41° CONGRESSO DELLA SOCIETA' ITALIANA PER LO STUDIO DEL CONNETTIVO/MATRIX BIOLOGY ITALY

SISC/MBIta 2025

MESSINA 2 – 4 OTTOBRE 2025

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Roberta Besio

Manuela Viola

Antonio J Lepedda

Michele Scuruchi

Maurizio Mongiat

Luisa Bracci

PROGRAMMA

Giovedì, 2 Ottobre 2025

PRIMA SESSIONE **Moderatori**: M. Onisto – A. Passi

13.00 – 14.30	Registrazione
14.30 – 15.00	Apertura del congresso e saluti di benvenuto
15.00 – 15.40	Opening Lecture *A. Theocharis (University of Patras)* **Versatile functions of serglycin in tumor cell phenotype and signaling**
15.40 - 16.00	*P. Brun (Università di Padova)* **Lactose-Modified Hyaluronic Acid Molecules attenuate in vitro chondrocyte inflammation**
16.00 - 16.15	*F. Bianchi (Università di Milano)* **Is antibiotic-induced gut dysbiosis associated to cardiac fibrosis?**
16.15 – 16.30	*V. Masola (Università di Padova)* **Metabolic control of ECM deposition and remodelling**
16.30 – 17.00	Coffee break

SECONDA SESSIONE **Moderatori**: D. Belotti – M. Mongiat

17.00 - 17.15	*A. J. Lepedda (Università di Sassari)* **Marine-derived sulfated polysaccharides enhance hemocompatibility and endothelialization of nanofibrous pcl scaffold for vascular graft applications**
17.15 - 17.30	*L. Depau (Università di Siena)* **HSPGs at the Frontline of Cancer Cell Migration: Functional and Therapeutic Insights**
17.30 - 17-45	*G. Schinello (CRO- Aviano)* **The Role of Multimerin-2 in Cancer: A Genome-Wide Transcriptomic Analysis Reveals a Novel Link to Immune Regulation**

20.00	Aperitivo e cena di benvenuto presso il ristorante il Giardino de La Durlindana, via Nicola Fabrizi 143, 98123 Messina

Venerdì, 3 Ottobre 2025

TERZA SESSIONE **Moderator**i: A. Rossi – L. Bracci

9.00 - 9.40	Invited Lecture N. Karamanos *(University of Patras, Greece)* **Harnessing the 3 Dimensional Cell Models to Evaluate the Multifaceted roles of Extracellular Matrix in Cancer**
9.40 - 10.00	*N. Gagliano (Università di Milano)* **AdipoRon modulates the invasive behavior of prostate cancer cells with distinct EMT phenotype**
10.00 - 10.20	*M. Franchi (Università di Bologna)* **Collagen forms a continuous skeleton connecting all adjacent extracellular matrices, from the epidermis to the bone marrow**
10.20-10.40	*L. Bracci (Università di Siena)* **A tetra-branched heparan sulfate proteoglycan binding peptide inhibits replication of different viruses**
10.40 - 11.10	Coffee Break

QUARTA SESSIONE **Moderatori:** M. Viola – R. Besio

11.10 - 11.25	*E. Carlessi (Mario Negri IRCCS)* **The matricellular protein thrombospondin-1 as a key regulator of breast cancer dormancy**
11.25 - 11.40	*N. Casagrande (CRO- Aviano)* **Aberrant Expression of Endothelial Multimerin-2 in Ovarian Cancer Cells Drives Chemoresistance and Predicts Poor Patient Outcome**
11.40 – 11.55	*N. Garibaldi (Università di Pavia)* **N-benzylglycine as a potential novel therapy for osteogenesis imperfecta: an *in vitro* and *in vivo* study**
11.55- 12.10	*A. Khan (Università di Pavia)* **Morphological and biochemical changes in the musculoskeletal phenotype of an animal model of diastrophic dysplasia**
12.10-12.25	*C. Fioravanti (Mario Negri IRCCS)* **Tumor-microenvironment interplay in pancreatic ductal adenocarcinoma metastasis**
12.25-12.40	*K. Spanopoulos (University of Patras)* **Uncovering Sulfated Hyaluronan–Mediated Changes in Matrix Effectors and Functional Properties of 3D Breast Cancer Cell Models**
12.40-12.55	*D. Henin (Università di Milano)* **Bone Regeneration in Peri-implant Defects Treated with the Subperiosteal Peri-implant Augmented Layer Technique Using Block and Particulate Deproteinized Bovine Bone Mineral: A Histological Evaluation**
12.55-15.00	Pranzo presso la sede del convegno

QUARTA SESSIONE **Moderatori**: M. Formato – G. Taraboletti

15.10 – 15.25	*L. Bizzotto (Università di Padova)* **FGF and FGFRs regulation in prostate cancer cells**
15.25 – 15.40	*E. Di Siena (CRO- Aviano)* **Loss of Multimerin-2 in Tumors Alters the ImmuneMicroenvironment**
15.40 – 15.55	*D. Antognoli (Università dell' Insubria)* **Molecular mechanism of the transition HA-collagen in wound repair**
16.10 – 16.25	*L. Anconelli (Università di Bologna)* **Osteogenic differentiation in 2d and 3d osteosarcoma models: role of the matrix and magnesium**
16.25 – 16.55	Coffee Break
16.55-18.25	**Assemblea Soci**
20.00	Cena sociale Ristorante Bellavista- Via Circuito, 36, Torre Faro (ME)

Sabato, 4 ottobre 2025

QUINTA SESSIONE **Moderatori**: N. Gagliano – M. Franchi

9.30 – 9.45	*F. Aliquò (Università di Messina)* **ESM1 is involved in crosstalk between activate fibroblasts and breast cancer cells to promote tumor progression**
9.45 – 10.00	*M. Candela (Università dell' Insubria)* **3D breast cancer cell culture in gelatin and hyaluronic acid scaffold: impact on gene expression**
10.00 – 10.15	*P. Cancemi (Università di Palermo)* **Evaluation of the role of the proteoglycan ESM1 in breast cancer**
10.15 – 10.30	*M. Scuruchi (Università di Messina)* **Endocan as a Biochemical Modulator of Inflammation, Oxidative Stress, Cardiac Remodeling, and Tumor-Related Signaling**
10.30 – 11.00	Coffee break
11.00 – 11.15	*F. Polito (Università di Messina)* **Multi-omics profiling in medication-related osteonecrosis of the jaw**
11.15 –11.30	*A. D'Ascola (Università di Messina)* **NOTCH signaling activation is part of the inflammatory response induced by LPS and small HA oligosaccharides in human chondrocytes**
11.30 – 12.00	**Cerimonia di Premiazione e Chiusura del Congresso**

COMUNICAZIONI ORALI

LACTOSE-MODIFIED HYALURONIC ACID MOLECULES ATTENUATE IN VITRO CHONDROCYTE INFLAMMATION

Paola Brun[1], Elisa Belluzzi[2], Valentina Masola[3], Pietro Ruggeri[2], Alice Cristina Donato[1]

1.Department of Molecular Medicine, University of Padova, Padova, Italy

2.Orthopaedics and Orthopaedic Oncology, Department of Surgery, Oncology and Gastroenterology (DiSCOG), University of Padova, Padova, Italy

3.Department of Biomedical Sciences, University of Padova, Padova, Italy

E-mail: paola.brun@unipd.it

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by macrophage-mediated inflammation that induces chondrocytes to begin to synthesize proinflammatory cytokines and galectins, molecules that bind specifically to β-galactoside sugars. Conventional treatment with pain relievers and NSAIDs or corticosteroids can be effective but it is often associated with significant side effects and toxicities. For this reason, viscosupplementation with hyaluronic acid (HA) has represented a notable advance in OA therapy, offering a local treatment option with a more favourable safety profile. Recently, in our laboratory we demonstrated that Hylach®, a hyaluronic acid (HA) derivative conjugated with lactose-based residues has a significantly broader anti-inflammatory effect. This study aims to evaluate the anti-inflammatory effects of Hylach®, on in vitro inflamed primary human chondrocytes. Methods. Changes in cell viability and pro-inflammatory mediators, at both gene and protein level, were analysed by means of MTT, qPCR and Elisa tests, using 2D and 3D human articular chondrocyte cultures exposed to the conditioned medium (CM) of PMA and LPS activated U937 monocytes and subsequently treated with Hylach or HA for different time points (4, 10, and 24 h). Statistical analyses were performed using the unpaired Student's t-test and the one-way ANOVA test with multiple comparisons. HA and Hylach® did not affect cell viability at any of the tested concentrations. Both molecules reduced the overexpression of Gal-3 and pro-inflammatory molecules in 2D inflamed cell cultures, at both gene and protein levels. Notably, IL-1β, IL-6 and Gal-3 showed a more pronounced inhibitory effect at 4 h, with Hylach demonstrating a stronger reduction compared to native HA. Moreover, in inflamed 3D chondrocyte cultures, Hylach® but not HA, significantly reduced IL-1β, TNF-α and Gal-3 gene expression.Our findings demonstrated that Hylach® exerts early and more potent, anti-inflammatory effects in inflamed 2D and 3D chondrocyte cultures when compared to HA. These data suggest that targeting Gal-3 through selective HA derivatives may represent a promising strategy for modulating both inflammation and matrix remodelling in OA.

IS ANTIBIOTIC-INDUCED GUT DYSBIOSIS ASSOCIATED TO ABERRANT INNATE IMMUNE RESPONSES AND CARDIAC FIBROSIS?

N. Gagliano[1], F. Arnaboldi[1], S. Vinci[1], L. Forleo[1,2], L. Sfondrini[1,3], D. Bosisio[4], F. Bianchi[1,5]

1.Università degli Studi di Milano, Milan, Italy;

2.IRCCS Fondazione Don Carlo Gnocchi Onlus, Milan, Italy;

3.Ss Microenvironment and Biomarkers of Solid Tumors, Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy;

4.Università degli Studi di Brescia, Brescia, Italy; 5 U.O. Laboratorio di Morfologia Umana Applicata, IRCCS Policlinico San Donato, Milan, Italy.

E-mail: francesca.bianchi1@unimi.it

The human gut microbiota (GM) is composed of trillions of microbial cells that play a crucial role in the host's biological functions through multiple mechanisms, including immune responses. Gut dysbiosis refers to an imbalance in the composition of the GM. Recent evidence has highlighted a pivotal contribution of gut dysbiosis to the development of non-communicable chronic diseases in which prolonged activation of the innate immune system plays a central pathogenic role, such as type I interferonopathies and cardiovascular diseases (CVDs). We established dysbiosis in a mouse model by administering vancomycin in drinking water. Control mice (CT) and animals receiving a high-fiber diet (HFiber) after antibiotic treatment were also included to assess a possible protective effect of dietary modulation. This approach is useful also for interferon-mediated disorders, since in both contexts, disruption of immune homeostasis by TLRs activation is the initiating event leading to chronic tissue damage. We already characterized immune profile by cytofluorimetric analysis (FACS) of mesenteric lymphnodes and spleens. Moreover, histological analysis using light microscopy showed no overt structural abnormalities, while transmission electron microscopy (TEM) revealed significant changes in the size and morphology of interfibrillar mitochondria as well as in their dynamics in treated animals compared with controls, together with alterations in oxidative stress pathways. Proteomic profiling suggested a potential role of dysbiosis in promoting extracellular matrix (ECM) remodeling, likely favoring deposition of collagen and cardiac fibrosis.
In the present study we quantified interstitial collagen in paraffin-embedded and Sirius Red–stained heart sections of CT and vancomycin-treated mice to assess the presence of cardiac fibrosis. Collagen content within the myocardial interstitium was expressed as fibrosis index.Our preliminary data indicate that dysbiotic mice displayed a higher fibrosis index compared with CT, supporting the hypothesis that vancomycin-induced dysbiosis may contribute to cardiac fibrosis. Notably, the fibrosis index in HFiber animals was comparable to CT, suggesting that a high-fiber

diet may counteract the detrimental cardiac consequences of gut dysbiosis.These findings are consistent with the idea that persistent activation of innate immunity chronic, originating from inappropriate activation of innate immune receptors such as TLRs, similar to those driven by aberrant type I interferon production in interferonopathies, may sustains ECM remodeling.

1.Nguyen HO et al. Front Immunol 2022, 12:797390
2.Salvi V et al. JCI Insight 2021
3.Bosisio D et al. Cancer Lett 2019, 452:59–65
4.Hou K et al. Signal Transduct Target Ther 2022, 7:135
5.Segura AM et al. Heart Fail Rev 2014, 19:173–85.

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METABOLIC CONTROL OF ECM REMODELLING AND DEPOSITION

V. Masola[1], L. Bizzotto[1], T. Prosdocimi[2], A. Arduini[2] and M. Onisto[1].

1.Department of Biomedical Sciences, University of Padova. 2) Iperboreal Pharma Srl, Pescara, ITALY.

E-mail: valentina.masola@unipd.it

The creation (deposition) and breakdown (remodelling) of the extracellular matrix (ECM) are fundamentally controlled by a cell's metabolic state. ECM Deposition is a constructive process that requires significant energy and specific molecular building blocks. Key metabolic inputs include: amino acids, essential for producing proteins like collagen, and glucose which provides energy (ATP) and is converted into sugars (glycosaminoglycans) that hydrate the matrix. ECM remodelling involves the degradation of the matrix by enzymes, primarily matrix metalloproteinases (MMPs). The activity of these enzymes is regulated by cellular energy levels sensors, such as AMPK which can suppress MMPs when energy is low, and redox state: indeed, oxidative stress can increase MMPs activity. Alterations in cellular metabolism are a hallmark of many diseases, and this dysregulation often has profound consequences for the ECM. In fibrotic diseases, such as peritoneal and pulmonary fibrosis, there is an excessive deposition of ECM, leading to tissue scarring and organ dysfunction. This is often driven by metabolic reprogramming in cells like fibroblasts and parenchymal cells, which increase their uptake of glucose and glutamine to fuel the massive production of collagen. Even the tumour microenvironment is characterized by a highly remodelled ECM that supports cancer cell growth and metastasis. Cancer cells often exhibit altered metabolism, such as the Warburg effect (aerobic glycolysis), which provides the necessary building blocks and energy for both rapid cell proliferation and the synthesis of ECM components that facilitate invasion.Our study on peritoneal fibrosis induced by peritoneal dialysis confirmed that glucose (the major component of the main peritoneal dialysis solutions on the market) induces a Warburg-like effect on mesothelial cells leading to fibrosis. Differentially we proved that the use of osmo-metabolic fluids, coupling glycolysis to Krebs cycle, not only prevent the induction of fibrosis but also induce a reworking of the matrix already deposited.

MARINE-DERIVED SULFATED POLYSACCHARIDES ENHANCE HEMOCOMPATIBILITY AND ENDOTHELIALIZATION OF NANOFIBROUS PCL SCAFFOLD FOR VASCULAR GRAFT APPLICATIONS

G Obino[1,2], G Nieddu[1], M Formato[1], AJ Lepedda[1], L Moroni[2]

1.Department of Biomedical Sciences, University of Sassari, Italy

2.Complex Tissue Regeneration Department, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht, Netherlands

E-mail: ajlepedda@uniss.it

Despite the clinical need for functional arterial substitutes, success has been limited to arterial replacement of large-calibre vessels (diameter > 6 mm), leaving the bulk of demand unmet. Besides adequate mechanical properties to sustain the hemodynamic flow forces, scaffold's properties should include biocompatibility, controlled biodegradability with non-toxic products, low inflammatory/thrombotic potential, porosity, and a specific combination of molecular signals allowing vascular cells to attach, proliferate and synthesize their own extracellular matrix. We recently purified a fucosylated chondroitin sulfate from two marine invertebrates, *Holothuria tubulosa* and *Sarcotragus spinosulus*, which showed strong anticoagulant activity and *in vitro* cytocompatibility[1]. These features were exploited in this study to improve the biocompatibility of nanofibrous poly(Ɛ-caprolactone) (PCL) electrospun scaffolds[2]. Furthermore, a functionalized multi-layered PCL-based small-caliber tissue-engineered vascular graft (sTEVG) was fabricated using a combination of electrospinning and 4-axis printing techniques, providing precise control over scaffold porosity, fiber alignment, and tunable mechanical properties and dimensions.The sTEVG supported a rapid formation of a mature endothelium on the inner layer, evaluated using human umbilical vein endothelial cells (HUVECs), whereas the middle layers enabled effective adhesion, orientation and infiltration by human coronary artery smooth muscle cells (HCASMCs), a crucial step to produce a functional tunica media regulating blood flow and maintaining blood pressure. The outer layer, consisting of random electrospun nanofibers, contributed significantly to the mechanical properties of the graft such as elasticity, toughness, burst pressure, and resistance to physiological vessel pressures.Overall, the developed construct, with its enhanced tunable mechanical and biological properties, shows promise in sTEVG applications representing a valuable customizable/off-the-shelf alternative to autologous grafts.

1.Nieddu G, et al. Mar Drugs. 2024, 22(3):139.
2.Obino G, et al., Cell Biomaterials. 2025, 1, 100155.

HSPGS AT THE FRONTLINE OF CANCER CELL MIGRATION: FUNCTIONAL AND THERAPEUTIC INSIGHTS

L. Depau[1], M. Garfì[1], M.F. Paolocci[1], G. Silvi[1], C. Falciani[1], J. Brunetti[1] and L. Bracci[1].

1.Department of Medical Biotechnologies, University of Siena, Siena; Italy

E-mail: lorenzo.depau@unisi.it

The role of heparan sulfate proteoglycans (HSPGs) in cancer cell differentiation, proliferation and migration has been well recognized and related to their ability to specifically interact with growth factors, morphogens, and extracellular matrix proteins, mainly through sulfated groups on their glycosaminoglycan (GAG) chains. However, a precise molecular characterization of the biological role of HSPGs in cancer remains incomplete. HSPGs, besides working as coreceptors for growth factors and morphogens, may have a determinant and autonomous role in cancer signaling events, regulating cell adhesion, migration, and invasiveness. Being overexpressed and over sulfated in cancer cells, HSPGs may become attractive tumor targets for cancer diagnosis and therapy. We had used the tetra-branched peptide NT4, which binds sulphated GAG chains of HSPGs, to validate HSPGs as potential tumor associated antigens in different human solid tumors. The same NT4 peptide had been tested as a tumor targeting agent for either cancer cell imaging or therapy, in vitro and in vivo[1]. Once identified the sulfated GAG specificity of NT4[2,3], the peptide has also been used as a specific tool for studying the role of HSPGs in cancer cell migration and invasiveness [2,4]. We found that NT4 inhibits adhesion, oriented migration, and colony formation in different human cancer cell lines. We investigated the role of HSPGs in two different human breast cancer cell lines that exhibit distinct migration behaviors: single-cell mesenchymal migration (MDA-MB-231) and collective migration (MCF-7). After assessing the expression of HSPG and of E- and N- cadherin in cell lines, their specific cellular distribution was detected by confocal microscopy in migrating and static cells. We observed that membrane distribution of E- or N- cadherins in migrating cells is complementary to that of HSPG, as detected by either NT4 peptide or an anti-HS monoclonal antibody. This is particularly evident in collective migration, where HSPGs are exclusively located at the migration front of leader cells, whereas cadherins are only expressed by follower cells[5]. Additionally, we recently found that syndecan 4 and PIP2 are also selectively localized at the migration front of collectively migrating MCF-7 cells. Our results suggest that HSPG may have a crucial role in defining the front of migrating cells, while cadherin-mediated cell-cell contacts on the opposite site may contribute to inducing protrusion formation at the front of migrating cells, as already suggested[6].

1.Brunetti et al. Sci. Rep. 2015, 5, 17736

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4.Depau et al. J Med Chem 2020, 63, 15997
5.Depau et al., Front Cell Dev Biol. 2025 12:1505680
6.Grimaldi et al Nat Commun 2020, 11, 5397

THE ROLE OF MULTIMERIN-2 IN CANCER: A GENOME-WIDE TRANSCRIPTOMIC ANALYSIS REVEALS A NOVEL LINK TO IMMUNE REGULATION.

Giorgia Schinello[1], Evelina Poletto[1], Emanuele Di Siena[1], Lucrezia Camicia L.[1], Naike Casagrande.[1], Matteo Braga[1]., Maurizio Mongiat[1]

[1]National Cancer Institute CRO Aviano (IRCCS), Italy.

E-mail: giorgia.schinello@unive.it

Multimerin-2 is an endothelial-derived extracellular matrix protein crucial for vascular stability and homeostasis. While present in both normal and tumor vasculature, MMRN2 is frequently downregulated in cancers such as ovarian, gastric, and colon, and its loss correlates with increased vascular permeability and reduced therapy efficacy[1,2,3]. To systematically investigate the functional consequences of Multimerin-2 loss in the tumor microenvironment, we conducted a genome-wide transcriptomic analysis using NanoString technology. We modeled this i*n vitro* by silencing Multimerin-2 in human umbilical vein endothelial cells (HUVECs) via adenoviral transduction. Our analysis revealed that Multimerin-2 knockdown significantly alters the expression of numerous genes, with a surprising and pronounced enrichment for pathways involved in immune response. Notably, the chemokine CXCL11 was among the most upregulated genes. We further demonstrated that this Multimerin-2-loss-driven CXCL11 upregulation enhances the migration of inflammatory cells through an IFN-γ-dependent mechanism. Crucially, this finding was validated in vivo, as high CXCL11 levels were also detected in tumors from Multimerin-2-deficient mice. Our results uncover a novel mechanism whereby loss of endothelial Multimerin-2 modulates the tumor immune microenvironment, providing a potential predictive biomarker for patient stratification and a new therapeutic target to improve cancer therapy.

1. Pellicani, R. et al. Matrix Biology 87, 11–25 (2020).
2. Lugano, R. Cell. Mol. Life Sci. 77, 1745–1770 (2020).
3. Andreuzzi, E. et al. IJMS 19, 3983 (2018).

ADIPORON MODULATES THE INVASIVE BEHAVIOR OF PROSTATE CANCER CELLS WITH DISTINCT EMT PHENOTYPE

Ana Luiza Romano Gabriel[1], Francesca Bianchi[2,3], Michele Sommariva[2], Stefano Vinci[2], Martina Anselmi[4], Daniela Bosisio[5], Sergio Luis Felisbino[1], Nicoletta Gagliano[2]

1. Institute of Biosciences, São Paulo State University (UNESP), Botucatu, SP, Brazil

2.Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy

3.U.O. Laboratorio di Morfologia Umana Applicata, IRCCS Policlinico San Donato, Milan, Italy

4.Unit of Microenvironment and Biomarkers of Solid Tumors, Department of Experimental Oncology, Fondazione IRCCS - Istituto Nazionale dei Tumori, Milan, Italy

5.Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

E-mail: nicoletta.gagliano@unimi.it

In prostate cancer (PCa) progression the epithelial-to-mesenchymal (EMT) allows cancer cells to acquire morpho-functional characteristics for invasion and metastasis. During EMT cancer cells lose their epithelial features and adopt mesenchymal traits, but also hybrid/intermediate phenotypes can occur, characterized by enhanced invasive properties.A reduction of serum adiponectin (APN) was detected in PCa patients with aggressive disease and, since AdipoRon (AR), an agonist of APN, was demonstrated to exert a potent antitumor effect in several types of cancers including PCa, we explored the effect of AR on the invasive potential of PCa cells having different EMT-related phenotypes. For this purpose, DU145 and PC3 cells were cultured in 3D-spheroids and treated with 150 µM AR for 48h or left untreated (CT). Expression for AdipoR1/R2 APN receptors and EMT markers was analyzed using real-time PCR and Western blot. To assess the invasive potential, MMP-2/-9 activity and TIMP-1/-2 gene expression were assessed by zymography and real time PCR, respectively.Gene expression analysis confirmed the "more epithelial" phenotype of DU145 compared to the "more mesenchymal" PC3 cells having high E-cadherin and N-cadherin expression, respectively. However, in both cell lines, vimentin was similarly expressed and unaffected by AR administration. AdipoR1 and AdipoR2 were expressed at higher levels in DU145 compared to PC3 cells and, in response to AR administration, they were markedly up-regulated only in DU145 spheroids, while their expression remained unchanged in PC3 spheroids.MMP-2 activity was similar in both CT and AR-treated DU145 and PC3 spheroids while MMP-9 activity, higher in PC3 compared to DU145 cells, was increased by AR in both cell types. While TIMP-2 mRNA levels are similar in the different experimental conditions, TIMP-1 mRNA levels were higher in PC3 compared to DU145 spheroids, but AR was not able to modify its gene expression. However, MMP-2/TIMP-2 ratio was strongly decreased in both AR-treated DU145 and PC3 spheroids, compared to their controls, and a similar pattern for MMP-9/TIMP-1 was observed only in DU145 cells. Overall, our

results indicate that AR seems more effective in reducing the invasive potential of PCa cells exhibiting a “more epithelial” than “more mesenchymal” phenotype, likely inducing an up-regulation of its receptors.

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2.Cui et al. Oncol Rep 2018;40:1330.
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COLLAGEN FORMS A CONTINUOUS SKELETON CONNECTING ALL ADJACENT EXTRACELLULAR MATRICES, FROM THE EPIDERMIS TO THE BONE MARROW

M. Franchi[1], M. Rossi[2], E. Malucelli[2], L. Franchi[3], L. Anconelli[2], C. Cappadone[2]

1.Department for Life Quality Studies, University of Bologna, Rimini; Italy.

2.Department of Pharmacy and Biotechnology, University of Bologna, Bologna; Italy.

3.School of Medicine, University of Bologna, Bologna; Italy

E-mail: marco.franchi3@unibo.it

Extracellular matrices (ECMs) of connective tissues regulate and support development, homeostasis and structural integrity of tissues and organs, and govern all reparative processes and diseases [1]. Structural and chemical connections between the epidermis, the basement membrane, and various ECM components suggest that ECM layers of the skin are primarily involved in sensing and transmitting mechanical loads from the environment [2]. We demonstrate that all ECMs including skin basement membrane, papillary and reticular dermis, hypodermis, fascia superficialis, subcutaneous tissue, deep fascia, epimysium, perimysium, endomysium, tendon, paratenon, mesotenon, epitenon, periosteoum, bone, and endosteoum are structurally connected to the bone marrow. In particular collagen, the main component of the ECMs, crossing all the connective tissues in the body, forms a continuous biomechanical skeleton connecting all the ECMs, from the epidermis to the bone marrow. In each ECM of the different connective tissues, both fibrillar collagen and interposed glycosaminoglycans (GAGs) and proteoglycans (PGs) linking and trapping large amount of water constantly adapt to respond to functional requests by transmitting tension and opposing slippage or pressure loads against gravity. In particular, we analyzed how the main fibrillar collagens in the body (type I and type III) form fibrils of different size, differently structured and oriented to resist and transmit both pressure and tensional forces from the environment to the deeper tissues [3]. The fibrillar collagens are immersed in a viscous matrix of GAGs and PGs which strongly contributes to the mechanical properties of the continuous collagen skeleton and modulates ECMs adaptations. These considerations strongly suggest a further role of the ECMs system in sensing and transmitting the environmental stimuli.

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A TETRA-BRANCHED HEPARAN SULFATE PROTEOGLYCAN BINDING PEPTIDE INHIBITS REPLICATION OF DIFFERENT VIRUSES.

L. Depau[1], L. Fiaschi[1], G. Bonucci[1], C. Falciani[1], I. Vicenti[1], M. Zazzi[1], J. Brunetti[1] and L. Bracci[1]

1.Department of Medical Biotechnologies, University of Siena, Siena, Italy

E-mail: luisa.bracci@unisi.it

Beyond several 'heparin-binding' endogenous ligands, Heparan Sulfate Proteoglycans (HSPG) can also bind many viruses, which use them as cell receptors or co-receptors[1] .Given their role in endocytosis and cellular vesicle transport, membrane HSPG may be used by viruses not only for concentrating at host cell membrane, but also for facilitating viral particles internalization. Viruses that use cell membrane HSPG as possible receptors include, Flaviviruses, herpes viruses, alphaviruses and others like HIV, HBV and coronaviruses like Sars-CoV, Mers-CoV and Sars-CoV-2 [2,3]. For some of the reported viruses, the binding to HSPG was reported in natural virus isolates, while for many others, the binding to HSPG was only detected in cell-adapted strains, indicating that binding to HSPG on host cells may be positively selected in evolution of virus strains[3]. With the aim of analyzing the role of HSPG in cell infection we used the HSPG-binding peptide NT4. NT4 is a tetra-branched peptide which specifically binds to highly sulfated HSPG on different human cell lines and can interfere with HSPG function in cell adhesion and migration, probing to be a useful tool for studying HSPG function and ligand binding [4-6]. Moreover, thanks to its branched form, NT4 is resistant to physiological degradation by proteases and peptidases [7], which is the main hindrance, limiting in-vivo use of linear monomeric peptides. Indeed, NT4 was efficient for targeting HSPG in different animal models of human cancers, resulting in a potential therapeutic use [8,9]. We checked the ability of NT4 to interfere with cell infection by different strains of Sars-Cov-2 and we found that NT4 can completely inhibit cell infection by Sars-Cov-2 omicron, while it has limited effect on infection by the original Wuhan variant, indicating a crucial role for HSPG in cell infection by omicron variants, which was not present in the original variant. We found that NT4 can also inhibit infection of different viruses, including HIV and West Nile Virus.

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THE MATRICELLULAR PROTEIN THROMBOSPONDIN-1 AS A KEY REGULATOR OF BREAST CANCER DORMANCY

E. Carlessi[1], P. Borsotti[1], C. Grasselli[2], M. Colombo[2], M. Marabese[2], M. Ripamonti[3], L. Carminati[1], G. Taraboletti[1]

1,2.Department of Experimental Oncology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Bergamo[1] and Milan[2], Italy;

3.Department of Biomedical Engineering, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Bergamo, Italy

E-mail: elena.carlessi@marionegri.it

Breast cancer (BC) remains one of the most diagnosed cancers in women worldwide. Although primary tumors can be effectively treated, distant metastases still occur in up to 30% of patients, often at late times. This phenomenon is attributed to the survival of dormant disseminated tumor cells (DTCs), that remain viable but non-proliferative, particularly within the bone marrow (BM). The matricellular protein thrombospondin-1 (TSP-1) plays a crucial role in mediating tumor-host interaction and has been reported to contribute to a dormancy-supporting niche. The exact mechanism of TSP-1 pro-dormancy effect is still unknown. We developed a breast cancer model (4T1.2-CVp27) to monitor dormant TCs both in vitro and in vivo settings, based on the detection of the $p27^{Kip1}$ quiescence marker fused to a fluorescent protein. In vitro, tumor cell quiescence was analyzed in coculture systems with freshly isolated murine BM cells or microvascular endothelial cells and in response to TSP-1 and TSP-1 recombinant fragments. Exposure of TCs to BM-derived cells resulted in increased tumor cell quiescence. This effect was mediated by TSP-1, as TSP-1-neutralizing antibodies prevented the induction of dormancy, highlighting the functional role of TSP-1 within the BM microenvironment. Among several cell populations present in BM, we found that endothelial-derived TSP-1 regulated dormancy in a context-dependent manner. In vitro, TSP-1 directly induced 4T1.2-CVp27 cell quiescence, without promoting cellular senescence. The activity was mediated by the interaction of the C-terminal region of TSP-1 with its receptor CD47 in association with αvβ3 integrin, leading to an increased p38/ERK ratio – a hallmark of dormancy. Additionally, TSP-1 treatment resulted in FAK activation and nuclear translocation of YAP, suggesting a pro-survival role for TSP-1 in maintaining the viability of the dormant tumor cells. These findings underscore the critical role of the BM niche in regulating tumor dormancy and identify the TSP-1/CD47/αvβ3 integrin complex as a key mechanism by which TSP-1 promotes dormancy in BC cells, through the promotion of cell survival and inhibition of cell proliferation.

These results lay the groundwork for the development of TSP-1–based therapeutic strategies aimed at maintaining DTCs in a dormant state and preventing metastatic relapse.

ABERRANT EXPRESSION OF ENDOTHELIAL MULTIMERIN-2 IN OVARIAN CANCER CELLS DRIVES CHEMORESISTANCE AND PREDICTS POOR PATIENT OUTCOME

Naike Casagrande[1], Evelina Poletto[1], Emanuele Di Siena[1], Greta Carobolante[1], Lucrezia Camicia[1], Matteo Braga[1], Giorgia Schinello[1], Maurizio Mongiat[1]

1.National Cancer Institute CRO Aviano (IRCCS) - Italy

E-mail: naike.casagrande@cro.it

Epithelial Ovarian Cancer (EOC) remains a lethal malignancy[1], largely due to the development of chemoresistance after standard therapy[2]. There is a critical need to identify novel biomarkers that predict drug sensitivity and guide treatment strategies. Here, we report the unexpected expression of the endothelial-specific glycoprotein Multimerin-2 in EOC cells[3].Immunohistochemical analysis of EOC tissues revealed Multimerin-2 not only in CD34+ blood vessels but also in distinct subpopulations of tumor cells. This aberrant expression was confirmed *in vitro* across a panel of EOC cell lines at both the mRNA and protein levels. Notably, Multimerin-2 was frequently expressed in models of high-grade serous ovarian carcinoma (HGSOC), suggesting a role in aggressive disease.To functionally characterize this finding, we genetically silenced Multimerin-2 using siRNA and CRISPR-Cas9 knockout. Dose-response assays demonstrated that Multimerin-2-expressing cells exhibited significantly lower sensitivity to cisplatin compared to controls. Supporting these experimental data, bioinformatic analysis of patient cohorts revealed that high Multimerin-2 expression is a powerful predictor of poor prognosis, correlating with reduced progression-free and overall survival in stage IV EOC patients.Collectively, our results identify the aberrant expression of endothelial Multimerin-2 as a novel mechanism of chemoresistance in ovarian cancer. This work positions Multimerin-2 as a promising predictive biomarker and a potential therapeutic target to overcome drug resistance and improve outcomes for EOC patients.

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N-BENZYLGLYCINE AS A POTENTIAL NOVEL THERAPY FOR OSTEOGENESIS IMPERFECTA: AN IN VITRO AND IN VIVO STUDY.

Garibaldi N.[1,2], Besio R.[1], Tonelli F.[1], Fernandez J.[2], Pirota V.[3], Doria F.[3], Albini B.[4], Galinetto P.[4], Carriero A.[2], Forlino A[1].

1.Dipartimento di Medicina Molecolare, Unità di Biochimica, Università di Pavia, Pavia, Italy

2.Department of Biomedical Engineering, The City College of New York, New York, United States

3.Dipartimento di Chimica, Università di Pavia, Pavia, Italy

4.Dipartimento di Fisica, Università di Pavia, Pavia, Italy

E-mail: nadia.garibaldi@unipv.it

Mutations in collagen type I chains or in proteins involved in their synthesis, post translational modifications, folding and secretion are the basis of the rare skeletal dysplasia Osteogenesis imperfecta (OI). These defects lead to the production of abnormal procollagen I molecules and to the deposition of a bone matrix with impaired structure and quality. Mutated collagen is also partly retained in the intracellular environment, due to the delay in its folding, thus impairing protein trafficking and causing endoplasmic reticulum (ER) stress, that plays a key role in OI phenotype and is a potential target for treatment. Recently, the chemical chaperone 4-phenylbutyrate (4-PBA) has proved its efficacy in improving OI cellular homeostasis[2, 3]. However, the limited half-life of the drug represents a limitation to its use. We designed and synthesized a new chaperone, namely N-benzylglycine (N-BG) through computational modelling of 4-PBA molecule focusing on increasing its intracellular stability. The aim of this work was to test its efficacy *in vitro* and *in vivo*. In osteoblasts from the dominant OI murine model *Brtl/+*, N-BG was non-toxic and metabolized less quickly than 4-PBA, furthermore it favoured protein secretion lowering intracellular protein aggregates and positively modulating osteoblast stress. N-BG also reduced cellular apoptosis. In *Brtl/+* matrix, N-BG improved collagen incorporation compared to 4-PBA and ameliorated collagen matrix quality. For the *in vivo* testing of the drug, two zebrafish models were used: the dominant OI *Chi/*$^{+}$ and the recessive *p3h1*$^{-/-}$, both displaying short length and abnormal bone properties. Caudal vertebrae analysis by synchrotron microtomography, second harmonic generation microscopy, Raman spectroscopy and nanoindentation revealed impaired bone structural parameters and porosity, irregular collagen distribution and abnormal compositional and mechanical parameters in both models compared to WT, with a more severe phenotype in *Chi/*$^{+}$. N-BG administration highlighted a genotype and sexual dimorphism, since it beneficially impacted only collagen fibrils organization limited to female *p3h1*$^{-/-}$ fish. Our study proposes N-BG a

potential complementation strategy for the current OI therapies, it also provides a thorough comparison between OI models with different inheritance and between sexes.

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MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN THE MUSCULOSKELETAL PHENOTYPE OF AN ANIMAL MODEL OF DIASTROPHIC DYSPLASIA

Asifa Khan[1], Alessandra Leone[1], Frank Zaucke[2], Zsuzsa Jenei-Lanzl[2], Michal Tamáš[3], Paolo Bonaldo[3], Matteo Signor[2], Giulio Gatto[2], Antonella Forlino[1], Antonio Rossi[1]

1.Dept. of Molecular Medicine, Biochemistry Unit, University of Pavia, Pavia, Italy

2.Trauma Surgery and Orthopedics, Goethe University Frankfurt, University Hospital, Frankfurt am Main, Germany

3.Dept.t of Molecular Medicine, University of Padova, Padova, Italy.

E-mail: asifa.khan01@universitadipavia.it

Diastrophic dysplasia (DTD) is a recessive chondrodysplasia caused by defects in SLC26A2, a sulfate/chloride antiporter of the cell membrane. Mutations in the transporter cause sulfate uptake impairment, thus affecting intracellular sulfate metabolism and proteoglycan (PG) sulfation. PG undersulfation alters the biochemical properties of these molecules impacting cartilage ECM structure and function. DTD patients are characterized by short trunk and limbs, hand and foot deformities, kyphoscoliosis and joint contractures. Degenerative changes in the joints are progressive, leading to deformities and early osteoarthritis. To better characterize the adult musculoskeletal phenotype, we studied at the morphological and biochemical level the knee joint, intervertebral discs (IVDs) and tibialis anterior (TA) muscles of an *in vivo* model of DTD, the *dtd* mouse, till 6 months of age. X-ray studies at 1, 2 and 6 months of age showed significant growth retardation and kyphosis in *dtd* mice. MicroCT revealed structural defects in the trabecular and cortical parameters of the tibia, and subchondral bone in the mutants. Proteoglycans and collagens from the femoral and tibial epiphysis of 2- and 4-months old wild-type and *dtd* mice were analysed by electrophoresis or immunoblotting. The expression of decorin was comparable; however, there was a shift to higher apparent molecular weight of glycanated decorin in *dtd* animals compared with wild-type, that might be due to reduced sulfation or to increased length of the glycosaminoglycan chain. No obvious difference was observed in the amount of collagen type II in the wild-type and dtd mice. Inflammatory biomarkers, TNFα and IL1ß, were increased in the epiphysis of *dtd* mice, prominently at 4-month timepoint. By immunohistochemistry, the IVDs of *dtd* mice exhibited signs of degeneration from the 2-month-old age point. In DTD patients as well as in the animal model movements are impaired due to the skeletal phenotype; for this reason, we studied the TA muscle of *dtd* which comprised a greater proportion of small muscle fibers, compared to wild-type. These results demonstrate that cartilage and bone impairment in adult mutant mice mimic the phenotype

of DTD patients in adulthood. Alterations in the muscle phenotype in the mutants also provide new insights on the long-term implications of diastrophic dysplasia on the musculoskeletal system.

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TUMOR-MICROENVIRONMENT INTERPLAY IN PANCREATIC DUCTAL ADENOCARCINOMA METASTASIS

Claudia Fioravanti[1], Federica Di Leva[1], Arianna Vallerga[2], Andrea Resovi[1], Giulia Garattini[1], Chiara Grasselli[3], Ilaria Craparotta[2], Elisa Longhi[1], Fabio Sangalli[4], Marco Bolis[2], Giulia Taraboletti[1], Dorina Belotti[1].

1.Laboratory of Tumor Microenvironment, Department of Oncology, Mario Negri Institute, Italy

2.Computational Oncology Unit, Department of Oncology, Mario Negri Institute, Italy.

3.Immunopharmacology Unit, Department of Oncology, Mario Negri Institute, Italy.

4.Laboratory of Medical Imaging, Department of Bioengineering, Mario Negri Institute, Italy

E-mail: claudia.fioravanti@marionegri.it

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related death in Western countries. It is characterized by an intense desmoplastic reaction, that fosters tumor growth and invasion. A hallmark of PDAC is its strong tendency to metastasize, particularly to the liver, driven by continuous interactions between tumor cells and the tumor microenvironment.To dissect the molecular drivers of tumor invasion and metastasis, we employed two murine PDAC cell variants derived from $Kras^{G12D/+}$; LSL-$Trp53^{R172H/+}$ Pdx-1-Cre mice, which exhibit distinct metastatic capabilities: FC1199 (non-metastatic) and FC1199_LV (metastatic to the liver). The invasive potential of the two cell lines in response to normal fibroblasts and cancer-associated fibroblasts (CAFs) conditioned media was compared using a 3D invasion assay in Matrigel. FC1199_LV cells exhibited markedly higher invasive capacity compared to FC1199, particularly in response to CAF-conditioned media. Transcriptomic analysis revealed an enrichment of ECM remodeling, cell adhesion and invasion-associated pathways in FC1199_LV. Pharmacological treatment with Batimastat (MMP inhibitor), Defactinib (FAK inhibitor), and Erdafitinib (FGFR inhibitor) reduced 3D tumor cell invasion, suggesting the involvement of multiple pathways in PDAC invasion. Tumor cell gelatinolytic activity measured by EnzChek™ gelatinase assay was higher in FC1199_LV tumors. Specifically, the levels of MMP-2, MMP-9, MMP-12, and TIMP-1 were higher in primary tumors and in metastatic livers of mice transplanted with FC1199_LV cells compared to those transplanted with FC1199 cells, supporting an active role of MMPs in PDAC metastasis. These findings revealed a critical role for ECM remodeling enzymes in PDAC invasion and metastasis suggesting their involvement as central regulators of multiple signaling pathways including those involved in epithelial-to-mesenchymal transition (EMT), cell adhesion, proliferation and motility. These results provide valuable insight for the identification of potential targets to inhibit different metastasis promoting pathways in pancreatic cancer and suggest that targeting

ECM remodeling enzymes could represent a promising therapeutic strategy to disrupt the metastatic cascade in PDAC.

UNCOVERING SULFATED HYALURONAN–MEDIATED CHANGES IN MATRIX EFFECTORS AND FUNCTIONAL PROPERTIES OF 3D BREAST CANCER CELL MODELS

K. Spanopoulos[1,3], S. Mangani[1], C. Koutsakis[1], Z. Piperigkou[1,2], A. Passi[3], E. Karousou[3], Nikos K. Karamanos[1,2]

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

2.Foundation for Research and Technology-Hellas (FORTH) / Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece

3.Dipartimento di Medicina e Chirurgia, Universitá degli Studi dell'Insubria, 21100 Varese, Italy

E-mail: kspanopoulos15@gmail.com

The extracellular matrix (ECM) critically regulates tumor development by influencing proliferation, invasion, and therapy response. [1]Hyaluronan (HA), a major ECM component, contributes to tumor progression via effects on cell adhesion, motility, and growth factor signaling. [2,3] Chemically modified derivatives, such as sulfated HA, have been proposed to disrupt HA–receptor interactions and thereby modulate malignant behavior. Notably, sulfated HA exhibits reduced recognition and degradation by hyaluronidases (HYALs), which may enhance its stability within the tumor microenvironment and prolong its modulatory effects on cellular behavior. While conventional two-dimensional (2D) cultures are widely used, three-dimensional (3D) spheroid models better mimic in vivo tumor architecture and ECM interactions, offering a more physiologically relevant platform for preclinical evaluation. [4].In our lab we have already seen different response to treatments in 3D cell cancer models, compared to conventional 2D cell cultures. [5,6] This study examines the impact of low-molecular-weight HA (50 kDa) and sulfated HA on MDA-MB-231 and MCF-7 breast cancer cell lines under both 2D and 3D conditions. Gene expression analysis of ECM-related and tumor progression markers, along with spheroid growth assessment, was performed to compare responses across models. Preliminary findings indicate that both HA derivatives alter gene expression profiles, with sulfated HA eliciting more pronounced effects. Moreover, differences between 2D and 3D culture systems highlight the importance of model choice in evaluating ECM–tumor interactions.These results lay the groundwork for continued investigation and suggest that sulfated HA may serve as a promising modulator of breast cancer progression and reinforce the value of 3D spheroid models for translational cancer research.

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BONE REGENERATION IN PERI-IMPLANT DEFECTS TREATED WITH THE SUBPERIOSTEAL PERI-IMPLANT AUGMENTED LAYER TECHNIQUE USING BLOCK AND PARTICULATE DEPROTEINIZED BOVINE BONE MINERAL: A HISTOLOGICAL EVALUATION

Dolaji Henin[1], Leonardo Trombelli[2,3], Gaia Pellegrini[1], Luca Denti[1], Mattia Severi [2,3], Chiara Franzini[2,3], Claudia Dellavia[1]

1.Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Italy

2.Research Centre for the Study of Periodontal and Peri-implant Diseases, Università degli Studi di Ferrara, Italy

3.Operative Unit of Dentistry, Azienda Unità Sanitaria Locale (AUSL), Ferrara, Italy

E-mail:dolaji.henin@unimi.it

Background and aim. The periosteum, when surgically isolated, can be used as a barrier and supportive membrane, further exploiting its osteogenic and angiogenic potential for bone regeneration1. The Sub-Periosteal Peri-implant Augmented Layer (SPAL) technique has been proposed to correct maxillary peri-implant bone defects2,3, namely peri-implant bone dehiscence (PIBD). To enhance clinical outcomes, SPAL has been combined with particulate (pDBBM) or block (bDBBM) deproteinized bovine bone mineral graft4. The present study aimed to histologically assess newly formed bone in sites treated with SPAL plus either pDBBM or bDBBM. Methods Patients with PIBD≥ 2 mm at implant placement in the posterior mandible were treated with the SPAL technique combined with either pDBBM (6 cases) or bDBBM (3 cases). At 6-month re-entry, bone biopsies were harvested from the regenerated sites. Samples were processed, histological ground sections were obtained and stained with toluidine blue and pyronin yellow. Qualitative and histomorphometrical analysis of the lamellar and woven bone, osteoid, medullary spaces, residual graft, and blood vessels were performed. Results PIBD correction was achieved in 5 out of 6 pDBBM cases and in all bDBBM cases. In cases treated with pDBBM, newly formed bone at different stages of maturation was observed around the graft. Samples treated with bDBBM showed pristine bone integrating the grafts, with woven bone deposited along the graft inner cavities. The proportion of lamellar bone varied among patients, ranging from 19.29% to 69.67%. In some cases, samples were mainly characterized by cortical lamellar bone, most likely due to biopsy collection near the pristine alveolar crest. Medullary spaces contained osteoblast-like cells actively depositing osteoid matrix, as well as blood vessels located adjacent to both the newly formed bone and the residual graft. No pathological inflammatory infiltrates were detected. Conclusion Histological evaluation revealed that the use of the periosteum as a containing,

supportive, and isolating membrane in regenerative surgery promoted the formation of well-structured supracortical bone with graft integration within 6 months.

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FGF AND FGFRS REGULATION IN PROSTATE CANCER CELLS

L. Bizzotto[1], *M. Onisto*[1], *V. Masola*[1].

1.Department of Biomedical Sciences, University of Padova.

E-mail: lorenzo.bizzotto@unipd.it

Among men, prostate cancer is the leading cause of death from a cancer that affects only one sex. The only endoglycosidase found in mammals, heparanase (HPSE), breaks down heparan sulfate (HS), which leads to the restructuring of the extracellular matrix and the release of bioactive mediators. HPSE is produced in excessive amounts in prostate cancer, which helps the tumor invade tissues and spread to other parts of the body. Fibroblast growth factor 2 (FGF2) and its receptors are key players in the development of prostate cancer. Research has shown that in various cancer cell lines, FGF2 promotes epithelial-to-mesenchymal transition (EMT), and that different forms of FGF2 receptors control this process in distinct ways. Building on our earlier research with an epithelial cell model, which showed that HPSE regulates the EMT induced by FGF2, this current study seeks to define the function of HPSE in EMT within prostate cancer. We investigated the impact of a specific heparanase (HPSE) inhibitor on three prostate cancer cell lines: DU145, PC3, and LNCaP. The cells were treated with the inhibitor both with and without FGF2 to determine its effect. To assess the results, we employed various molecular and cellular techniques, including PCR, real-time PCR, a viability assay, a colony assay, and a Western blot (WB). Our findings revealed that the different prostate cancer cell lines expressed varying levels of FGF2 and its receptor isoforms. We confirmed that FGF2 activates epithelial-to-mesenchymal transition (EMT) in prostate cancer cells. Most significantly, we demonstrated that inhibiting HPSE altered the expression of FGF2 and its receptors, which in turn modulated the EMT process. In summary, these results suggest that HPSE's involvement in EMT, partly through its effect on the FGF2-FGF2R pathway, could be a general mechanism. This is because EMT is a fundamental process not only in tumor progression but also in conditions like organ fibrosis. This link provides new insights into diseases of epithelial origin.

LOSS OF MULTIMERIN-2 IN TUMORS ALTERS THE IMMUNE MICROENVIRONMENT

Di Siena E. [1], *Schinello G.* [1], *Camicia L.* [1], *Carobolante G.*[1], *Poletto E.* [1], *Mongiat M.*[1]

1.National Cancer Institute CRO Aviano (IRCCS), Italy.

E-mail: emanuele.disiena@cro.it

The tumor microenvironment (TME) is a dynamic and complex network comprising immune cells, stromal cells, the extracellular matrix, and vasculature [1]. A key feature of the TME is its dysfunctional vasculature, characterized by disorganized, leaky, and inefficient vessels. We have previously demonstrated that the endothelial-specific extracellular matrix protein Multimerin-2, which is frequently lost in tumor-associated vessels, is a critical gatekeeper of vascular stability [2]. Preliminary observations in Multimerin-2 deficient (*Multimerin-2*$^{-/-}$) mice revealed altered immune cell infiltration within tumors [3], prompting us to investigate the mechanism by which Multimerin-2 loss influences immune recruitment and potentially modulates response to immunotherapy. To identify soluble mediators responsible for this altered immunity, we performed RNA-sequencing on control and Multimerin2-silenced human umbilical vein endothelial cells (HUVECs). Subsequent validation by qRT-PCR, ELISA, and Western blot analysis confirmed that Multimerin-2 loss significantly alters the expression of numerous immune-related genes. Notably, we identified a strong upregulation of the chemokine CCL8, a key recruiter of immunosuppressive monocytes and macrophages to inflammatory sites[4]. Mechanistically, we found that this increase in CCL8 expression is driven by the activation of the Toll-like receptor 4 (TLR4) pathway, a primary sensor of damage-associated molecular patterns (DAMPs) often activated in the stressed TME[5]. Our findings establish a novel link between the loss of an endothelial matrix protein and the creation of an immunosuppressive niche via chemokine dysregulation. We propose that assessing Multimerin-2 expression could serve as a valuable biomarker for predicting patient prognosis and response to immunotherapy.

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MOLECULAR MECHANISM OF THE TRANSITION HA-COLLAGEN IN WOUND REPAIR

D. Antognoli[1], P. Moretto[1], P. Suscetta[1], J. Karousou[1], D. Vigetti[1], A. Passi[1], M. Viola[1]

1.University of Insubria, Varese, Italy.

E-mail: d.antognoli@uninsubria.it

The wound healing process involves a transition of the extracellular matrix (ECM) from a hyaluronic acid (HA)-rich environment, crucial for cell migration and inflammation in the early stages[1], to a collagen-rich matrix that provides structural integrity. The molecular signals that orchestrate this HA-to-collagen switch in dermal fibroblasts are not fully understood, though central signaling hubs like mTOR are known to play key roles in tissue regeneration and repair[2].This study aims to elucidate how different molecular weights of HA influence ECM remodeling, with a specific focus on Collagens, HASs and mTOR .Fibroblasts (BJ-5ta) were treated with low (LMW-HA, ~200 kDa) or high (HMW-HA, >1000 kDa) molecular weight HA. The cellular response was profiled using RT-qPCR to analyze the expression of key genes including collagens (COL1a1, COL3a1); metalloproteinases (MMP1, MMP2, MMP3) and their inhibitors (TIMP1, TIMP2); prolyl-hydroxylase (PHD) and HA syntases (HAS2, HAS3). Zymography was used to assess the enzymatic activity of MMP2 and MMP9, while Western blotting was performed to detect the activation of mTOR. LMW-HA acted as a potent pro-fibrotic stimulus, nearly doubling the transcription of both Collagen 1 and 3. HMW-HA induced a moderate increase for Collagen 1. Both HA upregulated MMPs and TIMPs, with MMP2 showing the strongest response to HMW-HA. This was further supported by a strong upregulation of PHD expression, an enzyme essential for collagen maturation, which was most pronounced with HMW-HA. Interestingly, zymography results suggested that while LMW-HA increased MMP enzymatic activity, HMW-HA appeared to have an inhibitory effect, pointing to complex post-transcriptional regulation. Crucially, despite these significant modulations of the ECM secretome. The HAS3 was increased in both LMW-HA and HMW-HA treatments while HAS2 showed slight increase only with HMW-HA. The action of mTOR seems to be decreased by the treatment with LMW-HA.In conclusion, HA molecular weight is a critical factor in orchestrating the HA-collagen transition in fibroblasts. The effects on ECM synthesis appear to occur via mTOR-independent signaling pathways, highlighting the existence of alternative regulatory networks that could serve as novel therapeutic targets for modulating wound healing.

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OSTEOGENIC DIFFERENTIATION IN 2D AND 3D OSTEOSARCOMA MODELS: ROLE OF THE MATRIX AND MAGNESIUM

L. Anconelli[1], M.M. Rydzyc[1], F. Rossi[1], M. Rossi[1], E. Coschina1G. Ravegnini[1], S. Iotti[1], M. Franchi[2], C. Cappadone[1], E. Malucelli[1]

1.Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy

2.Department for Life Quality Studies, University of Bologna, Bologna, Italy

Email: lorenzo.anconelli3@unibo.it

Osteosarcoma is characterized by impaired osteogenic differentiation, with cells maintaining an immature, proliferative phenotype and producing an altered extracellular matrix. To investigate osteogenic potential, we induced differentiation in Saos-2 cells and mesenchymal stem cells (MSCs), initially in 2D cultures and then in advanced 3D systems that better recapitulate cell–matrix interactions[1,2]. Bicellular spheroids were developed as a refined 3D model, and differentiation was evaluated through cell viability assays, mineralized matrix deposition (Alizarin Red staining), and expression of key osteogenic markers, including collagen I, osteocalcin, and osteopontin. The role of magnesium as a differentiation modulator was explored in MSCs and Saos-2 cells in 2D, and in Saos-2 grown on collagen scaffolds[3]. Magnesium content was analyzed using multiple complementary techniques, including synchrotron radiation-induced X-ray fluorescence (SR-XRF) [4] and fluorescence-based biosensing approaches, employing a highly sensitive magnesium-specific fluorescent probe developed previously by our group [5,6]. Although this probe was not originally designed for this study, it was already available and particularly well-suited to quantifying the total intracellular magnesium pool, both free and bound, even in limited cell samples and complex 3D systems. Future work will extend magnesium analysis to bicellular spheroids and integrate multi-analytical approaches to correlate mineralized matrix formation with the regulation of organic matrix components. This strategy aims to elucidate mechanisms underlying osteogenic maturation and to identify novel approaches for modulating tumor cell behaviour within biomimetic 3D microenvironments.

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ESM1 IS INVOLVED IN CROSSTALK BETWEEN ACTIVATED FIBROBLASTS AND BREAST CANCER CELLS TO PROMOTE TUMOR PROGRESSION

F. Aliquò[1], A. Pantano[2], G. Giuffrè[3], A. Avenoso[1], G.M. Campo[2], S. Campo[1], M. Scuruchi[2], G. Mandraffino[2], A D'Ascola[2]

1.Department of Biomedical and Dental Sciences and Morphofunctional Images, University of Messina, Messina; Italy

2.Department of Clinical and Experimental Medicine, University of Messina, Messina; Italy

3.Department of Mathematical and Computer Sciences, Physical Sciences and Earth Sciences University of Messina, Messina; Italy

E-mail: faliquo@unime.it

The proteoglycan ESM1 (Endothelial cell-specific molecule 1) is involved in the pathogenesis of various diseases, including breast cancer (BC), where it is aberrantly expressed and contributes to cell proliferation, migration, and angiogenesis[1]. Stromal fibroblasts can be activated by tumor cells to differentiate into cancer-associated fibroblasts (CAFs), which promote tumor progression, metastasis, and invasion by secreting growth-promoting and proangiogenic factors [2]. Based on this evidence, we investigated the role of ESM1 in a co-culture model of mammary fibroblasts and BC cells to clarify its involvement in tumor progression. Mammary fibroblasts were seeded in the lower chamber of a transwell system, while either MCF-7 (non-aggressive) or MDA-MB231 (aggressive) breast cancer cells were placed in the upper chamber. Further groups of cells were treated with either ESM1-specific siRNA or control siRNA.After 24 h of co-culturing, a significant increase of ESM1 mRNA and protein levels was found in both BC cells and activated mammary fibroblasts. Furthermore, BC cells exhibited increased expression of MMP-9, vimentin, and other markers associated with proliferation, migration, and epithelial-mesenchymal transition. Notably, ESM1 knockdown in fibroblasts, as well as in BC cells, reduced all these parameters. These results suggest that ESM1 produced by both activated fibroblasts and BC cells plays a key role in enhancing breast cancer cell aggressiveness by modulating markers associated with tumor progression. This revealed the impact of ESM1 in the crosstalk between activated fibroblasts and breast cancer cells indicating a new mechanism through which the tumor microenvironment may drive tumor progression.

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3D BREAST CANCER CELL CULTURE IN GELATIN AND HYALURONIC ACID SCAFFOLDS: IMPACT ON GENE EXPRESSION

C. Morán Maidana[1], E. Karousou[2], M. Viola[2], P. Moretto[2], I. Sevic[1], L. Alaniz[1], A. Passi[2].

1.Laboratorio de Microambiente Tumoral, Centro de Investigaciones y Transferencia del Noroeste de la Pcia. de Bs. As. (UNNOBA-CONICET), Junín, Bs. As., Argentina.

2.Dipartimento di Medicina e Chirurgia, Università degli Studi dell'Insubria, Varese, Italia.

E-mail: cmoranmaidana@comunidad.unnoba.edu.ar

The extracellular matrix (ECM) plays a central role in tumor development, as its composition and architecture strongly influence cancer cell behavior[1]. Among its main components, collagen and hyaluronic acid (HA) can modulate tumor cell proliferation, invasion, and progression[1,2,3]. Gelatin, a hydrolyzed form of collagen, has been widely used for scaffold fabrication due to its biocompatibility and structural properties[4,5]. Moreover, three-dimensional (3D) culture systems, in contrast to conventional 2D models, provide a more physiologically relevant setting to investigate how tumor cells interact with each other and respond to ECM-derived signals[6]. In this work, we established scaffolds using gelatin crosslinked alone or in combination with high molecular weight hyaluronic acid (HA, 3.2 MDa), with the aim of optimizing the methodology and exploring their influence on breast cancer cell behavior. Two breast cancer cell lines (MCF-7 and MDA-MB-231) were cultured in these scaffolds in a 3D culture. The matrices were analyzed by scanning electron microscopy (SEM), and we also evaluated gene expression of HA metabolism-related genes and markers of invasion and tumor progression by RT-qPCR. SEM revealed larger pores in HA-containing matrices compared to gelatin scaffolds. In MCF-7 cells, RT-qPCR showed reduced expression of RHAMM and HYAL2 in gelatin scaffolds compared to control (without scaffold), and lower expression of HAS2-AS1 in gelatin scaffolds compared to those containing HA. In MDA-MB-231 cells, higher HAS2-AS1 expression was observed in both scaffolds compared to control, and reduced ICAM1 expression in HA-containing gelatin scaffolds compared to gelatin alone. Overall, these results suggest that the incorporation of very high molecular weight HA into scaffolds modifies matrix architecture and gene expression patterns, potentially contributing to the generation of a less pro-tumorigenic microenvironment in MDA-MB-231 cells. In contrast, in MCF-7 cells, gelatin alone appears to exert this effect. However, further analyses including additional invasion and progression markers are required to confirm these observations.

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EVALUATION OF THE ROLE OF THE PROTEOGLYCAN ESM1 IN BREAST CANCER

D. Mackiq[1], E. Peri[1], S. Feo[1], I. Pucci-Minafra[2], E. Roz[3], A. D'Ascola[4], P. Cancemi[1],

1.Department of Biological Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, 90128 Palermo, Italy;

2.Experimental Center of Onco Biology (COBS), 90145 Palermo, Italy;

3.La Maddalena Hospital III Level Oncological Department, Via San Lorenzo Colli, 312, 90145 Palermo, Italy;

4.Department of Clinical and Experimental Medicine, University of Messina, 98125 Messina, Italy.

E-mail: patrizia.cancemi@unipa.it

Breast cancer is associated with a complex interplay of genetic, epigenetic, hormonal, and environmental factors, and it is a highly heterogeneous disease with different manifestations in individual patients. Along with the intertumoral heterogeneity, significant diversity can exist in tumor cell subpopulations within the same patient, referred to as intratumoral heterogeneity. Endothelial cell-specific molecule 1 (ESM1), also known as endocan, is a secreted proteoglycan implicated in tumour progression through its roles in angiogenesis, inflammation, and metastasis[1]. High ESM1 expression has been observed in several cancer types[2–3]. However, its functional significance and molecular mechanisms in breast cancer, remain incompletely defined. In this study, we investigate the expression levels of ESM1 in breast cancer patients. Our findings demonstrate a potential correlation with HER2 expression status. Specifically, cERB-negative breast cancer patient sera samples exhibited elevated and more variable ESM1 levels compared to HER2 positive samples. Based on these findings we analysed the in vitro cell behaviour, after ESM1-silencing in two triple-negative breast cancer (TNBC) cell lines, highly aggressive. We conducted transcriptomic and proteomic analyses to evaluate downstream effects. Overall, our results suggest that ESM1 may play a particularly critical role in the aggressive phenotype of TNBC. These findings lay the groundwork for further functional studies and highlight ESM1 as a potential biomarker and therapeutic target in breast cancer.

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ENDOCAN AS A BIOCHEMICAL MODULATOR OF INFLAMMATION, OXIDATIVE STRESS, CARDIAC REMODELING, AND TUMOR-RELATES SIGNALING

M. Scuruchi[1], F. Aliquò[1], A. Pantano[1], G. Giuffrè[3], A. Avenoso[2], S. Campo[2], A D'Ascola[1] G.M. Campo[1]

1.Department of Clinical and Experimental Medicine, University of Messina, Messina; Italy

2.Department of Biomedical and Dental Sciences and Morphofunctional Images, University of Messina, Messina; Italy

3.Department of Mathematical and Computer Sciences, Physical Sciences and Earth Sciences University of Messina, Messina; Italy

E-mail: mscuruchi@unime.it

Endocan (endothelial cell-specific molecule-1, ESM1) has emerged as a pivotal proteoglycan involved in the biochemical regulation of inflammation, angiogenesis, and tumor progression. Our studies explored its role across different cellular contexts, highlighting its capacity to integrate cytokine-driven responses, oxidative stress, and oncogenic signaling pathways. In human chondrocytes, endocan expression was strongly induced by interleukin-1β (IL-1β), positioning it as a novel inflammatory marker.Functional experiments revealed that endocan knockdown attenuated IL-1β-mediated upregulation of angiogenesis-associated genes, suggesting its contribution to pathological neoangiogenesis in joint diseases[1]. In lung cancer cells, endocan promoted pro-tumorigenic phenotypes, including enhanced proliferation and migration, while regulating the expression of oncogenic long non-coding RNAs (lncRNAs) H19 and HULC [2]. In cardiac fibroblasts, endocan acted as a biochemical integrator of TGF-β/Smad and AKT/ERK signaling with lncRNA modulation, supporting its role in fibrotic remodeling. Finally, under oxidative stress conditions, NRF2 was shown to modulate endocan expression, linking redox homeostasis to endocan-dependent pathways. Collectively, these findings identify endocan as a biochemical hub orchestrating multiple signaling cascades across inflammatory, oxidative, and tumorigenic contexts. Targeting endocan or its downstream effectors may thus provide novel therapeutic strategies in inflammatory disorders, cancer, and cardiovascular disease.

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MULTI-OMICS PROFILING IN MEDICATION-RELATED OSTEONECROSIS OF THE JAW

Francesca Polito[1], Vincenzo Macaione[1], Laura Licitri[1], Irene Gasparo[2], Alessandro Allegra[2], Antonia Marcianò[3], Marco Ragusa[4], Selene Francesca Anna Drago[1], Marieme Khouyyi[3], Giacomo Oteri[3], Luca Bini[5], Laura Bianchi[5], M'hammed Aguennouz[1]

1.Department of Clinical and Experimental Medicine, University of Messina, Italy

2.Department of Human Pathology in Adulthood and Childhood "Gaetano Barresi", University of Messina, Italy;

3.Department of Biomedical and Dental Sciences, Morphological and Functional Images, University of Messina, Messina, Italy;

4.Department of Biomedical and Biotechnological Sciences, University of Catania, Italy;

5.Laboratory of Functional Proteomics, Department of Life Sciences, University of Siena, Italy

E-mail: francesca.polito@unime.it

Medication-related osteonecrosis of the jaw (MRONJ) is a complex condition associated with the use of antiresorptive drugs, such as bisphosphonates and denosumab. The condition is characterized by the presence of exposed bone in the maxillofacial region that fails to heal. MRONJ remains highly intractable, as its pathogenic mechanism are not yet fully understood. It is therefore essential to elucidate the molecular mechanisms underlying the disease. miRNoma analysis and proteomic studies were performed on a selected cohort of patients with MRONJ on jawbone tissue, using qRT-PCR and 2D electrophoresis followed by mass spectrometry.Nineteen miRNAs were overexpressed and 2 downregulated in jawbone tissue from all MRONJ patients. Notably, 5 of these dysregulated miRNAs are involved in the regulation of angiogenesis and desmosome functions, suggesting a potential link to the molecular alterations observed at the protein level. More specifically, analysis of upregulated miRNAs in the jawbone tissue of MRONJ patients revealed three miRNAs (i.e. miR-30b-5p, miR-204-5p, miR-222-3p) involved in the regulation of essential osteogenic transcription-factors, namely Runx1, Runx2, and nuclear factor of activated T-cells (NFAT). Complementary proteomic analysis identified some proteins with reduced abundance in MRONJ samples, i.e. PEDF, desmoglein-1, desmoplakin, and desmocollin-1, all of which are likewise implicated in osteogenic processes.The pathophysiology of MRONJ arises from a complex interplay of factors, including impaired bone remodeling, affected angiogenesis, and altered cell

adhesion and differentiation mechanisms, ultimately leading to necroptosis. Through proteomic analysis and validation of miRNA expression, our study proposes specific molecular alteration in MRONJ-compromised bone tissue, involving desmosomal component imbalance and angiogenesis inhibition. These molecular alterations collectively point toward a disruption in the transcriptional and structural jaw bone machinery governing osteoblast differentiation and bone matrix maintenance and thus might represent promising targets for more effective therapeutic strategies.

NOTCH SIGNALING ACTIVATION IS PART OF THE INFLAMMATORY RESPONSE INDUCED BY LPS AND SMALL HYALURONAN OLIGOSACCHARIDES IN HUMAN CHONDROCYTES

D'Ascola A[1], F. Aliquò[2], A. Pantano[1], M. Scuruchi[1], A. Avenoso[2], G. Giuffrè[3], S. Campo[2], and G.M. Campo[1]

1.Department of Clinical and Experimental Medicine, University of Messina, Italy

2.Department of Biomedical and Dental Sciences and Morphofunctional Images, University of Messina, Italy

3.Department of Mathematical and Computer Sciences, Physical Sciences and Earth Sciences, University of Messina, Italy

E-mail: adascola@unime.it

NOTCH signalling regulates numerous cellular processes including inflammatory response. TLRs might prime NOTCH activation indirectly through inducing the expression of NOTCH receptors and ligands. Stimulation of TLR4 leads to NF-kB and p38MAPK activation that increases JAGGED1 expression, responsible in turn of NOTCH receptors activation which through mediation of NICD, together with NF-kB, leads to the transcription of the pro-inflammatory cytokines and other detrimental mediators[1]. Previous investigations reported the enhancement of NOTCH pathway in patients with rheumatoid arthritis and other pathologies involving cartilage injury [2]. The present study aimed to investigate the involvement of NOTCH signalling in an experimental model in which inflammation was induced by the treatment of LPS and hyaluronan oligosaccharides (6-mer HA) in human chondrocytes.

Changes in TLR-4, p38MAPK, JAGGED1, NOTCH1, NOTCH2, NOTCH3, RBP-J, IRF8, HES1, IL-1beta and MMP-13 mRNA expression of LPS and 6-mer HA-stimulated chondrocytes were examined by PCR RealTime. Protein levels of TLR-4, p38MAPK, JAGGED1, NOTCH1, NOTCH2, NOTCH3, HES1 were evaluated by western blot, while IL-1beta, MMP-13, and p-NF-kB p65 subunit levels were assayed by ELISA kits. Specific NF-kB inhibitor and NOTCH1 siRNA were also used to clarify the inflammatory pathway.Treatment of chondrocytes with LPS produced NF-kB activation, as well as a significant increment in all the parameters reported above. The expression of NOTCH receptors was also increased with more significance for NOCH1. Stimulation of chondrocytes with 6-mer HA produced the same effect but with less intensity. The exposition of cells to both LPS and 6-mer HA resulted in an additive effect on the considered parameters. Specific siRNAs blocking NOTCH1 revealed the involvement of this pathway. The use of a selective NF-kB inhibitor clarify that NF-kB pathway was more effective than NOTCH

pathway in the transcription of inflammatory mediators. In conclusion, the activation of TLR4 induced by both LPS and HA oligosaccharides leads to the acute inflammatory response mediated both through the direct NF-kB/MAPK pathway and indirectly by the JAGGED1/NOTCH1 pathway.

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