor, with nuclear and cytoplasmic localization. Following HSE or HSEC treatment ERα localization became more cytoplasmic and this effect was more evident after HSEC induction. These data were also confirmed by ERα western blot analysis. Subsequently, we studied HSE and HSEC ability to alter migration and invasion capacity of ER positive MCF-7 cells. Using a scratch wound healing assay we did not observe any change in the migration of cells compared to untreated cells. On the contrary, in a Boyden chamber invasion assay, HSE, and especially HSEC, induced reduction of MCF-7 cell invasion. In conclusion, we have demonstrated that HS is able to reduce cell viability of ER positive MCF-7 and ER negative MDA-MB-231 cells. This effect is more evident in MCF-7 cells in which ER localization and reduced cell invasion were observed. These results are more evident after HSEC treatment. Further studies will be needed to better elucidate the involved mechanisms of action.

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Keywords

Hibiscus sabdariffa; human breast cancer cells.

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Correlation between Protein Kinase Cε expression and thrombotic risk in Primary Myelofibrosis (PMFs)

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Myelofibrosis (MF) - either primary (PMF) or arising from a previous PV or ET - is a Philadelphia-negative MPNs characterized by aberrant platelet production and consequent variable platelet count with altered hemostatic function (1). It has already been demonstrated that the risk of thrombotic events is one of the most common co-morbidities associated with PV and ET (2-5). However, risk of thrombotic events in PMF has not been investigated yet. We previously demonstrated that PKCepsilon (PKCε) is over-expressed in platelets from patients with acute myocardial infarction and accounts for their increased reactivity (6). Additionally, we recently showed that PKCε overexpression plays a crucial role in PMF MK impaired differentiation and that its levels correlated with the disease severity (expressed by the IPSS/DIPPS risk category) (7,8). On these bases, we analyzed PKCε expression in platelets from PMF patients, investigating a potential correlation with thrombotic risk and the aggressiveness of the disease. For this study, peripheral blood samples from 6 PMF patients and 3 healthy donors (HD) were collected in Na-citrate tubes. PKCε mRNA and protein levels were determined in platelets purified as described by Carubbi C, 2012. Finally, patients are stratified according to the history of cardio-vascular events and the IPSS/DIPSS risk category. PMF platelets showed significantly higher mRNA levels of PKCε as compared to HD. Protein analysis confirm PKCε over-expression in PMF platelets, almost reaching statistical significance. We then found that platelet from PMF patients who suffered from cardiovascular events display significantly higher levels of PKCε as compared to the one with a negative history. Finally, similarly to what observed in PMF magakaryocytes, we showed a positive correlation between PKCε platelets levels and IPSS/DIPSS risk category, with the lowest levels in low-risk patients and higher levels in high-risk patients. Collectively, our preliminary results indicate that PMF platelets show an aberrant expression of PKCε which correlates with the disease burden and a history of cardiovascular events. This suggests that the over-expression of PKCε may account for PMF platelet altered reactivity and function.

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PKC epsilon involvement in Th17 in vitro differentiation: implications in psoriasis pathogenesis

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Psoriasis is a noncontagious, arytematous-squamose dermatitits affecting both sexes and all races. Although its exact etiology is largely unknown, it is now recognized as one of the most common immune-mediated disorders and several studies demonstrate an impairment of regulatory T-cells (Tregs) function and an up-regulation of IL-17 levels produced by T-helper 17 lymphocytes (Th17)(1,2). Protein kinase C epsilon (PKCε) is a serine/threonine kinase which plays a key role in the proliferation and differentiation of epidermal cells. We have previously demonstrated a role for PKCε in the pathogenesis of the autoimmune disease Hashimoto's thyroiditis (3). PKCε is over-expressed in CD4+ T lymphocytes isolated from PBMC fraction in patients affected by this pathology and its forced down-modulation primed the TGF-mediated in vitro Treg polarization of human T CD4+ cells. Since it has been demonstrated that PKC-signalling is altered in psoriatic keratinocytes (4), we investigated the involvement of PKCε in Th17 in vitro differentiation and its potentially implication in immune response correlated to psoriasis. Using western blot and real time PCR, we have observed that PKCε protein levels and mRNA increase during Th17-lineage in vitro differentiation from naïve CD4+ T cells with a similar trend of Th17 markers of differentiation STAT3 and RoRγT. Moreover, PKCε overexpression significantly increases STAT3 and phosphorylated STAT3 levels, suggesting that PKCε boosts Th17 polarization. Thereafter, we sought to investigate PKCε expression in CD4+ lymphocytes obtained from peripheral blood of psoriatic patients and we observed that PKCε expression levels are significantly higher compared with healthy donors. Intriguingly, we observed a closely correlation of PKCε expression with PASI index, suggesting an involvement of the kinase with the severity of the disease. Collectively these data suggest that PKCε might be involved in Th17 differentiation, that it could be a key factor to regulate Th17 pathological expansion and therefore a potential psoriatic pharmacological target.

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Essential role for acid sphingomyelinase-inhibited autophagy in melanoma response to cisplatin

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In advanced stages, melanoma is still a therapeutic challenge, despite the large number of chemotherapeutic regimens so far developed. Single drug chemotherapy is in many cases ineffective and combinations of chemotherapeutic drugs have demonstrated response rates only marginally higher, and at the cost of systemic toxicity. The new targeted therapies and immunotherapies have shown better efficacy and have supplanted chemotherapy as first-and second-line therapy. However, since melanoma cells eventually become resistant also to these novel therapies, the quest for new, more effective and possibly less toxic approaches is still open. The sphingolipid metabolising enzyme Acid Sphingomyelinase (A-SMase) has been recently shown to inhibit melanoma progression and correlate inversely to tumour grade [1]. We have investigated the role of A-SMase in the chemo-resistance to anticancer treatment using mice with melanoma allografts and melanoma cells differing in terms of expression/activity of A-SMase. Furthermore, as autophagy is a crucial determinant of the melanoma sensitivity to chemotherapeutic drugs, we have also investigated whether an action of A-SMase in autophagy can explain its role [2]. Melanoma sensitivity to chemotherapeutic agent cisplatin in terms of cell viability/apoptosis, tumour growth, and animal survival depended directly on the A-SMase levels in tumoural cells. A-SMase action was due to inhibition of autophagy through activation of Akt/mammalian target of rapamycin (mTOR) pathway. Treatment of melanoma-bearing mice with the autophagy inhibitor chloroquine restored sensitivity to cisplatin of tumours expressing low levels of A-SMase while no additive effects were observed in tumours characterised by sustained A-SMase levels. In conclusion A-SMase, affecting mTOR-regulated autophagy and playing a central role in cisplatin efficacy, is an attractive target in anti-tumour strategy for melanomas and our data encourage preclinical testing of the modulation of A-SMase levels/activity as possible novel antineoplastic strategy.

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Keywords
A-SMase; melanoma; autophagy; mTOR; chemo-resistance.

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Magnesium homeostasis goes awry in chemoresistance -TRPM6, TRPM7 and MagT1 in colon carcinoma LoVo cells

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Chemoresistance is one of the most significant factors impeding the progress of cancer therapy (1). It is known that neoplastic cells accumulate magnesium and frequently upregulate one of its transporters, i.e.TRPM7 (2). We have investigated magnesium homeostasis in a model of chemoresistance i.e. colon carcinoma LoVo cells sensitive (LoVo-S) or resistant to doxorubicin (LoVo-R). We observed that LoVo-R have higher amount of total intracellular magnesium than LoVo-S. We studied the expression of some magnesium transporter (TRPM6, TRPM7 and MagT1) by Real Time PCR and Western Blot and found that TRPM6 and 7 are overexpressed in LoVo-S, while MagT1 is upregulated in LoVo-R. In LoVo-S, silencing TRPM7 retards cell growth and shifts the phenotype to one more similar to resistant cells. On the other hand, calpeptin, a calpain inhibitor, upregulates TRPM7, stimulated proliferation and enhances the sensitivity to doxorubicin of LoVo-R. Silencing MagT1 in LoVo-R markedly inhibited cell growth without affecting the response to doxorubicin. We conclude that alterations of magnesium homeostasis play a role in drug resistance.

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Keywords
Magnesium; drug resistance; colon carcinoma; magnesium transporters.

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Genotoxicity and cytotoxicity of Aloysia polystachia: an in vivo study in rabbits

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In the search of new compounds useful for the treatment of neurological disorders, medicinal plant research has progressed widely in the last decade, particularly because of the hypothesis that natural extracts possess a low or absent toxicity. Aloysia polystachia (Griseb.) Moldenke belonging to the family of Verbenaceae has been used in the traditional medicine for a variety of indications and as a sedative. Recently, some evidence reported that it exhibits some antidepressant- and anxiolytic-like effects involving the modulation of GABA system in preclinical studies in mice (1). However no investigations have been performed on its (geno)toxicity in long-term studies as it would be required for its use in neurological pathologies. In this study, we investigated the genotoxic potential of a water soluble extract of Aloysia polystachia leaves, administered orally every day for 90 days to 20 New Zealand white rabbits homogeneous for weight and age. Subjects were divided into four groups: the control group, and three experimental groups fed with a diet supplemented with 1g/kg; 1.5 g/kg and 2g/kg of Aloysia Polystachia, then peripheral blood was drawn from the vein auricularis marginalis before and after 45 and 90 days of treatment. Cytogenetic analysis was performed on each subject at each time point and chromosome aberrations (structural and numerical) and mitotic index were used as a measure of DNA damage and cytotoxicity (2). Results indicated that Aloysia polystachia extract induces a significant increase in the percentage of aberrant cells as well as in the aberration frequency (mainly chromatid breaks and fragments) associated with a decreasing trend of the mitotic index. The DNA damage was particularly higher after the first 45 days of treatment whereas it remains significantly high but almost unvaried until the end of the treatment. These data suggest that Aloysia polystachia extract has genotoxic and cytotoxic activity, even though further investigations are required to assess which compound of the extract could be responsible for the observed effects.

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Keywords
Aloysia polystachia; genotoxicity; rabbit.

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Ultrastructure of mouse granulosa cells exposed in vitro to the fungicide Mancozeb

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Mancozeb is an ethylene bis-dithiocarbamate widely used as fungicide, also due to a low reported toxicity in mammals. However, reproductive toxicity has been demonstrated in vivo and in vitro in mouse oocytes, by the alteration of spindle morphology [1] and impairment of fertilizability [2]. Mancozeb exerted on mouse GCs cultured in vitro a premalignant-like status, indicated by reduced p53 expression [3] and a mild oxidative stress [4]. However, presence and extent of ultrastructural alterations induced in vitro by Mancozeb on GCs were not yet studied. To this aim, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) were applied on mouse GCs cultured with increasing concentration of Mancozeb. GCs were obtained by puncturing antral follicles of PMSG-treated prepubertal CD1 female mice and cultured in vitro in DMEM+5%FBS+pen/strep without (control) or with increasing concentration of Mancozeb (0.001-to-1 μg/ml) for 48hrs, at 37°C and 5%CO2. At the end of the culture period, cells were washed in PBS, fixed in 2.5% glutaraldehyde/PBS and stored at 4°C until processing. GCs were, then, subjected to standard preparative for TEM [5] and SEM [6] observation. Results showed a dose-dependent toxicity of Mancozeb on mouse GCs. Ultrastructural data showed intercellular communication retraction, irregular nuclear membrane and chromatin marginalization at lower concentrations; chromatin condensation, membrane blebbing and cytoplasmic vacuolization at higher concentrations. In conclusion, Mancozeb showed a dose-dependent harmful effect on granulosa cells in vitro, probably due to the toxic breakdown product ethylenethiourea. TEM and SEM were again confirmed to be a valuable tool to study ultrastructural alterations after toxicants exposure.

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Keywords

Mancozeb; granulosa cells; TEM; SEM; ultrastructure.

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Effect of acute stress on the expression of BDNF in the hippocampus of the Roman rats, a genetic model of stress-induced depression-like behaviour

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The outbred Roman High- (RHA) and Roman Low-Avoidance (RLA) rats were psychogenetically selected for rapid vs. poor acquisition of active avoidance, respectively, and differ in many behavioural traits. Thus, RHA rats are impulsive, novelty seekers, and proactive copers, whereas RLA rats display behavioural traits that resemble some of the cardinal symptoms of depression (1). Beyond the monoamine hypothesis, compelling evidence suggests that mood disorders are characterized by reduced neuronal plasticity. Thus, it has been shown that exposure to stress and antidepressant treatments modulate the expression of neurotrophic factors, and that these changes show an anatomical specificity (2). To characterize the molecular and neuronal systems involved in the pathogenesis of stress-induced depression and in the mechanism of action of antidepressant treatments, we performed western blot (WB) and immunohistochemistry studies to assess the localization of the brain-derived neurotrophic factor (BDNF) in the hippocampus of RHA and RLA rats, both under basal conditions and after exposure to an acute stressor, i.e., the Forced Swim Test (FST). WB analyses showed that, under basal conditions, the relative levels of BDNF were lower in RLA vs. RHA rats, whereas, after FST, the relative levels of BDNF were markedly higher in the hippocampus of RLA vs. RHA rats. In brain tissue sections, BDNF-like immunoreactive material labeled neuronal cell bodies, proximal processes and varicose nerve fibers. Densitometric analysis used to compare immunostained brain sections from the two rat lines showed that, under basal conditions and upon FST, major differences were limited to the hippocampus proper, mostly to the CA3 and CA2 sectors, whereas the dentate gyrus (DG) showed no line-related differences. These results are at variance with previous studies showing that the expression of BDNF in the hippocampus is reduced in depressed patients and in rats exposed to stressors. In conclusion, the present study provides morphological evidence for an unexpected differential regional expression of BDNF in the hippocampus of RLA vs. RHA rats and supports the view that acute stress stimulates neuronal plasticity in genetic animal models of depression.

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Keywords

Depression; BDNF; hippocampus; western blot; immunohistochemistry.

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Cholesteatoma affected incus bone surface shows unusual iron-rich crystals, microvesicles and altered bone turnover

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Cholesteatoma is a noncancerous cystic lesion consisting in an abnormal growth of keratinizing squamous epithelium that invades the middle ear cavity. Due to its capacity of intracranial complications, cholesteatoma is cause of pediatric morbidity and death in countries with scarce hygiene and low possibility to access to advanced medical care (1). In order to understand cholesteatoma etiopathogenesis, we performed a SEM morphological analysis of 11 incus bones affected by cholesteatoma. Samples were fixed immediately upon recovery in 2.5% glutaraldehyde in PBS at 4°C for 48 h, then they were gently sonicated (to remove excess of keratinizing squamous epithelium, that would have prevented surface observation) and finally they were prepared with standard method for scanning electron microscopy observation. Five consecutive fields at 100X magnification aligned in 3 raws, the first one proximal and the last one distal to surgical removal point were analized. Images were obtained in secondary electron mode and in backscattering, bidimensional EDX analysis and mapping was also carried on. Incus bone surface analysis reveals the existence of an environment in which abnormal bone turnover takes place, in fact area of marked erosion were present together with areas of new bone formation. Resorbing bone surfaces with their characteristic lacunae were observed, resting surfaces (smooth and with collagen fibre bundles evident) were found and forming bone surface (collagen bundles in which calcium salts were just deposited) were also observed. Unusual flower-like apatite crystals rich in iron were uncovered in one sample. Iron presence may be due to cholesteatoma itself, being it made up of corneocytes that are iron-rich cells (2). Microvesicles of cellular origin, alone or clustered in groups or in about to fusing together, were found. Macrophages, lymphocytes osteoblast and osteoclast were observed in fully activated stage. The picture of these cell near to each other is the morphological representation of the complex cytochemical dialog existing among them. Taken all together our morphological results let us hypothesize that cholesteatoma creates an environment of chronic infection with peculiar biochemical characteristics that alters normal bone turnover on incus bone.

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Keywords

Cholesteatoma; scanning electron microscopy; microvesicle; crystals.

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Increased MG-63s invasion potential mediated by HFs

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During a malignant transformation, the crosstalk between the stroma and the cancer cells is described as a growing network of physical and paracrine signals, and it seems to have a direct influence on the phenotypic, genetic and epigenetic changes that affect the cells (1). In order to invade and metastasize to distant tissues, cancer cells transform themselves via ECM, induce tumor angiogenesis as well as undergo proliferation, detachment, migration, and invasion through secretion of various tumor derived factors (2). In this study we decided to analyze morphological and molecular aspects due to the coexistence between tumor cells MG-63s and fibroblasts HFs, verifying in particular the ability of MG-63s of invasion and microenvironment modulation. Monolayers of co-cultured cells were morphologically analyzed in time-laps by HR-SEM microscopy and a trans-well migration assay was performed over 24 h, 48 h, 72 h, and 96 h. The expression of several proteins, focusing on those involved in cancer cell invasion, inflammatory responses, and angiogenesis (TNF alpha, IL-6, YKL-40, MMP-1, MMP-9, and VEGF) was validated by Western blotting analysis. The images in time-laps for HR - SEM showed that fibroblasts in contact with MG-63 lost their spatial orientation, while the MG-63 quickly reached the confluence advancing towards HF cells, invading their space and overlying them. The increased MG-63s invasion mediated by the coexistence with HFs was confirmed by invasion assays in transwell co-culture. The protein levels of TNF-alpha, IL-6, YKL-40 and VEGF confirmed that tumor cells can regulate the development of a "tumor-stroma" via the aberrant expression of growth factors in the stromal compartment. Our results showed how tumor-stroma interactions play a significant role in tumor development and progression.

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Keywords

Tumor microenvironment; cell invasion; co-culture.

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Effects of methacrilyc thermosets coated with Silver-polysaccharide nanocomposite on HGFs adhesion in a S. mitis co-culture system

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Silver based medical products have been proven to be effective in retarding and preventing bacterial growth, being silver reported to control infections since ancient times (1). In the field of dentistry, the use of silver ions/nanoparticles has been explored to counteract bacteria in resins and implants, as silver can destroy bacterial cell walls by reacting with the thiol groups (–SH) of proteins exposed to the extracellular portion of the bacterial membrane. Conversely, eukaryotic cells lack these exterior binding sites, so nanoparticles are supposed to interact with them only upon metal internalization (2). To reduce both bacterial adhesion to dental devices and cytotoxicity against eukaryotic cells, we coated BisGMA/TEGDMA methacrylic thermosets with a new material, Chitlac-nAg, formed by stabilized silver nanoparticles with a polyelectrolyte solution containing Chitlac. Here we analyzed the proliferative and adhesive ability of human gingival fibroblasts (HGFs) on BisGMA/TEGDMA thermosets uncoated and coated with AgNPs in a co-culture model system with Streptococcus mitis. After 48 h, HGFs well adhered onto both surfaces, while S. mitis cytotoxic response was higher in the presence of AgNPs coated thermosets. After 24 h thermosets coated with Chitlac as well as those coated with Chitlac-nAg exerted a minimal cytotoxic effect on HGFs, while after 48 h LDH release rised up to 20%. Moreover, the presence of S. mitis reduced this release mainly when HGFs adhered to Chitlac-nAg coated thermosets. The reduced secretion of collagen type I was significant in the presence of both surfaces even more when saliva is added. Integrin β1 localized closely to cell membranes onto Chitlac-nAg thermosets and PKC α translocated into nuclei. These data confirm that Chitlac-nAg thermosets have a promising utilization in the field of restorative dentistry exerting their antimicrobial activity due to AgNPs without cytotoxicity for eukaryotic cells.

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Keywords

Human gingival fibroblasts; co-culture; Chitlac-nAg.

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Novel mechanisms of neuroprotective effects of Quercetin on human striatal neuroblasts

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Human striatal precursor (HSP) primary cell cultures were isolated from ganglionic eminence of 9-12 week old human fetuses and extensively characterized in vitro (1). Our studies demonstrated that these cultures consists of a mixed population of neural stem cells, neuronal-restricted progenitors and striatal neurons that express and are responsive to many trophic factors, as BDNF and FGF2, and possess an adaptive response to stress conditions as nutrient deprivation and hypoxia through mechanisms involving different factors and neurotrophins (1,2). In the last decades, several in vitro and in vivo studies have provided evidence for neuroprotective effects by Quercetin, a polyphenol widely present in nature, passively absorbed in the small intestine and able to traverse the blood brain barrier (3). However, the mechanisms through which Quercetin exerts its neuroprotective effects are not fully delucidated. Our study was aimed at investigating the effects of Quercetin on HSP cells and its contribution to cell survival in nutrient deprivation condition, obtained replacing culture medium with Phosphate Buffer Saline (PBS). Quercetin treatment significantly promoted cell survival and strongly decreased apoptosis induced by nutrient deprivation condition, as evaluated by MTT assay, Trypan Blue staining and western blot analysis of cell death and proliferation markers. Moreover, since the adhesive capacities of cells are essential for cell survival, we next analysed the expression of some adhesion molecules such as Pan-cadherin and Focal Adhesion Kinase; our results interestingly showed that PBS exposure determined a strong decrease in all the analysed adhesion molecules, while in presence of Quercetin the expression was significantly increased. Our results add new mechanicistic insights into the comprehension of neuroprotective action of Quercetin treatment, thus suggesting possible implications in sustaining striatal neuron survival during neurodegenerative disorders, such as Huntington Disease.

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Keywords

Quercetin; human striatal neuroblasts; neuroprotection.

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Sulforaphane prevents oxidative stress and cell death in rat cardiomyocytes

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Cardiovascular diseases (CVDs) are the major cause of death in developed countries. Oxidative stress plays a major role in the pathophysiology of cardiac disorders. Several studies have highlighted the cardinal role played by the overproduction of reactive oxygen species in the pathogenesis of ischemic myocardial damage and consequent cardiac dysfunction. Sulforaphane (SF) is a molecule within the isothiocyanate (ITC) group of organosulfur compound commonly found in cruciferous vegetables. It is reported to be capable of stimulating cellular antioxidant defenses and inducing phase 2 detoxifying enzymes, which can protect cells against oxidative damage. Although SF is known for its anticancer benefits, its role in cardioprotection is emerging. The aim of this study was to investigate the effects of SF in preventing cell damage induced by oxidative stress. Primary rat cardiomyocytes were exposed to different concentrations of SF for 24 h and subsequently treated with H2O2 to induce oxidative stress. Cell viability, and the expression of oxidative stress markers were studied. A transmission electron microscopy (TEM) analysis was carried out to evaluate the effect of SF on cell morphology. Results showed an higher cell viability and a lower level of oxidative stress in cells pre-treated with SF before peroxide exposure in respect to H2O2 treated cardiomyocytes. TEM analysis showed a well preserved morphology in cells pre-treated with SF before H2O2. These findings demonstrate that sulforaphane prevents H2O2 - induced oxidative stress and cell death in rat cardiomyocytes, suggesting a potential protective role of SF in CVDs

Keywords

Cardiomyocytes; sulforaphane; oxidative stress; cardiovascular diseases.

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Role of melatonin in HT22 cells challenged with serum deprivation

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In vitro serum deprivation (SD) is one model for investigating the molecular mechanisms underlying apoptosis as well as autophagy, which generally function as defense strategies upon cell injury by eliminating damaged organelles [1]. Furthermore, SD injury in vitro is widely used to mimic the ischemic environment [2]. In serum deprived conditions, cells show different parameters of apoptosis and autophagy. Melatonin (MLT), a lipophilic indole secreted by pineal and non-pineal cells, is a well-known potent free radical scavenger acting as neuroprotective molecule that prevents apoptotic cell death in several models of neurodegenerative diseases. In the present study we investigated the neuroprotective effects of MLT during SD condition on mouse hippocampal HT22 cells, considering that intracellular ROS are usually linked to autophagy and apoptosis. To explore potential effects of combining SD with melatonin we studied clonogenic survival of HT22 cells. Clonogenic assay demonstrated a significative ($p< 0.01$) reduction of HT22 total cell numbers challenged for 24h with SD, whereas the pre-treatment with 200nM of MLT for 24hr noticeably reduced this effect of about 30%. In HT22 starved cells the percentage of MitoTracker Red (MTR) positive cells doubled ($P< 0.05$) if compared to the control condition, suggesting that SD induced a remodelling of mitochondrial network. It is noteworthy that MLT pre-treatment produced a MTR positivity similar to that of controls. We next investigated whether melatonin was able to influence the autophagic pathway. Autophagy was detected by measuring the aggregation of LC3B protein coupled to green fluorescence protein (GFP). Confocal images show that SD induced an increase in the GFP-LC3 puncta, whereas the melatonin treatment reduces these aggregations. Taken together, our results suggest that MLT treatment may play protective roles against cellular modifications induced by SD treatment in HT22 cells.

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Keywords

Melatonin; HT22; serum deprivation.

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Biliary tree stem cells are involved in the pathogenesis of primary sclerosing cholangitis

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Biliary tree stem cells (BTSCs) are multipotent stem cells located in peribiliary glands (PBGs) of extrahepatic and large intrahepatic bile ducts (1). Primary sclerosing cholangitis (PSC) is characterised by fibro-stenosing strictures involving extrahepatic and/or large intrahepatic bile ducts. Mechanisms leading to bile duct injury are poorly understood (2). Our aims are to study the role of BTSC in the pathogenesis of biliary fibrosis in PSC. Specimens containing extrahepatic or large intrahepatic bile ducts were obtained from normal liver (n=6), liver explants from patients with PSC (n=11), and primary biliary cirrhosis (n=6). Specimens were processed for histology, immunohistochemistry and immunofluorescence. In PSC samples, progressive hyperplasia and mucinous metaplasia of PBGs were observed in large ducts with fibrosis, but not in inflamed ducts without fibrosis. PBG hyperplasia was associated with progressive biliary fibrosis and the occurrence of dysplastic lesions. Hyperplasia of PBGs was determined by the expansion of biliary tree stem cells, which sprouted towards the surface epithelium. In PSC, PBGs and myofibroblasts displayed enhanced expression of Hedgehog pathway components. PBGs in ducts with onion skin-like fibrosis expressed epithelial-to-mesenchymal transition traits associated with components of Hedgehog pathway, markers of senescence and autophagy. The biliary tree stem cell compartment is activated in PSC, its activation contributes to biliary fibrosis, and is sustained by the Hedgehog pathway. Our findings suggest a key role for peribiliary glands in the progression of bile duct lesions in PSC and could explain the associated high risk of cholangiocarcinoma.

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Keywords
Stem cells; biliary tree; hedgehog; liver cirrhosis.

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Study of the effects of different biomaterials on osteogenic differentiation of oral-periosteal cells

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Bone regeneration is currently one of the most important challenges for regenerative medicine and it is considered an ideal clinical strategy in the maxillo-facial area [1]. Bone resorption of alveolar crest occurring after tooth extraction leads to several risks for future treatments, including dental implants. For this reason, alveolar ridge preservation (ARP) has become a key component of contemporary clinical dentistry. Several clinical techniques and bone substitute materials can be used to fill the socket after tooth extraction. For all of them, the principle aim is to keep the shape and the size of the bone socket of the extracted tooth allowing inserting the dental implants [2]. The goal of our study was to compare different biocompatible scaffolds based on PLGA (Fisiograft®), Bioglass (Activioss®) and collagen (Sombrero®) in an in vitro model of tissue engineering for dental applications. The cells used in our study derived from Periosteum obtained from four different patients that underwent socket preservation selected by the School of Dentistry of the University of Pavia, previous informed consent. We created bio-complexes constituted by mesenchymal-periosteal cells seeded on different types of biomaterials and we performed adhesion, morphological, proliferative and bone differentiation analyses at different time points (7, 14 and 28 days of culture) in proliferative and osteogenic conditions. Bone differentiation was evaluated by qRT-PCR on genes involved in osteoblast development, like BMP-2, Osteocalcin and Periostin. Our results demonstrated that Sombrero® enhanced adhesion and proliferation of periosteal cells, as highlighted by Haematoxylin-Eosin staining and XTT test (3 and 7 days). Long-term studies (14 and 28 days) demonstrated that periosteal differentiation is about the same among the different materials tested. From these preliminary studies we can conclude that it could be advantageous the clinical use of both collagenic and PLGA scaffolds in order to ameliorate initial colonization and subsequent mechanical support in maxillo-bone regeneration.

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Keywords

Bone regeneration; socket preservation; PLGA scaffold; collagen scaffold; mechanical support.

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Alternative source of stem cells derived from human periodontal ligament: a new treatment for experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is categorized as an autoimmune disease and is potentially one of the most common causes of neurological disability in young adults. Formation of the sclerotic plaques of which the disease gets its name represents the end stage of a process involving inflammation, demyelination and remyelination, oligodendrocytes depletion, and astrogliosis as well as neuronal and axonal degeneration (1). MS damages the central nervous system and leads to a disabling condition. Recently, the potential role of mesenchymal stem cells (MSCs), derived in promoting tissue repair and disease control has been investigated by using an experimental autoimmune encephalomyelitis (EAE) model (2). The objective of the research was to investigate the product effects by mesenchymal stem cells derived from human periodontal ligament (hPDLSCs) when administered in an experimental model of autoimmune encephalomyelitis (EAE). EAE was induced by immunization with myelin oligodendroglial glycoprotein peptide (MOG)35-55 in C57BL/6 mice. Then, mice were observed every 48 hours for signs of EAE and weight loss. At the onset of disease, approximately 14 days after immunization, EAE mice were subjected to a single intravenous injection of hPDLSCs (10(6) cells/150 μl) into the tail vein. At the point of animal sacrifice on day 56 after EAE induction, spinal cord and brain tissues were collected in order to perform histological evaluation, immunohistochemistry and western blotting analysis. Obtained results reveal that treatment with hPDLSCs may produce neuroprotective effects against EAE, diminishing both clinical signs and histological score typical of the disease (lymphocytic infiltration and demyelination) probably through the production of neurotrophic factors (results focused on brain-derived neurotrophic factor and nerve growth factor expression). Furthermore, administration of hPDLSCs modulates expression of inflammatory key markers (tumor necrosis factor-α, interleukin (IL)-1β, IL-10, glial fibrillary acidic protein, Nrf2 and Foxp3), the release of CD4 and CD8α T cells, and the triggering of apoptotic death pathway (data shown for cleaved caspase 3, p53 and p21). In light of the achieved results, transplantation of hPDLSCs may represent a putative novel and helpful tool for multiple sclerosis treatment. These cells could have considerable implication for future therapies for multiple sclerosis and this study may represent the starting point for further investigations.

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Keywords

Stem cells derived from human periodontal ligament; multiple sclerosis; neurotrophic factors; apoptosis.

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An intriguing relation between periodontal and cardiovascular diseases

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Periodontitis is a chronic inflammatory condition promoted by bacterial colonization of the gingiva that causes alveolar bone and tooth loss. Under these conditions, bacteria, as well as bacterial products and inflammatory mediators can move from the gingival pocket to the well vascularised periodontal tissues and into the circulation. In fact, recently a possible connection between oral infection and cardiovascular disease was suggested (1). One of the major pathogens involved in the progression of periodontal disease is the Porphyromonas gingivalis (LPS-G). The aim of the study was to induce endothelial differentiation in human periodontal ligaments stem cells (hPDLSCs) (2) on decellularized pig heart valve as scaffold and evaluate the role of LPS-G on cell cultures in terms of reactive oxygen species (ROS) production and NFKB pathway. Many studies have shown that ROS provoked oxidative stress plays a critical role in the development of cardiovascular disease. Excessive generation of ROS can cause cellular dysfunction and injury by directly oxidizing and damaging proteins, DNA and lipids, which ultimately result in cell death. To induce endothelial differentiation, human periodontal ligament stem cells (hPDLSCs) were cultured with endothelial growth medium (EGM-2MV) supplemented with vascular endothelial growth factor (VEGF) and seeded on decellularized pig heart valve. Valve leaflets were incubated for 30 min with 10 μM DCFH-DA at 37°C in humidified incubator. At the end of loading, the valve leaflets were observed using multiphoton microscope. Results of time lapse experiments revealed that after treatment, with 2,5 mg/ml LPS-G, a rapid sustained increase in ROS generation was observed mainly in differentiated hPDLSCs. Comparing the average response of undifferentiated and differentiated hPDLSCs is clearly evident that the latter showed an six fold increase in ROS production after LPS-G exposure, while on undifferentiated appeared to be negligible. Moreover, in endothelial differentiated cells the NFKB nuclear translocation in presence of LPS-G was evident. A reasonable conclusion could be that the treatment of periodontal disease not only improve dental but presumably also cardiovascular health.

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Keywords

Stem cells; periodontitis; cardiovascular disease; endothelial differentiation.

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Absence of RAGE in an animal experimental model of Duchenne muscular dystrophy results in reduced muscle necrosis and inflammation

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Duchenne muscular dystrophy (DMD) is a lethal X-linked neuromuscular disorder characterized by progressive muscle degeneration due to lack of dystrophin, a protein essential for the integrity of sarcolemma during contraction. Chronic inflammation is a hallmark of muscles in DMD subjects, and contributes to progressive muscle wasting. RAGE (receptor for advanced glycation end-products) is a multiligand receptor of the immunoglobulin superfamily involved in physiological and pathological processes including inflammation and myogenesis [1]. While absent in healthy adult muscle tissue, RAGE is expressed in regenerating myofibers during muscle regeneration [2,3], in dystrophic muscles and activated immune cells. To have information about the role of RAGE in the pathophysiology of DMD we generated a double mutant mouse lacking dystrophin and RAGE (mdx/Ager–/– mouse) by cross-breeding dystrophic (mdx) mice with RAGE-null (Ager-/-) mice. Comparison of Quadriceps femoris of mdx and mdx/Ager–/– mice at different ages (i.e., 2, 3, 4 and 5 weeks, and 6 and 12 months of age) showed that the absence of RAGE in dystrophic mice did not affect the onset of the pathology. However, compared with age-matched mdx mice, muscles of 5 week- and 6 and 12 month-old mdx/Ager–/– mice showed i) significantly reduced numbers of necrotic myofibers, ii) a shift towards higher values of the cross-sectional areas (CSA) of myofibers, which was also evident in regenerating (centrally-nucleated) myofibers, and iii) reduced areas of immune cell infiltrate. The expression of MAC3, a marker of activated macrophages, was strongly reduced in muscles of mdx/Ager–/– mice compared with mdx mice. Moreover, muscles of mdx/Ager–/– mice exhibited significantly reduced PAX7+ve and myogenin+ve cell numbers, suggesting a reduced recruitment of muscle precursor cells and more efficient regeneration in dystrophic mice lacking RAGE. Our results suggest that RAGE may sustain inflammatory and degenerative processes in dystrophic muscles, and the inhibition of its expression/activity might represent a potential therapeutic approach in DMD patients.

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Keywords

Duchenne muscular dystrophy; muscle inflammation; RAGE; mdx mice; Ager–/– mice.

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S100B protein regulates myoblast and macrophage functions in skeletal muscle regeneration

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Regeneration of acutely injured skeletal muscles relies on a tightly controlled chain of cellular and molecular events, but a complete picture of factors concurring to the regeneration process is still missing. Extracellular S100B protein inhibits myoblast differentiation and stimulates myoblast proliferation by activating its canonical receptor, RAGE (receptor for advanced glycation endproducts), or bFGF/FGFR1 depending on myoblast density (1-4). S100B is released by damaged muscle tissue early after injury in advance of bFGF release, with declining release thereafter (4). We show that S100B is required for correct timing of skeletal muscle regeneration after acute injury. S100B expands the myoblast population, attracts macrophages to damage sites, promotes macrophage polarization into M2 (pro-regenerative) phenotype and reduces fibroblast proliferation. Also, S100B is transiently induced in and released by infiltrating macrophages under the action of proinflammatory and antiinflammatory cytokines, and effects of macrophage-derived S100B sum up with those of myofiber-released S100B. S100B's effects are mediated by RAGE during the first 3 days after injury, however during the myoblast proliferation phase/macrophage M2 phase (i.e. at days 4-6 post-injury) S100B also activates bFGF-FGFR1 to stimulate myoblast proliferation and macrophage M1/M2 transition. Thus, S100B is a major molecular determinant of timed muscle regeneration after acute injury by virtue of its regulatory effects on myoblasts and macrophages.

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Keywords

Muscle regeneration; S100B; RAGE; bFGF/FGFR1; myoblasts; macrophages.

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Effect of hypoxia-inducing ions on chondrogenic differentiation in adipose derived mesenchymal stem cells within alginate matrix

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Cartilage is a highly organized tissue with complex biomechanical properties, but since it has a poor intrinsic capacity of self-healing, injuries at this site usually lead to several problems, often ending in disabling symptoms. Although, different approaches have been proposed, even now cartilage repair represents a great challenge for orthopaedic surgeons (1, 2). One of the promising approach is given from tissue engineering, employing the combination of biomaterials and cell therapy to develop new therapeutic strategies. In this paper, we describe the behaviour of human adipose derived mesenchymal stem cells encapsulated into Ca/Co alginate beads as potential chondrogenic inducing biomaterial tacking advance on the synergy between alginate matrix and Co+2 ions without employing other expensive growth factors such as TGFbs or BMPs. The expression of chondrogenic markers such as sox9, collagen type II, and versican was investigated by Real Time PCR and Western blotting analysis. The expression of hif1mRNA was investigated to check the capability of Co+2 ions to induce a chemical hypoxia. Real Time PCR and WB data reveal a different cells behaviour on chondrogenic marker expression. In conclusion, the synergic effect of alginate and Co+2 ions can represent a valid strategy for chondrogenic differentiation of stem cells.

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Keywords
Stem cells; chondrogenic differentiation; alginate.

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hAFSC expressing a specific panel of stem cell markers give rise to fully differentiate cardiomyocytes

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Human amniotic fluid-derived stem cells (hAFSC) represent a novel class of multipotent stem cells sharing characteristics of both embryonic and adult stem cells. In fact, hAFSC proliferate rapidly, are able to differentiate into cells of all the three embryonic germ layers, but do not form teratoma. It has been already reported that hAFSC have a cardiac potential, but a high variability between hAFSC donors in differentiation efficiency has been described. Aim of this study was to phenotypically identify the hAFSC able to differentiate into mature cardiomyocytes. hAFSCs from 10 different donors were characterized for the immunophenotypic expression of stemness markers and then cultured in differentiatve conditions. hAFSC differed for both stemness markers expression and for differentiation efficiency. Only the hAFSC expressing specific stem cell antigens were able to differentiate into a homogeneous population of cells that highly express cardiac cytoskeletal proteins and the structural and functional sarcoplasmatic reticulum proteins. Our results demonstrate that only hAFSC showing a specific stem cell pattern phenotype can fully differentiate into myocytes giving rise to a homogenous population characterized by cardiac-specific molecular, structural, and functional properties.

Keywords

Human amniotic fluid-derived stem cells; stem cell pattern phenotyper; cardiac differentiation.

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Oxidative stress-induced S100B accumulation in myoblasts converts myoblasts into brown preadipocytes via an NF-κB/YY1/MIR-133 axis and NF-κB/YY1/BMP7 axis

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Muscles of sarcopenic people show hypotrophic myofibers and infiltration with adipose and, at later stages, fibrotic tissue. The origin of infiltrating adipocytes resides in fibro-adipogenic precursors, nonmyogenic mesenchymal progenitor cells, and satellite cells, the adult stem cells of skeletal muscles. Myoblasts and brown adipocytes share a common Myf5+ progenitor cell, and cell fate decision depends on levels of BMP7, a TGF-β family member; high BMP7 levels cause Myf5+ progenitor cells to differentiate in brown adipocytes. When expressed at relatively high levels as observed in myoblasts from sarcopenic humans, intracellular S100B, a Ca2+-binding protein of the EF-hand type (1), exerts anti-myogenic effects that are reversed by S100B knockdown (2,3). We show that ROS-activated NF-κB induces accumulation of S100B that causes myoblasts to convert into brown preadipocytes via 1) an NF-κB/YY1 axis that negatively regulates the promyogenic and anti-brown adipogenic miR-133 with consequent accumulation of the pro-brown adipogenic transcription factor, PRDM16, and 2) an NF-κB/YY1/BMP7 axis with resultant BMP7 autocrine activity. Also, culturing L6C8 (S100b-overexpressing) myoblasts (2) in adipocyte differentiation medium causes NF-κB-dependent upregulation of S100B expression, which precedes and is required for lipid droplet formation. Lastly, S100B knockdown in myoblast-derived brown adipocytes reconvert them into fusion competent myoblasts. Thus, S100B is a major molecular determinant of cell fate decision of proliferating myoblasts; while modulating myoblast differentiation (2,3), at high levels S100B promotes myoblast-brown adipocyte transition, which might have pathophysiological implications in sarcopenia.

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Keywords

Myoblast; brown adipocyte; chronic oxidative conditions; S100B; sarcopenia.

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Bone regeneration strategies in the elderly: the role of ageing and replicative senescence in periosteal-derived stem cells

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Periosteum contains resident progenitor cells (PDPCs) representing an attractive alternative source of mesenchymal stem cells (MSCs) for skeletal tissue engineering approaches based on cell recruitment (1). Increased in life expectancy point out the necessity for customized strategies to restore bone loss due to trauma and/or disease in elderly. Aim of the present research was the evaluation of the ageing impact on PDPCs isolated from differently aged subjects. Moreover, since long-term culture could lead MSCs to senescence, the effects of culture expansion method on young PDPC through sequential serial passages were examined. Age-related increase of p53 expression and impairment in proliferating capacity were observed; those findings were strictly related to nitric oxide (NO) release. Moreover, qRT-PCR analysis showed a greater expression of genes involved in bone remodelling in elderly donors. As far as replicative in vitro expansion was concerned, we observed that later PDPC passages exhibited the typical "replicative senescence" features (i.e. flattened and enlarged morphology, prolonged population doubling time and increased SA-βgal activity). In these cells, p16 rather than p53 seemed to be involved in senescence processes. Similarly to the elderly, the decrease in proliferating ability of in vitro senescent PDPCs was concomitant with a higher NO production, and the changes in the expression of genes involved in bone resorption and RANKL/OPG ratio were superimposable. Interestingly, the relationship between NO release and ageing could represent a cutting edge "replicative senescence index" as emerged by our System Biology approach. In conclusion, our findings suggest that in vivo cell ageing and in vitro subculturing must be taken into account when testing regenerative tissue strategies that use progenitor cells. Indeed, cells (e.g. MSCs and PDPCs) from the earliest subculture passages could be useful to validate any bone tissue engineering strategies, whilst the later ones could be used to test in vitro scaffolds for regenerative medicine approaches in elderly.

This work was supported by grants from MIUR (Project PRIN 2010, MIND-2010J8RYS7).

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Keywords

Periosteal cells; replicative senescence; aging; NO; tissue engineering.

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The inflamed microenvironment: role on MSCs immunobiology and cancer

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Inflammation and cancer are an inseparable binomial. The majority of cancers are triggered by somatic mutations and environmental factors with a common element: inflammation. Inflammation creates a microenvironment in which neoplastic cells can profit from the trophic factors secreted by inflammatory cells, useful to interfere with the anti-tumor response. Among the others, mesenchymal stem cells (MSCs) participate to microenvironment creation by a strong paracrine effect. The linkage between MSCs and inflammation is bidirectional: the inflamed microenvironment affects the complex MSCs immunobiology, but also MSCs can sustain inflammation. Here, we tried to clarify the influence of inflammation on the immunobiology of MSCs and deepen the paracrine effect of MSCs on tumor growth. MSCs were isolated from periprosthetic capsule caused by breast implant, affected by inflammation (I-MSCs). The contralateral part of the same patient, not inflamed, was used as control (C-MSCs). A panel of selected cytokines were analyzed by Real-Time PCR and ELISA. The cytokines expression was different in I-MSCs compared to C-MSCs, revealing that inflammation affects MSCs immunobiology. Then, C- and I-MSCs were indirectly co-cultured with MCF7 cells from breast adenocarcinoma. New analyses on proliferation rate and cytokines expression were performed. C- and I-MSCs gave almost the same results. The over-secretion of all the cytokines referred to the Th1 pathway and the decrease of those belonging to the Th2 pathway revealed the absence of a switch from Th1 to Th2 important to induce a chronic inflammation. The levels of TGF-β and G-CSF linked to the skill to damage the antigen-presenting cell function were decreased. In conclusion, even if MCF-7 proliferation increased after co-culture with I-MSCs, MSCs-derived paracrine effect does not sustain breast adenocarcinoma. These results absolve the breast implants from the insult to enhance adenocarcinoma onset.

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Keywords

Inflammation; mesenchymal stem cells; cancer; immunobiology.

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Preliminary observations on scleral ossicles in performing functionalized 3D vascularized scaffolds for "critical-size" bone defect healing

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The problem of "critical-size" bone defects occurs when a severe lesion is difficult to be self-recovered. Many strategies of regenerative medicine were used in the last decade, with translational approaches, to mimic both structure and function of the native bone tissue, making use of synthetic materials, nanotechnologies, bio/synthetic constructs or some of their combination. The main obstacle to engineering strategies is mostly due to the lack of a proper vascularization of the construct used. In this feasibility study, our attention is directed towards the main tissue engineering items: scaffolds, cells and conditioning factors. We propose the use of scleral ossicles of lower vertebrates (1), as natural scaffolds which will be functionalized to allow the best adhesion of endothelial cells along a geometrically controlled pattern on the bony surface of the construct; successively, on the functionalized scaffold, osteogenic cell lines will be cultured. In the preliminary phases of the study, the ossicles were scratched to remove soft tissue residues, variously flattened with different methods to reach a regular morphology on both sides, and finally autoclaved to eliminate cellular remnants and to annul antigenic properties. Ossicles were observed under SEM and subjected to micro-assay, to establish the best scaffold preparation and to characterize morphological properties more suitable for engineering phases. Functionalization will be made by immobilizing on the engineered ossicles specific growth factors for endothelial cells; later, mouse primary lung endothelial cells (ECs) and immortalized osteogenic cells (IDG-SW3) will be used. As expected results ECs should adhere to the ossicle surface and organize to form lumenized microvascular-like structures; later, supported by the vascular-like network, the osteogenic lineage should produce bone matrix on the construct. The production of newly-formed bone around vascular-like buds will be verify. 3D tissue constructs generated in vitro will be used in successive in vivo study for the healing of "critical-size" bone defects experimentally induced in mice.

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Keywords

Scleral ossicles; primary endothelial cells; immortalized osteogenic cells; bone scaffold.

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The amniotic membrane from the human placenta contains different subregions with different morpho-functional features

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Human Amniotic Membrane (AM) has been attributed anti-inflammatory, anti-angiogenic, anti-fibrotic and anti-microbial effects (1-2). A number of clinical studies have used AM for applications in regenerative medicine, but it has yet to be elucidated whether the different areas of the AM have the same plasticity and differentiation potential. Thus, the aim of our study was to map the AM from normal human placenta. AMs were obtained from 24 healthy women undergoing caesarean section. Four areas were considered with respect to the umbilical cord: the first area, closer to the umbilical cord, was named the central area; the second, in the middle, was the intermediate area; the third was the peripheral area (all the three areas were part of the chorion frondosum) and the fourth, the reflected area, corresponded to the chorion laeve. Our results demonstrate the presence of a multi-layered epithelium in different areas of AM, except for the intermediate area, with a number of budding or detaching cells as well as of apoptotic cells, especially in the central area. By means of immune-histochemistry and quantitative analysis with MetaMorph analysis software, we evaluated the in situ expression of different proteins observing that the peripheral area has the highest OCT-4, c-KIT and SOX-2 expression, well known indicators of pluripotency, and the highest levels of CREB and p-CREB, well known for its key role in proliferation, differentiation and apoptosis, whereas mainly the central area expresses high levels of α-fetoprotein, suggesting that it could be more prone to hepatic differentiation. Interestingly, amniotic epithelial cells (AECs) from the peripheral area display the highest quantity of granules in the cytoplasm, as shown in electron microscopy preparations. In addition, in vitro cultured AECs derived from the reflected area demonstrate the highest clonogenic capacity, whereas those from the intermediate area show the highest osteogenic potential, when induced to osteogenic differentiation. Our study is the first to propose the morpho-functional mapping of the AM as a useful tool to identify areas with different stemness properties and plasticity. This could increase the efficiency of human AM application within a therapeutic context.

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Keywords

Human term placenta; amniotic membrane; amniotic epithelial cells; placenta stem cells.

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Identification of telocytes in the lamina propria of pterygium: an immunohistochemical and transmission electron microscopy study

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Telocytes (TCs) are a novel type of interstitial cells already described in many tissues and organs (1). The name of these cells derives from their typical thin, long processes called telopodes (Tps). Since previous study provided evidences for TCs involvement in neoangiogenesis (2), our aim was to examine if TCs may be present also in pterygium, a common degenerative and hyperplastic disorder of bulbar conjunctival, characterized by an intense process of neovascularization. We performed a morphological and immunohistochemical analysis by light microscopy of thin and semithin sections and an ultrastructural study by transmission electron microscopy (TEM). Our results showed cells resembling TCs, most with very thin, long and irregular processes and typical dichotomic branching pattern. These processes were moniliform because of the alternation of thin segments and small dilatations accommodating caveolae. TCs and TPs appear in close spatial relationship with blood vessels, especially with neoangiogenetic elements. The immunohistochemical analysis, by using the specific markers for telocytes, showed a strong immunoreactivity for both cell body and telopodes in the lamina propria, frequently close to the vessels. This study confirms the presence of telocytes in the connectival stroma of pterygium and their close relationship to the newly formed vessels, but further investigations are required to clarify the role of these cells in pterygium angiogenesis.

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Keywords

Telocytes; pterygium; angiogenesis.

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Positional memory of fibroblasts may affect efficiency of iPSC reprogramming

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Induced Pluripotent Stem cells (iPSC) are pluripotent stem cells reprogrammed from adult somatic cells. Although iPSC hold great potential for applications in regenerative medicine, technical problems, mostly related to the low efficiency of reprogramming, are yet to be solved. Since the most used cells for iPSC reprogramming are skin fibroblasts (FB), and since FB preserve positional memory, we hypothesize that the anatomic origin of FB might influence iPSC reprogramming.

We isolated FB from skin of five different sites (neck, arm, thigh, breast, abdomen) of 13 patients undergoing plastic surgery or from heart wall or ascending aorta wall of the explanted heart of 3 patients receiving heart transplantation. FB from different anatomic sites and control FB from neonatal foreskin, were cultured for one week to evaluate morphology, proliferation rate and proneness to apoptosis. Additionally, expression of vimentin, cadherin, smooth muscle actin and Factor VIII was investigated to exclude the presence of other cell types. Transcriptome analysis including genes involved in stemness maintenance, embryogenesis, cell growth, activation and development, was performed by real-time PCR. Despite the similar morphology of FB from different sites, and immunopositivity for vimentin, along with the absence of other cell type markers, FB isolated from abdomen and heart had 1.5-fold higher doubling time, while FB from heart, abdomen and breast were less susceptible to apoptosis. Intriguingly, Real-Time PCR revealed that in abdomen, breast, neck, arm and heart FB genes involved in cell growth, development, proliferation, and migration, as TM4SF1, GPC4, CSPG2, DDIT4, ID1 were up-regulated, while genes regulating embryogenesis and tissue morphogenesis, like VCAN, FN1, HOXA5, CD49a were up-regulated in FB isolated from abdomen, arm and heart. However, all FBs had transcripts of markers of Mesenchymal Stem Cells (MSC), as CD105 and CD90. Our results provide evidence that human adult FB from different sites have different genetic program. Therefore, FB may respond to reprogram technology in different manner, thus affecting reprogramming efficiency. While offering novel perspective of the reprogramming technology, our study also demonstrates that abdomen and breast FB share cardiac genetic signature of cardiac FB while expressing markers of MSC and they might represent the ideal cell for cardiac reprogramming.

Keywords

iPSC; skin fibroblasts; direct reprogramming.

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Chondrogenic differentiation of adipose tissue-derived mesenchymal stem cells at different time points

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The aim of this study was to identify the most appropriate time point for the successful chondrogenic differentiation of adipose tissue-derived mesenchymal stem cells (AMSCs). For this purpose, the expression of some chondrogenesis markers, such as collagen type I, collagen type II, lubricin and RUNX2 have been investigated by immunohistochemical and Western blot analysis at different time points (7, 14, 21 and 28 days). The AMSCs chondrogenic differentiation in the natural self-assembling constructs, called 'cell pellets' has been also assessed by the histological (hematoxylin and eosin) and histochemical (alcian blue staining) methods. The results showed that the differentiated chondrocytes, after 21 days of differentiation process, were able to produce increased quantities of collagen type I, collagen type II, and lubricin, suggesting the hyaline cartilage formation, and reduced expression of RUNX2, a protein expressed by the hypertrophic chondrocytes in the late stages of differentiation and normally expressed by osteoblasts. Our study demonstrates that 21 days represents the optimum period for the potential implantation of AMSCs derived chondrocytes for the cartilage defects. This information could be useful for the future development of cell-based therapies for the articular cartilage degenerative diseases.

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Keywords

Adipose tissue; mesenchymal stem cells; chondrogenesis; lubricin; collagen; Runx2; cell pellets.

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Comparative characterization of human and equine Wharton's jelly derived mesenchymal stem cells

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Mesenchymal stem cells (MSCs) have the capability to differentiate into wide range of specialized cells of mesodermal origin such as osteocytes, chondrocytes, adipocytes, cardiomyocytes, muscle fibers. Due to these properties, MSCs are considered as a new emerging treatment option and therapeutic agent in regenerative medicine. Promising results have been obtained after application of MSCs for treating tendon and joint disease in the equine model, making it favorable for therapeutic application. While the horse is considered a highly suitable model for orthopedic diseases, knowledge is lacking regarding the level of analogy of equine MSCs and their human counterparts. Therefore, the aim of this study was to assess the properties of human and equine Wharton's jelly derived MSCs in a direct comparison. Obtained MSCs, were characterized for their staminal markers, proliferation and adhesion potential, ultrastructural morphology and their ability in differentiate towards osteogenic, chondrogenic and adipogenic lineages. Results showed a similar pattern in the expression of staminal markers, while a light difference was observed in the proliferation and adhesion potential. Ultramorphological analysis showed nuclear and citoplasmatic features comparable in human and equine MSCs. Finally, both MSCs were able to differentiate towards osteogenic, chondrogenic and adipogenic lineages. In conclusion, although revealing some potentially relevant differences, the study demonstrates a high level of analogy between human and equine MSCs, providing a basis for translational research in the equine model.

Keywords

Mesenchymal stem cells; human; horse; Wharton's jelly; comparative study.

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Domain-specific regulation of cerebellar morphogenesis by Zfp423 / ZNF423, a gene implicated in Joubert syndrome and cerebellar vermis hypoplasia

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The Zfp423 gene encodes a 30-Zn-finger transcription factor that acts as a scaffold for the assembly of complex transcriptional and cellular machineries regulating neural development. While null Zfp423 mutants feature a sharp decrease in the total number of cerebellar Purkinje cells (PCs), the underlying mechanisms remain unclear. Mutations of the human homolog ZNF423 have been identified in patients carrying cerebellar vermis hypoplasia (CVH) or Joubert Syndrome (JS), associated with other signs of classical ciliopathy outside the central nervous system. To further characterize the role of ZFP423 in cerebellar neurogenesis, we have performed morphological, cellular and molecular studies on two mutant mouse lines carrying allelic in-frame deletions of Zfp423. While both lines exhibit cerebellar hypoplasia, considerable differences are observed between the two mutants, with respect to neural progenitor differentiation, cell survival and morphogenesis. The results of this in vivo and in vitro structure-function analysis point to domain- and context-specific roles played by ZFP423 in different aspects of cerebellar development, and contribute to our understanding of its role as a disease / modifier gene in JS, CVH and other ciliopathies.

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Co-culture of Caco2 and HT-29 cells as an innovative method to mimic in vitro the morphology and permeability properties of human intestinal epithelium

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For investigating the complexity of the human intestinal epithelium, a valid experimental approach is represented by co-culture. In the present study an intestinal co-culture Caco2/HT-29 (70/30) was set up starting from the parental populations of differentiated cells as previously described [1, 2]. Co-culture was harvested at 0 (T0), 6 (T6), and 14 (T14) days of post confluence after plating. Transmission electron microscopy was carried out to monitor the morphological features of cell differentiation. Alkaline Phosphatase (ALP), Aminopeptidase N (APN) and Dipeptidyl Peptidase IV (DPP IV) activity were assayed as known markers of intestinal cell differentiation. The measure of TEER and the apparent permeability of Lucifer Yellow allows to monitor the integrity of the tight junctions and the permeability of the cell layer formed. At T0 a classical monolayer is present, with a mixed population of immature absorptive elements and secretive cells. At T6 and T14, cells are progressively organized in a multilayer with a parallel growth of microvilli. At T6, co-culture demonstrates good properties of permeability and barrier components, such as mucus, representing an appropriate model for absorption study. At T14, the brush border is even more developed respect to T6 and, together with the increase of the specific activity of ALP, APN, and DPP IV, indicate co-culture as a good model for digestion study. The advantage of this co-culture described is the use of the whole cell population without particular inducers of subclones and growth support In conclusion, the morphological and biochemical features of co-cultured parental cells change with time, strongly supporting i) an active interaction between the two parental cell lines and ii) the versatility of this model, with more than one prevalent cell type depending on the post confluent stage.

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Keywords

Differentiation; transepithelial electrical resistance; Transmission Electron Microscopy.

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Histogenesis of cardiac myxoma: the potential role of the cardiac neural crest

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Cardiac myxoma is the most common tumor of the heart. The myxoma arises in the region of the interatrial septum and the fossa ovalis, with the left atrium as the most common site of growth. The histogenesis of cardiac myxoma remains unclear. We have previously suggested that a clearer understanding of tumor origins can be achieved through a detailed investigation of heart development (1). In the heart, cardiac neural crest contributes to the septation of the outflow tract, the morphogenesis of the great arteries, and the maturation of the atrioventricular valves and the conduction system (2). We hypothesized that myxoma develops from resident cardiac neural crest cells, via a re-activation of the developmental programs that govern epithelial-mesenchymal transition. Immunohistochemical analysis for canonical markers calretinin, vimentin, α-SMA, CD31, CD34, S100, plus cardiac neural crest markers plexin A2 and semaphorin 3C was performed in one case of sporadic cardiac myxoma. Primary myxoma cell culture was obtained ex vivo via both single cell isolation and outgrowth methods. In vitro confocal microscopy confirms the presence of myxoma cells positive for calretinin, vimentin, α-SMA, CD31, CD34, S100, plexin A2 and semaphorin 3C. Expression of semaphorin 3C and plexin A2 was also confirmed by Western blot analysis. In order to characterize in vivo behaviour of cultured myxoma cells, $2{,}3 \times 10^6$ cells (cell culture passage 3) were subcutaneously inoculated in interscapular region of immunocompromised mouse. Newly formed tissue was processed for histological and immunohistochemical evaluations. Hematoxylin and eosin staining confirmed the myxoid background of the mass. Despite the presence of CD31-positive human cells, there was no evidence of neovascularization. Our preliminary findings confirm our hypothesis, suggesting that cardiac neural crest in adult heart could be involved in the pathogenesis of cardiac myxoma.

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Keywords

Cardiac myxoma; cardiac neural crest; plexinA2; semaphorin3C.

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Protein supplementation prevents etoposide-induced skeletal muscle damage

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Autophagy represents a physiological mechanism responsible for cell homeostasis and its deregulation is involved in several conditions related to muscle mass loss such as aging, inflammatory diseases and disuse [1]. In our previous work, double membrane vesicles, suggestive of autophagy, appeared after chemotherapeutic treatments in C2C12 myotubes [2]. Here, skeletal muscle cells have been exposed to Etoposide (Eto), a cell-death and oxidative stress inducer, as well as to protein supplementation before the trigger. Cytofluorimetric, morphological and molecular analyses revealed that Eto treatment increases cardiolipin peroxidation events, and induces lysosomal compartment and endoplasmic reticulum damage. Moreover, a peculiar accumulation of autophagic complex vacuoles resulted in LC3 localization into dot cytoplasmic structures, appeared in treated-differentiated cells, if compared to the diffuse cytoplasmic distribution observed in untreated cells. Protein supplementation, is able to prevent myotube damage, by reducing oxidative stress, improving the lysosomal degradation pathway, and, finally, by reactivating the protein synthesis. These findings suggest that a diet rich in protein could prevent the impaired autophagic degradation in a skeletal muscle model in vitro, exposed to a chemotherapeutic agent, thus contributing to delay the progression of several muscle disorders [3].

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Keywords

Skeletal muscle cells; etoposide; autophagy; protein supplementation.

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Reelin expression in liver and pancreas and its correlation with liver fibrosis

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Reelin is an extracellular glycoprotein secreted by a variety of cell types in both embryonic and adult tissue and plays a critical role during brain development (1,2). Reelin is up-regulated in experimental liver cirrhosis of rats in hepatic stellate cell(HSC)s, the cell type mainly implicated in liver fibrogenesis, supporting that reelin is involved in the pathogenesis of liver fibrosis (3). Pancreatic stellate cell(PSC) s share similar morphology and function to HSCs, in pancreatic fibrosis setting (4). Currently, the role of reelin in human liver and pancreas is still unclear. We investigated reelin expression in different stages of chronic liver disease in 81 liver biopsies of HCV affected patients and in pancreatic tissue near to tumoral lesions. The expression of Reelin, HSC markers (CRBP1, alpha-SMA) and Dab1, a Reelin adaptor protein, was investigated by immunohistochemistry and immunofluorescence. Reelin protein was expressed by HSCs and a strong correlation was found between Reelin expression and liver fibrosis stage ($p<0.05$). Reelin expression correlated with CRBP1 positive HSCs ($p<0.05$) but not with alpha-SMA positive ones, suggesting that Reelin should not be regarded as a marker of HSC differentiation but a functional protein expressed by HSCs in some phases of liver fibrosis. Dab1 was found expressed in ductular reaction (DR) cells and the number of Reelin positive HSCs correlated with DR ($p<0.05$), suggesting a paracrine role of Reelin during liver fibrosis. Furthermore we identified in the pancreas PSCs showing a variable degree of Reelin expression. A role of Reelin expression in liver fibrosis and in the remodelling pancreatic tissue surrounding tumoral lesions can be postulated on the basis of the present findings.

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Keywords

Reelin; liver fibrosis; hepatic stellate cells; pancreatic stellate cells; HCV.

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Expression and localization of Phosphoinositide-specific Phospholipase C enzymes in polarized macrophages

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The phenotypic and functional diversity of macrophages depends on differentiating programs being developed during the cells' lives. Great interest was addressed to identify the signal transduction pathways acting in macrophage polarization, including the phosphoinositide (PI) system and related phospholipase C (PLC) family of enzymes. Enzymes belonging to the PLC family are strictly tissue specific and the expression panel, as well as the subcellular localization differs in quiescent cells compared to the pathological counterpart. We analyzed the expression of PLC enzymes in unpolarized (M0), M1 and M2 macrophages to list the isoforms expressed in the polarized macrophages and their subcellular localization.

Our results confirmed that macrophages express a wide number of PLC isoforms. All PLC enzymes were detected within both M1 and M2 cells, but not in M0 cells. M0, as well as M1 and M2 cells own a specific panel of expression, different for both genes' mRNA expression and intracellular localization of PLC enzymes. PLC enzymes might play a complex role in macrophages during inflammation and probably also during polarization.

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Keywords

Macrophages; polarization; M1; M2; phosphoinositide; phospholipase C; signal transduction.

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Comparative morphological study of bone regeneration in different rabbit cranial osteotomies: traditional versus new generation osteotomes

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The aim of this study was to compare the bone regeneration after skull surgery by means of different recent osteotomy devices, piezosurgery medical (PM) and piezosurgery medical plus (PMP), versus a conventional rotary osteotomy device (OT) in 16 adult white New Zealand rabbits. After 14 days from surgery, the recovery of different resulting bone gaps were observed under structural and TEM-ultrastructural analyses. Our preliminary observations showed that the minimum distance from the osteotomy edge, at which it is possible to observe viable osteocytes in the pre-existent bone, was lower in PMP samples (average 38.2μm) with respect to PM (65.7μm) and OT (83.4μm) ones. Moreover, the size of osteotomy gap performed with OT was about twice in thickness with respect to those obtained by PM and PMP. In relation to our previous investigations (1) on two different types of bone formation occurring in sequence (static and dynamic osteogenesis), we observed in the present study that in PM and PMP samples the osteotomy gap is in a more advanced step of recovery with respect to OT ones: in OT samples numerous cords of stationary osteoblasts, forming preliminary bony trabeculae, were observed in the regenerating newly-formed bone (step of static osteogenesis); whereas, in PM and PMP samples bony trabeculae appeared mostly covered by typical prismatic osteoblasts, arranged in movable laminae (advanced step of dynamic osteogenesis). By histomorphometry, in PM and PMP samples with respect to OT ones: i) values of the regenerated bone area with respect to the total osteotomy area (BV/TV%) are about twice; ii) the number of TRAP positive osteoclasts per linear surface showed a significant increase, suggesting higher bone remodelling. Concerning SEM analysis, in all samples the regenerated bone displays, as expected, higher cell density and less mineralized matrix with respect to the pre-existent bone, independently from the device used. In conclusion, our results indicate that osteotomies performed with PM and PMP can be considered equivalent and show more advanced stages of healing compared to OT, in part due to the lower osteotomy thickness in PM and PMP with respect to OT.

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Keywords

Cranial osteotomy; bone regeneration; piezosurgery medical/medical plus.

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TGF-beta bioavailability is increased by a new interaction between megakaryocytes and fibrocytes activated in the Gata 1 low mouse

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Primary myelofibrosis is the most severe of the Philadelphia-negative myeloproliferative neoplasms and is associated with progressive TGF-β1-dependent scaring of the hematopoietic microenvironment which causes hematopoietic failure in the spleen. Nevertheless, the pathogenetic roleof TGF beta is still unclear because of the modest (2-fold) increases in its plasma levels, both in patients and in animal models. Transmission electron-microscopy (TEM) observations identified that spleen from PMF patients and Gata1low mice contained megakaryocytes with abnormally high levels of TGF-β and collagen fibres embedded in their cytoplasm. Additional immuno-TEM observations of spleen from Gata1low mice revealed the presence of numerous activated fibrocytes establishing with their protrusions a novel cellular interaction, defined as peripolesis, with megakaryocytes. These protrusions infiltrated the megakaryocyte cytoplasm releasing collagen that was eventually detected in its mature polymerized form. Megakaryocytes, engulfed with mature collagen fibres, acquired the morphology of para-apoptotic cells and, in the most advanced cases, were recognized as polylobated heterochromatic nuclei surrounded by collagen fibres strictly associated with TGF-β. These areas contained concentrations of TGF-β-gold particles ~1000-fold greater than normal and numerous myofibroblasts, an indication that TGF-β was bioactive. Loss-of-function studies indicated that peripolesis between megakaryocytes and fibrocytes required both TGF-β, possibly for inducing fibrocyte activation, and P-selectin, possibly for mediating interaction between the two cell types. Loss-of-function of TGF-β and P-selectin also prevented fibrosis. These observations identify that myelofibrosis is associated with pathological increases of TGF-β bioavailability and suggest a novel megakaryocyte-mediated mechanism that may increase TGF-β bioavailability in chronic inflammation.

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Keywords
Megakaryocytes; activated fibrocytes; neutrophils; TGF-β; P-selectin; myelofibrosis.

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H_2O_2 stress damage is reversed by melatonin in a spinal cord organotypic model

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Spinal cord injury (SCI) is characterized to be a two-step process: the primary lesion consisting of the initial trauma; the secondary damage, characterized by multiple processes including inflammation, oxidative stress and cell death that lead to a significant expansion of the original damage and to an increase of the functional deficit (1). Among the aforementioned processes, the oxidative stress plays a significant role in pathophysiology of SCI. In this study, we evaluated the role of the melatonin, an indoleamine recognized as a potent antioxidant and immunomodulator (2, 3)Reiter et al., 1995, Favero et al., 2015), on the oxidative stress, the tissue vitality and the neuritic plasticity in an experimental model of organotypic cultures of Sprague Dawley rat spinal cord slice (SPS) treated with hydrogen peroxide (H_2O_2) and/or melatonin. Five experimental protocols were performed: 1) control; 2) H_2O_2 exposure (50 μM); 3) melatonin treatment (5^{-10}M for 24 hours); 4) H_2O_2 exposure and post-treatment with melatonin; 5) H_2O_2 exposure after pre-treatment with melatonin. Cellular death was investigated by propidium iodide (PI) assay and the vitality by MTT assay. The total thiols (SH) levels, contrasting the oxidative stress, the neuronal specific nuclear protein (NeuN) and the synaptophysin (Syp) immunopositivity were also evaluated. Melatonin significantly decreases the number of dead cells and increases slice vitality, mainly in slices treated before H_2O_2 exposure. Moreover, melatonin attenuates total thiols decrease and NeuN and Syp immunopositivity reduction. Overall, these findings suggest that melatonin may exert a potential beneficial effect upon the progression of SCI secondary damage, protecting the tissue from a further degeneration.

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Keywords

Stress damage; melatonin; spinal cord organotypic model.

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Distribution of Tyrosine hydroxylase immunoreactivity in the CNS of the common carp Cyprinus carpio

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Catecholamines, including dopamine, are the principal neurotransmitters mediating a variety of functions in the CNS, such as motor control, cognition, emotion, memory processing, and endocrine modulation. Dysfunctional catecholamine neurotransmission is also implicated in neurologic and neuropsychiatric disorders. Human brain diseases, such as Parkinson's disease (1), have been recently approached by using fish models, especially cyprinid teleosts, given basic similarities of the fish brain to that of mammals. The distribution of the catecholaminergic system has been studied in the forebrain of several teleosts, but relevant information are not available for the common carp, Cyprinus carpio, which is a model species in several studies. In this study, we have analyzed the distribution of catecholaminergic neurons in the carp brain by immunohistochemistry using a specific antibody to tyrosine hydroxylase (TH) on transverse serial frozen sections of the whole brain. In the carp brain, TH-immunoreactive (ir) neurons were present in several nuclei. In particular, positive neurons were detected in the ventral nucleus of the ventral telencephalic area. In addition, neuronal bodies and varicose fibers were stained for TH in the preoptic region, from the anterior to the posterior nuclei, in the suprachiasmatic nucleus, in the ventrolateral and ventromedial talamic nuclei. Moreover TH-ir neurons were also distributed in the periventricular pretectum and locus coeruleus. TH-ir structures were localized not only in recognizable catecholaminergic nuclei, corresponding to those of mammalian brain, but also in regions that are uniquely organized in teleosts, including the ventral telencephalon, the anterior and posterior preoptic region, the ventromedial thalamus, suggesting that they may be useful in elucidating homologies between fish and mammal brain. The present study partially confirmed TH distribution in other CNS of cyprinids (2), and provided more detailed information to a better understanding of the evolution of catecholaminergic system in vertebrates.

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Keywords

TH; immunohistochemistry; cyprinid; CNS.

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Ketogenic diet: a nutritional protocol for a non-pharmacological treatment of Alzheimer's disease

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Alzheimer's disease (AD) is a progressively worsening disease that affects specific brain areas involved in spatial memory, short-term memory and has a negative impact on the individual, limiting his ability of independent life. AD remains asymptomatic for a considerable time before cognitive decline becomes clinically evident. For this reason, the establishment of appropriate nutritional protocols, in the early stage, may be more effective than any drug treatments in the fight against this disease. Ketogenic Diet (KD) is a diet high in fat, adequate-proteins and low carbohydrates producing ketone bodies (KBs) (alternative energy source to glucose for the brain). Nowadays, relations between KBs and cerebral Aβ accumulation are not clear. Most studies focus on the neuronal component and forget the vascular component of AD represented by the blood-brain barrier (BBB), located at the cerebral microvessels level. Consequently, it seems essential to focus on the BBB physiology, and in particular on the BBB's functions of the receptors/transporters and enzymes involved in Aβ peptide transport and metabolism, to better understand the influence of a KD on the onset and the evolution of this disease. For 4 weeks, Wild type mice (129SV) were maintained on KD and Control Diet (CD). Glucose and Beta-hydroxybutyrate (BHB) levels were assessed in blood sample. Microvessel fractions were isolated from total brain and qPCR analyses were performed to study expression of transporters, receptors and enzymes involved in amyloid transport and metabolism at the BBB level. KD fed animals showed increased levels of BHB which was accompanied by an increased expression of Monocarboxylate Transporters 1 (MCT1) and Glucose transporter 1 (GLUT1) at the BBB level. There were not changes in the level of Glucose and body weight in these mice. In addition we observed modifications in the expression of some transporters involved in Aβ exchanges such as Low density lipoprotein receptor-related protein 1 (LRP1) and Multidrug resistance-associated protein 1 (MRP1). The expression of Aβ synthesis enzymes remains unchanged, instead an increase for the Aβ degradation enzyme Endothelin Converting Enzyme 1 (ECE1) was observed. These preliminary results show that dietary factors and in particular KD can modulate the expression of some actors implicated in brain Aβ metabolism at the BBB level.

Keywords

Alzheimer's disease; brain-blood barrier; ketogenic diet.

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Acetylcholine induces nitric oxide production by inducing intracellular Ca2+ oscillations in mouse brain endothelial cells

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Basal forebrain neurons control intracortical arterioles by releasing acetylcholine (Ach), which stimulates endothelial cells (ECs) to produce the vasodilating gasotransmitter, nitric oxide (NO). Surprisingly, the mechanism by which Ach induces NO synthesis in brain ECs is still unknown. An increase in intracellular Ca2+ concentration ([Ca2+]i) recruits a multitude of endothelial Ca2+-dependent pathways, such as Ca2+/Calmodulin endothelial NO synthase (eNOS). The present investigation sought to investigate the role of intracellular Ca2+ signaling in Ach-induced NO production in bEnd5 cells, an established model of mouse brain microvascular ECs. Ach induced dose-dependent asynchronous Ca2+ oscillations in bEnd5 cells. Ach-evoked Ca2+ oscillations did not arise in the absence of external Ca2+ but rapidly resumed on Ca2+ restitution to the bath. However, nicotine, a selective agonist of the Ca2+-permeable nicotinic receptors, did not cause any detectable increase in [Ca2+]i. Pharmacological manipulation indeed revealed that Ach-induced Ca2+ spikes in bEnd5 cells are triggered by the interaction between intracellular Ca2+ release from InsP3 receptors (InsP3Rs) SOCE. Next, we found that Ach-induced NO production was hindered by L-NAME, a selective NOS inhibitor, and BAPTA, a membrane permeable intracellular Ca2+ buffer. Moreover, Ach-elicited NO synthesis was blocked by the pharmacological abrogation of the accompanying Ca2+ spikes. Ach stimulates bEnd5 cells by inducing a burst of intracellular Ca2+ spikes which is patterned by the interplay between ER-dependent Ca2+ mobilization and SOCE. Ach-elicited Ca2+ spikes result in NO production and are, therefore, predicted to control local CBF in mouse brain. Future experiments will assess whether this signaling pathway is altered in neurodegenerative disorders, such as Alzheimer's Disease.

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Keywords

bEnd5 cells; [Ca2+]I; acetylcholine; SOCE; mouse brain.

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Age-related structural changes of the peripheral nervous system in mice: a morphological study

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In animal models age-related changes occurring in the peripheral nervous system (PNS) have not been so far extensively investigated. In particular there are no studies evaluating the entire PNS sensory pathway, more frequently compromised in aged persons. In this study we describe the morphological modifications taking place in the different parts of PNS sensory pathway: intraepidermal nerve fibers (IENF), peripheral nerves and dorsal root ganglia (DRG) were examined in the same cohort of C57BL/6 mice over a period of 25 months. Estimate of IENF density is a diagnostic procedure that is gaining increasing interest, but its long-term time-course has not been so far described in relationship with other PNS changes. Sixty-six female mice aged 4 weeks at the beginning of the study were used. Every 2 months sciatic and ventral caudal nerve, L4-5 DRG and hind paw skin specimens were collected and processed for morphological and morphometric analysis from 3 randomly sacrificed mice. Morphological observations were performed at light and electron microscope. In all the samples morphological changes were evident in aged animals: diminished density and degenerative aspects were observed in myelinated fibers both in sciatic and caudal nerves; progressive reduction in IENF density, more striking in the last months of observation; vacuolation and polymorphic inclusions in neurons and satellite cells in DRG, with a significant increase in the nucleolar size. These morphological observations were corroborated also by neurophysiological evaluation. Our study describes changes occurring in healthy aging mice and they reflect the expected course of PNS aging in humans. Therefore, our data might provide the background for mechanistic studies designed to investigate on possible pathophysiological events at the basis of the observed age-related changes in healthy mice PNS allowing the selection of the most appropriate time points according to the investigation aims.

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Keywords

Peripheral nervous system; aging; animal model.

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Interactions between astrocytes and microglial cells in the hippocampus

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A great amount of data is currently available on the role played by astrocytes and microglial cells in normal and pathological conditions, due to their relevance in the progression of neurodegenerative diseases. It is known that astrocytes provide homeostasis for neuronal networks, regulate neuronal maturation and synapse formation, modulate neurotransmission, act as progenitor cells in the neurogenic zone, and may influence microglial phagocytosis. On the other hand, they may enhance an inflammatory condition by producing and releasing inflammatory cytokines and amyloid fibrils. Microglial cells are the immunocompetent cells of the central nervous system, they remove damaged neurons and dysfunctional synapses in normal and pathological brain. They constantly act as sensor cells in normal brain, their ramified processes constantly scanning brain environment and eventually extending toward their targets. The same molecular pathways characterizing these activities are also utilized by microglia to influence nervous system development and connectivity in the normal and developing brain. However, inflammation may lead to deregulation of microglial cells, resulting in aggravation of disease progression. In this scenario, the comprehension of the interactions occurring between astrocytes and microglial cells, could be essential to get an inclusive synthesis of the evidence on their functions which are constantly accumulating. This study is aimed to verify whether the contacts occurring between astrocyte and microglia processes may undergo significant changes in number and spatial distribution according to different functional states of glial cells. To this aim we performed 3D particle analysis on confocal optical volumes acquired in the CA1 hippocampal region of control- and chronically inflamed- young and old rats.

Keywords

Astrocytes; microglial cells; cell-cell interactions; inflammation; aging.

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Early administration of the phytoestrogen genistein induces sex specific permanent alterations of nitrergic and vasopressinergic systems

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Soy foods contain phytoestrogens as genistein (GEN) which may interfere with endocrine system and, during developmental critical periods, lead to permanent alterations of estrogen sensitive hypothalamic circuits. In a previous study, we demonstrated that GEN exposure through mothers resulted in an anxiolytic effect and a concurrent decrease of neural NO synthase (nNOS)+ cells in amygdale of male offspring [1]. This was consistent with both the role of NOS system in anxiety regulation and its sensitivity to gonadal hormones. In the present experiment, we analyzed anxiety levels and changes of neuronal circuits in mice directly fed with vehicle, Estradiol (E2) or GEN from birth (postnatal day 0, PND0) to PND8. Behavioral tests were conducted at PND60 and the mice were sacrificed at PND90. Coronal serial sections were processed for immunohistochemistry against nNOS and vasopressin (AVP). The GEN treatment had a dichotomic behavioral effects on sexes: anxiolitic on females while anxiogenic on males. Concurrently nNOS+ and AVP+ cell density in some hypothalamic nuclei was affected. Interestingly only a few of those effects were mimicked by E2 treatment suggesting that GEN may act trough different intracellular pathways. These results raise concerns about the possible long-term effects of soy-based food in livestock that largely use soy-based supplements and show hypo-fertility problems, as pigs. Similar concerns could involve the long-term use of soy-based formulas for babies.

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Keywords

Vasopressin; nitric oxide synthase; hypothalamus; anxiolitic; anxiogenic; sex differences; organizational effects.

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Immunohistochemical analysis of axillary skin biopsies for the detection of adrenergic innervation of sweat glands in normal subjects and Parkinson's disease patients

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Beside the typical motor symptoms Parkinson's disease (PD) is characterized, with varying severity, by autonomic dysfunction. Several studies have shed light on the anatomical and molecular changes that underlie the peripheral neuronal degeneration associated with PD and other Lewy body (LB) diseases (LBDs). By using skin biopsies from LBDs patients it was possible to detect misfolded phospho-α-synuclein (p-syn) deposits within dermal nerve fibers and correlate them with a reduced density of small nerve fibers. (1, 2). The skin biopsy approach is an inexpensive and minimally invasive technique. To date, there is not a standardized procedure for sampling site, tissue processing and nerve fibre assessment, so the goal of a diagnostic instrument for an early diagnosis of (LBDs) still remains a challenge. We have carried out a retrospective study setting up a novel protocol based on 10 μm thick serial sections from FFPE axillary skin biopsies. This choice take advantage from the presence of apocrine glands in the axillary region, as they receive a dense sympathetic adrenergic innervation, exploitable for a clear nervous fibers tracking. The biopsies were taken from 14 individuals who had been, in the first instance, diagnosed with various traits of motor and neurological dysfunction and two control subjects. Serial tissue sections were analysed by IHC (DAB chromogen) and by immunofluorescent labelling, using anti-p-α-synuclein (S129), anti-α -synuclein, anti-PGP9.5 and anti-tyrosine hydroxylase antibodies. This particular setting has proven useful to well highlight the adrenergic fibers surrounding the apocrine sweat glands and to visualize the fibers α -synuclein deposition. Our results enabled us to support the first diagnosis in various cases with probable PD but gave a negative p-Syn-S129 immunoreactivity results for samples from vascular Parkinson, multiple system atrophy, essential tremor and frontotemporal dementia. Our methodological setting is able to detect the adrenergic innervation of sweat apocrine glands and both the presence of Lewy bodies and Lewy neurites in axillary skin biopsies.

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Keywords
Parkinson's disease; apocrine sweat glands; skin biopsies; adrenergic fibers.

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Obesity-related nervous system injury: preliminary evidences in diet induced obesity (DIO) rats

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Increased food intake, reduced physical activity and altered metabolic processes are the variables that affect energy balance inducing obesity. Obesity is now considered an increasingly medical challenge. Actually, the prevalence of obesity has increased dramatically worldwide over the last decades and has now reached epidemic proportions. On the other hand, obesity is associated with the development of chronic diseases such as cerebrovascular disease promoting the cognitive decline. Caloric-dense diet induced obesity (DIO), provides a useful animal model sharing several common features with human obesity. DIO rats of 7 weeks of age are expose to high fat (45 %) diet ad libitum and after 5 weeks the obese phenotype starts to be develop. To clarify the possible relationships between obesity and nervous system changes, DIO rats were studied after 5 weeks and 17 weeks of hypercaloric diet compared to the control rats with not fat diet (Chow). Memory performance were measured using different cognitive tests. Moreover, ultrasonographic (US) and computed tomography (CT) evaluations were performed to detect adipose tissue changes. Magnetic resonance imaging (MRI) to highlight brain morphological alterations was used. Morphological changes of brain areas (frontal cortex, hippocampus) were evaluated by immunohistochemical analysis. The results confirmed the developed of obesity after 5 weeks of fat diet. At long-term (17 weeks) high fat diet exposure, rats increased significantly their body weight in comparison to the control group and the youngest DIO rats. The US and CT analysis indicated an increase of deposition of both visceral and subcutaneous adipose tissue and evidences a decrease of hepatic attenuation in the older DIO rats.MRI images did not show vascular and morphological alterations in brain. Instead, immuhistochemical and immunochemical analysis, revealed an increase expression of glial-fibrillary acidic protein (GFAP) in the older DIO rats compared to the age- matched Chow rats both in frontal cortex and in hippocampus. DIO rats showed a reduction of retention latency time in the emotional learning task. These preliminary findings indicate that the development of obesity, does not determined gross anatomy alteration in brain, but the occurrence of injury characterized by astrogliosis. The identification of neurodegenerative changes in DIO may represent the first insight to better characterize the neuronal involvement in obesity.

Keywords

Obesity; diet induced obesity rats; brain; astrogliosis.

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Repeated administration of the spasmolytic otilonium bromide counteracts functional and neurotransmitters' changes in the colon of rats underwent to wrap restraint stress

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Otilonium bromide (OB) is a quaternary ammonium derivative successfully used for the treatment of irritable bowel syndrome (IBS). Several in vitro experiments in human and rat colon demonstrated its spasmolytic capability due to the block of muscarinic and tachykinin receptors and L-type Ca2+ channels. Moreover, in vivo OB administrations showed interesting interaction with the enteric nervous system in healthy rats (1). The wrap restrain stress (WRS) is considered an adequate model of psychosocial stressor, able to induce most of the IBS signs and symptoms. WRS leads to important changes in the enteric neurotransmitters of rat colon, as recently demonstrated (2). Consequently, we chose this animal model to investigate whether a repeated, oral treatment with OB prevented the functional and neurotransmitters' changes reported in rats underwent to WRS. The results obtained by using multiple experimental approaches (in vivo colonic functional evaluations, routine histology, immunohistochemistry and western blot) showed that OB is able to counteract most of the morphological changes caused by WRS in the colonic wall. In particular, the drug prevents the decrease in SP-, NK1r-, nNOS-, VIP- and S100β-immunoreactivity (IR) and the increase in CGRP- and CRF1r-IR detected in WRS rats. On the contrary, OB does not interfere with the mild mucosal inflammation and does not affect the increase in CRF2r-immunoreactive neurons observed in WRS rats. Moreover, OB per se increases the muscarinic receptor 2 expression in the muscle wall and decreases the number of the myenteric ChAT-immunoreactive neurons. Functional findings show a significantly reduction in the number of spontaneous abdominal contraction in OB treated rats. The ability of OB to block L-type Ca2+ channels, also expressed by enteric neurons, might explain the drug efficacy in preventing excessive neuronal response to stress.

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Keywords

Wrap restrain stress; irritable bowel syndrome; nerve structures; rat colon.

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Synuclein expression in the african clawed frog Xenopus laevis

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The synuclein (syn) family comprises three proteins (α-, β- and γ- syns) encoded by different genes (snca, sncb and sncg). In mammals, α- and β- syn are primarily expressed in the brain where they are localized in pre-synaptic terminals while γ-syn is mainly expressed in the peripheral nervous system. In humans, synucleins are involved in neurodegenerative diseases such as Parkinson's disease and in tumors. However, the normal cellular functions of the three syns have not yet been fully clarified. Members of the syn family were sequenced in representative species of all vertebrates and the comparative analysis of amino acid sequences suggests that syns are evolutionarily conserved, but information about their expression in vertebrate lineages is still scarce. Our research focused on the evolution of syns with the aim of analyzing their molecular and cellular expression in the CNS of representative vertebrates such as the carp Cyprinus carpio for teleost fish (1,2) and the green lizard Anolis carolinensis for reptiles (3). Current model of our comparative analysis for amphibians is the african clawed frog Xenopus laevis. The only information available on syn expression in this species relate to embryonic stages but data on syn expression in the adult are still lacking. At larval stages, amphibian snca is expressed in the brain, branchial arches and somites, and sncb signals were detected in the entire brain and spinal cord whereas sncg was only expressed in the peripheral nervous system including trigeminal nerve and dorsal root ganglion (4). Preliminary data are here reported on syn expression in adult specimen of X. laevis, obtained by RT-qPCR, Western blot and IHC. The results demonstrated that syns are expressed both in neuronal and non-neuronal tissues suggesting differences in the expression pattern between developmental and adult stages.

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Keywords

Synuclein; amphibian; Anolis carolinensis; Western blot; RT-qPCR; immunohistochemistry.

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Expression of the ciliary neurotrophic factor and its receptor α in human placenta of first and third trimester of gestation

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The ciliary neurotrophic factor (CNTF) is a member of the IL-6 family of cytokines along with cardiotrophin-1, IL-11, leukemia inhibitory factor, oncostatin-M and IL-6 itself. These cytokines play an important role in the regulation of cellular processes such as gene activation and cell proliferation and differentiation. CNTF is a pleiotropic cytokine which effects are mediated via CNTF receptor α (CNTFRα). CNTF increases differentiation and/or survival in neuronal cells but it also has different effects on other cell types such as muscle cells, bone cells, adipocytes, retinal cells and pancreatic β-cells (1, 2). In addition, recent studies demonstrate that CNTF plays an important role in weight control since exogenously administration of CNTF has an anorectic effect in mice (3,4). Although many studies proved that CNTF plays different roles in many cell types, its role in the development of human placenta has never been investigated. In this study we investigated the expression of CNTF and CNTFRα in human trophoblast by, immunohistochemistry, immunocytochemistry and Western Blot analysis using normal first and third trimester human placentas and HTR-8/SVneo cell lines. Interestingly, using immunohistochemistry CNTF and CNTFRα were expressed in the cytotrophoblast and syncytiotrophoblast in the first and third trimester of gestation respectively. Moreover, the immunofluorescence analyses by confocal microscopy showed that CNTF is expressed in the cytoplasm and nuclei whereas CNTFRα is mainly expressed in the cell membrane and cytoplasm of HTR-8/SVneo cell line. In this study we demonstrated that CNTF and CNTFRα are normally expressed in human placenta and they may play an important role during placental development.

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Keywords

CNTF; CNTFRα; ciliary neurotrophic factor; placenta; HTR-8/SVneo; immunofluorescence.

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Morphology and viability of human spermatozoa vitrified with a new, cryoprotectant-free, artificial seminal fluid

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Cryopreservation is a process finalized to store tissues and cells at a very low temperature. The most common freezing protocols used for gamete preservation in Assisted Reproductive Technologies are slow freezing and vitrification (1). Vitrification combines ultrarapid cooling with high concentrations of cryoprotectants; it avoids, better than slow freezing, the formation of ice crystals. It has been demonstrated, however, that cryoprotectant addition may significantly reduce cell viability (2). This study was aimed to design a new, cryoprotectant-free, medium similar to normal human seminal fluid (SF) formulation (artificial seminal fluid; ASF), and to compare the cryoprotective potential of this medium with SF and Human tubal fluid (HTF) medium. Thirty normal ejaculates were processed with swim-up technique and sperm suspensions were divided in four groups: fresh (controls); vitrified in HTF (Vit HTF); vitrified in patients' SF (Vit SF); and vitrified in ASF (Vit ASF). To identify the effects of the different media we assessed sperm parameters of motility, viability and morphology after warming. Spermatozoa ultrastructure was also evaluated by scanning and transmission electron microscopy (SEM and TEM). The results showed that sperm motility, viability and normal morphology were significantly higher in Vit ASF than in Vit HTF. The same parameters were better in Vit ASF than in Vit SF, but only viability differed significantly. Deep cytoplasmic invaginations and folded tails were commonly observed by SEM in all vitrified sperms, but this alterations were more evident in Vit HTF and Vit SF than in Vit ASF. By TEM, acrosome damage, plasma membrane loss, chromatin vacuolation, disruption of mitochondria and adherence of several tail sections together were observed in all vitrified groups; the latter phenomenon, however, was more evident in Vit HTF and Vit SF than in Vit ASF. In conclusion, vitrification of human spermatozoa with ASF seems more effective in preserving sperm quality than Vit SF and, particularly, Vit HTF.

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Keywords

Spermatozoa; vitrification; artificial seminal fluid; human.

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Polydeoxyribonucleotide, an adenosine-A2A receptor agonist, preserves blood testis barrier from cadmium-induced injury

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Cadmium (Cd) impairs the blood-testis barrier (BTB) with changes of its junctional complexes [1]. Polydeoxyribonucleotide (PDRN), an adenosine A2A agonist, has positive effects on male reproductive system [2]. We investigated the effects of PDRN on the morphological and functional Cd-induced changes in mice testes. Swiss mice were divided into four groups: control animals treated with 0.9% NaCl (1 ml/kg, i.p., daily); control animals treated with PDRN (8 mg/kg, i.p. daily), animals challenged with Cd chloride (CdCl2) (2 mg/kg i.p, daily) and animals challenged with CdCl2 and treated with PDRN. The experiments lasted 14 days. At the end of experiment, the testes were processed for biochemical, structural and ultrastructural evaluation. CdCl2 increased pERK 1/2 expression and Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) levels, decreased testosterone (TE) and inhibin-B levels and induced structural damages in the extratubular compartment and in the seminiferous epithelium, with ultrastructural features of BTB disruption. Many TUNEL-positive germ cells were present in the peripheral parts of the tubules. CdCl2 increased also tubular TGF-β3 immunoreactivity and reduced claudin-11, occludin and N-cadherin immunoreactivity. PDRN administration reduced pERK 1/2 expression, FSH and LH levels, increased TE and inhibin-B levels, ameliorated germinal epithelium changes and protected BTB ultrastructure. Only few TUNEL-positive germ cells were present and the extratubular compartment was preserved, showing only a mild edema. Furthermore PDRN decreased TGF-β3 immunoreactivity and enhanced claudin-11, occludin and N-cadherin immunoreactivity. We demonstrate, for the first time, a protective effect of PDRN on Cd-induced BTB damages in mice testes. We suggest that the A2A agonist may play an important role against environmental Cd, and in particular against its harmful effects on gametogenesis.

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Keywords

Cadmium; PDRN; blood-testis barrier; pERK 1/2; TGF-β3; immunohistochemistry; transmission electron microscopy.

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The reduced content of estrogen and progesterone receptors in varicocele sperm may be indicative of the clinical surgery in the testicular varicocele

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The enigma of testicular varicocele has always attracted the researcher's attention as attested by the consistent literature, although conflicting data are reported (1). The detrimental role of varicocele in fertility is supported by the presence of an higher frequency of affected men in the infertile population (2). Varicocele influences male fertility in a variety of ways: spermatogenesis, semen quality and gamete biology. However, the mechanism/s by which the pathology impairs sperm production and activity, are not known yet. In spite of active interest, our knowledge about sperm molecular anatomy is very limited. Instead, it is important to fully elucidate the molecular sperm architecture, in order to clarify clinical cases of idiopathic infertility since not all the apparently normal sperm are able to fertilize. The presence of steroid/steroid receptor systems was demonstrated in human sperm, suggesting that both systemic and local steroids through sperm receptors, may influence male fertility. From our data, it emerged that varicocele causes a damage in the gamete at molecular level which includes a significant reduction of estrogen and progesterone receptors, opening a new chapter in the already multifaceted physiopathology of the disease. By the time of ovulation, estradiol and progesterone are almost everywhere in the egg microenvironment affecting ability of sperm to fertilize the oocyte. Therefore, the reduced responsiveness to these hormones as we observed in varicocele sperm, impedes their goal. Altogether, these studies constrain the need of further researches on the molecular anatomy of human male gamete both in healthy and in pathological conditions related 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 the reduction of steroid receptors in sperm implies a decline in the acquisition of fertilizing ability.

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Keywords

Testicular varicocele; steroid receptors; human sperm.

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A fitness index model for Italian adolescents living in Southern Italy. The ASSO project

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Strong relations between physical fitness and health in adolescents have been established in the last decades. The main objectives of the present investigation were to assess major physical fitness components in a sample of Italian school adolescents, comparing them with international data, and providing a fitness index model derived from percentile cut-off values of five considered physical fitness components. A total of 644 school pupils (15.9±1.1yrs; M=399; F=245) were tested using the ASSO-Fitness Test Battery (FTB), a tool developed within the Adolescents and Surveillance System for the Obesity prevention project, which included the handgrip, standing broad-jump, sit-up to exhaustion, 4×10m shuttle run and 20m shuttle run tests. Stratified percentile values and related smoothed curves were obtained. The method of principal components analysis (PCA) was applied to the considered five fitness components to derive a continuous fitness level score (the Fit-Score). A Likert-type scale on the Fit-Score values was applied to obtain an intuitive classification of the individual level of fitness: very poor (X<P20), poor (P20≤X<P40), medium (P40≤X<P60), good (P60≤X<P80) and very good (X≥P80). Boys had higher fitness levels compared to girls; they also showed an incremental trend amongst fitness levels with age in all physical components. These results could be overlapped with those related to European adolescents. Data revealed high correlations (r>0.5) between the Fit-Score and all the fitness components. The median Fit-Score was equal to 33 for females and 53 for males (in a scale of 0-100). The ASSO-FTB allowed the assessment of health-related fitness components in a convenient sample of Italian adolescents and provided a fitness index model incorporating all these components for an intuitive classification of fitness levels. If this model will be confirmed, the monitoring of these variables will allow early detection of health related issues in a mass population and hence will give the opportunity to plan appropriate interventions.

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Keywords

Physical fitness; fitness index; adolescents, Fitness Test Battery.

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Investigating body composition in wheelchair athletes

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Subjects with spinal cord injury (SCI) are at risk for adverse changes in body composition (BC), which are harmful for their health and relevant to sport performance. This study investigated whole-body and regional BC in wheelchair athletes (WA) by comparing tetraplegic and paraplegic WA with a larger sample of healthy males athletes. Dual-energy X-ray absorptiometry (DXA) was used by one operator to measure subtotal (total-body less head) and regional (arms, legs and trunk) body composition (lean mass [LM], bone mineral content [BMC], fat mass [FM] and fat mass percentage [%FM]) in twenty-seven male WA aged 30.0±9.4y with chronic SCI. WA were classified as tetraplegic (lesion above T1; Tetra, n=10) and paraplegic (lesion at T1 and below; Para, n=17) and matched each to three healthy males athletes (n=81) on the basis of DXA area and BMI. BC outcomes were compared in Tetra and Para as well as Tetra and Para, and their respective control with the t-test for independent samples. Alpha value was set at 0.05 and p-values corrected for multiple comparisons (pc; Benjamini and Hochberg procedure). Percent FM was significant higher in Tetra vs. Para at the subtotal and regional level ($0.024<pc<0.008$). Both Tetra and Para had significantly greater FM and %FM at the subtotal ($0.006<pc<0.001$) and regional level ($0.025<pc<0.001$) along with lower LM and BMC at the legs ($pc<0.001$ for all) vs. able-bodied athletes. At the subtotal level the Tetra group also showed significant lower BMC compared with control ($pc=0.016$). These results expand on previous findings in non-athlete SCI persons (1) by showing that WA show unfavourable changes in BC in comparison with able-bodied controls and such changes are worse in tetraplegic than paraplegic WA. These results prompt for strategies and innovative interventions aimed at preventing health risks associated with BC changes in immobilized athletes.

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Keywords

Paralympic; spinal cord injury; fat mass; lean mass.

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Importance of ACE polymorphisms for endurance performance

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The skeletal muscle renin-angiotensin system (RAS) plays an important role in exercise metabolism. A functional insertion (I)/deletion (D) polymorphism in the angiotensin I- converting enzyme (ACE) gene (rs4646994) has been associated with ACE activity. The ACE DD genotype is associated with increased circulating ACE levels, which are generally two times as high as those found for II genotypes. Although most reports suggest that the I allele predisposes to human endurance (1) the literature also contains some opposite data (2). These inconsistencies from genetic association studies relating to the ACE gene and its I/D polymorphism maybe attributable, partially, to epigenetic factors that have been reported to influence ACE activity. Indeed, if both copies of the ACE D/D gene are transiently methylated, ACE increased levels associated with this polymorphism, may be down-regulated. However, there have not been any reports that address whether epigenetic regulation of the ACE gene is specifically involved in modifying human endurance, but, importantly, the human ACE gene promoter has been shown to harbor CpG islands. It is well established that environmental factors may modify the epigenetic profile and that nutrition training, muscle unloading and mechanical stimulation significantly impact on performance, playing an epigenetic role . Hence, it is conceivable that some of these factors might influence the CpG islands within the ACE promoter affecting its expression. The present study is aimed at modulating the epigenetic pattern of the ACE promoter by administering arginine to professional soccer players. ACE (DD, ID, and II) will be genotyped by PCR, then the epigenetic profile of ACE (gene promoter methylation) will be determined by bisulfite method, the serum levels of angiotensin 2 will be measured by ELISA. Hence, we compare the performance capacity of soccer players exhibiting different ACE polymorphisms, by strength, speed, and endurance tests. Subsequently, we will split the subjects under study in 2 groups: group 1 will follow a mediterrean diet, group 2 will follow the same mediterrean diet supplemented with arginine. After six months we will analyze if an epigenetic regulation of the ACE gene has occurred, modulating endurance performance.

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Keywords

ACE polymorphisms; epigenetic factors; muscle metabolism; endurance.

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Anthropometric indices of sarcopenia in patients with Chronic Kidney Disease

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The Chronic Kidney Disease (CKD) is a kidney dysfunction for which the estimated Glomerular Filtration Rate (eGFR) results <90 ml/min. The primary causes are glomerulonephritis, nephrosclerosis, diabetic nephropathy, nephropathy due to obesity and unilateral kidney (1). CKD is associated with frailty, cardiovascular disease, hypokinesia, sarcopenia and disability (2). In this study participated 74 subjects with CKD (mean age ≥ 65 years), 45 males and 29 females. All subjects underwent to anthropometric measurements: height, weight, BMI, circumferences (waist, dominant wrist, right/left arm, right/left thigh, right/left leg), body folds (biceps right/left, triceps right/left), calculation of the arm areas (adipose area, lean area, total area and muscle circumference). We divided the subjects into 5 samples according to the classification of Walker (2) based on the eGFR (ml/min): stage I+II (n=1), stage III (n=14) stage IV (n=50) stage V (n=9). In particular we have taken into account the anthropometric data of the stage III and V: BMI (stage III 28.85 ± 6.2 vs stage V 26.47 ± 3.69); total arm area (stage III 72.33 ± 16.52 vs stage V 65.36 ± 18.1); muscle arm circumference (stage III 26.06 ± 3.05 vs stage V 24.46 ± 3.52); adipose arm area (stage III 16.80 ± 7.94 vs stage V 16.58 ± 6.39); lean arm area (stage III 55.37 ± 13.68 vs stage V 48.90 ± 15.09). These preliminary results show that there is a worsening trend in anthropometric data according to the disease progression, from III to V stage, though the statistical analysis did not reach the significance.

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Keywords

Chronic kidney disease; eGFR; anthropometry.

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Physical performance in high school students: effect of chronotype

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Morningness-Eveningness (M-E) is an individual characteristic, defined as chronotype. People are typically categorized in 3 different chronotypes: Morning, Evening and Neither types (M, E and N-types), which differ in the circadian rhythm of many physiological variables. M-types use to wake up and go to bed early and to have their best performances in the first part of the day, otherwise E-types go to bed and wake up late and they have the peak performances in the evening. The chronotype is influenced by both individual and environmental factors and gradually changes during human development. Many studies have demonstrated a trend beginning with a tendency toward morningness in children that gradually evolves into a shift toward eveningness. Approximately at the age of 20 years, this shift reaches its maximum and starts to decline, leading to a growing tendency toward morningness from midadulthood on. This more pronounced eveningness has been found to negatively affect adolescents' sleep and daytime functioning; even physical performance of adolescents can vary throughout the day because sleep pressure increases, the input from the circadian timing system is optimal or non-optimal to perform the task or both of them. In this study participated 216 students, 124 males and 92 females, attending the first two high school classes (mean age 14-15 years). For the assessment of chronotype, all students compiled the Morningness-Eveningness Questionaire (MEQ), validated by Horne and Ostberg in 1976. For all subjects we collected anthropometric data (weight, height, BMI). All the subjects were categorized as M (n=22), N (n=165), and E-types (n=29). To assess the relationship between chronotype and physical performance, from the sample we recruited 51 subjects, 22 M-type (14 males and 8 females) and 29 E-types (18 males and 11 females), who carried out three Eurofit tests (SHR, Shuttle Run; SBJ, Standing Broad Jump; Cooper endurance test). Preliminary results, although showing some differences in physical performance between E and M chronotype, have not detected statistically significant differences between the two groups.

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Keywords

Adolescent; chronotype; performance.

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Combining Core Training and Sensory Refinement: effects on physical performance

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The concept of "the Core" describes the complex of anatomical components of the trunk, pelvis and shoulder girdle that are responsible for maintaining the stability of the spine and pelvis and are critical for the transfer of energy from larger torso to the smaller extremities, during many sport and daily-living activities (1). This concept rooted in sport science and rehabilitation and recently Core Training (CT) became very popular as a method to prevent injuries and improve sport performance and physical fitness. It consists in the progressive training of the musculature of the Core with special emphasis in posture and lumbar spine stability. This aspect requires a fine coordination and body awareness that often are poor developed or regressed after an injury. Then, it is important to include exercises of Sensorial Refinement (SR) that may stimulate the refinement of perceptually neglected areas (2). The aim of the study was to evaluate the effect of combined CT and SR on physical performance and to compare these effects with traditional core training. Furthermore, the effect on retention after 4 weeks of detraining was evaluated. Two groups of participants were recruited (age >30 < 50) and assigned to experimental (CT and SR: EXP, n = 9) or control (CT: CON, n = 9) group. Both groups trained ten weeks, with a frequency of two sessions per week. Training consisted in 10′ of warm up, 40′ of workout and 10′ of cool down. Workout of EXP group consisted in 20′ of SR and 20′ of CT whereas CON group performs 40′ of CT. Participants where tested by: Star Excursion Balance Test (SEBT) for the dynamic balance of lower body, Upper Quarter Y Balance Test (YBT-UQ) to assess upper extremities function in a closed-chain position and McGill test to evaluate muscular endurance of the core. Both groups improved core endurance after training and worsen it at follow up; moreover, both groups ameliorate upper an lower body control (SEBT and YBT-UQ) after training but only EXP group improved or maintained it at follow up. Since the information about the movements of the body are elaborated in the somatomotor cortex for fine coordination, the combination of CT and SR should better promote the consolidation of motor memory and long-term body control.

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Keywords

Core Training; proprioception; physical performance.

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Neuro-degenerative and vascular diseases: methodology for functional recovery

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Posture refers to the position of the body in space that is expressed through the interaction of all the districts and systems such as the musculoskeletal system, the central and peripheral nervous system. Alterations in imbalances and associated diseases produce a structural and physiologic reorganization of the anatomical structures to improve postural dynamics. Generally, these changes can occur due to trauma or following the onset of neurodegenerative diseases or vascular problems that, in different ways, ranging to compromising the proper functioning of one of the components involved in postural processes. Currently postural diseases are treated by passive (brace and orthosis) and active (robotic device and traditional rehabilitation) methods according with the severity of imbalance (1). The aim of this study is to evaluate the effects of an innovative exoskeleton, called Human Body Posturizer (HBP), in rehabilitation of different neurodegenerative and vascular diseases. We recruited 37 subjects divided according to the pathology: 9 subjects with Parkinson's disease, 14 with multiple sclerosis, 10 post-stroke patients and 4 with infantile cerebral palsy. Subjects underwent 4 weeks HBP treatment, consisting of 30 minutes, with different timing and duration of treatment depending on the specific pathology. The samples were analyzed by using of Electronic Baropodometer, Stabilometric Platform and Sensorizer FreeSense. Each subject was sampled before and after treatment and differences between pre and post treatment were subjected to statistical analysis. In all groups, we found significant differences in the comparison between the measurements performed before and after treatment with HBP. These changes have allowed to pointing out the improvement in the parameters analyzed in the post-treatment tests. Thus, as demonstrated by other studies (2), the use of HBP could represent an integrative therapy for different postural diseases and it can be inserted as a supportive therapy during the rehabilitation process in neurodegenerative and vascular diseases.

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Keywords

Human Body Posturizer; posture; gait; balance.

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Ballistic differences in professional soccer players with and without visual impairment

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Vision is considered the most important sensory input modality for sport performance (1). Recent studies have shown the existence of developmental delays in motor and sport skills in individuals with visual impairment (2). The purpose of the current study is to examine the kinematic and ballistic differences during football match between professional players with visual impairment and players without visual impairment. During athletic preseason, all soccer players of Delfino Pescara 1936 were evaluated on the basis of visual and orthotic parameters. The sample was composed of 18 professional players including 8 with visual impairment (age -yrs- 23.5 ± 2,50 SD; height -cm- 181.75 ± 9.08 SD ; mass -kg- 78 ± 8.58 SD)and 10 with no visual impairment (age -yrs- 26.6 ± 5.29 SD; height -cm- 177.1 ± 5.95 SD; mass -kg-74.3 ± 6.78 SD). All players were subjected to Natural and Corrected Visual Acuity, Refractive Examination during Miosis and Cycloplegia, Intraocular Pressure (IOP), Extrinsic Ocular Motility, Examination of Convergence and Cover Tests. During all regular season (September 2015/ May 2016, 42 matches, 3780 minutes) for each player it was reported the number of passing under 10 meters, errors of passing under 10 meters (expressed in centimeters), number of passing over 10 meters, errors of passing over 10 meters (expressed in centimeters) and shots in the target and out the target. The results suggest that the players with visually impairment miss passing with a higher percentage than in the control group in both conditions (under the 10 meters and over 10 meters). No significance emerged on the parameter shots on target in both groups. Results showed that the vision is a fundamental proprioceptive channel for the performance in elite soccer players but the small samples analyzed and the lack of prior research studies on the topic subject suggest that further studies will be needed.

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Keywords
Vision; sport performance; soccer; ball speed; visual impairment.

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Can experience in karate be estimated by Principal Component Analysis?

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Karate is a Japanese martial art that involves repeated and technically demanding sequences of strikes and defences. In kata competition selected movements are organised in fixed sequences of varying duration and complexity. The evaluation of kata is usually made by subjective scoring from coaches and judges, who employ a qualitative, global assessment. Holistic quantitative evaluations of karate performance are still unavailable. Based on previous findings (1), we hypothesise that the multi-joint motion patterns that enable complex techniques are experience-dependent. If this were true, we would seek to find which motion pattern could be more sensible to the experience level. To test this hypothesis, a method based on the Principal Component Analysis (PCA) appears well-suited to detect the "synergies" or "coordinative structures" by which the motor system organises a movement (2). PCA can provide a quantitative global analysis of stylistic differences in technique. In this study we aimed at: describing the fundamental multi-joint synergies of a karate performance, under the hypothesis that the latter are skill-dependent; estimate karateka's experience level, expressed as years of practice. A motion capture system recorded traditional karate techniques of ten professional and amateur karateka. At any time point, the 3D-coordinates of body markers gave posture vector that were normalised, concatenated from all karateka and submitted to a first PCA. Five principal movements described both gross movement synergies and individual difference, explaining 91% of the overall variance. A second PCA followed by linear regression estimated the years of practice using principal movements (eigenpostures and weighting curves) and centre-of-mass kinematics (error: 3.71 ys; $R2=0.91$, $p<0.001$). Principal movements and eigenpostures varied among different karateka and as a function of experience. This approach provides a framework to develop visual tools for the analysis of motor synergies in karate, allowing to detect the multi-joint motor patterns that should be specifically trained to increase performance.

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Keywords

Martial arts; biomechanics; coordinative structures; principal movements.

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Core stability in young female dancers

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Core has been described as a corset stabilizing the trunk area, bordered by the diaphragm upwardly, the abdominal muscular complex anterior-laterally, the spinal and gluteal muscles posteriorly and by the pelvic floor and the muscles of the pelvic girdle inferiorly (1). Core Stability (CS) is intended as a neuromuscular skill of trunk control, assisted by passive (ligaments) and active (muscles) elements. The effects of CS training on the strength, endurance and balance of young female dancers were measured. Thirty three young women participated (22 amateur dancers; 11 sedentary people; age 21±5.4yr) were asked to perform endurance (2), strength, and balance (3) tests (Session I) that were repeated after 10 weeks (Session II). During this period, 11 dancers (experimental group, EG) were randomly selected to attend specific training for CS in addition to traditional dancing exercises; 11 dancers only performed regular dance training (dance group, DG); the sedentary people did not carry out any exercise (control group, CG). Within each group and session, descriptive statistics of test performances were computed. Differences between groups and sessions were assessed, setting the level of statistical significance at 5% ($p \leq 0.05$) for all comparisons. In both sessions, EG and DG were stronger and more resistant than CG and demonstrated better balance in two balance tests ($p < 0.05$). In Session II, performances generally improved in both EG and in DG. EG dancers significantly improved their endurance performances in Session II ($p < 0.01$). Hence dance training in young female amateurs could be considered an effective exercise to enhance strength, endurance and balance. Specific CS training seemed to have favourable effects while improving endurance in female dancers. Further studies should be conducted on a larger sample of dancers, either men or women, to determine the efficacy of CS both in performance and in injury prevention.

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Keywords

Balance; core stability; core strength; dance; injuries.

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Actigraphy-based activity levels and anthropometric measurements in breast cancer survivors: effects of aerobic physical activity

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The research investments for the identification of modifiable factors associated with BC recurrences is increasing. Adiposity and other anthropometric indices have been acknowledged as factors involved in BC recurrences and mortality (1). Physical activity (PA) has the potential to counterbalance all of these risk factors (2). We designed a randomized controlled trial to test the effect of an aerobic PA program on anthropometric indices of adiposity and circadian rhythm activity level, evaluated by actigraphy, in BC women included in a dietary intervention trial for prevention of BC recurrences. 40 BC women, aged 35-70 years, were randomized into an intervention (IG=19) and a control group (CG=21). The IG participated in a 3-month active PA program that included two sessions of one-hour brisk walking per week. At baseline and after 3 months, both IG and CG were evaluated for the following parameters: height, weight, BMI, waist circumferences, % fat mass, % lean mass; energy expenditure and motion level (Total Energy Expenditure-TEE, number of steps, PA level, Metabolic Equivalents-METs) using a SenseWear Pro 3 Armband; and activity level circadian rhythm using the Actigraph Actiwatch. At the end of the 3-month PA program, IG showed a significant reduction in fat mass % while CG improved weight and BMI. The population mean cosinor applied to IG and CG at PRE and POST revealed the presence of a significant circadian rhythm in two groups ($p<0.001$). MESOR, Amplitude and Acrophase were not different in the two groups in pretest conditions. Amplitude decreased significantly between PRE and POST ($F(1,38)=6.4$, $p=0.02$). MESOR and acrophase remained unchanged. Our results suggest that a standardized PA program in BC survivors reduces anthropometric indices of adiposity and may represent an integrative intervention therapy able to modify behaviour.

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Keywords

Breast cancer; physical activity; actigraphy; anthropometry.

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Anthropometric and performance differences among playing positions between Italian and American high school football players

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Height, body weight and body composition are different among American football players and this is due to the different movement demands for each playing position (1). The results of the National Football League (NFL) Combines are different for playing position and they are used by coaches and scouts to assess players' physical abilities as a determinant of their success in the professional level (2). The purpose of this study was to examine anthropometric and performance differences among young football players of a top Italian team (Rhinos Milan) and to compare these values with the American high school football players. Participants (N=62) were categorized by position in 3 groups based on playing position: Skill players (SP) included wide receivers, cornerbacks, safeties, and running backs; Big skill players (BSP) consisted of fullbacks, linebackers, tight ends, and defensive ends; Lineman (LM) included centers, offensive guards, offensive and defensive tackles. Body weight and percentage of body fat were determined using the TANITA Body Composition Monitor BC-418 and we obtained performance results of the following Nike SPARQ Combine drills: 40-yard dash, vertical jump, 20-yard shuttle and the kneeling power ball toss (KPBT). The one-way ANOVA followed by the Tukey-Kramer post-hoc test showed significant differences for all the variables among the 3 playing categories: LM had higher anthropometric and body composition values than SP ($p<0.001$) and BSP ($p<0.01$) while they performed significantly worse in the physical tests, except for the KPBT ($p<0.01$). We calculated and compared the 95% confidence limits for each anthropometric and physical test parameter of Italian and American players: American high school players had higher values for all the anthropometric and physical tests variables than Italian players ($p<0.001$). Administrators of professional football teams in Italy need to improve players' physical attributes so the gap that currently exists between Italy and USA can be reduced.

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Keywords

Anthropometrics; American football; Nike SPARQ Combine, high school.

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The future of didactics in Anatomy from the point of view of students

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The learning of Anatomy is based traditionally on books, 2D atlases, anatomical physical models and – if accessible – cadaver dissections (1). Emerging tools, like multimedia contents, 3D virtual models or the new head-mounted displays (e.g. Oculus Rift, Samsung Gear VR), which allows to dive into virtual reality environments, are rarely taken into account even if they are nowadays available at affordable prices (2). In order to assess students' point of view on these latest technologies, we prepared an anonymous questionnaire of 9 questions based on a five-point Likert scale. The questionnaire was randomly proposed to 61 students, enrolled in the preclinical years and who had completed the course of Anatomy. The students were asked to evaluate the usefulness of different tools in preparing the exam of Anatomy and to indicate which didactic tools should be available for the study of Anatomy. They also evaluated the importance given to morphology, relations and variations of organs during the exam study. The results showed that most students found very useful (answer point: 4 or 5) multimedia sources (61%) and 3D virtual models (66%). According to students, the most important tools at disposal for learning Anatomy should be 3D virtual models (26%) and 3D models in immersive virtual reality (25%) rather than physical models (21%) or other tools. Moreover, students stated they focused on morphology (74%) and relations between organs (92%) much more than anatomical variations (17%), although patient-specific anatomy would be essential in clinical practice (3). Therefore, the results can be useful to steer didactic activities and underline the importance of considering new technologies like 3D virtual models as effective tools to improve the learning of Anatomy and to focus on inter-individual variants from the very beginning.

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Keywords
Virtual models; anatomy; education; student perceptions.

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Methodological aspects of the study branch of science histology (as electron microscopy) under the formation of a new technological order (Russia, the second half of the XX century - the beginning of the XXI century)

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After analyzing the obtained during the investigation, the information we can talk about the existence of several major periods in the development of electron microscopy of the XX century in Russia. The first period is descriptive, on the basis of works on the history of certain biological sciences, cytology, histology, as well as the basic approach to the presentation of their main methods [E.M. Vermel, 1970; R.F. Kapustin, A.A. Gorbach, 2014], which states that no matter how improved the microscope, he would not allow to penetrate the secrets of cell morphology, if not at the same time to improve the technique of material processing, machinery manufacturing "microscopic sample» [Z.S. Katsnelson, 1963]. Destinations many studies have allowed through the use of modern methods of microscopy to link all stages of development of an organism with certain cell structures [L.Y. Blyakher, 1968]. The next period is associated with the development of technologies in the improvement and dissemination of electron microscopy. There has been a real surge of new directions in mikropostroenii are absorbed microscopes that meet all modern technical requirements [B.K. Ioannisiani, 1960; M.P. Panfilov, 1970]. Out of electron microscopy in the sphere of large-scale production and export to the world markets, identified the third stage of development of electron microscopy. Methodological analysis of the results allows us to determine the following main tendencies of development: use of nanoelectronics, the ability to explore the structure of organisms; in the formation of a new technological order play an important role public investment in microscopy, which form the basis of the spread of this method in the world and Russian economy [S.Yu. Glazyev, 2009]; scientific justification for ways to optimize physical resources and production processes, which are of a purely applied character, its practical importance can be manifested in the following forms: nanoelectronics, nanomaterials, nanobiotechnology and nanoequipment [S.Yu. Glazyev, 2010]. Thus, such a timely practical development can provide investment and the leading position of this sector in the crest of the wave of a new economic order.

Keywords

Electron microscopy; economic order.

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Protective effects of melatonin against nicotine-induced oxidative damage of kidney

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Several studies demonstrated that melatonin treatment prevents tissue damage in various models of oxidative stress (1). Experiments have shown that chronic nicotine administration caused oxidant damage in various organs by increasing lipid peroxidation products and decreasing the activity of endogenous antioxidants (2). The aim of this study was to investigate the effects of melatonin treatment on nicotine-induced oxidative changes in rat kidney and to explore the possible mechanisms of action. Three groups of rats were used as controls (the first without treatment, the second with melatonin alone and the third with nicotine alone). The last group of rats was orally treated with nicotine and melatonin for 28 days. Morphological changes in kidney were evaluated by histological procedures and immunohistochemical analysis using inflammation (NFkB and IL-6) and oxidative stress (SOD, CAT and iNOS) markers. Experiments performed demonstrated that nicotine administration increases inflammation and oxidative stress. Melatonin has a protective effect against nicotine kidney toxicity through an inhibition of inflammation and consequent oxidative damage. These data suggest that melatonin supplementation effectively counteracts the deleterious effect of chronic nicotine administration on kidney and attenuates oxidative damage possibly by its anti-inflammatory and antioxidant effects.

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Keywords

Kidney; nicotine; melatonin; inflammation; oxidative stress.

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Anatomical network analysis reveals centralities in human biomechanical structure

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The anatomical structure of the human body is a highly complex system for the large amount of components and the relationships and interactions between these. Today there are tools that allow us to deal with this complexity and the network is certainly one of the most used. The use of the network model for the analysis of complex systems has already been used in the nineteenth century by Etienne Geoffrey Saint Hilaire as part of the morphological biology. Recently they have been reconsidered because of the possibilities of analysis and interpretation of mathematics that they offer still nowadays (1,2). The anatomical network presented here consists of 2294 anatomical parts interconnected by 7196 links between bones, muscles, fasciae, joint capsule, ligaments and tendons. Hence, all this structures refers to the biomechanical structure of the locomotor system. The topological analysis of the network allows us to develop new applications in the context of the study and the anatomical evaluation of the locomotor system related to reachability, connectivity and brokerage of the various body parts.

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Keywords

Anatomical network; biomechanics; locomotor system.

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Effects of melatonin long-term treatment in aging mice

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Aging is a complex and progressive process involving every organ in the body and it is the result of coordinated biology events that can span decades (1). At cardiovascular level, age-related degeneration and functional decline are quite heterogeneously, nevertheless oxidative stress is a well known pathological process involved. The aim of this study was to investigate the effects of a chronic and long-term treatment with melatonin on functional responses of small mesenteric arteries and on the expression of oxidative stress markers at aorta level of senescence-accelerated prone mice (SAMP8), a model of age-related vascular dysfunction and cognitive decline (2), respect relative controls, senescence-accelerated resistant mice (SAMR1). In the present study were investigated SAMP8 and SAMR1 mice orally treated or not treated for 10 months with melatonin (MelapureTM by Flamma S.p.A.). It was observed that the anticontractile effect of perivascular adipose tissue is impaired in untreated SAMP8, compared with SAMR1. On the contrary, the chronic treatment with melatonin decreased the contractile response to norepinephrine in mesenteric small arteries of SAMP8, restoring an anticontractile effect, probably through melatonin antioxidant mechanisms. In untreated SAMP8 mice was observed at aorta level also an overexpression of oxidative stress and inflammatory markers compared with controls; whereas the long-term treatment with melatonin in SAMP8 was able to increase the expression of some markers of vasculoprotection and to decrease oxidative stress and inflammation. A reduced expression of adiponectin and adiponectin receptor 1 was also observed at visceral fat level of untreated SAMP8 respect SAMR1, while a significant increase was observed after melatonin treatment. In conclusion, melatonin exhibited marked antioxidant and vasculoprotective effects, underlining its potential anti-aging properties at cardiovascular level.

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Keywords

Aging; aorta; melatonin; mesenteric small arteries; oxidative stress.

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Heart morphology in Zucker-obese rat

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Obesity represents the central and causal component of the metabolic syndrome (MetS), which is a growing medical challenge in western countries as a result of changes in lifestyle. Obesity is also associated with an increased incidence of arterial hypertension and of cardiovascular disease burden. In animal models of diet-induced obesity, endothelial inflammatory activation, demonstrated by changes in adhesion molecule expression, is one of the earliest manifestation of vascular and cardiac damage. The intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin (Ig) superfamily present on the surface of several other cell types, including endothelial cells. Adhesion molecules [e.g., ICAM-1, vascular cell adhesion molecule 1 (VCAM-1) and platelet-endothelial cell adhesion molecule-1 (PECAM-1)] if in contact with an activated endothelium could represent attractive targets for delivery of drugs and imaging probes to vascular pathological sites. The present study was designed to investigate, with morphological, immunochemical and immunohistochemical techniques, changes of heart and coronary arteries in Obese Zucker rats (OZR) compared to the lean Zucker rats (LZRs). The OZRs, with a mutation in leptin receptors, is a model of Type II diabetes mellitus, characterized by the presence of obesity, hyperglycemia, hyperinsulinemia, hyperlipidemia and moderate hypertension similar to MetS. The heart of OZRs of 12, 16 and 20 weeks was processed for microanatomical analysis and ICAM-1, VCAM-1 and PECAM-1 and pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) immunohistochemistry. OZRs at the different age, developed ventricular hypertrophy, characterized by increase size of cardiomyocytes but not fibrosis compared to LZRs. This phenomenon was more evident in 20-weeks-old OZRs. VCAM-1 was more expressed in the coronary arteries compared to other adhesion molecules, and increased in the OZRs of 20-weeks of age. In the same age, IL-6 expression was significantly increased. These results suggest that the obesity leads to heart tissue changes and coronary inflammation. Myocardial vascular inflammation, induced by metabolic comorbidities, could contribute to the development of heart failure. Protective strategies in obesity may be focussed versus body weight loss and countering of metabolic alterations induced by obesity.

Keywords

Obesity; heart morphology; inflammation; obese Zucker rats.

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Expression of the estrogen and relaxin receptors in human fascial tissue

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Fascia is a tissue that interact with different structure in a very precise manner. It creates a structural continuity that give form and function to every tissue and organ. It plays a significant role in mechanical tension, transmitting force, correct motor coordination so altered structure of specific components layers could be generate a clinical problem. Recent studies have shown the possible role of the fascial nociceptors to mechanical and chemical stimuli may contribute to myofascial or musculoskletal pain (1). Many epidemiologic, clinical, and experimental evidence points to sex differences in myofascial pain, and generally adult women more often have myofascial problems than do men (2). It is possible that one of the stimuli to sensitization of fascial nociceptors could come from hormonal factors such as estrogen and relaxin that are involved in extracellular matrix and collagene remodelling (3). Relaxin-2 (RLX-2) is recognized as anti-fibrotic factor that is the ligand for RXFP1. Estrogens and in particular 17β-estradiol (E2) regulate a widespread of physiological functions and the actions are mediated by two estrogen receptor isoforms, ERα and ERβ. We hypothesized that E2 and relaxin contribute on metabolism and function of myofascial tissue. Immunohistochemical and molecular investigation (real-time PCR analysis) for RXFP1 and ERα localization were carried out in human fascia of different districts (peroneal, abdomen rectum, hip and low back fascia) and in fibroblasts isolated from the same districts, with the aim of describing both protein and RNA expression. ERα and RXFP1 are expressed on fibroblasts of human fascial tissues and RXFP1 expression was particular intense on vessels and nerves. These results are confirmed in isolated fibroblasts derived from the same fascial districts. Not all the cells have the same reactivity but the positive reaction was evident in the cytoplasm of cells for RXFP1 and with more intensity on nuclei of cells for ERα. Our results are the first demonstration that the fibroblasts of different districts of the muscular fasciae express sex hormone receptors. These findings could represent a new target for the care of myofascial pain and the possible stimulation during manipulative treatments and exercises. More studies about the interactions between fibroblasts, extracellular matrix and hormone receptors (estrogen, progesteron, relaxin) could help to understand the role of these receptors on myofascial pain.

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Generation of spheroids from human primary myofibroblasts: an experimental system to study myofibroblasts deactivation

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Fibroblasts represent a heterogeneous cell population, that in adult body maintains the homeostasis of the extracellular matrix (ECM) and can acquire an immunoregulatory phenotype. Indeed, activated fibroblasts produce large amounts of cyclooxygenase-2 (COX-2) and proinflammatory cytokines (1). The activation of fibroblasts is represented by their differentiation into myofibroblasts. This process, either in wound healing or cancer tissue, is associated with the expression of alpha-smooth muscle actin (alpha-SMA), increased levels of growth factors and ECM-degrading proteases (2). Moreover, myofibroblasts form clusters in wound healing process and hypertrophic scars. In particular, cell clusters of hypertrophic scars are represented by nodules of myofibroblasts (3). It is known that human dermal fibroblasts established from neonatal foreskin, and forced in vitro to form clusters named spheroids, are activated to produce massive amounts of COX-2, prostaglandins and proinflammatory cytokines: this process leads to a programmed necrosis, designated "nemosis" (1). In the present study we generated spheroids from human primary myofibroblasts of skin, to evaluate necrotic, inflammation and activation markers during myofibroblasts clustering. Western blotting analysis, showing low levels of COX-2 and a significant decrease of alpha-SMA in protein extracts of spheroids, led to hypothesize that myofibroblasts have undergone a deactivation process within spheroids. This hypothesis is confirmed by cytostatic effect exerted by spheroids conditioned medium on both normal and cancer cell lines, by confocal immunofluorescence analysis of connexin 43 and immunohistochemical evaluation of proliferation marker Ki-67. This work could represent an experimental model to study myofibroblasts deactivation and highlights an alternative process regulating the turnover of myofibroblasts.

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Keywords

Myofibroblasts; spheroids; deactivation.

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Histopathological rearrangements of the colonic wall following dopaminergic nigrostriatal neurodegeneration

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Parkinson's disease (PD) is a degenerative neurological disorder, which is often associated with gastrointestinal disturbances (e.g., constipation and defecatory dysfunctions), whose mechanisms are still unknown [1]. Recently, an inflammatory pathogenesis has been proposed to explain these colonic disorders, but low literature are available. This study aims to analyze whether the central dopaminergic denervation, induced by intranigral injection of 6-hydroxydopamine (6-OHDA), can alter the morphological arrangement of colon in rats. Animals were euthanized 4 and 8 weeks after 6-OHDA injection. Histological, histochemical and immunohistochemical analysis were carried out on formalin-fixed, paraffin-embedded colonic samples in order to evaluate: histology, inflammatory cells (eosinophils and mast cells) and collagen fibers in the whole wall; glial fibrillary acidic protein (GFAP), immunoperoxidase, alpha-smooth muscle actin (alpha-SMA) and vimentin immunofluorescence by confocal microscopy. Malondialdehyde (MDA, colorimetric assay), TNF and IL-1β (ELISA assay) levels were also examined. 6-OHDA-induced nigrostriatal denervation was associated with the following histopathological changes observed in the colonic wall: eosinophil and mast cell infiltration, collagen deposition, activation of myenteric glial cells (GFAP+), increased vimentin immunostaining associated with alpha-SMA decrease in the tunica muscularis, enhanced colonic tissue levels of MDA, TNF and IL-1β. On the basis of the present results it is possible to conclude that the induction of central nigrostriatal dopaminergic denervation is followed by inflammation and fibrotic rearrangement of the colonic wall.

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Keywords

Parkinson's disease; experimental dopaminergic nigrostriatal neurodegeneration; colonic rearrangement.

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Connexin 26 expression in mammalian cardiomyocytes

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Connexins (Cxs) are a family of membrane-spanning proteins named according to their molecular weight. They have been known to form membrane channels mediating cell-cell communication, which play an essential role in the propagation of electrical activity throughout the heart. So far, expression of seven isoforms, namely Cx30.2, Cx37, Cx40, Cx43, Cx45, Cx46 and Cx57, have been found in cardiac myocytes (1,2). Cx26 has been described in a number of tissues but not yet in the heart, and its mutations are frequently associated with deafness and skin diseases (3,4). To our knowledge, the expression of Cx26 also in human, pig, rat and mouse cardiomyocytes has been demonstrated for the first time in the present study. Interestingly, this Cx was found as scattered throughout cell cytoplasm but not at level of the intercalated disks where the other cardiac Cxs are mainly located. Furthermore, in cardiomyocytes of a pig model of left ventricular dysfunction (LVD), Cx26 expression was modulated and dipyridamole treatment, which was previously demonstrated to have a protective action on left ventricular function (5), was associated to an increased Cx26 expression. Dipyridamole induced the same effect in cardiac rat cell line H9c2. For our study, paraffin embedded sections of human auricle, pig ventricle, mouse whole heart and H9c2 cells were used. Several methods were employed to test the expression of Cx26. In particular, different immunohistochemical and molecular biology techniques were performed by using two types of primary anti-Cx26 antibodies to ascertain the specificity of cardiomyocyte immunopositivity for Cx26 avoiding analysis-dependent artifacts.

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Keywords

Connexin 26; connexin 43; cardiomyocytes; human; pig; mouse; immunohistochemistry; dipyridamole.

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CHI3L1 and lubricin expression in osteoarthritic cartilage

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Osteoarthritis (OA) is the most common human arthritis characterized by degeneration of articular cartilage. Several studies reported that levels of human cartilage glycoprotein 39 (GP-39) are known as a potential marker for the activation of chondrocytes and the progression of OA, whereas lubricin appears to be chondroprotective. The aim of this study was to investigate the co-expression and co-localization of CHI3L1 and lubricin in normal and osteoarthritic rat articular cartilage to correlate their modified expression to a specific grade of OA. Samples of normal and osteoarthritic rat articular cartilage were analyzed by the Kellgren-Lawrence OA severity scores, the Kraus' modified Mankin score and the Histopathology OARSI system for histomorphometric evaluations, and through CHI3L1 and lubricin gene expression, immunohistochemistry and double immuno-staining analysis. The immunoexpression and the mRNA levels of lubricin increased in normal cartilage and decreased in OA cartilage. By contrast, the immunoexpression and the mRNA levels of CHI3L1 increased in OA cartilage and decreased in normal cartilage. Our findings are consistent with reports suggesting that these two glycoproteins are functionally associated with the development of OA and in particular with grade 2/3 of OA evidenced in histomorphometric analysis of our samples, so that they could have a role in the daily clinical practice in staging the severity and progression of the disease.

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Keywords

Lubricin; CHI3L1; osteoarthritis; anterior cruciate ligament transection (ACLT); immunohistochemistry; mRNA.

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Activation of anti-inflammatory cell pathways in skin ulcers upon photodynamic therapy

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This study was aimed at assessing the variations in skin histology in ulcers caused by chronic venous insufficiency of the lower extremities, upon photodynamic therapy (PDT). The study was approved by the ethical committee of Azienda Sanitaria Firenze. Patient assessment included clinical history, physical examination and echo-Doppler sonography. Four to five sessions of photodynamic therapy (20% 5-aminolevulinic acid gel application followed by 3 min irradiation at 630 nm, total 180 J/cm2) were administered to 15 patients refractory to previous conventional treatments. Skin biopsies were embedded in freezing tissue medium and quick frozen. Cryosections were post fixed in cold acetone. Sections from each case were stained with hematoxylin and eosin or labelled with primary antibodies against the following antigens: MHC-II class, DC-SIGN, CD68, CD163, BDCA2, CD4, CD25, TNF alpha. In some instances avidin and Ulex europaeus lectin were used to tag mast cells and vessels respectively. Upon treatment, MHC-II signal intensity per positive cell and TNF alpha signal in mast cells increased, as well as the numbers of CD68 positive/CD163 positive cells (M2 macrophages), BDCA2 positive (plasmacytoid dendritic) cells and CD4 positive/CD25 positive (Treg) lymphocytes number. Diffuse tissue TGF beta positivity also increased. DC-SIGN positive cells decreased in number. Mast cells were found in proximity of dendritic cells and of vessels; plasmacytoid dendritic cells were found in proximity of T reg cells. Clinically, mild decrease in ulcer size and granulation at ulcer borders were observed. Therefore treatment apparently led to the activation of cells and of intercellular communication pathways possibly down-regulating the inflammatory response. The same treatment had been shown to increase mast cell expression of basic fibroblast growth factor and fibroblast number (1), potentially responsible for increased production of extracellular matrix. Both types of effects could by synergistically beneficial for ulcer repair.

This work was supported by the Italian Ministry of Education, MIUR FIRB 2010 and MIUR PRIN-2009; University and Research, Ente Cassa di Risparmio di Firenze (Grant n. 3681 year 2012) and Foemina Foundation.

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Keywords

Dendritic cells; mast cells; photodynamic therapy; PDT; T cells.

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Morphological changes in the bladder of spinal cord injured patients affected by detrusor overactivity

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It is well known that the neurogenic detrusor over activity (NDO) represents one of the most disabling condition in patients with suprasacral spinal cord injury (SCI). The treatments with anti-muscarinic drugs and botulinum toxin local injection show a temporary efficacy in controlling the bladder impairment; after that, the typical NDO urodynamical signs, such as reduced bladder capacity, high intravesical pressure with potentially upper urinary tract damage, reoccurs (1). At this stage, these patients can choice to perform a cystectomy with or without bladder augmentation. In order to investigate the pathological remodelling of bladder wall, we collected bladder specimens from SCI patients underwent to cystectomy. Control specimens were obtained from patients underwent to surgery for bladder cancer. Data obtained from clinical records, histology and immunohistochemistry were paralleled. The group of SCI patients were uniform on the basis of age, legion level, previous therapies and urodinamic parameters. These latter indicated a strong reduction of bladder capacity and reflex volume. Histologically, SCI bladder showed an important inflammatory state with oedema and congestive blood vessels in the submucosa and lamina propria; in the most serious cases, phlogosis was present also in the detrusor and characterized by eosinophilic infiltrate. Moreover, an important alteration of smooth muscle cell organization was appreciable. The immunohistochemical experiments demonstrated a redistribution of alpha-smooth muscle actin filaments (αSMA) in the smooth muscle cells associated with an altered distribution of caveolin 1 (cav1). Present findings show consistent alteration of the contractile structures and molecules that might explain the recurrence of NDO sign and symptoms in SCI patients. It remains to understand whether these changes are side effects of pharmacological therapies or instead the typical evolution of the disease that the therapies, eventually, postponed.

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Keywords

Spinal cord injury; neurogenic bladder over activity; actin remodeling.

VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI SIAI, 2015

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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 Chimico/Biomedico (MAMMUT) dell'Università degli Studi di Ferrara

In data 18 Settembre 2015, 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 Chimico/Biomedico (Mammut) dell'Università degli Studi di Ferrara, in occasione del 69° 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 2014 e sulla previsione finanziaria per il 2016. 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 2016; 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 il Presidente del Congresso, Prof. Silvano Capitani, nonché i suoi Collaboratori, per l'impegno profuso nell'organizzazione del 69° Congresso SIAI.

Il Prof. Gaudio comunica ai Soci che in data 17 Settembre 2015 si è svolto il Consiglio Direttivo della SIAI, che ha preso atto delle dimissioni presentate dal Prof. Lucio Cocco. Il Prof. Gaudio comunica all'Assemblea che cercherà di far recedere il Prof. Cocco dal suo proposito, visto il suo alto profilo scientifico e l'importante contributo dato negli anni alla SIAI.

Il Prof. Gaudio passa quindi alla trattazione degli altri punti all'O.d.G., che sono stati discussi in seno al Consiglio Direttivo.

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. Orlandini commemora il **Prof. Giuseppe Carlo Balboni**, scomparso nel mese di Settembre 2014. Il Prof. Cristiano Rumio commemora il **Prof. Paolo Castano**, scomparso nel mese di Novembre 2013. La Prof. Elisabetta Caramelli commemora il **Prof. Gian Paolo Bagnara**, scomparso nel mese di Luglio 2014. La Dott. Claudia Dolci commemora la **Prof. Laura Vizzotto**, scomparsa nel mese di Settembre 2014. Il Prof. Paolo Clavenzani commemora il **Prof. Ruggero Bortolami**, scomparso nel mese di Luglio 2014. La Prof. Stefania Montagnani commemora la **Dott. Maria Pia Cinelli**, scomparsa nel mese di Marzo 2015.

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. Rosa Alba Rana, Prof. Anto De Pol e Prof. Marco Vitale.

Il giorno 18 Settembre 2015 si è riunita la Commissione dei Revisori dei Conti designata in seno alla Società Italiana di Anatomia e Istologia e costituita dai Proff.: Rana Rosalba, De Pol Anto e Vitale Marco.
Dopo aver valutato attentamente il conto Consuntivo relativo all'anno 2014 e il conto di previsione relativo all'anno 2016, 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 2014, qui di seguito riportato assieme alla relazione di accompagnamento.

Bilancio consuntivo anno 2014

Causale delle entrate	Entrate Euro	Causale delle uscite	Uscite Euro
Quote sociali incassate nel corso dell'anno 2014 (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)	**19.207,42**		
		Elenco spese per attività statutarie	
Donazione in memoria del Prof. R.A. Stockwell	1.248,71	Premi poster, anno 2013	**854,00**
		Contributo al 68° Congresso SIAI, anno 2014	**3.000,00**
		Contributo al Convegno G.I.S.N, anno 2014	**500,00**

		Premio alla Carriera, anno 2014	**573,40**
		Spese varie (mantenimento conto corrente postale e bancario, spese bollo e commissioni bancarie, anno 2014)	**640,60**
		Contributo per l'organizzazione del Convegno in onore di Clemente Susini, anno 2014	**1.300,00**
		Partecipazione alla riunione EFEM, anno 2014	**1.344,84**
		Spese impreviste: storno somme erroneamente versate a SIAI, anno 2014	**160,00**
		Spese premio Giovane Ricercatore, anno 2014	**2.000,00**
		Spese per Iscrizione EFEM, anno 2014	**520,00**
		Spese premio migliore comunicazione orale al Congresso Nazionale SIAI, anno 2014	**1.000,00**
		Partecipazione riunione IFAA, anno 2014	**1.321,78**
		Totale spese attività statutarie	**13.214,62**
		Elenco spese di funzionamento	
		Spese per consulenza del Commercialista, anno 2013	**1.500,60**
		Versamento deleghe fiscali per compensi Commercialista, anni 2012 e 2013	**572,57**
		Spese per utilizzo server UNIFI per sito web SIAI, anno 2013	**272,06**
		Spese per il funzionamento del Consiglio Direttivo, anno 2014	**1.381,28**
		Totale spese di funzionamento	**3.726,51**
Totale entrate	**20.456,13**	**Totale uscite**	**16.941,13**
Avanzo d' esercizio finanziario 2014	**3.515,00**		
Saldo Conto corrente Bancario al 31/12/2013	**14.700,36**		
Saldo Conto corrente postale al 31/12/2013	**4.630,57**		
Totale saldo finanziario al 31/12/2013	**19.330,93**		
Avanzo gestione esercizio 2014	**3.515,00**		
Saldo finanziario al 31/12/2014	**22.845,93**		

Stanziamenti impegnati Al 31/12/2014	**Euro**	**Euro**
Accantonamento premi poster e comunicazione orale, anno 2014 (rimane premio migliori poster 2014)		**1.000,00**
Contributo all'It. J. Anat. Embryol., anno 2014		**4.000,00**
Spese per il funzionamento della Presidenza, anno 2014		**1.000,00**
Spese per il funzionamento della Tesoreria, anno 2014		**1.000,00**
Consulenza Commercialista, anno 2014		**1.500,00**
Ritenuta d'acconto		**280,80**
Spese per il sito web della Società, anno 2014		**400,00**
Spese per ECM, anno 2014		**500,00**
Totale impegno di spesa		**9.680,80**
Saldo disponibile	**13.165,13**	

*Relazione di accompagnamento al rendiconto economico e finanziario per l'anno **2014***

Come risulta dal Bilancio Consuntivo il saldo finanziario al 31/12/ 2014 è pari ad **€ 22.845,93** ed è costituito dal saldo finanziario al 31/12/2013 pari a **€ 19.330,93** e dall'avanzo dell'esercizio 2014 pari a **€ 3.515,00**.

A tale importo devono essere sottratti **€ 9.680,80** impegnati nel Bilancio Previsionale del 2014, ma non ancora effettivamente utilizzati alla data del 31/12/2014, per le seguenti voci di spesa:

- **Accantonamento premi poster e comunicazione orale, anno 2014:**
  Per questa voce risultano stanziati, nel previsionale 2014, **€ 2.000,00** che in parte (**€ 1.000,00**) sono stati utilizzati nel corso del 68° Congresso Nazionale della Società del 2014 per il premio alla migliore comunicazione orale e nella parte rimanente (**€ 1.000,00**) saranno utilizzati per il pagamento delle quote di iscrizione al 69° Congresso Nazionale della Società del 2015 di due Soci risultati vincitori dei premi poster nel Congresso societario del 2014;
  - **Contributo all'It. J. Anat. Embryol.,** anno 2014: **€ 4.000,00;**
  - **Spese per il funzionamento della Presidenza,** anno 2014: **€ 1.000,00;**
  - **Spese per il funzionamento della Tesoreria,** anno 2014: **€ 1.000,00;**
  - **Consulenza Commercialista,** anno 2014: **€ 1.780,80;**
  - **Spese per il sito web della Società,** anno 2014: **€ 400,00;**
  - **Spese per ECM,** anno 2013: **€ 500,00.**

  Pertanto l'anno 2014 si è chiuso con un saldo disponibile di **€ 13.165,13**.

Durante il 2014 le quote associative incassate sono state **320** comprese alcune quote arretrate ed integrazioni di versamenti di quote non corretti, per un totale di **€**

19.207,42 che sommate al saldo finanziario al 31/12/2013 (**€ 19.330,93**) e alla somma ricevuta come donazione in memoria del Prof. R. A. Stockwell pari a **€ 1248,71**, hanno dato la disponibilità di **€ 39.787,06**.

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

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

La rispondenza dei Soci ai solleciti da parte del Tesoriere in merito alla regolarizzazione dei pagamenti delle quote associative si mantiene discreta, tuttavia, al 31 dicembre 2014, 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 di tutte le quote arretrate consentirebbe alla SIAI di intraprendere nuove iniziative.

Il Presidente, nel ringraziare il Prof. Dolfi per l'accuratezza del rendiconto pone in votazione il Bilancio Consuntivo 2014.

L'Assemblea approva all'unanimità.

Il Presidente dà quindi la parola al Tesoriere per illustrare la Previsione Finanziaria per il 2016, qui di seguito riportata assieme alla relazione di accompagnamento.

SOCI NEL 2014: 517
SOCI NEL 2015: 526
SOCI ORDINARI 2015: 499 (5 soci hanno versato la quota del 2016)

ENTRATE

Quote sociali anno 2015 **(n. 494)**	€	29.640,00
Quote sociali arretrate 2007 - 2014	€	6.000,00
Totale entrate	**€**	**35.640,00**

USCITE

Contributo al convegno nazionale 2016, atti di convegni, altri contributi a convegni, partecipazione convegni, organizzazione eventi scientifici, borse di studio, etc.	€	12.000,00
Accantonamento per premi poster e comunicazione anno 2016	€	2.000,00
Accantonamento per premi SIAI (Carriera e Ricercatore under 40) anno 2016	€	4.000,00
Contributo all' It. J. Anat. Embryol., anno 2016	€	4.000,00
Spese per sito web della Società, anno 2016	€	300,00
Spese per ECM, anno 2016	€	530,00
Spese per la partecipazione Meeting Comitato Internazionale per la Terminologia Anatomica e Istologica, FICAT, anno 2016	€	3.500,00
Quota adesione all'European Federation for Experimental Morphology, EFEM, anno 2016	€	530,00

Spese varie (bancarie, postali, necrologi, etc.), anno 2016	€	1.000,00
Spese impreviste, anno 2016	€	1.000,00
Totale spese per attività statutarie	***€***	***28.860,00***
Spese per il funzionamento della Presidenza, anno 2016	€	1.000,00
Spese per il funzionamento della Segreteria, anno 2016	€	1.000,00
Spese per il funzionamento della Tesoreria, anno 2016	€	1.000,00
Spese per il funzionamento del Consiglio Direttivo, anno 2016	€	2.000,00
Spese per consulenza Commercialista, anno 2016	€	1.780,00
Totale spese di funzionamento	***€***	***6.780,00***
Totale uscite	**€**	**35.640,00**

Relazione di accompagnamento alla previsione finanziaria per l'anno 2016

La chiusura del bilancio consuntivo del 2014 con un saldo disponibile di **€ 13.165,13** ha permesso al Tesoriere di sostenere anche alcune spese indicate nella previsione finanziaria del 2015.

Il Tesoriere, nel corso di questo anno, oltre a cercare di riscuotere le quote associative del 2015, ha continuato l'azione di recupero di quelle arretrate. Tuttavia, al 31 agosto '15, sono state incassate soltanto 49 quote sociali (anno 2015 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 **€ 4.140,00** e comprende le quote riscosse finora.

Comunque il piano previsionale del 2015 prevedeva entrate pari a **€ 35.280,00** dovute alla riscossione delle quote dell'anno in corso, più una cifra forfettaria concernente il recupero delle quote arretrate. In particolare, in tale previsione, come in quelle degli anni precedenti, è stata indicata questa cifra forfettaria sulla base dell'esperienza relativa alle difficoltà di ottenere il pagamento degli arretrati da tutti i Soci non in regola.

La Società conta attualmente **526** Soci, di cui **499** Soci ordinari e **27** Soci Emeriti o Onorari (esonerati dal pagamento della quota sociale).

Allo stato attuale, dei 499 Soci che sono tenuti a pagare la quota associativa:

- 5 Soci sono in regola fino al 2016
- 44 Soci sono in regola fino al 2015
- 11 nuovi Soci devono la quota 2015
- 164 Soci devono la quota 2015
- 53 Soci devono la quote del 2014 e del 2015
- 43 Soci devono le quote del 2013, 2014 e 2015
- 54 Soci devono le quote del 2012, 2013, 2014 e 2015
- 18 Soci devono le quote del 2011, 2012, 2013, 2014 e 2015

- 34 Soci devono le quote del 2010, 2011, 2012, 2013, 2014 e 2015
- 27 Soci devono le quote del 2009, 2010, 2011, 2012, 2013, 2014 e 2015
- 26 Soci devono le quote del 2008, 2009, 2010, 2011, 2012, 2013, 2014 e 2015
- 20 Soci devono le quote del 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014 e 2015

Il Tesoriere fa presente che cercherà di raggiungere la parità di bilancio e di fare previsioni finanziarie quanto più possibile aderenti alla realtà. Rimane ancora un certo numero di Soci che non hanno adeguatamente risposto ai solleciti di pagamento; il Tesoriere si impegna ad esercitare a sua volta una azione di richiamo ricordando 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 il recupero delle quote arretrate e l'incasso puntuale delle quote annuali permetterebbero alla SIAI di raggiungere meglio questi scopi.

Il Presidente ringrazia il Prof. Amelio 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 2016.

L'Assemblea approva all'unanimità.

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

Il Prof. De Caro, Presidente del Collegio dei Docenti di Anatomia, presenta una breve relazione sull'attività svolta nell'anno accademico in corso. Le riunioni del Collegio si sono svolte nel Dicembre 2014 in seduta plenaria; nel Febbraio 2015 in seduta ristretta; nel Giugno 2015 in seduta plenaria e in riunione telematica del Consiglio Direttivo. I principali temi affrontati sono stati: a. relazioni sulla partecipazione all'attività della Conferenza dei Presidenti dei Collegi dell'Area Medica; b. informative su: Manifesto sulla Formazione in Medicina, Corsi a numero programmato e aggiornamento su situazione dell'M-EDF. I principali documenti prodotti sono stati: a. risultati della ricognizione CFU e ripartizione esami di Anatomia nel Corso di Laurea in Medicina e Chirurgia per anno/semestre nelle diverse sedi; b. documento sulla abilitazione scientifica nazionale; c. posizione dell'Anatomia nella suddivisione delle risorse negli Atenei e nei Dipartimenti: indice di produttività; d. Scuole di Specializzazione e Anatomia. Il Prof. De Caro informa che la prossima riunione del Collegio è programmata per Novembre 2015.

La Prof. Sica comunica che durante l'Assemblea del Collegio dei Docenti di Istologia ed Embriologia tenutasi il 6 Febbraio 2015 è stata riconfermata per acclamazione Presidente del Collegio dei Docenti di Istologia ed Embriologia, per il triennio 6/2/2015 – 6/2/2018, e che nella stessa Assemblea è stata ratificata la carica di Segretario e Tesoriere nella persona del Prof. Gianpaolo Papaccio. Si sono svolte inoltre le elezioni di 3 Membri della Giunta nelle persone dei Proff. Anto De Pol, Fulvia Ortolani ed Elio Ziparo. In seno al Collegio sono state affrontate le seguenti importanti tematiche: modifica della Declaratoria del Settore Scientifico Disciplinare BIO 17 e analisi della situazione del Settore; problematiche inerenti all'Abilitazione Scientifica Nazionale; rapporti con la Conferenza Permanente dei Collegi di Area Medica.

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. Giuseppe Musumeci**, Ricercatore presso il Dipartimento di Scienze Biomediche e Biotecnologie dell'Università di Catania (Indice H: 8), candidato già menzionato lo scorso anno durante il 68° Congresso della SIAI.

Il Presidente consegna il premio al Dott. Giuseppe Musumeci.

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. Francesco Osculati**, di cui traccia un breve profilo. Il Prof. Osculati ha dato un rilevante contributo alla comunità scientifica manifestando un impegno costante e duraturo sul piano scientifico nonché organizzativo. Ha creato il Laboratorio di Anatomia Umana delle Università di Ancona e di Verona, entrambi attrezzati con strumenti all'avanguardia, che hanno consentito ai suoi collaboratori ed allievi di produrre un elevato numero di lavori scientifici di livello internazionale. In entrambe le sedi il Prof. Osculati ha svolto l'importante ruolo di Preside della Facoltà di Medicina. A livello nazionale, il Prof. Osculati si è sempre battuto per mantenere l'identità e lo spazio accademico delle discipline morfologiche.

Pertanto, a nome di tutta la SIAI, il Presidente consegna una Targa d'argento ed una pergamena al Prof. Osculati che, con un breve discorso, ringrazia per l'onore riservatogli.

8. Assegnazione premi poster.

Il Presidente riferisce che la Commissione per l'attribuzione dei premi poster, formata dai Proff. S. Montagnani, R. Di Primio e M. De Mattei, nominata dal Consiglio Direttivo, si congratula per l'elevato livello scientifico raggiunto dai vari gruppi di ricerca, e, dopo un'attenta valutazione, ha deciso all'unanimità di segnalare i seguenti poster:

- *Frontal sinus drainage pathway and uncinate process: a single morphofunctional unit with a complex anatomy*
  Marco Ferrari, Luca Pianta, Paolo Rondi, Riccardo Nocini, Vittorio Rampinelli, Piero Nicolai, Barbara, Buffoli (Brescia).

- *Bilayered scaffolds colonized with dental pulp stem cells for osteochondral tissue engineering application*
  Laura Bertoni, Manuela Zavatti, Elisa Resca, Tullia Maraldi, Francesca Beretti, Gianluca Carnevale, Alessandra Pisciotta, Anto De Pol (Modena).

Il Presidente comunica che la premiazione avverrà alla fine del Congresso e il verbale della Commissione Poster verrà spedito a tutti i Soci SIAI.

9. Assegnazione premio migliore comunicazione orale.

Il Presidente riferisce che la Commissione nominata dal Consiglio Direttivo e

costituita dai Proff. M. Bentivoglio, G. Cavaletti, e G. Sica, dopo aver stabilito i criteri ai fini della valutazione, ha deciso all'unanimità l'assegnazione del premio di Euro 1.000,00 alla **Dott.ssa Grazia Maugeri**, per la migliore comunicazione orale dal titolo "Parkin Interferes With Hypoxia- Inducible Factors Expression in Glioblastoma Cells" nell'ambito della Sessione "Neuroscienze (II)".

Il Presidente consegna il Premio alla Dott.ssa Maugeri.

10. Prossimi Congressi nazionali della SIAI e Congressi nazionali ed internazionali previsti per l'anno 2014; proposte di temi di relazione.

Il Presidente ringrazia il Prof. Capitani che ha dato la sua disponibilità ad organizzare per il 2015 il 69° Congresso Nazionale della SIAI nella sede di Ferrara e ricorda che il 70° Congresso Nazionale della SIAI si terrà presso l'Università Cattolica del Sacro Cuore in Roma.

In merito ai temi di relazione, il Presidente riferisce che in seno al Direttivo, relativamente alle relazioni da tenere al 70° Congresso SIAI, la Prof. G. Sica ha proposto di invitare a tenere una delle due letture magistrali la Dott. Ornella Parolini, Direttore del Centro di Ricerca Ettore Menni, presso la Fondazione Poliambulanza di Brescia. La Dott. Parolini è autrice di numerose pubblicazioni su riviste impattate e molte di queste sono dedicate allo studio delle cellule staminali derivate da annessi fetali ed alla loro applicazione nel settore della Medicina Rigenerativa. La Dott.ssa Parolini è Responsabile di numerosi progetti di ricerca nazionali ed internazionali e fondatrice dell'International Placenta Stem Cell Society.

Il Prof. S. Adamo propone che la seconda relazione possa essere tenuta dal Prof. Antonio Filippini sul tema del ruolo dei segnali del calcio nell'angiogenesi.

Il Presidente ha invitato la componente anatomica ad avanzare ulteriori proposte e si è deciso all'unanimità di prendere delle decisioni nel prossimo Consiglio Direttivo.

Il Presidente comunica che il Prof. Anastasi ed il Prof. Papaccio hanno presentato la candidatura delle sedi di Messina e di Napoli rispettivamente per il 71° ed il 72°Congresso Nazionale della SIAI.

11. Problemi relativi all'Italian Journal of Anatomy and Embryology: relazione dell'Editor in Chief, Prof. Paolo Romagnoli.

Il Prof. Gaudio dà la parola al Prof. Romagnoli, il quale riferisce che nel corso del 2015 è stato recuperato in buona parte il ritardo accumulato nel 2014 per via di problemi interni alla casa editrice e alla stamperia. La puntualità è maggiore per l'edizione on line che per quella cartacea. E' stata inoltre riavviata la recensione su PubMed, che aveva avuto un arresto per il fallimento dell'intermediario che trasmetteva l'indice alla banca dati. Adesso provvede direttamente la FUP e la recensione è tempestiva, con l'inevitabile attesa per la registrazione dei dati.

L'amministrazione non presenta problemi, grazie al contributo della SIAI e al pagamento delle spese da parte degli autori. La richiesta a questi ultimi è assai contenuta: la tariffa è sempre 40,00 euro a pagina + IVA e comprende sia la stampa in bianco e nero sia la pubblicazione on line a colori in open access.

Ciò nonostante rimane viva la difficoltà dei pagamenti da parte di autori stranieri - soprattutto di alcuni Paesi con restrizioni economiche o finanziarie - e permangono alcuni problemi di contabilizzazione da parte della FUP anche per autori italiani, che

il Prof. Romagnoli sta cercando di risolvere. D'altra parte il contributo di autori stranieri è fondamentale per assicurare non solo la vita della Rivista, ma anche una sua valida collocazione internazionale.

D'intesa con gli esperti della Firenze University Press è stato deciso di attendere ancora per richiedere di essere presi in esame per un impact factor: poiché la selezione è competitiva una mancata domanda non nuoce, mentre un eventuale rifiuto - anche per ragioni organizzative, come per una non puntuale cadenza di stampa - verrebbe inevitabilmente risaputo nell'ambiente scientifico e ridurrebbe il credito della Rivista.

Il Prof. Romagnoli rileva che nella pubblicazione degli articoli sussiste un certo sbilanciamento a favore dell'Anatomia macroscopica, compresa la casistica anatomica, rispetto alla biologia della cellula, dei tessuti e dello sviluppo.

La Rivista è tuttavia apprezzata e molto giova l'avere ogni articolo un "doi" (digital object identifier), il che semplifica e promuove la diffusione degli articoli stessi e aumenta il loro peso ai fini della valutazione anche da parte degli Atenei e delle Agenzie nazionali.

Il punteggio nella valutazione internazionale secondo Scopus (SCImago Journal Ranking) è modesto anche se non irrisorio, ed è necessario impegnarsi per migliorarlo: la posizione è nel terzo quartile per l'Anatomia e nel quarto per l'Embriologia, indicatore SJR 0,18.

Lo SCImago Journal Rank o SJR indicator è un indicatore che misura il grado di influenza scientifica delle riviste accademiche; utilizza il numero di citazioni ricevute da un giornale e l'importanza o il prestigio delle riviste da cui tali citazioni provengono mediante un algoritmo simile a PageRank e fornisce un'alternativa all'impact factor (IF) che invece si basa sui dati del Science Citation Index. Utilizza la stessa formula del journal impact factor della Thomson Reuters.[Butler D., Free journal-ranking tool enters citation market. Nature 451: 6, DOI:10.1038/451006a - Falagas M.E. et al, Comparison of SCImago journal rank indicator with journal impact factor. FASEB J. 22: 2623-2628, DOI:10.1096/fj.08-107938].

Chi volesse vedere in dettaglio il ranking secondo SCImago e le altre statistiche correlate può andare all'indirizzo web specifico per la nostra Rivista:

http://www.scimagojr.com/journalsearch.php?q=9500154001&tip=sid&clean=0.

12. Proposta di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.

Sono pervenute 11 domande di ammissione a Socio SIAI da parte di:

Cacciola Alberto
Corvino Valentina
Favero Gaia
Geloso Maria Concetta
Lauriola Mattia
Marcenaro Emanuela
Montaruli Angela
Moretta Alessandro
Ongaro Alessia
Parolini Ornella
Sivori Simona

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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