european journal of histochemistry
a journal of functional cytology

ISSN 1121-760X
volume 60/supplement 2
2016

PROCEEDINGS OF THE XXXVI NATIONAL MEETING OF THE ITALIAN SOCIETY FOR THE STUDY OF CONNECTIVE TISSUES (SISC)

Padova, 30th September – 1st October, 2016
University of Padova - Complesso Vallisneri

President
Antonio Rossi

Local Organizing Committee
Maurizio Onisto, Michele Spina, Barbara Zavan, Gloria Bellin, Valentina Masola, Maria Francesca Secchi

Scientific Committee
Antonio Rossi, Mario Raspanti, Maria Enrica Tira, Patrizia Cancemi, Salvatore Campo, Federica Boraldi, Marilena Formato, Marco Franchi, Michele Spina, Maurizio Onisto

under the auspices of
the University of Pavia, Italy

Published by PAGEPress, Pavia, Italy

Editorial Office:
Via A. Cavagna Sangiuliani 5, 27100 Pavia, Italy
Phone: +39.0382.464340 - Fax: +39.0382.34872
E-mail: info@pagepress.org

Printed quarterly by:
Press Up s.r.l.
via La Spezia, 118/C 00055 - Ladispoli (RM)
Phone and Fax: +39.076.15.27.351

Annual Subscriptions
Europe: Euro 200
All other Countries: $ 250

Subscriptions, cancellations, business correspondence and any enquiries must be sent to PAGEPress Publications, Pavia, Italy.
Cancellations must be received before the end of September to take effect at the end of the same year.

Reg. Tribunale di Pavia n. 289/23.2.1984.

Recognized by the Ministero per i Beni e le Attività Culturali, Italy as a publication of high cultural value.

Associato all'USPI
Unione Stampa Periodica Italiana

european journal of histochemistry
a journal of functional cytology

ISSN 1121-760X
volume 60/supplement 2
2016

ejh

under the auspices of
the University of Pavia, Italy

European Journal of Histochemistry
a journal of functional cytology

The *European Journal of Histochemistry* was founded in 1954 by Maffo Vialli and published until 1979 under the title of *Rivista di Istochimica Normale e Patologica*, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is published under the auspices of the University of Pavia, Italy.
The *European Journal of Histochemistry* is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC).

The Journal publishes Original Papers, Technical Reports, Reviews, Brief Reports, Letters to the Editor, Book Reviews, Views and Comments, concerning investigations performed with the aid of biophysical, biochemical, molecular-biological, enzymatic, immunohistochemical, cytometric, and image analysis techniques.
Areas of particular interest to the *European Journal of Histochemistry* include:
- functional cell and tissue biology in animals and plants;
- cell differentiation and death;
- cell-cell interaction and molecular trafficking;
- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.

2015 Impact factor: 2.421. ©JCR Thomson Reuters

table of contents

PROCEEDINGS OF THE XXXVI NATIONAL MEETING OF THE ITALIAN SOCIETY FOR THE STUDY OF CONNECTIVE TISSUES (SISC)

european journal
of histochemistry
ISSN 1121-760X
volume 60/supplement 2
2016

INVITED LECTURES

HEPARANASE: FROM BASIC CANCER RESEARCH TO THERAPEUTIC APPLICATIONS

I. Vlodavsky

Cancer and Vascular Biology Research Center, Rappaport Faculty of Medicine, Technion, Haifa 31096, Israel

E-mail: vlodavsk@mail.huji.ac.il

Heparan sulfate proteoglycans (HSPGs) are primary components at the interface between virtually every eukaryotic cell and its extracellular matrix (ECM). HSPGs not only provide a storage depot for heparin-binding molecules (i.e., growth factors, chemokines, enzymes) in the tumor microenvironment, but also decisively regulate their accessibility, function and mode of action. As such, HSPGs are intimately involved in modulating cell invasion and signaling loops that are critical for tumor growth. Heparanase, the sole heparan sulfate degrading endoglycosidase, regulates multiple biological activities that enhance tumor growth, angiogenesis and metastasis. Much of the impact of heparanase on tumor progression is related to its function in mediating tumor-host crosstalk, priming the tumor microenvironment to better support tumor take and growth. Immunohistochemistry, *in situ* hybridization, qPCR and western blotting demonstrate that heparanase expression is enhanced in almost all cancers examined including various carcinomas, sarcomas and hematological malignancies. The repertoire of heparanase activities is expanding. Heparanase regulates gene expression, activates cells of the innate immune system, promotes the formation of exosomes and autophagosomes, and stimulates signal transduction pathways via enzymatic and non-enzymatic activities. These effects dynamically impact multiple regulatory pathways that together drive tumor progression and inflammatory responses. Numerous clinical association studies have consistently demonstrated that upregulated heparanase expression correlates with increased tumor size, tumor angiogenesis, enhanced metastasis and poor prognosis. Knockdown of heparanase expression or treatments of tumor bearing mice with compounds that inhibit heparanase enzyme activity markedly inhibit tumor progression further underscoring the potential of anti-heparanase therapy for multiple types of cancer. Importantly, there is only a single, enzymatically active form of heparanase in humans, it is expressed in very low levels in normal tissues and heparanase knock-out animals exhibit no obvious deficits. These characteristics imply that inhibition of heparanase will cause minimal side effects in patients, together stirring heparanase as a highly desirable and druggable target for anti-cancer therapy. Development of heparanase inhibitors focused on carbohydrate-based, heparin-like compounds of which four are being evaluated in clinical trials for various types of cancer, including myeloma, pancreatic carcinoma and hepatocellular carcinoma. Heparanase neutralizing monoclonal antibodies were recently found to inhibit myeloma and lymphoma tumor growth and dissemination in preclinical models. Heparanase-inhibiting small molecules are being developed based on the recently resolved crystal structure of the heparanase protein. Collectively, the overarching premise guiding our work is that heparanase is a master regulator of the aggressive phenotype of cancer, an important contributor to the poor outcome of cancer patients and a prime target for therapy.

NOVEL PROTEOLYSIS- DEPENDENT AND INDEPENDENT ROLES OF MEMBRANE-TYPE 1 MATRIX METALLOPROTEINASE (MMP-14)

P. Mignatti

Dept. of Medicine and Cell Biology, New York University School of Medicine, NewYork, USA

E-mail: paolo.mignatti@nyumc.org

Membrane-type 1 matrix metalloproteinase (MT1-MMP, MMP-14), a transmembrane proteinase with an extracellular catalytic domain and a short cytoplasmic tail, degrades extracellular matrix components and controls diverse cell functions through proteolytic and non-proteolytic interactions with extracellular, intracellular and transmembrane proteins. We found that in tumor cells MT1-MMP downregulates fibroblast growth factor-2 (FGF-2) signaling by a proteolytic mechanism that reduces the amount of FGF-2 bound to the cell surface through high and low affinity receptors. This effect modulates tumor cell migration and invasion *in vitro*. In addition, MT1-MMP activates intracellular signaling by non-proteolytic mechanisms mediated by its cytoplasmic tail. We found that binding of tissue inhibitor of metalloproteinases-2 (TIMP-2) to MT1-MMP induces rapid and sustained activation of both the ERK1/2 and AKT pathways. FGF receptor-1 mediates TIMP-2 induction of ERK1/2 but not of AKT activation; however, Ras activation is necessary to transduce the TIMP-2-activated signal to both the ERK1/2 and AKT pathways. ERK1/2 and AKT activation by TIMP-2 binding to MT1-MMP protects tumor cells from apoptosis induced by serum starvation. Conversely, TIMP-2 upregulates apoptosis induced by three-dimensional type I collagen in epithelial cancer cells. Thus, TIMP-2 interaction with MT1-MMP provides tumor cells with either pro- or anti-apoptotic signaling depending on the extracellular environment and apoptotic stimulus. MT1-MMP plays a key role in postnatal skeletal development. The genetic deficiency of MT1-MMP in the mouse causes dwarfism, osteopenia, severe arthritis and lipodystrophy. In humans mutation of MT1-MMP causes the multicentric osteolysis and arthritis disease, Winchester syndrome, which recapitulates the abnormalities caused by MT1-MMP deficiency in the mouse. It has been proposed that the phenotype of MT1-MMP$^{-/-}$ mice results from the lack of the proteolytic activity of MT1-MMP. In light of the proteolysis-independent signaling functions of MT1-MMP, we hypothesized that the phenotype of MT1-MMP$^{-/-}$ mice is mediated, at least in part, by proteolysis-independent mechanisms. The unique tyrosine (Y573) in the MT1-MMP cytoplasmic tail appears to be fundamental for the control of intracellular signaling. We have shown that Y573 controls activation of the Ras-ERK1/2 pathway. Y573 substitution with aspartic acid (D), a negatively charged amino acid like phosphotyrosine, blocks this function without affecting MT1-MMP proteolytic activity. Based on these observations we generated a mutant mouse with the Y573D substitution in the cytoplasmic tail of MT1-MMP (MT1-MMP Y573D). The analysis of the phenotype of this mouse shows that, surprisingly, MT1-MMP Y573D mice have increased bone mass, defects in articular cartilage and growth plate, and decreased adipose tissue. The bone marrow-derived mesenchymal stem cells (MSC) of these mice show increased osteoblast and decreased chondrocyte and adipocyte differentiation, an effect mediated by Wnt signaling. Thus, MT1-MMP is a bifunctional protein that controls a variety of cell functions *in vitro* and *in vivo* through an extracellular proteolytic activity that controls ECM remodeling and growth factor receptor activation, and a cytoplasmic tail that activates diverse intracellular signaling pathways.

THE CRITICAL ROLES OF ERS, GROWTH FACTOR RECEPTORS, MIRNAS AND ECM EFFECTORS IN BREAST CANCER CELLS AGGRESSIVENESS AND PROPERTIES

N. Karamanos

Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, 26110, Greece

E-mail: n.k.karamanos@upatras.gr

In order to evaluate how estrogen receptors (ERα/β) and key matrix effectors are implicated in breast cancer development and progression, several different breast cancer cell lines have been established; MCF-7 SP10+ following ERα deletion and the triple negative MDA-MB-231shERβ. Following the suppression of ERs the expression profile of various ECM macromolecules and the functional properties of cells were significantly altered.

Notably, the expression of syndecans is associated with ERα in ERα+ breast cancer cells. Suppression of ERα causes epithelial-to-mesenchymal transition (EMT) in MCF-7 cells and at the same time causes the loss of cell surface syndecan-1 and syndecan-4. Re-establishing expression of syndecan-4, through full-length cDNAs, converted the mesenchymal phenotype back to epithelial (MET), to resemble wild-type low invasive MCF-7 cells. Syndecans seems to have particularly role also in the regulation of cell behavior in the case of triple negative cell line before and after the depletion of ERβ. Specifically, ERβ increase the levels of syndecan-1 and syndecan-4 in combination with a less aggresive phenotype. Moreover, following extended evaluation of the growth factor receptors expression profiles we have clarified that ERs are key players in controlling the expression levels of EGFR and insulin growth factor receptor IGFR. At the level of epigenetics using transfection of specific microRNA (antisense and precursors) their role in matrix expression and properties of cancer cells were evaluated.

These data indicated that ERs are of crucial importance for the expression of ECM molecules and cell phenotype in breast cancer cell, highlighting their involvement in EMT and/or MET behavior of cancer cells in cancer progression and might provide a potential target for the design of advanced treatments of breast cancer.

PRESENTATIONS

HEPARANASE REGULATES RENAL INFLAMMATION INDUCED BY ISCHEMIA /REPERFUSION

V. Masola[1,3]*, G. Zaza[1]*, G. Bellin[1], G. Gambaro[2], G. Vischini[2], A. Lupo[1], M. Onisto[3]

[1]Renal Unit, Department of Medicine, Verona, Italy; [2]Renal Unit, Columbus-Gemelli Hospital, Catholic University of the Sacred Heart, Roma, Italy; [3]University of Padova, Department of Biomedical Sciences Padova, Padova, Italy

**These authors contributed equally to this work*

E-mail: valentina.masola@unipd.it

Ischemia/reperfusion (I/R) injury has a significant clinical impact in the setting of renal transplantation both at short time when it is related to delayed graft function as well at long time when it promote fibrosis. I/R injury is characterized by inflammation as well as cell death.[1] Heparanase (HPSE) is an endoglycosidase that cleaves heparan sulfate and it is a regulator of epithelial to mesenchymal transition (EMT) induced by growth factors and by I/R.[2,3] HPSE plays also a significant role in inflammation.[4] Given the importance of inflammation on I/R damage we investigated whether and how HPSE and its inhibitor SST001 can modulate these events.

In-vitro studies showed that HPSE regulated viability, apoptosis and damage associated molecular patterns (DAMPs) generation of HK2 cells (human renal proximal tubular cells) together with TLRs and pro-inflammatory cytokines expression. HPSE modulated also U937 derived macrophages phenotype. Results from the present study showed that HPSE modulate a cross-talk between I/R injured tubular cells and activated macrophages. In detail, I/R injured cells shifted macrophages to more inflammatory phenotype and in turn they sustain EMT of tubular cells. On the contrary, HPSE inhibition reduced macrophages activation with the consequent reduction of tubular cells transdifferentiation.

By targeting inflammatory activation and macrophages phenotype the results from the present study may represent a fruitful approach for the treatment and management of I/R injury and subsequent fibrosis.

1. Menke J, et al. Curr Opin Organ Transplant. 2014;19(4):395-400
2. Masola V, et al. J Transl Med. 2012;10:213
3. Masola V. et al. PLoS One. 2016;11(7):e0160074.
4. Goldberg R, et al. Matrix Biol. 2013;32(5):234-40.

HEPARANASE EXPRESSION IN CHRONIC LIVER DISEASE

M.F. Secchi[1], M. Crescenzi[2], G. Pesavento[1], F.P. Russo[2], A. Floreani[2], M. Onisto[1]

[1]Department of Biomedical Science, University of Padova, Padova; [2]Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Italy

E-mail: mfrancesca.secchi@studenti.unipd.it

Regardless of the etiology, chronic liver disease is characterized by inflammatory damage and progressive matrix deposition. If damage persists, the pathological process may lead to cirrhosis and liver failure.[1] Heparanase (HPSE) is an endo-glucuronidase that cleaves heparan sulfate chains of proteoglycans and participates in the remodelling of extracellular matrix and basement membranes.[2] Although the role of HPSE in kidney fibrosis is documented,[3] its involvement in liver fibrosis is unknown. Therefore, we aimed to measure circulating plasma levels of HPSE in patients with different stages of chronic liver diseases and to investigate HPSE expression in the (CCl_4)-induced mouse model of chronic liver injury. HPSE plasma activity was significantly higher in patients with mild, significant and severe liver fibrosis as compared to healthy controls. Interestingly, HPSE activity decreased in progression of liver disease and negatively correlated with the organ stiffness. Following 1 and 2 weeks of CCl4 treatment, murine liver tissue showed centrolobular necrosis with extensive inflammatory cells infiltration and mild fibrosis. Minimal cell infiltration and micronodular cirrhosis were observed after 8 and 12 weeks of CCl_4 treatment. HPSE protein and mRNA levels were significantly up-regulated in murine liver tissue after 1 and 2 weeks of CCl_4 exposure. However, HPSE expression decreased to equal control mice levels after 8 and 12 weeks of treatment. Immunofluorescence revealed a strong immunopositivity for HPSE after 1 and 2 weeks of CCl4 administration. HPSE protein was restricted to centrolobular necrotic area and it co-localized with F4/80, a macrophage marker. U937 macrophage cell line was used to study HPSE expression upon inflammatory activation. Among different cytochines, TNF-α cell treatment increased HPSE mRNA and protein expression as well as HPSE secretion. Overall, our data indicated an up-regulation of HPSE in early chronic liver disease suggesting its possible involvement in the initial phase of fibrotic process. TNF-α-activated macrophages could be a relevant source of HPSE in the injured liver.

1. Hernandez-Gea V et al. Annu Rev Pathol 2011;6:425-56.
2. Secchi MF et al. Biomol Concepts 2015;6(5-6):415-21.
3. Masola et al. J Transl Med 2015;13:181.

EPIGENETIC CONTROL OF HYALURONAN SYNTHASES

A. Passi, D. Vigetti, M. Viola, E Karousou, I. Caon, E. Caravà, G. De Luca

Department of Surgery and Morphological Sciences, University of Insubria, Varese, Italy

E-mail: alberto.passi@uninsubria.it

Epigenetics has emerged as a key aspect in gene expression also in in extracellular matrix (ECM). The synthesis of hyaluronan (HA) is controlled by gene expression of hyaluronan synthases 1, 2 and 3 and in particular, the HAS2 shows an intriguing and complex regulation with epigenetic relevance. Hyaluronan is a major component of ECM regulating cell migration and proliferation. Smooth muscle cells in the presence of different stimuli, as inflammation, oxLDL, mechanical stress, produced an altered ECM where HA is abundant in areas of atherosclerotic lesions. The control of the HA synthesis is therefore critical not only in ECM assembly but also in various pathologies. In contrast with other glycosaminoglycans, which are synthesized in the Golgi apparatus, HA is produced on the plasma membrane by HA synthases (HAS1-3), using UDPGlcUA acid and UDPGlcNAc as substrates. UDP-sugar availability as well as the cellular energy are critical for the synthesis of HA and for HAS2 activity. The AMP activated protein kinase, a sensor of the energy status of the cell, leads to HAS2 T110 phosphorylation, which specifically inhibits HA secretion.[1] However, the most general sensor of cellular nutritional status is the UDPGlcNAc produced by hex-

osamine biosynthetic pathway. This metabolic pathway is influenced by protein, fatty acid, nucleotide and glucose metabolisms and when activated leads to intracellular protein glycosylation (O-GlcNAcylation). We described that O-GlcNAcylation of serine 221 residue of HAS2 induces a dramatic stabilization of the enzyme on the membranes and an increase of HA production.[2] Eventually we found a long non-coding RNA (NAT) positively controls in cis the HAS2 expression involving p65 and NFkB pathway.[3] Beside the antisense effect, another epigenetic control has been described for P300 and histone acetylation. In fact transfection of P300 increased the HAS2 expression and HA synthesis whereas transfection of HDAC1 has opposite effects, indicating that this epigenetic control plays a role in this context involving the sirtuin1 activation.

1. Vigetti D et al. (2011) J Biol. Chem. 286(10):7917-24.
2. Vigetti D et al. (2012) J Biol. Chem. 287(42):35544-55.
3. Vigetti D, et al. (2014) J Biol Chem. 289(42):28816-26.

GLICOCALIX IN ENDOTHELIUM INFLAMMATION: CHANGES IN BOTH GLYCOSAMINOGLYCANS AND PROTEOGLYCANS

M. Viola, E. Caravà, D. Vigetti, E. Karousou, P. Moretto, G. De Luca, A. Passi

Department of Surgical and morphological Sciences University of Insubria, Varese, Italy

E-mail: manuela.viola@uninsubria.it

In the arterial vessel wall, endothelial cells regulate the vascular tone, the thrombosis/fibrinolysis balance, and the adhesiveness of inflammatory cells; most of these abilities are due to molecules of the glycocalyx. Inflammation is an extremely complex series of events that tissues undertake after harmful stimuli; besides the alteration of the cellular behavior, inflammation also causes the modification of tissue architecture and of the extracellular matrix (ECM). Noteworthy ECM modifications caused by inflammatory cytokines (*e.g.* TNF-alfa or IL-1) and/or altered molecules such as oxidized LDL are also involved in the recruitment/activation of inflammatory cells.

HUVEC cells were used as a model of inflammation after treatment with TNF-alfa. HS/HE polymerization enzymes (EXT1, EXT2, NDST1) and syndecans core protein expression were evaluated by qPCR, and HS/HE disaccharides by means of HPLC techniques. HA metabolism was investigated by the expression of the synthetic enzymes HAS2 and HAS3 as well as the quantification of HA by ELISA assay.

The HUVECs response to TNF-alfa was modulated within the 48 hours: EXT1, EXT2, NDST1 increased their expression at 24 hours and EXT1 and EXT2 were back to control levels at 48. In parallel, syndecans core proteins had an increase in syndecans -3 and -4 expression at 24 hours that were changed by syndecans -1 and 4 at 48 hours; moreover, HS/HE disaccharide showed a higher amount of N-sulfation. The main HA synthetic enzymes HAS2 was upregulated, while HAS3 role in HA accumulation is still under evaluation.

The inflammation of the endothelial cells induced dramatic changes in the ECM they produce, in particular, on the composition and expression of the HS/HE PGs, in a specific temporal order, starting with GAG polymeric enzymes and syndecans-1 core protein to the syndecans-4 PG. Those events are also involved in the monocyte recruitment within the inflammatory site. As reported in several studies syndecans-4 expression is related to NO production and release from endothelial cells, we observed that in TNF-alfa stimulated HUVEC, the expression of NOS enzymes is highly increased, accounting for the changes in permeability and adhesiveness in *in vivo*.

ANIMAL MODELS OF DESBUQUOIS DYSPLASIA TYPE 1 DEMONSTRATE CANT1 ROLE IN PROTEOGLYCAN METABOLISM

L. Monti[1], C. Paganini[1], R. Costantini[1], S. Lecci[1], S. Maruelli[1], M. Biggiogera[2], V. Cormier-Daire[3], A. Forlino[1], A. Rossi[1]

[1]Dept. Molecular Medicine, Unit of Biochemistry, University of Pavia, Italy; [2]Dept. Biology & Biotechnology, Cell Biology Lab, University of Pavia, Italy; [3]Dept. Genetics and INSERM U781, Hôpital Necker Enfants Malades, Paris, France

E-mail: luca.monti02@universitadipavia.it

Desbuquois dysplasia (DBQD) is a rare recessive chondrodysplasia, characterized by growth retardation, multiple dislocations and advanced carpal ossification. Two forms of DBQD have been described on the basis of the presence (type 1) or absence (type 2) of characteristic hand anomalies. DBQD type 1 is caused by mutations in the *Calcium-Activated Nucleotidase 1* gene (*CANT1*), while DBQD type 2 is caused by mutations in the *xylosiltransferase 1* (*XYLT1*) gene. CANT1 is a nucleotidase of the ER/Golgi that hydrolyzes UDP, suggesting its involvement in protein glycosylation; for this reason its role in proteoglycan metabolism has been hypothesized. To better characterize CANT1 role in the etiology of DBQD, we generated a *Cant1* knock-in mouse carrying the R302H substitution reproducing the R300H mutation detected in patients. Morphometric analyses demonstrated that mutant mice are smaller with shorter and thinner tibiae, femurs and ilia compared to wild type animals. Limb extremities of KI mice reproduced the hand anomalies described in patients: additional carpal ossification centers and the delta phalanx. Thus the KI mouse develops a skeletal phenotype reminiscent of DBQD type 1. To better investigate CANT1 role in proteoglycan (PG) synthesis we generated a *Cant1* knock out mouse by excision of exon 3 and 4. The KO mouse showed the same growth defects and hand anomalies of patients already observed in the KI mouse. To study PG synthesis, rib chondrocytes were metabolically labeled with ^{35}S-sulfate and the amount of newly synthesized PGs was evaluated. KO cells showed reduced PG synthesis compared to wild types both in presence and in absence of β-D-xyloside, an enhancer of glycosaminoglycan (GAG) synthesis. Gel filtration chromatography of GAGs released from newly synthesized PGs after β-elimination demonstrated that the hydrodynamic size of GAG chains was reduced in KO chondrocytes compared to the controls. Ultrastructural analysis of KO and wild type cartilage and cultured chondrocytes by TEM demonstrated the presence of dilated vacuoli containing electrondense proteinaceus material suggesting a role of CANT1 in protein secretion. Pulse-chase labeling of cells with ^{35}S-sulfate demonstrated reduced PG secretion in mutant cells compared to the controls.

In conclusion we generated and validated two different mouse models for the study of DBQD type 1 and we demonstrated that CANT1 play a role in PG synthesis.

Work supported by Telethon-Italy (grant n. GGP11079) and the EU (FP7, "Sybil" project, grant n. 602300).

Nominated by the Italian Society for the Study of Connective Tissue for the Dick Heinegard European Young Investigation Award.

SILENCING RNA: APPROACING A TREATMENT FOR CLASSICAL OSTEOGENESIS IMPERFECTA

S. Maruelli[1], R. Besio[1], R. Gioia[1], A. Renaud[2], V. Trichet[2], P. Layrolle[2], A. Rossi[1], A. Forlino[1]

[1]Dep. Molecular Medicine, University of Pavia, Biochemistry Unit; [2]INSERM U957 – LPRO , University of Nantes

E-mail: silvia.maruelli01@universitadipavia.it

Osteogenesis Imperfecta (OI) is a rare heritable bone disease characterized mainly by frequent fractures throughout life, bone deformities and growth retardation. In its classical form OI is caused by dominant negative mutations in collagen type I genes (*COL1A1, COL1A2)*. Nowadays, no decisive cure for this pathology is available.

The aim of this study is to develop a gene therapy approach for the treatment of classical OI caused by mutation in the *COL1A2* gene. It is known that in humans a glycine substitution in *COL1A2* leads to OI, while null mutations in both *COL1A2* alleles, impairing protein synthesis, lead to a milder form of Ehler-Danlos Syndrome (EDS) without skeletal phenotype.[1] Thus specific suppression at bone level of both *COL1A2* alleles is expected to ameliorate the OI bone outcome in a large number of patients. For the project the OI murine model Amish, carrying a Gly610Cys substitution in the 2 chain of collagen type I and affected by a moderate form of OI, was used.[2] A gene silencing approach based on silencing RNAs (siRNA) was developed to suppress both the mutant and wild type *Col1a2*.

Three different siRNAs for the murine *Col1a2* were selected *in silico* and their efficiency was tested *in vitro* using primary murine embryonic fibroblasts (MEF). The specificity against *Col1a*2 was evaluated for the more efficient siRNA as well as its ability to suppress α2(I) at protein level.

Furthermore the efficiency and specificity of this siRNA *in vivo* was evaluated. To this purpose 3D osteogenic scaffolds Biphasic Calcium Phosphate (BCP) loaded with murine mesenchymal stem cells (MSCs) were implanted in muscles of nude mice. The selected siRNA was injected three times a week for three weeks at the level of implants. The analysis of *Col1a2* expression at molecular and protein level in the MSCs-BCP implants confirmed the specificity and efficiency of the siRNA without impairing bone formation.

Specific delivery systems to direct the siRNA to the bone cells will need to be investigated to evaluate in the OI murine model the goodness of the proposed gene therapy approach for OI treatment.

The work was supported by Care4Brittlebones Foundation and the EU (FP7, "Sybil" project, grant n. 602300).

1. Malfait F et al. J Med Genet 2006:43:e36

2. Daley E. et al. J. of Bone and Mineral Research 2010;25:247-261

COMPARATIVE PROFILING BETWEEN STROMAL FIBROBLAST-LIKE STEM CELLS (F-LSCS) AND ADULT HUMAN FIBROBLASTS

R. Musso[1], G. Di Cara[1], L. Tomasello[2], C. Giordano[1,2], I. Pucci-Minafra[1]

[1]Centro di OncoBiologia Sperimentale, University of Palermo, La Maddalena Cancer Center, Palermo and [2]Section of Cardio-Respiratory and Endocrine-Metabolic Diseases, Biomedical Department of Internal and Specialist Medicine (Di.Bi.M.I.S.), University of Palermo, Palermo, Italy

E-mail: rosi82.m@libero.it; ida.pucci@unipa.it

The human limbus is a highly specialized region of the eye located at the junction of the cornea and the conjunctiva of the ocular surface. This region is characterized by stromal invaginations, surrounded by a rich extracellular matrix, that provides the favorable microenvironment for the maintenance of the undifferentiated phenotypes. Each invagination hosts two classes of the limbal stem cells: the limbal epithelial stem cells (LESCs), which are responsible for the corneal renewal, and the stromal fibroblast-like stem cells (f-LSCs) which are characterized by a high level of cellular plasticity and regenerative potential. The f-LSCs are scarcely immunogenic, as they do not express HLA-DR molecules, and are able to differentiate into several cytotypes of mesenchymal origin. For their peculiar features, it is assumed that the f-LSCs could be employed in the regenerative medicine.[1] In a previous work by our group,[2] the proteomic approach was used to outline a potential signature for the f-LSCs and to investigate on the possible influences exerted by external factors either technical (*i.e.* sub-cultivation passages) or biological (*i.e.* age of donors). In order to assess the reliability of the limbal stemness signature, we performed the comparative proteomic analysis between the undifferentiated f-LSCs and a differentiated human fibroblast cell line. Indeed, the fibroblastic phenotype represents one of the natural end-points of the differentiation process of the f-LSCs. Both cell cultures were individually processed and submitted to 2D-IPG electrophoresis (IPG strips 18 cm long with pH range 3.0–10). Protein spots were identified by MALDI-TOF spectrometry and grouped into functional categories, as previously reported.[3,4] Qualitative and quantitative comparison of the proteomic profiles of the two cell populations showed a high percentage of similarity (~54%) as expected for the existence of a common phenotypic lineage. Moreover, the majority of the differentially expressed proteins, in terms of relative abundance (vol%), showed a higher expression level in the f-LSCs with respect to the differentiated fibroblasts. Among the highly differentially expressed proteins in the stem cells it is worth to note the presence of GRP78, which is known to be essential for embryonic cell growth and pluripotent cell survival, and of RABP2 for which it has been hypothesized a role as a marker of pluripotency. We believe that the results on the proteomic profile of the limbal stem cells are a further confirmation of the validity of this cell population, easily accessible with minimally invasive techniques, to be used for multiple applications in regenerative medicine.

1. Criscimanna A et al. Cell Transplant 2012;21:73-90.

2. Tomasello L et al. Stem Cell Res Ther 2016;7(1):83.

3. Pucci-Minafra I et al. Proteomics 2004;4:849–860.

4. Pucci Minafra I et al. J Proteomics Bioinform 2016;9:3.

CHONDROCYTE MATURATION CYCLE AND ENDOCHONDRAL OSSIFICATION IN HUMAN FETAL CARTILAGE

UE. Pazzaglia[1], V. Sibilia[2], L. Casati[2], G. Zarattini[1], T. Congiu[3], M. Reguzzoni[3], D. Quacci[3]

[1]Department DSMC, University of Brescia; [2]Department of Biomedical Technology and Translational Medicine, University of Milan; [3]Department of Surgical and Morphological Sciences, University of Insubria

E-mail: ugo.pazzaglia@unibs.it

The relationship between the chondrocyte maturation cycle and the endochondral ossification process was studied in human long bone cartilage Anlagen. A topographic zonal classification was used for a morphometric evaluation in light microscopy of chondrocyte lacunar area and total matrix area. The hypertrophic chondrocyte zone corresponded to the mineral deposition on the interterritorial of the cartilage Anlage.

SEM observations of the same slides allowed a morphometric analysis of the interfibrillar empty area between fibrils which substantiated an hypothesis of the transport of water from the matrix to the swelling chondrocytes where mineral deposition was occurring.

The pattern of the primary diaphyseal ossification center was compared with that of the epiphyseal center in the post-natal age.

COMPARATIVE STUDY OF MINERAL DEPOSITION IN CARTILAGE AND BONE MATRIX IN HUMAN BONE ANLAGE IN THE FETAL DEVELOPMENT PERIOD

UE. Pazzaglia[1], M. Raspanti[2], AG. Salvi[1], M. Protasoni[2], A. Minnini[1], C. Dell'Orbo[2], T. Congiu[2]

[1]Department DSMC, University of Brescia; [2]Department of Surgical and Morphological Sciences, University of Insubria

E-mail: ugo.pazzaglia@unibs.it

The initial calcification and the progression of the mineral phase depositing in cartilage and bone matrix was studied in human, fetal cartilage Anlagen at a developmental stage when the calcified cartilage resorption had not yet started. The model allowed a morphological, comparative analysis of the calcification process in both cartilage and osteoid which represented the basic steps of endochondral calcification. The study was carried out with light microscopy and SEM-Edax (combined with hest deproteination and morphometry) on a sequence of thin sections, cut on the same paraffin inclusions. This allowed to analyzed corresponding fields with the different microscopy techniques. In cartilage focal, globular mineral deposits (randomly distributed) represented the initial phase of calcification which progressed with an eccentrical growth of the single deposit. With the increase of calcified globules size and of their density, aggregation led to the extensive mineralization of the inter-territorial matrix. Mineral deposition in osteoid below the periosteal osteoblasts occurred shortly after the collagen fibrils at thin extruded from the osteoblast cell membrane and packed in compact bundles. The fibrils themselves acted to orient and to compact the mineral deposits along a direction of the fibrils. This study documented the progression of the mineral deposition in both cartilage and osteoid and integrated the actual knowledge of the initial Calcium Phosphate nucleation in endochondral ossification.

SEM STUDY OF HEAT-DEPROTEINATED HUMAN CORTICAL BONE

T. Congiu[1], UE. Pazzaglia[2], M. Reguzzoni[1], AG. Salvi[2], P.Basso[1], M. Raspanti[1]

[1]Department of Surgical and Morphological Sciences, University of Insubria; [2]Department DSMC, University of Brescia

E-mail: mario.raspanti@uninsubria.it

The primary effect of cortical bone samples thermic treatment is to burn and vaporize the organic phase including cells, the collagen and not-collagenic proteins of the matrix. The present study were observed with a Scanning electron microscope the effects of heat deproteination in the range of 350-600°C. In the whole interval the lamellar texture, the vascular canal surface and the inner surface of lacunae were preserved.

XRD diffraction analysis documented that below 600°C the hydroxyapatite crystalline structure was not altered, therefore this method in association with sample fracturing allowed to feature the mineral cast of the collage texture. The organic phase was only partially removed at lower temperature (350°C) but it was almost removed at 400°C. This condition allowed the visualization of the hydroxypatite cast of the collagen fibrils. No difference was documented between the treatments at 400°C and 450°C.

At 500°C the collagen was completely removed with an initial remodelling of the crystal features. At 600°C could no longer be recognizable the original crystal feature. A comparison with the same bone samples deproteinated with collagenase for 1 to 3 weeks were used as control.

THE NATURAL ANTISENSE TRANCRIPT HAS2-AS1 REGULATES AGGRESSIVENESS OF BREAST CANCER CELLS

D. Vigetti[1], I. Caon[1], M. Gotte[2], M. Viola[1], E. Karousou[1], E. Caravà[1], P. Moretto[1], G. De Luca[1], A. Passi[1]

[1]Dept. of Surgical and Morphological Sciences, University of Insubria, Varese, Italy, [2]Dept. of Gynecology and Obstetrics, University of Munster, Germany

E-mail: davide.vigetti@uninsubria.it

Hyaluronan is a ubiquitous glycosaminoglycan of extracellular matrix important for tissue homeostasis and development that can regulate different cellular behaviors like adhesion, motility, growth and inflammation. Its presence is critical in tumor microenvironment, where the up-regulation of HA synthase 2 (HAS2) and the overproduction of HA are often associated with tumor progression and metastasis.[1] Recently, it has been discovered that the natural antisense transcript for hyaluronan synthase 2 (HAS2-AS1) can modulate the expression of HAS2 and the production of (HA) in different pathologies.[2,3] HAS2-AS1 is a long-non coding RNA (lncRNA) transcribed in the opposite strand of HAS2 gene on chromosome 8. It has an alternative splicing site which generates two RNA isoforms of different lengths (HAS2-AS1 long and HAS2-AS1 short), that have 257 or 174 nucleotides of perfect complementary sequence to the first exon of HAS2, respectively. LncRNAs play important roles in cancer, like chromatin remodeling, as well as transcriptional and post-transcriptional regulation, through a variety of chromatin-based mechanisms and the interaction with other RNA species.[4] Here we show that the knockdown of HAS2-AS1 in the aggressive triple negative breast cancer cells (MDA-MB-231) increases proliferation respect to the estrogen receptor (ER)

positive breast cancer cell line MCF-7. Furthermore, we report that the silencing of HAS2-AS1 in MDA-MB-231 is linked to a higher migration and invasion rate. Moreover, quantitative PCR analysis reveals that the abrogation of HAS2-AS1 brings to higher levels of HAS2, HAS3, CD44 and hyaluronidase 2 (HYAL2) mRNA, suggesting a possible role of HAS2 antisense in tumor progression. Since little is known about HAS2-AS1, in our future experiments we want to elucidate the cellular pathway linked to its abrogation and to study how it is involved in tumor development.

1. Auvinen P et al. Am. J. Pathol. 2000;156: 529-36.
2. Vigetti D. Passi A. Adv Cancer Res. 2014;123:95-119.
3. Vigetti D et al. J. Biol. Chem. 2014;289:28816-26
4. Vigetti D et al. FEBS J 2014;281:4980-92

BIOLOGICAL ACTIVITIES OF AGNPS-EPS ON BREAST CANCER CELLS *IN VITRO*

M. Buttacavoli[1], NN. Albanese[2], G. Gallo[1], AM. Puglia[1], C. Faleri[3], M. Gallo[4], F. Baldi[4], G. Pizzolanti[5], C. Giordano[5], S. Feo[1], P. Cancemi[1,2]

[1]Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Italy; [2]Centro di Oncobiologia Sperimentale (C.OB.S.), Dipartimento Oncologico La Maddalena, Palermo, Italy; [3]Dipartimento Scienze della Vita, Università di Siena, Siena, Italy; [4]Dipartimento di Scienze Molecolari e Nanosistemi, Università Cà Foscari Venezia-Mestre, Italy; [5]Dipartimento Biomedico di Medicina Interna e Specialistica, Sezione di Endocrinologia, Diabetologia e Malattie del Metabolismo, Università degli Studi di Palermo, Italy

E-mail: miriambuttacavoli@libero.it

Silver nanoparticles (AgNPs) are an important class of nanomaterials used in a wide range of industrial and biomedical applications. Recently, several efforts have been made to use AgNPs as anticancer compounds with positive outcomes[1]. Several approaches are available for the synthesis of AgNPs, including chemical, electrochemical, photochemical and biological methods. The latter have received great attention due to the growing need of developing eco-friendly and green technologies in material synthesis. Bacterial production of AgNPs generally represent a defense mechanism for neutralization of the toxic metal ions through the action of the intra or extracellular bioactive components, such as exopolysac-charides (EPS) acting as metal reducers and/or stabilizers. The EPS of *Klebsiella oxytoca* DSM 29614 has a peculiar structure consisting of a branched heptasaccharide with metal-binding capability[2]. In the present study, we explored the antitumoral activities of AgNPs produced by *Klebsiella oxytoca* DSM 29614 under aerobic (AgNPs-EPS^{aer}) or anaerobic (AgNPs-EPS^{anaer}) conditions, against the human breast cancer cell line SK-BR3. The AgNPs-EPS treatments caused a dose dependent behavior resulting in a conspicuous inhibition of cell proliferation rate and colony-forming capability, dramatic morphological changes with apoptotic features and reducing of migratory ability and MMPs activity. Proteomic analysis showed modulation of several proteins of endoplasmic reticulum lumen and mitochondria, response to oxidative stress and other proteins involved in glycolysis and regulation of apoptosis. Cellular uptake of AgNPs, detected by TEM analysis, suggested to occur through endocytosis process and voltammetric analysis revealed a major accumulation of Ag^+ into mitochondria, suggesting a possible crosstalk between mitochondrial dysfunction, ER-Stress, oxidative stress and autophagy. Infact, recently, autophagy activation was shown to correlate with AgNPs exposure[3]. These findings suggest the possible application of AgNPs-EPS as anticancer agent; further efforts are necessary to analyze the effects on oxidative stress-ER stress, ER-mitochondria connectivity and apoptosis/autophagy.

1. Wei L et al. Drug Discov Today. 2015;20(5):595-601.
2. Battistel D et al. Talanta 2015;132:294-300.
3. Mao BH et al. Nanotoxicology 2016;10 (8):1021-40.

NOVEL BIOMARKER PANEL FOR COLON CANCER UNVEILED BY PROTEOMICS

M. Buttacavoli[1], NN. Albanese[1,2], G. Di Cara[2], R. Musso[2], S. Bonfardeci1, E. Roz[3], I. Pucci-Minafra[2], S. Feo[1,4], P. Cancemi[1,2,4]

[1]Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Italy; [2]Centro di Oncobiologia Sperimentale (C.OB.S.), Dipartimento Oncologico La Maddalena, Palermo, Italy; [3]Dipartimento Oncologico La Maddalena, Palermo, Italy, [4]ATeN (Advanced Technologies Network Center), Università degli Studi di Palermo, Italy

E-mail: patrizia.cancemi@unipa.it

Colorectal cancer (CRC) is one of the most common types of cancer. Recent advances indicate that the extensive use of biomarkers represent the promise for next major advance in the diagnosis and management of CRC. Biomarker discovery is increasing with the modern throughput of medical research in genomic and proteomics. In present study, by using 2D-DIGE combined with MALDI-TOF-MS/MS and molecular network relationship, we perform a comparative proteomic profile of pooled colon cancer tissues paired with adjacent non-tumoral tissues, to investigate potential target proteins correlated with carcinogenesis. Our analysis showed 111 differentially-expressed proteins, including 73 up-regulated and 38 down-regulated protein spots in CRC. The differentially expressed proteins, functionally classified, have been suggested to act at multiple tumor progression steps, affecting cell proliferation, apoptosis, metabolic pathways, oxidative stress, cell motility and invasion. Interestingly, we identified 4 different isoforms of Transgelin-2, a 22 kDa actin-binding protein, collectively down-regulated in colon cancer tissues. Transgelin is involved in controlling cell motility and podosome formation and is also a direct target of transforming growth factor β (TGF-β)/Smad3-dependent epithelial cell migration.[1] In cancer the role of Transgelin is quite controversial: a number of studies suggest that transgelin can act as a tumor suppressor,[2] while several others indicate its pro-tumorigenic role.[3]The spectrometric characterization of TAGL isoforms by MALDI-TOF MS, identified two short forms in the C-terminal end. Interestingly, this portion contains the actin-binding site and two different phoshoserine residues. It is reasonable to believe that these isoforms may exert diversified functions in the cell. We believe that this is an important contribution to the knowledge of TAGL isoforms as a possible tumor suppressor and biomarker for CRC.

1. Ali NA et al. Mol Biosyst. 2010;6(11): 2332-8.
2. Yeo M et al. Cancer. 2010;116(11): 2581-9.
3. Zhou HM et al. BMC Cancer 2016;4(1):1-11.

UBIQUITOUS AND ENIGMATIC PRESENCE OF 14-3-3 GAMMA IN BREAST CANCER TISSUES

G. Di Cara[1], R. Musso[1], N.N. Albanese[1], P. Cancemi[1,2], A.E. Iacò[1], S. Minafra[1], I. Pucci-Minafra[1]

[1]Centro di OncoBiologia Sperimentale, University of Palermo, La Maddalena Cancer Center, Palermo; [2]Department of Biological, Chemical, and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo; Italy

E-mail: lucadicar@gmail.com

The discovery of new prognostic and/or predictive biomarkers is a major aim of the current anti-cancer research, in support of a correct classification and subtype prediction of tumors, crucial to improve the clinical and therapeutic approach to the patients. Our research group has been historically focused on the identification of putative biomarkers for breast cancer (BC).[1] For the present research we applied the proteomic analysis to one hundred BC tissues, collected at the La Maddalena Hospital from informed consenting patients, and characterized for the clinical and molecular parameters.

Analytically, the tissue samples were washed in PBS and properly homogenized for the protein extraction. The protein amount of each sample was quantified by Bradford assay and subjected to IPG-2D electrophoresis. The protein spots were revealed by ammoniacal silver staining (analytical gels) and by Coomassie blue (preparative gels) and the interesting protein spots were identified by mass spectrometry.

The identified proteins (about 450) were classified into functional clusters and grouped on the basis of their occurrence in the proteomic maps of the selected patients.[2] Worthy of note was the peculiar expression of members of the 14-3-3 protein family.

The 14-3-3 proteins are specific phospho-serine and -threonine binding proteins, integrated into the phospho-regulatory signaling pathways controlling many biological processes, crucial for the normal and tumorigenic cell behavior: cell cycle regulation, protein trafficking, metabolic regulation, cell proliferation, cell migration and apoptosis. Recently a new role for the 14-3-3 proteins was highlighted in the epithelial-mesenchymal transition.[3]

To date, 7 members of the 14-3-3 family in the mammalian have been identified. In our proteomics maps we have recognized 4 out of 7 members of the family (η, γ, σ, ζ), having different occurrence in cancer tissues. Among these protein members, the 14-3-3 γ resulted ubiquitously expressed in our collection of BC samples, suggesting a key role for this protein in the tumorigenic process. The bioinformatics approach revealed an intriguing protein network having the 14-3-3 γ as central node, so confirming and strengthening the involvement 14-3-3 γ in the molecular processes critical for tumor progression.

In conclusion, our data candidates 14-3-3 proteins, and particularly the 14-3-3 γ, as potential biomarkers for the prediction and the monitoring of breast cancer progression.

1. Pucci-Minafra I et al. Ann N Y Acad Sci 2002;963:122-39.

2. Pucci-Minafra I et al. J Proteomics Bioinform 2016;9;3. http://dx.doi.org/10.4172/jpb.1000392

3. Freeman AK et al. Semin Cell Dev Biol 2011;22:681-7.

THE NOVEL PROTEIN C10ORF118 SECRETED BY MCF-7 INDUCES HYALURONAN SYNTHESIS IN STROMAL FIBROBLASTS

E. Karousou[1], M.L. D'Angelo1, P. Cancemi[2], D. Vigetti[1], M. Viola[1], I. Caon[1], E. Caravà[1], G. De Luca[1], A. Passi[1]

[1]Department of Surgical and Morphological Sciences, University of Insubria, Varese, Italy; [2]Centro di Oncobiologia Sperimentale (C.OB.S.), Oncology Department, La Maddalena, Palermo, Italy

E-mail: jenny.karousou@uninsubria.it

Extracellular matrix (ECM) is a complex network of macromolecules and secreted factors that ensures the tissue integrity and elicits signals to and from the embedded cells. Dysregulation of the composition of the ECM is associated with several pathologies, including cancer. Among various ECM glycosaminoglycans, hyaluronan (HA) has a remarkable structural importance but also a role in regulating cellular processes through a binding with membrane receptors and activation of signaling pathways[1]. The role of HA in tumor cells' functions depends on its molar mass which is regulated by the enzymes that synthesize HA, i.e. hyaluronan synthases (HAS), and hyaluronidases (HYALs). Alterations of these metabolic enzymes are correlated with breast cancer progression.

Induction of HAS2 in the cells of the stroma on the breast tumor surroundings and increase of HA in the tumor microenvironment showed a stimulation of metastasis and proliferation. Recently, in our laboratory we discovered a new protein in the conditioned medium of the low invasive breast tumor cell line BC8701, called "Uncharacterized protein of c10orf118" or "Q7z3e2". Further studies on the two well-known breast cancer cell lines MCF-7 (low invasive cells) and MDA-MB231 (high invasive cells) demonstrated a higher expression and secretion of Q7z3e2 in MCF-7 cells. As reported in the literature, co-culture of breast cancer cells with fibroblasts results to an induction of HAS2 in fibroblasts and an increase of the secreted HA. In a recent experiment performed in our laboratory, it was noticed that when fibroblasts were treated with a recombinant protein of Q7z3e2 (part of protein sequence 1-211 a.a.) or with the conditioned medium of MCF-7 which was previously treated with anti-Q7z3e2 that blocks this protein, HAS2 was induced and HA was increased.

Thus, we hypothesize that this novel protein is implicated in breast tumor cellular mechanisms and its secretion is concerned in a cross-talk of breast tumor and stromal cells.

1. Karousou E et al. Matrix Biol In press.

INHIBITION OF HEPARANASE REVERSED HIGH-GLUCOSE-INDUCED EPITHELIAL – TO - MESENCHYMAL TRANSITION IN MESOTHELIAL CELLS

G. Bellin[1], V. Masola[1], S. Granata[1], G. Gambaro[2], M. Onisto[3], C. Rugiu[1], A. Lupo[1], G. Zaza[1]

[1]Renal Unit, Department of Medicine, University/Hospital of Verona, Verona, Italy; [2]Division of Nephrology and Dialysis, Columbus-Gemelli Hospital Catholic University, School of Medicine Rome, Italy; [3]Department of Biomedical sciences, University of Padova, Italy

E-mail: gloria.bellin@gmail.com

High glucose (HG)-induced epithelial-to-mesenchymal transition (EMT) of peritoneal mesothelial cells is a major biological mechanism leading to myofibroblasts accumulation in the omen-

tum of peritoneal dialysis (PD) patients.[1] We suppose that heparanase (HPSE), an endoglycosidase involved in the EMT of several cell lines,[2,3] may have a central role in this pro-fibrotic process.

To assess the role of HPSE in the HG-induced mesothelial EMT process, rat mesothelial cells were grown for 6 days in HG (200 nM) culture medium in presence or absence of SST0001, a specific HPSE inhibitor. Subsequently, EMT markers (VIM, alpha-SMA, TGF-beta) and VEGF were evaluated by RT-PCR and Immunofluorescence/western blotting. We also measured the trans-epithelial resistance of mesothelial cells and the permeability to albumin by using Millicell-ERS ohmmeter and spectrophotometer, respectively.

Our results revealed that 200 nM of glucose induced a significant up-regulation of all markers of EMT and VEGF after 6 days of culture and the introduction of SST0001 at day 3 reversed all these biological effects. Moreover, the inhibition of HPSE restored the normal trans-epithelial resistance and permeability lost during HG treatments. All together our data confirmed that HPSE has a central role in the induction of EMT process in HG condition. They suggest that in future the pharmacological inhibition of HPSE could represent a valuable therapeutic tool to minimize fibrosis and to avoid a rapid loss of the dialytic efficiency in patients undergoing peritoneal-dialysis treatment.

1. Ito T. et al. J Am Soc Nephrol. 2000;11(11):1969-79
2. Tomei P. et al. J Nephrol. 2016 Mar 29; doi:10.1007/s40620-016-0295-4
3. Masola V. et al. J Transl Med. 2015;13:181

INVOLVEMENT OF RIBOSOMAL RIBONUCLEIC ACIDS IN MINERALIZATION OF AORTIC VALVE INTERSTITIAL CELLS IN EXPERIMENTAL AND PATHOLOGICAL CONDITIONS

A. Bonetti, A. Della Mora, M. Contin, M. Marchini, F. Ortolani

Lab. of Histology and Embryology, Dept. of Experimental and Clinical Medicine, University of Udine, Udine, Italy.
E-mail: antonella.bonetti@uniud.it

As described for actual aortic valve mineralization,[1] metastatic-like calcification of primarily cultured aortic valve interstitial cells (AVICs) was previously found to consist in intracellular release of acid-phospholipid-rich material (PPM) followed by its layering (PPLs) at the edges of dying cells and cell-derived debris, and acting as major hydroxyapatite nucleator.[2] Since calcifying AVICs often showed electrondense particles resembling free ribosomes embedded within PPM, immunocytochemical and immunogold labelling detection of ribosomal ribonucleic acid (rRNA) was performed on AVICs untreated or treated for 3 up to 9 days with 3.0 mM inorganic phosphate, 100 ng/ml bacterial lipopolysaccharide (LPS), and 20% (v/v) conditioned medium derived from LPS-stimulated macrophages, so simulating metastatic calcification. Immunolocalization of rRNA was also performed on human aortic valve leaflets affected by severe calcific stenosis. Under light microscope, immunopositivity to rRNA resulted for both untreated cells and calcifying AVICs, with negligible differences depending on cell treatment or incubation times. In pro-calcific AVIC cultures, immunopositivity to rRNA was also appreciable at level of calcific nodules. Ultrastructurally, antibody-conjugated gold particles were found to decorate all ribosomes in untreated AVICs as well as PPM/PPLs and still recognizable ribosomes in calcifying AVICs. In stenotic aortic valve leaflets, immunopositivity to rRNA was clearly detectable around calcific nodules, with ultrastructural analysis revealing gold particles to be mainly localized at level of PPLs lining degenerating cells and cell-derived byproducts. As observed for cultured AVICs, free and membrane-bound ribosomes were additional immunopositive sites in suffering cells populating valve areas affected by incipient mineralization. In conclusion, these results suggest that rRNAs derived from ribosome degradation may contribute to aortic valve mineralization in both *in vitro* and *in vivo* conditions, with their acidic nature possibly enhancing PPM/PPL capacity to nucleate hydroxyapatite. The present results also strengthen the requirement of nucleic acid removal in preparing decellularized aortic valve scaffolds to attain calcification-free heart valve substitutes before surgical implantation.[3]

1. Kim KM Scanning Microsc 1995;9:1137–78.
2. Bonetti A et al. Anat Rec 2012;295:1117-27.
3. Iop L et al. PLoS One 2014;9:e99593.

HYPOXIA-INDUCIBLE FACTOR-1 PROMOTES UPREGULATION OF EXTRACELLULAR MATRIX IN ARTICULAR CHONDROCYTES

A. D'Ascola[1], M. Scuruchi[2], A. Avenoso[1], A. Calatroni, S. Campo[1], G.M. Campo[1]

[1]Department of Biomedical Sciences and Morphological and Functional Images; [2]Department of Clinical and Experimental Medicine, University of Messina, Italy

E-mail: adascola @unime.it

Cartilage homeostasis is regulated by multiple mechanisms including growth factors, cytokines, oxygen supply and mechanical force. Hypoxia is a stimulus for articular cartilage development and regeneration. These processes are regulated by the transcription factor hypoxia-inducible factors-1α (HIF-1α). Under normoxia, HIF-1α is hydroxylated by prolyl hydroxylase, ubiquinated and then degradated by proteosoma. Hypoxia inhibits the activity of the HIF-targeting prolyl hydroxylase enzymes, therefore HIF-1α traslocates into the nucleus and dimerizes with HIF-1β to induce transcription of target genes.[1] Recent study reported that HIF-1a is able to promotes the synthesis of relevant extracellular matrix (ECM) components.[2]

In this study we investigate the effect of hypoxia on gene expression of extracellular matrix components, such as collagen type II, aggrecan, biglycan and decorin, in primary articular chondrocytes. Cells cultured under normoxia were treated with deferoxamine mesylate, which inhibits HIF-1α hydroxilation, to simulate hypoxia, and transfected with siRNAs targeting HIF-1α. Treatment of cells with deferoxamine mesylate increased the levels of HIF-1αprotein. The levels of collagen type II, aggrecan, biglycan and decorin mRNAs and proteins assessed by real time RT-PCR and Western blotting, respectively, were significantly higher under simulated hypoxia than under normoxia. The specific siRNA knocked down mRNA and protein levels of ECM gene under hypoxia.

These data seem to confirm that hypoxia, by activating HIF-1α, promotes the anabolic activity of chondrocytes that increase ECM production. Particularly our goal has been shown that also biglycan and decorin were upregulated under simulate hypoxia, since these SLRP are reported to be key molecules in modulat-

ing the physiological processes, such as cartilage regeneration. The results suggest that HIF-1α has an important role in cartilage metabolism and modulation of HIF pathway could be a useful strategy to promote articular cartilage repair through regulating chondrocyte ECM synthesis.

1. Schofield CJ et al. Nat Rev Mol Cell Biol 2004;5:343–54.
2. Brucker PU et al. Arthritis Rheum 2005;52:3181–91.

THE BYSSUS THREADS OF PINNA NOBILIS: A HISTOCHEMICAL AND ULTRASTRUCTURAL STUDY

A. Diana[1], M. Reguzzoni[2], T. Congiu[2], A. Rescigno[1], F. Sollai[1], M. Raspanti[2]

[1]Department of Biomedical Sciences, University of Cagliari, [2]Department of Surgical and Morphological Sciences, University of Insubria, Varese; Italy
E-mail: diana@unica.it

In the history of textiles the byssus coming from Pinna nobilis (the largest bivalve mollusc in the Mediterranean sea) has covered only a marginal section but the fashinating recall of the alternative name "sea-silk" has crossed the centuries between myths and legends. Such a circumstance can only partially explain the fragmented and not systematic investigation of the biochemical and morphological properties of this precious and threatened with extinction material. The byssus is a tensile structure present in the larval stage of all bivalve molluscs.[1] As already mentioned, research data on the byssus threads are both relatively sparse and mostly dealing with few species of molluscs belonging to mytiloids.[2,3,4,5] but apparently no scientific report has been published on the *Pinna* byssus since 1970. Thus, we have collected enough byssus strands in order to perform a histochemical and ultrustructural study by means of integrated microscopic approach. Light microscopy observations revealed at low magnification a distinctive elliptical shape in cross-section, with a typical size close to 50×25 micron and a featureless glassy appearance. Histochemical experiments indicated the presence of elastic domains but a lack of collagen, which is known, by contrast, to be the main molecule in byssus from other species. Ultrastructural analysis by TEM showed, within the byssus thread, at least two components often associated to an inner arrangement of straight, tightly packed longitudinal streaks. SEM analysis not only confirmed the inner overlapping of straight, parallel subfibrils but made possible to detect the presence of unidentified material along the fracture surfaces. This was likely to be responsible for cementing together the same subfibrils and remarkably with tendency to be removed by exposure to extreme pH values. Finally, AFM micrographs were consistent with previous results in terms of tight subfibrillar packing and provided further evidence of orthogonal, barely visible connecting structures and again, HCl or NaOH treatment produces a subfibril patterns clean and free from any other component. Additional studies will be necessary to determine the biochemical nature of subfibrils and ground substance.

1. Yonge M . J Mar Biol Ass UK 1962;42:113-125.
2. Pearce T et al. J Exp Biol 2009;212:1442-1448.
3. Hagenau A et al. Macromol Biosci 2009;9:162-8.
4. Harrington MJ et al. J Struct Biol 2009;167:47-54.
5. Patel MB et al. Int J Curr Res Aca Rev 2015;3:20-27.

POSTERS SESSION

EFFECTS OF AN ANTIOXIDANT MIX SUPPLEMENTATION ON OXIDATIVE STRESS AND *IN VITRO* MINERALIZATION IN PRIMARY CELL CULTURES

F. Boraldi, S. Costa, D. Quaglino
Department of Life Sciences, University of Modena and Reggio Emilia, Modena; Italy
E-mail: federica.boraldi@unimore.it

Oxidative damages of biomolecules is often mediated by reactive oxygen species (ROS) generated by a wide range of biological processes (*i.e.* mitochondrial respiration, immune defence mechanisms, enzymatic chemical reactions) and by environmental factors (*i.e* tobacco smoke). ROS contribute to the aging process and to the development of pathologic conditions (i.e. diabetes and atherosclerosis). Antioxidants, through their ability to "neutralize" ROS consequences are considered to be protective and beneficial for cellular homeostasis. However, a growing literature is recently suggesting that an antioxidant molecule may exhibit anti- or pro-oxidant properties depending for instance on cell type, environmental factors, concentration and length of treatment. Therefore, we have investigated the effects of an antioxidant mix (quercetin, keampferol, vitamins C and E at low and high concentration) in fibroblasts isolated from patients affected by a mild chronic oxidative stress condition. Moreover, since altered redox balance has been associated to pathologic calcification, we have evaluated the effects of the same antioxidants in an *in vitro* mineralization model. Data indicate that: i) the antioxidant mix at high concentration induced a reduction of hydrogen peroxide and superoxide anion; ii) lipid peroxidation was decreased by both doses of antioxidant mix. After 10 and 25 days of culture in a pro-calcifying medium, the mineralization process appeared favoured by treatment with antioxidants at low doses. The antioxidant mix at high concentration induced cell death already after 10 days of treatment and therefore the extent of calcification observed at 25 days could be consequences of the accumulation of necrotic debris acting as nucleation site of mineral deposition. Results suggest that antioxidant mix is effective in reducing ROS and lipid peroxidation, however concentration and length of treatments may lead to cumulative effects with deleterious consequences on cell homeostasis, as demonstrated by the occurrence of cell death, increased phosphatase alkaline activity and calcification. Since ROS act also as signalling molecules, a strong ROS reduction may drive cells into a highly reducing state that is likely harmful. Therefore, synergic and cumulative effects of antioxidants should be taken into consideration especially when these molecules are self-administered without a real need.

Work supported by FCRMO (#2015-0306).

PRESENCE OF BOUND ANIONIC DETERGENT LIGANDS MIGHT PROMOTE ELASTIN DEGRADATION AFTER IMPLANTATION OF CELL DEPLETED HEART VALVE SCAFFOLDS

M. Spina[1], F. Naso[2], G. Gerosa[2], A. Zamuner[3], M. Dettin[3]
[1]Department of Biomedical Sciences, University of Padova; [2]Department of Cardiac, Thoracic and Vascular Sciences, University of Padova; [3]Department of Industrial Engineering, University of Padova, Italy
E-mail: mspina41@gmail.com

Facing the increasing incidence of aortic valve diseases, there is almost no alternative when replacement surgery is required in spite of the shortcomings of the currently used glutaraldehyde-treated (GAT) xenogenic bioprostheses that have a limited lifespan (1-2 decades) following incoming calcific degeneration. Tissue engineering (TE) procedures hold promise to overcome these limitations by repopulating cell-depleted scaffolds with patients own cells. However in case of xenogenic scaffolds these putative devices still share some biocompatibility problems (alpha-Gal epitopes) with the GAT substitutes as well as they might acquire new shortcomings in common with the cell depleted homografts in case of similar detergent-based scaffold preparation procedures.

Many detergent-based decellularization methods have been proposed. Anionic surfactants, and among them bile acids, have been widely used, even if toxicity of residual detergent might present limitations to the survival and/or the functional expression of the repopulating cells. In addition, bile acids like deoxycholate (DOC), cholate (COL) and taurodeoxycholate (TDOC) bound to elastin have been found to increase significantly the rate of its enzymatic degradation. Elastin degradation products in turn are known to promote myofibroblastic and osteogenic differentiation in fibroblasts and to recruit inflammatory cells *in vivo.*

Following a new HPLC-based procedure we have quantified bile acid residues entrapped in the decellularized scaffold of porcine aortic and pulmonary valves. Residual detergent was detected in all cell depleted samples . With respect to different treatments, DOC-based preparations exhibited the highest residual content, less than half of this value being detected in the TritonCOL-based one and the lowest in the TritonTDOC preparation. However, with respect to the same detergent its residual concentration was significantly lower in the pulmonary with respect to the aortic preparation. Thus suggesting that the difference might be related to the lower thickness of the pulmonary leaflets thus allowing a better washout of surfactant's excess.

In the perspective of a tissue-guided regeneration it has not been assessed if the presence of surfactant entrapped in the ECM could interfere with cell-matrix interactions in the promotion of cellular adhesion, migration as well as matrix-induced cell differentiation. Moreover it remains to be investigated whether residual detergents are evenly distributed within the ECM scaffolds or preferentially concentrated in the main collagen or elastin components.