EVALUATION OF GALECTIN-3 IN THE CROSS-TALK BETWEEN MUSCLE AND BONE

Emanuela AMORE[1], Carla PALUMBO[2], Jessika BERTACCHINI[1]

[1]Department of Surgery, Medicine Dentistry and Morphological Sciences with Interest in Transplant, University of Modena and Reggio Emilia, Modena; [2]Department of Biomedical, Metabolic Science and Neuroscience, Section of Human Morphology, University of Modena and Reggio Emilia, Modena, Italy

Galectin-3 (Gal-3) is a pleiotropic lectin that has been recognized over the past two decades as being involved in many physiological and pathological processes. Although the galectin field has been a lot studied remain incompletely understood, in particular the molecular mechanisms of their actions in intra and extracellular space and the relationship between these activities, as well as an associated regulatory loop. Since it is well described that Gal-3 is widely distributed both in the developing and mature bone and that its expression is under control of the master regulator of bone growth RUNX2, it could suggest that Gal-3 may be a key player in all stages of bone biology. Many types of bone pathologies are strikingly related to severe damage inputs affecting the bone-associated tissues. In particular, it is well recognized that bone and muscle have recently been identified as endocrine organs, that secrete cytokines and chemokines, through which they interact to promote bone formation, repair and maintenance in the bone-muscle cross talk. For that, the general aim of our study is to define the role of Gal-3 on bone tissue and its possible involvement in skeletal muscle crosstalk. First, we found an increase of Gal- 3 expression during myogenic differentiation and that it was secreted in the culture medium. Through a coculture experiments between myogenic cells and osteoblast cells we documented that recombinant Gal-3 inhibited osteoblast differentiation and since it may be argued that it may have a different conformation state in comparison to endogenous Gal-3, we next tested whether the silencing of Gal-3 secreted by muscle cells affect osteoblast differentiation at the same manner. In order to investigate the effects induced by extracellular Gal-3 on bone cells, focusing on signaling events unleashed by the recombinant protein, by a mass spectrometric approach, we analyzed the entire proteome obtained by differentiated osteoblasts differently affected by the presence of recombinant Gal-3. Moreover, through an *ECM* Cell *Adhesion* Array *kit* we monitored the impact of Gal3 on the extracellular matrix complex, evaluating specific cell surface Integrins and Adhesion molecules upon Gal-3 silencing, inhibition and overexpression.

EFFECTS OF SIMULATED MICROGRAVITY ON HUMAN PANCREATIC ADENOCARCINOMA (PDAC) MULTICELLULAR SPHEROIDS

Valentina BONETTO[1], Marcello MANFREDI[2], Emilio MARENGO[1], Maria Angela MASINI[1]

[1]DiSIT, University of Eastern Piedmont, Alessandria; [2]CAAD Center, Novara, Italy

Gravity is a well-known physical force that represents a fascinating area of interest in biology and medicine, considering relevant physiological changes in organisms during space exploration. Spaceflight opportunities are rare and expensive and access to space experiments is limited, but studies on the effect of gravity variation can be carried out using devices able to simulate microgravity, like Random Positioning Machine (RPM, Airbus, Defence & Space, ADS, Dutch Space, Leiden, NL) used in ours experiments. The aim of this project was to evaluate the effect of simulated weightlessness on human pancreatic adenocarcinoma cell line, PaCa-44 (Neuroscience, Biomedicine and Movement department, University of Verona). Pancreatic adenocarcinoma remains a major unsolved health problem nowadays, with conventional cancer treatments that have little impact on disease course. Pancreatic cancer co-opts multiple cellular and extracellular mechanisms to create a complex cancer organ with an unusual proclivity for metastasis and resistance to therapy. Exploiting simulated microgravity (SM), we designed a reproducible and easy method for scaffold-free PaCa-44 spheroids formation, that better reproduce the physical communications and signaling pathways observed in firsts stages of solid tumors. Morphological analyses were performed at different time-points to evaluate cell's aggregation and spheroid's structure under SM. LC-MS/MS proteomics analyses were performed on spheroids obtained by SM and on ground control samples. Results showed variations in protein's expression related to the process of epithelial mesenchymal transition (EMT) coupled with an increased glycolysis pathway related to Warburg effect, typical in cancer cells, as confirmed by quantitative RT-PCR assay. Moreover, both proteomic and PCR results showed an increase in expression levels of genes and proteins related to stemness pathway in spheroids samples compared to ground control non-spheroid cells. All these data suggest that PaCa44 cells exposed to gravitational unloading changed their growth behavior and started growing in a three dimensional (3D) manner in RPM. Moreover, obtained data showed that simulated microgravity plays a role in activation of molecular pathways implicated in stemness and in overexpression of genes involved both in stemness and in EMT under SMG condition, supporting the assumption that microgravity induces cancer cell transformations towards the acquisition of cancer stem cells-like features.

COMBINING TRANSLATION READTHROUGH INDUCING DRUGS AND NONSENSE MEDIATED DECAY PATWHAY INHIBITION TO THE CFTR RESCUE IN CYSTIC FIBROSIS CELL MODEL SYSTEM

Roberta BONGIORNO, Laura LENTINI, Andrea PACE, Ivana PIBIRI

Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Universita degli Studi di Palermo, Italy

Nonsense mutations affect 10% of patients with cystic fibrosis and produce a premature termination codon in *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) mRNA causing early termination of translation and leading to lack of CFTR function. A potential therapy for nonsense mutations provides the use of small molecules able to overcome the premature stop codon (PTC) by a readthrough mechanism that lead to synthesis a complete CFTR protein. Despite the good results obtained from this approach, TRIDs efficiency is considerably reduced by the poor amount of target transcript, that is the mRNA containing the PTC. The readthrough, indeed, does not occur on the totality of target transcripts because of their degradation due to the nonsense mediated decay pathway (NMD). This pathway provides the degradation of mRNA harboring premature stop codon to prevent the production of altered polypeptides. In contrast, the activity of this pathway interferes with the effectiveness of the readthrough drugs, limiting the mRNA concentration of the target protein. Thus, a promising strategy for nonsense mutation treatment is a com-

bined use of readthrough agents and factors that attenuate the nonsense mRNA decay. By silencing the UPF1 mRNA/protein, the activity of the NMD pathway was reduced, in FRT cells CFTR ^{W1282X}. Alternatively, caffeine was used as specific inhibitor of the UPF1 activity, to increase the efficiency of readthrough molecules (NV848 and NV914) in FRT cells CFTR^{W1282X} cells. In both cases, the combined treatment: NV848 or NV914/caffeine and NV848 or NV914/UPF1siRNA caused an increase of CFTR^{W1282X} mRNA level followed by the rescue of the CFTR expression and functionality. However, unexpectedly, despite the higher CFTR^{W1282X} mRNA level in caffeine treated samples, both expression and functionality CFTR rescue resulted slightly lower than the recovery achieved by UPF1 silencing. Our results indicate that modulation of NMD pathway, although still to be optimized, could be a promising approach in order to increase TRIDs effects in presence of stop mutations.

BIOCHEMICAL RAMAN SPECTRAL SIGNATURE OF PLASMA LIPOPROTEINS

Arianna BONIZZI[1], Alessandra RICCIARDI[2], Gabriele PIURI[1], Matteo DELLA PORTA[1], Serena MAZZUCCHELLI[1], Marta TRUFFI[2], Marta SEVIERI[1], Raffaele ALLEVI[1], Fabio CORSI[1,2], Roberta CAZZOLA[1], Carlo MORASSO[2]

[1]Department of Biomedical and Clinical Sciences "Luigi Sacco", Università degli Studi di Milano, Milano; [2]Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy

Lipoproteins (LPs) are complex macromolecules, consisting of a hydrophobic lipid core, rich in triglycerides and cholesterol esters, surrounded by an envelope formed by amphipathic molecules, such as phospholipids, non-esterified cholesterol and apolipoproteins. Plasma lipoproteins are classified into five main classes: chylomicrons, VLDL (very low-density lipoprotein), IDL (intermediate-density lipoprotein), LDL (low-density lipoprotein) and HDL (high-density lipoprotein), based on their density and play a key role in the transport lipids throughout the circulation. Lipoprotein fractionation, as well as the quantification of the amount of cholesterol and triglycerides carried by LPs, has become one of the main clinical approaches to assess the increased risk of cardiovascular complications in individuals. However, conventional lipidomics approaches used to characterize LPs are notoriously *laborious* and extremely time-consuming. In this context, Raman spectroscopy (RS) represents an innovative, optical technique that allows to analyze biological samples without any sample preparation. Moreover, this technique allows to obtain simultaneous information about both the biomolecules composing lipoproteins and the relative amount. Therefore, the aim of the present work is to demonstrate the suitability of RS to rapidly get access to an impressive amount of valuable information about the lipids and proteins of the main classes of LPs present in blood – such as VLDL, LDL and HDL – thus providing new tools that could be effectively used in clinic to perform LPs analysis. We investigated spectra differences between major lipoproteins classes isolated from plasma of six fasting healthy donors, by ultracentrifugation in discontinuous KBr density gradient. All spectra were acquired and analyzed in two different regions, the low frequency region (between 400 and 1800 cm^{-1}) and the high frequency region (between 2600 and 3200 cm^{-1}). The obtained spectra showed peaks related to the different biomolecules that compose the lipoproteins: cholesterol, triglycerides, membrane lipids, unsaturated fatty acid, carotenoids, proteins, and their intensity well reflects their relative composition. Moreover, the typical structure of VLDL, LDL and HDL were evaluated and confirmed by transmission electron microscopy. Our preliminary results, obtained on a limited number of subjects, suggesting that Raman spectroscopy could be a viable and reproducible approach to provide a biochemical signature of the main classes of LPs. Since the composition of LPs is known to be altered in many pathological conditions, we are conducting a new study to demonstrate the possible application of RS in diagnostics, in order to identify the changes in lipoprotein composition.

REFERENCE

Ricciardi, *et al. Vibrational Spectroscopy 109 (2020) 103073.*

DEVELOPMENT OF A SCREENING STRATEGY FOR THE IDENTIFICATION OF NLRP3 SELECTIVE INHIBITORY COMPOUNDS

Paola DINO[1], Marco BUSCETTA[2], Giovanni ZITO[2,3], Fabio BUCCHIERI[1], Chiara CIPOLLINA[2,4]

[1]Dipartimento di Biomedicina Sperimentale, Neuroscienze e Diagnostica Avanzata (Bi.N.D), University of Palermo; [2]Fondazione Ri.MED; [3]Research Department, IRCCS ISMETT (Istituto Mediterraneo per i Trapianti e le Terapie ad alta Specializzazione); [4]Istituto per la Ricerca e l'Innovazione Biomedica (IRIB), Consiglio Nazionale delle Ricerche (CNR), Palermo, Italy

NOD-like receptor pyrin domain-containing 3 (NLRP3) is the central component of the signalling complex "inflammasome" that promotes caspase-1 activation leading to processing and release of IL-1β and IL-18. NLRP3 activation is involved in different chronic age-related inflammatory diseases, therefore selective inhibition of NLRP3 is a promising strategy for the treatment of these diseases. The aim of this project is the discovery of novel NLRP3 selective inhibitors by using a semi-automated screening approach. To this purpose a primary screening assay must be developed, miniaturized and validated. Also, the development of an orthogonal assay is required for the validation of the primary actives (compounds that give positive results in the primary assay). Then, the real screening campaign can start. Herein we present the results of the development and validation of the primary and the orthogonal assays that will be used for the screening campaign to find selective NLRP3 inhibitors. A robust phenotypic primary assay was developed using human THP-1 macrophages stimulated with lipopolysaccharide (LPS) followed by nigericin, to induce selective activation of the NLRP3 inflammasome. At the end of the experiments the release of IL-1β was measured using Homogeneous Time-Resolved Fluorescence (HTRF) technology. This primary assays was first tested and validated in 6-well plates. The selective NLRP3 inhibitor MCC950 was used as positive control and three additional reference compounds, 3,4-methylenedioxy-β-nitrostyrene (MNS), parthenolide and glyburide, known to modulate NLRP3 activity, were included in the assay. Herein we report half maximal inhibitory concentration (IC_{50}) and IC_{max} for MCC950 and IC_{50} curves for the three reference compounds. We also report the results of assay miniaturization in 384-well plates and validation of the protocol using an automated liquid handling system following a stringent statistical evaluation of results reproducibility and variability. Finally, we present the development of the orthogonal assay that allows to measure NLRP3 activation using an independent approach compared to primary assay in order to discriminate false positives. The proposed orthogonal assay measures the