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ADVANCES IN MOLECULAR THERAPIES

AMT1. *In Vitro* Evaluation of Type II Ribosome-Inactivating Proteins (RIPs) for Experimental Chemoablation of Muscle Cells in Strabismus and Eye-Movement Disorders

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Background: Today the treatment with botulinum toxin (BTX) is the most used molecular surgery of strabismus and other eye-movement disorders, as alternative to traditional surgery. However the temporary effect of BTX treatment requires the research of alternative therapies. To this purpose the *in vitro* effects of three type II RIPs from plants (lanceolin, stenodactylin and ricin)and of the skeletal muscle-specific immunotoxin saporin-mAb73 were evaluated on muscle cells. **Methods:** The RIPs and the immunotoxin were tested for their cytotoxicity in three cell lines: L6E9 (myoblasts), TE671 and RD/18 (rhabdomyosarcoma), both undifferentiated and differentiated. The aspecific toxicity was evaluated on conjunctival IOBA-NHC cell line. Protein synthesis inhibition, viability and apoptotic changes were assayed. **Results:** All substances showed a strong cytotoxic effect on protein synthesis and viability, with IC50 and LC50 ranging from 0.1 nM to 0.01 pM. Lanceolin and stenodactylin were 1-2 logs more toxic than ricin and 2-3 logs more toxic than the immunotoxin. Myoblasts were particularly susceptible to stenodactylin (IC50<0.01pM). All RIP-treated cells showed typical morphological apoptotic changes and no signs of necrosis. In further experiments miming *in vivo* treatment, no toxic effects were reported on conjunctival IOBA-NHC cell line. **Conclusions:** The strong cytotoxicity observed for stenodactylin at very low dose could be compatible with loco-regional treatments in strabismus and eye-movement disorders. It could be possible to modulate the effect on muscle fibers and to obtain a complete ablation of myoblasts, gaining more durable effects as compared to BTX treatment. Moreover, the absence of necrosis should avoid flogistic side effects.

AGING

AGE1. The Role of Dermal Fibroblasts in the Development of Ectopic Calcifications

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Background: Ectopic calcifications (EC) represent a deleterious consequence of diabetes, renal disorders and aging, being a key determinant of cardiovascular morbidity and mortality. Although the molecular pathways leading to the undesired mineralization of soft connective tissues have been largely investigated in smooth muscle cell cultures (SMC), no effective treatments are available. **Methods:** To further investigate EC, dermal fibroblasts (DF) from healthy individuals and from patients affected by Pseudoxanthoma elasticum, a disease characterized by progressive calcification of elastic fibres, were grown up to 30 days in a standard or in a calcifying medium. **Results:** Degree of mineralization was evaluated after Von Kossa staining, whereas markers of calcification (ALP, ANKH, BMP2, ENPP1, MGP, SPP1) were assessed by RT-PCR and Western Blot. **Conclusions:** Data demonstrate that: 1) DF can be responsible for ectopic calcifications *in vivo*, but, as all other mesenchymal cells, require a specific medium to mineralize *in vitro*; 2) in contrast to SMC, cultured DF do not develop a calcifying signature resembling that of osteoblasts; 3) changes in osteogenic markers are mostly related to the duration of cell cultures; 4) development of a calcified matrix is tightly dependent on the characteristics of the extracellular environment and the availability of phosphate donor substrates; 5) increased ALP activity is necessary but not sufficient to have mineral deposit formation; 6) the complex balance between pro- and anti-calcifying factors, including circulating factors as fetuin, plays a significant role in the occurrence of ectopic calcifications *in vivo*. Work supported by FCRMO(EctoCal).

AGE2. Vascular Aging Effect on Medial Aorta Degeneration: Focus on Blood Leukocyte Telomere Length in Hypertensive and Old Patients with Sporadic Thoracic Aortic Aneurysm

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Background: Aging is a well recognised factor in the development of cardiovascular diseases (i.e. sporadic thoracic aortic aneurysm). The physiological aging process determines various changes and a progressive deterioration in structure and function of heart and vascular system, i.e. thoracic aorta. As consequence of these age-related modifications having catalyst and accelerator effect for sporadic thoracic aortic aneurysm (S-TAA), medial degeneration occurs. This pathological entity leads to weakening of aorta wall, which in turn results in aortic dilatation, aneurysm, increased risk for aortic dissections and ruptures. Thus, S-TAA risk increases with chronological as well as biological aging. One optimal marker of this might be peripheral blood leukocyte telomere content. It accurately reflects that of vascular wall and its decrease is associated with premature vascular disease. Thus, the aim of this study was the evaluation of mean blood leukocyte telomere length as predictor for S-TAA. **Methods:** Peripheral blood samples were collected from TAA patients and age- and gender matched controls. Genomic DNA was extracted from leukocytes and telomere length was determined using a chemioluminescence technique. We examined patients and controls selected randomly, but considering the same age and gender. **Results:** A significant lower mean telomere length was detected in TAA group, significantly correlated with age, smoking, hypertension, inflammatory cellular infiltrate and genetic inflammatory variants **Conclusions:** Thus, telomere assay could contribute to identify individuals at risk for S-TAA. Accordingly, our results should seem to suggest that vascular biological aging might have a strong role in the S-TAA pathogenesis.

AGE3. Trafficking Profile in Naive and Memory B Cells in Young and Old Subjects

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Background: The impairment of humoral immune response in the elderly human population has been extensively demonstrated. We have reported the increase of a memory B cell (IgG+IgD-CD27-, double negative, DN) population in the elderly, in which there is also a typical inflammatory micro-environment. To evaluate whether this pro-inflammatory status could influence the trafficking phenotype of naive/memory B cells, we have assessed the expression of CCR7, CCR6, CXCR3, CXCR4, CXCR5 and CD62L on naive/memory B subpopulations in young and elderly subjects. **Methods:** We evaluated the expression of some receptors involved in trafficking on different naive/memory B cell subpopulations by flow cytometry approach, using a FACSCALIBUR Cytometer (BD, CA, US). **Results:** In young donors naive/memory B lymphocytes express different chemokine receptors according to the stage of peripheral maturation, whereas the DN B population