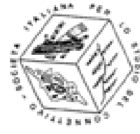

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MATRIX BIOLOGY EUROPE 2022

FLORENCE
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ABSTRACT BOOK

PLENARY SESSIONS

PLENARY SESSION 1 - LATE-BREAKING TOPICS ON THE MATRIX

Emerging concepts in tumor extracellular matrix functions and targeting

Nikos Karamanos
Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Lab. of Biochemistry, University of Patras, Patras, Greece

Extracellular matrices (ECMs) as highly dynamic three-dimensional structural networks of macromolecules, such as proteoglycans/glycosaminoglycans (PGs/GAGs), collagens, laminins, elastin, (glyco)proteins, and matrix-degrading enzymes. ECMs exhibit high structural complexity and heterogeneity. Their complex networks dynamically communicate with cells serving as regulators of several homeostatic and pathological processes, including cancer. The mechanistic aspects governing cell–matrix interactions are of critical importance to understand the matrix-mediated cancer pathobiology and to discover novel therapeutic approaches. In this presentation the emerging concepts in tumor extracellular matrix functions and targeting will be discussed. Focus will be given on the effective matrix macromolecules, including PGs/GAGs, matrix-remodeling enzymes, membrane receptors and epigenetics, all of key importance in the development of breast cancers with different estrogen status (ERalpha/ERbeta/Triple negative), as well as in paracrine interactions among cancer cells and tumor stroma that modulate cancer cell aggressiveness.

Systemic abrogation of hyaluronan synthesis by induced gene knockout in mice leads to enlargement of dermal white adipose tissue with a pro-inflammatory gene expression signature

Tom Wippold[1], Anja Saalbach[1], Ulf Anderegg[1]
[1]*Klinik f. Dermatologie, Venerologie und Allergologie, Medizinische Fakultät, Universität Leipzig, Leipzig, Germany*

Purpose: Hyaluronan (HA) represents a high molecular weight, linear polymer of repeating disaccharides of glucuronic acid and N-Acetylglucosamine. HA is a major part of the extracellular matrix of the skin. HA is synthesized by three HA-synthases (Has1-3) through cell membrane and many of HA functions depend on the individual synthase and the length of the HA-chain. HA content in skin changes during ageing, inflammation and repair. We analyse the functional impact of individual HA-synthases and strongly reduced HA content in skin with respect to homeostasis, inflammation and tissue repair.
Methods: We crossed constitutive Has1,3 double KO mice with an inducible Has2-knockout strain under the control of the UbiquitinC-promoter (Has$^{2loxp/loxp}$-UBC-cre$^{ERT+/-}$). The resulting Has1,3$^{-/-}$ Has2$^{loxp/loxp}$-UBC-creERT$^{+/-}$ mice lack activities of all known HA synthases upon induced recombination. These mice were analysed in resting state by immunohistochemistry and ELISA, as well as by microarray gene expression analysis of whole skin. Single cell RNAseq of acute inflamed skin was performed.
Results: Knockout of all HA synthases leads to a 90% reduction of skin HA, but the mice are viable with normal weight, motility and behaviour. The loss of skin HA results in epidermal thickening, a thinner dermis and a significant increase of the dermal white adipose tissue. Microarray analysis showed that the absence of HA-synthesis leads to differently expressed pathways, confirming increased adipogenesis and epidermal dysregulation. Moreover, changes in metabolic pathways and activation of cytokine receptor-mediated signaling pathways were significant. In inflamed skin of Has-knockout mice, a preferred M1-macrophage polarization was detected leading leads to more severe inflammation.
Discussion: Our data indicate that HA loss is compensated by the organism. However, the matrix rearrangement of HA depleted skin leads to adipogenesis in the skin as well as enhanced and dysregulated inflammatory reactions suggesting a connection between changes of ECM composition and the inflammatory status of the skin.
Conclusion: The novel mouse model enables comprehensive investigations of HA metabolism and its impact on various processes in the organism.

CRELD2 is a novel modulator of calcium release and calcineurin-NFAT signalling during osteoclast differentiation and bone resorption

Adam Duxfield[1], Ella Patricia Dennis[1], Jennifer Munkley[1], Michael Briggs[1]
[1]Biosciences, United Kingdom

Purpose: Cysteine rich with EGF-like domains 2 (CRELD2) is an endoplasmic reticulum (ER)-stress regulated gene that has been implicated in the pathogenesis of skeletal dysplasias and has previously been shown to play an important role in the differentiation of chondrocytes and osteoblasts. Despite CRELD2 having an established role in skeletal development and bone formation, its role in osteoclasts is currently unknown.
Methods: CRELD2 was overexpressed in RAW264.7 pre-osteoclast cells and osteoclastogenesis was performed to understand the role of CRELD2 in osteoclast differentiation. Osteoclastogenesis was performed and analysed by a range of molecular biology techniques including histology, proteomic/transcriptomic analysis and activity assays.
Results: We show for the first time that CRELD2 plays a novel role in trafficking transforming growth factor beta 1 (TGF-β1), which led to an upregulation of Nfat2 expression, the master regulator of osteoclast differentiation. Despite this finding, we show that CRELD2 is dispensable for osteoclastogenesis. Indeed, the overexpression of Creld2 impaired osteoclast differentiation due to a reduction in the activity of the calcium-dependant phosphatase calcineurin. This led to a subsequent block in the dephosphorylation of nuclear factor of activated T cells 1 (NFATc1), preventing its nuclear localisation and activation as a pro-osteoclastogenic transcription factor.
Discussion and Conclusion: Our results show that the overexpression of Creld2 in osteoclasts impaired calcium release from the ER which is essential for activating calcineurin and promoting osteoclastogenesis. Our data proposes a novel inhibitory role for this calcium-binding ER-resident chaperone in modulating calcium flux during osteoclast differentiation which has important implications in our understanding of bone remodelling and the pathogenesis of skeletal diseases.

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Antibody-based therapy against microfibrillar-associated protein 4 inhibits retinal neovascularization and vascular leakage

Bartosz Pilecki[1], Anders Schlosser[1], Claire Allen[2], Andrew Benest[2], Jing Hua[2], Nikita Ved[2], Zoe Blackley[2], Thomas Andersen[1;3], Dorle Hennig[1], Jonas Graversen[1], Soren Moller[4], Sofie Skallerup[1], Maria Ormhøj[1], Jakob Grauslund[5], Steffen Heegaard[6], Wenzheng Hu[7], Matthew Lawrence[7], Nick Laursen[8], Gregers Andersen[8], Uffe Holmskov[1], David Bates[2], Grith Lykke Sorensen[1]
[1]Institute of Molecular Medicine, University of Southern Denmark, Denmark; [2]Cancer Biology, Division of Cancer and Stem Cells, School of Medicine, University of Nottingham, Nottingham, United Kingdom; [3]Clinical Cell Biology, Department of Pathology, Odense University Hospital, Odense, Denmark; [4]OPEN - Open Patient data Explorative Network, [5]Department of Ophthalmology, Odense University Hospital, Odense, Denmark; [6]Department of Pathology, Department of Ophthalmology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [7]Virscio Inc., New Haven, CT, USA; [8]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark

Purpose: Neovascular age-related macular degeneration (nAMD) and diabetic macular edema (DME) are leading causes of vision loss and blindness in the elderly and working-age populations, respectively. The main pathological hallmarks of both nAMD and DME include retinal neovascularization and leakage, and integrins $\alpha V\beta 3$ and $\alpha V\beta 5$ have been established as potent stimulants of angiogenesis. Microfibrillar-associated protein 4 (MFAP4) is an integrin $\alpha V\beta 3/5$ ligand expressed in the vascular extracellular matrix. We envisioned that therapeutic blocking of MFAP4 might constitute a beneficial treatment strategy for nAMD and DME. Therefore, we have produced MFAP4-blocking antibody and tested its potential effects in vitro and in vivo to obtain the preclinical proof-of-concept of efficacy.
Methods: Immunohistochemistry and in situ hybridization were used to detect and localize MFAP4 expression within the human eye. Humanized monoclonal anti-MFAP4 antibody hAS0326 was tested in vitro in retinal endothelial cell adhesion, proliferation and migration assays with concomitant MFAP4 and VEGF stimulation. Moreover, we investigated the efficacy of hAS0326 treatment in vivo in a mouse model of laser-induced choroidal neovascularization, rat model of streptozotocin-induced retinopathy, and African green monkey model of DL-2-aminoadipic acid-induced chronic retinopathy.
Results: MFAP4 was expressed in retina and choroid of the human eye. hAS0326 blocked integrin-dependent endothelial cell adhesion, proliferation and migration in vitro. Furthermore, hAS0326 efficiently inhibited retinal vascular leakage with efficacy comparable to anti-VEGF positive control in all three in vivo retinopathy models. In the non-human primate model, one intravitreal dose of hAS0326 exerted a prolonged duration of efficacy of over 3 months in reduction of vascular leakage.
Discussion: These data suggest that hAS0326-mediated inhibition of the extracellular matrix protein MFAP4 constitutes a promising stand-alone therapy with future translational potential for the treatment of vision-threatening retinal vascular eye diseases such as nAMD and DME.
Conclusion: Antibody-based MFAP4 inhibition efficiently attenuates retinal cell activation, neovascularization and vascular leakage in vitro and in vivo.

Mechanisms and functional importance of neural ECM remodeling

Alexander Dityatev[1]
[1]*German Center for Neurodegenerative Diseases (DZNE), Molecular Neuroplasticity, Magdeburg, Germany*

Purpose: This presentation is to highlight the recent progress in the identification of canonical and non-canonical signaling pathways that govern the remodeling of neural extracellular matrix (ECM) and regulate the plasticity of neural cells in an ECM-dependent manner.
Methods: Immunohistochemistry, dendritic spine measurements, electrophysiology, and behavioral studies.
Results: Neural ECM is formed and remodeled in a manner dependent on neuronal activity and activation of neuromodulatory systems. Signaling via 5-HT7 receptors promotes activation of MMP9 and cleavage of CD44 (the major receptor to the neural ECM backbone, hyaluronic acid), triggering elongation of dendritic spines [1]. Activation of D1-like dopamine receptors results in ADAMTS4/5-mediated cleavage of lecticans as major ECM proteoglycans [2]. Presynaptically released protease neurotrypsin cleaves the ECM molecule agrin, a small fragment of which promotes the formation of dendritic spines during learning. In addition to ECM proteolysis, recent studies highlight the roles of microglial phagocytosis and integrin-based ECM recycling in the remodeling of perisynaptic ECM [3, 4]. Neural ECM remodeling leads to structural plasticity and changes in the neuronal excitability and modulation of GluN2B-containing NMDA receptors that control functional synaptic plasticity [5; unpublished data]. Forced ECM remodeling by neuronal overexpression of ADAMTS5 could "rejuvenate" the brain of two-year-old mice in many aspects, including learning and synaptic plasticity.
Discussion: The presented signaling mechanisms contribute to the regulation of diverse forms of learning and memory and are involved in the pathophysiology of epilepsy, schizophrenia, mental retardation and dementia.
Conclusion: Experience-dependent remodeling of neural ECM may reactivate the GluN2B-dependent developmental mechanism for gaining synaptic plasticity.

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PLENARY 3 - DICK HEINEGARD AWARD

PLENARY SESSION 4 - MATRIX IN TRANSLATIONAL MEDICINE

Two-fraction extracellular matrix extracts to bestow functional mimicry on bioprinted 3D tissue models

Daniel Reis[1,2], **Mariana Malta**[1,2], **Lucília Silva**[1,2], **Luca Gasperini**[1,2], **Alexandra Marques**[1,2]
[1]3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal; [2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal

Purpose: Extracellular matrix (ECM) has been inspiring the design of bio-instructive materials to bioengineer tissues and biological microenvironments. ECM's role is not exclusively defined by direct cell-ECM interactions and biomechanics, but also by its capacity to store soluble biochemical cues. In this work, we develop bioinks based on structural ECM components (strECM) enriched in soluble ECM-derived factors (sECM), both extracted using an in-house method, to enhance the functionality of in vitro bioprinted 3D tissue models.
Methods: To proof the concept we considered the skin dermis and used human dermal fibroblasts (hdFBs) to obtain the ECM extracts. Bioinks were prepared by mixing the strECM with gellan gum(GG), and the sECM with GG functionalized with divinyl sulfone(GG-DVS) to retain smaller proteins of interest. Extracts were analysed by mass spectrometry and then regarding their functionality - angiogenic potential (human dermal endothelial cells), human keratinocytes (hKCs) and hdFBs migration, hKCs differentiation, ECM remodelling - was assessed in 2D cultures and 3D bioprinted structures.
Results: Proteomic analysis showed a complementarity between strECM and sECM and the complete preservation of the native ECM protein profile. The GO accessions linked to each fraction allowed pinpointing the specific cues provided by either of them. In vitro cell studies confirmed that the combination of strECM and sECM confer complementary functionalities to the developped bioinks.
Discussion: Our results validate the hypothesis that each ECM fraction effectively triggers different biological functions. Their reproduction in 3D printed constructs also confirms the ECM extract's benefit in engineered biological microenvironments.
Conclusion: ECM-based bioinks that better mimic the complex functionality of the dermal ECM are herein validated as a way to develop biologically relevant 3D tissue models.

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Targeting myofibroblast transformation using phenotypic screening to find novel treatments for fibrosis

Marcus Ilg[1], Alice Lapthorn[1], Peter Dziewulski[1,2], David Ralph[1,3], Selim Cellek[1]
[1]Medical Technology Research Centre, Chelmsford, Anglia Ruskin University, United Kingdom; [2]St Andrew's Centre for Plastic Surgery and Burns, Broomfield Hospital,Chelmsford, United Kingdom; [3]Department of Urology, University College London Hospital,London, United Kingdom

Purpose: Fibrosis is the excess accumulation of extracellular matrix in response to injury, accounting for 45% of mortality in the Western world. Despite this, medical treatments for fibrosis are limited. Decades of target-based drug discovery have failed in identifying novel treatments for fibrosis; therefore, we have developed a phenotypic screening approach targeting the key cell responsible for matrix production, the myofibroblast.
Methods: Human primary fibroblasts were isolated from patients with Peyronie's disease (PD; penile fibrosis) and hypertrophic scars (HS; skin fibrosis). Phenotypic screening assays which quantify myofibroblast transformation were developed and validated using these cells. The assays were utilised to screen 1,954 FDA-approved drugs at 10 μM. Hits were defined as eliciting > 80% inhibition of myofibroblast transformation while retaining > 80% cell viability. The PD and HS screening campaigns revealed 26 and 90 hits, respectively. Hits were triaged according to safety profiles and suitability for disease indication.
Results: The HS screen revealed hydroxypyridone anti-fungals as hits; drugs capable of achieving the desired tissue concentrations deep in the skin after topical administration at safe doses. Two drug classes, phosphodiesterase type 5 inhibitors and selective estrogen receptor modulators, identified in the PD screen were shown to synergize in vitro and in vivo and to slow the disease progression in patients with early PD.
Discussion: Our phenotypic screening campaigns identified drugs that can prevent myofibroblast transformation and subsequent extracellular matrix production in cells derived from PD and HS. These drugs have the potential to be repurposed for the treatment of the respective fibrotic indications.
Conclusion: These results suggest phenotypic screening for fibrosis as a viable alternative to target-based screening, with myofibroblast transformation presenting a translatable phenotype for successful drug repurposing.

Locking the door instead of capturing all the intruders. Blocking HSPG by a specific peptide ligand is more effective than using soluble heparin for inhibiting cancer cell adhesion and migration

Lorenzo Depau[1], Elisabetta Mandarini[1], Jlenia Brunetti[1], Marta Zanchi[1], Chiara Falciani[1], Luisa Bracci[1]
[1]Department of Molecular Biotechnologies, University of Siena, Siena, Italy

Purpose: Despite promising preclinical results indicating possible effectiveness of heparin for cancer therapy, different clinical trials on the use of Low MW heparins in different cancers, reported no significant benefit in tumor progression and overall survival, indicating that using heparin as a soluble decoy for the many HSPG ligands, does not appear to be the best possible approach [1, 2]. Using a specific peptide ligand of HSPG sulfated GAG chains [3, 4], we intended to test the effectiveness of an alternative approach, by targeting HSPG for interfering with their functions.
Methods: The tetra-branched peptide NT4 and heparin were compared for their effect on adhesion of different cancer cell lines to extracellular matrix (ECM) supports, as well as on migration and in vitro invasiveness of the same cell lines.
Results: We found that inhibition by NT4 of cancer cells adhesion and migration is variable in different cancer cell lines and ECM supports, whereas heparin cannot inhibit adhesion or migration of any cancer cell line on all the tested ECM supports.
Discussion: In previous papers we had found that NT4 binding to human cancer cell lines resulted in either inhibition or increase of oriented migration in cells with different migration phenotypes (4,5). Here we demonstrated that heparin can reverse the effect produced by NT4 on migration of different cancer cells, whereas it cannot modify cell migration, when used alone. A possible explanation may lie in the high redundancy of heparin binding sites in ECM proteins, which requires very high concentration of heparin to be completely saturated and blocked.
Conclusion: In many invasive cancers, targeting sulfated GAGs of HSPG may be a more efficient and personalized anti-metastatic strategy compared to soluble heparin analogues.

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Tissue engineered fibrous caps - A new model platform to study plaque mechanics and matrix failure mechanism

Tamar Wissing[1,2,3], Kim van der Heiden[1,2], Sheila Serra[1], Anthal Smits[2,3], Carlijn Bouten[2,3], Frank Gijsen[1,4]
[1]Cardiology, Biomedical Engineering, Erasmus University Medical Centre, Rotterdam, The Netherlands; [2]Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands; [3]Institute for Complex Molecular Systems, Eindhoven, The Netherlands; [4]Biomechanical Engineering, Technical University Delft, Delft, The Netherlands

Purpose: Many stochastic thrombotic events are caused by the rupture of the fibrous caps that overlie atherosclerotic plaques. Here, we introduce a tissue engineered collagenous matrix as a first 3D cap analog to systematically scrutinize the reciprocal relationships between cap composition, mechanics, adaptation and ultimately failure.
Methods: Human myofibroblasts were cultured for 21 days in 1 x 1.5 cm-sized fibrin-rich constrained gels. The gels were exposed to static and dynamic (i.e. intermittent and continuous loading) culture protocols to vary collagen composition (e.g. amount, type and organization) [1]. A soft 2 mm ∅ fibrin inclusion was introduced in the centre of each tissue at day 7 to mimic the soft lipid core, simulating the heterogeneity of a plaque. Cell and collagen content, type, crosslinking and organization were assessed via tissue assays and immunohistochemistry. Tissue mechanics were determined via tensile testing.
Results: At day 21, consistent tissues with integrated soft inclusions were formed. IHC analyses showed the presence of both collagen type I and III, the main types present in the fibrous cap. In addition, similar lysyl oxidase expressions, an enzyme for collagen crosslinking, were observed between the created tissues and the fibrous cap positive control. Anisotropic collagen configurations were detected in the shoulder and mid-cap regions of the dynamically loaded tissues, where the top and base regions as well as the statically cultured samples exhibited a random collagen configuration. Mechanical analyses revealed that all analogs mimicked plaque mechanics found ex vivo [2].
Discussion / Conclusion: Reproducible collagenous tissues were created that vary in collagen composition due to the presence of an integrated soft inclusion and the culture protocol applied. The analogs can be deployed to assess tissue composition, mechanics, evolution and failure of fibrous caps but also more complex heterogeneous tissues in general. Current initiatives focus on integrating calcifications and macrophages to study their impact on tissue mechanics and failure.

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WORKSHOPS

WORKSHOP A - MATRIX SYNTHESIS AND REMODELING

Proteomic identification of modified extracellular matrix proteins in symptomatic atherosclerotic carotid plaques

Lasse Lorentzen[1], Karin Yeung[2], Henrik Sillesen[2], Nikolaj Eldrup[2], Jonas Eiberg[2], Michael Davies[1]
[1]Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; [2]Rigshospitalet, Copenhagen, Denmark

Purpose: Atherosclerotic plaque formation is driven by lipid accumulation in the artery wall. This can be asymptomatic for many years, but destabilization and rupture can occur suddenly causing myocardial infarction or stroke. Stable (hard) plaques are characterized by fibrosis and calcification, whilst unstable (soft) plaques have a high inflammatory activity, a thin fibrous cap, a large necrotic lipid core, and often intraplaque haemorrhage. We hypothesized that proteolytic degradation of extracellular matrix (ECM) proteins drives plaque destabilization and rupture.
Methods: Patients with recent symptomatic carotid artery disease (stroke, transient ischaemia or ocular ischaemia) had plaques removed surgically. 21 lesions (3 groups of 7) macroscopically categorised as hard, soft or mixed were solubilised to extract proteins. These were then subjected to proteolytic digestion and LC-MS proteomics to examine the total proteome and detect fragments with new (non-canonical) N-termini.
Results: More than 5000 proteins were identified and quantified in lesions in a reproducible manner, including 211 ECM species. 622 proteins were differentially abundant between hard and soft lesions. An enrichment of inflammatory proteins, proteolytic enzymes involved in ECM remodelling, and a loss of ECM proteins was detected in soft lesions compared to hard lesions. N-terminal peptide proteomics, identified 3,118 N-terminal peptides. 1085 of these were more abundant in soft compared to hard plaques, with 941 of these having new (non-canonical) N-termini, consistent with these arising from proteolytic cleavage of parent proteins. These include multiple collagens, fibronectin and other ECM proteins.
Conclusion: The data presented offer unique insights into inflammatory and proteolytic mechanisms of plaque destabilization, and identifies targets and mechanisms of proteolytic ECM degradation in soft lesions. The identification of peptides from specific ECM proteins are consistent with a weakened plaque structure. This study provides a framework for identification of blood biomarkers of unstable plaques, and the proteolytic enzymes involved. The latter may be therapeutic targets.

Interaction of ADAMTSL2 with the heparin binding region of fibronectin suggests a role in matrix assembly

Mukti Singh[1], Stuart A. Cain[1], Alan R.F. Godwin[1], Michael P. Lockhart-Carins[1], Holly L. Birchenough[1], Thomas A. Jowitt[1], Alan M. Roseman[1], Clair Baldock[1]

[1]School of Biological Sciences, University of Manchester, Manchester, United Kingdom

Purpose: A disintegrin and metalloprotease with thrombospondin type 1 motifs-like- 2 (ADAMTSL2) is an extracellular matrix (ECM) molecule, mutations in which phenotypically mimic geleophysic dysplasia typically caused by mutations in fibrillin-1 [1]. ADAMTSL2 interacts with fibrillin-1 [2, 3] suggesting that ADAMTSL2 may contribute to the structural assembly and maintenance of microfibrils.
Methods: Surface plasmon resonance (SPR) has enabled us to study interactions of ADAMTSL2 with ECM proteins. Immunofluorescence (IF) microscopy was used to visualise the deposition of ADAMTSL2 in ECM of human dermal fibroblasts (HDFs). We have used cryo-electron microscopy (cryoEM) and small-angle X-ray scattering (SAXS) to determine the structural properties of ADAMTSL2.
Results: We report a novel interaction of ADAMTSL2 with fibronectin (FN) using SPR which was corroborated by cell surface co-localisation in the ECM of HDFs. A high affinity interaction of ADAMTSL2 was fine-mapped to the heparin-binding domains of FN, and an interaction with heparan sulphate (HS) was reported. We show that fibrillin-1 and FN compete for ADAMTSL2 binding. CryoEM has revealed ADAMTSL2 has an elongated C-shaped 3D structure. SAXS modelling has determined the shape and flexibility of ADAMTSL2 suggesting that the C-terminal region is flexible.
Discussion: Our investigation reports the first structural data on ADAMTSL2. We present novel data showing the interaction of ADAMTSL2 with the heparin binding region of FN and HS suggesting a role for ADAMTSL2 in fibrillin-1 microfibril assembly.

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Impairment of Calcium-activated nucleotidase 1 affects proteoglycan synthesis and cell homeostasis

Chiara Gramegna Tota[1], Chiara Paganini[1], Alessandra Leone[1,2], Marco Biggiogera[3], Jean-Marc Schwartz[4], Valérie Cormier-Daire[5], Antonella Forlino[1], Antonio Rossi[1]

[1]Department of Molecular Medicine, [3]Department of Biology & Biotechnology, University of Pavia, Pavia, Italy; [2]Scuola Universitaria Superiore IUSS, Pavia, Italy; [4]Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom; [5]Department of Genetics and INSERM UMR1163, Hôpital Necker Enfants Malades, Paris, France

Purpose: Desbuquois dysplasia type 1 (DBQD1) is a recessive chondrodysplasia caused by mutations in the CANT1 gene, encoding for Calcium-Activated Nucleotidase 1 (CANT1) that hydrolyses UDP in the Golgi. To study CANT1 involvement in DBQD1 pathogenesis, a Cant1 knock-out mouse ($Cant1^{-/-}$) was generated and characterized. Using the animal model, the CANT1 role in proteoglycan (PG) synthesis has been recently demonstrated. In this work, we investigated the role of CANT1 in PG processing and in organelle homeostasis.
Methods: PG secretion was analysed by pulse-chase labelling of chondrocytes with 35S-sulfate. Expression level of BiP and ATF4 was measured by western blots, while the spliced form of Xbp1 (Xbp1s) was studied by RT-PCR. Aggrecan western blot analysis were performed after digestion with chondroitinase ABC to unmask the aggrecan core protein. Nuclear translocation of TFE3 was analysed by western blot and confocal microscopy.
Results: PG secretion was reduced in $Cant1^{-/-}$ chondrocytes compared with wild-type cells. Moreover, only glycanated aggrecan was secreted by $Cant1^{-/-}$ chondrocytes as in wild-type cells. TEM analysis of $Cant1^{-/-}$ chondrocytes showed ER enlargement not due to ER stress since the markers BiP, ATF4 and Xbp1s were normal. A higher nuclear translocation of TFE3 was observed in $Cant1^{-/-}$ chondrocytes compared with wild-type cells.
Discussion: Even if CANT1 impairment affect PG secretion, only glycanated aggrecan was secreted. Since the level of ER stress markers such as BiP, ATF4 and Xbp1s were normal in $Cant1^{-/-}$ chondrocytes, the ER enlargement observed by TEM analysis was not due to ER stress, but more likely to impaired GAG synthesis in the Golgi. This observation is further supported by increased TFE3 translocation to the nucleus, a marker of altered Golgi homeostasis.
Conclusion: CANT1 impairment causes not only defective PG synthesis and secretion in cartilage, but also affects cell homeostasis.

Linking cilia to WNT signaling in osteogenesis imperfecta

Mirko Rehberg[1], Nicola Stevenson[2], Alice Stephan[1], Roberta Besio[3], Nadia Garibaldi[3], Nadine Reintjes[4], Matthias Mörgelin[5], Antonella Forlino[3], Christian Netzer[4,6], David Stephens[2], Jorg Oliver Semler[1,6], Frank Zaucke[7], Julia Etich1,7
[1]Department of Pediatrics and Adolescent Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany; [2]Cell Biology Laboratories, School of Biochemistry, University of Bristol, Bristol, United Kingdom; [3]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [4]Institute of Human Genetics, University Hospital, University of Cologne, Cologne, Germany; [5]Colzyx AB, Medicon Village, Lund, Sweden; [6]Center for Rare Diseases, University Hospital, University of Cologne, Cologne, Germany; [7]Dr. Rolf M. Schwiete Research Unit for Osteoarthritis, Department of Orthopedics (Friedrichsheim), University Hospital, Goethe University, Frankfurt/Main, Germany

Purpose: Osteogenesis imperfecta (OI) is a rare, hereditary connective tissue disorder clinically characterized by bone fragility, bone deformities and small stature. 85% - 90% of patients have a mutation in one of the collagen I genes. The remaining individuals harbor mutations in non-classical OI-causing genes that, however, have been implicated in collagen type I biosynthesis, osteoblast homeostasis and bone matrix mineralization. Despite the advanced molecular understanding if this disease there are still OI patients with unknown genetical cause.
Methods: We now identified a novel OI-causing gene in a consanguine family by whole exome sequencing that encodes a cilia-associated protein with unknown function. Using a cellular model of patient-derived fibroblasts, we explored the underlying pathomechanism.
Results: We could confirm that collagen I secretion, deposition and assembly were disturbed. Moreover, increased expression of PTCH1, a direct target gene of the cilia-dependent hedgehog signaling (HH) pathway, was observed. To our knowledge, HH signaling deregulation has not been linked to OI yet but it was described to regulate via transcriptional regulation of secreted frizzled receptor protein 1 (sFRP1) expression the WNT/β-catenin signaling whose impairment is known to cause OI. We showed that expression of sFRP1 was persistently induced to potentially inhibit WNT signal transduction essential for bone remodelling. Indeed, the clinical phenotype of the patient supports a bone remodelling defect characteristic for OI patients with WNT1 mutations.
Discussion: sFRP1 is known to inhibit the WNT signaling pathway. Although we do not know yet how a mutated cilia-associated protein can induce sFRP1 expression, it molecularly links the cilium to WNT signaling regulation.
Conclusion: Here, we expand the spectrum of WNT-related OI forms and potentially open a new channel for tailored therapeutic approaches.

WORKSHOP B - MATRIX IMMUNITY AND AUTOIMMUNITY

148 - Matrix, immune system and pathologies

Immune modulatory effects of collagen type I

Marie-Louise Thorseth[1], Anne Mette Askehøj Rømer[1], Daniel Hargbøl Madsen[1]
[1]National Center for Cancer Immune Therapy, Department of Oncology, Copenhagen University Hospital - Herlev, Herlev, Denmark

Purpose: The extracellular matrix (ECM) can affect numerous biological processes. However, little attention has been given to the possibility that the ECM can have immune modulatory functions. During cancer progression, the ECM is extensively remodeled and the generated tumor-specific ECM is known to have pro-tumorigenic functions. Here, we investigated if collagen type I, which is the main ECM component in solid tumors, can affect the activity of immune cells.
Methods: T cells or macrophages were 3D-cultured for investigations of the effects of different collagen densities on cellular function. The effects were evaluated through a combination of whole-transcriptome analysis and a number of functional assays. The importance of collagen for pancreatic cancer progression and on the tumor immune environment in vivo was investigated using two different transgenic mouse strains that allowed for specific manipulation of collagen type I levels.
Results: In a 3D cell culture system, a high collagen density reduced proliferation of primary T cells compared to culture in a low-density collagen matrix and induced a gene expression profile indicating that the T cells became less cytotoxic and more immune regulatory. Consistently, tumor-infiltrating T cells from melanoma patients were less efficient at killing autologous cancer cells after culture in high-density collagen compared to low-density. Additionally, a high collagen density reprogrammed macrophages towards a more immune suppressive phenotype. In vivo, reduction of collagen levels using inducible conditional collagen type I knockout mice led to increased infiltration of NK cells and CD8 T cells and reduced tumor growth. Opposite effects were observed in transgenic Col^{R} mice, which accumulated higher levels of collagen in the tumors.
Discussion: We show that collagen type I can modulate T cell and macrophage activity in vitro and that manipulation of collagen levels in cancer in vivo affects the immune environment in the tumors.
Conclusion: Collagen-induced immune suppression could be a novel and conceptually different immune modulatory mechanism with importance in cancer and in other pathologies.

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Investigating versican as an immunotherapeutic target in triple negative breast cancer

Priyanka Hirani[1], Daniel Pennington[2], Pedro Cutillas[3], Thomas Wight[4], Oliver Pearce[1]
[1]Barts Cancer Institute, Tumour Microenvironment, London, United Kingdom; [2]Blizard Institute, London, United Kingdom; [3]Barts Cancer Institute, London, United States; [4]Benaroya Research Institute, Seattle, USA

Purpose: The tumour extracellular matrix can act as a barrier to immunotherapy response, and from previous analysis [1] we identified the proteoglycan versican as a component of this barrier. Our hypothesis is that versican interferes withh T-cell invasion of tumour islands leading to the failure of immunotherapy response [2].
Methods: FFPE TNBC human tissues were stained for CD8, CD68, pancytokeratin, and versican (RNA and protein). Areas were defined as either the epithelial zone (EZ) or stromal zone (SZ) and analysed for the number of CD8+ and CD68+ immune cells. Based on the ratio of immune cells, tissues were classified as either inflamed (EZ^{hi}, $SZ^{hi/lo}$), excluded (EZ^{lo}, SZ^{hi}) or cold (EZ^{lo}, SZ^{lo}). The levels of versican as well as the structural arrangement through TWOMBLI [3] were analysed.
Results: In tissues with an excluded immune phenotype that associate with poor prognosis and failure of therapy response, versican was expressed highly within epithelial zones, whereas in inflamed tumours versican expression was restricted to stromal areas. Structural arrangement analysis showed excluded tissues to have a more open and disordered display of versican in comparison to the tight dense expression seen in inflamed tissues.
Discussion: Associations between versican accumulation and expression and the localisation of CD8+ T cells may be a result of the interactions between the protein and the immune cells. This interaction may be impacted by the structure of versican and the arrangement of versican within the ECM.
Conclusion: By identifying the spatial relationship between versican accumulation and immune cell infiltration in different regions of tumours will add to the development of strategies leading to restoration of immune cell motility and immunotherapy response in the treatment of cancer.

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Autonomic neurotransmitters influence human osteoarthritic chondrocyte function in vitro

Rebecca Sohn[1], Markus Ehnert[2], Sebastian Braun[2], Andrea Meurer[2], Frank Zaucke[1], Zsuzsa Jenei-Lanzl[1]
[1]Department of Orthopedics, Dr. Rolf M. Schwiete Research Unit for Osteoarthritis, [2]Department of Orthopedics, University Hospital Frankfurt, Frankfurt am Main, Germany

Purpose: The sympathetic (SNS) and parasympathetic nervous systems (PNS) exhibit opposing effects in the periphery via the respective neurotransmitters norepinephrine (NE) and acetylcholine (Ach) and might therefore contribute to osteoarthritis (OA) pathogenesis. The autonomic balance is often disturbed in pathological situations and for this reason, we examined the response of human articular chondrocytes to different SNS/PNS neurotransmitter combinations in vitro.
Methods: Chondrocytes of OA patients were cultivated under physioxic conditions (2% $O_{2)}$ with or without IL-1β (0.5 ng/ml) for 7 days. Additional treatment with different combinations and concentrations of NE and Ach mimicked low and high SNS and/or PNS tones. Gene expression changes (ACAN, COL1A1, COL2A1, MMP13, IL6) were determined by qRT-PCR and activation of major intracellular signaling pathways was analyzed via Western blotting.
Results: Cells treated with IL-1β demonstrated a significant increase in IL6 and MMP13 expression compared to cells without IL-1β treatment ($p < 0.05$). The IL-1β-induced increase in IL6 expression was slightly diminished by Ach. Similarly, the IL-1β-induced increase in MMP13 expression was reversed by high concentration of Ach, or equal concentrations of Ach and NE. Overall, Ach and/or NE treatment decreased IL-1β -induced ERK1/2 phosphorylation. This effect was more pronounced when Ach and NE were administered alone than in combination. PKA, p38 and NfkB phosphorylation was not altered by any treatment.
Discussion: Predominantly Ach counteracted IL-1β-induced increase in inflammation- and matrix degradation-related gene expression in human OA chondrocytes. This finding indicates that PNS activity might beneficially influence chondrocyte function during OA pathogenesis likely via the ERK1/2 pathway.
Conclusion: Our results provide a first insight into the complex interplay of SNS and PNS during OA progression and might open exciting avenues for future strategies for OA prevention and therapy.

Macrophages and dermal fibroblasts-own ECM: a good neighborhood?

Sara Chaves[1,2], Joana Macedo[1,2], Daniel Reis[1,2], Lucília Silva[1,2], Alexandra Marques[1,2]
[1]3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics, Guimarães, Portugal; [2]2ICVS/3B's–PT Government Associate Laboratory2ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal, Guimarães, Portugal

Purpose: Immunocompetent 3D skin models allow studying molecular pathways that otherwise are only possible to explore using animal models. The human biology representation in existing 3D in vitro models is limited by the minimal extracellular matrix (ECM) mimicry [1]. This can be overcome by combining, from the onset of the generation of the model, a relevant subset of cells in a 3D microenvironment formed by cells-own ECM. In this work we hypothesised that this ECM was not able to promote macrophage activation, ruling out a response that would compromise the physiologic phenotype of the model.
Methods: Structural and soluble ECM components were extracted from fibroblasts cultures using an in-house established approach. Blood monocyte-derived macrophages (MDMs) isolated from different donors were primed with a range of ECM concentrations (5, 25 and 50 μg/mL soluble, or 5000 and 1000 μg/mL structural). After 48h, the supernatants were collected for multiplexing (IL-6, CXCL10, CCL8) while cells were processed for flow cytometry (CD14, CD86, CD206, CD197, CD319) and RT-PCR (NF-kB, STAT1, PPARG and JMJD3) analyses.
Results: Exposure to the ECM extracts, led to downregulation of transcription factors associated with pro-inflammatory pathways (NF-kB, STAT1) and unchanged mRNA expression of anti-inflammatory (PPARG and JMJD3) genes, in relation to unstimulated MDM. Moreover, lower/unchanged levels of pro-inflammatory markers (CD86, CD197, CD319) and increased expression of anti-inflammatory CD206 were detected. Pro-inflammatory cytokines IL-6 and CXCL10 were significantly reduced while the levels of anti-inflammatory CCL18.
Discussion: Fibroblast-derived ECM support MDMs basal or anti-inflammatory phenotype even if obtained from different donors confirming our hypothesis.
Conclusion: This study shows that cells-own ECM do not activate macrophages, warranting the representation of the physiologic skin phenotype.

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WORKSHOP C - MATRIX IN DEVELOPMENT AND AGEING

Collagen VI ablation in zebrafish causes neuromuscular defects and abnormal signaling during development

Valentina Tonelotto[1], Chiara Consorti[1], Valeria Trapani[1], Nicola Facchinello[1], Paolo Bonaldo[1]
[1]Department of Molecular Medicine, University of Padova, Padova, Italy

Purpose: Collagen VI (COL6) plays a broad range of critical functions in different tissues. In humans, mutations of COL6 genes cause inherited disorders affecting skeletal muscles and collectively known as COL6-related myopathies [1]. To get insight into the pathogenic mechanisms underlying COL6-related diseases, diverse animal models were produced [1-3]. However, the roles exerted by COL6 during embryogenesis remain unknown.
Methods: We generated a zebrafish COL6 knockout line through CRISPR/Cas9 site-specific inactivation of the col6a1 gene and characterized its phenotype during development and in adult life.
Results and Discussion: COL6 ablation leads to neuromuscular defects and motor dysfunctions at both embryonic and larval stages. These defects are linked to altered signaling during early development and affecting adaxial cells, with defective muscle patterning and impaired motor axon elongation. Such phenotypic features are maintained in adult fish, which display altered muscle organization and impaired swimming capabilities. Furthermore, COL6 null fish exhibit impaired autophagy and organelle defects at both embryonic and adult stages, thus recapitulating the main features of patients affected by COL6-related myopathies [3-5]. Treatment of COL6 null embryos with the β_2-adrenergic receptor agonist salbutamol elicits a significant amelioration of the neuromuscular and motility defects.
Conclusions: Altogether, these findings allow to throw light on the roles exerted by COL6 during development and demonstrate that the col6a1 null fish is a valuable in vivo platform for dissecting the mechanisms underlying COL6-related diseases and for the screening of drugs and prospective therapeutic strategies.

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Structural and functional changes in the development of the extrahepatic bile duct interstitium

Jessica Llewellyn[1], Woowon Lee[2], Emilia Roberts[3], Richard Assoian[3], Kimani Toussaint[4], Neil Theise[5], Rebecca Wells[1]
[1]Gastroenterology, University of Pennsylvania, Philadelphia, USA; [2]Mechanical Engineering, University of Colorado Boulder, Boulder, USA; [3]Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia, USA; [4]Engineering, Brown University, Providence, USA; [5]Pathology, NYU Langone Health, New York City, USA

Purpose: The interstitial space, found throughout the body but still poorly characterized, is composed of a network of collagen bundles lined with fibroblasts. We have previously shown that in the extrahepatic bile duct (EHBD), the interstitium drastically changes postnatally. Key matrix components such as collagen and elastin are deposited within the first weeks of life during which neonatal interstitial cells are highly active (collagen production, contractile, proliferative) in contrast to adult cells. Here we further characterize the postnatal structural and functional changes of the EHBD interstitium during development.
Methods: EHBDs were isolated from rodents at birth to adulthood. Mouse EHBDs were imaged using second harmonic generation. Interstitial cells were isolated from mouse EHBDs and cultured on substrates of different stiffness. Rat EHBDs were used for mechanical testing by pressure myography.
Results / Discussion: We identified significant matrix, mechanical, and cellular changes that appear to be related and have important functional relevance. We found that collagen fibers are organized into an aligned and crimped network postnatally suggesting a lack of structure and tissue integrity in the neonatal duct. Pressure myography was used to test this. At high pressures, neonatal EHBDs stretched more suggesting a poor response to luminal pressure (e.g. obstruction). Both the matrix deposition and organization during normal development as well as obstruction during disease lead to an increase in tissue stiffness. We therefore assessed the effect of stiffness on interstitial cells. Both neonatal and adult cells were found to be highly mechanosensitive (nuclear YAP). However, stiff substrates lead to a loss of contractility (αSMA) in neonatal cells which was not observed in adult interstitial cells, suggesting differential pathway activation.
Conclusion: The EHBD is a low pressure tissue, which allows for the majority of matrix deposition to occur postnatally. However, the lack of an organized matrix, increased stretch during pressure myography and altered response of cells to mechanical cues suggest that neonatal EHBDs may be less tolerant to mechanical stress such as obstruction and uniquely susceptible to disease (e.g. biliary atresia) when compared to adults.

Conserved role of matrix metalloproteases 2 and 9 in promoting the migration of neural crest cells in avian and mammalian embryos

Rotem Kalev-Altman[1,2], Erez Hanael[1], Einat Zelinger[3], Martin Blum[4], Efrat Monsonego-Ornan[2], Dalit Sela-Donenfeld[1]
[1]The Koret School of Veterinary Medicine, [2]The Institute of Biochemistry, Food Science and Nutrition, [3]Center for Scientific Imaging, The Faculty of Agriculture, Food and Environment, Rehovot, Israel; [4]Hohenheim University, Stuttgart, Germany

Purpose: Neural crest cells (NCCs) are a unique embryonic cell population that migrate in the embryo and differentiate into multiple types of derivatives [1]. To acquire motility, NCCs undergo epithelial-to-mesenchymal transition and invade the surrounding extracellular matrix (ECM) [2]. Matrix metalloproteases (MMPs) are a family of proteases that regulate migration of various cell types via ECM remodelling [3]. Here we explored whether the gelatinase's subgroup of MMPs, MMP2 and MMP9 has a role in executing NCC migration, by determining whether they regulate migration across species in singular, combined, or redundant manners.
Methods: Chick and mouse embryos were utilized to compare expression and activity of both MMPs using genetic and pharmacological approaches in multiple in vivo and ex vivo assays.
Results: Both MMPs were found to be expressed and active in mouse and chick NCCs. Inhibition of each MMP was sufficient to prevent NCC migration in both species. Yet, NCC migration was maintained in MMP2-/- or MMP9-/- mouse mutants due to compensation between the gelatinases indicated by increased mRNA levels of one MMP in the absence of the other. However, reciprocal pharmacological inhibition in each mutant prevented NCC migration.
Discussion: The increased mRNA levels of MMP9 in MMP2-/- mice together with the higher concentrations of MMP9 inhibitor that were needed to block NCC migration in the MMP2-/-, compared to WT, both suggest that the lack of MMP2 gene is being overridden by over expression of MMP9, which rescues NCC migration.
Conclusion: This study reveals for the first time that both gelatinases are expressed and active in avian and mammalian NCCs, and demonstrates their fundamental and conserved role in promoting embryonic cell migration.

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Intake of avian eggshell membrane powder can reduce inflammation and attenuate skeletal muscle aging

Sissel Beate Rønning[1], Harald Carlsen[2], Sergio Rocha[2], Nina Solberg[1], Vibeke Høst[1], Eva Veiseth-Kent[1], Silje Bergum[1], Bente Kirkhus[1], Nada Abedali[3], Amanda Rundblad[3], Pia Bålsrud[3], Kirsten Holven[3], Stine Ulven[3], Mona Pedersen[1]

[1]Nofima AS, Raw Materials and Process Optimisation, Ås, Norway; [2]Biotechnology and Food Science, University of Life Science, Ås, Norway; [3]Institute of Basic Medical Sciences, University of Oslo, Norway

Purpose: Eggshell membrane (ESM) is a extracellular matrix consisting of collagens, glycoproteins, proteoglycans, and hyaluronic acid. We have previously shown anti-inflammatory properties in vitro, and that ESM regulates MMP-activity during wound healing processes in vivo. The purpose of the study was to investigate if oral intake could attenuate skeletal muscle aging.
Methods: Aged mice (14 mo) were fed regular mouse diet suppl. with 0, 0.1 and 8% ESM for 14 weeks and compared with young mice (Ctr). Blood serum was analyzed for inflammatory markers. Rotarod test and grip-test were used to analyze aging phenotypes. Tibialis anterior skeletal muscle was analyzed using immunofluorescence histology analysis, real-time PCR, and proteomic analysis. A pilot-clinical trial of healthy home-dwelling elderly (> 70 years) receiving capsules with either placebo or 0.1 % ESM for 1 month was performed, and inflammation markers in blood was investigated. The protein digestibility of ESM was investigated using the INFOGEST in vitro model simulating human digestion
Results: ESM in the diet lowered the expression of the inflammation marker TNF-α in the mice. Several hallmarks of sarcopenia were observed in old mice compared with young mice, i.e., reduced number of muscle fibers, centronucleated fibers, changed fiber type proportion, reduced gene expression of satellite cell markers (Sdc3 and Pax7) and increased expression of FBXO32. Interestingly, old mice who digested 8% ESM had lost many of these hallmarks, with a phenotype resembling young mice. A significant reduction in the inflammation marker CRP was observed in the elderly receiving ESM capsules.
Discussion: ESM has immune modulating effects after oral intake in humans and mice. Furthermore, eating ESM seemed to attenuate skeletal muscle aging. Since ESM was to poorly digested (when analyzed with the INFOGEST in vitro model), further studies should focus on possible microbiota effects.
Conclusion: ESM is interesting as nutraceutical.

WORKSHOP D - CELL-ECM SIGNALLING AND REGULATION

Zebrafish tmem38b Knock out unveils the in vivo role of trimeric intracellular cation (TRIC) channel B on cell homeostasis

Francesca Tonelli[1], Laura Leoni1, Valentina Daponte1, Cecilia Masiero1, Roberta Gioia1, Silvia Cotti2, Daria Larionova2, Imke A.K. Fiedler3, Bjoern Busse3, P. Eckhard Witten2, Antonella Forlino1

[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [2]Department of Biology, Ghent University, Belgium; [3]Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Germany

Purpose: TMEM38B encodes the endoplasmic reticulum (ER) potassium channel TRIC-B, which indirectly modulates Ca^{2+} exit from the ER. Ca2+ is required for collagen type I assembly and as cofactor for enzymes responsible for collagen type I post-translational modifications. Mutations in TMEM38B cause recessive osteogenesis imperfecta (OI) type XIV characterized by under-modified collagen type I and ER stress. OI type XIV patients show a wide range of phenotypes whose molecular basis is still unknown. To better understand the link between TRIC-B activity and OI tmem38b mutant zebrafish were generated.
Methods: tmem38b mutants were generated by CRISPR/Cas9. Alizarin red staining was performed to evaluate mineralization. MicroCT and nanoindentation were used to evaluate bone properties. Transmission electron microscopy was used to evaluate ER cisternae size. Collagen was analysed by SDS-PAGE. The expression of the collagen specific chaperone heat shock protein 47 (Hsp47) was determined by whole mount immunohistochemistry with or without 4-phenylbutyrrate. Tail regeneration assay was employed to investigate bone modelling.
Results: Two tmem38b zebrafish mutants were generated: one carrying a frameshift mutation resulting in a premature stop codon ($tmem38b^{-/-}$) and one with an in-frame deletion, which impairs pore channel domain. $tmem38b^{-/-}$ show significant growth retardation at 21 dpf and 1 mpf linked to a reduced size of vertebral centra. Shape and mechanical properties remained normal. The enlarged ER cisternae size and the increased Hsp47 expression observed in both mutants suggest collagen retention in the ER, partially rescued by 4-phenylbutyrrate. Defects in bone cells activity were evaluated in regenerated tails.
Discussion: tmem38b mutants recapitulate the intracellular stress condition reported in OI type XIV patients' cells and in knock out murine model.
Conclusion: The intracellular stress condition reported in our models suggest ER as potential target for pharmacological treatment for OI type XIV.

Latent TGFβ complexes are transglutaminase cross-linked to fibrillin to facilitate TGFβ activation

Michael P. Lockhart-Cairns[1], Stuart A. Cain, Rana Dajani[1], Ruth Steer[1], Jennifer Thomson[1], Yasmene F. Alanazi[1], Cay M. Kielty[1], Clair Baldock[1], Clair Baldock[1]

[1]Wellcome Centre for Cell-Matrix Research, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom

Purpose: TGFβ superfamily members are potent growth factors in the extracellular matrix with essential roles in all aspects of cellular behaviour. Latent TGFβ binding proteins (LTBPs) are co-expressed with TGFβ to form large latent complexes, essential for correct folding and secretion of the growth factor. These large latent complexes bind fibrillin in the matrix, essential for normal tissue function, and dysregulated TGFβ signalling is a hallmark of many fibrillinopathies. Transglutaminase-2 (TG2) cross-linking of LTBPs is known to play a role in TGFβ activation but the underlying molecular mechanisms are not yet resolved.

Methods: In this study, we used TG2 cross-linking assays and a nano-luciferase reporter cell-based assay to probe the impact of TG2 cross-linking. Small angle X-ray scattering was used to determine the structural consequence of cross-linking and the shape of the formed complex. SPR binding assays determined the affinity of complexes for integrin αVβ6.

Results: Fibrillin is a matrix substrate for TG2 and TG2 cross-linked complexes can be formed between fibrillin and LTBP-1 and -3, and their latent TGFβ complexes. The structure of the fibrillin-LTBP1 complex shows that the two elongated proteins interact in a perpendicular arrangement, which would allow them to form distal interactions between the matrix and the cell surface. Formation of the cross-link with fibrillin does not change the interaction between latent TGFβ and integrin αVβ6 but does increase TGFβ activation in cell-based assays, which can be ameliorated by heparin sulphate.

Conclusion: These data suggest that TGFβ activation is enhanced by covalent tethering of LTBPs to the matrix via fibrillin. The activating effect may be due to direction of the latent complexes to the cell surface by fibrillin, as competition with heparan sulphate can ameliorate the activating effect.

The extracellular matrix glycoprotein ADAMTSL3 has anti-fibrotic and cardio-protective effects in the failing heart

Karoline Bjarnesdatter Rypdal[1], Arne Olav Melleby[2], Emma Louise Robinson[3], Jia Li[4], Deborah Seifert[5], Sheryl Palmero[4], Daniel Martin[5], K. Andreassen[4], Christen Peder Dahl[2], I. Sjaastad[4], T. Tønnessen[4], M.K. Stokke[4], W.E. Louch[4], S. Heymans[3], Geir Christensen[4], Suneel S. Apte[5], Ida Gjervold Lunde[1]

[1]Division for Diagnostics and Technology (DDT), Akershus University Hospital (Ahus), Lørenskog, Norway; [2]University of Oslo, Oslo, Norway; [3]Maastricht University, Maastricht, The Netherlands; [4]Institute for Experimental Medical Research (IEMR), Oslo University Hospital, Oslo, Norway; [5]Cleveland Clinic Lerner Research Institute, Cleveland, USA

Purpose: The ADAMTSL family of extracellular matrix (ECM) glycoproteins is upregulated in heart failure with unknown function [1]. Molecular evidence suggest that they may regulate TGFβ2, a major pro-fibrotic driver of heart failure 3. Here, we examined the role of ADAMTSL3 in heart failure.

Methods: ADAMTSL3 expression was investigated in heart failure patients, and Adamtsl3 knock-out (KO) mice were generated and were subjected to heart failure by aortic banding (AB) for a total of six weeks. Mice were followed with echocardiography and cardiac MRI, and hearts were harvested for molecular analyses at one and six weeks. Molecular mechanisms of ADAMTSL3 was investigated by overexpression in human cardiac fibroblasts (CFBs) with an extensive ECM, followed by functional assays and mRNA and protein analysis.

Results: ADAMTSL3 was upregulated in patients with heart failure of different aetiologies. Adamtsl3 KO mice had higher mortality (40%) vs WTs (4%) after AB, and developed exacerbated heart failure with reduced contractility and increased cardiac dilatation. RNA sequencing revealed 233 differentially expressed genes in KO vs WT, with Col1a1 and Postn among the most upregulated. KO hearts had increased TGFβ signalling (pSMAD) and increased ECM expression (Spp1, Lox, Eln). ADAMTSL3 inhibited TGFβ signalling and type I collagen protein synthesis by 25% in cultured CFBs, as well as expression of pro-fibrotic genes, including COL1A1, LOX, FN1, FBN1, ELN, CTGF and POSTN. Furthermore, ADAMTSL3 reduced α-SMA mRNA and protein by 25%, and attenuated SPP1 expression, to 6% of control, with inhibited CFB differentiation, proliferation and contraction.

Discussion: Our results show that ADAMTSL3 is important for survival and heart function upon cardiac stress. As lack of ADAMTSL3 in the heart caused increased TGFβ signalling, and increased ADAMTSL3 in CFBs inhibited TGFβ signalling, collagen production and myofibroblast differentiation, this indicates that ADAMTSL3 regulates the cardiac fibrotic response by inhibiting TGFβ in the ECM.

Conclusion: The ECM glycoprotein ADAMTSL3 has a cardio-protective role in the failing heart, with anti-fibrotic effects on CFBs.

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Stiff substrate inhibits collagen accumulation via integrin α2β1, FAK and Src kinases in human cardiac fibroblasts derived from patients with aortal stenosis

Malgorzata Gaudyszynska[1], Radosław Zwoliński[2], Lucyna Piera[1], Jacek Szymanski[3], Ryszard Jaszewski[2], Jacek Drobnik[1]

[1]General and Experimental Pathology, Department of Pathophysiology, [2]Department of Cardiosurgery, [3]Central Scientific Laboratory, Lodz, Poland

Purpose: The purpose of the study was assessment whether stiffness of the cell environment may participate in regulation of fibrosis. The involvement of integrin a2b1, Focal Adhesion Kinase (FAK) and Src kinase in signal transmission will be investigated.
Methods: The experiments were carried out on myofibroblasts derived from right atrium of patients with aortal stenosis. The cells were cultured on soft (2.23 ± 0.8 kPa) or stiff (8.28 ± 1.06 kPa) polyacrylamide gels.
Results: The isolated cells (stained positively with a smooth muscle actin, vimentin, desmin, and α2 integrin) were identified as myofibroblasts. Culture of the myofibroblasts on the stiff gel decreased intracellular collagen and collagen, type I telopeptide (PICP) but this effect is not accompanied with modification of a1 chain of procollagen type I and III expression. Integrin α2β1 inhibition by TC-I15 (10^{-7} and $^{10-8}$ M) and α2 integrin subunit silencing increased intracellular collagen level. Inhibitors of FAK or Src kinase augment collagen content within the myofibroblasts culture. Inhibition of the tissue inhibitor of matrix metalloproteinase-4 (TIMP4) secretion by myofibroblasts cultured on stiff gel was observed. Matrix metalloproteinase-2 and TIMPs 1, 2 and 3 secretion were not modified by stiff substrate. Secretion of interleukin-6 but not Fibroblasts Growth Factor was inhibited in cultures on stiff gel.
Discussion: The stiff substrate exerts inhibitory control on collagen accumulation by cardiac myofibroblasts. This effect is dependent on integrin a2b1 as well as FAK and Src kinase activity. The final effect of stiff substrate on collagen content comprises of both collagen synthesis and catabolism modification. The described above mechanism is postulated to be involved in control of collagen accumulation in the hypertrophied or fibrotic heart.
Conclusion: Stiff substrate inhibits collagen accumulation via integrin α2β1, FAK and Src kinases in human cardiac fibroblasts.

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WORKSHOP E - MATRIX IN CANCERS

Analysis of extracellular matrix network dynamics in cancer using the MatriNet database

Valerio Izzi[1,2,3], Juho Kontio[1,2], Valeria Rolle Soñora[4], Vilma Pesola[1], Rijiuta Lamba[1,2], Annalena Dittmann[1], Ander Diaz Navarro[5], Jarkko Koivunen[1], Taina Pihlajaniemi[1]

[1]Faculty of Biochemistry and Molecular Medicine, [2]Faculty of Medicine, University of Oulu, Oulu, Finland; [3]Foundation for the Finnish Cancer Institute, Helsinki, Finland; [4]Biostatistics and Epidemiology Platform, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain; [5]Dpto. de Bioquímica y Biología Molecular, IUOPA-Universidad de Oviedo, Oviedo, Spain

Purpose: Changes in the quantity of extracellular matrix (ECM) components and their balance within the tumor microenvironment (TME) accompany and fuel all steps of tumor development, growth and metastasis, and a deeper and more systematic understanding of these processes is fundamental for the development of future therapeutic approaches [1]. The wealth of "big data" from numerous sources has enabled gigantic steps forward in the comprehension of the oncogenic process, also impacting on our understanding of ECM changes in the TME. Most of the available studies, however, have not considered the network nature of ECM and the possibility that changes in the quantity of components might be regulated (co-occur) in cancer and significantly "rebound" on the whole network through its connections, fundamentally altering the matrix interactome.
Methods: To facilitate the exploration of these network-scale effects we have implemented MatriNet (www.matrinet.org), a graphical database enabling the study of structural changes in ECM network architectures as a function of their protein-protein interaction strengths across 20 different tumor types.
Results: The use of MatriNet is intuitive and offers new insights into tumor-specific as well as pan-cancer features of ECM networks, facilitating the identification of similarities and differences between cancers as well as the visualization of single-tumor events and the prioritization of ECM targets for further experimental investigations.
Discussion: Matrinet supports the identification and visualization of megascale (network-level) probabilistic events in the ECM interactome in cancer, highlighting tumor-specific and pancancer events providing new clues into the functional architecture of ECM networks.
Conclusion: Matrinet, though in its infancy, provides an unprecedented possibility to investigate a whole new "layer" of complexity in the ECM interactome.

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Fc-fused decoy receptor remodels ECM microenvironment and attenuates metastasis

Nikolaos Afratis[1,2], Shivang Parikh[2,3], Idan Adir[2], Roma Parikh[3], Inna Solomonov[2], Carmit Levy[3], Irit Sagi[2]
[1]Department of Agricultural Development, Agrofood & Management of Natural Resources, National and Kapodistrian University of Athens, Psachna, Greece; [2]Department Biological Regulation, Weizmann Institute of Science, Rehovot, Israel; [3]Department of Human Genetics and Biochemistry, Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv, Israel

Purpose: Cancer is a fibrotic disease and the excessive extracellular matrix (ECM) turnover drives disease progression, immune suppression and affects treatment response. Here, we propose that bispecific targeting of lysyl oxidase (LOX) and heat shock protein 70 (HSP70) affects ECM microenvironment and controls the metastatic ability of melanoma cancer cells
Methods: In this work we used human biopsies in order to validate the expression of LOX and HSP70 in melanoma tissues and we developed in vitro and in vivo melanoma models in order to evaluate the effect of our bispecific Fc-fused decoy receptor
Results: Development of our Fc-fused bispecific inhibitors (AS1 & AS2) and their in vivo evaluation diminishes melanoma to lung metastasis formation. Thorough characterization of inhibitors proved their high binding affinity and inhibitory capacity against both biomarkers. In addition, bispecific decoys inhibit in vitro migration and invasion and in vivo melanoma metastasis to lung and diminished the circulated melanoma cells. Combinational treatment with immune checkpoint inhibitor dramatically increased the CD8+ T-cell-induced cytotoxicity.
Discussion: In the present study we revealed two new molecules, LOX and HSP70, for targeting fibrosis in malignant melanoma and this evidence led us to develop a bispecific inhibitor in order to eliminate their activity. The successful inhibition of LOX and HSP70 by the bispecific inhibitors led to rearrangement of tumor microenvironment and immune system profile, while they are preventing the melanoma to lung metastasis formation.
Conclusion: Our findings suggest that dual-inhibition of LOX and HSP70 have the potential to be a new strategy for melanoma treatment by enhancing the efficacy of existing immunotherapies and propose the adaptation of this strategy to other cancers.

Colon cancer cells morphological phenotype, EMT markers and matrix effectors are related to type and concentration of the substrate, time of culturing and cell free motion

Marco Franchi[1], Konstantinios Athanasios Karamanos[2], Concettina Cappadone[3], Natalia Calonghi[3], Nicola Greco[4], Leonardo Franchi[5], Maurizio Onisto[4], Valentina Masola[4]
[1]Department for Life Quality Studies, Bologna, Italy; [2]Department of Pharmacy, Bologna, Italy; [3]Department of Pharmacy and Biotechnologies, Bologna, Italy; [4]Department of Biomedical Sciences, Padua, Italy; [5]Department of Medicine, Bologna, Italy

Purpose: To better understand doxorubicin resistant CRC cells we investigated the behavior of LoVo-R and LoVo-S cells, resistant and sensitive to doxorubicin respectively, when they try to or they are free to cross the basement membrane (BM) and lamina propria (LP) [1].
Methods: We evaluated how different concentrations of Matrigel (BM) or type I collagen (normal or desmoplastic LP) covering both 5 or 8µm pore Millipore filters, could affect the gene expression of epithelial-to-mesenchymal (EMT) markers and phenotypes at scanning electron microscope (SEM) after 24 hours. To evaluate how cell movement and culturing time affect LoVo cells we also cultured them on 8µm pore filter covered by same substrates for 3 and 24 hours.
Results / Discussion / Conclusion: When LoVo-S were cultivated on 5µm pore filter for 24 hours the cells adapted to invade the substrates but they couldn't cross the small pores. Increasing of aggressive markers and E-cadherin expressions suggest that collagen induces a hybrid EMT phenotype in LoVo-S cells. Differently, in LoVo-R cells preparing to invade the substrates both type and concentration of substrate do not affect the behavior of doxorubicin resistant cells. Evaluating the effect of cell movement, data of 8µm pore filter after 24 hours vs. 5µm pore filter after 24 hours showed a decrease of MMP2,9,14 associated to epithelial phenotypes in LoVo-S cells on collagen. Differently, MMP2 and 9 strongly increased in LoVo-R cells on concentrated collagen as confirmed by EMT phenotypes showing invadopodia at SEM. The effect of cell movement attenuates LoVo-S aggressiveness, whereas it improves malignity in LoVo-R cells. Evaluating the effect of culturing time, LoVo-S cells on 8µm pore filter after 24hours vs. 8µm pore after 3 hours showed a decrease of MMP2 and heparanase expressions associated to epithelial phenotypes on collagen, whereas MMP2 and 9 strongly increased in LoVo-R cells on the same substrate. The effect of time of culturing differently affect the aggressiveness of LoVo-S and LoVo-R cells: extending time of culturing attenuates LoVo-S aggressiveness, whereas it improves malignity in LoVo-R cells.

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Spock1 supports the development of hepatocellular cancer. Competition with syndecan-1

Ilona Kovalszky[1], Lorand Vancza[1], Kornelia Baghy[1]
[1]Semmelweis University, Department of Pathology & Experimental Cancer Research, Budapest, Hungary

Purpose: Testican 1 is considered as a hardly known extracellular matrix proteoglycan , but publications indicate its role in the development and progression of cancers. The aim of the present work is to find mechanistic details as Spock-1/Testican-1 promotes the development and progression of liver cancer.
Methods: Testican-1 immunohistochemistry on human liver specimens, and analysis of hepatoma cell lines after modification of Testican expression by siRNA, or expression vector transfection, was carried out to detect the proliferation, invasion and cellular signaling of tumor cell lines.
Results: Spock1 is an extracellular matrix proteoglycan, but, its presence increases in the cytoplasm of normal, cirrhotic and tumorous human livers. Similar phenomenon was observed in the experimental hepatocarcinogenesis. Syndecan-1, the major proteoglycan of the liver, and Spock1 are in an inverse correlation there. In vitro analysis of hepatoma cell lines revealed, that Spock1 localizes with mitochondrial marker, and TOMM20, a protein of the outer membrane of mitochondrion. Spock1 downregulation by siRNA inhibits cell proliferation, upregulates p21, p27, whereas inhibits pAkt and CDK4 expression. Inhibition of Spock1 alters the activity of regulatory proteins relating to the aggressiveness of the hepatoma cell lines.
Discussion: Testican-1 is involved in the development of malignant phenotype of hepatocellulat cancer, and the literature indicates its implication of the behavior of several other cancers.
Conclusion: Our data suggests, that Testican-1 is an active player of malignant transformation and tumor progression in general. Its mitochondrial localization raises the possibility, that it also has physiological function in epithelial cells, which requires further study to evaluate.

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WORKSHOP F - MATRIX IN DEGENERATIVE DISEASES

Silencing of endocan down-regulates the expression of angiogenesis-associated genes in IL-1ß activated chondrocytes

Michele Scuruchi[1], Angela Avenoso[2], Giuseppe Mandraffino[1], Federica Aliquò[2], Aurelio Minuti[2], Salvatore Campo[2], Giuseppe Maurizio Campo[1], Angela D'Ascola[1]
[1]Clinical and Experimental Medicine, University of Messina, Messina, Italy; [2]Biomedical and Dental Sciences and Morphofunctional Images, University of Messina, Messina, Italy

Purpose: To investigate endocan expression and the underlying molecular mechanisms in human chondrocytes stimulated with IL-1β.
Methods: Articular human chondrocytes were stimulated with IL-1ß; mRNA and related proteins were measured for endocan, VEGF-A, VEGF-R and MMP-9. A separate set of chondrocytes was also treated with a specific small interfering RNA against endocan mRNA to block its expression.
Results: Endocan, VEGF-A, VEGF-R and MMP-9 expression was significantly up-regulated by IL-1ß induced inflammation; notably in endocan knockdown chondrocytes their expression was significantly affected.
Discussion: Endocan was found highly expressed in arthritic synovial tissues from patients with rheumatoid arthritis or osteoarthritis. In this setting, endocan could be involved in the mechanisms that stimulate cell invasion, cell migration, and angiogenesis in the pannus of arthritic joints. Our data, clearly demonstrate that endocan is part of inflammatory response in chondrocytes and in turn is involved in the modulation of proangiogenic factors.
Conclusion: Endocan released by activated chondrocytes may be involved in the mechanisms that stimulate cell migration and invasion as well as angiogenesis in the pannus of arthritic joints.

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Single cell RNA-sequencing of energy storing tendons reveals heterogeneity and complexity of interfascicular cell populations

Danae Zamboulis[1], Neil Marr[2], Luca Lenzi[3], Shumeng Duan[4], Binyao Zhang[4], Helen Birch[4], Hazel Screen[1], Peter Clegg[3], Chavaunne Thorpe[2]
[1]School of Engineering and Material Science, Queen Mary University of London, London, United Kingdom; [2]Royal Veterinary College; [3]University of Liverpool; [4]University College London

Purpose: The interfascicular matrix (IFM) binds tendon fascicles and contributes to the mechanics of energy storing tendons such as equine superficial digital flexor tendons (SDFT). IFM resident cells likely maintain IFM structure and tendon function, and deficits in IFM cell function with ageing may contribute to increased injury risk. The aim of this study was to use single cell RNA-sequencing (scRNAseq) and immunolabelling to profile IFM cells and establish how they are affected by ageing.
Methods: Forelimb SDFTs were harvested from young (3-7 y) and old horses (>17 y); 4/group. Tendons were digested and 6,500 cells from each sample underwent scRNAseq.~5x10^8 reads were generated across samples and aligned on the horse genome. Following quality control and data normalization, principal component analysis and integration was performed. Differentially expressed genes (DEGs) between age groups within each cluster were identified. SDFTs were also immunolabelled for cell markers.
Results: 11 cell clusters were identified; 3 tenocyte clusters of 2 tenocyte populations, 2 endothelial, 2 mural and 4 immune clusters. 1 tenocyte population, and the endothelial, mural and immune cells localised to the IFM, and 1 tenocyte population localised to the fascicles (FM). The IFM and FM tenocytes had distinct matrisomes. IFM tenocytes and mural cells also had the highest number of DEGs with ageing, with many DEGs associated with the matrisome, senescence, proteostasis loss and inflammation.
Discussion: We identified 9 cell types within the IFM, the largest being tenocytes that likely regulate IFM maintenance and repair and are disproportionately affected by ageing, indicating that senescence may result in loss of proteostasis and ability to regulate inflammation.
Conclusion: This study uncovers the heterogeneity of tendon cells, with much of this complexity due to IFM cell populations. Loss of IFM cell phenotype and associated matrisome changes with ageing may contribute to increased injury risk.

Matrix remodelling in parkinsonian mice alters nanoscale organization and diffusion in the brain extracellular space

Federico Soria[1,2], Chiara Paviolo[3], Evelyne Doudnikoff[2], Marie-Laure Arotcarena[2], Benjamin Dehay[2], Laurent Cognet[3], Erwan Bezard[2]
[1]Achucarro Basque Center for Neuroscience, Leioa, Spain; [2]Institute des Maladies Neurodegeneratives, CNRS UMR 5293 and Université de Bordeaux, Bordeaux, France; [3]Laboratoire Photonique Numerique et Nanosciences, CNRS UMR 5298 and Université de Bordeaux, Talence, France

Purpose: The extracellular space (ECS) is a dynamic compartment where molecules travel mostly by diffusion. It holds a plastic extracellular matrix which in the brain is composed mainly by hyaluronan, a versatile polymer with structural and signalling properties. Here we study the nanoscale organization and diffusion in pathological ECS in adult mice under neurodegenerative conditions [1].
Methods: We used an in vivo mouse model of alpha-synuclein (a-syn)-induced neurodegeneration as a proxy of Parkinson's disease. We probed the substantia nigra of these mice with electron microscopy in cryofixed tissue, single-nanotube tracking coupled to super-resolution image analysis in live tissue and confocal microscopy in immunostained fixed slices, to analyse both ECS properties and matrix and glia status.
Results: We found enlarged widths and an uneven distribution of the ECS in the parkinsonian brain, along with increased nanoscale diffusion. We also found a degraded hyaluronan matrix, phagocytosed by reactive microglia. Depleting hyaluronan artificially replicated the increased diffusion observed in pathological brain, and partially prevented cell death by activating microglia and reducing a-syn load.
Discussion: Our results suggest that pathological alterations in the ECS are rather local than global. Changes in ECS width are driven mostly by cell death, while changes in diffusion depend mostly on hyaluronan. Strikingly, hyaluronan manipulation can alter microglia homeostasis and modify disease-progression.
Conclusion: These findings highlight the interplay of ECS, matrix and glia in pathology, unravelling ECS features relevant for the α-syn propagation hypothesis and suggesting matrix manipulation as a disease-modifying strategy.

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The defective collagen biosynthesis in kyphoscoliotic Ehlers-Danlos syndrome due to pathogenic variants in PLOD1 and FKBP14: further insights into the common pathophysiological mechanisms and comparison of clinical characteristics

Colman Marlies[1], Robin Vroman[1], Zoë Malfait[1], Sofie Symoens [1], Fransiska Malfait[1], Delfien Syx[1]
[1]Center for Medical Genetics, Department of Biomolecular Medicine, Ghent University, Ghent, Belgium

Purpose: The autosomal recessive kyphoscoliotic Ehlers-Danlos syndrome (kEDS) is caused by the deficiency of either lysyl hydroxylase 1 (LH1) or the peptidyl-prolyl cis-trans isomerase FKBP22. Deficiency of LH1 (encoded by PLOD1), a protein crucial for collagen crosslinking, was the first biochemically elucidated congenital error of the collagen metabolism. Defects in FKBP22 (encoded by FKBP14), a molecular chaperone of type III, IV, VI and X collagens, were discovered only relatively recently in a subset of individuals presenting a kEDS phenotype with a normal LH1 function. Clinical characteristics of kEDS include congenital muscle hypotonia, early onset kyphoscoliosis, joint hypermobility, and vascular fragility. Despite the phenotypic resemblance of kEDS-PLOD1 and kEDS-FKBP14, the common pathophysiological pathway remains poorly understood and functional studies on patient-derived material are scarce.
Methods: This study reports the clinical and molecular characteristics of 14 individuals with kEDS-PLOD1 and 3 individuals with kEDS-FKBP14. We reviewed and compared the clinical characteristics of all hitherto reported cases of kEDS-PLOD1 and kEDS-FKBP14. Using fibroblast cultures, we performed intra- and extracellular immunocytochemistry of collagens type I,III,VI and V, studied protein (western blotting) and gene (qPCR) expression of proteins/genes involved in the unfolded protein response and autophagy, and analyzed fibroblast migration with scratch wound assays.
Results: To date, 109 individuals with kEDS-PLOD1 and 40 with kEDS-FKBP14 have been reported. For PLOD1-kEDS, the functional data using fibroblast cultures is being collected. For kEDS-FKBP14, we found the first evidence for intracellular retention of collagens type III and VI with immunocytochemistry, the scratch wound assays showed an initial delay in fibroblast migration and no significant upregulation was found of proteins/genes involved in the unfolded protein response and autophagy.
Discussion and Conclusion: This study brings significant new insights into the underlying pathophysiological mechanisms of these defects of the collagen biosynthesis and is the first to compare clinical and functional findings of kEDS-PLOD1 and kEDS-FKBP14.

WORKSHOP G - MATRIX IN STEM CELLS AND TISSUE REGENERATION

Dental pulp mesenchymal stem cells' response to fibrin hydrogel

Alicia Reuzeau[1], David Tong[1], Marianne Leveque[1], Marielle Pasdeloup[1], Frederic Delolme[2], Adeline Page[2], Cécile Hilpert[2], Frédéric Mallein-Gerin[1], Jean-Christophe Farges[1,3], Maxime Ducret[1,3], Mourad Bekhouche[1]
[1]LBTI - Tissue Biology and Therapeutic Engineering Laboratory, UMR5305 CNRS / Université Lyon 1, Lyon, France; [2]Protein Science Facility - UAR3444/US8, Lyon, France; [3]Odontology Faculty of Lyon, Lyon, France

Purpose: Current treatment of dental pulp (DP) irreversible damage leads to a devitalized tooth unable to detect new invasions by oral bacteria [1]. Implantation of mesenchymal stem cells (MSCs) associated to a fibrin scaffold has been proposed to regenerate a living DP [2]. Major scientific lock are residual bacteria that may remain after disinfection and uncontrolled MSCs-dependent mechanisms resulting in dysfunctional tissues. Our objectives were to design a scaffold providing an aseptic environment and to investigate the mechanisms initiated by DP-MSCs in fibrin.
Methods: DP-MSCs were collected from human third molars [3]. Poly(D,L)lactic acid nanoparticles (NPs) loaded with clindamycin were combined with a fibrin hydrogel [4]. DP-MSC viability was assessed in this nanocomposite hydrogel. Molecular mechanisms initiated by DP-MSCs in fibrin were investigated by quantitative proteomics and transcriptomics (RNAseq).
Results: The nanocomposite hydrogel displayed antibacterial properties without affecting DP-MSC viability. NPs reduced antibiotic release from the hydrogel compared to free antibiotic. Time-course omic investigations of DP-MSC response to fibrin hydrogel revealed a two-step mechanism beginning with fibrinolysis and extracellular matrix synthesis followed by cell morphology modification. Classical actors of innate immunity were involved at all stages.
Discussion: Stem cell-dependent tissue regeneration is the sum of biomolecular events poorly characterized in most tissues. Our multi-omic approach allowed to identify key molecular checkpoints to guide proper DP regeneration. This study points out the importance of early steps occurring during the first two days and that imply cell-derived matrix and the innate immune system.
Conclusion: This project proposes an innovative nanocomposite hydrogel for dental pulp regeneration. It also provides fundamental knowledge about potentially important molecular mechanisms initiated by DP-MSCs in fibrin hydrogel.

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Dermal fibroblasts linked via ultrafine dendrites form a network that regulates dermal aging - 3D digital reconstruction of skin enables high-resolution computer analysis

Tomonobu Ezure[1], Kyoichi Matsuzaki[2], Nobuhiko Ohno[3]
[1]Shiseido Co., LTD.; [2]International University of Health and Welfare; [3]Jichi Medical University, Japan

Purpose: Dermal fibroblasts regulate the condition of the dermal layer by secreting collagen and controlling its tension. However, the nature of the regulatory system, which is crucial for skin aging and related clinical disorders, remains largely unknown. This is because it is technically difficult to observe the actual status of fibroblasts in skin. We aimed to establish a method to observe fibroblasts in whole skin, and used it to clarify the role of fibroblasts in regulating skin condition.
Methods: We found that electron-conductive treatment made it possible to observe whole skin three-dimensionally (3D) by means of serial-block-face scanning electron microscopy (SBF-SEM), without interference from charged collagen fibers. We applied an artificial intelligence (AI) deep-learning system to identify skin structures in the SEM images, and used the data to reconstruct skin with ultrahigh resolution on computer. This digitally reconstructed skin (digital skin) can be freely manipulated on computer (digital anatomy, sorting and measurement).
Results: Digital skin analysis revealed that fibroblasts are interlinked via ultrafine dendrites in skin, forming a network, in contrast to the conventional view that they are isolated. Comprehensive gene expression analysis followed by histological observation showed that N-cadherin plays a key role in the dendrite linkages. Knockdown of N-cadherin (siRNA) inhibited network formation in cell culture, and the fibroblasts showed an aged phenotype (decreased collagen production; increased senescence markers). We confirmed that the network was significantly decreased with aging in 20 female skin specimens.
Discussion: Thus, our results suggest that dermal cells construct a network structure that inhibits skin aging.
Conclusion: This work provides novel technology for computer analysis of internal structures of whole skin, and identifies the dermal cell network as a critical target of skin aging.

Basement membrane is molecularly and structurally specialized for inter-tissue interactions

Ko Tsutsui[1,2], Hiroki Machida[2], Asako Nakagawa[2], Hironobu Fujiwara[2]
[1]Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; [2]RIKEN, Center for Biosystems Dynamics Research (BDR), Kobe, Japan

Purpose: Inter-tissue interaction is essential for all organs. Although the basement membrane (BM) is located at the border of most tissues, its roles in inter-tissue interactions remain poorly understood. We aimed to identify the tissue distribution of BM molecules and to elucidate its roles in distinct inter-tissue interactions using mouse hair follicle (HF) as a model, because its epidermis has three different connections with muscle tissue, nerve tissue and connective tissue, dermal papilla (DP).
Methods: To understand the region-specific components of BM, we combined transcriptome and immunohistochemical analyses. Structural characterization of BM was performed using TEM. Functional study was performed using epidermis-specific Lama5 cKO mice.
Results and Discussion: We uncovered the ECM distribution landscape around mouse HF and identified several ECMs specific to the distinct interaction sites. Intriguingly BM at the epidermis-DP possesses structural characteristics such as protrusions toward the dermis side (named hook BM). DP cells interact with the hook BM through integrin α6. In the mutant mice deleted lama5 gene in the epidermis, both laminin α5 deposition and integrin α6 accumulation disappeared from the hook BM, indicating that laminin α5 is the major ligand of integrin α6 at the hook BM. Furthermore, the mutant mice showed abnormal activation of TGF-β signaling pathway at resting phase of the hair cycle, followed by precocious entry to the growing phase. These results suggested that the hook BM plays significant roles in the interaction between the epidermis and DP.
Conclusion: Compositional and structural BM heterogeneity provides HF niches for distinct inter-tissue interactions.

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Unravelling motor neurons identity and new cell-extracellular matrix interplay in zebrafish motor axon development and regeneration

Laurie Nemoz-Billet[1], Nathan Gil[1], Florence Ruggiero[1], Sandrine Bretaud[1]
[1]Institut de Génomique Fonctionnelle de Lyon, Université de Lyon, ENS de Lyon, CNRS UMR5242, Lyon, France

Purpose: During development and nerve regeneration, axons of motoneurons (MN) follow stereotypical trajectories to their muscle targets, guided by various molecular cues including extracellular matrix (ECM) proteins. In zebrafish, every trunk hemisegment is innervated by three different MN. Motor axons first extend through a common path, pause at the choice point and then diverge to arborize in a specific territory of the myotome. The common path is made of ECM proteins known to provide guidance to developing axons. Their absence often arrests axon growing beyond the choice point. Among them is the myotomal matrix protein collagen XV-B (COLXV-B)1. The mechanisms underlying motor axon navigation and MN-specific divergence are poorly documented. We thus aim at characterizing [1] the motor axon identity using single cell transcriptomic analysis (scRNA-seq) and [2] the key ECM proteins that orchestrate this process.
Methods: We used MN-specific transgenic line mnx1:gfp to isolate MN by FACS and perform scRNA-seq and to carry out real-time monitoring of nerve regeneration as a tunable model of axonogenesis.
Results: Clustering analysis revealed a pMN-specific gene expression signature that distinguishes the three pMNs indicative of a pMNs subtype identity. We then developed a laser ablation method to injure the ventral nerve of mnx1:gfp and col15a1b-/-;mnx1:gfp larvae. Using video-microscopy and lysotracker dyes, we showed that COLXV-B, which is enriched in the common path, may have additional roles to those shown in development1 by acting more specifically on Wallerian degeneration, clearing-cells recruitment and the formation of the regenerated nerve.
Discussion: We are now investigating the behavior of Schwann cells, macrophages and neutrophils by using diverse transgenic lines in control and mutant larvae.
Conclusion: This study opens new leads on the interplay between nerve-derived actors, ECM cues and inflammatory cells in motor axon growth in development and regeneration.

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WORKSHOP H - MATRIX AND PATHOLOGIES

Heparanase modulates FGF2/FGFRs effects in prostate cancer

Valentina Masola[1], Marco Franchi[2], Nicola Greco[1], Maurizio Onisto[2]
[1]Department of Biomedical Sciences, University of Padova, Padova, Italy;
[2]Department of Medicine, University of Bologna, Bologna, Italy

Purpose: Prostate cancer (PC) is responsible for more gender-specific cancer-related deaths in men than any other cancer. Heparanase (HPSE) is the sole mammalian endoglycosidase that cleaves heparan sulphate (HS) resulting in in extracellular matrix remodeling and release of these bioactive mediators [1]. HPSE is overexpressed in prostate cancer facilitating tumor invasion and metastasis [2]. Fibroblast growth factor 2 (FGF2) and the expression of its receptors has a central role in prostate cancer progression. It has been demonstrated that in cancer cells models FGF2 induces epithelial to mesenchymal transition (EMT) and that isoforms of FGF2 receptors differentially regulates EMT [3]. Since our previous studied in an epithelial-cells model proved that FGF2-induced EMT is regulated by HPSE [4], with the present project we aim to characterize the role of the HPSE in prostate carcinoma EMT.
Methods: The effect of a specific HPSE inhibitor was assayed in presence or absence of FGF2 treatment in three PC cell lines: DU145, PC3 and LNCaP. Several biomolecular techniques were used: PCR, real-time PCR, viability assay, colony assay and WB
Results: Results showed that the different PC cell lines display different expression levels of FGF2 and different FGF2R isoforms. We confirmed that FGF2 activate EMT in PC and we proved that HPSE inhibition modulated the expression of FGF2 and FGFR isoforms resulting in a modulation of the EMT process.
Conclusion: In summary, since EMT is a common process shared by organ fibrosis but also by tumor progression, these data proved that the involvement of HPSE in EMT, also by affecting FGF2-FGF2Rs, could be a general mechanism involved in cancers of epithelial origin.

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Lack of collagen XVIII leads to lipodystrophy and perturbs hepatic glucose and lipid homeostasis

Tiina Petäistö[1], David Vicente[1], Kari Mäkelä[2], Mikko Finnilä[2], Jarkko Koivunen[1], Valerio Izzi[1], Ritva Heljasvaara[1], Taina Pihlajaniemi[1]

[1]Faculty of Biochemistry and Molecular Medicine, [2]Faculty of Medicine, University of Oulu, Oulu, Finland

Purpose: Basement membrane collagen, ColXVIII, regulates adipocyte differentiation, and the lack of this collagen leads to a reduced fat mass and ectopic lipid accumulation [1]. Here we investigated the metabolic consequences of the reduced adiposity on whole-body glucose and lipid metabolism.
Methods: Mice were fed with a high fat diet and body composition was analyzed by micro computed tomography (µCT). Glucose tolerance and insulin sensitivity tests were performed for mice fed with standard diet. Metabolic performance was analysed at room temperature and during cold exposure.
Results: µCT analysis revealed significantly reduced adipose tissue volumes in the Col18a1$^{-/-}$ mice compared to the wild-type controls. The Col18a1$^{-/-}$ mice showed elevated blood triglyceride levels and larger steatotic areas in livers than the controls. Consequently, these mice developed insulin resistance and glucose intolerance even on a standard chow. Additionally, the Col18a1$^{-/-}$ mice showed increased heat production and reduction of high blood triglyceride levels at low temperatures likely via non-shivering thermogenesis [2].
Discussion: ColXVIII deficient mice are lipodystrophic and develop symptoms similar to the type 2 diabetes. We suggest that the lack of ColXVIII primarily causes defects in adipose tissue development1 and that the observed lipodystrophy causes secondary effects, i.e. fatty liver and impaired insulin sensitivity as also detected in other lipodystrophic mice models3. However, better understanding of how the ColXVIII disturbs adipose tissue development and functions requires still further studies.
Conclusion: The results shown here reveal a new role for collagen XVIII in the regulation of glucose and lipid metabolism and expand the understanding of the development of metabolic disorders.

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IL-13-mediated hyaluronan accumulation in the lungs

Rebecca Dodd[1,2,3], Alexandra N. Donlan[4], Sheona Drummond[1,3], Caroline M. Milner[3], William E. Petri[4], Anthony Day[1,2,3], Judith E. Allen[1,2,3], Tara E. Sutherland[1,2,3]

[1]Wellcome Centre for Cell Matrix Research, [2]Lydia Becker Institute of Immunology and Inflammation, [3]Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom; [4]University of Virginia, Charlottesville, USA

Purpose: Recent work identified a role for the type 2 cytokine IL-13 in SARS-CoV-2 infection. Using a mouse model, it was found that SARS-CoV-2 infected mice showed accumulation of hyaluronan (HA) in the lungs. Accumulation of HA could also be induced by direct delivery of IL-13 [1]. In other lung pathologies where HA is elevated (e.g., asthma or influenza infection [2, 3]), the secreted glycoprotein TSG-6 catalyses modification of HA with heavy chains (HC) from the inter-alpha-trypsin inhibitor (ITI) family. Such HC•HA matrices are crosslinked (via HC-HC and HC-PTX3 interactions) and have distinct properties, e.g., increased leukocyte adhesion in some contexts [4]. Our aim is to investigate how IL-13 leads to HA accumulation and establish if HA is modified with HCs.
Methods: Murine lung tissue (after IL-13 delivery) was processed for immunofluorescence staining with antibodies against HC1, HC2 and HC3 and TSG-6. qtRT-PCR was performed on whole lung extracts and corresponding samples analysed by western blot and ELISA.
Results: Intranasal delivery of IL-13 resulted in increased expression of Tnaifp6 (TSG-6). IL-13 also increased whole lung Has2 expression but reduced expression of Hyal1 and Hyal2. In lung tissue, colocalisation of HA and HCs was observed; however, individual HCs were found to have discrete staining patterns.
Discussion: IL-13 results in altered lung HA metabolism, leading to HA accumulation. Identification of ITI HCs and TSG-6 within the lung supports our hypothesis that the IL-13-induced HA matrix is likely to be modified with HCs.
Conclusion: Modulation of HA by IL-13 represents a novel mechanism by which immune-mediators influence the extracellular matrix. Further characterisation of these matrix changes will allow us to understand how IL-13 influences the composition of HA matrices in different lung pathologies including SARS-CoV-2 and influenza infection.

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Lumican accumulates with fibrillar collagen in cardiac fibrosis

Chloe Rixon[1,2], K. Andreassen[1,2,3], Xin Shen[1,2], Pugazhendhi Murugan Erusappan[1,2], Vibeke Almaas[3], Sheryl Palmero[1,2], Christen Peder Dahl[4], I. Sjaastad[1,2], W.E. Louch[1,2], M.K. Stokke[1,2,3], T. Tønnessen[1,5], Geir Christensen[1,2], Ida Gjervold Lunde[1,2]

[1]Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo, Oslo, Norway; [2]K. G. Jebsen Center for Cardiac Research, University of Oslo, Oslo, Norway; [3]Department of Cardiology, [4]Research Institute of Internal Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway; [5]Department of Cardiothoracic Surgery, Division of Cardiovascular and Pulmonary Diseases, Oslo University Hospital, Oslo, Norway

Purpose: The extracellular matrix (ECM) proteoglycan lumican is upregulated in the heart during development of fibrosis. However, lumican's role in cardiac fibrosis remains unknown. Hypertrophic cardiomyopathy (HCM) is a cardiac disease where fibrosis is an important aspect of pathophysiology. We have therefore examined lumican in HCM.
Methods: Left ventricular (LV) samples were collected from n = 15 patients and n = 10 mice with HCM. N = 3 human fetal cardiac fibroblast (hfCFB) cultures were treated with recombinant human lumican. LV tissues and cell cultures were analyzed with molecular biology and advanced microscopy techniques.
Results: Lumican mRNA was increased 3-fold (LUM $p < 0.05$), correlating with fibrillar collagen production in patients with HCM (COL1A2 $p = 0.0012$, COL3A1 $p = 0.0015$). Cellular lumican protein increased 2-fold ($p < 0.01$) in HCM patients and 3-fold ($p < 0.05$) in HCM mice, yet deposited lumican protein in the ECM increased 20-fold in HCM mice ($p > 0.001$). We revealed lumican located throughout regions with fibrosis (interstitial, focal and perivascular), colocalizing more with collagen I in diseased tissue (+13%, $p < 0.01$). We utilized direct stochastic optical reconstruction microscopy (dSTORM) to show that collagen I located closer to (-15 nm, $p < 0.05$) and overlapped more with (+7.3%, $p < 0.05$) lumican in diseased, but non-fibrotic tissue. Adding lumican to hfCFBs resulted in thicker (+53.8 nm, $p < 0.001$), longer (+345.9 nm, $p < 0.001$) and fewer (-8.9%, $p < 0.001$) collagen fibers.
Discussion: We show that lumican colocalizes with collagen I in cardiac fibrosis. We suggest a role in accumulation of collagen fibrils, as collagen fibers were fewer, thicker and longer in hfCFB cultures with lumican. We employ dSTORM in a novel manner to quantify subtle alterations in the cardiac ECM.
Conclusion: Lumican is present throughout fibrotic areas in HCM hearts, colocalized with collagen I. We suggest a role for lumican in collagen fibril accumulation into larger, pro-fibrotic fibers.

POSTERS

P001
Identification of molecular and cellular markers for detection of biological traces for genetic and criminal identification

Mathilde Recipon[1,2], Rémy Agniel[1], Johanne Leroy-Dudal[1], Franck Carreiras[1], Francis Hermitte[2], Sylvain Hubac[2], Olivier Gallet[1], Sabrina Kellouche[1]

[1]Laboratoire ERRMECe (Equipe de Recherche sur les Relations Matrice Extracellulaire-Cellules), University of Cergy, Neuville-Sur-Oise, France; [2]Institut de Recherche Criminelle de la Gendarmerie Nationale, Cergy-Pontoise, France

Purpose: At a crime scene, investigators face a multitude of traces. Among them, traces of biological nature are of primary interest for the rapid identification of individuals. Biological traces are of two types: visible [1] (blood) and invisible (such as contact traces) [2]. Our study focused on biological traces left by the simple contact of a person's skin onto objects which are commonly called "touch DNA" [3]. Touch DNA are invisible and undetectable to the naked eye. Currently no satisfactory detection method is available in the field to detect them mainly because the goal is not well defined: is it a question of touch DNA or touch biological molecules or touch cell? This study aimed to detect touch traces targeting both DNA and cell biological molecules.
Methods: Using keratinocytes or fingermarks, the expression of cellular and DNA markers (by targeting matrix proteins, carbohydrate motifs or nucleic acids) were analyzed on shedded, desiccated, fragmented cells with a combination of antibodies, DNA probes and lectins to improve biological traces detection. Different parameters (temperature, hygrometry, ...) were tested to be able to correspond as possible to the on-field reality.
Results: With keratinocytes or fingermarks, some cellular markers as laminin, keratin and DNA remained detectable even after 2 months. Cells can be specifically visualized in an environment unfavorable to their preservation indoor or even more amazing directly outdoor in contact with all kinds of contamination
Discussion: The study of the stability and robustness of our proteins over time is a major advance to detect touch traces. Now, it is necessary to develop this research for application in situ on field.
Conclusion: Identification of new molecular targets and optimisation of several tools to detect touch DNA are a real challenge to help investigators and justice.

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P002
Lack of evidence for a role of anthrax toxin receptors as surface receptors for collagen VI and for its cleaved off C5 domain (endotrophin)

Matthias Przyklenk[1], Steffen Lütke[1], Alvise Schiavinato[1,2], Gerhard Sengle[1,345], Manuel Koch[1,6], Mats Paulsson[1,4,5], Raimund Wagener[1,4,5], Heumüller Stefanie-Elisabeth[1]
[1]Center for Biochemistry, [3]Department of Pediatrics and Adolescent Medicine, [4]Cologne Center for Muscoloskeletal Biomechanics (CCMB), Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany; [2]Istituto di Patologia Clinica, Azienda Sanitaria Universitaria Integrata di Udine (ASUID), Udine, Italy; [5]Center for Molecular Medicine Cologne (CCMC), University of Cologne, Cologne, Germany; [6]Institute for Dental Research and Oral Musculoskeletal Biology, Medical Faculty, University of Cologne, Cologne, Germany

Purpose: The microfibril-forming collagen VI is subject to proteolytic cleavage and it has been proposed that the cleaved off C-terminal Kunitz domain (C5 or "endotrophin") of the α3 chain is an adipokine important for tumor progression and inflammation. However, the biochemical mechanisms behind endotrophin activity remain elusive. Given the proposed physiological role of endotrophin we aimed to determine how the endotrophin signal is transmitted to the recipient cells [1]. In earlier studies, the anthrax toxin receptor 1 (Antxr1) was found to bind to C5 [2], but this interaction has not been further studied.
Methods: Interaction between recombinant C5 and the VWA-domains of Antxr 1 or -2 was evaluated via surface plasmon resonance spectroscopy. Anthrax receptor overexpressing cells were incubated with recombinant C5 or collagen VI enriched conditioned media and binding of interaction partners to the cell surface was investigated by immunofluorescence microscopy and immunoblotting. Pull-down assays were performed with recombinant protein or cell lysate and investigated by immunoblotting.
Results: We could not detect any interaction between endotrophin and Antxr1 or -2, while a control, the protective antigen, the receptor binding moiety of the anthrax toxin, was found to bind to both receptors in all assays used. Moreover, we could not detect binding of fully assembled collagen VI to either anthrax toxin receptor. In contrast, we could confirm that NG2 is a collagen VI receptor that binds to assembled collagen VI, but not to the C5/endotrophin fragment.
Discussion: Our results exclude both anthrax toxin receptors as interaction partners for endotrophin. Our results are consistent with the proposed role of endotrophin during the complex assembly of collagen VI. However, it remains to explore how endotrophin influences lipogenesis, lipolysis and inflammation, how it influences pro-fibrotic and pro-inflammatory genes or how it acts as a chemokine augmenting tumor growth.

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P003
Development of novel collagen NC1 domain peptides with anti-angiogenetic properties for future treatments of both cancer and fibrosis

Stine Jansen[1], Kim Henriksen[1], Morten Karsdal[1]
[1]Nordic Bioscience, Peptide Discovery, Herlev, Denmark

Purpose: Endostatin is one of the best-known anti-angiogenic collagen NC1 domain derived peptides [1]. However, there is a need for a better understanding of how the potential of endostatin fragments can be realized [2]. This study was conducted to identify and develop novel endostatin derived fragments and explore the therapeutic possibilities of these in fibrosis and cancer by looking at their ability to inhibit angiogenesis.
Methods: We fragmented endostatin into novel peptides and validated their effect in an in vitro matri-gel tube formation assay using human umbilical cord endothelial cells (HUVEC). We quantified tube-length as a function of treatment. As unstimulated control we used minimal media without FBS or any growth factors. Media with VEGF was used as positive control as the VEGFR-2/KDR receptor is known to stimulate cells to proliferate and migrate.
Results: Endostatin and a VEGF-antibody (bevacizumab) were added as reference inhibitors for our peptides in the tube formation assay. We found that one long acting novel col XVIII peptide named AAP-04A significantly inhibited the total length of tube formation compared to the positive control when used in concentrations of 40 uM, 20 uM and 10 uM. Furthermore, AAP-04A also inhibited tube formation almost completely. AAP-04A inhibition at 40uM was similar to the inhibition by 2 uM endostatin or 40 ng/ml VEGF-antibody.
Discussion: AAP-04A shows great potential in terms anti-angiogenetic properties. However, the concentration of AAP-04A is high compared with endostatin and further assessment of the peptide in other assays is needed to elucidate the specific mode of action.
Conclusion: AAP-04A demonstrated a great inhibition of tube formation by HUVEC cells which was significantly inhibited when comparing to both endostatin and VEGF-antibody.

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P004
Visualized type I procollagen α1 demonstrated the intracellular processing of propeptides

Koji Moriya[1], Toshiaki Tanaka[1]
[1]Department of Life Science and Technology, Tokyo Institute of Technology, Midori-ku, Yokohama, Japan

Purpose: The processing of type I procollagen is essential for fibril formation; however, the steps involved remain controversial. To elucidate the mechanisms underlying its processing, transportation, secretion, and fibril formation, we constructed a live imaging system with type I procollagen α1 by inserting two fluorescent protein tags. Here, we show intracellular processing of propeptides based on live imaging and biochemical analysis.
Methods: We constructed expression vectors, in which GFP and mCherry tags are inserted into type I procollagen α1 protein. We transfected cells with the vectors and examined biosynthetic steps of collagen with biochemical and imaging analyses.
Results: The visualized type I procollagen α1 was shown to be assembled into multimers more than trimers in cells with BN-PAGE analysis followed by Western blotting. Immunoelectron microscopy showed GFP signals on collagen fibrils with D-periodic structures secreted from cells with the visualized collagen, indicating construction of regular collagen bundles including the tagged collagen. Live imaging analysis revealed that C-propeptide (C-pp) is intracellularly cleaved from procollagen and accumulated in the perinuclear region. We showed that the processed C-pp is co-localized with lysosome and degraded. N-propeptide (N-pp) was also intracellularly cleaved; however, it was directed towards a pseudopodium and secreted into the extracellular space. The processed collagen (the repeating structure domain) was transported with N-pp.
Discussion: Our results showed that visualized type I procollagen α1 assembles into proper structures as endogenous procollagen does. The visualized procollagen demonstrated its intracellular processing, and the different fates of processed propeptides. Our results also suggested that the rate-limiting step for collagen secretion is trimerization, processing, transportation, and/or secretion from the plasma membrane.
Conclusion: Visualized type I procollagen α1 demonstrated the intracellular processing of propeptides. The visualized procollagen system is useful for analyses of intractable diseases caused by an aberration of collagen protein such as osteogenesis imperfecta and organ fibrosis.

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P005
Design and investigation of proteins inspired by natural adhesive matrices

Amel Benabdi[1], Charlotte Vendrely[2,3], Cédric Picot[2], Olivier Gallet[2], Franz Bruckert[3], Marianne Weidenhaupt[3], Rémy Agniel[2], Andrey Zaytsev[2]
[1]Equipe de Recherche sur Les Relations Matrice Extracellulaire-Cellules (EA1391), Institut des Matériaux I-MAT (FD4122), Cergy Paris University, [2]Equipe de Recherche sur Les Relations Matrice Extracellulaire-Cellules (EA1391), Institut des Matériaux I-MAT (FD4122), Neuville sur Oise, France; [3]Laboratoire des Matériaux et du Génie Physique, CNRS UMR 5628, University of Grenoble Alpes, France

Purpose: Some animals and plants produce various adhesive matrices necessary for their survival. Among them, marine barnacles secrete a glue, named cement composed of proteins which are able to self-assemble into fibers for their adhesion under water [1]. The proteins forming the adhesive matrix of the barnacle Megabalanus rosa have been previously identified and sequenced. Their sequence are particularly rich in repetitions providing an ideal template for biomimetic protein design [2] as a mean to develop new biomimetic and biocompatible adhesive matrices for medical applications.
Methods: By genetic engineering, we developed two protein models derived from Cp19k, one of the proteins composing the natural cement of M. rosa. Then, the self-assembly of the recombinant proteins was studied in different conditions. The nanoscale morphology and the secondary structure of the proteins under soluble and assembled states were characterized by AFM and CD. Then, the adhesion of the protein self-assemblies has been analysed by a lap shear test. Finally, their in vitro cytocompatibility was also tested.
Results: Biomimetic proteins inspired from an insoluble cement could be produced recombinantly and purified. They are able to form fibers and the fiber formation depends on the pH, and the material surface in contact with the protein. Moreover, the proteins show interesting adhesive properties and potential cytocompatibility.
Discussion: The material surface in addition to chemical parameters seem to be important in the control of the self–assembly of the proteins. This behavior could be related to the adsorption of the protein onto the surface.
Conclusion: Our results give insight into the structure-function relationships of one natural adhesive protein and could provide an advanced class of biomaterials of interest for medical purposes as tissue engineering.

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P006
Electroactive PolyHIPE-PEDOT 3D porous scaffolds as dynamic in vitro cell culture platform

Franziska Hahn[1,2,3], Ana Ferrández Montero[1,2,3], Mélodie Culot[1,2,3], Cédric Vancaeyzeele[2,3], Cédric Plesse[2,3], Johanne Leroy-Dudal[1,3], Rémy Agniel[3,4]

[1]ERRMECe - Equipe de Recherche sur les Relations Matrice Extracellulaire-Cellule / Mec'up group, CY Cergy Paris University - Biology, Cergy, France; [2]LPPI - Laboratoire de Physicochimie des Polymères et des Interfaces, CY Cergy Paris University- Chemistry, Cergy, France; 3I-MAT - Fédération Institut des Matériaux, CY Cergy Paris University, Cergy, France; 4ERRMECe - Equipe de Recherche sur les Relations Matrice Extracellulaire-Cellule, Cergy, France

Purpose: The cell microenvironment is a 3D- and dynamic molecular scaffold that determines the cell behaviors in physio-pathological processes. To better mimic this living 4D-environment, the development of relevant in vitro cell culture platforms is a long-standing challenge with perspectives in advanced cell biology, drug screening and tissue engineering [1]. Promising in vitro 3D-porous polyHIPE-based scaffolds [2] as well as the interest of electronic conducting polymers to turn them from passive to active scaffolds were recently described [3]. This work evaluates the opportunity of such electroactive 3D-porous supports as new cell culture platform.
Methods: PolyHIPE 3D scaffold was synthetized, functionalized by the electroactive conducting polymer PEDOT (poly(3,4-ethylenedioxythiophene)) and then seeded with fibroblasts to study cell behavior.
Results: PolyHIPE displays a high porosity with ~70 µm-interconnected voids. After its functionalization, resulting PolyHIPE/PEDOT scaffold is cytocompatible. Fibroblasts adhere on the scaffold, colonize it and spread into voids. A preliminary imaging set-up was developed to monitor cell fate during actuation of the scaffold. The electrostimulation of polyHIPE-PEDOT scaffold leads to a mechanical response consisting in contraction/expansion and pores' size variation (~10%) allowing the concomitant stimulation of cells without apparent cytotoxicity (cell detachment and LDH release).
Discussion: This PolyHIPE/PEDOT dynamic could influence cell phenotype, that is currently studied.
Conclusion: PolyHIPE/PEDOT are promising dynamic cell culture supports that pave the way to a better understanding of cell responses to the dynamic of their microenvironment.

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P007
Investigating biomaterials, structure and cell mechanics in tissue engineering scaffolds

Dimitar Stamov[1], Tanja Neumann[1], André Koernig[1], Torsten Mueller[1], Heiko Haschke[1]
[1]Bruker Nano GmbH, JPK BioAFM, Berlin, Germany

Purpose: Development of novel biomaterials for tissue engineering is driven by the biomechanical and molecular cues provided to cells by their environment. The topography and mechanical properties of biomaterials are crucial parameters that influence motility, behavior, and the fate of progenitor cells [1, 2]. Thus, processes such as the self-assembly of single matrix molecules, and adhesion-induced structural changes in living cells must be further explored.
Methods: We applied high-speed imaging atomic force microscopy, with a temporal resolution on the second to millisecond scale to resolve dynamic processes such as the collagen fibrillogenesis and cytoskeletal dynamics in living cells. As a tool for analyzing the complex cellular mechanobiology, we went beyond purely elastic models, and performed sine oscillations (up to 1 kHz, amplitude 5-60 nm) in Z while in contact with the surface to probe the frequency-dependent response of living fibroblasts.
Results: We will provide insight into the structural formation of collagen type I, emphasizing the intermediate steps in the process [4]. We will demonstrate how cell spreading and migration in living KPG-7 fibroblasts and CHO cells, can be associated with spatially resolved cytoskeletal reorganization events [5].
Discussion: We will further discuss how to calculate the viscoelastic properties, characterized by the dynamic storage and loss modulus (E', E") distribution in living fibroblast cells.
Conclusion: AFM can be successfully applied to study the mechanobiology of living cells during tissue engineering, and to evaluate the structure of and the interaction with their cell culture substrates.

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P008
Novel oncosuppressive functions of decorin proteoglycan

Renato Iozzo[1]
[1]Thomas Jefferson University, Pathology, Anatomy and Cell Biology, Philadelphia, USA

Among the class I of the small leucine-rich proteoglycan gene family, decorin is the most widely investigated. In addition to its original eponym signifying a functional role in collagen fibrillogenesis during development, decorin is also a soluble and circulating proteoglycan involved in cell regulation, angiogenesis and tumor suppression. Most of its activities are mediated by a hierarchical affinity for tyrosine kinase receptors (RTK). Through an outside-in signaling network, decorin binds to multiple RTKs, including EGFR and Met, and, following a transient activation, it downregulates receptor activity. Decorin also inhibits VEGFR2 and evokes protracted autophagy of endothelial cells in a non-canonical, energy-independent manner and a concurrent autophagic degradation of the pro-angiogenic VEGFA. Both activities lead to overall suppression of angiogenesis. Using an unbiased RNAseq approach, we discovered that orthotopic breast carcinoma allografts systemically treated with recombinant decorin showed a marked downregulation of a cluster of genes involved in lymphangiogenesis, including podoplanin and Lyve1 the main hyaluronan receptor of lymphatic vessels. We further validated these in vivo findings with a refined ex vivo lymphatic duct ring assay in 3D collagen. We found that decorin potently suppressed lymphatic sprouting and promoted autophagic degradation of Lyve1 in lymphatic endothelial cells. We also discovered that decorin bound to and physically downregulated VEGFR3, the main lymphatic RTK, and evoked rapid and sustained AMPK activation. These findings provide the first evidence that a soluble proteoglycan can modulate in vivo cancer growth by suppressing both angiogenesis and lymphangiogenesis with concurrent activation of autophagy in a nutrient- and energy-independent manner.

P009
The female syndecan-4-/- heart has an elevated insulin/pSer473-Akt/pSer9-GSK-3β signaling pathway and lowered pThr308-Akt/Akt and GLUT4 levels

Thea Støle[1,2], Marianne Lunde[1,2], Xin Shen[1,2], Marita Martinsen[1,2], Per Kristian Lunde[1,2], Jia Li[1,2], Francesca Lockwood[1,2], I. Sjaastad[1,2], W.E. Louch[1,2], Jan Magnus Aronsen[1,3], Geir Christensen[1,2], Cathrine Rein Carlson[1]
[1]Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo, Oslo, Norway; [2]K.G. Jebsen Center for Cardiac Research, University of Oslo, Oslo, Norway; [3]Department of Molecular Medicine, Institute of Basic Medical Sciences, Oslo, Norway

Purpose: In the cardiac muscle, the proteoglycan syndecan-4 is involved in the hypertrophic response to pressure overload. Protein kinase Akt signaling, which is known to regulate hypertrophy, has been found to be reduced in the heart of exercised male syndecan-4$^{-/-}$ mice, and in contrast elevated in the skeletal muscle of female syndecan-4$^{-/-}$ mice. To determine if the differences in Akt signaling are sex specific, we have investigated Akt signaling in the heart of sedentary and exercised female syndecan-4$^{-/-}$ mice.
Methods: Left ventricles (LVs) from sedentary and exercised female syndecan-4$^{-/-}$ and WT mice were analyzed by immunoblotting and real-time PCR. Phosphorylated Ser473-Akt was also analyzed in isolated adult cardiomyocytes by confocal imaging. Glucose levels were measured by a glucometer and fasting serum insulin by ELISA. LVs from sedentary male mice were immunoblotted with Akt antibodies for comparison.
Results: Compared to WT female hearts, sedentary female syndecan-4$^{-/-}$ hearts had higher levels of pSer473-Akt and its downstream target pSer9-GSK-3β, and lower levels of pThr308 and GLUT4. The pSer473-Akt inhibitory phosphatase SCOP was lowered, hypothesized to be in response to the elevated serum insulin levels. The pAkt levels were unaltered in the cardiac and skeletal muscle from sedentary male syndecan-4$^{-/-}$ mice.
Discussion: Female syndecan-4$^{-/-}$ have hyperphosphorylated pSer473-Akt, likely due to the elevated insulin lowering SCOP levels. Whereas acute Akt signaling can promote physiological adaptations, chronic Akt signaling is considered pathological and thus raises the question whether the changes we observe protect or adversely affects female syndecan-4$^{-/-}$ mice in a disease state.
Conclusion: Our data indicate an elevated insulin/pSer473-Akt/pSer9-GSK-3β signaling pathway and lowered pThr308-Akt and GLUT4 levels in the female syndecan-4$^{-/-}$ heart. In contrast, Akt signaling was unaltered in male syndecan-4$^{-/-}$ mice, suggesting important sex differences.

P010
Development of a membrane yeast two hybrid screening for the identification of membrane neuraminidase-1 interaction partners in human macrophages

Digne Tembely[1], Olivier Bocquet[1], Christine Terryn[2], Amar Bennasroune[1], Sébastien Blaise[1], Hervé Sartelet[1], Laurent Duca[1], Béatrice Romier[1], Maurice Pascal[1]
[1]UMR CNRS 7369 Matrice Extracellulaire et Dynamique Cellulaire (MEDyC), Team 2 "Matrix Aging and Vascular Remodelling", UFR Sciences Exactes et Naturelles, [2]PICT Platform, Université de Reims Champagne Ardenne (URCA), Reims

Purpose: Vascular aging is associated with elastin remodeling and production of elastin-derived peptides (EDP) that bind to the elastin receptor complex (ERC). Data from our lab have shown the critical role played by its catalytic subunit, neuraminidase-1 (NEU1), in the biological effects mediated by EDP and the ERC in vascular and metabolic diseases (Wahart et al, FEBS J. 2019). We recently identified a common action mechanism by which, by stimulating the catalytic activity of NEU1, EDP binding to the ERC induces desialylation of key membrane glycoproteins (CD36, β2 integrin, ICAM-1) and leads to modulation of major events occurring during atherosclerosis development [1, 2]. We developed a Membrane Yeast Two Hybrid screening for the search of new interaction partners of membrane NEU1 from a human macrophage cDNA library.
Methods: cDNA library of prey proteins was subcloned into the pNX32-DEST vector encoding the expression of the N-terminal end of ubiquitin (Nub). The bait (NEU1) was subcloned into the pMetYC-DEST vector for expression of the C-terminal end of ubiquitin (Cub) fused to LexA and VP16.
Results: Characterization of the cDNA library showed good quality and abundance (average insert size: 1.7 kb, % recombinants: 100%). Expression of the bait (NEU1-Cub-LexA-VP16) as well as its membrane localization and preservation of its catalytic activity were confirmed in yeast. Mating the cDNA library (preys) with the bait revealed 155 potential interactions which are being sequenced. These potential interactions will be then individually validated and further characterized.
Conclusion: This study will definitely help in better understanding the roles played by the ERC, through its NEU1 catalytic subunit, in monocytes/macrophages, and should open new avenues for pharmacological strategies targeting the ERC and its biological effects in vascular diseases associated with elastin remodeling.

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P011
Initiation of fibronectin fibrillogenesis is an enzyme-dependent process

Shay Melamed[1], Shelly Zaffryar-Eilot[1], Elisabeth Nadjar-Boger[1], Rohtem Aviram[1], Huanning Zhao[2], Wesal Yaseen-Badarne[1], Rotem Kalev-Altman[3], Dalit Sela-Donenfeld[3], Oded Lewinson[1], Sophie Astrof[2], Peleg Hasson[1], Haguy Wolfenson[1]
[1]Technion, Medicine; [2]Rutgers; [3]Hebrew University, Jerusalem, Israel

Purpose: Initiation of fibronectin (FN) fibrillogenesis is considered to be a force-dependent process that is triggered by cytoskeletal forces applied through integrin-FN adhesions [1]. However, multiple lines of evidence demonstrate that FN fibrils are observed also within early embryos where the tissues are very soft [2] and do not support transmission of high cellular forces. We have previously demonstrated that Lysyl oxidase-Like3myofibers have to stretch and anchor at the myotendinous junction (MTJ (LOXL3) oxidizes FN, facilitating the induction of integrin-mediated cell adhesion and affecting FN matrix organization [3]. Therefore, we set out to test an alternative hypothesis where the initiation of FN fibrillogenesis is dependent on the enzymatic activity of the Lox family of enzymes rather than on force.
Methods: FN treated with Lox enzymes was subjected to mass-spec analyses and superresolution microscopy. Treated FN was further used as a substrate for cells in monitoring their adhesion, integrin activation and FN fibril formation.
Results: We find that Lox enzymes directly oxidize three specific lysine residues that are crucial for FN fibrillogenesis. This oxidation is force-independent and leads to bigger FN clusters, better early cell force transmission, adhesion, and migration. Strikingly, FN fibril formation was significantly decreased following mutagenesis of the oxidation sites or knockdown of Lox.
Discussion: Our results reveal an alternative and novel way of initiation of cell adhesion and matrix formation.
Conclusion: We find that the initiation of FN fibrillogenesis is an enzyme-dependent process that is essential for regulating cell behavior.

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P012
A new Smad4I500V/+ mouse model unveils the role of the extracellular matrix in the development of the Myhre syndrome

Zakaria Mougin[1], Huguet Herrero Julia[1], Arnaud Pauline[1,2], Hanna Nadine[2], Jondeau Guillaume[1,3], Boileau Catherine[1,2], Bailly Sabine[4], Le Goff Carine[1]
[1]INSERM U1148 - Laboratory for Vascular Translational Science, PARIS, France; [2]AP-HP, Bichat Hospital, Département de Génétique, Paris, France; [3]APHP, Bichat Hospital, National Reference Center for Marfan Syndrome and related disorders, Paris, France; [4]Université Grenoble Alpes, CEA, INSERM - Laboratory BioSanté, Grenoble, France

Purpose: Myhre syndrome (MS) is a part of acromelic dysplasias (AD) and is characterized by short stature, thick skin, hearing loss, and cardiovascular defects. We identified the molecular basis of MS as SMAD4 mutations localized at the isoleucine 500 residue1. As the co-mediator of the TGFβ and BMP signaling pathways, SMAD4 is essential for the proper development and homeostasis of the whole organism.
Methods: A new Smad4 mouse model has been generated, introducing the human mutation p.Ile500Val. The skeletal and cardiovascular phenotypes analysis has been performed using biochemical, histological assays, and next-generation RNA sequencing (RNA-seq).
Results: The mutation induces a longer half-time of the protein Smad4, with some TGFβ and BMP target gene dysregulation. The new Smad4I500V/+ mouse model is viable and presents stature retardation associated with growth plate and chondrocyte differentiation defects. Homozygotes mice (Smad4I500V/I500V) present a more severe phenotype with a perinatal lethality possibly due to septal defects. Using RNAseq, we established the transcriptomic profile of mutant chondrocytes, and identified that the majority of differentially expressed mRNAs is linked to the ECM (structural proteins, integrin-reacting proteins, matrix-degrading enzymes, and cytokines).
Discussion: This newly generated mouse model mimicking MS-like phenotype presents some of the chondrocyte's differentiation aberration found in other mouse models mimicking other AD2,3. The involvement of the ECM in the physiopathology of MS echoed the physiopathology of other AD with mutations in FBN1 and ADAMTSL2, both encoding ECM proteins.
Conclusion: The generation of this new mouse model suggests that the dysregulation of SMAD4 in MS leads to a disruption of the ECM, causing skeletal and cardiac defects.

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P013
The role of extracellular landscape in germ cell fate in vivo

Sandeep Gopal Pakideeri Karat[1]
[1]Lund Stem Cell Center, Department of Experimental Medical Sciences, Lund University, Sweden

Purpose: The extracellular matrix (ECM) serves as a physical force inducer and a repository of ligands and inorganic ions - three potent regulators of cell fate 1, 2. Investigating the in vivo functions of ECM during cell fate decisions is challenging due to the complex nature and continuous remodelling of ECM. In this study, we employed Caenorhabditis elegans germline as a model to study the role of ECM in cell fate and behaviour.
Methods: *C. elegans* expresses 467 evolutionary conserved ECM and ECM-associated proteins. By using the germ cell model, we studied the role of ECM in germline stem cell proliferation, differentiation and apoptosis. We systematically silenced the post-developmental expression of ECM proteins and examined the role of individual ECM molecules in germ cells in vivo.
Results: Our study revealed ~200 novel ECM molecules control the cell fate in the germline- thus providing further insight into the biochemical and biomechanical roles of the ECM in germ cell fate.
Discussion: Investigating the collective role of ECM is important as cell fate decisions are a result of total ECM and their interactions. Due to the conserved nature of ECM composition in *C. elegans*, this study will be relevant to other research models.
Conclusion: Our findings revealed a regulatory landscape involving extracellular matrix molecules, matrix remodelling enzymes and receptors that control germ cell fate.

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P014
Targeting hyaluronan-CD44 signalling in cancer

Paraskevi Heldin[1], Theodoros Karalis[1], Athanasios Chatzopoulos[1,2], Konstantinos Kolliopoulos[1], Spyros Skandalis[2], Carl-Henrik Heldin[1]
[1]Department of Medical Biochemistry and Microbiology, Biomedical Center, Uppsala University, Uppsala, Sweden; [2]Laboratory of Biochemistry, Department of Chemistry, Patras University, Greece

Purpose: We aim to elucidate the molecular mechanisms that contribute to the excessive accumulation of hyaluronan in certain tumors, resulting in over-activity of CD44 signaling.
Methods: We use 2D and 3D experimental models to evaluate cell proliferation, stemness, migration and invasion.
Results: Our data revealed a correlation between tumor progression and growth-factor-mediated hyaluronan synthesis and CD44 over-activity. We have identified a thymidine analog, DDIT, as a new small molecule inhibitor of hyaluronan synthesis. DDIT inhibits tumor progression and preferentially suppresses hyaluronan synthase 2 (HAS2)-synthesized hyaluronan in cancer cells. We have also investigated the proteolytic cleavage of CD44, which leads to the liberation of its intracellular domain (ICD). We demonstrate that growth factors promote CD44 cleavage and transcriptomic analyses revealed several genes whose expression is dependent on CD44-ICD, and may promote glioblastoma multiforme progression. Finally, we have screened for CD44 binders that inhibit the adhesion of cancer cells to hyaluronan coated-dishes.
Discussion: Perturbed hyaluronan-CD44 signaling contributes to the progression of diseases. Our screening of potent inhibitors of hyaluronan-engaged CD44 signaling may be useful for treatment of malignancies.
Conclusion: Our data support the notion that HAS2-synthesized hyaluronan binding to CD44 contributes to the progression of certain tumors.

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P015
OMICS approaches to dissect phenotypic variability in osteogenesis imperfecta

Nadia Garibaldi[1,2], Laura Bianchi[3], Luca Bini[3], Gabriella Tedeschi[4], Elisa Maffioli[4], Roberta Besio[1], Antonella Forlino[1]
[1]Dipartimento di Medicina Molecolare - unità di Biochimica, Università degli Studi di Pavia, Pavia, Italy; [2]Department of Biomedical Engineering, The City College of New York, New York, USA; [3]Dipartimento di Scienze della Vita, Università degli Studi di Siena, Siena, Italy; [4]Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Lodi, Italy

Purpose: The wide range of clinical outcomes is a hallmark of classical osteogenesis imperfecta (OI), caused by mutations in type I collagen α chains. Phenotype variability can be due to the mutated gene, the position and type of mutation, the individual genomic background and the combination of these factors. Being the intracellular mechanisms underneath OI variability still mainly obscure, we took advantage of multiple -OMICS approaches to address this topic.
Methods: Proteomic analysis of fibroblasts from OI patients with lethal and non lethal phenotypes was performed by mass spectrometry and bioinformatic processing. Transcriptomic and secretomic studies were performed in osteoblasts from two classical OI murine models, Brtl and Amish.
Results: Mass spectrometry in OI patients pointed out the differential expression of proteins involved in cell signalling, cytoskeleton and nuclear organization, metabolism and vesicle trafficking. A specific response to stress emerged from murine osteoblasts transcriptome, with Brtl being more prone to apoptosis, more consistently in lethal mice, and Amish preferentially upregulating Unfolded Protein Response. A deep analysis of Brtl and Amish osteoblasts secretome is underway and preliminary data showed model specific alteration in mRNA and protein processing, intracellular trafficking and cellular metabolism.
Discussion: The comparison between lethal and surviving outcomes both in patients and murine models suggested that lethal individuals are less able to cope with impaired homeostasis, displaying also hampered cytoskeletal components expression. -OMICS analyses of murine cells showed how the type of mutated chain significantly affects cellular signalling, secretion and metabolism.
Conclusion: We provided an insight into phenotype severity determination, identifying novel modulators of the intracellular machinery as well as the expression and secretion of different sets of proteins depending on the type of collagen mutations.

P016
Multimerin-2 affects endothelial cell functionality impinging on the YAP and Notch pathways

Greta Carobolante[1], Evelina Poletto[1], Eva Andreuzzi[1], Lucrezia Camicia[1], Maurizio Mongiat[1]
[1]National Cancer Institute Aviano (CRO), IRCCS, Department of Translational Research, Division of Molecular Oncology, Aviano, Italy

PURPOSE: Vascular homeostasis is achieved through the coordination of several signalling pathways engaged by endothelial cells (ECs). In this context, we found that the extracellular matrix (ECM) glycoprotein Multimerin-2, which is specifically expressed by ECs, is key in modulating vascular junction stability and permeability via the VEGFR2 signalling axis [1] its role in modulating vascular homeostasis remains largely unexplored. Here we identified Multimerin-2 as a key molecule required to maintain vascular stability. RNAi knockdown of Multimerin-2 in endothelial cells led to cell-cell junctional instability and increased permeability. Mechanistically cell-cell junction dismantlement occurred through the phosphorylation of VEGFR2 at Tyr951, activation of Src and phosphorylation of VE-cadherin. To provide an in vivo validation for these in vitro effects, we generated Multimerin-2$^{-/-}$ (Mmrn2$^{-/-}$. Here, we aimed at identifying additional signalling pathways through which Multimerin-2 could affect EC functionality.
Methods: Multimerin-2 was knocked-down in ECs by siRNA and the expression of basement membrane (BM) ECM molecules assessed by immunofluorescence (IF). YAP and the Notch Intracellular Domain (NICD) fragment were analysed by IF and subcellular fractionation. Activation of Notch pathway was assessed by WB, qRT-PCR and verified in spheroid-based assays.
Results: Upon Multimerin-2 knockdown, ECs showed an altered organization of the BM. In accordance with the altered stiffness, Multimerin-2 depleted ECs showed increased YAP nuclear localization. Consistently, retinal vessels from Multimerin-2$^{-/-}$ pups displayed higher YAP levels. On the other side, knockdown ECs showed a reduced nuclear expression of NICD, and decreased expression of the Notch target genes. In line with the above results, Multimerin-2 depleted ECs acquired the tip cell phenotype.
Discussion: Our results suggest that Multimerin-2 affects different signalling pathways involved in vascular functionality. We suppose that Multimerin-2, through the maintenance of a proper ECM organization, affects EC behaviour impinging on the activation of the YAP and Notch signalling pathways [2].
Conclusion: These results provide novel insights into the possible mechanisms by which Multimerin-2 can regulate EC biology and vascular function.

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P017
PLX8394, an oral serine-threonine kinase inhibitor, prevents TGF-β induced laminin-332 synthesis, cell invasion and tumour growth in cutaneous squamous cell carcinoma

Elina Siljamäki[1], Pilvi Riihilä[2], Ujjwal Suwal[1], Liisa Nissinen[2], Pekka Rappu[1], Veli-Matti Kähäri[2], Jyrki Heino[1]
[1]Department of Life Technologies, University of Turku, Turku, Finland;
[2]Department of Dermatology, University of Turku and Turku University Hospital, Turku, Finland

Purpose: Cutaneous squamous cell carcinoma (cSCC) is the most common metastatic skin cancer, with increasing incidence worldwide [1]. The prognosis of metastatic cSCC is poor which stresses the urgent need for new therapies.
Methods: RT3 (H-Ras-transformed immortalized keratinocytes) and UT-SCC-7 cells (human cSCC cell line) were cultured as 3D spheroids with human skin primary fibroblasts. Spheroids were harvested for western blotting or used in invasion assays on collagen I. For in vivo assays, UT-SCC-7 cells were injected subcutaneously into the back of SCID/SCID mice. The mice were fed with PLX8394 (150 mg/kg) by oral gavage once daily for 18 days.
Results: 10 mM PLX8394 inhibits SMAD2 and TGFbRII phosphorylation and consequently decreases TGF-b -dependent laminin-332 accumulation in RT3 and UT-SCC-7 cells. PLX8394 simultaneously targets ERK1/2 and p38 activation and thus further decreases laminin-332 synthesis. PLX8394 treatment significantly decreases RT3 cell invasion out of coculture spheroids. In mouse xenograft model orally administered PLX8394 significantly inhibits UT-SCC-7 tumour growth in vivo.
Discussion: Following PLX8394 treatment, TGF-b signalling and ERK1/2 and p38 activation are blocked, and laminin-332 synthesis is decreased. This consequently inhibits cancer invasion and tumour growth both in vitro and in vivo. We show that low micromolar concentrations of PLX8394 suppress an optimal pattern of signalling pathways that have previously been linked to the progression of cSCC [2].
Conclusion: We introduce PLX8394 as a novel serine-threonine protein kinase inhibitor, which blocks the promoting effect of laminin-332 on cSCC tumour progression. Our results indicate that it is possible to find inhibitors for serine-threonine kinases that have a favorable target spectrum in experiments aiming to prevent the growth of cSCC tumours.

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P018
EMILIN-1 deficiency promotes chronic inflammatory condition through TGFβ signaling alteration and impairment of the gC1q/α4β1 integrin interaction

Eliana Pivetta[1], Alessandra Capuano[1], Maddalena Vescovo[1], Eugenio Scanziani[2,3], Maurizio Mongiat[1], Paola Spessotto[1]
[1]Unit of Molecular Oncology, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano, Italy; [2]Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy; [3]Mouse and Animal Pathology Lab (MAPLab), Fondazione UniMi, Milan, Italy

Purpose: Alterations in extracellular matrix (ECM) components have been shown to be early signs of various diseases such as fibrosis and cancer (1). We have shown that the ECM glycoprotein EMILIN-1 exerts a homeostatic role in skin and supports lymphangiogenesis (2,3). We investigated whether the loss of EMILIN-1 or its changes could provide a favorable environment for further disease development.
Methods: We used the imiquimod (IMQ)-induced mouse model of psoriasis, a skin disease characterized by a conspicuous inflammatory infiltrate and vascular changes. Histological and immunophenotypic analyzes were performed.
Results: We found that EMILIN-1 deficiency was associated with psoriasis severity, dilated lymphatic vessels (LVs), and increased inflammatory infiltrate. Importantly, also in the transgenic (TG) mouse model carrying the E933A mutation in the gC1q domain of EMILIN-1, which abolishes the interaction with α4/α9-integrins, a higher incidence of a myofibroblast phenotype was observed as in KO mice, leading to a fibrotic microenvironment. Using the TG model, we demonstrated that the observed changes in TGFβ signaling were due to both the EMI and gC1q domains of EMILIN-1. Lower levels of EMILIN-1 were present in human psoriatic lesions and were very rarely associated with LVs, underscoring the multifuncional role of this ECM protein in the skin.
Discussion: gC1q may exert multiple functions by controlling skin homeostasis, by interacting with both keratinocytes and fibroblasts, by affecting non-canonical TGFβ signaling, and by most likely acting on the structure and function of LVs. The more consistent inflammatory response to IMQ treatment in KO and E933A TG mice may also be due to structural and functional dysregulation of the lymphatic system.
Conclusion: In the skin, EMILIN-1 deficiency results in a fibrotic phenotype leading to worsening of the inflammatory state.

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P019
Role of Multimerin-2 in pericyte recruitment and vascular stability

Lucrezia Camicia[1], Eva Andreuzzi[1], Evelina Poletto[1], Greta Carobolante[1], Maurizio Mongiat[1]
[1]National Cancer Institute Aviano (CRO), IRCCS, Department of Translational Research, Division of Molecular Oncology, Aviano, Italy

Purpose: The delivery of nutrients and oxygen to the tissues is ensured by the presence of a continuous vascular endothelial cell (EC) layer embraced by mural cells. Multimerin-2 is an extracellular matrix glycoprotein deposited along the blood vessels in juxtaposition between ECs and mural cells, where it exerts homeostatic functions [1]. Our study aimed to investigate the role of Multimerin-2 in modulating the behaviour pericytes, a key mural cell type [2].
Methods: Human brain vascular pericytes (HBVP) were challenged with recombinant Multimerin-2 and cell viability assessed by MTT. The molecular pathways were assessed by phospho-arrays and Western blot. Haptotaxis assays were performed using transwells. Pten$^{-/-}$ and p53$^{-/-}$ ID8 ovarian cancer cells were injected in wild type and Multimerin-2$^{-/-}$ C57BL/6 mice and pericyte coverage was assessed by immunofluorescence.
Results: Multimerin-2 increased the proliferation of HBVP cells and, consistently, this associated with a prominent activation of AKT. Multimerin-2 represented a significant haptotactic stimulus for pericytes. Accordingly, pten$^{-/-}$ and p53$^{-/-}$ ID8 xenograft tumors developed in Multimerin-2$^{-/-}$ mice displayed an impaired pericyte coverage compared to that of wild type mice.
Discussion: These new evidences demonstrate that Multimerin-2$^{-/-}$ not only represents an adhesion substrate for pericytes1 and a direct stimulus for pericytes recruitment, but it also affects their proliferation, possibly mediated by the activation of the AKT pathway. Furthermore, our preclinical experiments demonstrate that loss of Multimerin-2$^{-/-}$, frequently observed in tumors, may impinge on pericyte recruitment, further exacerbating the tumor-associated vascular aberrations.
Conclusion: Multimerin-2$^{-/-}$ affects vascular efficiency impinging on pericyte recruitment and proliferation, and its loss results in a defective vasculature.

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P020
Dermatan sulfate can induce necroptosis in breast cancer cells

Adam Pudelko[1], Grzegorz Wisowski[1], Krystyna Olczyk[1], Monika Paul-Samojedny[2], Ewa Koźma[1]

[1]Department of Clinical Chemistry and Laboratory Diagnostics, Medical, [2]Department of Medical Genetics, University of Silesia in Katowice, Sosnowiec, Poland

Purpose: The aim was to assess the effects of dermatan sulfate (DS) structural variants on breast cancer cells.
Methods: DS variants were isolated from normal human fascia (NF), human fibrosis-affected fascia (DF) and porcine skin (PS). Moreover, commercial DS from porcine intestinal mucosa (PM) was also used. All variants were characterised in respect to their sulfation pattern and glucuronate content. The impact of these variants on the viability, proliferation, and cell count was tested in several lines of breast cancer cells including luminal and triple-negative types. Potential mechanisms underlying the observed biological effects were assessed by immunocytochemical and/or fluorescence analyses of cell death, activation of necroptotic effector MLKL as well as the mitochondrial transmembrane potential and oxidative stress.
Results: NF, DF and/or PM were able to significantly reduce the viability and cell number only in luminal breast cancer cells. Moreover, these variants quickly induced oxidative stress in the cytoplasm of these cells and influenced the function of their mitochondrion. Most likely via the first of these mechanisms the active variants triggered necroptosis in the luminal breast cells. However, dynamics of necroptosis was strongly dependent on the structure of the DS variant. Moreover, the intensity of necroptosis was also well correlated with DS-mediated effects on the viability and number of cancer cells.
Discussion: Our study for the first time delivers direct evidence that DS can trigger necroptosis of the luminal cancer cells in its structure - and concentration-dependent manner during short-term exposition. This mechanism can be responsible for the regulation of the viability and number of cancer cells.
Conclusion: DS may significantly affect the progression of luminal breast cancer cells in vivo because these variants that shared structural features with this glycan from the tumour niche had especially strong biological effects.

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P021
Cartilage-specific respiratory chain inactivation alters extracellular matrix homeostasis through metabolic signaling

Kristina Bubb[1,2], Tatjana Holzer[1,2], Janica Lea Nolte[3], Marcus Krüger[3], Richard Wilson[4], Ursula Schlötzer-Schrehardt[5], Jürgen Brinckmann[6], Janine Altmüller[7,8,9], Attila Aszodi[10], Lutz Fleischhauer[10], Hauke Clausen-Schaumann[11], Kristina Probst[1,2], Bent Brachvogel[1,2]

[1]Department of Pediatrics and Adolescent Medicine, Experimental Neonatology, Cologne, Germany; [2]Center for Biochemistry, Medical Faculty and University Hospital Cologne, Cologne, Germany; [3]Institute of Genetics and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Cologne, Germany; [4]Central Science Laboratory, University of Tasmania, Hobart, Australia; [5]Friedrich-Alexander University of Erlangen-Nürnberg, Department of Ophthalmology University Hospital Erlangen, Erlangen, Germany; [6]Institute of Virology and Cell Biology, University of Lübeck, Department of Dermatology, Lübeck, Germany; [7]Cologne Center for Genomics, University of Cologne, Cologne, Germany; [8]Berlin Institute of Health at Charité, Core Facility Genomics, Berlin, Germany; [9]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany; [10]Department for Orthopaedics and Trauma Surgery, Musculoskeletal University Center Munich (MUM), University Hospital, Ludwig-Maximilians-University (LMU), Munich, Germany; [11]Center for Applied Tissue Engineering and Regenerative Medicine, Munich University of Applied Sciences, Munich, Germany

Purpose: During endochondral ossification chondrocytes mediate matrix deposition and drive skeletal growth. Due to cartilage avascularity the common belief is that mitochondrial respiratory chain (mtRC) is of minor importance for chondrocyte metabolism. We recently showed that mtRC is surprisingly orchestrating postnatal skeletal growth despite the avascular nature of cartilage. However, the connection between chondrocyte metabolism and extracellular matrix (ECM) homeostasis is currently still poorly understood. Here, we provide a link between mtRC, metabolic signaling and ECM homeostasis in cartilage.
Methods: Single cell RNA sequencing (scRNA-seq) and mass spectrometry (MS) were used to characterize the load of mtRC dysfunction and the ECM composition in 28 days old femoral head cartilage from mutant mice with cartilage specific mitochondrial dysfunction. Signaling networks were studied in chondrocytes using immunoblot and MS analysis. Properties of the ECM were characterized by histomorphological analysis, collagen crosslinking and ECM stiffness measurements.
Results: scRNA-seq analysis demonstrates that mitochondrial DNA encoded genes are specifically decreased in the nonarticular chondrocyte population of mutant mice accompanied by a changed expression of ECM-related genes (MATN1, THBS1). This inactivation of the mtRC results in a disorganized expanded resting zone in femoral head cartilage of mutant mice, characterized by deposition of MATN1, THBS1 and $p62^+$ autophagic vesicles. These changes are accompanied by activation of mTORC1 and an increase of ECM crosslinking and stiffness.
Discussion: These results indicate that mtRC dysfunction in chondrocytes stimulates mTORC1 signaling to inhibit autophagy related processes and to disturb matrix formation and its biomechanical properties.
Conclusion: mtRC dysfunction in cartilage induces a metabolic signaling leading to a disturbed ECM organization. This provides novel understanding of ECM homeostasis in endochondral ossification.

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P022
Could isoflavone quercetin be used in the treatment of eye pterygium?

Maria Elpida Christopoulou[1,2], Christina Sotiropoulou[1], Zoe Kordou[1], Elena Neofotistou[1], Alexios Aletras[1]
[1]Department of Chemistry, University of Patras, Greece, Patras, Greece; [2]Medical Center, Department of Pneumology, University of Freiburg, Freiburg, Germany

Purpose: Eye pterygium is an abnormal tissue growth on the conjunctiva and the adjacent cornea. Chronic sun (Ultraviolet, UV) exposure is thought to be the major cause that triggers inflammation and extracellular remodeling leading to eye pterygium. Quercetin is a member of flavonoids that protects the cells from oxidative stress induced by reactive oxygen species. The aim of this study was to explore the effect of quercetin on TNF-α and IL-1β induced expression of inflammatory molecules.
Methods: The effect of quercetin on ECM molecules was studied in cultures of primary pterygium fibroblasts (PFs) that were established from biopsies by ELISA, RT-PCR and western blotting.
Results: Quercetin significantly inhibited the secretion and gene expression of metalloproteinases (MMPs) and their tissue inhibitor TIMP-1. The levels of IL-6 and IL-8 were also downregulated after quercetin treatment. Quercetin at a dose of 100 mM reduced the expression of COX-2, but increased the levels of thereodoxin protein (TXNIP) resulting in reduced activity of nuclear factor-kB. Moreover, quercetin decreased the UVB-induced MIF expression resulting in the enhancement of the proteasome.
Discussion: The suppressive effect of quercetin on the IL-1β, TNF-α, TGF-β1 and UVB induced expression of various inflammatory factors is considered beneficial for tissue homeostasis in the eye. Quercetin´s anti-inflammatory activity may be exerted through regulation of AMPK/NF-κB pathway by both the TXNIP up-regulation and MIF down-regulation, respectively, since it is known that MIF interacts with TXNIP thereby promoting the NF-κB activation. The down-regulation of UVB-enhanced expression of MIF by quercetin may be also responsible for the stimulatory effect of flavonoid on the expression and activation of β5 proteasome subunit, since the UVB-enhanced expression of MIF has been correlated with the UVB-induced down-regulation of β5 subunit of proteasome through the phosphorylation of Nrf2 by src kinases.
Conclusion: These results are of great importance for the emergence of quercetin as an anti-inflammatory molecule and thus it may be potentially used in the treatment of eye pterygium.

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P023
Mitochondria and signaling pathways in elastic fiber calcification

Francesco Lofaro[1], Maria Garcia-Fernandez[2], Daniela Quaglino[1], Federica Boraldi[2]
[1]Department of Life Science, University of Modena and Reggio Emilia, Italy; [2]Department of Human Physiology, University of Malaga, Spain

Purpose: The local microenvironment may influence the susceptibility of matrix components to mineralize, either by changing the ionic context, by increasing the number of accessible hydroxyapatite nucleation sites or by modulating the behavior of mesenchymal cells 1,2 Moreover, oxidative stress can be associated to the ectopic calcification (EC) observed in chronic diseases and the aging process. Interestingly, Pseudoxanthoma elasticum (PXE), a still untreatable genetic disease also regarded as a premature aging syndrome3, is characterized by fragmentation and mineralization of elastic fibers and by a mild oxidative stress condition4. Beside the role of sequence variants of the causative gene, it is still unclear which pathogenic mechanisms can contribute to the occurrence and progression of EC, thus representing possible druggable targets. Therefore, we have investigated SMAD signaling pathways and mitochondria structure and function to better elucidate their role in the development of EC.
Methods: We have integrated different technical approaches (i.e., proteomic, biochemical, morphological analyses) to investigate PXE and control mitochondria. Furthermore, SMAD signaling pathways were assessed by immunohistochemistry on dermal tissue sections.
Results: Data indicate that i) pSMAD 2,3 and pSMAD 1,5,8 are activated in PXE fibroblasts, ii) PXE mitochondria have: a) several differentially expressed proteins involved in redox balance, oxidative phosphorylation, and calcium homeostasis, b) a low ability to cope with a sudden increased need for ATP exhibiting a structure and an organization different from controls.
Discussion: Data underline the importance of SMAD activation in modulating mitochondria dynamics in the development of a pro-osteogenic context.
Conclusion: Ameliorating mitochondrial functions and metabolism could open new strategies to modulate signaling pathways associated to pathologic calcification.

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P024

Implication of pleiotrophin and its receptors: receptor protein tyrosine phosphatase zeta 1 and alpha v beta 3 integrin in mTORC1 signaling

Eleni Mourkogianni[1], Evangelia Papadimitriou[1]

[1]Department of Pharmacy, University of Patras, Patras, Greece

Purpose: Pleiotrophin (PTN) is a secreted growth factor that modulates the function of endothelial cells through its receptor protein tyrosine phosphatase zeta 1 (PTPRZ1) and alpha v beta 3 ($\alpha_v\beta_3$) integrin [1]. The present work aims to elucidate the implication of PTN and its receptors in mTORC1 signaling.

Methods: Mouse lung microvascular endothelial cells (LMVEC) isolated from Ptprz1$^{+/+}$ and Ptprz1$^{-/-}$ mice and human umbilical vein endothelial cells (HUVEC) were cultured in the presence of exogenous PTN and/or inhibitors. Extracted proteins were analyzed by SDS-PAGE. All data are expressed as mean ±SD of at least three independent experiments. Unpaired t test or one-way ANOVA was used as appropriate.

Results: PTN enhances translation and the phosphorylation of 4EBP1 and p70S6 kinase, an action abolished by the mTOR inhibitor rapamycin. However, rapamycin did not have any impact on PTN induced migration. Ptprz1$^{-/-}$ LMVEC have decreased expression of $\alpha_v\beta_3$, as well as increased translation rate and phosphorylation status of p70S6K, compared to Ptprz1$^{+/+}$ LMVEC. In the same line, the PTPRZ1 tyrosine phosphatase inhibitor MY10 and the anti- $\alpha_v\beta_3$ integrin monoclonal antibody LM609 induced translation in endothelial cells, similarly to the effect of PTN.

Discussion: Our data highlight a potential interplay among PTN/PTPRZ1/$\alpha_v\beta_3$ implicated in the regulation of translation. Activation of mTOR does not seem to be involved in PTN-induced endothelial cell migration.

Conclusion: Both PTPRZ1 and $\alpha_v\beta_3$ are involved in the regulation of protein translation by PTN and may contribute to the adaptation of endothelial cells to numerous extracellular stimuli beyond PTN.

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P025

Low-density lipoprotein receptor-related protein 1 (LRP-1) expression in fibroblasts or cancer-associated fibroblasts differentially modulate endothelial cells functions through paracrine signals and extracellular matrix landscaping

Julie Martin[1], Sara Faour[1], Christine Terryn[2], Damien Rioult[3], Cathy Hachet[1], Stéphane Dedieu#[1], Stéphanie Salesse[1], Benoît Langlois[1]

[1]UFR SEN, [2]Cellular and tissular imaging platform (PICT),University of Reims Champagne Ardenne, UMR 7369 CNRS MEDyC, Reims, France; [3]UFR SEN, University of Reims Champagne Ardenne, MOBICYTE, Reims, France

Purpose: Low-density lipoprotein receptor-related protein 1 (LRP-1) is a multifunctional endocytic receptor specific for functionally diverse ligands. It explains the versatile functions it can modulate during development or during cancer progression. The role of the receptor was reported in the regulation of tumor growth and aggressiveness, in stromal reaction and angiogenesis [1]. In mammary cancer microenvironment, cancer-associated fibroblasts (CAF) are intimately associated to the control of matrix landscape and tumor bed composition [2]. Our data suggest that LRP-1 is highly expressed in CAF but its expression functional impact on their paracrine and desmoplasic signals, specially towards endothelial cells behavior, remains elusive.

Methods: We used two different models to address the stromal LRP-1 impact on endothelial cells features: murine embryonic fibroblasts wild type or knock-out for LRP-1 (MEF-1 and MEF-2) and CAF$_2$ [3] treated with LRP-1 blocking agents. We implemented two approaches to study the angiogenic cues mediated by fibroblasts: i) conditionned media (CM) and ii) fibroblasts-derived matriced (F-DM).

Results: MEF-2 secretome impaired morphogenic, migratory and invasive behavior of HUVEC as compared to LRP-1 expressing cells. It also stabilized VE-Cadherin positive adherens junctions of endothelial cells cultivated as monolayer. LRP-1 ablation causes drastic modifications of F-DM composition and topography. Video-tracking of HUVEC seeded on F-DM revealed that MEF-2-DM limits migration and invasion compared to controls. Conversely, LRP-1 silencing facilitated endothelial motility on CAF$_2$-DM.

Discussion / Conclusion: Altogether, our results suggest that LRP-1 loss of function in embryonic (MEF) or adult activated (CAF) models of fibroblast results in opposite angio-modulatory functions in vitro. Experiments are ongoing to decipher the molecular drivers modulated by LRP-1 in fibroblasts secretome and its differential impact on the endothelial cell membranome by proteomic approaches.

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P026
A computational approach to generate an extracellular matrix atlas of LGR5+ stem cells from high-content imaging data

Marco Franzoni[1], Sigita Malijauskaite[1], Séamus Hickey[1], Cathal King[1], Kieran McGourty[1,2]
[1]Bernal Institute, [2]Health Research Institute, Chemical Sciences, Limerick, Ireland

Purpose: The presented method allows for an automated, hands-off analysis of large immunofluorescence images and the quantification of the relative abundance and localization of ECM proteins.
Methods: Serially obtained bovine intestinal tissues slices are stained for adult stem cell marker (LGR5), one of 17 ECM proteins and an activity reporter (Ki67). Immunofluorescent images are automatically acquired and analysed using CellProfiler [1] to identify cells positively stained and to filter artifacts. The individual images and results are recomposed and analysed using RStudio. The results can be interactively explored using a dedicated application created with Shiny.
Results: The proposed approach allows to spatially reconstruct and explore large datasets of individually identified cells [Fig.1].

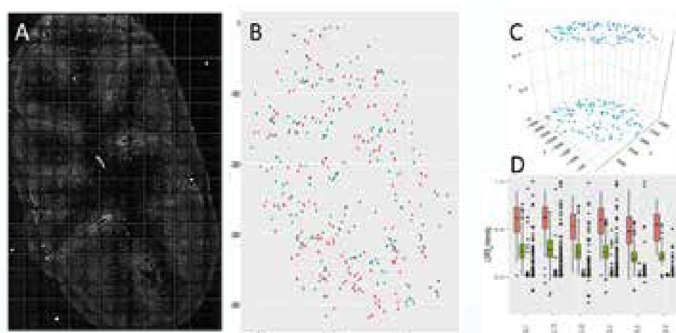

Figure 1. Examples of results of the workflow. A) Reconstruction of the immunofluorescent images, B) 2D plot of the identified LGR5+ cells, C) 3D visualization of two sequential slides and D) evaluation of LGR5 intensity of different LGR5+ cell subclasses.

Discussion: The proposed methods provide a workflow to analyse and reconstruct full slices of biological tissues maintaining high spatial resolution and individual cell identity. The filtration algorithms allow to generate clean datasets with minimal human intervention. The image reconstruction coupled with information-rich dataset allows to obtain an informative atlas of the investigated tissue. This method can be used for any type of biological tissue and staining combinations with minimal modifications.
Conclusion: Image based biology allows for quick and relatively inexpensive investigation of healthy and diseased tissues. In this context the proposed method adds an important tool to post process high magnification images coupled with information of the individual cells. The result is an easy and intuitive application that can be used to explore biological tissue with at a multiscale resolution.

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P027
Transcriptomic analysis of the matrisome in dorsal root ganglion of wild-type and Col5a1$^{+/-}$ mice

Delfien Syx[1], Robin Vroman[1], Rahel Hunter[2], Zoë Malfait[1], Anne-Marie Malfait[2], Fransiska Malfait[1], Rachel Miller[2]
[1]Center for Medical Genetics, Department of Biomolecular Medicine, Ghent University, Ghent, Belgium; [2]Division of Rheumatology, Rush University Medical Center, Chicago, USA

Purpose: The extracellular matrix (ECM) plays pivotal roles in physiological and pathological processes in many tissues, including the nervous system. Pain is a frequent complaint in patients with monogenic disorders affecting the ECM, including Ehlers-Danlos syndromes (EDS). Classical EDS (cEDS) is a heritable connective tissue disorder caused by type V collagen defects. We demonstrated that haploinsufficient Col5a1$^{+/-}$ mice, a model for cEDS, show hypersensitivity to mechanical stimuli, in combination with altered nociceptive innervation in skin. We aim to expand the knowledge on ECM composition in the peripheral nervous system and its role in pain, by investigating the expression and cellular origin of matrisome genes in murine dorsal root ganglia (DRG), which contain the cell bodies of sensory neurons. In addition, we want to see how the matrisome is altered in Col5a1$^{+/-}$ DRG.
Methods: We performed bulk RNA sequencing (wild-type (WT) and Col5a1$^{+/-}$) and single cell RNA sequencing (scRNAseq, WT) on RNA extracted from murine lumbar DRG (L3-L5) as well as immunohistochemistry (IHC) on DRG.
Results: Bulk RNAseq analysis showed that 65% of murine matrisome genes are expressed in WT DRG. ScRNAseq revealed that collagens are mainly expressed by vascular cells while matrisome-associated genes are primarily expressed by neurons in the DRG. Sex-specific transcriptional alterations in matrisome genes were observed in WT mice, with limited overlap in differential gene expression between both sexes in Col5a1$^{+/-}$ mice. IHC showed a similar distribution of collagen types I, III and VI and decorin around the DRG cell bodies in WT and Col5a1$^{+/-}$ mice and severely reduced type V collagen in Col5a1$^{+/-}$ DRG. Interestingly, increased αvβ3 integrin staining was found in Col5a1$^{+/-}$ DRG cell bodies.
Discussion and Conclusion: A large portion of matrisome genes are expressed in DRG. Col5a1$^{+/-}$ mice provide a useful tool to study how ECM alterations affect pain mechanisms associated with cEDS.

P028

Ablation of the FACIT collagen XII disturbs musculoskeletal ECM organization and causes patella dislocation and myopathy

Mengjie Zhu[1], Fabian Metzen[2], Janina Betz[1], Mark Hopkinson[3], Juliane Heilig[4], Thomas Imhof[1], Anja Niehoff[4], Andrew Pitsillides[3], Gudrun Schreiber[5], Manuel Koch[1], Bent Brachvogel[6]

[1]Institute for Dental Research and Oral Musculoskeletal Biology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany; [2]Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany; [3]Skeletal Biology Group, Comparative Biomedical Sciences, The Royal Veterinary College; [4]Institute of Biomechanics & Orthopaedics,German Sport University Cologne, Germany; [5]Department of Pediatrics, Klinikum Kassel, Kassel, Germany; [6]Department of Pediatrics and Adolescent Medicine, Experimental Neonatology, Faculty of Medicine and University Hospital Cologne, Germany

Purpose: Mutations in human Col12a1 gene are the causes of Ehler-Danlos syndrome (EDS), a rare disorder that is characterized by the destabilization connective tissues. Here Col12a1 deficient mice model was used to elucidate the functions of Collagen XII in musculoskeletal system and its role in the disease.
Methods: Whole leg from control and Col12a1-deficient mice were isolated and characterized by both micro-CT and histomorphological analysis. Single cell sequencing and matrisome analysis in isolated P7 whole legs was conducted to identify further molecular changes.
Results: Fluorescent in situ hybridization and immunofluorescent staining results identified expression of Collagen XII in bone, articular cartilage, muscle and tendon. Col12a1 deficient mice showed: i) Delayed endochondral ossification, with decreased body size and weight. ii) Quadriceps muscle loss, which was observed from postnatal 7 days onward. iii) Developmental patella growth retardation and subluxation due to facies patellaris femoris groove malformation which was observed in 7-day and 1-month mice by micro-CT and histological analysis, but not in newborns. Further, single cell sequencing was conducted at legs of WT and KO mice at the age of postnatal 7 days. In differentially regulated genes in the tenocytes subpopulations, 11 out of 274 genes in the core matrisome and 22 out of 836 matrisome-associated genes were differently expressed. In 2 mutant patients, X-ray analysis of their knees revealed the similar clinical feature, which in one knee a complete absence of the facies patellaris femoris groove, and in the other knee the grooves were less pronounced.
Discussion: The phenotypes observed in our mice model mimic the clinical features of patients. In the future we aim to understand the molecular mechanisms of how loss of collagen XII expression destabilizes extracellular matrix (ECM) organization and the potential signaling pathway involved in patella and muscle differentiation and homeostasis to induce an Ehler-Danlos syndrome-like phenotype in our mice model.
Conclusion: Our results indicate that collagen XII mediated extracellular and cellular mechanisms are important for the proper establishment of patella-muscle unit in the knee.

P030

A redox-dependent thiol-switch and a Ca2+ binding site within the hinge region hierarchically depend on each other in α7β1 integrin regulation

Johannes A. Eble[1], Michele F. Caliandro[1], Luca Matteo Todesca[2], Matthias Mörgelin[3], Albrecht Schwab[2]

[1]Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, Münster, Germany; [2]Institute of Physiology II, University of Münster, Münster, Germany; [3]em3, Anderslöv, Sweden

Purpose: Integrin-mediated cell contacts with the extracellular matrix (ECM) are essential for cellular adhesion, force transmission, and migration. Several effectors, such as divalent cations and redox-active compounds, regulate ligand binding activities of integrins. The aim of this study was to investigate the role of the Ca2+ binding site within the hinge region of the integrin α7 subunit in context to the closeby thiol-switch.
Methods: We genetically abrogated the Ca2+ binding site in the α7hiΔCa mutant. We compared it with the α7hiΔSS mutant, in which the redox-regulated thiol-switch is deleted [1], in transfected WM115 melanoma cells with respect to cell spreading, adhesive forces, and migration. Soluble integrin ectodomains with the same mutations were expressed in insect cells and used for protein chemical analyses, such as binding ELISAs, crosslinkage assays, and electron microscopic imaging.
Results: Ca2+ complexation and redox-regulation within the hinge interdepend on each other. Moreover, integrin activation via the subunit α hinge is primed by the formation of the cysteine pair–based crosslinkage. Subsequently, this allows Ca2+ complexation within the hinge, which results in the conformational extension of the integrin ectodomain. This results in integrin activation and enhanced ligand binding [2].
Conclusion: The α hinge is an allosteric integrin regulation site, in which both effectors, Ca2+ and redox-active compounds, synergistically and hierarchically induce far-ranging conformational changes, such as the extension of the integrin ectodomain, resulting in integrin activation of ECM ligand binding and altered integrin-mediated cell functions. By changing their redox environment locally at sites of adhesion [3], cells regulate their integrin-mediated adhesion strength via a mechanism that involves Ca2+ complexation of their integrins.

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P030
YAP1 mediates anoikis through failed integrin adhesion reinforcement

Lidan Shi[1], Elisabeth Nadjar-Boger[1], Haguy Wolfenson[1]
[1]Technion - Israel Institute of Technology, Rappaport Faculty of Medicine, Haifa, Israel

Purpose: Mechanosensing of extracellular matrix (ECM) rigidity drives various cell-fate decisions [1]. Cells from healthy tissues are rigidity-dependent, and they undergo anoikis (detachment-induced apoptosis) on soft matrices when focal adhesions (FAs) fail to mature [2]. In contrast, cancer cells display aberrant mechanosensing, which allows them to evade anoikis. Here we studied the signaling pathways linking ECM mechanosensing and the anoikis cell fate decision.
Methods: We restored the mechanosensing ability of the breast cancer cell line MDA-MB-231 by inducing Tropomyosin 2.1 (Tpm) expression [3], and studied the resulting change in apoptosis-inducing signals from cell-matrix adhesions during mechanosensing. To that end, we used a combination of live-cell imaging, confocal microscopy, force measurements, apoptosis assays, small molecule inhibitors, and site-directed mutagenesis.
Results: We find that it is not the rigidity of the ECM per se, but rather the balance between the forces generated by cells and the resistance that the ECM provides which determines cell fates. Slight reduction of the forces of MDA-Tpm cells by Myosin II inhibition leads to assembly of mature FAs and survival of the cells on a soft ECM. Accordingly, elevating Myosin II activity leads to FA disassembly and increased cell death on a stiff ECM. Moreover, Yes-Associated Protein 1 (YAP1) is recruited and phosphorylated at the Y357 site in nascent adhesions, but not in mature FAs.
Discussion: We propose that accumulation of pY357-YAP1 due FA maturation failure triggers the YAP-p73 apoptotic pathway. These results thus provide a direct link between mechanosensing and anoikis and show that cellular decisions can be switched based on the cell-matrix force balance.
Conclusion: These results place YAP1 as a binary switch in the decision to grow or apoptose based on ECM signals.

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P031
ADAMTS9 is critical for the development and survival of primary ovarian follicles and conversion of female sex to males in zebrafish

Yong Zhu[1]
[1]East Carolina University, Biology, Greenville, USA

Results: To further understand the mechanisms of Adamts9 in zebrafish ovarian development, we investigated Adamts9 expression, role in primordial germ cell (PGC) migration, gonad development, and sexual differentiation in zebrafish. We found adamts9 was widely expressed during embryonic and larval development and is maternally deposited at the one cell stage. We found strong expression in the developing retina, that moved to the ciliary marginal zone at 72hpf. We also found expression in somites surrounding the PGCs during migration, and in primary follicles in juvenile and adult ovaries. In contrast to invertebrate models, we only saw migration delay of PGCs in Adamts9 KOs. But we saw slower development of juvenile gonads in Adamts9 KO, and significantly reduced size and number of primary oocytes in Adamts9 KO. Adamts9 KO had no effect on primary sex determination, but in female Adamts9 KO the ovary remained dramatically underdeveloped compared to wildtype control siblings. Rescuing general growth defects by overfeeding did increase female percentage but did not rescue the ovarian phenotype. Further, follicles in rescued Adamts9 KO females remained at Stage IB and only few follicles could continue maturation. We also found morphological evidence for sex reversal in Adamts9 KO at 90 days old, including coexistence of Stage IB oocytes and sperm in the same tissue section. As the fish age, the male biased sex ratio continued to increase, indicating that female Adamts9 KOs are sex reversing into males. Furthermore, blocking TP53 apoptosis pathway completely recused ovarian defects.
Conclusion: We show that Adamts9 is essential for proper ovarian development and that loss of Adamts9 leads to folliculogenesis deficiency, follicle arrest, loss of ovarian follicles, eventual female to male sex conversion in zebrafish.

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P032
Involvement of jagged/ notch pathway in human chondrocytes exposed to lps: effect of high molecular weight hyaluronan treatment

Giuseppe Maurizio Campo[1], Angela D'Ascola[1], Angela Avenoso[2], Aurelio Minuti[2], Federica Aliquò[2], Giuseppe Bruschetta[3], Salvatore Campo[2], Michele Scuruchi[1]

[1]Department of Experimental and Clinical Medicine, [2]Department of BIOMORF, [3]Department of Veterinary Science, Unversity of Messina, Messina, Italy

Purpose: Since previous studies revealed the activation of JAGGED/NOTCH signaling in inflammatory processes, including cell treatment with lipopolysaccharides (LPS), we aimed to investigate the involvement of JAGGED/NOTCH pathway and the effects of high molecular weight hyaluronan (HMW-HA) treatment in human chondrocytes stimulated by (LPS).
Methods: Chondrocytes were stimulated with LPS and then the mRNA expression of TLR-4, NOTCH1/2, JAGGED1, MAPK, RBP-J, IL-6, IL-1β, and MMP-13 was evaluated. A further set of plates were treated with NOTCH1/2 siRNAs before LPS treatment. NF-kB (p65) activation was also determined by a commercial assay. The same parameters were assayed after HMW-HA treatment.
Results: LPS induced high expression of TLR-4, MAPK, JAGGED1, NOTCH1/2, RBP-J, IL-6, IL-1β and MMP-13, as well as NF-kB activation. The treatment of chondrocytes with HMW-HA, previously stimulated with LPS, reduces significantly the inflammatory response.
Discussion: Stimulation of TLR-4 triggers activation of downstream signaling molecules such as MAPK and finally NF-kB. Furthermore, this receptor may prime NOTCH pathway through JAGGED1 that in turn activated pro-inflammatory cytokine transcription and consequently a strong inflammatory response. The treatment with HMW-HA reduced the expression of pro-inflammatory mediators, as well as NF-kB activation. In addition, HMW-HA was able to reduce MAPK, JAGGED1, NOTCH1/2, and RBP-J activity. As HMW-HA acts by blocking TLR4 activation, specific NOTCH1/2 siRNAs were used to verify if the inflammatory response could be further influenced. Results proved that IL-6, IL-1β and MMP-13 were further reduced, those suggesting that NOTCH-1/2 signaling can be also activate independently by TLR-4 activation
Conclusion: These results suggest that HMW-HA exert a protective effects on human primary chondrocytes by indirectly reducing JAGGED/NOTCH pathway trough TLR-4 modulation.

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P033
The mannose-binding lectin and the extracellular matrix

Andreas Kungl[1], Lisa Weinheimer[1]

[1]Institute of Pharmaceutical Sciences, University of Graz, Graz, Austria

Purpose: The carbohydrate binding domain (CRD) of C-type-lectins enables these proteins to participate in immunologic cascades through the extracellular matrix (ECM) interacting with adjacent cells [1, 2]. Several lectins are upregulated during tissue remodeling, hence it is assumed that these proteins are enrolled in untangling complex processes. Recent work has shown that CLEC14 (C-type lectin domain family 14 member A), has a heparan sulfate binding site which indicates a link with glycosaminoglycans (GAGs) in the ECM [3]. Mannose-binding lectin (MBL) is a soluble protein distributed as oligomer via blood flow. Besides its important role in the innate immune system where it opsonizes pathogens via mannose-binding, little research has been done on interactions of MBL with ECM components such as GAGs. Especially. Here we were particularly interested in the CRD domain of MBL.
Methods: MBL-CRD was expressed in E. coli and purified with affinity-chromatography. Isothermal fluorescence titration (IFT) was used to determine the binding affinities (KD) of MBL-CRD and full length MBL for heparan sulfate and other ECM components.
Results: Reducing MBL to its CRD domain left intact its intrinsic mannose binding affinity. Furthermore, IFT experiments revealed a KD value in the nanomolar range for both MBL-CRD and MBL with the GAGs HS and heparin.
Discussion: Binding of MBL to glycans in the ECM via its CRD is supposed to immobilise the lectin and thereby support its matrix remodelling function.
Conclusion: Our results indicate a participation of MBL in ECM function which will be further explored in the direction of immune cell (re-)direction.

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P035
Pulsed electric fields as promising tool for treating skin fibrosis

Gouarderes Sara[1], Camille Ober[2], Layal Doumard[1], Jany Dandurand[2], Patricia Vicendo[1], Alexander Golberg[3], Valérie Samouillan[2], Gibot Laure[1]
[1]IMRCP, CNRS UMR5623, Toulouse, France; [2]CIRIMAT, UMR5085, Toulouse, France; [3]Porter School of Environment and Earth Sciences Studies, Tel Aviv University, Tel Aviv, Israel

Purpose: Physical stimuli appear as attractive tools to remodel extracellular matrix (ECM) [1]. For that purpose, we assessed the potential of pulsed electric field technology (electroporation), classically applied to drug and plasmid delivery, to remodel collagen at tissue scale.
Methods: Gene electrotransfer and electrochemotherapy electric parameters were applied without addition of any drugs onto a self-assembled tissue-engineered human dermal substitute model devoid of exogenous material [Fig. 1]. ECM remodelling was examined through genes modulation by transcriptomic and proteins synthesis, Fourier-Transform Infrared spectroscopy, Differential Scanning Calorimetry as well as MMPs activity.

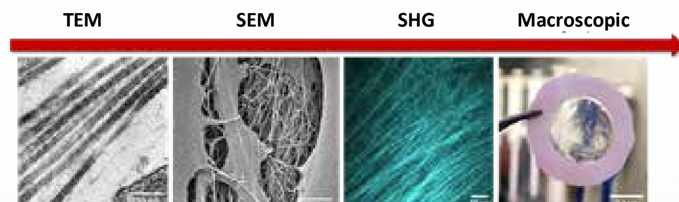

Figure 1: Multi-scale analyse of ECM in human dermal substitute.

Results: Electroporation induced 1) a rapid modulation of mRNA's genes composing the matrisome, particularly a down-regulation of pro-collagens and ECM maturation's enzymes; 2) a transient decrease in pro-collagens production and hydroxyproline tissue content; 3) a long-lasting ROS-dependent over-activation of MMPs, especially collagenase's family and 4) a down-regulation of TGF-β1 [2].
Discussion: Since electroporation is already used today in clinics, validated equipment (generator, electrodes, dosimetry) is available. Our research therefore has a strong applicative potential.
Conclusion: Our results open up realistic and relevant prospects for pulsed electric field technology as a local and effective treatment of skin abnormal ECM.

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P036
Extracellular matrix degradation and matrix metalloproteinases' dysfunction drive hypermobile Ehlers-Danlos syndrome fibroblast-to-myofibroblast transition: potential therapeutic targets from proteome profiling

Nicoletta Zoppi[1], Nicola Chiarelli[1], Daniele Capitanio[2], Marina Venturini[3], Cecilia Gelfi[2], Marco Ritelli[1], Marina Colombi[1]
1Department of Molecular and Translational Medicine, Division of Biology and Genetics, University of Brescia, Brescia, Italy; 2Department of Biomedical Sciences for Health, Department of Biomedical Sciences for Health, University of Milan, Segrate (MI), Italy; 3Division of Dermatology, Department of Clinical and Experimental Sciences, Spedali Civili University Hospital, Brescia, Brescia, Italy

Purpose: During tissue injury and regeneration, myofibroblasts are involved in extracellular matrix (ECM) remodeling/reabsorption and inflammation's resolution. Their persistent activation induces aberrant ratio of secreted ECM proteins, ECM-degrading matrix metalloproteinases (MMPs), and inhibitors TIMPs, contributing to prolonged immune response, chronic inflammation, and pain. Hypermobile Ehlers-Danlos syndrome (hEDS) is a heritable connective tissue disorder orphan of genetic etiology and specific therapies mainly characterized by generalized joint hypermobility and musculoskeletal complaints. In patients' dermal fibroblasts we reported a widespread ECM disarray and myofibroblast-like traits including α-smooth muscle actin (α-SMA) fibers organization, cadherin-11 expression, and increased levels of secreted MMP9. Control fibroblasts treated with hEDS cells-derived conditioned media (hEDS-CM) acquire this pathological phenotype [1].
Methods: A comprehensive proteomic study based on top-down and bottom-up approaches was performed by comparing the intra- and extracellular proteome of patients' and controls' fibroblasts. We also performed in vitro studies to define the effect of the MMP inhibitor doxycycline on ECM organization and fibroblast-to-myofibroblast transition (FMT).
Results: Cellular proteome analysis disclosed protein changes involved in actin cytoskeleton dynamics, proteostasis, intracellular trafficking, and secretion [2]. Secretome profiling revealed MMP1 upregulation and decrease of TIMP2, related to fibronectin, type I collagen, and tenascins fragmentation in hEDS-CM. MMPs inhibition with doxycycline rescued in hEDS cells a control-like ECM, partly reverted the myofibroblast phenotype, and abolished ECM disarray and FMT induced in control cells by hEDS-CM [3].
Discussion: MMPs dysfunction and ECM degradation coupled with FMT contribute to hEDS pathogenesis.
Conclusion: We provide evidence on putative disease targets for the development of therapeutic strategies with a potential benefit for patients' management.

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P037

Curcumin inhibits endoplasmic reticulum stress induced by the retention of the mutant matrix protein matrilin-3 in multiple epiphyseal dysplasia

Ella Patricia Dennis[1], Robyn Watson[1], Michael Briggs[1]
[1]Biosciences, United Kingdom

Purpose: Multiple epiphyseal dysplasia (MED) is a clinically and genetically heterogenous genetic skeletal dysplasia (GSD) that presents in childhood with disproportionate short stature, limb deformities and painful joints. Chondrocyte endoplasmic reticulum (ER)-stress in the cartilage of growing bones caused by the accumulation of misfolded mutant protein is a core disease mechanism in a range of GSDs, including MED resulting from mutations in the structural extracellular matrix protein matrilin-3 (MED-MATN3). As such, targeting ER-stress is an effective therapy to restore homeostasis and improve long bone growth. Indeed, the anti-epileptic drug carbamazepine (CBZ) has proven effective in reducing ER-stress in the treatment of a related GSD (MCDS) and is now being used in an EMA-approved clinical trial. Although CBZ has been shown to be successful in treating MCDS, it is ineffective in MED. Besides surgical intervention and joint replacement in early adulthood there are no treatments for MED. This study therefore focused on identifying novel treatments for MED-MATN3 and addressing an area of unmet clinical need.
Methods: Using a pre-validated ER stress luciferase reporter we determined the ability of compounds to reduce pathological ER-stress in our fully characterized MED-MATN3 cell model. The effects of successful candidates on ER-stress pathways, mutant MATN3 aggregation and cell phenotype were then analysed.
Results: Our data show that curcumin, a derivative of turmeric, is not only able to reduce ER-stress caused by the intracellular aggregation of mutant matrilin-3 in MED, but also reduces the ER accumulation of the mutant protein itself.
Discussion and Conclusion: To date, no tested chemicals have proven successful at restoring ER homeostasis in MATN3-MED. We show for the first time that curcumin has therapeutic potential to improve quality of live by restoring chondrocyte homeostasis, bone growth and cartilage integrity in MED-MATN3.

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P038

Nanobodies directed against procollagen C-proteinase enhancer 1 efficiently slow down the proteolytic maturation of fibrillar procollagens

Priscillia Lagoutte[1], Bourhis Jean-Marie[2], Gueguen-Chaignon Virginie[3], Mariano Natacha[1], David Vandroux[4], Catherine Moali[1], Sandrine Vadon-Le Goff[1]
[1]Tissue Biology and Therapeutic Engineering Laboratory (LBTI), UMR5305 CNRS / University of Lyon, Lyon, France; [2]Institut de Biologie Structurale, University Grenoble Alpes, CEA, CNRS, Grenoble, France; [3]Protein Science Facility, SFR BioSciences, UAR3444/US8, Lyon, France; 4NHV Medicinal, Dijon, France

Purpose: The excessive deposition of fibrillar collagens is a hallmark of fibrosis. Their biosynthesis involves different enzymes but procollagen N- and C-proteinases (PNPs and PCPs) play a major role by triggering the final step of fibril formation. PCPE1 (Procollagen C-Proteinase Enhancer1), a regulatory protein which activates the C-terminal maturation of procollagens by the main PCPs, is widely overexpressed in fibrotic contexts and could be a relevant target to develop novel anti-fibrotic strategies. The aim of our project was thus to develop and evaluate PCPE1 antagonists.
Methods: Nanobodies against PCPE1 were generated by two different strategies: an in vivo selection based on lama immunization and an in vitro selection using a synthetic library. Their affinity for PCPE1 and their capacity to inhibit PCPE1/procollagen interaction were analyzed by SPR. Finally, their ability to modulate procollagen maturation in vitro and in cellulo was also measured.
Results / Discussion: Two nanobodies, I5 and H4, showed a high affinity for PCPE1 and were able to inhibit PCPE1 interaction with procollagens. Moreover, they had the capacity to simultaneously interact with PCPE1. The structure of the complex formed by H4, I5 and CUB1CUB2 (the active part of PCPE1) showed that H4 binds to CUB1 whereas I5 binds to CUB2. Combining I5 and H4 in a bivalent fusion (diabody D1) improved the affinity for PCPE1 (K_D = 0.32 nM). Better still, diabody D1 was able to prevent the stimulation of procollagen cleavage by PCPE1 in vitro. In cellulo, treatment of cardiac or skin fibroblasts with diabody D1 led to a decrease in C-propeptide release, suggesting inhibition of procollagen I processing.
Conclusion: The selected nanobodies are the first antagonists of PCPE1. They display a high affinity for PCPE1 and have the capacity to prevent the stimulation of procollagen processing in vitro and in cellulo. In future work, we will evaluate the potency of PCPE1 antagonists as novel anti-fibrotic molecules in an animal model of cardiac fibrosis.

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P039
Post-natal N-acetylcysteine treatment improves the skeletal phenotype in an animal model of diastrophic dysplasia

Chiara Paganini[1], Chiara Gramegna Tota[1], Alessandra Leone[1,2], Antonella Forlino[1], Morgane Bourmaud[3], Martine Cohen-Solal[3], Antonio Maurizi[4], Anna Maria Teti[4], Simona Villani[5], Andrea Superti-Furga[6], Antonio Rossi[1]
[1]Deparment of Molecular Medicine, University of Pavia, Pavia, Italy; [2]Scuola Universitaria Superiore IUSS, Pavia, Italy; [3]Inserm U1132 and University Paris 7, Hopital Lariboisiere, Paris, France; [4]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy; [5]Department of Public Health, Experimental & Forensic Medicine, Unit of Biostatistics, University of Pavia, Pavia, Italy; [6]Division of Genetic Medicine, Lausanne, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Switzerland

Purpose: Diastrophic dysplasia (DTD) is a recessive chondrodysplasia caused by mutations in the SLC26A2 gene encoding for a sulfate transporter. Its impairment leads to reduced intracellular sulfate level causing cartilage proteoglycan (PG) undersulfation. Since intracellular sulfate in part may come from the catabolism of sulfur-containing amino acids and other thiols (i.e. N-acetylcysteine, NAC), we investigated the effect of post-natal NAC treatment in a murine model of DTD (dtd mouse).
Methods: Wild-type and dtd mice were injected with 250 mg NAC/Kg body weight twice a day for 7 or 21 days starting from birth. The skeletal phenotype was studied by X-ray imaging, microCT and DEXA analysis, while PG sulfation was measured by HPLC analysis. Growth plate morphology was studied by conventional histology.
Results: PG sulfation and morphometric measurements of long bones showed only an amelioration trend in dtd mice treated with NAC for 7 days, while both parameters were significantly increased in dtd mice after 21 days treatment. Moreover, after 21 days, growth plate architecture was improved in treated dtd mice compared with the placebo group. The improvement of trabecular bone and of bone mineral content (BMC) was observed in dtd mice treated with NAC for 21 days.
Discussion: In a short-term NAC treatment (7 days) only an amelioration trend in the skeletal phenotype and in cartilage PG sulfation was observed in dtd mice suggesting that a longer treatment could be more effective. Indeed after 21 days drug treatment, PG sulfation and length of long bones were increased in dtd mice. The improvement of endochondral ossification was confirmed by the amelioration of growth plate morphology. Moreover, the bone quality was improved as demonstrated by the increase of trabecular bone parameters and of BMC in treated dtd mice compared with untreated animals.
Conclusion: Our results demonstrated that NAC might pave the way for a pharmacological treatment of DTD.

P040
Identification of potential non-invasive biomarkers in diastrophic dysplasia

Chiara Paganini[1], Ricki Carroll[2,3], Chiara Gramegna Tota[1], Alessandra Leone[1,4], Andrea Schelhaas[2], Angela Duker[2], Ryan Coghlan[5], Brian Johnstone[5,6], Carlos Ferreira[7], Stefania Boni[8], Riccardo Albertini[9], Antonella Forlino[1], Luisa Bonafè[10], Andrea Superti-Furga[10], Andreas Zankl[11], Michael Bober[2,3], Antonio Rossi[1]
[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [2]Nemours Children's Hospital, Wilmington, USA; 3Thomas Jefferson University, Philadelphia, USA; [4]Scuola Universitaria Superiore IUSS, Pavia, Italy; [5]Shriners Hospitals for Children, Portland, USA; [6]Oregon Health and Science University, Portland, USA; [7]National Human Genome Research Institute, NIH, Bethesda, USA; [8]Medical Genetic Unit, S. Martino Hospital, Belluno, Italy; [9]Laboratory of Clinical Chemistry, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [10]Division of Genetic Medicine, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland; [11]The Children's Hospital at Westmead and Garvan Institute for Medical Research, University of Sydney, Sydney, Australia

Purpose: Diastrophic dysplasia (DTD) is a recessive chondrodysplasia caused by mutations in the SLC26A2 gene encoding for a sulfate/chloride antiporter of the cell membrane crucial for sulfate uptake and glycosaminoglycan (GAG) sulfation. In view of future therapeutical approaches to DTD the identification of non-invasive biomarkers is crucial to assess the efficacy of the treatment. In this work, we have investigated urinary GAG sulfation and N-terminal fragment of collagen X (CXM) in blood as potential biomarkers.
Methods: Urine was collected from patients affected by DTD and age matched controls and from dtd (an animal model of DTD) and wild type mice at 1 and 2 months of age and from newborn treated with acetylcysteine (NAC) or placebo during fetal period. Urinary GAGs sulfation was analyzed by HPLC. CXM was assessed in human blood samples or in dried blood spots.
Results: The percentage of non-sulfated disaccharide in urinary GAGs was higher in dtd mice compared with wild-type animals at all age points. After NAC treatment in fetal period, urinary GAG sulfation was increased in dtd newborns compared with the placebo group. Urinary GAGs were undersulfated in DTD patients compared with age matched controls and unaffected family members. CXM level was lower than normal in 73% of DTD patients.
Discussion: Dtd mice showed a significant GAG undersulfation compared with wild-type animals, that was partially recovered after NAC treatment during the fetal period. In humans, among the 16 DTD patients, 13 individuals presented a z score > 1. In control samples GAG sulfation slightly decreased from 0 to 12 years of age suggesting no differences related to pubertal growth. In 5 individuals among 11 patients analyzed for CXM a z score < -1 was observed.
Conclusion: Urinary GAG sulfation and CXM are two reliable biomarkers of different aspects of the DTD pathology. Urinary GAGs point to the overall metabolism of sulfated GAGs, while CXM is a specific marker of endochondral ossification.

P041
Superoxide dismutase 3 increases the integrity of extracellular matrix in fibroblasts

Jin Hyung Kim[1], Hae Dong Jeong[1], Min Ji Song[2], Dong Hun Lee[2], Jin Ho Chung[2], Seung-Taek Lee[1]
[1]Department of Biochemistry, Yonsei Unversity, Seoul, South Korea;
[2]Department of Dermatology, Seoul National University College of Medicine, Seoul, South Korea

Purpose: The superoxide dismutase (SOD) family functions as a reactive oxygen species (ROS)-scavenging system by converting superoxide anions into hydrogen peroxide in the cytosol (SOD1), mitochondria (SOD2), and extracellular matrix (SOD3) [1-3]. In this study, we examined the potential roles of SOD family members in skin aging.
Methods: The expression levels of Sod members were analyzed in the skin tissues of young and aged mice and humans. Changes in MMP-1 and type I collagen levels induced by SOD3 were analyzed in fibroblasts. Under three-dimensional culture conditions of fibroblasts mimicking the dermis, effect of SOD3 on the synthesis and breakdown of type I collagen was examined.
Results: We found that SOD3 expression levels were significantly reduced in the skin tissues of old mice than in young mice and human counterparts, but SOD1 and SOD2 expression levels remained unchanged with aging. Treatment of foreskin fibroblasts with recombinant SOD3 reduced secretion of matrix metalloproteinase (MMP)-1, while increasing the secretion of type 1 collagen. The effects of SOD3 were greater in fibroblasts treated with the tumour necrosis factor (TNF)-α. In a three-dimensional culture of foreskin fibroblasts, SOD3 decreased the amount of type I collagen fragments produced by MMP-1 and increased the amount of nascent type 1 procollagen.
Discussion: The expression of SOD3 decreases with age. In addition, SOD3 plays a role in maintaining ECM integrity in fibroblasts in the absence and presence of TNF-α. Therefore, SOD3 may have an effect of delaying both intrinsic and extrinsic skin aging.
Conclusion: These results demonstrate that SOD3 suppresses MMP-1 expression and induces type I collagen expression in fibroblasts. Therefore, we suggest that SOD3 plays a role in delaying or preventing skin aging.

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P042
Imaging of intestinal inflammation with iron oxide based contrast agent depends on hyaluronic acid content in tissue

Laura Golusda[1]
[1]Charité – Universitätsmedizin Berlin, Medical Department of Gastroenterology, Infectiology and Rheumatology, Berlin, Germany

Purpose: We used VSOP enhanced magnetic resonance imaging (MRI) to visualize intestinal inflammation in mouse models of colitis (dextran sodium sulfate induced colitis and transfer colitis). In inflammatory bowel diseases (IBD) inflammation brings along excessive changes in the extracellular matrix (ECM) of the intestine. MRI is used for diagnosis of fibrotic strictures. As studies raised concerns about the safety of gadolinium based contrast agents an iron oxide based contrast agent called very small iron oxide nanoparticles (VSOPs) is currently studied as an alternative. Studies suggest that VSOPs specifically bind to ECM components.
Methods and Results: MRI showed VSOP accumulation in colon wall of DSS-induced colitis mice while no accumulation was detected in transfer colitis mouse model. With high pressure liquid chromatography (HPLC) hyaluronic acid (HA) and chondroitin sulfate 4S (CS-4S) content was analyzed in colon tissue samples from DSS-induced colitis and transfer colitis mice. This showed an increase in HA in DSS-induced colitis while HA in transfer colitis decreased. Histological staining for HA and CSPG4 showed higher levels of HA in DSS-induced colitis and decrease of CSPG4. While in transfer colitis the levels of HA did not change but CSPG4 significantly increased. Moreover, imaging mass cytometry (IMC) confirmed higher amount of VSOPs in colon tissue from DSS-induced colitis mice. Further, the analysis showed uptake of VSOPs by macrophages expressing the hyaluronic receptor CD44.
Discussion: The application of VSOPs in MRI for intestinal inflammation needs further validation until clinical application. As ECM changes at the onset of inflammation, these changes might be detected with VSOPs as accumulation is already visualized 2 days after DSS-treatment.
Conclusion: Higher levels of HA lead to better accumulation of VSOP-filled macrophages in the inflamed tissue due to interaction of CD44 with HA in the colon tissue.

P043
A panel of high affinity chondroitin sulfate antibodies for targeting solid tumors

Elena Vidal-Calvo[1,2], Anne Martin Salazar[1,2], Ali Salanti[1,2]
[1]Institute of Immunology and Microbiology, Center of Medical Parasitology, University of Copenhagen, Copenhagen, Denmark; [2]VAR2 Pharmaceuticals Aps, Copenhagen, Denmark

Purpose: Onco-fetal Chondroitin Sulfate (ofCS) is a long sulfated hybrid glycosaminoglycan (GAG) expressed on the cell surface, secreted or shedded into the tumor microenvironment. We have described ofCS to be present in tumors and absent from normal tissues and to play a role in cell growth, angiogenesis, and migration. A malaria protein, VAR2CSA, binds with high affinity to ofCS, yet a monoclonal antibody (mAb) with same specificity could provide an optimal cancer targeting therapeutic compound.
Methods: 5 phage display libraries were used to select binders. Biosensor, ELISA and flow cytometry evaluated the affinity, specificity and binding to chondroitin sulfates (CS) and cancer cell lines, respectively. Tumor specificity was validated by immunofluorescence on human tissues. Pull downs from human biopsies were analyzed by mass spectrometry (MS) for identification of mAb-binding proteoglycans (PG). Tumor localization was assessed in vivo in mice and the anti-tumor efficacy of the best candidates was tested as Antibody-Drug-Conjugates (ADC).
Results: The panning on ofCS generated 20 mAb with distinct specificities to CSA, CSB or CSC. Few antibodies displayed strong tumor specificity, and these mostly recognized long hybrid structures with low nanomolar affinities. MS pull downs identified tumor relevant PG as targets. Staining of multi-organ arrays showed that one mAb specifically bound most tumor sections with limited binding to normal tissues. Intravenous injection in tumor bearing mice confirmed tumor specificity and MMAE-conjugated ADC effectively restricted tumor growth in spontaneous breast cancer.
Discussion: The difficulty of developing GAG-targeting mAb was circumvented by the present method which opens a window for the generation of new high affinity GAG-binders.
Conclusion: We have developed and validated a panel of antibodies targeting cancer-associated CS. Several of the antibodies holds the promise to be developed into novel cancer targeting therapies.

P044
Target intracellular stress with a new chemical chaperone to treat osteogenesis imperfecta

Nadia Garibaldi[1,2], Roberta Besio[2], Francesca Tonelli[2], Cecilia Masiero[2], Carla Aresi[2], Filippo Doria[3], Alessandra Carriero[1], Antonella Forlino[2]
[1]Department of Biomedical Engineering, The City College of New York, New York, USA; [2]Dipartimento di Medicina Molecolare - Unità di Biochimica, [3]Dipartimento di Chimica Organica, Università degli Studi di Pavia, Pavia, Italy;

Purpose: Mutations in collagen I α chains, causing classical Osteogenesis Imperfecta (OI), lead to abnormal collagen assembly, structure and secretion, impairing bone intracellular homeostasis and bone extracellular matrix. We recently demonstrated that the treatment with the chaperone 4-phenylbutyrate (4-PBA) restores OI murine osteoblasts (OBs) morphology and function. Nevertheless, its limited half-life in vivo together with the lack of a bone specific delivery system limit its efficacy. Thus, identifying molecules that overcome these drawbacks is particularly relevant for patients.
Methods: Molecular modelling starting from 4-PBA structure allowed the identification of a more stable derivative, MOD-4-PBA. Following toxicity evaluation, in vitro assessment of its efficacy was performed in primary OBs isolated from the OI murine model Brtl using thioflavin T labelling of intracellular protein aggregates, qPCR-based arrays, western blot and immunofluorescence.
Results: MOD-4-PBA administration was well tolerated and non-toxic in Brtl OBs up to 5 mM. Thioflavin T fluorescence, which was increased in Brtl OBs compared to controls due to mutated collagen accumulation, was reduced following treatment. The increased expression of chaperones and ER quality control proteins in treated OBs suggests an attempt of the cells to respond to intracellular collagen accumulation and was associated to a decreased expression of the apoptosis marker cleaved caspase 3. Ongoing protein secretion assay will allow to confirm the drug effect on OBs proteostasis. In vivo MOD-4-PBA stability, impact on bone quality and its effects on the extracellular matrix composition is under evaluation in OI zebrafish models.
Discussion and Conclusion: MOD-4-PBA administration ameliorated proteostasis, induced an ER response that stimulates protein folding and quality control and protected cells from apoptosis.

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P045
Fibulin-5 as a potential blister-preventive therapy in dystrophic epidermolysis bullosa

Mariana D. Malta[1,2], Maria G. Fernandes[1,2], António Andre[3], Pedro Martins[3,4], Ricardo A. Pires[1,2] Rámon Novoa-Carballal[1,2], Hugo Osório[5,6,7], Christina Guttmann-Gruber[8], Thomas Kocher[8], Mariana T. Cerqueira[1,2], Alexandra Marques[1,2]
[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Barco, Portugal; [2]ICVS/3B's–PT Government Associate Laboratory , Braga/Guimarães, Portugal; [3]INEGI, Porto, Portugal; [4]ARAID, i3A, Universidad de Zaragoza, Spain; [5]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [6]Ipatimup - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal; [7]FMUP – Faculdade de Medicina da Universidade do Porto, Porto, Portugal; [8]EB House Austria, Research Program for Molecular Therapy of Genodermatoses, Department of Dermatology and Allergology, University Hospital of the Paracelsus Medical University, Salzburg, Austria

Purpose: COL7A1 mutations cause dystrophic epidermolysis bullosa (DEB), an incapacitating disease with varying degrees of severity ranging from a severe variant with widespread blistering and chronic wounds (recessive) to a milder form with localized blistering (dominant). Current treatment relies on wounds/pain management, therefore novel therapeutic approaches to prevent skin blisters are urgently needed. In this work, we uncovered biological and mechanical differences in the dermal ECM of DEB variants, and, for the first time, we explored fibulin-5 as a potential blister-preventive therapy.
Methods: Immortalized fibroblasts from three DEB variants were obtained from EB House Austria and cultured for 14 days with ascorbic acid to maximize ECM deposition. The ECM was analyzed regarding their composition (mass spectrometry), organization (TEM), and mechanical properties (AFM and uniaxial tensile testing). Based on the results recombinant fibulin-5 was added to the cell culture to evaluate a potential beneficial effect over the ECM mechanical features.
Results: Extracellular proteome analysis revealed that each DEB variant has their unique fingerprint. Despite this, all variants presented a decrease in fibulin-5 expression and a significant reduction in the mechanical stability and stiffness of the ECM. These features were enhanced after addition of fibulin-5 due to a significant increase of elastic fibers crosslinking in all DEB variants.
Discussion: Our findings show that in all DEB variants ECM mechanical fragility is associated to lower expression of fibulin-5. Moreover, fibulin-5 supplementation reverts that debility, enhancing the mechanical strength of the ECM.
Conclusion: This study highlights the use of fibulin-5 as a potential blister-preventive therapy in DEB to enhance skin mechanical resistance minimizing blistering.

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P046
Tumor and stroma targeted and MMP14 active hyaluronic acid nanoparticles carrying chemotherapy drugs for targeted therapy of pancreatic cancer by overcoming stromal cell and extracellular matrix barriers

Lily Yang[1], Lei Zhu[1]
[1]Emory University, Surgery, Atlanta, USA

Purpose: The presence of extensive stromal cell and extracellular matrix in pancreati cancer creates a drug delivery barrier that limits therapeutic response. To overcome the stromal barrier, we have developed a stroma penetrating recombinant protein as a targeting ligand. Urokinase plasminogen activator receptor (uPAR) is highly expressed in tumor and stromal cells. Pancreatic cancer cells also express a very high level of MMP14 to cleave fibrillar collagens and other extracellular matrix proteins to facilitate cancer cell invasion through dense extracellular matrix.
Methods: We produced a fusion protein containing the amino terminal fragment (ATF) of uPA and the catalytic domain of MMP14 (ATF-MMP14). To develop an efficient nanoparticle drug delivery system, we produced hyaluronic acid nanoparticles (HANP) encapsulated with SN38, an active metabolite of irinotecan. Conjugation of ATFmmp14 ligand to HANP/SN38 resulted in a dual uPAR and CD44 targeted and MMP14 protease active nanoparticle drug (ATFmmp14-HANP/SN38).
Results: Systemic delivery of ATFmmp14-HANP led to significant increase in intratumoral drug delivery in an orthotopic pancreatic cancer patient derived xenograft (PDX) model. Most importantly, ATFmmp14-HANP/SN38 could migrate through the dense stromal cellular and extracellular matrix barriers to efficiently enter into cancer cells lining the ductal lumen. Following five treatments of ATFmmp14-HANP/SN38 at equivalent dose of 5 mg/kg of SN38 in the PDX tumor bearing nude mice, we found significant tumor growth inhibition compared to the no treatment control, free drug, and non-protease active HANP/SN38 treated mice. We further found that ATFmmp14-HANP/SN38 treatment at 10 mg/kg dose significantly prolonged survival of the mice bearing pancreatic PDX tumors compared with currently used combination therapies for pancreatic cancer.
Discussion: ATFmmp14-HANP/SN38 is the first targeted nanodrug that reduced levels of FAP+ fibroblasts and collagen, but retained overall stromal structure that prevents tumor cell invasion.
Conclusion: ATFmmp14-HANP/SN38 is a promising stroma penetrating nanodrug for targeted therapy of pancreatic cancer.

P047

Dioxin-like pentachlorobiphenyls promote extracellular matrix remodeling by increasing the expression of endothelial cell-specific molecule 1 and metalloproteinases, and induce epigenetic modifications in thyrocytes

Aurelio Minuti[1], Federica Aliquò[1], Rosaria Ruggeri[2], Martina Laganà[3], Marta Ragonese[3], Angela Avenoso[1], Giuseppe Maurizio Campo[2], Fiorenza Gianì4, Salvatore Campo[1], Salvatore Cannavò[3], Michele Scuruchi[2], Angela D'Ascola[2]
[1]Biomedical and Dental Sciences, and Morpho-functional Images, University of Messina, University Hospital G. Martino, Messina, Italy; [2]Dept Clinical and Experimental Medicine, [3]Dep Human Pathology of Adulthood and Childhood "G.Barresi", University of Messina, Messina, Italy; [4]Dept Clinical and Experimental Medicine, University of Catania, Catania, Italy

Purpose: The present study was aimed at investigating the effects of dioxin-like pentachlorobiphenyls (PCBs) on aryl hydrocarbon receptor (AHR)/ nuclear factor erythroid 2–related factor 2 (NRF-2) pathway and related long noncoding RNAs (lncRNAs) and endothelial cell-specific molecule 1 (ESM-1) in thyrocytes.
Methods: Primary human thyrocytes were exposed to increasing doses (5 and 10 μM) of 2,3',4,4',5-pentachlorobiphenyl (PCB-118) and 3,3',4',4',5 pentachlorobiphenyl (PCB-126) for 24 h. Cell culture and medium were collected to assess: mRNA levels of AHR, NRF-2, CYP1A1 lncRNAs (HOTAIR, MALAT-1, MEG-3), ESM-1, metalloproteinases (MMP) -3, MMP-9 and IL-1β by qPCR; protein levels of AHR and ESM-1 by western blot and ELISA, and ROS production by a commercial kit.
Results: Treatment with PCB-126 and PCB-118 at both doses significantly increased mRNA levels of AHR, NRF-2, CYP1A1, MMP-3, MMP-9, IL-1β and ROS production. Furthermore, PCBs, especially PCB-118, enhanced ESM-1, HOTAIR and MALAT-1 expression and reduced MEG-3 mRNA levels.
Discussion: PCBs are chemical pollutants able to promote inflammation and carcinogenesis. Our data demonstrated that PCB-118 and PCB-126 may induce AHR/NRF-2 pathway activation with a consequent ROS production and inflammatory responses in thyrocytes. This condition led to an increase in ESM-1, a circulating proteoglycan involved in angiogenesis and overexpressed in several tumors, and MMP-3 and MMP-9 that cause ECM remodeling. Furthermore, PCBs can induce epigenetic modifications by increasing lncRNAs MALAT-1 and HOTAIR expression, associated with cell proliferation and tumor growth, and reduce anti-oncogenic lncRNA MEG3.
Conclusion: These results suggest new mechanisms underlying toxic effects of PCBs exposure on thyrocytes, indicating ESM-1 and lncRNAs as putative key factors of thyroid tumorigenesis.

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P048

Catelysine, a molecule capable of regulating the tumor stroma and offering new therapeutic options to fight breast cancer?

Daphnée Bériot[1], Erik Maquoi[2], Agnès Noël[2], Alain Colige[1]
[1]Laboratory of Connective Tissues Biology, [2]Laboratory of Tumor and Development Biology, University of Liège, Giga-Cancer, Liège, Belgium

Purpose: In breast cancer, tumor growth, progression and metastasis are correlated with stromal formation and abundance1. This tumor micro-environment (TME) is made of extracellular matrix macromolecules, especially fibrillar collagens, immune cells and fibroblasts which are responsible for collagen synthesis and remodeling. Although TME is a prime target for treating cancer, molecules efficiently modifying its structure and turnover are lacking.
Methods: The catechin is a molecule able to modify collagen remodeling2,3 and to regulate mesenchymal cell activation4, suggesting its potential capacity to regulate the TME and tumor progression. By using in vitro and in vivo models, we quantify the impact of Catelysine (Cat:Lys), a catechin molecule conjugated to two lysines on tumor progression.
Results: We first investigated in vitro the effect of Cat:Lys on the architecture of collagen fibers. Cat:Lys dose-dependently increased collagen polymerization kinetics without involving covalent bond formation. Confocal reflection microscopy imaging revealed that high Cat:Lys concentrations prevented collagen fiber formation. Next, the impact of Cat:Lys on cell behavior was investigated. Live cell imaging performed on 2D cancer cell lines cultures demonstrated that Cat:Lys modulated cell proliferation and altered cell morphology in a cell-line and dose-de¬pendent manner. In vivo, Cat:Lys administrated per os dose-dependently decreased the complexity of the mammary epithelial tree during the mammary gland development.
Discussion: Currently, the effect of Cat:Lys on tumor progression as a single agent is being evaluated in MMTV-PyMT transgenic mice. In the future, we plan to study its impact as an adjuvant to conventional chemotherapy in breast cancer.
Conclusion: These results suggest that Cat:Lys affects dose-dependently the in vitro collagen fiber formation, the cell behavior in 2D and the mammary development of normal mice.

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P049
A new role for HAS2-AS1 in regulating apoptosis independently from hyaluronan metabolism in triple-negative breast cancer cells

Arianna Parnigoni[1], Paola Moretto[2], Manuela Viola[2], Evgenia Karousou[2], Alberto Passi[2], Martin Goette[1], Davide Vigetti[2]
[1]Department of Gynecology and Obstetrics, University of Münster, Münster, Germany; [2]Medicine and Surgery, University of Insubria, Varese, Italy

Purpose: In aggressive cancers, hyaluronan (HA) is accumulated in the tumour niche; although several studies report that HA favours tumour growth and spreading [1], the molecular mechanisms behind this cancer-promoting effect are not fully clear. The long non-coding RNA HAS2-AS1 is critical to controlling HAS2 gene expression via epigenetic modification, at least in aortic smooth muscle cells [2]. Yet, this mechanism does not apply to MDA-MB-231 cells, in which HAS2 expression levels remain unaltered after overexpressing HAS2-AS1, as well as secreted and pericellular HA levels. We recently described that HAS2-AS1 has a role in lowering MDA-MB-231 cell aggressiveness via modulating several genes involved in cell proliferation, invasion, survival, and mesenchymal-to-epithelial transition, but not related to HA metabolism [3]. The objective of this study is to better understand the molecular mechanisms underneath HAS2-AS1 modulation of triple-negative breast cancer (TNBC) cells lines aggressiveness. An intriguing cytoplasmic function of lncRNAs is their ability to bind miRNAs, creating a competition for the interaction between miRNA, lncRNA and other regulatory targets (ceRNAs).
Methods: We evaluated the capability of HAS2-AS1 to sponging miRNAs both in silico and in vivo. We also measured apoptosis induction in MDA-MB-231 stable clones overexpressing HAS2-AS1 via TUNEL assay, western blot and qPCR.
Results: In silico analysis revealed that HAS2-AS1 exon 2 transcript contains several putative binding sites for different miRNAs, among which miR186-3p. By luciferase assays, we confirmed the interaction between HAS2-AS1 and miR186-3p. Deep analysis of apoptosis induction upon HAS2-AS1 overexpressing stable clones revealed that HAS2-AS1 increases apoptosis in MDA-MB-231 cells via the activation of caspases 3, 7and 9, but not 8. Interestingly, HAS2-AS1/miR186-3p interaction was confirmed to be involved in apoptosis induction, likely via the purinergic receptor P2RX7 [4].
Conclusion: Our data suggest that the sponge effect of HAS2-AS1 can antagonize the function of the miR186-3p on its downstream targets inducing, among all, apoptosis pathway and lowering TNBC cells' aggressiveness.

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P050
Unveil the extracellular matrix signature from colorectal cancer patients-derived tissues: side matters?

Ângela Marques-Magalhães[1,2,3,] Marta Laranjeiro Pinto[2,3], Tânia Cruz[2,3], Joana Caldeira[3,4], Hugo Osório[5,6,7], Pedro Amoroso Canão[8], Elisabete Rios[8], Fátima Carneiro[3,8,7], Ana Patrícia Cardoso[2,3], Raquel M. Gonçalves[3,4,9], Maria José Oliveira[2,3,9,10]
[1]BiotechHealth Doctoral Programme, ICBAS - School of Medicine and Biomedical Sciences, [2]Tumour and Microenvironment Interactions Group, [4]Microenvironments for New Therapies Group, INEB - Institute of Biomedical Engineering, [3]i3S - Institute for Research and Innovation in Health, [5]Faculty of Medicine, [6]Proteomics, i3S - Institute for Research and Innovation in Health, [7]IPATIMUP – Institute of Molecular Pathology and Immunology, [9]ICBAS - School of Medicine and Biomedical Sciences, [10]Department of Pathology and Oncology, University of Porto, Porto, Portugal; [8]Department of Pathology, Centro Hospitalar Universitário São João, Porto, Portugal

Purpose: The extracellular matrix (ECM) is a main component of the tumor microenvironment (TME) with an active role in colorectal cancer progression [1]. This study aims to characterize the ECM signature of colon normal/tumor tissues, considering the anatomical locations (right and left) to further exploit the impact of tumor region in the crosstalk between the TME components.
Methods: Paired normal and tumor tissues from right- and left-sided colon were decellularized and analyzed by mass spectrometry (MS). Bioinformatic analysis was performed with DAVID software, and PCA plots and heat maps obtained through ClustVis software. Biomechanical properties were evaluated by rheology and SEM.
Results: The MS revealed 27 ECM proteins (5 up and 21 down) differentially expressed between normal and tumor samples from right-sided colon, and 28 proteins (23 up and 6 down) from left-sided colon. While clusters of gene ontology terms related to elastic fibers and calcium binding were found in upregulated proteins from both locations, downregulated proteins were related to proteoglycans and collagen binding in right- and left-sided tumors, respectively. Moreover, tumor decellularized ECM from left colon are stiffer than the normal counterpart, contrarily to what is observed in the right-sided colon.
Discussion: The altered proteins between normal and tumor tissue are mainly up-regulated in left-sided colon and downregulated in right-sided colon, which might indicate that throughout tumorigenesis, the protein expression mechanisms differ with the tumor location. Moreover, the higher overall stiffness in tumors compared to colon normal tissues was shown to be dependent on the tumor anatomical location, since this difference was only observed for left-sided tumors.
Conclusion: The colon matrisome displays distinct ECM signatures and biomechanical properties according to the location. Given the impact of the ECM features on TME dynamics and disease progression, it is crucial to consider these differences when studying disease biology and searching for new therapeutic targets/biomarkers.

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P051
Ascites microenvironment conditions the peritoneal pre-metastatic niche to promote the implantation of ovarian tumor spheroids: involvement of fibrinogen/fibrin and αV and α5β1 integrins

Carine Laurent-Issartel[1], Rémy Agniel[1], Alexandra Landras[1], Florence Giffard[2], Cécile Blanc-Fournier[2], Elisabete Da Silva[3], Johanne Leroy-Dudal[1], Franck Carreiras[1], Sabrina Kellouche[1]
[1]Université de Cergy Paris, Laboratoire Relations Matrice Extracellulaire-Cellules (ERRMECe); [2]Université de Normandie, Inserm U1086, Caen, France; [3]Université de Strasbourg, CNRS UMR 7021, Illkirch, France

Purpose: At least one-third of patients with epithelial ovarian cancer (OC) present ascites at diagnosis and almost all have ascites at recurrence [1] especially because of the propensity of the OC cells to spread in the abdominal cavity leading to peritoneal metastasis. Our previous studies demonstrate that ascites -accumulated in the abdominal cavity during the OC dissemination- is a unique tumoral microenvironment which regulates cancer cells behavior [2,3]. We investigated the ascites impact on the development of premetastatic niches and on the biological mechanisms leading to OC peritoneal colonization.
Methods: The effect of ascites on cell junctions, cytoskeleton and extracellular matrix proteins of mesothelial cells was assessed using immunofluorescence, western-blot and pull-down, respectively. The implantation and dispersion of OC spheroids on the mesothelium were analysed and quantified by an in vitro three-dimensional model of co-culture using imagery approaches.
Results: Ascites causes destabilization of the integrity of mesothelium with a modification of the organization of N-cadherin and ZO-1 cell junctions without affecting their synthesis. Moreover, ascites causes actin cytoskeletal reorganization dependent on the activity of Rac1. Ascites allows the organization of fibrinogen/fibrin network on the mesothelium and surrounding OC spheroids. Fibrin leads to OC spheroids adhesion to the mesothelium, and the ascites promotes their disaggregation and the clearance of mesothelial cells. Both αV and α5β1 integrins are involved.
Discussion: Destabilization of the actin cytoskeleton of mesothelial cells under ascitic conditions appears to be due to participation of Rho proteins.
Conclusion: Ascites and its fibrinogen/fibrin composition weaken the mesothelium and promotes peritoneal implantation of OC spheroid. These findings may be critical for a better understanding of the recurrence of OC disease.

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P052
Multimerin-2 represents a barrier to cancer cell dissemination

Evelina Poletto[1], Greta Carobolante[1], Eva Andreuzzi[1], Lucrezia Camicia[1], Maurizio Mongiat[1]
[1]Department of Translational Research, Division of Molecular Oncology, National Cancer Institute of Aviano (CRO), IRCCS , Aviano, Italy

Purpose: Tumor vessels are instrumental to support cancer progression. However, due to the aberrant functionality of endothelial cells (ECs), they are inefficient and leaky. We have found that the extracellular matrix glycoprotein Multimerin-2, specifically expressed by ECs, is required for the maintenance of vascular homeostasis [1] its role in modulating vascular homeostasis remains largely unexplored. Here we identified Multimerin-2 as a key molecule required to maintain vascular stability. RNAi knockdown of Multimerin-2 in endothelial cells led to cell-cell junctional instability and increased permeability. Mechanistically cell-cell junction dismantlement occurred through the phosphorylation of VEGFR2 at Tyr951, activation of Src and phosphorylation of VE-cadherin. To provide an in vivo validation for these in vitro effects, we generated Multimerin-2$^{-/-}$ (Mmrn2$^{-/-}$. Here, we hypothesised that Multimerin-2 loss in ECs could impact on cancer cell dissemination, promoting their passage through the EC barrier.
Methods: Multimerin-2 expression was analysed through IHC in human tumors. The effects of Multimerin-2 loss were assessed via transmigration assays and the mechanism verified by WB and qRT-PCR. B16F10 cells were intravenously injected in wt and Multimerin-2$^{-/-}$ mice and the metastatic foci evaluated.
Results: We found that Multimerin-2 expression is frequently lost in tumor vessels from ovarian, breast and colon cancer patients. ECs showed reduced Multimerin-2 expression under tumor-mimicking conditions. Multimerin-2-devoid ECs challenged with cancer cell-derived conditioned media displayed increased expression of the VCAM1, a key regulator endothelial transmigration. Accordingly, Multimerin-2 loss favored the passage of tumor cells through the ECs barrier. Consistently, Multimerin-2$^{-/-}$ mice displayed a higher number of lung metastatic lesions following the injection of tumor cells.
Discussion: These results suggest that cancer cells cause Multimerin-2 down-modulation favouring their passage through the EC barrier. This is achieved through the increase of VCAM1expression in Multimerin-2-devoid vessels, thus generating a more favourable microenvironment for metastatic dissemination [2].
Conclusion: Multimerin-2 emerges as a key molecule supporting vascular stability and halting the escape of cancer cells. Thus, Multimerin-2 devoid vessels could be the preferential route for metastatic dissemination, opening new therapeutic opportunities.

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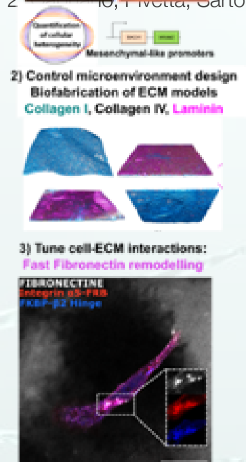
P053
miR125a-5p affects the protective role of EMILIN-1 in gastric cancer microenvironment

Alessandra Capuano[1], Maddalena Vescovo[1], Eliana Pivetta[1], Renato Cannizzaro[2], Roberto Doliana[1], Maurizio Mongiat[1], Paola Spessotto[1]

[1]Molecular Oncology, [2]Oncological Gastroenterology, Centro di Riferimento Oncologico di Aviano (CRO), Aviano, Italy

Purpose: Gastric cancer (GC) is one of the most frequent carcinomas world-wide. Tumor microenvironment gives a peculiar contribution to the aggressive biology of this malignant disease. The extra-cellular matrix (ECM) protein EMILIN-1 controls cell proliferation and represents a key structural element in the maintenance and function of Lymphatic Vessels (LVs) (1,2); here we analyze its possible role as a molecular regulator in in the context of GC development and progression.
Methods: EMILIN-1 expression and the association with LVs were analyzed with immunofluorescence (IF) in gastric biopsy samples. Normal stomach mucosa cells and several GC cell lines were employed for in vitro experiments. EMILIN-1 expression levels were evaluated in Western Blot, IF and Real Time PCR. miRNAs expression was quantified by TaqMan assays and their functions verified using miRNA mimics and inhibitors.
Results: EMILIN-1 is diffusely deposited in normal gastric mucosa and is significantly decreased in malignant and pre-neoplastic samples; this finding correlates with the presence of aberrant LVs. To unveil the main mechanisms responsible for EMILIN-1 reduction, in vitro models were employed. The treatment of fibroblasts and lymphatic endothelial cells (the major cellular sources of EMILIN-1 in the gastric microenvironment) with conditioned media derived from GC cells lines (but not from normal gastric cells) dramatically impacts on EMILIN-1 expression (at mRNA and protein levels). Among several candidates, we identified miR125a-5p as negative regulator of EMILIN-1.
Discussion: The observation that EMILIN-1 levels are reduced in malignant samples and in pre-neoplastic lesions suggests that loss of this ECM molecule may affect microenvironment changes and promote tumorigenesis in the stomach.
Con... r results demonstrate a novel regulatory mechanism of EMILIN-1 expression in GC via miR-125a-5p.



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P054
Cell-microenvironment interactions and cell heterogeneity modulation toolbox: correlation is not causation?

Kamboj Sahil[1], Alphonse Boché[1], Chen Changchong[2], Mathieu Morel[2], Olivier Gallet[1], Franck Carreiras[1], Sabrina Kellouche[1], Carole Aimé[2], Stephen J. Royle[3], Ambroise Lambert[1]

[1]Institute of Advanced Studies CY Cergy Paris Université, ERRMECe lab, Group MEC-uP, Neuville sur Oise, France; [2]École Normale Supérieure, PSL University, Sorbonne Université, CNRS, P.AS.T.E.U.R Lab, Paris, France; [3]Warwick Medical School, Royle Lab, Coventry, United Kingdom

Purpose: Ovarian cancer is the deadliest gynecological malignancy. Ovarian cancer cases often have excess fluid in the peritoneal cavity called ascites, which is a complex microenvironment. Matrix proteins like fibronectin (Fn) in the ascites interact with α5β1 integrin. Cell-microenvironment interactions, more specifically cell- extra-cellular matrix (ECM) interaction, modulate cancer cell heterogeneity, which may contribute to cancer drug resistance [1]. How to demonstrate causative link between cell-microenvironment interactions and cell heterogeneity modulation?
Methods: We designed a toolbox to 1) measure cell heterogeneity 2) control microenvironment and 3) modulate cell-matrix interactions. Ovarian carcinoma cell model (SKOV3) is used as a high Epithelial to Mesenchymal Transition (EMT) plasticity.

Results: 1. We developed a multi-parametric pipeline to measure EMT shifts in cell behavior. 2. We built ECM in vitro models to control the biochemical, mechanical and topological characteristics of the cell microenvironment [2]. 3. We enable rapid, fine tuning of cell-ECM interactions. We use an inducible system to tune α5β1 integrin trafficking, by initiating endocytosis of integrins (hotwiring). Hotwiring is highly effective in HeLa and SKOV3 cells.

Discussion: We can track the EMT plasticity using EMT-promoter reporter levels under Transformed Growth Factor B (TGF-B) induction. We show that topology of connective tissues impacts nucleus size and shape independently of stiffness and protein composition [3]. Cell-microenvironment interaction is tuned in few minutes effectively: hotwired SKOV3 cells increase the internalization of Fn through clathrin-mediated endocytosis.

Conclusion: This strategy allows modulation of cell response in controlled, tunable biological matrices to test drug response and broader effectiveness.

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P055
Role of Thrombospondin-1 in the microenvironment of osteolytic bone metastases

Laura Carminati[1], Elisa Longhi[1], Chiara Urbinati[2], Davide Scarpetta[3], Enrico Cabri[3], Patrizia Borsotti[1], Fabio Sangalli[4], Maddalena Fratelli[3], Marco Rusnati[2], Giulia Taraboletti[1]

[1]Oncology, [3]Biochemistry and Molecular Pharmacology, [4]Biomedical Engineering Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Bergamo, Italy; [2]Molecular and Translational Medicine, Università degli Studi di Brescia, Brescia, Italy

Bone is a common site of metastases from diverse tumors, particularly breast and prostate cancer. Breast cancer frequently develops osteolytic bone metastases, characterized by the upregulation of the bone-resorbing activity of osteoclasts, which favor excessive bone degradation. The matricellular protein thrombospondin-1 (TSP-1) plays a complex role in the tumor microenvironment and bone remodeling. This study aims at investigating the role of TSP-1 in bone metastasis, and particularly in osteolytic metastases generated by breast cancer by integrating different experimental approaches.
We have collected two sets of bone metastatic samples transcriptomics data from breast and prostate tumors – to compare osteolytic and osteoblastic lesions - available in public datasets. Pathway analysis of those gene sets, selected by (direct or inverse) significant correlation of expression in the two tumor types, reveals distinct patterns of (potential) functional involvement of TSP in processes related to bone formation/degradation.
In solid phase binding assays and SPR analysis, the TSP-1 domains showed variable ability to interact with cell receptors and soluble factors involved in osteoclast differentiation and signaling, including RANK and OPG.
To investigate the role of TSP-1 and its different domains on bone remodeling, we analyzed the effects of TSP-1 recombinant fragments on osteoclastogenesis. Recombinant TSP-1 fragments differently affected the differentiation of osteoclasts form bone marrow precursors or from the RAW 264.7 monocyte/macrophage cell line. Finally, overexpression of a TSP fragment by tumor cells was effective in modulating the formation of osteolytic bone metastasis in vivo, in a preclinical model of breast cancer metastatic to the bone.
Through this multidisciplinary approach, our findings point to a role of TSP-1 as a relevant player in the microenvironment of bone metastasis and a potential basis to investigate new approaches for therapies.

P056
Collagen XIII in breast cancer

Charlotta Henriksson[1], Raman Devarajan[2], Anne Heikkinen[2], Jarkko Koivunen[3], Ritva Heljasvaara[2], Taina Pihlajaniemi[2]

[1]Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; [2]University of Oulu, Finland; [3]University of Turku, Finland

Purpose: Collagen XIII (ColXIII) is a transmembrane collagen whose extracellular domain can be proteolytically shed and incorporated into the pericellular matrix. ColXIII is overexpressed in many mesenchymal and epithelial human cancers including breast cancer (BC), especially in the triple negative (TNBC) subtype. This study investigates the roles and mechanisms of action of ColXIII in BC.
Methods: Transgenic mice expressing the Polyoma middle T-antigen in mammary epithelial cells (the MMTV-PyMT mice) were crossed with ColXIII knockout mice and mice expressing only the transmembrane form of ColXIII. The tumor growth and formation of metastases was monitored. In addition, reciprocal orthotopical tumor cell transplantations were performed between these mice.
Results: In the MMTV-PyMT mouse model the proportion of shed ColXIII increased along tumor progression. Deficiency of ColXIII in MMTV-PyMT model resulted in delayed primary tumor growth, reduced metastasis formation, and prolonged overall survival. The lack of shed ColXIII reduced metastasis in the MMTV-PyMT model but did not significantly affect primary tumor growth. The absence of ColXIII led to cystic lesions in the hyperplastic mammary tumors. In addition, the mammary tumors of these two Col13a1 mutants were more differentiated than those in the wild type MMTV-PyMT mice. Reciprocal orthotopical tumor cell transplantations suggested microenvironmental roles for ColXIII in breast cancer.
Discussion: We found that the lack of ColXIII in mice reduced tumor growth at an advanced stage and decreased the formation of lung metastases. In contrast, the presence of the transmembrane form of ColXIII in combination with a lack of the shed ColXIII form increased the primary tumor growth while metastasis formation was reduced. This interesting discovery suggest diverse roles for transmembrane and shed form of ColXIII in BC development.
Conclusion: ColXIII plays a vital role in BC and its transmembrane and shed forms seem to have different functions in mammary carcinogenesis.

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P057
Multi-omics analysis of epithelial-to-mesenchymal transition mediators in breast cancer

Miriam Buttacavoli[1], Emanuela Peri[1], Elena Roz[2], Ida Pucci[3], Salvatore Feo[1,3], Patrizia Cancemi[1,3]
[1]Department of Biological Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy; [2]La Maddalena Hospital III Level Oncological Department, Palermo, Italy; [3]Experimental Center of Onco Biology (COBS), Palermo, Italy

Purpose: Multi-omics approach to identify a cluster of genes/ proteins and their interrelated pathways involved in epithelial-to-mesenchymal transition (EMT) to outline a breast cancer (BC) EMT-signature.
Methods: Bioinformatics analysis and Western blotting validation.
Results: Using the EMTome database, 144 EMT BC-related genes were selected and analyzed using the UALCAN and the Kaplan-Meier Plotter databases, to assess their expression levels and the prognostic value in BC. Among these, 49 genes were differentially expressed, significantly associated with prognosis and functionally associated with syndecan-2. Finally, proteomic analysis was performed by Western blotting on 99 tumoral mammary tissues, 9 normal tissues adjacent to the cancer and 13 sera to verify the expression of two mesenchymal phenotype's markers (N-cadherin and Vimentin) and the expression of E-cadherin, epithelial phenotype's marker.
Discussion: The EMT is a complex and high regulated process involved in embryogenesis, wound healing processes and in the progression of different cancer types, including BC. Recently, it has been reported that the EMT and the mesenchymal-epithelial transition (MET) are the main mechanisms for BC metastasis During EMT, BC cells lose epithelial characteristics and acquire mesenchymal traits, including motility and invasiveness. In this study, we analyzed in silico a possible EMT-gene signature in BC and its biological interconnectivity and verified in BC tissues and sera a remarkable heterogeneity of expression, including the expression of several isoforms, of three master proteins involved in EMT: Vimentin, N-cadherin and E-cadherin.
Conclusion: In conclusion, it may be useful to identify predictive or prognostic markers and possible molecular targets to be used for personalized therapies. Further studies on the link between EMT markers and BC will contribute to identify the clinical significance of this heterogeneity.

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P058
The role of osteoblasts in the leukemic matrisome interface

Annalena Dittmann[1], Vilma Pesola[1], Jarkko Koivunen[1], Ujjwal Suwal[2], Pekka Rappu[2], Jyrki Heino[2], Valerio Izzi[1]
[1]Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; [2]Department of Life Technologies, University of Turku, Turku, Finland

Purpose: Osteoblasts (OB) and their precursors are essential building blocks of bones and bone marrow and one of the main producers of extracellular matrix in those tissues [1, 2]. During leukemogenesis the matrisome plays a crucial role in transitioning from normal haematopoietic- to leukemic stem cells (LSC). In the bone marrow, LSC interact with osteoblasts and actively create a leukemic niche to evade chemotherapy. Hence, OB play an important role in supporting LSC to evade chemotherapy and thus allowing relapse [3, 4]. We aim to investigate the composition of the leukemic matrisome interface (LMI) established between AML cells (AMLs) and OB, the intercellular communication network responsible for LMI formation and how OB change in contact with AML and under drug treatment.
Methods: We established an AML-OB co-culture (CC) model with human KG1a-GFP AMLs and murine MC3T3-dTomato OB. The CCs are treated with the anti-leukemic drug Ara-C, analysed via confocal microscopy, flow cytometry and qPCR for AML and OB marker expression and investigated for LMI changes by mass-spectrometry.
Results: Our results show that OB undergo morphological and physiological changes when in contact with AMLs. When cultured together, the formation of different specialized layers becomes evident. OB, especially, acquire a pleiomorphic shape at the OB-AML interface which is accompanied by drastic qualitative and quantitative changes in the matrisome they produce and hindered osteogenic differentiation.
Discussion: These findings are in line with previous observations of reduced osteogenic capacity of osteoblast lineage cells in leukemia in vivo [5, 6].
Conclusion: These findings validate our in vitro model of the patients' bone marrow minimal AML niche, deepen our understanding of the role of OB-derived matrisome in leukemogenesis and will help unveil new therapeutic targets.

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P059
Targeting acute myeloid leukemia by the leukemic matrisome interface

Vilma Pesola[1], Annalena Dittmann[1], Jarkko Koivunen[1], Ujjwal Suwal[2], Pekka Rappu[2], Jyrki Heino[3], Valerio Izzi[1]
[1]Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; [2]Department of Life Technologies, [3]Biochemistry University of Turku, Turku, Finland

Purpose: Acute myeloid leukemia (AML) is the most fatal of haematological cancers [1]. The Extracellular Matrix together with many extracellular enzymes, cytokines and growth factors - an ensemble known as the matrisome - has an important role when it comes to transitioning from normal haematopoietic stem cells to leukemic stem cells (LSC) [2]. LSCs are also in contact with the surrounding bone marrow cells such as osteoblasts (OB) and create a leukemic matrisome around them [2,3]. We are interested in the composition of the leukemic matrisome interface (LMI) between AML cells and OBs, the intercellular communication networks responsible for LMI formation and how the different cells influence each other upon drug treatment.
Methods: We co-culture (CC) human KG1a-GFP AML cells and murine MC3T3-dTomato osteoblasts. The CCs are treated with Ara-C, imaged via confocal microscopy, analysed using flow cytometry and qPCR for the expression of AML specific markers, and investigated for LMI-specific events using mass-spectrometry.
Results: We have noticed, that during co-culture AML cells form different layers with distinct properties: a top layer with floating AML cells, a middle layer where AMLs and OBs are in physical contact and a bottom layer with only a few, flat, macrophage-like AMLs attached to the OBs. AMLs on the top layer show a typical round morphology whereas at the AML-OB interface and within the OB layers, morphological changes of AMLs are evident. Interestingly, the change of morphology seems to accompany an increase in stemness and expression of cell binding markers in AML which is enhanced upon Ara-C treatment.
Discussion: AML continues to have a poor outcome and understanding the matrisome around it allows the discovery of potential novel approaches to treatment [4].
Conclusion: This in vitro model provides an inexpensive way to deepen our understanding of leukemogenesis in AML and the role of the leukemic niche in it.

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P060
Investigating the role and mechanisms of microRNAs in chondrosarcoma: a small RNA sequencing approach

Megan Heague[1,2], Yalda Ashraf[1], Panagiotis Balaskas[1], Mandy Peffer[1]
[1]Institute of Life Course and Medical Sciences, Liverpool, United Kingdom; 2Leeds School of Medicine, Worsley Building, Leeds, United Kingdom

Purpose: Chondrosarcoma (CS) is the second most common primary malignant bone tumour. The molecular mechanisms underlying CS are poorly understood, yet evidence suggests microRNAs (miRNAs) provide good candidates as therapeutic targets. We used an unbiased sequencing approach to define altering miRNAs in CS.
Methods: Small RNA sequencing (RNA-seq) was undertaken on RNA extracted from CS of the distal femur/knee (n = 5) and normal knee cartilage (n = 5). Differentially expressed (DE) miRNAs were validated with qRT-PCR in the same samples (n = 10). MiR-143-3p, miR-21-5p and miR-140-3p were overexpressed and knocked-down in SW1353 cells. Expression of selected predicted target genes of miR-143-3p and miR-21-5p were then measured with qRT-PCR. The effect of miR-143-3p overexpression and knockdown on the SW1353 proteome was measured using liquid chromatography tandem mass spectrometry (LC-MS/MS). The effect of miR-143-3p and miR-21-5p on SW1353 proliferation and migration were assessed using CCK-8 and migration assays.
Results: RNA-seq revealed 135 DE miRNAs, and validation revealed five DE miRNAs in CS compared to normal cartilage ($p < 0.05$). qRT-PCR revealed miR-143-3p knockdown increased KRAS and MMP13 expression, and miR-21-5p overexpression increased HECTD1 expression (all $p < 0.05$). Overexpression of miR-140-3p revealed four DE proteins, such as N-ethylmaleimide sensitive factor, and knockdown revealed 22 DE proteins such as Replication Protein A1 (all $p < 0.05$). Bioinformatic analysis revealed pathways such as IL-15 production and DNA damage response. Overexpression of miR-143-3p was found to inhibit SW1353 cell migration ($p < 0.05$).
Discussion: Validated gene targets of miR-143-3p and miR-21-5p are frequently mutated in cancer [1]. Additionally, miR-140-3p overexpression and knockdown revealed DE proteins and pathways linked to cancer [2].
Conclusion: This study provided data on the aberrant expression and potential involvement of miRNAs in CS. The pleiotropic nature of miRNAs makes an attractive drug target for CS.

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P061
Biglycan regulates WNT signaling pathway activation to control MG63 osteosarcoma cells' autophagy and response to chemotherapy

Eirini-Maria Giatagana[1], Aikaterini Berdiaki[1], John Aggelidakis[1], Dragana Nikitovic[1]
[1]University of Crete, Medical School, Heraklion, Greece

Purpose: Biglycan, a class I small leucine-rich proteoglycan (SLRP), is implicated in carcinogenesis. The WNT signaling pathway is found to participate in bone formation. We have previously shown that biglycan is a low-density lipoprotein receptor-related protein 6 (LRP6) binding partner and a positive regulator of MG63 osteosarcoma cell growth. Indeed, biglycan enhances WNT/LRP6/β-catenin signaling axis and upregulates canonical downstream β-catenin signaling. Furthermore, we demonstrated that biglycan facilitates β-catenin/insulin-like growth receptor -I (IGF-IR) signaling crosstalk to protect osteosarcoma cells from doxorubicin-induced growth arrest. The purpose of the study is to examine biglycans' role in autophagy.
Methods: Cell culture, western blot, immunoprecipitation, Real-Time PCR, immunofluorescence, cell growth assay, specific inhibitors, and siRNA transfection were utilized.
Results: Utilizing western blot, we show that biglycan treatment attenuated LC3-II protein expression levels ($p \leq 0.001$) of MG63 osteosarcoma cells. Furthermore, real-time PCR experiments demonstrated that the mRNA expression of p62 in biglycan-treated cells was increased ($p \leq 0.001$), indicating that biglycan negatively affects autophagy. The utilization of rapamycin at concentrations that trigger autophagy in the MG63 osteosarcoma model led to decreased biglycan mRNA and protein expression levels ($p \leq 0.001$, $p \leq 0.05$). Furthermore, as demonstrated by immunofluorescence, treating cells with rapamycin resulted in decreased co-localization of biglycan with the Wnt co-receptor, LRP6 ($p \leq 0.05$).
Discussion: This study has shown that biglycan reduces autophagy through WNT/β-catenin signaling, while rapamycin-induced autophagy attenuates LRP6 receptor co-localization with biglycan. The correlation of these pathways indicates their synergistic action to control cellular function.
Conclusion: Biglycan attenuates autophagy in an in vitro osteosarcoma cell model.

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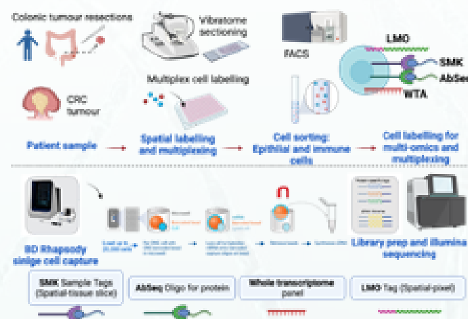
P062
Hierarchicheal barcoding for single cell spatial multi-omics for investigation of Tumour microenvironment (TME) in colorectal cancer

Olwyn Mahon[1,2], Marco Franzoni[3], Séamus Hickey[3], Eanna Fennell[2], Cathal King[3], Cliona McCarthy[4], Colin Peirce[5], Patrick Kiely[1], Kieran McGourty[2,3]
[1]School of Medicine, [2]HRI and Bernal Institute, [3]Chemical Sciences, [4]School of Engineering, [5]Gastroenterology, University of Limerick, Ireland

Purpose: Colorectal cancer (CRC) is one of the leading causes of mortality worldwide. The tumour microenvironment (TME) is a heterogenous system consisting of a plethora of cell types including, tumour, immune and stromal cells, alongside distinct extracellular matrix (ECM) compartments [1]. Unfortunately, current immunotherapeutic strategies have been hampered by a lack of understanding of the full complexity of the tumour biology which further confounded by a lack of understanding of the contributions of Extracellular Matrix (ECM) dynamics in the TME [2]. The overall aim of this study is to characterize the cancer cell and immune cell phenotypes in human colonic tumors in relation to their spatiotemporal position in the tumour in order to understand how their local tissue microenvironments and ECM architecture can contribute to cancer and immune cell behaviour and phenotype.
Methods: CRC patient tumour samples were obtained from colonic resection surgeries and using spatial grid-referencing methodologies spatial transcriptomics was performed. Specifically,



cells were labelled using BDs multiplexing sample tags and a novel barcoding strategy, using Lipid modified oligonucelotides (LMOs) developed in UCSF, to allow for hierarchicheal barcoding for sample multiplexing.
Results: Single cells were then captured using BDs Rhapsody capture system before single cell multiomics NovaSeq paired-end sequencing was performed to evaluate gene and protein expression.
Discussion & Conclusions: Overall, we believe that these findings may lead to novel insights into the immune and cancer cell heterogeneity in CRC patients and the role of the TME on CRC cancer progression. Furthermore, this spatial information can be used to develop an architecturally accurate TME using state of the art 3D bioprinting strategies where cellular environments can be precisely tailored with various ECM and biomechanical composition to monitor cancer and immune cell dynamics.

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P063
The roles of collagen synthesis enzymes in tumor progression and metastasis, with a focus on the tumor stroma and pancreatic cancer as a model

Linda Birgisdóttir[1], Anu Laitala[1], Antti Salo[1], Johanna Myllyharju[1]

[1]Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland

Purpose: Pancreatic ductal adenocarcinoma (PDAC) is a highly deadly disease with collagen-rich dense extracellular matrix (ECM). Tumor microenvironment (TME), including the ECM, is an important regulator of the tumor growth. Cancer-associated fibroblasts are the most abundant cell type in tumor stroma and are mainly responsible for the ECM remodeling during cancer progression. Collagen prolyl 4-hydroxylases (P4Hs) and lysyl hydroxylases (LHs) have important roles in collagen synthesis. In this project, we concentrate on the roles of these enzymes in pancreatic tumor stroma by using cP4H and LH deficient mouse lines and syngeneic PDAC cells (KPC).

Methods: Plod1$^{-/-}$ and compound P4ha1$^{+/-}$;P4ha2$^{-/-}$ mouse lines were used. Three groups for compound mice: P4ha1$^{+/+}$;P4ha2$^{+/-}$ (wt), P4ha1$^{+/+}$;P4ha2$^{-/-}$ (ko) and P4ha1$^{+/-}$;P4ha2$^{-/-}$ (dmut). We used mouse intrasplenic injection model to achieve liver metastases with the KPC cells and followed the signal with in vivo imaging system (IVIS) for both mouse lines. In addition to that, we used subcutaneous mouse tumor model in Plod1 mice and orthotopic model in the compound mice.

Results and Discussion: Subcutaneous tumors grew slower in Plod1$^{-/-}$ mice when compared to Plod1$^{+/+}$ mice. At the endpoint, tumors were smaller, but the total collagen amount was the same. When Plod1$^{-/-}$ mice were injected intrasplenically, they had enhanced signal in IVIS and increased liver weight indicating higher tumor burden. However, no difference was seen in the collagen amount of the liver metastases samples. Intrasplenic injections in compound P4ha1$^{+/-}$;P4ha2$^{-/-}$ mouse lines showed both ko and dmut had decreased signal in IVIS and a decreased liver weight when compared to control mice. Total collagen amount was significantly reduced in dmut liver metastasis. No difference was seen in the primary tumor growth with the orthotopic model in compound P4ha1$^{+/-}$;P4ha2$^{-/-}$ mouse line.

Conclusion: The role of the ECM has been shown to play important role in PDAC progression and fibrillar collagens are estimated to comprise 90% of the total ECM mass. Here we show that the stromal collagen P4ha and Plod1 deficiency do play a role in PDAC liver metastasis, but they seem to have opposed effects. One probable cause for the P4ha mediated difference can be transmitted via the reduced collagen amount, but further studies are needed.

P064
The mutational landscape of the tumor matrisome

Rijuta Lamba[1,2], Valerio Izzi[1,2,3], Vilma Pesola[2]

[1]Faculty of Medicine, [2]Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; [3]Foundation for the Finnish Cancer Institute, Helsinki, Finland

Purpose: The matrisome, computationally defined as an ensemble of approximately 1000 genes encoding for the core-matrisome and matrisome associated proteins 1, plays a key role in cancer. We wish to delve into large, open-access, public datasets containing all the mutations in cancer to unveil patterns and trends that may be characteristic to defining the role of the matrisome in cancer.

Methods: In our ongoing studies, we perform bioinformatics and statistical analysis focused on the pan-cancer mutational landscape of the tumor matrisome from The Cancer Genome Atlas (TCGA), evaluating the patterns of frequencies of non synonymous mutations for all matrisome families across cancer. Furthermore, we explore the interaction of the mutations with the immune mechanisms active in the tumour microenvironment (TME).

Results: From our studies so far, we found that the matrisome accumulates a significantly higher number of mutations as compared to the rest of the cancer genome. Furthermore, on analysing the single nucleotide substitution profiles as per the Catalogue of Somatic Mutations In Cancer (COSMIC)2 we found strong indications of the relevance of the tissue of origin in the accumulation of mutations in the cancer matrisome. Finally, we found proteoglycans to display a strong deviation in the pattern of distribution of mutations as compared to the rest of the matrisome families.

Discussion: The ECM is important in cancer , all the “hallmarks of cancer” that fuel cancer growth3 are arguably modulated by the biochemical and biomechanical signals from the peri-cancerous ECM 4,5. Our studies initiate an annotation of trends of the ECM mutation in cancer.

Conclusion: Recurring, conserved mutations in specific ECM families point to a non-random accumulation in the tumour matrisome which calls for investigating further the role that the matrisome mutations play in cancer and their interactions with other mechanisms active in the TME.

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P065
N-terminomics, a new “omic” for the identification of the substrate repertoire of ADAMTS2 and the evaluation of the functional implications of their cleavage during tumor progression

Loïc Joannes[1], Laura Dupont[1], Agnès Noël[2], Alain Colige[1]
[1]Laboratory of Connective Tissues Biology, Liège, Belgium; [2]Laboratory of Biology of Tumor and Development, GIGA-R, Liège, Belgium

Purpose: Preliminary studies from our laboratory [1] indicate that ADAMTS2 displays both pro- and anti-antitumor properties. Our working hypothesis is that these opposite observations are explained by the cleavage of some of still unknown ADAMTS2 substrates which could affect tumor growth.
Methods: First, we identified experimental cancer models, in vitro and in vivo, which are the most affected by the presence of ADAMTS2. Next, these models were further investigated by Terminal Amine Isotopic Labeling of Substrates (TAILS) analysis, a proteomic approach dedicated to the identification of substrates of proteases in complex samples.
Results: The survival, proliferation and migration of cancer cells in vitro are not affected by recombinant ADAMTS2. By sharp contrast, tumors forming after subcutaneous injection of cancer cells grow faster in Adamts2-K0 mice as compared to their wild type littermate whereas, conversely, forced expression of ADAMTS2 by cancer cells represses tumor growth. To find ADAMTS2 substrates whose cleavages would explain these observations, different models in vitro and in vivo were used for TAILS analyses. Several potential substrates were identified.
Discussion: Already known substrates [2], such as collagens, LTBP1 and TGFβR3, were repeatedly identified, validating our experimental approach. Among the substrates never identified before, osteopontin was found to be cleaved at an identical site in different experimental models, making it a likely ADAMTS2 substrate. Osteopontin is a glycoprotein involved in tumor cell chemo-resistance and in “Cancer Associated Fibroblasts” and “Tumor Associated Macrophages” reprogramming. Periostin, laminin receptor and thrombospondin 2 are amongst the other potential substrates that were identified.
Conclusion: Our data clearly suggest that the antitumor properties of ADAMTS2 are indirect and do not result from direct effects on cancer cells. The newly identified substrates are currently being validated through in vitro cleavage and western blotting. The corresponding recombinant proteins cleaved or not by ADAMTS2 will be evaluated for their respective effects on cancer development.

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P066
Biomechanical characterisation of colorectal tumour tissue and healthy adjacent tissue at the microscale

Cliona McCarthy[1], Sinead Connolly[1], Colin Peirce[2], Michael Walsh[1], Kieran McGourty[3], John Mulvihill[1]
[1]School of Engineering, [2]General Surgery, [3]Chemical Sciences, University of Limerick, Limerick, Ireland

Purpose: Cancer is among the leading obstacles to improving life expectancy worldwide. Specifically, colorectal cancer accounts for 1 in 10 cancer cases and deaths [1]. There is increasing evidence to suggest that mechanical changes to microenvironment of the extracellular matrix (ECM) can elicit an oncogenic response. However, this has yet to be thoroughly investigated [2] and there has been no robust mechanical analysis of the colon at a microscale to date. The aim of this study is to perform a biomechanical characterisation of the microenvironment ECM for colorectal tumour tissue and healthy adjacent tissue.
Methods: Atomic force microscopy is a popular method for measuring tissue at this microscale that aims to mimic the physiological environment [3]. A probe is lowered into contact with the tissue and stiffness value is generated using the Hertz contact model. For this study, a probe with cantilever stiffness of 0.32N/m and a 0.52μm spherical tip was used. A 5x5 matrix scan was performed which provides a surface area map of the stiffness [Fig 1].

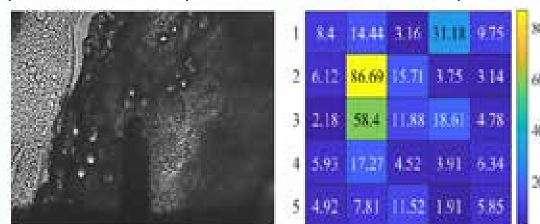

Figure 1. Example of probe measuring tissue stiffness (left). Stifness heatmap generated from atomic force microscopy measurements (right).

Results: The preliminay data [Fig 2] indicate that the median stiffness of the tumour (0.208 ± 0.019kPa) is less than the healthy adjacent tissue (0.511 ± 0.291kPa).

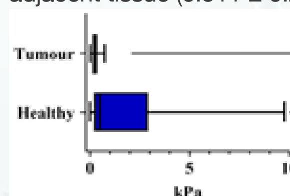

Figure 2. Preliminary results showing statistically significant difference between tumour and healthy tissue.

Discussion: The preliminary data indicate there is a statistically significant change in the microenvironment. This finding could lend itself to early disease detection in the form of diagnostic tools and targeted therapies.
Conclusion: A robust microscale characterisation of tissue could revolutionise cancer diagnostics, treatments and improve patient outcomes.

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P067
Investigating the contribution of the extracellular matrix to heterogeneic expression of cancer stem cells in colorectal cancer through single-cell RNA-sequencing

Séamus Hickey[1], Christina Enright[1], Cathal King[1], Patrick Kiely[2], Kieran McGourty[1]
[1]Chemical Sciences, [2]School of Medicine, University of Limerick, Limerick, Ireland

Purpose: Cancer stem cells (CSCs) provide invaluable information for the prognosis of colorectal cancer (CRC). It has been postulated that the microenvironment surrounding these cells can regulate dormancy, proliferation and differentiation [1]. However, difficulty exists in the characterisation and evaluation of the CSCs' cellular expression programme. Therefore, the aim of this project is to overcome these challenges by exploring the influence of the Extracellular Matrix (ECM) on CSCs gene expression in CRC.
Methods: Using 6 publically available CRC single-cell RNA-seq (scRNA-seq) datasets, CSCs were isolated and integrated. After performing batch correction and normalisation, clusters were investigated. Differentially expressed genes (DEGs) associated each cluster were cross-referenced with a list of ECM proteins drawn from the Matrisome Project [2] to create a list of ECM proteins associated with clusters of CSCs in CRC to evaluate whether the clusters were correlated with unique ECM fingerprints. Biological pathway analysis from each cluster can also be associated to a unique ECM fingerprint using this analysis, thereby forming the basis of targeted causal investigation through selected perturbations of key ECM proteins.
Results: This approach has led to identification of multiple clusters of CSCs in CRC [Fig 1]. These same cells types exhibit distinct gene expression programs with independent associations with the ECM based on their DEGs indicating that cells of the same type can behave differently conditional to their microenvironment.

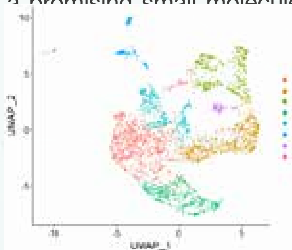

Figure 1. UMAP showing different CSC populations of the same type.

Discussion: CSCs have been implicated in many cancer related processes. By investigating the ECM association with cell behaviours in each cluster, we can begin to elucidate the effect of the ECM on various CSC behaviours.
Conclusion: Publically available datasets are a powerful tool to investigate rare cells beyond the scope of primary researchers' aims. We have established a list of key ECM proteins driving cellular expression heterogeneity in the same cell type of CSCs in CRC. The ECM microenvironment surrounding these cells may yield invaluable insight into the mechanisms governing CSC behaviour.

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P068
Identification of a small molecule inhibitor of hyaluronan synthesis, DDIT, targeting breast cancer cells

Theodoros Karalis[1], Andrew K. Shiau[2], Spyridon Skandalis[3], Carl-Henrik Heldin[1], Paraskevi Heldin[1*]
[1]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden; [2]Ludwig Cancer Institute, San Diego, USA; [3]Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece

Purpose: Breast cancer is a highly heterogenous disease and one of the most common cancers in women. Breast cancer cells synthesize large amounts of hyaluronan to assist their proliferation, survival, migration and invasion. Currently, the only known small molecule inhibitor of hyaluronan synthesis is 4-methylumbelliferone (4-MU). Due to the importance of hyaluronan for breast cancer, our aim was to identify more potent inhibitors of its synthesis.
Methods: Cell invasion, proliferation, migration and 3D culture assays were employed for investigation of the inhibitor effect on breast cancer cell aggressiveness.
Results: Upon screening a list of candidate compounds, we identified one compound (DDIT) as a potent hyaluronan synthesis inhibitor in several cell types. Further investigation of this compound suggests that is non-toxic and more potent than 4-MU. Treatment of Hs578T triple-negative breast cancer cells with DDIT reduced the expressions of the main hyaluronidase TMEM2 and hyaluronan receptors CD44s and RHAMM. On the other hand, no effect was evident in the expression of hyaluronan synthases. Upon further investigations, we found that DDIT reduced breast cancer cell proliferation by arresting cells in G0/G1 phase, as evidenced by FACS analysis. DDIT was also able to reduce migration of breast cancer cells in normal lung microenvironment. Importantly, DDIT abrogated breast cancer stem cell self-renewal and reduced the expression of TMEM2 in cells grown in low-attachment conditions.
Conclusion: Collectively, the novel compound DDIT seems to be a promising small molecule for breast cancer treatment through [illegible] ...nthesis.

P069
SPOCK1 overexpression suggests poor prognosis of ovarian cancer

Lorand Vancza[1], Lee Seungyeon[1], Gabor Petovari[1], Martin Goette[2], Ilona Kovalszky[1], Kornelia Baghy[1]
[1]Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary; [2]Department of Gynecology and Obstetrics, University of Munster, Munster, Germany

Purpose: The sparc/osteonectin, cwcv, and kazal-like domains proteoglycan 1 (SPOCK1) is an extracellular matrix chondroitin sulfate-heparan sulfate proteoglycan. The first study reporting its oncogenic role was published in 2011. Since then, SPOCK1 was detected in several malignant tumors, and its implication in poor outcome was reported in almost all types of cancer. Our research group aimed to explore the role of SPOCK1 in ovarian cancer.
Methods: Ovarian cancer cell lines SKOV3 and SW626 were transfected with full length SPOCK1 cDNA construct or empty vector using electroporation. Transfected cells were studied by immunostaining and Western blot. Their proliferative and migratory capacity were tested by BrdU uptake and wound healing assays. SPOCK1 expression in human ovarian cancer specimens and plasma were also studied by immunostaining and ELISA. With in silico studies, we analysed the survival of patients with tumors exhibiting low and high SPOCK1 expression.
Results: Tumor cells were successfully transfected with SPOCK1 constructs. Interestingly, the transfected cells did not retain the excessive SPOCK1 in their cytoplasm, it was secreted into the culture medium. SPOCK1 overexpression stimulated DNA synthesis and enhanced migration, together with pAkt, pEGFR and pERK1/2 activity. Although $p21^{CIP1}$ level was also elevated, its cytoplasmic localization reflected on its antiapoptotic/oncogenic action. In line with the previous, tumor cells of human ovarian cancer tissues also express high level of SPOCK1. Increased SPOCK1 serum levels seem to associate with the lack of chemotherapy. In silico analyses revealed that high SPOCK1 expression correlates with shorter survival of patients with ovarian cancer.
Discussion: Here, we call attention to our first experiences with a hardly known proteoglycan, which seems to be a predictor for poor outcome of ovarian cancer.
Conclusion: Our results raise the possibility that SPOCK1 could be utilized as a tumor prognostic factor in the near future.

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P070
Spock1 supports the development of hepatocellular cancer. Competition with syndecan-1

Ilona Kovalszky[1], Lorand Vancza[1], Andrea Reszegi[1], Kornelia Baghy[1]
[1]Department of Pathology & Experimental Cancer Research, Semmelweis University, Budapest, Hungary

Purpose: Although Testican 1 is still considered as a hardly known extracellular matrix proteoglycan , fast growing number of publications indicate its outstanding role in the development and progression of cancers. The aim of the present work is to find mechanistic details as Spock-1/Testican-1 promotes the development and progression of liver cancer.
Methods: Testican-1 immunohistochemistry on human liver specimen, and analysis of hepatoma cell lines after modification of Testican expression by si-RNA, or expression vector transfection, was carried out to detect the proliferation, invasion and cellular signaling of tumor cell lines.
Results: Spock1 is an extracellular matrix proteoglycan, however, its presence keeps increasing in the cytoplasm starting from normal, toward cirrhotic and tumorous human livers. Similar phenomenon was observed in the experimental hepatocarcinogenesis. Syndecan-1, the major proteoglycan of the liver, and Spock1 are in an inverse correlation in this event. In vitro analysis of hepatoma cell lines revealed, that Spock1 colocalizes with mitochondrial marker, and TOMM20, a protein of the outer membrane of mitochondrion. Spock1 downregulation by siRNA inhibits cell proliferation, upregulates p21, p27 and caspase3, whereas inhibits pAkt and CDK4 expression. Inhibition of Spock1 alters the activity of regulatory proteins relating to the aggressiveness of the hepatoma cell lines.
Discussion: Testican-1 is involved in the development of malignant phenotype of hepatocellulat cancer, and the literature indicates its implication of the behavior of several other cancers.
Conclusion: Our data suggests, that Testican-1 is an active player of malignant transformation and tumor progression in general. Furthermore its mitochondrial localization raises the possibility, that it also has physiological function in epithelial cells, which requires further study to evaluate.

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P071
Loss of collagen-binding α1- and α2-integrins correlates with prostate cancer progression

Sara Cruz[1], Qin Zhang[1], Raman Devarajan[1], Tomasz Wenta[1], Gonghong Wei[2], Aki Manninen[1]
[1]Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; [2]Fudan University, Shanghai, China

Prostate cancer (PCa) is the most diagnosed cancer in men around the world. The current screening methods often fails to differentiate benign prostate lesions from aggressive prostate cancers, which needs immediate treatment intervention. Therefore, more accurate diagnostic methods need to be developed. Extracellular Matrix (ECM)-binding integrins in its active form regulate several cellular functions that are crucial to the initiation, progression, and metastasis of solid tumours, making them an appealing target for cancer diagnosis and treatment. In the current study, we performed a meta-analysis with different PCa cohorts and show that simultaneous loss of α1- and α2-integrin expression associates with higher recurrence rate and poor prognosis. Moreover, in vitro models show that the dual loss of integrin α1 and α2 in prostate cells (RWPE1) modulates the tumorigenic potential by enhancing the invasiveness via Epithelial-to-Mesenchymal Transition (EMT). EMT is driven by enhanced secretion and activation of TGFβ by the α1/α2-double-negative cells. In vivo metastasis experiments showed that α1/α2-deficient cells were capable of inducing micro-metastases in mice. Moreover, we identified TEA Domain Transcription Factor 1 (TEAD1), as a regulator of ITGA1 and ITGA2 expression. TEAD1 ChIP-qPCR analysis confirmed multiple functional TEAD1-binding sites in the ITGA1/ITGA2 genomic loci. Ablation of TEAD1 in RWPE1 cells led to downregulation of α1 and α2 and resulted in similar functional traits of α1α2 deprived cells. Taken together, we show that PCa progression is correlated with reduced α1-and α2-integrin levels and implicate TEAD1 as one of the key regulators of their expression. The α1-integrin/α2-integrin/TEAD1-signaling axis could potentially be used as a novel prognostic biomarker for prostate cancer.

P072
Integrative proteomics analysis of extracellular matrix identifies association of fibulins 2, 3 and 5 with liver fibrosis and tumor aggressiveness

Célia Thomas[1], Dominique Bonnier[1], Vincent Legagneux[1], Nathalie Theret[1]
[1]Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail), UMR_S 1085, Rennes, France

Purpose: Hepatocellular carcinoma (HCC) arises in the context of chronic liver diseases (CLD). CLD are associated with the development of liver fibrosis, characterized by excessive accumulation of extracellular matrix (ECM)1, promoting tumor progression. The activated hepatic stellate cells (aHSC) are the main producers of ECM components1. In order to identify new markers involved in the progression of CLD, we characterized the proteomic profile of fibrotic microenvironment in HCC patients.
Methods: A proteomic screening was conducted using ECM-enriched samples2 from adjacent non-tumor tissues from 20 patients with HCC. Integrative analyses and survival studies were performed using TCGA database. qRT-PCR and western blot were achieved in tissues from HCC patients and in cultured hepatic cell lines.
Results: The proteomic study revealed 29 upregulated and 77 downregulated proteins in at least one grade of fibrosis. Among the upregulated proteins, we observed an association of three fibulins, FBLN2, FBLN3 and FBLN5, with the stage of fibrosis. Using tissues from patients with HCC, we demonstrated that increase of fibulin mRNA levels was associated with fibrosis grade and tumor aggressiveness. We identified aHSC as a major source of fibulins, whose expression was differentially regulated by TGF-β and IL-1β treatment. Comparative analysis of protein-protein interaction networks of these fibulins suggests different functional roles in CLD.
Discussion: Proteomic profiling allowed us to identify three members of fibulin protein family, FBLN2, FBLN3 and FBLN5, associated with the severity of liver fibrosis and tumor aggressiveness. We demonstrated for the first time that FBLN3 is mainly expressed by aHSC and that its regulation and function differ from FBLN2 and FBLN5.
Conclusion: Our preliminary results highlight specific functions of fibulins in CLD and identifies FBLN3 as a new actor of HSC-dependent fibrosis.

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P073
Microfibrillar-associated protein 4 is secreted by activated fibroblasts and promotes tumorigenesis of lung cancer

Ali Mohammadi[1], Anders Schlosser[1], Kim Ravnskjær[2], Bartosz Pilecki[1], Niels Marcussen[3], Behzad Baradaran[4], Grith Lykke Sorensen[1]
[1]Department of Cancer and Inflammation, Institute of Molecular Medicine, [2]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark; [3]Department of Clinical Pathology, Odense University Hospital, Odense, Denmark; [4]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Purpose: Microfibrillar-associated protein 4 (MFAP4) is an extracellular matrix protein. High MFAP4 expression is associated to the adverse prognosis of patients with advanced stages of lung cancer. MFAP4 induces cellular migration and proliferation via Focal adhesion kinase (FAK) signaling activation in human aortic vascular SMCs. These effects are through integrin αvβ3 and αvβ5 ligation. The purpose of this study was to investigate the hypothesis that MFAP4 is an essential component of the tumor microenvironment (TME) and can induce proliferation and migration of lung cancer cells.
Methods: MFAP4 expression was assessed in lung cancer tissues by immunohistochemistry. Further, mRNA in situ hybridization was performed to evaluate the colocalization of MFAP4 synthesis with alpha-smooth muscle actin. The proliferation of lung cancer cells in the presence and absence of MFAP4 was evaluated by WST-1 assay. The migration of NSCLCs was evaluated by trans-well assay. RNA-sequencing was used for identification of relevant signaling pathways.
Results: Patient samples supported that MFAP4 is expressed in cancer assosiated fibroblasts; MFAP4 mRNA expression was found in the TME and was overlapping with alpha-smooth muscle actin expression pattern. Moreover, recombinant MFAP4 induced lung cancer cell proliferation and migration in vitro, and the latter was reduced after anti-MFAP4 Ab treatment blocking MFAP4-integrin interaction. RNA-sequencing supported that MFAP4 treatment of cancer cells induced pro-oncogenic signaling pathways.
Discussion: Our data support that MFAP4 in TME may be involved in growth and migration of NSCLC integrin dependently. Integrin αvβ3 and αvβ5 are further involved in angiogenesis. On this basis it is now warranted to set up anti-MFAP4 interventional studies in NSCLC models in vivo.
Conclusion: Our results demonstrate that MFAP4 has capacity to induce proliferation and migration of lung cancer cells in vitro, and modulates cancer related signalling pathways, and is a new candidate target for intervention in NSCLC.

P074
The cell surface heparan sulfate proteoglycan syndecan-3 promotes ovarian cancer pathogenesis

Lara Hillemeyer[1], Nancy Adriana Espinoza-Sanchez[1], Burkhard Greve[2], Nourhan Hassan[2], Anca Chelariu-Raicu[3], Martin Götte[2]
[1]Department of Gynecology and Obstetrics, [2]Department of Radiotherapy-Radiooncology, Münster University Hospital, Münster, Germany; [3]Department of Obstetrics and Gynecology, LMU, Munich, Germany

Purpose: Syndecans are transmembrane heparan sulfate proteoglycans that integrate signaling at the cell surface [1]. By interacting with cytokines, signaling receptors, proteases, and extracellular matrix proteins, syndecans regulate cell proliferation, metastasis, angiogenesis, and inflammation [2]. Here, we investigated a possible role for syndecan-3 (Sdc-3) in ovarian cancer pathogenesis.
Methods: We analyzed public gene expression datasets to evaluate the potential prognostic impact of Sdc-3 in ovarian cancer. Moreover, we performed functional in vitro analysis in Sdc-3-siRNA-treated SKOV3 and CAOV3 ovarian cancer cells.
Results: In silico analysis of public gene array datasets revealed that Sdc-3 mRNA expression was significantly increased in ovarian cancer tissues and metastases compared with controls. Sdc-3 siRNA knockdown impaired 3D spheroid growth and colony formation as stemness-related readouts in SKOV3 and CAOV3 cells. In SKOV3, but not in CAOV3 cells, Sdc-3 depletion reduced cell viability both under basal conditions and under chemotherapy. In vitro, reduced Stat3 activation and changes in the expression of Wnt and notch signaling constituents were observed. KM Plotter analysis of 1435 ovarian cancer patients revealed that high Sdc-3 expression was associated with reduced survival in patients treated with taxol and platin.
Discussion: Our study establishes a novel prognostic role for Sdc-3 in ovarian cancer. The dysregulation of stemness-related factors is in line with the phenotypic observation of reduced sphere formation capability and reduced cell viability. In contrast, the response to chemotherapy in vitro was confounded by the basal decrease in cell viability induced by Sdc-3-depletion.
Conclusion: Our study suggests that up-regulation of Sdc-3 promotes the pathogenesis of ovarian cancer by modulating stemness-associated pathways.

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P075
The heparan sulphate proteoglycan Syndecan-1 (CD138) regulates tumour progression in a 3D model of ductal carcinoma in situ of the breast

Christopher D'Arcy[1], Clarissa Constanze Zimmermann[1], Nancy Adriana Espinoza-Sanchez[1], Burkhard Greve[2], Ludwig Kiesel[1], Marie-Kristin von Wahlde[1], Martin Götte[1]

[1]Department of Gynecology and Obstetrics, [2]Department of Radiotherapy-Radiooncology, Münster University Hospital, Münster, Germany

Purpose: Ductal carcinoma in situ (DCIS) is a form of breast cancer that is restricted to the lactiferous ducts and has not yet invaded the surrounding breast tissue. Dysregulation of the transmembrane heparan sulphate proteoglycan Syndecan-1 (Sdc-1) plays a role in tumour progression of invasive breast cancer (IBC) [1]. In DCIS, Sdc-1, c-Met and E-cadherin are part of a proangiogenic expression signature [2]. Here, we investigated a role of Sdc-1 in progression of DCIS in a 3D cell culture system.
Methods: We employed a siRNA knockdown approach in the DCIS model cell line MCF10A DCIS.com n a Matrigel-based 3D cell culture model.to investigate a potential connection between Sdc-1 and epithelial mesenchymal transition (EMT), proteolysis and the Rho kinase pathway.
Results: Analysis of public gene expression data revealed that Sdc-1 expression was higher in primary breast tumours compared to metastases. Sdc-1 depletion in vitro resulted in formation of larger spheroids and invasive protrusions. Application of matrix metalloproteinase (MMP) and Rho kinase inhibitors could block the Sdc-1-induced phenotype. qPCR analysis of Sdc-1-depleted cells revealed upregulated expression of the EMT-markers CDH1, FN-1, CLDN1, the proteolysis markers MMP3, and MMP9, and HPSE, while MMP2, VIM and ROCK-2 were downregulated. Immunocytochemistry confirmed upregulation of MMP9 and fibronectin, the latter being particular prominent after ROCK inhibition. STRING analysis confirmed an interaction of the investigated gene products at the protein level.
Discussion: While Sdc-1 is overexpressed in breast cancer, its downregulation in metastases compared to primary tumors may suggest a dose-dependent molecular function.
Conclusion: Our results suggest that diminished Sdc-1 expression plays a role in DCIS progression to IBC through deregulation of proteolytic factors and a partial EMT.

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P076
Modulation of the tissue factor pathway by syndecan-1 in breast cancer

Nourhan Hassan[1], Janes Efing[1], Eyyad Nassar[1], Ludwig Kiesel[1], Burkhard Greve[2], Martin Goette[1]

[1]Department of Gynaecology and Obstetrics, [2]Department of Radiotherapy, Münster University Hospital, Muenster, Germany

Purpose: Breast cancer is one of the most common causes of cancer death in women worldwide. Many cancer patients show a hypercoagulable status leading up to frequent severe complications, such as thromboembolism and haemorrhage. Tissue factor (TF) is a potent stimulator of the extrinsic coagulation cascade. It also enhances cell proliferation and migration [1]. Angiogenesis and haemostasis are two of the most reliable host responses linked with cancer [2]. Poor prognosis is associated to dysregulation of the cell surface heparan sulfate proteoglycan and signaling co-receptor Syndecan-1 (Sdc-1).
Methods: We silenced and overexpressed Sdc-1 in TNBC MDA-MB 231 cells utilizing a 3D human umbilical vein endothelial cell (HUVEC) co-culture system to examine the role of Sdc-1 in angiogenesis and the modulation of the tissue factor pathway. Furthermore, we used multiple molecular and functional experiments to examine the potential link between Sdc-1 knockdown and TF pathway inhibitor (TFPI).
Results: Sdc-1 siRNA depletion reduced HUVEC tubule network formation in MDA-MB-231 cells. In the Sdc-1-silenced secretome, angiogenesis array indicated lower levels of VEGF-A and TF. Altered expression of F3, F7, F2R/PAR1, F2RL1/PAR2, VEGF-A, EDN1, IGFBP1, and IGFBP2 in cell lines was validated by qPCR. Sdc-1 knockdown resulted in lower secreted EDN1 and TF levels, while TFPI treatment inhibited angiogenesis. Moreover, TFPI stimulation was found to induce apoptosis and cell cycle arrest in G2/M phase. Survival analysis of 3951 patients demonstrated that high expression of F3 and F7 are associated with better relapse-free survival, whereas poor survival was observed in TNBC and p53 mutant basal breast cancer (F3) and in ER-negative and HER2-positive breast cancer (F2R, F2RL1).
Discussion: Our study demonstrates a novel link between TFPI and Sdc-1 in breast cancer cells. Our group also suggested in previous work that TNBC Syndecan-1 regulates angiogenesis via the TF and additional angiogenic pathways and marks its constituents as novel prognostic markers and therapeutic targets [3].
Conclusion: TFPI and Sdc-1 may have potentials as novel prognostic markers and therapeutic targets.

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P077
The role of syndecan1 proteoglycan in the resistance of irradiation of triple-negative breast cancer cells

Nancy Adriana Espinoza-Sanchez[1,2], Nourhan Hassan[1], Ludwig Kiesel[1], Hans Theodor Eich[2], Burkhard Greve[2], Martin Goette[1]
[1]Department of Gynecology and Obstetrics, University Hospital Münster,
[2]Department of Radiotherapy-Radiooncology, Münster, Germany

Purpose: Breast cancer (BrC) is the most common cancer in women. Cell-surface-derived proteoglycans like Syndecan-1 (Sdc-1) contribute to tumor progression. We previously showed that downregulation of Sdc-1 in the triple-negative BrC cell line (TNBC) MDA-MB-231 further increases cellular invasiveness and increases resistance to radiation therapy [1, 2]. Here, we want to identify the underlying molecular mechanisms and pathways of the Sdc-1-dependent radiation resistance phenotype of BrC cells.
Methods: Using a siRNA Sdc-1 was downregulated in three human TNBC cell lines. Both control and Sdc-1 KD cells were irradiated with the therapeutically relevant dose of 2Gy, and cell apoptosis, cell cycle progression, and DNA repair were analyzed by flow cytometry. Gene expression profile by qPCR and protein levels of FAK and CDK6 by western blot was performed. Wound healing, collagen contraction, and spheroid formation were analyzed on Integrin β1 KO and Sdc1-KD cells.
Results: We observed no association between Sdc1 and radiation on apoptosis, cell cycle, and DNA repair of triple-negative cells. Differential expression of DK6, CDC20, CCNB1, AKRIC3, and PMS1, changes in the morphology and in the levels of cytoskeletal proteins were observed in the Sdc1 KD cells and after irradiation. FAK phosphorylation, lower protein levels of CDK6, as well as changes in sphere formation, collagen contraction, and wound healing in triple-negative cells were observed after Sdc1 KD and Integrin β1 KO.
Discussion: Based on these preliminary results, it is important to analyze the role of Sdc1 and radiotherapy on integrin recycling. We suggest that Sdc-1 regulates the resistance of BrC cells to irradiation in an integrin-FAK-CDK6-dependent manner.
Conclusion: A mechanistic understanding of this process could provide the basis for a selected targeting of signaling pathways in order to improve the response to radiotherapy.

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P078
Presence of structurally relaxed fibronectin fibers in human breast tumors

Arnaud Mieville[1], Charlotte Fonta[1], Cornelia Leo[2], Gad Singer[2], Viola Vogel[1]
[1]ETH Zurich, Health Science and Technology, Zürich, Switzerland;
[2]Kantonsspital Baden, Institute of Pathology, Baden, Switzerland

Purpose: Upon tumor growth and metastasis, the extracellular matrix (ECM) undergoes major structural and biochemical transformations that seriously alter the ECM composition and the tensional state of its protein fibers. This tumor-specific matrix is highly involved in shaping the progression of the disease but also plays a major role in influencing the response toward therapy. The literature reports stiffer ECM and altered physical features in the tumor stroma, however, the lack of a probe to visualize the tensional states of ECM fibers limits the understanding of how tensional state alterations modify the cell-ECM crosstalk. Our lab developed a mechanical probe that binds specifically to fibronectin (Fn) in a relaxed tensional state [1]. Previous studies in our lab on murine tissues showed a higher accumulation of relaxed Fn fibers in tumor tissues, however, nothing is known about human breast tumors and their impact on immune cells [2].
Methods: We are investigating the presence of relaxed Fn in human cancer tissues. By doing proximity analyses, we aim to characterize ECM components and immune cells neighboring relaxed Fn fibers in healthy and tumor breast tissues using immunohistochemistry.
Results: We observed a significant accumulation of relaxed Fn fiber in invasive human tumors, compared to healthy breast tissues. Furthermore, as shown in mice, there is spatial proximity between relaxed Fn fibers and fibrotic markers, such as Tenascin C, dense and aligned bundles of collagen fibrils, and contractile myofibroblasts.
Discussion: The ability to detect differential Fn fibers tensional states is of great interest, as a change in Fn fibers tensional state will have a direct impact on the availability of cryptic sites for their interaction with components of the tumor microenvironment.
Conclusion: With this mechanical probe, we provide the first tool to measure ECM fiber tension in human ex vivo tissues, opening new possibilities to study cell-ECM interaction and potentially improve tumor therapy.

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P079
Extracellular domain of syndecan-2 interacts directly with pro-domain of MMP7 for activation

Weontae Lee[1], Bohee Jang[2], Ji-Hye Yun[1], Eok-Soo Oh[2]
[1]Biochemistry, Yonsei University, Seoul, South Korea; [2]Life Sciences, Ewha Womans University, Seoul, South Korea

Purpose: We tried to identify the detail structural basis of direct interaction of syndecan-2 extracellular domain with pro-domain of MMP-7, and syndecan-2-medaited cancer activity regulation.
Methods: Flow cytometry-cells were washed with PBS, released by the addition of 5% FBS and 1 mM EDTA in PBS, collected by centrifugation, resuspended in PBS, and incubated with anti-syndecan-2. Colony forming assays - Each well of a 6-well culture plate was coated with 3 ml of bottom agar mixture. After the bottom layer had solidified, 1 ml of top agar mixture containing HT-29 cells was added to each well and the cultures were incubated. Colony formation was monitored daily with a light microscope.
Results: Our data showed that the direct protein-protein interaction was mediated through the extracellular domain of syndecan-2 with helix-loop-helix motif at MMP-7, strongly suggesting that syndecan-2 extracellular domain mediates its interaction and processing of pro-MMP-7 into active enzyme, regulating syndecan-2 function dependent on MMP-7 activity. We further investigated the effect of the reduced activation ability of syndecan-2 on tumorigenic activity of colon cancer cells. Expectedly, overexpression of Y51A mutants reduced syndecan-2-mediated colon cancer cell migration and colony forming activity.
Discussion: In this report, we investigated Tyr51 residue-mediated interaction is involved in the regulation of cancer activity. Consistently, defective syndecan-2 mutants decreased syndecan-2-mediated anchorage independent growth of HT-29 cells. These data provide that syndecan-2-mediated cancer activity regulation is closely associated with the direct interaction with pro- MMP-7.
Conclusion: Our data revealed that Tyr51 residue in the extracellular domain of syndecan-2 mainly mediated interaction with helix-loop-helix motif at the pro-domain of MMP-7. These findings provide important new insights into colon cancer regulation mediated by syndecan-2 as a docking receptor at the cancer cell surface.

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P080
The cross-talk between endothelial cell-specific molecule 1 and long non-coding RNAs HULC and H19 through HIF/VEGF pathway

Federica Aliquò[1], Aurelio Minuti[1], Angela D'Ascola[2], Angela Avenoso[1], Giuseppe Bruschetta[3], Giuseppe Maurizio Campo[2], Salvatore Campo[1], Michele Scuruchi[2]
[1]Department of Biomedical and Dental Sciences and Morphofunctional Images, [2]Department of Clinical and Experimental Medicine, [3]Department of Veterinary Sciences, Biochemistry Unit, University of Messina, Messina, Italy

Purpose: The aim of this study is to elucidate the potential role of endothelial cell-specific molecule 1 (ESM-1) in tumor progression by evaluating the effect of ESM-1 silencing on HIF/VEGF pathway in non-small-cell lung carcinoma (NSCLC). Furthermore, the cross-talk between ESM-1 and selected lncRNAs was investigated.
Methods: The A549 cells were transfected with ESM-1 siRNA and cells were treated with deferoxamine to simulate the hypoxic conditions. mRNA levels of ESM-1, VEGFR-2, HIF-1α, lncRNAs HULC and H19 were evaluated by qPCR; protein levels of ESM-1,VEGFR-2 and AKT were assayed by ELISA and WB, respectively; cell migration and proliferation were evaluated by scratch assay and MTT, respectively.
Results: The hypoxia enhanced the expression levels of ESM-1, VEGFR-2, HIF-1α, HULC and H19, whereas the silencing of ESM-1 decreased mRNA and protein levels of VEGFR-2, AKT and both lncRNAs. Furthermore, ESM-1 silencing reduced cell migration and proliferation.
Discussion: ESM-1 is a proteoglycan considered as a potential biomarker in inflammatory disorders and tumor progression [1]. In hypoxic conditions, the upregulation of HIF-1α stimulates the VEGF pathway, which is a positive regulator of ESM-1 in angiogenesis. HULC and H19 are angiogenesis-related lncRNAs influencing HIF/VEGF pathway [2]. Our data demonstrated that ESM-1 silencing reduce mRNA and protein levels of VEGFR-2 and AKT, indicating the modulatory effect of ESM-1 in HIF/VEGF pathway, with a consequent decrease in cell migration and proliferation. Notably, ESM-1 knockdown reduced HULC and H19 expression, demonstrating a regulatory mechanism that could affect tumor angiogenesis.
Conclusion: In this context, ESM-1 seems to play a role in the activation of HIF/VEGF pathway also through the modulation of HULC and H19.

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P081
Oxidized LDL adversely affect tendon matrix synthesis and remodelling via TGF-β regulation

Rouhollah Mousavizadeh[1], Erin DeBruin[1], Charlie M. Waugh[1], Robert G. McCormack[2], Vincent Duronio[3], Alex Scott[1]
[1]Department of Physical Therapy, [2]Department of Orthopaedics, [3]Department of Medicine, The University of British Columbia, Vancouver, Canada

Purpose: To investigate the impact of hypercholesterolemia on tendon pathology by dissecting the role of oxLDL on the mechanism that regulates matrix synthesis and remodelling.
Methods: Isolated human tendon cells were cultured and incubated in the presence or absence of oxLDL and TGF-β. Collagen gel contraction and tenocyte migration assays were conducted to compare tenocyte motility and matrix remodelling in the oxLDL and control conditions. The influence of oxLDL on the expression level of key mRNA and proteins was examined using real-time quantitative PCR and Western blots. Finally, the activities of enzymes relevant to collagen synthesis and breakdown (lysyl oxidase and matrix metalloproteinases) were quantified using fluorometry.
Results: This study showed that oxLDL disturbed a number of important processes in human tendon cells, including the remodeling, synthesis and cross-inking of key tendon extracellular matrix components. The expression and activity of degrading enzymes such as MMP1 were increased while collagen, TGF-β and LOX were reduced. Adding TGF-β abrogates the effects of oxLDL on collagen and MMPs expression.
Discussion: Our study demonstrated detrimental effects of oxLDL on regulation of MMP activity in human tendon cells. This effect, in conjunction with the diminished LOX activity may explain the poor mechanical properties of tendon tissues in animals with high cholesterol [1,2]. A balance of LOX and MMP is essential for tendon homeostasis, which involves both crosslinking and degradation of the ECM, respectively [3]. TGF-β is crucial for tendon cell migration and collagen synthesis [4]. Decreased activity and expression of TGF-β by oxLDL may play an important role in dysregulation of tendon matrix.
Conclusion: The adverse effects of oxLDL on the homeostasis of tendons is potentially through downregulation of TGF-β activity which changes tendon matrix degradation, crosslinking and synthesis.

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P082
Sclerostin is targeted to the fibrillin scaffold

Annkatrin Correns[1,2], Zimmermann Laura-Marie[1,2], Ariane Gießler-Furlan[2], Gerhard Sengle[1,2,3]
[1]Department of Paediatrics and Adolescent Medicine, University Hospital Cologne, [2]Center for Biochemistry, University Hospital Cologne, Faculty of Medicine, Cologne, Germany; [3]Center for Molecular Medicine Cologne (CMMC), Cologne, Germany

Purpose: The intricate extracellular matrix (ECM) microenvironment of the aorta controls the bioavailability of growth factors of the TGF-β superfamily. This is achieved by direct targeting and sequestration of growth factors or their inhibitors (Correns et al., 2021). Structural deficiency of ECM components such as fibrillin microfibrils may lead to failed sequestration of growth factor inhibitors and thereby to disbalanced tissue homeostasis and regeneration processes. Here, we found the BMP / Wnt inhibitor sclerostin to be implicated in the molecular pathways underlying aortic aneurysm formation in a mouse model of Marfan syndrome.
Methods: Mouse aortas were stained and examined with confocal and light microscopy. Surface plasmon resonance (SPR) technology and ELISA were utilized to monitor protein-protein interactions.
Results: Mass spectrometry analysis of Fbn1 GT8 Marfan aortas revealed SOST as the most upregulated protein. Co-localization of fibrillin-1 and SOST was found in the aorta and ECM of vascular smooth muscle cells. Direct interaction of fibrillin-1 and SOST was revealed via SPR. Disruption of the microfibril ultrastructure in the Fbn1 GT8 mouse model was shown to increase SOST expression, whereas genetic ablation of Sost in GT8 Marfan mice appears promising in ameliorating aortic aneurysm formation.
Discussion: Fibrillin deficiency leads to structurally compromised microfibrils which may release BMP inhibitors such as SOST that turn off BMP signalling and thus promote TGF-β signalling. This may explain the wide clinical spectrum of patients with FBN1 mutations.
Conclusion: Our data highlight the structural importance of fibrillin microfibrils as signalling platform. Integrating growth factor and antagonist signalling, microfibrils play a key role in tissue homeostasis.

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P083
BMP driven mechanisms in aortic aneurysm formation in a mouse model of Marfan syndrome

Laura-Marie Zimmermann [1,2], Ariane Gießler-Furlan[2], Dennis Mehrkens[3], Stephan Baldus[3], Paola Zigrino[4], Gerhard Sengle[1,2,5]
[1]Department of Pediatrics and Adolescent Medicine, Faculty of Medicine, [2]Center for Biochemistry, [3]Heart Center, Faculty of Medicine, [4]Department of Dermatology, University Hospital of Cologne, Cologne, Germany; [5]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

Purpose: Fibrillin microfibrils target and sequester growth factors of the TGF-β superfamily within the extracellular microenvironment of aortic resident cells. Bone morphogenetic proteins (BMPs) interact with microfibrils via their prodomains which renders them latent. Recently, we demonstrated that prodomain degradation by matrix metalloproteinases (MMPs) leads to activation of BMPs (Furlan, 2021). To assure tissue homeostasis, a fine-tuned balance between BMP and MMP activity is crucial. In disease situations characterized by ECM degradation, such as Marfan syndrome, that is caused by fibrillin-1 deficiency, small amounts of active BMP or MMP may initiate a vicious feed-forward cycle where MMP-mediated BMP release from ECM-targeted pools further promotes MMP production, ultimately resulting in severe ECM destruction [1].
Methods: Aortas from GT8 Fbn1 knock-in Marfan mice expressing a fibrillin-1 C-terminal truncation mutation were analyzed by echocardiography and microscopy. Cell culture and genetic breeding experiments were conducted.
Results: GT8 Fbn1 mice showed significant aortic root enlargement at P10 which correlated with the onset of aberrantly increased BMP signaling and MMP-13 expression levels. BMPs upregulated MMP-13 expression in VSMCs. Breeding the Fbn1 GT8 Marfan allele onto a Mmp13 null background prevents aortic root enlargement implicating the relevance of this mechanism in aortic aneurysm formation in MFS.
Discussion: Non-sequestered BMPs due to fibrillin-1 deficiency trigger a destructive feed-forward cycle by turning on MMP-13 activity which activates more fibrillin-bound BMPs but also other MMPs leading to gross destruction of the aortic architecture including collagen and elastin networks.
Conclusion: Here we identified a new activation mechanism of BMPs from ECM-bound pools and MMP-13 as a new potential therapeutic target which could be relevant for aortic disease progression in MFS.

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P084
Peroxynitrous acid causes changes to the structure and function of versican

Sara Marthedal Jørgensen[1], Christine Chuang[1], Michael Davies[1]
[1]Biomedical Sciences, University of Copenhagen, København N, Denmark

Purpose: Versican (VCAN) is a proteoglycan found in the arterial extracellular matrix (ECM) associated with hyaluronic acid (HA) and vascular smooth muscle cells (VSMCs). In the development of atherosclerosis, the deposition of VCAN in the arterial ECM increases. At the same time, activated macrophages accumulate in the same space and produce damaging oxidants, including peroxynitrous acid (ONOOH). Extracellular proteins are major targets of oxidative modification, and we hypothesized that ONOOH-induced damage to VCAN might modulate its composition, structure and biological function. These changes might enhance the development of atherosclerosis. We have previously shown that VCAN produced by human coronary artery smooth muscle cells (HCASMCs) is modified by ONOOH, but little is known about the nature of the modifications and their consequences for protein function.
Methods: Recombinant VCAN isoform 3 (V3) treated with ONOOH was subjected to SDS-PAGE and LC-MS (after hydrolysis to amino acids) to detect modifications. Silver staining or immunoblotting, probing for VCAN or damage-associated epitopes, was used to examine both changes to parent V3 VCAN and the formation of modifications. HA binding, cell adhesion, proliferation and migration were used to assess the effect of modifications on protein function.
Results: Silver stains and immunoblotting revealed structural modifications to V3, including loss of parent monomer and aggregate formation, on ONOOH treatment. Levels of the damage biomarkers 3-nitrotyrosine and di-tyrosine increased with exposure to increasing oxidant concentrations. A further product, 6-nitrotryptophan was also detected at high ONOOH concentrations. ONOOH-treatment reduced binding of VCAN to HA and adhesion of HCASMC, but stimulated HCASMC proliferation and migration.
Discussion and Conclusion: Exposure to the inflammatory oxidant ONOOH induces multiple modifications to V3 with consequences for protein structure and function. These changes modulate interactions with HA, and affect cell adhesion, proliferation and migration. Whilst native V3 is associated with anti-atherogenic smooth muscle cell behavior, ONOOH-modified V3 promotes pro-atherogenic behavior suggesting a link between oxidant-modified ECM components and disease progression.

P085
Hypoxia promotes the synthesis of a versican-rich extracellular matrix by human coronary endothelial cells

Song Huang[1], Sara Marthedal Jørgensen[1], Lasse Lorentzen[1], Michael Davies[1], Christine Chuang[1]

[1]Department of Biomedical Sciences, Panum Institute, University of Copenhagen, Copenhagen N, Denmark

Purpose: During the development of atherosclerosis, the vascular wall accumulates lipids and becomes thickened, which results in a hypoxic environment due to limited O_2 diffusion and increased demand. Increasing evidence suggests that hypoxia may be a driver of further extracellular matrix (ECM) modification, contributing to development and instability of the lesion. This increases the risk of lesion rupture and subsequent myocardial infarction or stroke. The aim of this study was to investigate whether exposure of human coronary artery endothelial cells (HCAEC) to 1 versus 20% O_2 resulted in an altered synthesis and composition of the ECM and whether this modulated HCAEC activation, adhesion, proliferation, gene expression of ECM proteins and generation of reactive oxidants.
Methods: HCAECs were cultured for 7 days at 1 or 20% O_2 and gene expression of ECM and cell activation markers were examined by qPCR, while ECM protein levels were detected by enzyme-linked immunosorbent assay (ELISA), immunoblotting and mass spectrometry. Cell adhesion and proliferation were quantified using calcein-AM and MTS assay respectively. Flow cytometry was used to investigate the expression of ICAM-1, VCAM-1 and production of reactive oxidants.
Results: Changes in mRNA expression and protein synthesis of ECM components were observed in response to HCAEC exposure to 1% O_2 for 7 days, with versican levels markedly elevated. This increase in versican was confirmed by immunoblotting and mass spectrometry. Decreased mRNA expression of cell adhesion molecule, VCAM-1, as well as increased oxidant generation were detected. Despite reduced HCAEC adhesion to the versican-rich ECM generated under 1% O_2, the adherent cells demonstrated increased metabolic activity.
Discussion and Conclusion: These data indicate that exposure of HCAEC to 1%, compared to 20% O_2, alters the ECM generated by the cells. The increased production of versican may exacerbate the progression of atherosclerosis, as this proteoglycan is a well-established binding site for lipoproteins, and therefore may contribute to lipoprotein accumulation within lesions. The concurrent increase in oxidant production may exacerbate lipoprotein modification, and contribute to the unregulated accumulation of lipids within macrophage and other cells in developing lesions.

P086
Extracellular thrombospondin-1 is regulated by the endocytic collagen receptor, urokinase plasminogen activator receptor-associated protein (uPARAP/Endo180)

Kirstine S. Nørregaard[1], Henrik J. Jürgensen[1], Signe Z. Ingvarsen[1], Signe S. Hansen[1], Christina E. Hagensen[2], Henrik Gårdsvoll[1], Daniel Hargbøl Madsen[3], Ole N. Jensen[2], Lars H. Engelholm[1], Niels Behrendt[1]

[1]Rigshospitalet/Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen N, Denmark; [2]Department of Biochemistry & Molecular Biology and VILLUM Center for Bioanalytical Sciences, University of Southern Denmark, Odense, Denmark; [3]National Center for Cancer Immune Therapy, Department of Oncology, Copenhagen University Hospital – Herlev and Gentofte, Herlev, Denmark

Purpose: UPARAP (Endo180/CD280) is an endocytic receptor with a homeostatic regulator function. This receptor undertakes the endocytosis of various ligands for intracellular degradation and takes part in ECM remodelling in bone development, fibrosis and cancer invasion (1). The proteins subject to regulation by uPARAP include structural collagens but the complete ligand spectrum of the receptor is not known. The purpose of this study was to find novel ligands for uPARAP.
Methods: We first performed an unbiased, mass spectrometry based comparison of the endosomal content of cells differing only by the presence or absence of uPARAP. A uPARAP-depleted SaOS-2 osteosarcoma cell line was created using CRISPR/CAS-9. These cells were compared with wild-type SaOS-2 cells. Protein endocytosis was studied in vitro using proteins labelled with I-125 or using quantitative ELISA. For analyses in vivo, fluorescence-labeled ligands were injected s.c., followed by analysis by confocal microscopy and flow cytometry.
Results: The matricellular protein thrombospondin-1 (Tsp-1) was found as one of very few extracellular proteins displaying a difference when comparing the endosome content of uPARAP wild-type and uPARAP depleted cells. In cellular uptake studies with recombinant Tsp-1 we then demonstrated a pronounced uPARAP-dependent endocytosis and degradation of Tsp-1 in several cultured cell lines. uPARAP could also modulate the levels of endogenous, secreted TSP-1. Finally, comparing wild-type and uPARAP knock out mice, we found that uPARAP was capable of cellular Tsp-I uptake in vivo.
Discussion: We show that uPARAP is a specific receptor for Tsp-1, that it has a regulator function for the extracellular TSP-1 levels and that this process is relevant in vivo.
Conclusion: uPARAP is a novel receptor and homeostatic regulator of TSP-1, a crucial determinant in the regulation of angiogenesis and the organization of the ECM.

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P087
Matrix turnover quantified in adult mouse tendon by isotope labelled proteomics

Anna Hoyle[1,2], Joan Chang[1,2], Ronan O'Cualain[3], David Knight[3], Quing-Jun Meng[1,2], Karl Kadler[1,2], Joe Swift[1,2]
[1]Wellcome Centre for Cell-Matrix Research; [2]Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, [3]BioMS Facility, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester, United Kingdom

Purpose: Heavy carbon isotopes in the tendons of people who grew up in the age of nuclear bomb testing have shown that the extracellular matrix, assembled during development, stays with us for life [1]. However, recent work suggests that type-I collagen, in mouse tendon, exists in two pools: a permanent matrix, and a more soluble, circadian-regulated matrix [2]. Here, we use stable isotope labelling coupled with mass spectrometry to quantify the half-lives of tendon matrix proteins.
Methods: Tail tendon was harvested from 22-week old C57/BL6 mice fed on a heavy-lysine (13C-Lys; CK Isotopes) diet for either 1, 2, 4, or 8 weeks (n = 3). Protein was extracted from tissues using a sequential two-step protocol: fraction 1 (F1), in sodium laurate/deoxycholate; and fraction 2 (F2), in sodium dodecyl sulphate with ultra-sonication (Covaris). Solubilized fractions were analysed by liquid-chromatography coupled tandem mass spectrometry (Thermo).
Results: The soluble F1 fraction was found to contain intracellular proteins, and a range of core and associated extracellular matrix proteins, including a circadian-regulated pool of type-I collagen [1]. The F2 fraction contained primarily collagens, including type-I collagen which did not show rhythmicity. Matrix proteins extracted in the F1 pool had significantly shorter half-lives than F2, including type-I collagen which had half-lives of 4 ± 2 days in F1, compared to 700 ± 100 days in F2.
Discussion: Circadian-regulated matrix proteins were found to have significantly faster turnover. More generally, matrix protein half-lives could be placed in a hierarchy of collagens > glycoproteins and proteoglycans > matrix-associated proteins.
Conclusion: This work provides further evidence for the circadian/permanent model of tendon matrix. Moreover, it establishes a pipeline for quantitative and systematic study of matrix turnover.

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P088
The structural asymmetry of the epiphyseal plate

Piero Antonio Zecca[1], Marcella Reguzzoni[1], Marina Protasoni[1], Mario Raspanti[1]
[1]Department of Medicine and Surgery, Insubria University, Varese, Italy

Purpose: The epiphyseal plate is basically a cartilage layer sandwiched between two bony segments in long bones. On the epiphyseal side the chondrocytes are small and rounded in a sort of resting zone; toward the diaphysis they gradually blend into a proliferating zone and then a hypertrophic zone, that eventually calcifies [1]. The molecular mechanisms underlying these processes are poorly known. This study aims to elucidate the structure and ultrastructure of the two bony interfaces of the epiphyseal plate.
Methods: The epiphyseal plates from bovine proximal femoral epiphyses were investigated by light microscopy (Nikon Eclipse 300), scanning electron microscopy (FEI XL-30 FEG) and microCT (Stratec XCT-Research SA+).
Results: On the diaphyseal side the chondrocytes become hypertrophic and express collagen types X and I, easing the endochondral ossification of the cartilage matrix. The calcification proceeds very actively with a thick solid mineralization front but leaves behind a constellation of small cartilage islets interspersed with the newformed bone, with which they will subsequently be remodelled. The calcification is apparently initiated by matrix vesicles and proceeds with no visible involvement of the collagen fibrils. In stark contrast, the other interface of the epiphyseal plate shows no trace of ossification in progress, and SEM and microCT consistently confirm just normal, mature cancellous bone.
Discussion: The two sides of the epiphyseal plate show an opposite situation, with endochondral ossification occurring very actively on one face of the cartilage layer, and nothing at all on the other side. No cause-effect relationship is evident at the ultrastructural level.
Conclusion: Other research is necessary to elucidate the role of the adjoining bone on such opposite behaviors occurring a few micrometers apart.

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P089
Collagens contribute to the endothelial glycocalyx barrier-function

Shailey Twamley[1,2], Héctor Eduardo Sánchez-Ibarra[1], Victoria Isakzai[1], Heike Heilmann[1], Anke Stach[1], Tobias Christaller[1], Berit Soehl-Kielczynski[1], Niclas Gimber1[1], Agnieszka Muenster-Wandowski[1], Antje Ludwig[1,2]
[1]Charité - Universitätsmedizin Berlin, Berlin, Germany; [2]DZHK (German Centre for Cardiovascular Research), partner site Berlin, Germany

Purpose: The endothelial glycocalyx (GCX) is a hydrogel-barrier on the apical side of the endothelium. Inflammation and low wall shear stress disrupt the GCX. Such alternations increase GCX permeability and initiate vascular disease. It is assumed that the GCX is mainly composed of glycoproteins, proteoglycans, and glycosaminoglycans, and that their density alone determines the strength of the GCX barrier-function (BF). In this study, we tested the hypothesis that structural proteins contribute to the GCX BF.
Methods: Human endothelial cells (ECs) were cultivated under laminar shear stress (LSS) using the Ibidi pump system. We cryo-preserved the GCX for immuno-transmission electron microscopy (TEM) and confocal laser scanning microcopy (CLSM) analysis. Spinning disc confocal microscopy with FluoSpheres was used to study the permeability of the GCX under LSS. Gene expression was assessed by mRNA microarray, qPCR and Western blot. GCX composition was modified by treatment with halofuginone (HF) and Col type V (ColV) expression was inhibited with siRNAs. Very small superparamagnetic iron oxide nanoparticle (VSOP) uptake was measured to test BF strength.
Results: LSS stimulation increases GCX thickness and the pericellular exclusion region of FluoSpheres in ECs. Additionally, LSS increased the expression of Col I and V (Col). Immuno-TEM and CLSM confirmed the presence of Col as filamentous structures within the GCX. HF treatment and ColV-siRNA inhibited the LSS-induced increase of Col expression and augmented the uptake of VSOP in LSS cultivated ECs.
Discussion: We describe here Col as a previously neglected component of the GCX. Col are structural proteins that may determine the dimension and mechanical properties of the GCX. We provide initial evidence that Col contributes to the BF and that TGF-β signaling is involved in their LSS-induced expression.
Conclusion: Lack of LSS reduces GCX density and Col expression within the GCX. A decrease in Col weakens the BF.

P090
Endocytosis of extracellular molecules in respiratory health and disease

Maria Martina Meschis[1], Kazuhiro Yamamoto[1], George Bou-Gharios[1], Carsten Scavenius[2], Jan J. Enghild[2], Sonya Craig[3]
[1]Musculoskeletal & Ageing Science, University of Liverpool, Liverpool, United Kingdom; [2]Department of Molecular Biology and Genetics Aarhus University, Aarhus, Denmark; 3Aintree Hospital, Liverpool, United Kingdom

Purpose: Low-density lipoprotein receptor-related protein 1 (LRP1) is ubiquitously expressed and mediates endocytosis of various molecules. The large genome-wide association studies identified LRP1 is relevant for pulmonary function1 but little is known about its role in lung tissue. We aimed to identify molecules regulated by LRP1 and to investigate the impact of LRP1 loss on lung tissue homeostasis.
Methods: Lung primary cells were incubated with soluble from of LRP1 (sLRP1) that blocks the binding of LRP1 ligands to membrane-bound LRP1. The molecules bound to sLRP1 were isolated and identified by anti-sLRP1 beads and mass-spectrometry, respectively. Cell viability in the presence of sLRP1 was assessed by cell counting and colorimetric MTS assay.
Results: We found that LRP1 is abundantly expressed in the lung cells and tissue. We identified total 67 ligand candidates including 50 previously unreported novel ligands. Importantly, we found that sLRP1 and LRP1 ligand-binding antagonist induce cell death (> 60%) after 24 hours.
Discussion: The newly identified LRP1 ligands are involved in cell survival, inflammation and extracellular matrix remodelling. Considering that inhibition of LRP1-mediated endocytosis induced cell death, prolonged presence of LRP1 ligands could be detrimental to the tissue. We tested this hypothesis using tissue-specific conditional Lrp1 knockout mice.
Conclusion: A number of extracellular molecules are rapidly taken up by the cells and degraded intracellularly via LRP1 in lung tissue. An increased ectodomain shedding of LRP1 alters the homeostatic balance and may become a cause of pulmonary diseases.

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P091

Estradiol inhibits endoplasmic reticulum stress-induced apoptosis in chondrocytes and reduces age-related osteoarthritis

Yvonne Rellmann[1], Ising Thomas[1], Ramroth Markus[1], Eidhof Elco[1], Uwe Hansen[2], Rita Dreier[1]
[1]Insitute of Physiological Chemistry and Pathobiochemistry, Münster, Germany; [2]IMM, Münster, Germany

Purpose: Aging and endoplasmic reticulum (ER) stress in chondrocytes are risk factors for osteoarthritis (OA). C28/I2 ERp57 knock-out cells and cartilage-specific ERp57-KO mice served as models to investigate ER stress in cartilage homeostasis.
Methods: ER stress was confirmed by transmission electron microscopy and immuno-fluorescence/-blot analysis of ER stress marker proteins. TUNEL assays and cell death ELISA detected apoptosis in chondrocytes. The severity of OA in mice +/- forced treadmill training was determined by OARSI scoring. In vitro studies +/- estradiol elucidated sex differences.
Results: Human knee cartilage samples showed an increased ERp57 staining with rising OA severity. This confirms that ERp57 is involved in OA development, possibly in ER stress management. With age, male ERp57-KO mice developed more ER stress and apoptosis in chondrocytes than females, which was associated with more pronounced osteoarthritic cartilage degeneration and osteophyte formation in knee joints. In contrast, young mice did not exhibit ER stress and were not susceptible to OA, even when exposed to forced exercise. Cell culture studies demonstrated a reduction in ER stress and apoptosis in C28/I2 cells by physiological estradiol concentrations, consistent with less OA development in aged female mice compared to male littermates.
Discussion: This study demonstrates that estradiol reduces ER stress and ER stress-induced apoptosis in articular chondrocytes, thus minimizing critical events in osteoarthritic cartilage degeneration.
Conclusion: Regulation of ER stress on estradiol signaling pathways has potential for OA therapy.

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P092

The gelatinases, matrix metalloproteinases 2 and 9, play individual roles in mouse skeleton development

Rotem Kalev-Altman[1,2], Jerome Nicolas Janssen[2], Nir Ben-Haim[1], Tamar Levy[1], Astar Shitrit[2], Josh Milgram[1], Ron Shahar[1], Dalit Sela-Donenfeld[1], Efrat Monsonego-Ornan[2]
[1]Food and Environment, The Faculty of Agriculture, The Koret School of Veterinary Medicine, Rehovot, Israel; [2]Food and Environment, The Faculty of Agriculture, The Institute of Biochemistry, Food Science and Nutrition, Rehovot, Israel

Purpose: Recently, the gelatinases, a subgroup of the matrix metalloproteinases (MMPs) superfamily, were found to be necessary for neural crest cell migration and to compensate for each other loss in these cells1,2NCCs undergo epithelial-to-mesenchymal transition and invade the surrounding extracellular matrix (ECM. To characterize their involvement in the skeletal system, and to better reveal their individual or common roles, we have generated double knockout (dKO) mice, lacking both MMP2 and MMP9.
Methods: We µCT-scanned and analysed the skeleton morphological and mechanical parameters at postnatal day 21, 3 months (M) and 8M of age and performed transcriptomic analysis on different skeletal elements, the cortical bone, the growth plate and the skull of the single and double-KO.
Results: An unexpected distinct role for each gelatinase was revealed; MMP2 was found to be involved merely in intramembranous ossification which led to a smaller skull and inferior cortical parameters upon its loss, while MMP9 was found to affect only the endochondral ossification process, which led to shorter long-bones in its absence. Importantly, the dKO mice demonstrated a combination of both the skull and long bone phenotypes as found in the single-KOs, and not a severer additive phenotype. Transcriptome analysis reinforced the specific and distinct role of each gelatinase in each bone type.
Discussion: The distinct roles of each gelatinase imply for their different role of action in effecting different genes and bone/cartilage cells.
Conclusion: The results suggest that although both gelatinases share the same substrates and are highly expressed in flat and long bones, they are indispensable and control separately the development of different bones.

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P093
Mapping proteolytic events in extracellular matrix proteins using a label-free quantitative proteomics approach

Salvatore Santamaria[1], Daniel Martin[2], Suneel S. Apte[2]
[1]Immunology and Inflammation, London, Imperial College London, United Kingdom; [2]Department of Biomedical Engineering, Cleveland Clinic Lerner Research Institute, Cleveland, USA

Purpose: Proteolysis is a major mechanism of post-translational regulation of extracellular matrix (ECM) proteins. Identification of cleavage sites is important to elucidate the substrate specificity of a certain protease; additionally, the resulting neopeptides can be further investigated as matrikines and/or biomarkers of specific diseases. Here, we aimed to map proteolytic events generated by members of the A Disintegrin And Metalloproteinase with ThromboSpondin motifs (ADAMTS) family on chondroitin sulfate proteoglycans (CSPGs) and osteopontin (OPN), major components of the ECM in a variety of tissues.
Methods: Recombinant purified CSPGs (versican and biglycan) and OPN were digested with recombinant full-length ADAMTS1, 4, 5 and 8, using catalytically inactive mutant proteases in control digests. Semi-tryptic peptide abundance ratios determined by liquid-chromatography-tandem mass spectrometry (LC-MS/MS) in ADAMTS: control digests were compared to the mean of all identified peptides to obtain a z-score by which outlier peptides were ranked.
Results: We confirmed that ADAMTS1, 4, and 5 cleave versican at the Glu441-Ala442 bond [1]. Additionally, we identified several novel cleavage sites in versican and biglycan, supporting a cleavage site preference for a P1-Glu residue. Finally, we identified multiple cleavage sites by ADAMTS8 in OPN.
Discussion: The ability of ADAMTS1, 4, and 5 to cleave at multiple sites in versican may explain the relatively mild phenotype exhibited by mouse strains where the versican Glu441-Ala442 bond was mutated to make it uncleavable by ADAMTS proteases [2, 3] compared to that of Adamts knockout strains [4].
Conclusion: Digestion of ECM proteins in vitro and application of our z-score approach is potentially widely applicable for mapping protease cleavage sites using label-free proteomics.

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P096
The role of extracellular matrix in brain remodeling post stroke

Egor Dzyubenko[1], Dirk Hermann[1]
[1]Center for Translational Neuro- & Behavioral Sciences (C-TNBS), University Hospital Essen, Essen, Germany

Purpose: Understanding the mechanisms underlying brain remodeling post stroke is key for the development of novel neurorestorative therapies [1]. Emerging evidence indicates that the brain extracellular matrix (ECM) is essential for the maintenance of neuronal networks [2], but the role of ECM in neurological recovery after brain injury remains unknown. Here, we investigated how ultrastructural changes in perineuronal nets (PNNs), the condensed ECM layers on the surface of fast-spiking neurons, contribute to synaptic remodeling and functional recovery after stroke.
Methods: Stroke was induced in wild-type C57BL/6 mice as described previously [3, 4]. PNN morphology was analyzed using three-dimensional superresolution structured illumination (3D SR-SIM) and stimulated emission depletion (3D STED) microscopy and quantified using our in-house computational method [5]. Synapse density and neuronal marker expression were measured by immunohistochemistry and high-resolution confocal microscopy. Neurological recovery was analyzed by behavioral testing.
Results: Our data revealed that PNNs in the motor cortex layer 5 are reversibly degraded and remodeled during the first two weeks after stroke. The loosening of PNNs can be modulated by inflammatory preconditioning and precedes glutamate- and GABAergic synapse remodeling associated with post stroke recovery.
Discussion: Our findings indicate a novel neuroplasticity mechanism involving ultrastructural ECM changes and synaptic rewiring for post stroke recovery.
Conclusion: Reversible ECM remodeling is essential for brain remodeling and neurological recovery post stroke.

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P097
Degeneration-related extracellular matrix changes in human facet joint cartilage

Annika Kohler[1], Panagiotis Diaremes[1], Andrea Meurer[1], Zsuzsa Jenei-Lanzl[1], Frank Zaucke[1]
[1]Orthopedics (Friedrichsheim), University Hospital Frankfurt, Goethe University, Germany

Purpose: With increasing degree of degeneration, proteoglycan content is reduced and water is lost from the extracellular matrix (ECM) in articular cartilage of facet joints. Previous studies on matrix degeneration focused on type I and II collagens as well as on proteoglycans, the major ECM components. However, minor components that interconnect collagen fibrils, link the collagen network to the aggrecan matrix and determine functional properties have not been analyzed systematically.
Methods: Human facet joints were harvested from patients undergoing spondylodesis. One half of the tissue was decalcified and embedded in paraffin, the other half was used for protein isolation. To visualize tissue morphology and proteoglycans, sections were stained with 1,9-dimethylmethylene blue (DMMB). Further ECM components were detected with specific antibodies directed against collagen XII, thrombospondin-4 (TSP-4) and -5 (COMP), matrilin-3, and nidogen-1 by immunohistochemistry and immunoblotting.
Results: We observed distinct degeneration-associated changes in matrix protein expression. In degenerating facet joint sections, reduced DMMB indicated the expected proteoglycan loss. In addition, a decreased expression of type XII collagen, TSP-4, matrilin-3 and nidogen-1 was detected, while COMP staining intensity was only slightly reduced. A similar degeneration-dependent loss of ECM molecules was detected in protein extracts by immunoblotting.
Discussion: We detected degeneration-related changes in the expression of minor ECM components. The rearrangement and/or loss of minor interconnecting proteins might destabilize higher order structures in the ECM. This would explain altered mechanical properties and an increased susceptibility for tissue degeneration.
Conclusion: Further studies analyzing the cleaved fragments will help to understand the complex pathogenesis of facet joint degeneration.

P098
Syndecans as potential regulators of the severe skeletal muscle myopathy Wooden breast in chicken

Lucie Pejšková[1], Sissel Beate Rønning[1], Cathrine Rein Carlson[2], Vibeke Høst[1], Nina Solberg[1], Svein Olav Kolset[3], Mona Pedersen[1]
[1]Nofima AS, Raw Material and Process Optimization, Ås, Norway; [2]Institute for Experimental Medical Research, Oslo University Hospital, University of Oslo, Norway; [3]Department of Nutrition, Institute of Basic Medical Science, University of Oslo, Oslo, Norway

Purpose: Skeletal muscle deformities in commercial chickens, known as the Wooden breast (WB), is a recent fibrotic myopathy of unknown etiology. For a better understanding of the biological mechanisms triggering WB it is essential to study the biological mechanisms. Fibrosis is caused by dysfunctional skeletal muscle satellite cell activity (MuSC) in combination with an imbalanced regulation of fibroblast/myofibroblast activity. Myopathies of the skeletal muscle are known to involve a family of proteoglycans – syndecans. They were showed to regulate fibrosis in cardiac muscle and to have crucial functions in normal MuSC and fibroblast activity. There has been no research into syndecans' possible role in WB. And more importantly, how can these multifaceted molecules be modified to avoid development of the muscle disease and retain essential functions for key cellular processes? This is the foundation of this study, where we will try to answer some of these questions.
Methods: Breast muscle samples were collected from Ross308 chickens fed by a palatable diet with high energy for 36 days and classified as WB or non-affected using NIR-spectroscopy and palpation after slaughter. Histology and immunofluorescence microscopy were used for analysis of samples. qPCR was used for gene expression with focus on ECM components in general with special emphasis on syndecans, followed up with western blotting.
Results: Morphological characteristics of WB demonstrated increased fibrosis with heavily collagen staining and fatty infiltration in wooden breast affected samples. The collagen staining pattern was unorganized in WB affected samples compared to a more regular pattern in non-affected samples. SMA expression was detected in extracellular matrix production (fibrosis), and degeneration of muscle tissue with apoptosis/necrosis was visible.
Discussion / Conclusion: The morphology of WB identified extensive fibrosis, inflammation, and extracellular matrix production. This elucidation of molecular mechanisms of ECM proteins and their role in WB contributes to fundamental research of myopathy, and further research will be necessary for a comprehensive explanation.

P099
Differential MMP-14 targeting by biglycan, decorin, fibromodulin and lumican unraveled by in silico approach

Romain Rivet[1], Rajas Mallenahalli-Rao[1,2], Pierre Nizet[1], Nicolas Belloy[1,2], Louise Huber1, Manuel Dauchez[1,2], Laurent Ramont[1,3], Stéphanie Baud[1,2], Stéphane Brézillon[1]

[1]CNRS UMR 7369, Matrice Extracellulaire et Dynamique Cellulaire (MEDyC), [2]P3M, Multi-Scale Molecular Modeling Platform, Université de Reims Champagne Ardenne, Reims, France; [3]CHU Reims, Service Biochimie Pharmacologie-Toxicologie, Reims, France

Purpose: Small leucine-rich proteoglycans (SLRPs) are major regulators of extracellular matrix assembly and cell signaling. Lumican, a member of the SLRPs family, and its derived peptides were shown to possess anti-tumor activity by interacting directly with the catalytic domain of MMP-14 leading to the inhibition of its activity. The aim of the present report was to characterize by in silico 3D modeling the structure and the dynamics of four SLRPs [Biglycan (BGN), Decorin (DCN), Fibromodulin (FMOD), Lumican (LUM)] including their core protein and their specific polysaccharide chains to assess their capacity to bind to MMP-14 and to regulate its activity.
Methods: Molecular docking experiments were performed to identify the specific amino acids of MMP-14 interacting with each of the four SLRPs using the Hex software. The inhibition of each SLRP (100nM) on MMP-14 activity was measured and the constants of inhibition (Ki) were evaluated. The impact of the glycan chain number, structures and dynamics of lumican on the interaction with MMP-14 was assessed in silico by molecular dynamics simulations using GROMACS software.
Results: Molecular docking analysis showed that all SLRPs bind to MMP-14 through their concave face, but in different regions of the catalytic domain of MMP-14. Each SLRPs inhibited significatively the MMP-14 activity (BGN: 92%, Ki: 21 ± 7.2nM; DCN: 76%, 39 ± 28.4nM; FMOD: 83%, Ki: 19 ± 7.9 nM; LUM : 86%, Ki: 19 ± 11.3nM). Finally, molecular dynamics showed the role of glycan chains in interaction with MMP-14 and shielding effect of SLRPs.
Discussion: Altogether, the results demonstrated that each SLRP exhibited inhibition of MMP-14 activity. However, the differential targeting of MMP-14 by the SLRPs was shown to be related not only to the core protein conformation but also to the glycosaminoglycan chain structures and dynamics. These results might explain, at least in part, the differential effects of SLRPs in tumor progression due to the differential regulation of SLRPs on MMP-14 activity.
Conclusion: These results might explain, at least in part, the differential effects of SLRPs in tumor progression due to the differential regulation of SLRPs on MMP-14 activity.

P100
Characterization of collagen turnover pathways in primary muscle fibroblasts derived from a dystrophic mouse model of Duchenne muscular dystrophy

Matteo Giovarelli[1], Silvia Zecchini[1], Laura Brigida Cornaghi[2], Cecilia Barbieri[1], Ambra Nava[2], Luca Denti[3], Michele Sommariva[2], Francesca Arnaboldi[2], Nicoletta Gagliano[2]

[1]Department of Biomedical and Clinical Sciences, [2]Department of Biomedical Sciences for Health, [3]Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy

Purpose: In Duchenne muscular dystrophy (DMD), skeletal muscles undergo a progressive degeneration and fibrosis. We aimed at characterizing COL turnover pathways in cultured primary muscle fibroblasts obtained from the quadriceps of young and adult wild type (wt) and mdx mice (the most employed model for preclinical DMD research).
Methods: Muscle fibroblasts were obtained from young (1 month) and adult (5 months) mdx mice. COL-I, COL-III and matrix metalloproteinases (MMP)-1 levels and MMP-2 activity were assessed, respectively, by Slot blot and SDS-zymography in cell culture supernatants. Gene expression for lysyl oxidase (LOX) and lysyl hydroxylase 2 (LH2b), and TIMP-1 and 2 were analyzed by real time PCR.
Results: COL-I and COL-III secreted by wt and mdx fibroblasts were not significantly affected by mdx in young and adult mice. LOX tended to be up-regulated whilst LH2b mRNA levels were significantly increased in both young and adult mdx compared to wt mice. MMP-1 and 2 were not affected, but TIMP-1 and 2 increased in fibroblasts of 5-month-old mdx mice.
Discussion: We show that increased collagen cross-linking and MMPs inhibition could contribute to muscular fibrosis. Interestingly, these two mechanisms could differently contribute to COL accumulation in the progression of DMD. In fact, in young mice fibrosis seems be dependent on increased collagen-crosslinking. Conversely, in adult mice, both increased collagen cross-linking as well as the inhibition of its degradation could act as major molecular mechanisms to furtherly favour the expansion and the persistence of the fibrotic remodeling of dystrophic muscles.
Conclusion: These findings could offer new insight in understanding the mechanisms responsible for muscular fibrosis in DMD in order to find new therapeutic targets to effectively prevent its progression and potentially reverse its course.

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P101
The proteoglycan versican regulates the development of cardiac fibrosis

Athiramol Sasi[1,2], Andreas Romaine[1,2], Arne Olav Melleby[1], Almira Hasic[1], Pugazhendhi Murugan Erusappan[1], Christen Peder Dahl[3], Hideto Watanabe[4], Kaspar Broch[2,5], Vibeke Almaas[5], Ida Gjervold Lunde[1,2], I Sjaastad[1,2], Maria Vistnes[1,6], Geir Christensen[1,2]
[1]Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo, Oslo, Norway; [2]K.G. Jebsen Center for Cardiac Research, University of Oslo, Oslo, Norway; [3]Research Institute of Internal Medicine, Oslo University Hospital, Oslo, Norway; [4]Institute for Molecular Science of Medicine, Aichi Medical University, Nagakute, Japan; [5]Department of Cardiology, Oslo University Hospital Rikshospitalet, Oslo, Norway; [6]Department of Cardiology, Oslo University Hospital Ullevål, Oslo, Norway

Purpose: Cardiac fibrosis, excessive deposition of extracellular matrix (ECM), is a central pathological feature in heart failure. We and others have shown that proteoglycans are involved in the development of cardiac fibrosis [1]. Versican, an ECM proteoglycan, has important roles in tissue hydration and maintaining ECM integrity. The aim of this study was to investigate the production and role of versican in cardiac fibrosis.
Methods: To study the production of versican and its localization during the development of fibrosis, we used a pressure overload mouse model that mimics aortic stenosis. The role of versican in cardiac fibrosis was examined in a conditional versican knockout mouse model. Finally, we studied the myocardial expression of versican and its cleaved fragment in endomyocardial biopsies from patients with dilated and hypertrophic cardiomyopathy.
Results: In the pressure overload model, the mRNA expression of V0 and V1 isoforms of versican increased in parallel with collagen I and III after 24 hours. The expression remained higher than in controls 3-56 days post aortic banding. Immunofluorescence showed that versican spreads from the perivascular area to the surrounding interstitium from 14-56 days after aortic banding. In the versican conditional knockout model, mRNA expression of collagen I and III, and protein expression of phospho-smad2 and NF-kB, were reduced. In biopsies from patients, versican was higher than in normal cardiac tissue and localized in fibrotic regions.
Discussion: In patients and the pressure overload model, versican increased and accumulated in the fibrotic regions. Moreover, cardiac deletion of versican reduced the expression of pro-fibrotic genes and NF-kB, suggesting that versican regulates inflammation and fibrotic responses in heart disease.
Conclusion: Versican is a potential regulator of fibrotic and inflammatory responses in heart disease.

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P102
Amylin perturbs the organisation of collagen network

Asrafuddoza Hazari[1], Ashutosh Kumar[1], Shamik Sen[1]
[1]IIT Dept. of Biosciences and Bioengineering, Bombay, Mumbai, India

Purpose: The extracellular matrix (ECM) is a dynamic network of proteins and proteoglycans which provides physical support to cells, and regulate growth factor signalling by tethering bioactive molecules. Distinct ECM alterations have been documented in several tissues associated with Type 2 Diabetes Mellitus (T2DM) and hyperamylinemia. While a lot of attention has been focused on understanding how amylin perturbs cell function, relatively less is known about its interactions with the surrounding ECM.
Methods: To understand the entanglement of amylin into ECM, we have started looking at the interaction of most abundant ECM protein- collagen with different forms of amylin. We document a direct interaction between amylin with collagen monomers and fibrils by biophysical techniques like FITC based fluorescence intensity measurement, SPR, temperature-sensitive CD and Solid & liquid-state NMR.
Results: As per the SPR, measurement of change in FITC intensity & CD data shows amylin binds to collagen in the micromolar range and also leading a conformational alteration in Collagen monomers and perturbing collagen fibrillation. Our solid-state NMR data suggest that amylin binds to Proline, Hydroxyproline, Arginine and carbonyl carbon region of fibrillar collagen
Discussion: Based on our findings, we posit that interaction of amylin with collagen and structural perturbation might play a role in altered cellular signalling in pancreatic endocrine tissue which leads to T2DM complications.
Conclusion: We conclude that Amylin interaction with collagen I simultaneously might cause alteration in the ECM architecture & turnover and such interactions result in amyloid formation associated with collagen matrix during hyperAmylinemia in T2DM.

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P103
The role of O-glycosylation in pancreatic cancer matrix degradation

Rebecca Bennion[1,2], Martin Humphries[1], Frederic Bard[3]
*[1]Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, Manchester, United Kingdom; [2]A*STAR, Institute of Molecular Cell Biology, Singapore, Singapore; [3]Marseille Cancer Research Centre, GLYNTERA, Marseille, France*

Purpose: In cancer, endoplasmic reticulum (ER)-residents become hyperglycosylated by relocalisation of O-GalNAc glycosyltransferases from Golgi-to-ER via the GalNAc transferase activation pathway (GALA). Hyperglycosylated ER proteins can locate to the plasma membrane (PM) where they interact with extracellular matrix (ECM) proteins1. Glycosylated ER-residents Calnexin and PDIA3 at the PM reduce disulphide bonds within the ECM to drive degradation1. This project aims to investigate the role of GALA in pancreatic cancer and how GALA activation influences ECM degradation.
Methods: A DQ collagen, collagen I and fibronectin ECM degradation assay was used to assess the effects of GALA and calnexin inhibition in pancreatic cancer cells (PCCs). O-GalNAc glycoproteomics identified GALA targets, which were validated by immunoblotting and immunofluorescence. PCCs reduced human foreskin fibroblast cell derived matrices (CDM) in the presence of protease inhibitors. Reduced cysteines were detected by alkylation with N-ethylmaleimide (NEM) and anti-NEM immunoblotting.
Results: GALA PCCs showed increased ECM degradation. Glycoproteomics analysis identified that ER-residents were glycosylated with GALA activation. GALA targets included calnexin, TXNDC5 and PDIA3. In GALA PCCs calnexin was present at the cell surface. Knockdown or antibody blocking of calnexin reduced PCC ECM degradation 2-fold. GALA PCCs showed greater reduction of disulphide bonds which was inhibited with anti-calnexin antibody treatment.
Discussion: Calnexin is a mediator of ECM degradation in PCCs. Blocking calnexin may reduce pancreatic tumour growth and invasion through the ECM. ER O-glycosylation is observed in many cancers, suggesting that calnexin may provide a suitable target for multiple tumours. Future work includes mapping the ECM substrates of calnexin/PDIA3 and investigating the blocking of tumour development with anti-calnexin treatment.
Conclusion: Calnexin promotes ECM degradation by PCCs via reduction of disulphide bonds within ECM.

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P104
More healthy bone: alternating dietary phosphorus increases bone volume in zebrafish

Silvia Cotti[1;2], Ann Huysseune[1], Wolfgang Koppe[3], Antonella Forlino[2], P. Eckhard Witten[1]
[1]Ghent University, Department of Biology, Gent, Belgium; [2]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [3]SimplyFish AS, Stavanger, Norway

Purpose: Dietary phosphorus (P) is essential for mineralisation of the collagen type I based bone matrix. Low-P dietary conditions result in reduced bone mineralisation, but increased formation of non-mineralised bone matrix in zebrafish1 and salmon2,3. We test the mineralisation potential of the large non-mineralised matrix volume formed in low-P fed zebrafish, by providing the animals with adequate dietary P. The aim is to mineralise the formerly non-mineralised matrix and to increase the volume of mineralised bone.
Methods: WT zebrafish (1 month old) were fed a low-P diet (0.5% P, LP) for 8 weeks. Subsequently, animals continued for 6 weeks on a LP, regular-P (1.0% P, RP) or high-P (1.5% P, HP) diet. Controls received RP diet throughout. Alizarin red, X-rays, microCT and histology were used to analyse bone phenotype and mineralisation.
Results: Zebrafish with LP history have increased total bone area at vertebral endplates compared to controls ($p < 0.001$). The non-mineralised matrix formed during the LP period eventually mineralises in LP-RP and LP-HP fish, the mineralisation extent depends on dietary P intake. A dramatic increase in the volume of vertebral centra bone (mineralised and non-mineralised) is observed in all fish with LP history. All fish have vertebral centra without malformations and intact intervertebral spaces.
Discussion: The large non-mineralised bone matrix volume formed during 8 weeks of low-P intake retains the ability to mineralise if adequate dietary P is provided. The result is a dramatic increase of the mineralised bone volume. Different from human diseases with pathological increased bone mass, no skeletal malformations occur in zebrafish.
Conclusion: This zebrafish model shows that bone formation and mineralisation are independent processes. Our protocol has already been applied to partly rescue the Chihuahua zebrafish osteogenesis imperfecta model4. Low-P conditions have the potential to mitigate bone loss, e.g. caused by osteoporosis or aging.

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P105
Characterization of collagen prolyl 4-hydroxylase III

Emma Karjalainen[1], Antti Salo[1], Pekka Rappu[2], Ujjwal Suwal[2], Ilkka Miinalainen[3], Jyrki Heino[2], Johanna Myllyharju[1]
[1]Faculty of Biochemistry and Molecular Medicine, [3]Biocenter Oulu, University of Oulu, Finland; [2]Department of Life Technologies, University of Turku,Turku, Finland

Purpose: 4-hydroxyproline (4Hyp) is essential for the procollagen triple helix folding and thermal stability. It is catalyzed by C-P4Hs that are $\alpha_{2\beta2}$ tetramers with three known isoforms of the catalytic α subunits (encoded by P4HA1, P4HA2 and P4HA3). C-P4Hs are promising targets for treating diseases involving excess collagen production and accumulation. Very little is currently known of the roles of C-P4H-III in collagen synthesis.
Methods: We first investigated the expression of P4ha3 in mouse by digital droplet PCR. We then established P4ha3$^{-/-}$ and P4ha1$^{-/-}$;P4ha2$^{-/-}$ mouse embryonic fibroblast (MEF) cell lines using CRISPR/Cas9 gene editing. Various methods such as C-P4H activity assay, collagen extraction assay and ultrastructural analysis was performed to characterize the C-P4H deficient MEFs.
Results and Discussion: During embryogenesis, P4ha3 expression was highest at 12.5 days post coitum and postnatally mainly in growth plates, bone, muscle, heart and lung. We also noticed that P4ha3 expression decreases by age. In most cases, P4ha3 expression was lower than that of P4ha1 or P4ha2, and P4ha1 was typically the predominant isoform. P4ha3$^{-/-}$ MEFs had no significant difference in total CP4H activity, while no detectable P4H activity was present in P4ha1$^{-/-}$;P4ha2$^{-/-}$ MEFs. Very few collagen fibrils with decreased diameter were observed in ultrastructural analysis of P4ha1$^{-/-}$;P4ha2/ MEF cultures. No reduction in 4Hyp was detected in collagen I from P4ha3$^{-/-}$ MEFs. Surprisingly, P4ha1$^{-/}$-;P4ha2$^{-/-}$ MEFs secreted procollagen polypeptides outside the cells, but they were mainly pepsin sensitive suggesting that they did not have a stable triple-helical structure. However, a small amount of pepsin-resistant collagen was detected with reasonably high hydroxylation status.
Conclusion: This study strengthens the hypothesis that C-P4H-III is a prolyl 4-hydroxylase, although its capacity to hydroxylate at least type I collagen is remarkably low.

P106
Substrate specificity of collagen prolyl 4-hydroxylases

Antti Salo[1], Pekka Rappu[2], Kati Drushinin[1], Kristian Koski[1], Jarmo Käpylä[2], Jyrki Heino[2], Johanna Myllyharju[1]
[1]Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; [2]Department of Life Technologies, University of Turku, Turku, Finland

Purpose: Collagen prolyl 4-hydroxylases (C-P4H-I, -II and -III) catalyze the formation of 4-hydroxyproline (4Hyp) in -XPG-sequences. The enzymes are $a_{2\beta2}$ tetramers with two identical catalytic a subunits (encoded by P4HA1, P4HA2 and P4HA3). Loss of P4ha1 in mouse leads to early embryonic lethality, whereas P4ha2 knockout has no obvious phenotypic outcome. Loss of P4ha2 together with loss of one allele of P4ha1 leads to chondrodysplasia and extracellular matrix defects in various tissues. C-P4H isoforms clearly have different functions. In this study, we wanted to answer the long-standing question: why we have multiple C-P4H isoforms and what are their functions.
Methods: We extracted collagen from P4ha1 and P4ha2 mutant mice and cells in order to identify, by tandem mass spectrometry, prolines with affected hydroxylation. We also performed activity assays with (XPG)5 peptide substrates, in vitro transcription/ translation produced procollagen substrates, and purified recombinant C-P4H enzymes.
Results: Deletion of P4ha1 led to substantial decrease in 4Hyp in type I collagen and affected many different hydroxylation sites. In contrast, loss of P4ha2 affected only few sites. Interestingly, these sites were prolines that are next to X-position aspartate (D) or glutamate (E). This suggested that CP4H-I is very poor in catalyzing hydroxylation of prolines that follow negatively charged amino acid D or E, whereas C-P4H-II can efficiently hydroxylate those. This was further supported by in vitro activity assays. No strict collagen type or α-chain specificity was observed.
Discussion: We noticed a critical role for the X-position amino acid to following proline in the sequence specificity for C-P4H-I and -II. Our data suggest that bulk hydroxylation requires C-P4H-I, in contrast to -DPG- and -EPG- sites that need C-P4H-II. The catalytic domain of C-P4Hs seems to have selectivity for different hydroxylation sites.
Conclusion: Results explain the existence of multiple isoforms. We suggest that substrate proline selection is based on collagen sequence rather than a collagen type and that at least two enzyme isoforms, with different assets, are needed for the full prolyl 4-hydroxylation of a procollagen a-chain.

P107
The circadian endosome control of collagen fibrillogenesis, in health and disease

Joan Chang[1], Jeremy Herrera[2], Stephen High[3], Richa Garva[2], Donald Gullberg[4], Karl Kadler[2]
[1]Wellcome Centre for Cell-Matrix Research, Division of Molecular and Cellular Function, Manchester, United Kingdom; [2]Wellcome Centre for Cell-Matrix Research, Manchester, United Kingdom; [3]Division of Molecular and Cellular Function, Manchester, United Kingdom; [4]Norwegian Center of Excellence, Department of Biomedicine and Centre for Cancer Biomarkers, Bergen, Norway

Purpose: Despite collagen's importance, therapeutics for collagen pathologies is lacking due to limited understanding on how collagen is assembled and removed. Here, we found an unexpected role for endocytosis that feeds into collagen fibrillogenesis and not degradation.
Methods: We used flow cytometry and immunofluorescence to study collagen uptake, fibril formation and trafficking; Biotin cell surface labeling paired with mass spectrometry (Biotin-MS) for protein identification; CRISPR-Cas9/siRNA to manipulate protein expression.
Results: Extracellular collagen-I was endocytosed in a circadian manner and repurposed to form new fibrils (endocytic recycling), without requiring endogenous collagen production. Endocytosis inhibition reduced collagen-I fibrils. Endosomal protein VPS33b [1] correlated with collagen-I fibrillogenesis but not secretion. Split-GFP demonstrated VPS33b co-traffics with collagen. Biotin-MS identified integrin-a11 as central to fibrillogenesis. Lung fibroblasts from idiopathic pulmonary fibrosis (IPF) patients had elevated VPS33b/integrin-a11 levels and endocytic recycling capabilities.
Discussion: Circadian endocytosis peak before maximum fibril assembly, and inhibition led to loss of fibrils, suggesting a concentration step to overcome the nucleation threshold for effective fibril formation. VPS33b levels correlate with fibril numbers and not secretion, showing collagen secretion and fibril assembly are controlled separately. VPS33b and integrin-a11's prevelance in pathologies indicate endocytic recycling is hijacked in fibroproliferative diseases.
Conclusion: Fibroblasts assemble collagen fibrils via endocytic recycling of external collagen alone, without need for endogenous collagen production, a circadian process central to normal homeostasis through VPS33b and integrin-a11 subunit. This is elevated in fibrotic tissues, suggesting a disease-potentiating mechanism.

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P108
Fibrillin microfibril structure identifies long-range effects of inherited pathogenic mutations affecting a key regulatory binding site for latent TGFβ

Alan R.F. Godwin[1], Jennifer Thomson[1], David F. Holmes[1], Christin S. Adamo[2], Gerhard Sengle[2], Michael J. Sherratt[1], Alan M. Roseman[1], Clair Baldock[1]
[1]School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, M13 9PT, United Kingdom; [2]Center for Biochemistry, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany

Purpose: Genetic mutations in fibrillin microfibrils cause a range of serious inherited diseases such as Marfan syndrome (MFS) and Weill-Marchesani syndrome (WMS). These diseases typically show major dysregulation of tissue development and growth, particularly in skeletal long bones, but links between the mutations and the diseases are unknown. Despite their biological importance, structural analysis of fibrillin microfibrils is lacking due to their complex hierarchical assembly.
Methods: In this study, we have analysed the structure of native fibrillin microfibrils purified from mammalian tissue by cryo-electron microscopy. Fibrillin microfibrils purified from two mouse models with domain deletions that either cause WMS or perturb the latent TGFβ-binding site were also imaged.
Results: The cryo-EM structure of native fibrillin microfibrils reveals the molecular ultrastructure of the microfibril at high resolution. The bead region has pseudo 8-fold symmetry and a buried protease resistant N-terminal core. Microfibrils with the WMS-causing deletion induces a rearrangement with long-range effects blocking interaction with latent TGFβ-binding protein (LTBP)-1 at a remote site. Separate deletion of the LTBP1 binding domain resulted in the assembly of shorter fibrillin microfibrils with structural alterations.
Conclusion: These results establish that in complex extracellular protein assemblies, such as in fibrillin, mutations may have long-range structural consequences to disrupt growth factor signalling and cause disease.

P109
Extracellular targeting of hemicentins to the fibrillin microfibrillar network

Steffen Lütke[1,2], Douglas Keene[3], Matthias Hammerschmidt[4,5,6], Gerhard Sengle[1,2,6,7]
[1]Center for Biochemistry, [2]Department of Pediatrics and Adolescent Medicine, Faculty of Medicine, Cologne, Germany; [3]Micro imaging center, Shriners Hospital for Children, Portland,; [4]Institute of Zoology, Developmental Biology Unit, [5]Cluster of Excellence Cellular Stress Responses in Aging-Associated Diseases (CECAD), [6]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany; [7]Cologne Center for Musculoskeletal Biomechanics (CCMB), Cologne, Germany

Purpose: Fibrillins (FBN1 and FBN2) assemble into microfibrillar networks (FMF), which endow tissues with specific biomechanical properties and target/ sequester essential growth factors to specialized cellular microenvironments. Hemicentins (HMCN1/ HMCN2) are large 600 kDa proteins belonging to the fibulin family whose members have been characterized as functional components of the elastic fiber/ fibrillin network. However, little is known about how HMCNs may exert their extracellular functions which have been mainly studied in C. elegans and zebrafish.
Methods: Murine tissues were investigated by immunofluorescence and immunogold electron microscopy employing new antibodies raised against recombinant HMCN1 and HMCN2 protein fragments. To investigate whether the ECM anchorage of hemicentins depends on FMF, tissue extraction experiments of fibrillin deficient murine tissues were performed. Protein-protein interaction assays were conducted to characterize the binding repertoire of HMCNs to other ECM molecules.
Results: HMCN1 localizes in the dermis, while HMCN2 specifically localizes to the endomysium of skeletal muscle. Both HMCNs co-localize with FBN1 and FBN2. Immunogold electron microscopy revealed that HMCN1 is targeted to FMF and elastic fibers. Tissue extracts from fibrillin deficient mice showed that proper anchorage of HMCNs depends on structurally intact FMF. Solid-phase interaction assays displayed that the fibulin-like module of HMCNs mediates the interaction with the N-terminal region of FBNs.
Discussion: HMCN 1/2 directly interact with FBN1/FBN2 and other ECM components suggesting a new structural role of HMCN in elastic fibers of vertebrates while depending on FMF. Moreover, our interaction analysis identified a new interaction of HMCNs with the basement membrane components nidogen-1 and -2.
Conclusion: Our data points to a so far unknown structural role of HMCNs in the ECM, potentially linking FMF with basement membranes, cell surface proteins and elastogenesis.

P111
Investigation of the structural and biochemical properties of mammalian tolloid proteinases

Mark Becker[1], Rana Dajani[1], Michael P. Lockhart-Carins[1], Stuart A. Cain[1], Alan R.F. Godwin[1], Clair Baldock[1]
[1]Faculty of Biology, Medicine and Health, Wellcome Centre for Cell-Matrix Research, Manchester, United Kingdom

Purpose: The family of bone morphogenetic protein 1 (BMP1)/ Tolloid-like proteases (BTPs) is involved in a variety of important functions in mammalian tissue development and homeostasis [1]. BTPs process procollagens to allow their assembly in the extracellular matrix (ECM) [2]. Additionally, BTPs are involved in the regulation of transforming growth factor-beta (TGF-β) and BMP growth factors, which regulate a variety of cell signalling processes during the formation of tissues [3].
Methods: This study aims to understand how BTPs function on a molecular level. As sample preparation presents a critical step in the pipeline for cryogenic electron microscopy (cryoEM), a major focus of this study is to optimise the expression of recombinant proteins and the preparation of specimens for cryoEM imaging, in order to obtain high-resolution structures of BTPs on their own, as well as in complex with their substrates.
Results: This study reveals a high variability of expression levels of BTPs between different mammalian cell-based systems. Optimisation of the expression provided sufficient recombinant protein for specimen preparation for cryoEM imaging. Additionally, we found that BTP substrates and enhancers form stable complexes in solution, making them suitable targets for structural and biochemical analyses.
Discussion: Specimen preparation remains one of the most challenging steps for cryoEM applications. This study provides an insight into how different systems can affect this process and show that optimisation of protein expression allows for the preparation of better specimens, enabling the collection of high-quality cryoEM data.

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P112
Role of evolutionary conserved lysine-1185 in the integrin α11 cytoplasmic tail

Pugazendhi Murugan Erusappan[1,2], Raúl Pérez Mato[3], Nazia Islam Disha[3], Ning Lu[3], Moses Musiime[3], Jon Vidar Helvik[4], Donald Gullberg[3]
[1]Institute for Experimental Medical Research, [2]KG Jebsen Cardiac Research Center, University of Oslo, Oslo, Norway; [3]Department of Biomedicine, [4]Department of Biological Sciences, University of Bergen, Bergen, Norway

Purpose: Integrin α11 is a collagen-binding integrin highly expressed in stromal connective tissue cells. Like most integrin α chains, α11 is composed of a large extracellular part, a transmembrane part and a short cytoplasmic tail. Analysis of conserved residues of α11 tail identified two evolutionary conserved residues corresponding to Arg1174 and Lys1185 in human α11($_{Hu}$α11). Our aim was to further analyse the functional role of these conserved residues.
Methods: cDNAs encoding wildtype human $_{Hu}$α11, as well as variants with mutations replacing Arg1174 or Lys1184 with alanine; or a deletion of the final 17 residues, were generated. Following transfection of cDNAs into C2C12 cells, cell adhesion, focal adhesion formation, collagen gel contraction and cell migration from spheroids in 3D collagen I gels, was analysed.
Results: Mutation of Arg1174 did not affect α11 function, but deletion of the whole cytoplasmic tail or mutation of Lys1185 impaired the ability of integrin α11β1 to form focal contacts, to remodel collagen gels and to migrate from 3D collagen spheroids. The ability to adhere to collagen was not affected by mutations or the deletion of the α11 cytoplasmic tail.
Discussion: Previous studies showed an effect of the integrin α11 cytoplasmic tail on collagen interactions [1]. Our further experiments now demonstrate a conserved role for Lys1185 in mediating α11 chain functions and suggest that important α11 partners may interact with the distal part of the α11 cytoplasmic tail.
Conclusion: Integrin α11 cytoplasmic tail contributes to focal adhesion formation, reorganization of collagen gels and cell migration, and that this activity depends on the evolutionary conserved Lys1185.

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P113
Healthy salmon bone: matrix formation and mineralisation can be uncoupled

Lucia Drábiková[1], Silvia Cotti[1,2], Per Gunnar Fjelldal[3], Thea Morken[4], Matthew A.G. Owen[4], Ann Huysseune[1,5], P. Eckhard Witten[1]
[1]Department of Biology, Ghent University, Gent, Belgium; [2]Department of Molecular Medicine, Biochemistry Unit, University of Pavia, Pavia, Italy; [3]Institute of Marine Research, Matre Research Station, Matre, Norway; [4]Skretting Aquaculture Research Centre, Stavanger, Norway; [5]Department of Zoology, Charles University, Prague, Czech Republic

Purpose: Phosphorus (P) is critical for bone matrix mineralisation. Chronic P-deficiency causes severe bone malformations in humans and other vertebrates. A series of studies on Atlantic salmon examines the effect of periodic P-deficiency and P-oversupply on bone and the potential to recover from P-deficiency [1-4].
Methods: Salmo salar received three diets for 7 weeks: low-P, regular-P, and high-P (0.5%, 1.0%, 1.5% of total P, respectively). Low-P fish were subsequently fed regular or high-P diets. Analyses included: Growth data, plasma phosphate (Pi), bone minerals, expression of fgf23, high resolution X-rays, vertebral body morphometrics, mechanical testing, histology to visualise bone cells and mineralised matrix, and Alizarin red S whole mount staining for 3D imaging [1-4].
Results: Low-P intake causes abrupt stop of matrix mineralisation. Bone matrix formation continues, albeit it remains non-mineralised. Bone P and Ca decrease by 50%, plasma Pi decreases 3-fold. Expression of fgf23 is down-regulated. P-sufficient diets restore the bone mineral content. Mineralisation resumes distant from the osteoblasts at the last mineralised matrix. Non-mineralised bones are not deformed. Bones with restored mineral content show normal load/deformation curves.
Discussion: Under P-deficient conditions osteoblasts produce large amounts of non-mineralised bone matrix. Findings that this matrix retains the capacity to mineralise, and mineralisation starts far distant from the osteoblasts, triggers questions about the cells' involvement in bone mineralisation.
Conclusion: Bone formation and bone mineralisation are independent events. Current studies translate the salmon model to zebrafish in the frame of biomedical research [5-6].

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P114
Characterization of a novel gain of malfunction mutation in an intron of COL6A1

Carolin Freiburg[1], Herimela Solomon-Degefa[1], Veronique Bolduc[2], Carsten Bönnemann[2], Patrick Meckelburg[3], Elmar Behrmann[3], Matthias Mörgelin[4], Alvise Schiavinato[1], Raimund Wagener[1], Mats Paulsson[1]
[1]Center for Biochemistry, Medical Faculty, Cologne, Germany; [2]Neuromuscular and Neurogenetic Disorders of Childhood Section, National Institutes of Health, Bethesda, USA; [3]Institute of Biochemistry, Faculty of Mathematics and Natural Sciences, Cologne, Germany; [4]Colzyx AB, Medicon Village, Lund, Switzerland

Purpose: Collagen VI is a microfibril forming extracellular matrix protein that connects large interstitial structures and is important for proper skeletal muscle function. It is mainly composed of three α chains encoded by COL6A1, COL6A2 and COL6A3. Mutations result in Bethlem Myopathy and Ullrich Congenital Muscular Dystrophy (UCMD). Here we characterized a UCMD causing mutation in an intron of COL6A1, resulting in the insertion of 24 amino acid residues into the triple helical region.
Methods: We used recombinant proteins, cultures of patient dermal fibroblasts and patient muscle sections and performed immunoblot analysis, immunofluorescence staining, electron microscopy (EM) and CD spectroscopy. COL6A1 knockout (KO) WI-26 cells were generated and retransfected with either the wildtype α1 or the mutant chain to analyze the assembly into collagen VI tetramers in a cell line under standardized conditions.
Results: The patient muscle structure is massively disrupted and the mutant chain is found in the endomysium and perimysium surrounding muscle fibers where it appeared to coincide with highly fibrotic areas. In EM, the mutant α1 chain secreted by patient fibroblasts displays clustered tangles at the ultrastructural level. Moreover, the recombinant wildtype and mutant α1 chains aggregate. While patient fibroblasts secrete wildtype collagen VI tetramers, the mutant α1 chain is secreted as a single chain that is not incorporated into collagen VI tetramers. Similar results were obtained in the COL6A1 KO cell line carrying the mutant α1 chain. In contrast, the cells retransfected with the wildtype α1 chain lay down a collagen VI network similar to that observed in the original WI-26 cells.
Discussion and Conclusion: Patient fibroblast form a collagen VI network, which is exclusively formed by the wildtype α1, α2 and α3 chain. In parallel the mutant chain is also secreted, but not assembled into collagen VI microfibrils. The colocalization of the mutant chain with fibrotic areas in the muscle and its tendency to aggregation points to a highly dominant gain of misfunction phenotype. Potentially, the progressively aggravating condition of the patients is caused by a continuous accumulation of mutant protein reminiscent to what can be observed in amyloid related diseases and points to a toxic extracellular pathomechanism.

P115
Organization of lamellae in the corneal stroma: mouse models for better understanding of the pathomechanism of keratoconus

Uwe Hansen[1], Melanie Timmen[1], Martin Goette[2], Marie-Claire Schanne-Klein[3], Latour Gael[4], Schmeltz Margaux[3], Clothilde Raoux[3], Heiduschka Peter[5], Richard Stange[1], Thomas Pap[1], Nicole Eter[5], Alnawaisheh Maged[6]
[1]Institute for Musculoskeletal Medicine, [2]Department of Gynaecology and Obstetrics, University Hospital Münster, Muenster, Germany; [3]Laboratory for Optics and Biosciences, École Polytechnique, Palaiseau Cedex, Germany; [4]Université Paris-Saclay, Orsay, Germany; [5]Department of Ophthalmology, University of Münster Medical Center, Muenster, Germany; [6]Department of Ophthalmology, Hospital Fulda, University of Marburg, Fulda, Germany

Purpose: In keratoconus (KC), structural and compositional changes lead to disruptions of the lamellar organization with thinning and scarring of the cornea. Both genetic and environmental factors have been associated with KC and recent studies suggest that inflammation could trigger the onset. Therefore, human corneas as well as corneas of hTNFtg and syndecan-1 and -4 deficient mice were analyzed.
Methods: Corneas were analyzed by TEM and collagen degradation was visualized by the collagen hybridizing peptides B-CHP. Moreover, 3D-cell cultures of human keratocytes were analyzed by TEM and for activity of tissue transglutaminase (TG). Furthermore, collagenous structures of healthy and KC corneas were analyzed by P-SHG microscopy and OCT was used for imaging of hTNFtg and wt corneas.
Results: Sheets of orthogonally arranged collagen fibrils were found in the stroma of wt mice and human controls. In contrast, lamellae were disrupted and fibril diameter increased in hTNFtg and syndecan-deficient mice. Interestingly, stromal morphology of hTNFtg mice was similar to that of KC samples. We found macrophages and apoptotic keratocytes. 3D-cell cultures of KC keratocytes generated an altered ECM with reduced TG-activity. Moreover, binding of B-CHP was stronger in KC and hTNFtg mice. In addition, OCT analysis revealed a slightly altered shape of the hTNFtg corneas. Alterations in corneal epithelium were found in all mouse models. P-SHG analysis confirmed changes in lamellae orientation distribution in KC.
Discussion: Disruptions of the lamellar organization of collagen fibrils in hTNFtg and syndecan-1 and -4 deficient mice are similar to KC patients. Thus, inflammation and altered cross-links could be crucial factors for onset of KC. Moreover, strong binding of B-CHP supports our hypothesis of enhanced stromal collagen degradation.
Conclusion: Thus, hTNFtg mice as model for KC will help to understand the pathomechanism of KC and P-SHG analysis provides new options for a better characterization of lamellar distribution in KC.

P116
Integrin α11β1 localizes to fibrillar adhesions and participates in collagen fibrillogenesis

Moses Musiime[1], Pugazhendhi Murugan Erusappan[2], Ning Lu[1], Edna Cukierman[3], Anders Molven[4], Karl Kadler[5], Joan Chang[5], Uwe Hansen[6], Zeltz Cedric[1], Donald Gullberg[1]
[1]Department of Biomedicine and Centre for Cancer Biomarkers, University of Bergen, Bergen, Norway; [2]Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo, Oslo, Norway; [3]Cancer Biology Department, Fox Chase Cancer Center, Temple Health, Philadelphia, USA; [4]Department of Clinical Medicine, University of Bergen, Bergen, Norway; [5]Wellcome Centre for Cell-Matrix Research,Faculty of Biology, Medicine & Health, University of Manchester, Manchester, United Kingdom; [6]Institute for Musculoskeletal Medicine, University Hospital of Münster, Münster, Germany

Purpose: Integrin a11b1 is a collagen receptor expressed on a subset of fibroblasts. Although integrin a11b1 has been shown to contribute to cell adhesive interactions with collagen, the potential role of integrin a11b1 in integrin-mediated collagen fibrillogenesis is not clear [1].
Methods: Fresh-frozen tumor tissue sections from patients diagnosed with pancreatic ductal adenocarcinoma (PDAC), wildtype cancer-associated fibroblasts (CAFs) previously isolated and integrin α5 knockout (α5 KO) CAFs generated using CRISPR/Cas9 were used. For imaging electron microscopy, high-resolution TIRF and confocal microscopy were used.
Results: We aimed to determine the involvement of integrin a11b1 in the formation of the fibrillar collagen matrix by CAFs in PDAC desmoplastic stroma. Integrin a11b1 was expressed in PDAC tumor stroma in vivo, translocated into fibrillar adhesions in CAFs in vitro and colocalized with deposited collagen I fibrils.
Discussion: Co-expression of a11 with tensin 1 and collagen I in CAFs in desmoplastic areas of PDAC tumors promoted us to analyze its role during matrix assembly in fibroblasts at the cellular level in relation to a2b1 and a5b1 integrins. In agreement with an independent role for a11b1 in fibrillar adhesions, tensin 1 and collagen I continued to co-distribute with a11b1 in fibrillar adhesions in a5 KO CAFs deficient in a5b1 and fibronectin assembly. Knockdown of a11 by siRNA in these a5 KO CAFs reduced the number of tensin 1-containing fibrillar, but also reduced the amount of assembled collagen I.
Conclusion: Our data demonstrate the presence of a11b1 in fibrillar adhesions and supports a fibronectin-independent role of integrin a11b1 in collagen fibrillogenesis in vitro and during desmoplastic stromagenesis.

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P118
The interaction network of four small leucine rich proteoglycans

Romain Rivet[1], Sylvie Ricard-Blum[2], Laurent Ramont[1,3], Stéphane Brézillon[1]
[1]CNRS UMR 7369, Matrice Extracellulaire et Dynamique Cellulaire (MEDyC), Université de Reims Champagne Ardenne, Reims, France; [2]Institute of Molecular and Supramolecular Chemistry and Biochemistry, UMR 5246, University Claude Bernard Lyon 1, CNRS, Villeurbanne Cedex, France; [3]Service de Biochimie-Pharmacologie-Toxicologie, CHU Reims, Reims, France

Purpose: Small Leucine Rich Proteoglycans (SLRP) are composed of a core protein containing leucine-rich repeats and glycosaminoglycan (GAG) chains, namely chondroitin sulfate, dermatan sulfate, heparan sulfate or keratan sulfate. GAG structural heterogeneity is age- and tissue- dependent. SLRPs are involved in remodeling of the Extracellular Matrix (ECM). They interact with numerous proteins, and chemicals via their core proteins and/or their GAG chains. Here, we present the interactome of four SLRPs: biglycan, decorin, fibromodulin and lumican.
Methods: The interactome was built by merging data extracted from MatrixDB, IntAct (mammalian interactions), and STRING databases and from the BioPlex 3.0 dataset. Interactions with a score higher than 0.9 were obtained from BioPlex 3.0 and STRING. Only experimental interactions with the first and second partners and to those reported in curated databases were extracted from STRING. Domain-domain interactions were extracted from the 3did database, and GAG interactions from MatrixDB and IntAct databases. The network was visualized with Cytoscape and enrichment analyses of Gene Ontology terms were performed with the BINGO plugin.
Results: The interactome comprises 162 first partners and 426 second partners. All SLRP partners contribute to ECM organization. Most of them were involved in GAG biosynthesis, development, angiogenesis, and in the regulation of chemotaxis, migration and division of endothelial cells, and of kinase signaling pathways. BGN partners are more implicated in NF/kappaB cascade, and DCN partners in system and tissue development. SLRP interactions were mediated by LRR (Toll Like Receptor) or TSP_1 (thrombospondin) domain or by the GAG chains.
Discussion: GAG biosynthesis, cancer progression and angiogenesis are enriched in the SLRP network. Transcriptomics and proteomics data could be integrated into this network to build cell-, tissue- or disease-specific SLRP subnetworks.

P119
Molecular dynamics reveals that collagen (I) homotrimerisation is favoured in low calcium conditions

Emily Johnson[1], Elizabeth Laird[2], Agnieszka Bronowska[3]
[1]Computational Biology Facility, Faculty of Health and Life Sciences, Liverpool, United Kingdom; [2]Institute of life course and medical science, Musculoskeletal & Ageing Science, Liverpool, United Kingdom; [3]School of Natural and Environmental Sciences, Chemistry, Newcastle upon Tyne, United Kingdom

Purpose: Type I collagen is the main component of human connective tissues. It is almost exclusively produced as a heterotrimer, composed of two α1(I) chains and an α2(I) chain, but an aberrant homotrimeric isoform - comprised of three α1(I) chains - can be produced. The homotrimer is implicated in ageing, fibrosis and neoplastic metastasis. The mechanisms that drive homotrimer production are poorly understood. Structural calcium has been shown to play an essential role in the trimerisation of type I collagen in the C-propeptide region. Dysregulated calcium levels in the endoplasmic reticulum (ER) may thus affect type I collagen homeostasis.
Methods: To investigate this, molecular dynamics simulations were carried out on 3D structures of the C-propeptide region. Equilibrium simulations and enhanced sampling techniques were employed in the presence and absence of structural calcium.
Results: Heterotrimeric and homotrimeric type I collagen C-propeptides were both destabilised in the absence of structural calcium. Both isoforms showed disruption of hydrogen bonds, salt bridges and disulphide-forming cysteines. The α1(I) monomer has a higher affinity for structural calcium than the α2(I) monomer. The calcium-bound heterotrimeric α2(I) chain has a higher affinity for neighboring α1(I) chains than the corresponding calcium-bound homotrimeric α1(I) chain.
Discussion: ER calcium is essential for type I collagen trimerisation and is dysregulated in various conditions. By employing atomistic simulations, these results reveal a novel mechanism through which abberant calcium homeostasis could lead to homotrimeric type I collagen being favoured.
Conclusion: It is likely that when ER calcium is limited, α1(I) chains sequester what calcium is available, preventing α2(I) chains from binding structural calcium and forming stable heterotrimers, and thus driving pathological processes such as fibrosis and metastasis.

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P120
The tendon interfascicular basement membrane provides an endothelial-like niche for CD146+ cell subpopulations

Neil Marr[1], Dirk Werling[2], Jayesh Dudhia[3], Andrew Pitsillides[1], Chavaunne Thorpe[1]
[1]Comparative Biomedical Sciences, Royal Veterinary College, London, United Kingdom; [2]Pathobiology And Population Sciences, [3]Clinical Sciences And Services, Hatfield, Royal Veterinary College, Hatfield, United Kingdom

Purpose: The tendon interfascicular matrix (IFM) plays a substantial role in the mechanical adaptations and response to load in energy-storing tendons, such as the human Achilles and equine superficial digital flexor tendon (SDFT) [1, 2]. In this study, we hypothesized that the IFM is a tendon progenitor cell niche housing an exclusive cell subpopulation.
Methods: 2D and 3D immunolabelling of equine SDFT (n = 5, ages: 3-8 years) were used to identify the IFM niche3. CD31 (endothelial cells), CD146 (IFM cells) and LAMA4 (IFM basement membrane) were labelled alongside network-predicted cell markers. Magnetic-activated cell sorting (MACS) was employed to isolate CD146+ and CD146- subpopulations and their in vitro properties were compared using differentiation assays.
Results: We found that CD146 demarcated an exclusive interfascicular cell subpopulation that resides proximal to a basal lamina which forms interconnected vascular networks. In vitro CD146+ isolates exhibited limited mineralization (osteogenesis) and lipid production (adipogenesis).
Discussion: This study demonstrates that the interfascicular matrix is a unique tendon cell niche, containing a perivascular-rich network of basement membrane, CD31+ endothelial cells and CD146+ cell populations that are likely essential to tendon structure-function.
Conclusion: Interfascicular CD146+ subpopulations did not exhibit stem cell-like phenotypes and are more likely to represent a pericyte lineage. Further investigation into the role of CD146+ interfascicular cells and their niche is required to determine their physiological role in tendon homeostasis, disease, and ageing.

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P121
CS-4S degradation and hyaluronic acid synthesis following altered glycosaminoglycan metabolism characterize the brain matrix in a multiple sclerosis model

Rafaela Vieira da Silva[1;2;3], Karina Biskup[4], Jessica Katherine Zabala Jouvin[4;5], Clara Batzdorf[1;2], Caroline Stellmach[4;6], Anna Sophie Morr[7], Ingolf Sack[7], Antje Ludwig[8], Véronique Blanchard[4], Carmen Infate Duarte[1;2]
[1]Experimental and Clinical Research Center, Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany; [3]Einstein Center for Neurosciences Berlin, [4]Institute of Laboratory Medicine, Clinical Chemistry and Pathobiochemistry, [7]Department of Radiology, [8]Department of Cardiology and Angiology, Charité Universitätsmedizin Berlin, Berlin, Germany; [5]Institute of Chemistry, [6]Institute of Biology, Freie Universität Berlin, Berlin, Germany

Purpose: Our previous work points to specific alterations of CNS glycosaminoglycans (GAG) in neuroinflammation1. Here, we investigated how inflammation influences the expression of genes involved in GAG metabolism as well as the composition of GAGs during experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS).
Methods: Alterations in genes of GAG metabolic pathways were determined by microarray and RT-qPCR; CS/DS and hyaluronic acid (HA) disaccharides were quantified by HPLC following chondroitinase ABC digestion on cerebellum, mid- and forebrain of mice at different EAE phases (n = 4-6) and sham-immunized control mice (n = 4-5).
Results: Transcriptional changes were found specially in the cerebellum. Gene expression data showed upregulation of Has2, Dse, Dcn as well as of key enzymes for GAG degradation (Hexb, Gusb, Hpse) in EAE brains compared to controls, while core proteins of CS proteoglycans were downregulated (Cspg5, Bcan). Changes in gene expression were detected in the pre-symptomatic phase and increased during EAE progression. GAG disaccharide profile revealed a 26.1% increase in HA-0S and a 3.6% reduction of CS/DS-4S at peak, which was less pronounced at remission.
Discussion: HA accumulation in EAE brain was preceded by the upregulation of Has2. As in MS, HA was found in the inflamed brain and may prevent remyelination[2]. CS-4S reduction may result from the inflammation-induced degradation of GAGs, which could be supported by the upregulation of degrading enzymes such as HEXB and GUSB. Taken together, our results indicate that transcriptional changes occur in early disease stages and precede alterations in GAGs in later EAE phases.
Conclusion: Neuroinflammation-induced GAG remodeling in the brain during EAE may contribute to disease initiation and progression and should be evaluated as therapeutic targets.

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P122
Identification and localization of gene expression patterns by spatial transcriptomics in scleroderma

Katrin Schönborn1, Pia Moinzadeh2, Ann-Helen Rosendahl1, Till Baar3, Niklas Kleinenkuhnen3, Achim Tresch3, Beate Eckes1, Thomas Krieg1
1Translational Matrix Biology,3Institute of Medical Statistics and Computational Biology, University of Cologne, Cologne, Germany; 2 Dermatology, Universty Clinic of Cologne, Cologne, Germany

Purpose: Localized scleroderma is a skin disease in which a vicious cycle of vasculopathy, inflammation and fibroblasts activation leads to the excessive deposition of extracellular matrix (ECM) and a subsequent stiffening of the tissue. Lesions from localized scleroderma patients offer a unique insight into the dynamics and underlying mechanisms of fibrosing diseases as they contain distinct areas that are in different stages of lesion development.
Methods: We performed spatial transcriptomics on localized scleroderma lesions. Our computational analysis identified clusters with specific gene expression patterns and characterized the cluster-specific genes and the underlying biological processes involved. These methods enable us to correlate specific gene expression patterns and biological functions to distinct histological regions.
Results: We were able to identify multiple gene expression clusters that aligned with areas exhibiting a distinct histology. Amongst those were three clusters in the lower dermis: 1. Lesion-adjacent regions that still displayed a normal histology and high expression of genes involved in ECM deposition, 2. Inflamed areas with high leucocyte numbers and upregulation of immune response-related genes, and 3. Central fibrotic regions with densely packed ECM but low cell numbers and weak specific transcriptional activity.
Discussion: Our findings indicate that the inflamed and lesion-adjacent regions still actively propagate the fibrotic process. On the other hand, the central lesion appears to be transcriptionally inactive. This suggests that the fibrotic ECM was produced during earlier stages of lesion development, possibly comparable to the lesion-adjacent regions with the high ECM-related gene expression. The lack of cells and transcriptional activity in the center could furthermore prevent fibroblast-mediated ECM degradation and stall lesion resolution.

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Role of integrin α11β1 in activation of fibroblast-like synoviocytes in rheumatoid arthritis

Isabel Zeinert[1], Anna De Giuseppe[2], Donald Gullberg[3], Thomas Pap[2], Adelheid Korb-Pap[2], Thomas Krieg[1], Beate Eckes[1]

[1]Translational Matrix Biology, University of Cologne, Cologne, Germany; [2]Institute of Musculoskeletal Medicine, University Hospital Münster, Münster, Germany; [3]Centre for Cancer Biomarkers, Bergen University, Bergen, Norway

Purpose: Rheumatoid arthritis (RA) is a chronic autoimmune disorder that leads to progressive destruction of cartilage and bone by activated fibroblast-like synoviocytes (FLS). Upon activation, FLS become migratory and adhere to and invade cartilage extracellular matrix (ECM). We showed that RA-FLS express higher levels of the collagen-binding integrin α11β1 and want to understand if and how α11β1 contributes to activation.

Methods: FLS were isolated from wild type (wt) C57BL/6N, integrin α11 knockout ($\alpha 11^{-/-}$) mice and mice expressing the human TNFα transgene (hTNFtg) that develop RA-like disease in their hind paws. FLS morphology, adhesion and migration were assayed in response to fibronectin and collagen I and II, representing the most abundant ECM proteins in joints.

Results: In contrast to healthy (wt) FLS, diseased (hTNFtg) FLS form more focal adhesions and stress fibers leading to increased cell spreading on all three substrates. hTNFtg FLS also display enhanced migration, in particular on collagen I. Adhesion of wt or hTNFtg FLS to collagens was comparable. Deletion of α11β1 from wt FLS did not result in significant alterations in morphology, adhesion or migration.

Discussion: Enhanced migration by hTNFtg FLS is compatible with elevated α11β1 expression and can contribute to the activation of these cells in RA. Surprising was the observation that lack of α11β1 in wt FLS appears not to influence adhesion to or migration on collagen. This observation is in agreement with our results showing only low expression of α11β1 by wt FLS. We hypothesize that α11β1 is of particular importance for FLS activation in the pathological context and suggest to study the response of hTNFtg FLS with additional deletion of α11β1 to collagens, in which we expect to see reduced adhesion and migration.

Conclusion: Our results are based on monocultures of FLS, in which paracrine signals from other cell types present in the tissue are absent. They show that contact of wt (healthy) FLS with collagens through α11β1 is not sufficient to induce an activated (migratory) phenotype. We conclude that inflammatory signaling may be influenced by the presence or absence of integrin α11β1, potentially by receptor crosstalk at the cell surface.

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EMILIN-2 expression by tumor-associated macrophages mediates the crosstalk with endothelial cells and represents a liquid biopsy marker for colorectal cancer patients

Eva Andreuzzi[1], Lucrezia Camicia[1], Albina Fejza[1], Evelina Poletto[1], Greta Carobolante[1], Renato Cannizzaro[2], Paola Spessotto[1], Maurizio Mongiat[1]

[1]Dept. of Cancer Research and Diagnosis, Division of Molecular Oncology, Aviano, Italy; [2]Oncological gastroenterology, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano, Italy

Purpose: Colorectal cancer (CRC) development is profoundly influenced by tumor-associated macrophages which exert immuno-stimulatory (M1) or immuno-suppressive (M2) functions and represent a prognostic/predictive marker[1]. The extracellular matrix glycoprotein EMILIN-2 promotes the polarization of macrophages towards the M1 phenotype2 and preliminary evidences indicate that macrophages themselves are a source of EMILIN-2. This lead to hypothesize that macrophage-derived EMILIN-2 may trigger autocrine and/or a paracrine effects impacting on other components of the tumor microenvironment.

Methods: Monocytes and endothelial cells (ECs) were used to perform polarization and angiogenic assays (spheroid sprouting and matrigel tube formation). EMILIN-2 expression was modulated by means of shRNA. EMILIN-2 levels were analyzed in buffy coats and plasma by qPCR and ELISA. Cytokine expression assessed by qPCR and Western blot.

Results: EMILIN-2 is expressed at higher levels by M2 compared with M1 macrophages. M2 subtypes further differ in terms of EMILIN-2 expression. Macrophage-derived EMILIN-2 was shown affect their polarization in an autocrine manner and to affect EC function stimulating the prodoction of pro-angiogenic molecules (paracrine function). Promising preliminary results highlighted a high EMILIN-2 expression in liquid biopsies from CRC patients compared with healthy donors, consistent with the higher presence of M2-like cells.

Discussion: For the first time, in this study we unveiled the biologicals function of macrophages-derived EMILIN-2. The evaluation of EMILIN-2 in liquid biopsies represent a promising approach to analyze the dynamicity of macrophages during CRC progression and therapy.

Conclusion: EMILIN-2 is a key molecule mediating the crosstalk between immune and vascular cells.

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P125
Collagen prolyl 4-hydroxylase I and II in bleomycin-induced pulmonary fibrosis

Tapio Röning[1,2], Anniina Mertaniemi[1,2], Kati Drushinin[1,2], Ilkka Miinalainen[2], Antti Salo[1,2], Johanna Myllyharju[1,2], Joni Mäki[1,2]
[1]Faculty of Biochemistry and Molecular Medicine, Universtiy of Oulu, Oulu, Finland; [2]Center for Cell-Matrix Research, Biocenter Oulu, Oulu, Finland

Purpose: Idiopathic pulmonary fibrosis (IPF) is a chronic progressive disease with altered extracellular matrix (ECM) remodelling and has no curative therapy. IPF is characterized by uncontrolled accumulation of type I and III collagens. Hydroxylation of collagen proline residues by collagen prolyl 4-hydroxylases (C-P4H-I, II and III) is essential for collagen stability and hence synthesis and accumulation of collagen. Our aim is to evaluate the potential role of C-P4H-I and II as therapeutic targets in IPF.
Methods: Bleomycin-induced mouse pulmonary fibrosis model was used in mouse lines lacking C-P4H-II (P4ha2$^{-/-}$) or C-P4H-II and one allele of C-P4H-I (P4ha1$^{+/-}$;P4ha2$^{-/-}$). Bleomycin was administered via oropharyngeal aspiration in a single dose (3.5 or 1.75 U/kg). The mice were sacrificed 14, 21 or 28 days after induction of fibrosis.
Results: The depletion of C-P4H-II significantly reduced the accumulation of 4-hyproxyproline (4-Hyp) in the P4ha2$^{-/-}$ lung tissue compared to wild-type (wt) animals. Difference in the 4-Hyp between genotypes was observed also in the baseline mice. The Sircol assay showed decreased accumulation of insoluble collagen in P4ha2$^{-/-}$ lungs relative to wt at 28-day timepoint. Soluble collagen amount was also significantly reduced in P4ha2$^{-/-}$ lungs at baseline and 14-day time point. P4ha2$^{-/-}$ mice showed a trend towards faster resolution of bleomycin induced pulmonary fibrosis in histological evaluation using Modified Ashcroft scale and reduced amount of collagen in the lesion areas by automated analysis with Visiopharm software. Furthermore, scanning electron microscopy showed structural differences in collagen fiber structure in the lesions. The depletion of one allele of C-P4H-I in P4ha1$^{+/-}$;P4ha2$^{-/-}$mice seemed to have a marginal additional beneficial effect over P4ha2$^{-/-}$ mice.
Conclusion: Taken together, our results show that inhibition of C-P4H-I and C-P4H-II may have protective effects in pulmonary fibrosis.

P126
The FASCInAting function of skin resident LYVE-1+ macrophages in extracellular matrix maintenance

Shu Wen Tan[1,2], Debabrata Biswas[3,4], Kimberly Xuan Zhen Lim[3,4], Thet Tun Aung[1], Yingrou Tan[5], Syaza Hazwany Mohammad Azhar[1,2], Hwee Ying Lim[1,2], Sheau Yng Lim[1,2], Joan Joo Eng Lau[1,2], Benoit Malleret[1,5], Lai Guan Ng[5], Emanuel Hanski[3,4], Veronique Angeli[1,2]
*[1]Immunology Translational Research Programme, Yong Loo Lin School of Medicine, Department of Microbiology and Immunology, [2]Immunology Programme, Life Sciences Institute, National University of Singapore, Singapore, Singapore; [3]Department of Microbiology and Immunology, Singapore-HUJ Alliance for Research and Enterprise (SHARE), Singapore, Singapore; [4]Department of Microbiology and Molecular Genetics, Jerusalem, The Hebrew University of Jerusalem, Faculty of Medicine, Israel; [5]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore*

Purpose: Recently, lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) has emerged as a prominent marker for resident macrophages. In most tissues, LYVE-1$^+$ macrophages show characteristic influence on homeostatic collagen regulation [1, 2]. Yet, distribution and functions of LYVE-1$^+$ macrophages in skin remains underexplored.
Methods: To study LYVE-1$^+$ macrophages, our group generated LYVE-1$^+$ macrophages-specific depleted mice (Lyve1wt/creCsf1rflox/flox). With this mouse model, we profiled for changes in extracellular matrix (ECM) using flow cytometry and imaging techniques. Also, we used the Group A Streptococcus bacterial infection mouse model to study the effect of ECM changes on immune cell trafficking during skin bacterial infections.
Results: Here, we revealed LYVE-1$^+$ macrophages as the major macrophage subset in mouse skin. In Lyve1wt/creCsf1rflox/flox mice, skin fascia collagen levels was reduced, along with increase collagenase activity. Furthermore, we observed fascia ECM remodelling, whereby collagen fibers lose their anisotropic alignment. Upon bacterial challenge, reduced bacterial load, associated with increase immune cell presence was seen in Lyve1wt/creCsf1rflox/flox mice as compared to control mice.
Discussion: In this study, we showed that LYVE-1$^+$ macrophages are vitally involved in skin fascia collagen content and organization, likely via collagen degradation pathways. Additionally, we propose that skin fascia ECM changes in Lyve1wt/creCsf1rflox/flox mice promoted immune cell infiltration during GAS bacterial infection, conferring protection against bacterial pathogenesis.
Conclusion: The study of fascia remains at a nascent stage despite it being a functionally relevant connective tissue in clinical settings. With this study, our group may be the first to address the role of resident macrophages in skin fascia ECM regulation and introduced LYVE-1$^+$ macrophages as key regulators.

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P127
How lack of trimeric intracellular cation channel B affects bone

Roberta Besio[1], Barbara Contento[1], Nadia Garibaldi[1], Marta Filibian[2], Marisa Brini[3], Stephan Sonntag[4], Doron Shmerling[4], Antonella Forlino[1]
[1]Department of Molecular Medicine, [2]Centro Grandi Strumenti University of Pavia, Pavia, Italy; [3]Department of Biology, University of Padua, Padua, Italy; [4]PolyGene AG, Rümlang, Switzerland

Purpose: Ubiquitous lack of trimeric intracellular cation channel B (TRIC-B), an ER channel specific for K^+ ions necessary as counter ions for intracellular calcium flux, is associated to a recessive form of Osteogenesis Imperfecta (OI), a group of collagenopathies characterized by reduced bone mass and bone fragility. In order to address TRIC-B function in bone, an osteoblast specific conditional knock-out mouse of TRIC-B (Runx2Cre;Tmem38bfl/fl) was generated.
Methods: Tmem38b early osteoblasts conditional knock-out mouse (Runx2Cre;Tmem38bfl/fl), under the Runt-related transcription factor 2 (Runx2) promoter, was generated by Cre-loxP system and its phenotyping was undertaken by X-ray, micro computed tomography and skeletal staining. Primary calvarial osteoblasts were employed to evaluate the impact of loss of TRIC-B function on calcium flux and osteoblasts activity.
Results: The mutant skeleton showed bone deformations and frequent calli. Delay in mineralization was also evident. Importantly, mutant mice showed a significant smaller size and > 80% lethality within the first 38 days, indicating the impact of the bone fracture and deformation on the severity of the disease. Micro computed tomography analysis demonstrated the presence of an osteoporotic phenotype affecting both the cortical and the trabecular compartments. A decreased calcium concentration in mitochondria, reflecting an impairment in calcium flux through the ER, was found in primary mutant osteoblasts that showed a decreased mineralization and alkaline phosphatase activity, as well as a delay in cell differentiation. Importantly, these alterations were associated to a decreased collagen synthesis and a reduced collagen incorporation into the matrix.
Discussion and Conclusion: The osteoporotic phenotype found in the Runx2Cre;Tmem38bfl/fl mouse proves the primary function of osteoblast TRIC-B for bone development.

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P128
Regional distribution of LYVE-1+ macrophages and extracellular matrices across human umbilical cord

Natalie Lim[1], Hou-Chun Lin[1], Joanne Tze Chin Lim[1], Hui Ting Ong[2], Anne Beghin[2], Celes Maria Catherine Dad[3], Virgile Viasnoff[2], Shiao-Yng Chan[3], Veronique Angeli[1]
[1]Department of Microbiology and Immunology, [2]Mechanobiology Institute, [3]Department of Obstetrics & Gynaecology, National University of Singapore, Singapore

Purpose: Perivascular macrophages expressing the hyaluronan receptor LYVE-1 play an essential role in maintaining the extracellular matrix (ECM) protecting adult mouse arteries from becoming stiff [1, 2]. In humans, the umbilical cord is the only readily available vessel which provides a snapshot of the human fetal cardiovascular system. This study explores the distribution of LYVE-1+ macrophages and various ECM components across healthy human umbilical cords and the effect of pathological conditions like gestational diabetes on LYVE-1+ macrophage distribution.
Methods: Transverse cross-sections of the cord were segmented into seven regions. Region specific LYVE-1+ macrophage and ECM content was analysed and normalised to nuclei area.
Results: Abundant LYVE-1 + macrophages were present in the cord with region specific densities. Elastin, collagen (I, III, VII), glycosaminoglycans (GAG) and tenascin-C were markedly higher in the intermediate compared to intravascular Wharton's jelly (WJ) region. Collagens III and VII were also expressed by LYVE-1+ macrophages. In gestational diabetes, LYVE-1 + macrophage content was reduced in the intermediate and perivascular WJ regions. This decrease was masked when the cross-section was analysed as a whole.
Discussion: This study supports the concept that the umbilical cord has region-specific vascular support functions3. Elevated levels of GAGs and fibrous protein observed in the intermediate WJ confers tensile and elastic strength which serves to dissipate compressive forces away from vessels while the softer intravascular WJ core allows for an unimpeded transmission of arterial stresses to the vein to boost venous backflow3. Pericellular collagen expressed by LYVE-1 + macrophages may be involved in regulating matrix micromechanical properties and alterations in region-specific LYVE-1 + macrophages composition may be associated with vascular abnormalities.
Conclusion: This study highlights a need for a standardized methodology to study regional changes in LYVE-1+ macrophages and extracellular matrix content in umbilical cord vessels.

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P129
ADAMTS2 and ADAMTS14 can substitute for ADAMTS3 in adults for pro-VEGFC activation and lymphatic homeostasis

Laura Dupont[1], Joannes Loïc[2], Florent Morfoisse[3], Silvia Blacher[1], Christine Monseur[2], Christophe Deroanne[2], Agnès Noël[1], Alain Colige[2]
[1]Laboratory of Tumor and Developmental Biology, [2]Laboratory of Connective Tissues Biology, University of Liège, Liège, Belgium; [3]Institute of Metabolic and Cardiovascular Diseases of Toulouse, INSERM Toulouse, Toulouse, France

Purpose: The capacity of ADAMTS3 to cleave pro-VEGFC into active VEGFC able to bind its receptors and to stimulate lymphangiogenesis has been clearly established during embryonic life. However, this function of ADAMTS3 is unlikely to persist in adulthood because of its restricted expression pattern after birth. Because ADAMTS2 and ADAMTS14 are closely related to ADAMTS3 and are mainly expressed in connective tissues where the lymphatic network extends, we hypothesized that they could substitute for ADAMTS3 during adulthood in mammals allowing proteolytic activation of pro-VEGFC.
Methods: To investigate a potential role of ADAMTS2 and ADAMTS14 in lymphangiogenesis, we first evaluated their capacity to activate pro-VEGFC in vitro. Then, we analyzed their functionnal activity in physiological and pathological lymphangiogenesis in vivo by using mice lacking Adamts2 and/or Adamts14.
Results: We demonstrated that ADAMTS2 and ADAMTS14 are able to process pro-VEGFC into active VEGFC as efficiently as ADAMTS3. In vivo, adult mice lacking Adamts2 developed skin lymphedema due to a reduction of the density and diameter of lymphatic vessels, leading to a decrease of lymphatic functionality, while genetic ablation of Adamts14 had no impact. In a model of thermal cauterization of cornea, lymphangiogenesis was significantly reduced in mice lacking Adamts2 or Adamts14 and further repressed in double knockout mice.
Discussion: The absence of ADAMTS2 or ADAMTS14 does not involve massive edema in our mouse models, suggesting that mutations in these genes should not be responsible for primary lymphedema. However, some reduction of their activity could be part of the numerous factors increasing the sensitivity to secondary lymphedema occurring after diverse types of injuries or trauma. In this line the development of ADAMTS2, 3 and 14 inhibitor could help to fight conditions in which excessive or abnormal lymphangiogenesis is part of the pathological mechanisms.
Conclusion: In summary, we have demonstrated that ADAMTS2 and ADAMTS14 are as efficient as ADAMTS3 in activation of pro-VEGFC and are involved in the homeostasis of the lymphatic vasculature in adulthood, both in physiological and pathological processes [1].

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P130
How macrophages and circadian clocks affect homeostasis of collagen

Katie Lowles[1], Karl Kadler[1], Joan Chang[1], Tracy Hussell[2], Quing-Jun Meng[3], Marie Cutiongco[1]
[1]Division of Cell Matrix Biology & Regenerative Medicine, [2]Division of Infection, Immunity & Respiratory Medicine, [3]University of Manchester, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom

Purpose: Fibrosis is associated with 45% of all deaths in the developed world and yet no effective treatments exist to treat it. Fibrosis is driven by dysregulated collagen production and it is of great clinical importance to determine how collagen regulation is achieved in homeostasis and what drives pathology-associated collagen production. Both circadian rhythms and macrophages have been shown to influence collagen regulatory pathways. Recent discoveries have shown that a 'sacrificial' pool of collagen exists which is regulated by the circadian clock [1] and macrophages themselves can deposit collagen in wound sites, where previously it was thought only myofibroblasts possessed this function [2].
Methods: To record circadian rhythms in fibroblasts, we used fibroblasts expressing PER2-luciferase fusion protein; the luciferase signal reflects expression of the clock gene, PER2. Immunofluorescence (IF) imaging of macrophage:fibroblast co-cultures were used to study the effects immune cells have on collagen fibril deposition.
Results: Macrophages induced a circadian rhythm in fibroblasts. Addition of macrophage conditioned media also induced a circadian rhythm in fibroblasts. IF imaging of co-cultures showed that macrophages increase collagen fibril deposition by fibroblasts.
Discussion: Conditioned media experiments suggested macrophages induce a circadian rhythm in fibroblasts via soluble cell-secreted factors. IF data demonstrated macrophages are positive regulators of collagen production by fibroblasts and indicated macrophage:fibroblast cross-talk is highly regulated to ensure collagen production results in a healthy and functional matrix.
Conclusion: These data show macrophages interact with fibroblasts to regulate their circadian rhythm and collagen fibril deposition, suggesting macrophages are an integral part of the collagen regulation mechanism.

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P131
Functional characterization of a novel disease-causing human P4HA1 mutation

Sofia Sova[1], Ajith Kumar[2], Emma Clement[3], Sandra Donkervoort[4], Ying Hu[4], Carsten Bönnemann[4], Francesco Muntoni[5], Antti Salo[1], Johanna Myllyharju[1]

[1]Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; [2]Great Ormond Street Hospital; [3]Great Ormond Street Hospital for Children NHS Foundation Trust; [4]NIH/NINDS, Neuromuscular and Neurogenetic Disorders of Childhood Section; [5]UCL Institute of Child Health, Developmental Neurosciences Dept

Purpose: Collagen prolyl 4-hydroxylases (C-P4H) catalyze the formation of 4-hydroxyproline, a post-translational modification that is needed for collagen thermal stability. P4HA1 encodes the most abundant isoform of three C-P4H α-subunits that form $\alpha_{2}\beta_{2}$ tetramers with protein disulfide isomerase (PDI) as β subunits. Previously, Zou and co-workers (2017) identified a patient with compound heterozygous mutations in P4HA1. That caused decreased P4HA1 protein and C-P4H enzyme activity levels. The disorder was congenital-onset and manifested at joint hypermobility and contractures, muscle weakness, bone dysplasia and high myopia. Here we describe a second family with mutations in P4HA1. Mutation is bi-allelic and causes p.Arg136Cys. Disease presentation is very similar to the first described case.

Methods: Recombinant proteins were produced in insect cells with PDI co-expression. In addition to p.Arg136Cys mutant, Arg was replaced by Ala, Ser and Lys for detailed characterization of residue 136 by activity assays. Circular dichroism spectroscopy and nanoscale differential scanning fluorometry were used to study the p.Arg136Cys mutant. Patient skin fibroblasts were also analyzed by Western blot and C-P4H activity assay.

Results: Recombinant C-P4H with p.Arg136Cys in P4HA1 had significantly (about 90%) decreased enzyme activity. Equally low activity in p.Arg136Ala and p.Arg136Ser mutants suggested that introduced cysteine is not causing defects for example via novel disulfide bridges. Lys at 136 cannot efficiently rescue Arg136 suggesting an important role for Arg in that position. Arg136 is in the α5 helix and AlphaFold structure prediction suggests that it binds to catalytic domain of P4HA1. Arg136Cys causes increase in Km for (PPG)10 substrate, decrease in Vmax and affects thermal stability and protein structure. Patient skin fibroblasts exhibited decreased C-P4H enzyme activity and P4HA1 protein level.

Discussion and conclusion: We have established that p.Arg136Cys causes significant C-P4H activity reduction that underlies the disease. Skin fibroblast C-P4H activity in the patient is at similar level to previously described case, which is expected in the light of similar disease presentation.

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P132
TIMP1 and TIMP2 re-educate TGFβ induced pro-inflammatory/pro angiogenic decidual-like NK cells Phenotype

Douglas Noonan[1,2], Adriana Albini[3], Maria Teresa Palano[4], Valentina Carlini[1], Riccardo Ricotta[5], Antonino Bruno[4], William Stetler Stevenson[6]

[1]Unit of Molecular Pathology, Biochemistry and Immunology, [4]Laboratory of Innate Immunity, Unit of Molecular Pathology, Biochemistry and Immunology, IRCCS MultiMedica, Milan, Italy; [2]Immunology and General Pathology Laboratory, Department of Biotechnology and Life Sciences, University of Insubria,Varese, Italy; [3]European Institute of Oncology IEO-IRCCS, Milan, Italy; [5]IRCCS MultiMedica, Milan, Italy; [6]National Institute of Health, Extracellular Matrix Pathology Section, Laboratory of Pathology, National Cancer Institute, Bethesda, USA

Purpose: As inhibitors of Matrix metalloproteases (MMP) TIMPs downregulate invasion, metastasis and angiogenesis. However TIMP1 levels have been found increased in patients with different types of cancer. Natural Killer cells have been found to be functionally compromised in several cancers. We were the first in characterizing NK pro-angiogenic phenotype in the peripheral blood and tumor tissues of patients with Non-small Cell Lung cancer, colorectal cancers, prostate of patients. Also, we found that pro-angiogenic NK cells in the peripheral blood of colorectal cancer patients release of TIMP-1 and TIMP-2. Here, we investigated the effects of TIMP-1 and TIMP-2 recombinant proteins, using a TGFb-mediated NK cell polarization model, in vitro.

Methods: We investigated the effects of TIMP1 and TIMP2 recombinant proteins in hindering decidual-like markers in NK cells, generated by polarizing cytolytic NK cells with TGFβ. The effects of TIMP1 or TIMP2 on NK cell surface antigens were determined by multicolor flow cytometry.

Results: We found that TIMP1 and TIMP2 were effective in interfering with TGFβ induced NK cell polarization towards a decidual-like-phenotype. TIMP1 and TIMP2 counteracted the effect of TGFβ in increasing the percentage of CD56bright, CD16-, CD9+ and CD49a+, and restoring normal levels for TIMP 1 and 2 also inhibited decrease levels of the activation marker NKG2D induced by TGFβ and decreased the TGFβ upregulated exhaustion marker TIM-3. TIMP1 treatment could also partially restore degranulation marker CD107a expression decreased by TGFβ.

Discussion: Our results suggest a potential role of TIMPs in controlling the tumor-associated cytokine TGFβ-induced NK cell polarization.

Conclusion: Given the heterogeneity of released factors within the TME, it is clear that TGFβ stimulation represents a model to prove TIMP's new properties, but it cannot be envisaged as a soloist NK cell polarizing agent. Therefore, further studies from the scientific community will help defining TIMPs immunomodulatory activities of NK cells in cancer, and their possible future diagnostic-therapeutic roles.

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P133
Hyaluronan accumulates in cortical areas in the ageing brain: interplay with microglia

Irene Tomé Velasco[1], Federico Soria[2], Carlos Matute[2,3]
[1]Achucarro Basque Center for Neuroscience, Leioa, Spain; [2]Faculty of Medicine and Nursery, [3]Science Park of the UPV/EHU, Neuroscience, Leioa, Spain

Purpose: Beyond neurons and glia, the Central Nervous System (CNS) holds a plastic scaffold known as the extracellular matrix (ECM). In the brain, the interstitial matrix consists mainly of long chains of the glycan polymer hyaluronan. Protein components of the ECM bind to hyaluronan forming a self-assembled matrix that functions as structural framework and signalling hub. Microglia, the never-resting immune cell of the CNS, constantly survey the brain parenchyma, interacting with cells and the surrounding extracellular microenvironment [1, 2]. Current data suggest a link between hyaluronan and neuroinflammation, although most results derive from in vitro studies and there is scarce information on the microglia-matrix interplay in vivo [3], especially in ageing.
Methods: We performed immunofluorescence on brain tissue from Cx3Cr1$^{eGFP/+}$ mice of 1, 12 and 18 months, followed by high-resolution confocal imaging and script-assisted image analysis. These tools describe structural alterations and changes in the distribution pattern of the hyaluronan matrix in ageing.
Results: We observed that Hyaluronan accumulates in the aged mouse brain in M1 and barrel cortex cortical regions. Hyaluronan accumulation correlates positively with the increase in morphological complexity of microglia.
Discussion and Conclusion: The pattern of distribution of hyaluronan changes during mouse ageing, followed by small morphological changes in some isolated microglial populations. These results shed light on the structure of the extracellular matrix in ageing and its interplay with microglial cells, a critical stepping-stone towards exploring this duo in CNS disorders.

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P134
Adrenergic anti-hypertensive and anti-asthmatic drugs influence human osteoarthritic chondrocyte function in vitro

Rebecca Sohn[1], Bastian Grütter[1], Victoria Reiser[1], Sebastian Braun[2], Markus Ehnert[2], Andrea Meurer[2], Frank Zaucke[1], Zsuzsa Jenei-Lanzl[1]
[1]Department of Orthopedics, Dr. Rolf M. Schwiete Research Unit for Osteoarthritis, [2]Department of Orthopedics, University Hospital Frankfurt, Frankfurt am Main, Germany

Purpose: The sympathetic neurotransmitter norepinephrine aggravates catabolic processes in healthy but anabolic effects in osteoarthritic (OA) chondrocytes mostly via the β2-adrenoceptor (AR). Recently, anti-hypertensive drugs often targeting ARs, have been linked to cartilage degeneration and pain. To further elucidate this association, we investigated the effect of anti-hypertensive (β-sympatholytic) as well as anti-asthmatic (β-sympathomimetic) drugs on human articular chondrocytes.
Methods: Human OA chondrocytes were treated with adrenergic anti-hypertensive (Propranolol: β1/2-AR blocker, Carvedilol: β1/2- and α1-AR blocker, Metoprolol: β1-AR blocker) or anti-asthmatic (Salbutamol, Formoterol: β2-AR agonists) drugs (in low and high serum concentrations during medication) with or without IL-1β (0.5 ng/ml). After 7 days, gene expression changes (COL1A1, ACAN, MMP13, IL6) were determined by qRT-PCR and activation of intracellular signaling pathways (ERK1/2, PKA) was analyzed via immunoblotting.
Results: Salbutamol without IL-1β significantly increased ACAN ($p = 0.029$), IL6 ($p = 0.029$) and slightly increased COL1A1 ($p = 0.057$) expression. IL6 expression was also significantly enhanced by Formoterol ($p= 0.026$) and Carvedilol ($p = 0.021$). As expected, IL-1β significantly decreased ACAN ($p = 0.026$) but increased MMP13 ($p < 0.001$) and IL6 expression ($p < 0.001$). IL-1β-mediated ACAN decrease was significantly reversed by Metoprolol ($p = 0.028$) and by trend by Salbutamol ($p = 0.057$). All substances activated only the ERK1/2 pathway.
Discussion: Anti-hypertensive and anti-asthmatic medication might act pro-inflammatory in a non-inflamed joint (increased IL6 expression). In contrast, these drugs seem to counteract IL-1β-induced catabolic effects (reversal of inhibited ACAN expression).
Conclusion: Our results suggest that anti-hypertensive and anti-asthmatic medication should be taken into consideration during the treatment of OA patients with different inflammatory states.

P135
The hyaluronan receptor RHAMM is Critical for toll like receptor 7 and SARSCoV2-mediated increases in lung cytokines and inflammation

Rashmin Savani[1], Christopher Longoria[1], Jie Liao[1], Naeun Cheong[1], Elias Coutavas[2], Sojin Kim[2], Patty Lee[2], Len Luyt[3], Eva Turley[3], Stavros Garantziotis[4]
[1]Center for Pulmonary & Vascular Biology, University of Texas Southwestern, Pediatrics, Dallas, USA; [2]Allergy, Pulmonary & Critical Care, Duke University Medical Center, Durham, USA; [3]Lawson Health Research Institute, University of Western Ontario, London, Canada; [4]National Institute for Environmental Health Sciences, Matrix Biology Group, Research Triangle Park, USA

Purpose: SARSCoV2 causes COVID19 with cytokines, inflammation, and respiratory failure. Hyaluronan (HA) and the HA synthases (HASs) are elevated in COVID19. The SARSCoV2 host sensor, TLR7, causes increased interferons and interleukin1 beta (IL1ß). Receptor for HAMediated Motility (RHAMM) promotes lung inflammation after injury and RHAMMbased peptides block injury-induced inflammation and fibrosis. We wanted to determine if TLR7 activation mimics COVID-19, whether SARS-CoV-2 infection in mice causes increased cytokines, HA and HASs and inflammation, and if RHAMMbased peptide abrogates these responses.
Methods: We developed a non-infectious model of COVID19 using intratracheal (IT) TLR7 agonist SM324405 in C57Bl/6 mice and used K18hACE2 transgenic mice given intranasal (IN) SARS-CoV2.
Results: IT SM324405 increased lung neutrophils and macrophages maximally at 72 hours. Increased mRNA expression of Tlr2, Tlr4, Tlr7 and Nlrp3 was at four hours. Increased lung Il1ß mRNA was at 4 hours and IL1ß protein at 8 hours. Rhamm, Has1, Has2 and Has3 as well as Ifng and Il6 mRNA were increased at 4 hours. Plasma HA was maximal at 16 hours and lung HA was maximal at 72 hours. Subcutaneous treatment with a RHAMM-based peptide resulted in decreased caspase 1 activity, plasma and lung HA content and IL1ß protein. Peptide also inhibited inflammation. Administration of IT SM324405 to Rhamm/ mice did not result in inflammation. K18hACE2 receptor transgenic mice given IN SARSCoV2 had increased mRNA expression of Tlr2, Tlr7, Il1ß, and Nlrp3, as well as increased Rhamm, Has1, Has2, and Has3 expression, mimicking the changes seen with IT TLR7 agonist.
Discussion and Conclusion: We conclude that IT SM324405 in mice is an excellent non-infectious model that could be used to screen potential therapeutics for single stranded RNA viral-mediated lung inflammation. Further, RHAMM and HA are mechanistically involved in lung inflammation in COVID19, and RHAMMbased peptide may be important in limiting inflammation in COVID19.

P136
Myelodysplastic syndrome associated alterations in the bone marrow mesenchymal cell-derived extracellular matrix

Amanpreet Kaur Bains[1], Lena Behrens Wu[2], Mandy Richter[1], Sandra Rother[3], Valentina Magno[4], Victoria Menger[1], Julia Schulz[1], Carsten Werner[4], Michael Cross[1], Manja Wobus[2], Uwe Platzbecker[1]
[1]Department of Hematology and Cell Therapy, Faculty of Medicine, University of Leipzig, Leipzig, Germany; [2]Technical University of Dresden, University Hospital Carl Gustav Carus, Dresden, Germany; [3]Preclinical Center for Molecular Signal Processing, Saarland University School of Medicine, Homburg (Saar), Germany; [4]Max Bergmann Center of Biomaterials, Leibniz Institute of Polymer Research Dresden, Dresden, Germany

Purpose: The mesenchymal stromal cell (MSC) derived extracellular matrix (ECM) plays a key role in coordinating both physical and biochemical hematopoietic interactions in the bone marrow (Domingues et al. 2017). However, the involvement of ECM in myelodysplastic syndrome (MDS), a bone marrow hematopoietic cell disorder, has yet to be determined. Hence, we aimed to functionally characterise the ECM in MDS.
Methods: MSC from low-(LR) and high-(HR) risk MDS patients and healthy donors (HD) were cultured for 10 days on poly-octadecene-alt-maleic anhydride and human fibronectin-coated slides, which were then decellularized using 20mM ammonia and DNase. Matrix structure was analysed by scanning electron microscopy and atomic force microscopy, sircol assay, lectin staining, immunostaining and gel electrophoresis. CD34+ hematopoietic stem and progenitor cells (HSPC) from HD were cultured for 7 days on ECM before determination of their differentiation potential by colony assay.
Results and Discussion: We report differences in both structure and composition between ECM derived from HD, LR-MDS and HR-MDS MSCs. The ECM from both LR- and HR-MDS was thicker, softer and of a higher density than that produced by HD MSCs. A high overall collagen content in MDS ECM was detected by sircol assay and confirmed by immunostaining for the most prevalent collagen subtypes 1 and 4. Lectin staining and gel electrophoresis of the extracted matrix revealed disease stage-specific differences in glycosaminoglycans (GAGs). Most markedly, high levels of low molecular weight hyaluronic acid (LMW-HA) were observed in ECM from LR-MDS. Culture of HSPCs on ECM derived from MDS MSCs specifically suppressed their subsequent potential to form hematopoietic colonies.
Conclusion: The observed changes in ECM composition, together with the decreased support of HSPCs on MDS ECM suggest that MSC-derived ECM may contribute to the pathogenesis of MDS and therefore offer potential new targets for therapy.

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P137
An atlas of matrix expression reveals complex ecosystems of immune regulation and dysregulation in rheumatoid arthritis

Jean-Baptiste Richard[1], Christopher Buckley[1], Kim Midwood[1]
[1]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Kennedy Institute of Rheumatology, Oxford, United Kingdom

Purpose: Rheumatoid Arthritis (RA) is an autoimmune disease characterised by immune dysregulation in the synovial tissue. While the cellular basis of RA has been extensively characterised, poor understanding of the matrisomal composition of synovial tissue in health and disease proves to be an obstacle to the development of effective therapeutic interventions in all patients. This study aims to address this gap in knowledge by creating an atlas of matrix expression in the RA synovium.
Methods: Published single-cell RNA sequencing datasets of synovial tissue biopsies [1, 2] were analysed for differential expression of matrisome genes. Selected proteins of interest were imaged via multiplexed immunofluorescence for additional spatial resolution.
Results: Matrisome-focused gene set enrichment analysis was performed across all synovial cell subsets, showing fibroblasts to be the predominant source of core matrix, with matrix-associated molecule production shared by fibroblasts and macrophages. Spatially and functionally distinct fibroblast subsets were found to have distinct matrix expression profiles, revealing differential composition of the vascular and lining-layer basement membranes and key novel insights into tertiary lymphoid structure architecture.
Discussion: This work explores a heterogeneous tissular landscape hitherto unappreciated. The findings highlight the microenvironmental diversity of RA synovium, which can only be resolved by combining current understanding of stromal, immune, and matrisomal networks at a sub-synovial niche level.
Conclusion: This atlas provides a community resource designed to inform further investigation of the role of key cell-matrix networks in the context of RA immune dysregulation, with a view to a more detailed understanding of disease pathology and the development of better treatments for people with RA.

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P138
A matrisome-enriched co-expression module associated with the perivascular niche is upregulated in inflammatory bowel disease and is predictive of treatment non-response

Libby Jennings[1], Mathilde Pohin[1], Matthias Friedrich[1], Kim Midwood[1]
[1]Kennedy Institute of Rheumatology, University of Oxford, United Kingdom

Purpose: Extensive tissue remodelling occurs in the pathogenesis of inflammatory bowel disease (IBD) pathogenesis. However, the genetic and molecular changes that occur in disease are not fully understood, nor are the effects of this changing microenvironment on cellular behaviour. Characterizing these processes holds potential to uncover novel mechanisms of ECM-mediated pathology in IBD.
Methods: Differential expression of matrix and matrix-related (matrisome) genes was analysed in a bulk RNA sequencing (RNAseq) dataset of IBD and non-IBD patients. Weighted gene correlation network analysis (WGCNA) was used to identify co-expression modules1. Module expression was compared in public single cell RNAseq datasets of IBD and selected gene expression was validated in murine dextran sodium sulphate (DSS) colitis by qPCR.
Results: Bulk RNAseq indicates transcriptional regulation of 167 matrisome genes. A WGCNA module comprising 43.6% matrisome correlates with IBD occurrence, Nancy Index, macroscopic inflammation and predicts non-response to anti-TNF and corticosteroid therapies. In single cell RNAseq datasets, expression of this module is restricted to endothelial cells, pericytes, fibroblasts, activated/inflammatory fibroblasts and was upregulated in disease. Analysis of colon tissue from murine DSS colitis shows conservation of this module, with a number of genes upregulated before peak colitis.
Discussion: These data suggest 1) tissue remodelling is orchestrated by a local cellular ecosystem of diverse stromal cells, 2) remodelling events precede, and may be required for, inflammation, and 3) dysregulation of this niche is associated with inflammation and treatment-nonresponsive IBD.
Conclusion: Transcriptional regulation of a matrisome-enriched coexpression module in perivascular stromal cells points to novel mechanisms of IBD pathology and presents new therapeutic targets in patients who are non-responsive to current therapies.

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P139
Rebalancing immune checkpoints in rheumatoid arthritis by targeting the synovial microenvironment

Linda Mies[1], Kim Midwood[1], Christopher Buckley[1]
[1]Kennedy Institute of Rheumatology, NDORMS, University of Oxford, Oxford, United Kingdom

Purpose: Tenascin-C (TNC) is an immune-regulatory extracellular matrix protein, that is upregulated in the synovium during the onset of rheumatoid arthritis (RA). High serum levels of TNC are associated with hard-to-treat, erosive disease and can predict RA patients whose pain will not improve with anti-TNF therapy, whilst synovial TNC levels decrease in people with spontaneously resolving synovitis. TNC blockade prevents disease progression and tissue destruction in experimental arthritis. The aim of this project is to better understand the role of TNC in human RA pathology.
Methods: Single cell RNA sequencing data was used to investigate the cellular source of TNC in the OA and RA synovium, and CellDIVE multiplexed imaging was used to determine the localization, and cellular targets, of TNC in synovial biopsies.
Results: TNC is expressed exclusively by stromal cells in the synovium, in particular by fibroblasts. Gene expression amongst fibroblast subsets changes with disease type and/or stage. In OA tissue, TNC protein is absent from the synovial lining layer, and predominantly located in the sub-lining layer and perivascular niche. Two distinct groups of RA patient were observed, characterized by high or no TNC in the lining layer, concomitant with perivascular staining and diffuse distribution in the sub-lining. TNC-rich niches in the sub-lining are populated by immune cells and sub-lining fibroblasts, whilst TNC in the lining coincides with disordered tissue architecture, loss of barrier function, and hyperplasia.
Discussion: Synovial expression of TNC in RA and OA demonstrates both shared, and disease-specific, remodelling of tissue geography.
Conclusion: Differential pathogenic cell-matrix neighbourhoods in the synovium lead to different types of joint disease.

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P140
Investigating atherosclerotic plaque rupture mechanics using a 3D tissue-engineered human disease model

Hanneke Crielaard[1], Tamar Wissing[1], Su Guvenir Torun[1], Pablo de Miguel[1], Ranmadusha Hengst[1], Gert-Jan Kremers[2], Frank Gijsen[1], Ali Akyildiz[1], Kim van der Heiden[1]
[1]Cardiology, Biomedical Engineering, [2]Optical imaging Centre, Erasmus University Medical Centre, Rotterdam, The Netherlands

Purpose: Atherosclerotic plaque cap rupture is difficult to predict due to the heterogeneous biological composition, the unknown material properties, and the stochastic nature of the event. We tissue engineered collagenous plaque analogs with controllable collagen composition [1], to scrutinize the reciprocal relationships between composition, mechanical properties, and rupture mechanics. Here we studied collagen orientation and local mechanical properties in these analogs.
Methods: Ten cap analogs with a soft inclusion (SI), mimicking the plaque lipid core, were created as before [1]. The analogs were exposed to multiphoton microscopy with second harmonic generation to obtain local fiber orientation, using a fiber orientation analysis tool (FibLab) [2]. After imaging, the analogs were mechanically tested until rupture. Local (Green-Lagrange) strains were measured through digital image correlation (DIC) using the software Ncorr [3].
Results: Collagen fibers were mostly oriented in the y-direction, which is the stretch direction during analog culturing and corresponds to the circumferential direction in human plaques. At the analogs edges, fibers were oriented closer to the y-direction than in the center ($\Delta\theta$ was 13° at the edges and 35° in the center). Center sections showed a more dispersed fiber distribution, compared to the edges (SD: 24° vs. 18°). Tensile testing resulted in ruptures that initiated in the SI and propagated in a -slightly tilted-horizontal direction. Local tensile strains (Eyy) at rupture initiation, demonstrated elevated tensile strain near the rupture location (maximum vs mean strain: 60% ± 20% vs 15% ± 5%).
Discussion / Conclusion: Collagen fibers were mainly oriented in the circumferential direction (y-direction). In the center, larger deviations in predominant fiber orientation from the y-direction and a more dispersed fiber architecture were found compared to the edges. Rupture initiated near high tensile strain regions within the SI, suggesting that rupture initiation does not always occur at the luminal surface of an atherosclerotic plaque, as is generally assumed.

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P141
Interaction of CX3CL1 with extracellular matrix components affects CX3CL1-mediated monocyte migration

Andreas Kungl[1], Maria Ennemoser[1]
[1]Institute of Pharmaceutical Sciences, Univerisity of Graz, Graz, Austria

Purpose: For its dual function, the membrane-bound CX3CL1 holds a special position among chemokines. While its constitutively expressed form acts mainly as adhesion molecule, inflammation leads to shedding of the soluble domain by ADAM-proteinases, causing CX3CL1 to act chemotactically on monocytes via its receptor CX3CR1 [1]. CX3CL1 has been found in the extracellular matrix (ECM) where it controls, e.g., the directional migration of trophoblasts through the maternal decidua during early pregnancy [2]. CX3CL1 is therefore supposed to interact with ECM-components such as proteoglycans, glycosaminoglycans (GAGs), structural and other ECM-proteins.
Methods: Here we investigated the binding of the chemokine domain of CX3CL1 to ECM-glycans heparan sulfate (HS) and chondroitin sulfate (CS) by Isothermal Fluorescence Titration [3]. Monocyte chemotaxis induced by CX3CL1 and by a mutant with 2x higher GAG-binding affinity, was determined in a Boyden chamber assay in the presence/absence of HS-masking antibodies and HS-degrading enzyme.
Results: We observed CX3CL1 binding to GAGs in the low nanomolar range. We showed that CX3CL1-induced cell migration appears to be HS-dependent, as neutralization of HS resulted in reduced chemotaxis, while CX3CL1 with enhanced GAG-binding led to increased cell migration.
Discussion: Since CX3CL1 is present in the ECM, it evidently interacts with ECM components and biological processes will be dependent on this interaction network.
Conclusion: Having shown that this network is relevant to chemotactic behavior, it is reasonable to propose that other ECM molecules, e.g., collagen 4 and/or fibronectin and/or tenascin C, might also show a previously unknown impact. Such studies are currently ongoing in our labs.

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P142
Tenascin-C orchestrates an immuno-suppressive tumor microenvironment with potential for targeting

Thomas Loustau[1], Gertraud Orend[1]
[1]Inserm U1109, Strasbourg, France

Purpose: The extracellular matrix (ECM) is a major component of the tumor microenvironment (TME) that interacts and provides instructions to immune cells. Different therapeutic strategies are being evaluated to restore protective immunity in tumors, however the ECM can induce signals that impair immune responses and thus are potential targets for new antitumor strategies.
Methods: A tongue and breast tumor models were used in mice with engineered TNC levels.
Results: In TNC knockout mice with carcinogen-induced tongue tumors, dendritic cells (DC) invaded the tumor nests whereas DC accumulated within the matrix in tumors of wild-type mice. By binding to the chemokine CCL21 and upregulating its expression in lymphatic endothelial cells, TNC retains DCs. This is further enhanced by TNC inducing expression of CCR7 in DCs. TNC also upregulated expression and organization of ECM molecules in the stroma. Altogether, TNC orchestrated an immune suppressive TME, that we did not only observe in the murine tumor model, but also found to be generated in human tongue cancer. We discovered that blocking CCR7 inhibited the interaction of the DC with TNC thus releasing them from the matrix and facilitating their entry into the tumor nests causing tumor remission and reduced lymph node metastasis [1]. In breast cancer, TNC again orchestrated an immune suppressive TME. Here, CD8 T cells were trapped by TNC binding to CXCL12. Inhibition of CXCR4 released the CD8 T cells from TNC causing reduced tumor growth and metastasis. The described mechanism of tumor infiltrating leukocyte (TIL)-matrix retention is also relevant in human breast cancer as we observed that the levels of TNC and CXCL12 and the abundance of CD8 T cells inside the stroma correlated with worsened breast cancer patient survival [2].
Discussion: As TNC is crucial in the TIL-matrix retention mechanism causing immune suppression we developed novel tools targeting TNC.
Conclusion: We demonstrated that peptides [3] and nanobodies against TNC [4] blocked chemoretention of DCs by TNC offering novel opportunities for inhibiting the TNC immune suppressive properties in vivo.

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P143
Identifying disease mechanisms of vascular Ehlers Danlos syndrome

Ramla Omar[1], Neil Bulleid[2], Fransiska Malfait[3], Tom Van Agtmael[1]
[1]Institute of Cardiovascular and Medical Sciences, [2]Institute of Molecular Cell and Systems Biology, University of Glasgow, Glasgow, United Kingdom; [3]Centre for Medical Genetics, University of Ghent, Ghent, Belgium

Purpose: Vascular Ehlers Danlos Syndrome (vEDS) is the most severe type of EDS caused by COL3A1 mutations [1]. The majority of mutations are glycine substitutions, but the disease mechanism is largely unknown and there are no treatments [1]. Missense mutations in secreted proteins such as collagen can lead to the misfolded protein accumulation in the endoplasmic reticulum (ER) and ER stress 1. This project focuses on investigating the disease mechanism of vEDS to guide future treatments.
Methods: We investigated patient fibroblast cells harbouring COL3A1 glycine mutations. ER stress and collagen III retention were analysed by western blot. Collagen III sensitivity to trypsin digestion was used as a measure of collagen folding quality. To determine if targeting ER stress is a therapeutic strategy, cells were treated with the chemical chaperone 4-Phenylbutyric acid (PBA).
Results: COL3A1+/G906R and COL3A1+/G189S cells presented collagen III intracellular retention and ER stress. The ER stress levels was increased in COL3A1+/G906R compared to COL3A1+/G189S and apoptosis indicated activation of chronic ER stress. Secreted collagen III from mutant cells was more sensitive to trypsin digestion compared to WT. PBA reduced intracellular collagen III, ER stress and apoptosis. PBA was also able to rescue the trypsin sensitivity of secreted collagen III.
Discussion: Glycine mutations affect stable folding of collagen III, causing its retention and activation of ER stress. COL3A1+/G906R mutation is closer to the C-terminal where collagen folding initiates. This causes an earlier delay in folding causing over modification, which could explain the higher activation of ER stress. Interestingly, trypsin sensitivity assay showed that mutant collagen III was secreted into the extracellular matrix (ECM), which could affect ECM organization. PBA treatment allowed for better folded collagen III, that was able to bypass ER quality-control system and be successfully secreted into the ECM.
Conclusion: The disease mechanism of vEDS involves ER stress and targeting it through PBA represents a treatment strategy for both intracellular and matrix defects of VEDS.

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P144
Bone cell differentiation is differentially affected in two zebrafish models of dominant and recessive osteogenesis imperfecta

Valentina Daponte[1], Francesca Tonelli[1], Cecilia Masiero[1], Chloé Exbrayat-Héritier[2], Marilyne Malbouyres[2], Florence Ruggiero[2], Antonella Forlino[1]
[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [2]Institut de Génomique Fonctionnelle, ENS de Lyon, Lyon, France

Purpose: The dominant OI Chihuahua (Chi/+) and recessive OI $p3h1^{-/-}$ zebrafish models are characterized by collagen type I retention in the ER, with consequent cellular stress [1, 2]. Chi/+ is characterized by a glycine substitution in collagen type I α1 chain, while $p3h1^{-/-}$ lacks the endoplasmic reticulum (ER) resident enzyme prolyl 3-hydroxylase 1 (P3H1), involved in collagen type I folding. In this work, zebrafish caudal fin regeneration ability was exploited to study the effect of altered homeostasis on bone cell differentiation in vivo.
Methods: Caudal fins of adult WT and mutant fish were amputated. Regenerated area and fin rays were measured after 7 and 14 days post amputation (dpa) to evaluate bone growth rate. RNA was collected at day 0, 3 and 5 dpa from pools of WT and mutants' caudal fins to evaluate the expression of osteoblastic and osteoclastogenic markers. Bone resorption activity was investigated through Tartate-resistant acid phosphatase (TRAP) assay. Bone cell differentiation was investigated through confocal imaging.
Results: A significant reduction in caudal fin regeneration was detected in both OI zebrafish models compared to WT. The expression and enzymatic activity of TRAP were impaired in both mutants respect to WT. Significantly reduced expression of sp7 and bglap was detected in Chi/+ respect to WT, confirmed also by confocal microscopy analysis performed on Tg(Ola.Sp7:NLS-GFP) and Tg1(Ola.Bglap:EGFP) transgenic lines.
Discussion: $p3h1^{-/-}$ and Chi/+ OI zebrafish models display reduced bone regeneration ability. $p3h1^{-/-}$ show impaired osteoclast activity but no significant alterations in osteoblastogenesis. On the other hand, a distinctive impairment of bone cells expression and activity is detected in the dominant OI model Chi/+.
Conclusion: Altered homeostasis causes a delay in bone formation, regardless of the mutation. However, only Chi/+ display impaired bone cell differentiation, suggesting a "gene mutation-specific" effect on this process.

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P145
Functionalization of electrospun polycaprolactone scaffolds with acidic glycans from marine sponges for tissue regeneration applications

Gabriele Nieddu[1], Gabriele Obino[1], Antonio Junior Lepedda[1], Tiziana Cubeddu[2], Giacinta Angela Stocchino[2], Renata Manconi[2], Cristina Ciampelli[1], Claudia Crosio[1], Ciro Iaccarino[1], Lorenzo Moroni[3], Marilena Formato[1]
[1]Department of Biomedical Sciences, [2]Department of Veterinary Medicine, University of Sassari, Sassari, Italy; [3]Complex Tissue Regeneration Department, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht, The Netherlands

Purpose: One of the most important goals of regenerative medicine is the production of biocompatible and biodegradable scaffolds, combining the mechanical properties of natural tissue with specific molecular signals. Porifera represent a rich source of bioactive molecules with a wide spectrum of activities [1] and are well known for their ability to self-heal and regenerate [2]. In this context, the aim of this work was to evaluate the potential of synthetic electrospun scaffolds, functionalized with acidic glycans purified from marine sponges, as active support for cell growth, suitable for tissue engineering applications.
Methods: Four fractions of acidic glycans, purified from Sarcotragus spinosulus by anion-exchange chromatography, or standard GAGs were used for the functionalization of electrospun polycaprolactone scaffolds, by chemical EDC-NHS crosslinking. Human fibroblasts were cultured on scaffolds for 7 days. The metabolic activity and cell proliferation were evaluated at different time points by PrestoBlue® and CyQUANT™ assays, respectively. The potential of scaffold to guide cell adhesion and spreading was assessed by fluorescence microscopy using anti-α-actin antibody and DAPI staining.
Results: Preliminary results showed that electrospun scaffolds were successfully functionalized, with yields ranging from 45 to 63%. Moreover, fibroblasts grown on scaffolds functionalized with sponge acidic glycans showed a remarkably increased metabolic activity and improved cell adhesion and spreading along functionalized fibres.
Discussion: Functionalization of electrospun polycaprolactone scaffolds with acidic glycans from marine sponges ameliorates cell adhesion and proliferation, suggesting a role for these bioactive molecules in promoting scaffold population.
Conclusion: Although preliminary, these results suggest a potential usefulness for these compounds in mimicking the natural extracellular matrix environment. Thus, the application of these functionalized scaffolds in different fields of regenerative medicine deserves to be further investigated.

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P146
Embigin deficiency leads to delayed embryonic lung development and high neonatal mortality

Salli Talvi[1], Johanna Jokinen[1], Kalle Sipilä[2], Pekka Rappu[1], Fu-Ping Zhang[3], Matti Poutanen[4], Pia Rantakari[4], Jyrki Heino[1]
[1]Department of Life Technologies, [4]Institute of Biomedicine, University of Turku, Turku, Finland; [2]Centre for Stem Cells and Regenerative Medicine, King's College London, London, United Kingdom; [3]Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland

Purpose: Embigin (Emb, Gp70) is a novel fibronectin receptor that regulates stem cell niches in the sebaceous gland [1] and bone marrow [2] in adult mice. The aim was to study Emb expression in mice and function during mouse embryogenesis.
Methods: Generation of Emb knockout ($Emb^{-/-}$) mouse line and motoring timed pregnancies. Morphological analysis of the mice organs with histological stainings. Fluorescent immunohistochemistry from embryonic and adult mice samples. RNA sequencing from lungs and VetScan analysis from amniotic fluids at embryonic day 17.5 (E17.5).
Results: Emb protein is highly expressed from early embryogenesis until E10.5, but it is still present in later gestation. $Emb^{-/-}$ embryos have high neonatal mortality (72%), and the embryos are smaller than WT. Based on both morphological observations, RNA sequencing data, and Ki67 staining, in addition to the observed low alkaline phosphatase activity in amniotic fluid, the maturation of the $Emb^{-/-}$ mice lung is significantly delayed at E17.5. A few $Emb^{-/-}$ mice that survive to adulthood have a normal lifespan and fertility without apparent pathologies. In adult mice, Emb is located in the epithelial cells lining tubular structures in the lung, kidney, and epididymis, along with skin and testis. In the lungs, Emb is expressed in stem-like club cells lining bronchioles.
Discussion: High Emb expression in early mouse embryogenesis suggests that the main effect of Emb may occur during the early phases of lung development. The compromised lung maturation of the $Emb^{-/-}$ embryos explains the markedly increased mortality during the neonatal period. Furthermore, the Emb expression in stem-like club cells lining bronchioles indicates that Emb may participate in the maintenance of stem cell niche also in the lungs.
Conclusion: We show that Emb plays an essential role during mouse embryogenesis, most notably affecting the development of the lungs, where it may function in stem cell niche regulation.

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P147

Bio-engineering a hybrid material for heart regeneration: combining a synthetic hydrogel with fetal cardiac extracellular matrix

Atze van der Pol[1], Simone de Jong[1,2], Paula Everts[1], Peter van der Meer[3], Patricia Dankers[1,2], Carlijn Bouten[1,2]
[1]Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands; [2]Institute for Complex Molecular Systems, Eindhoven, The Netherlands; [3]Department of Cardiology, University Medical Center Groningen, Groningen, The Netherlands

Purpose: Tissue homeostasis is perturbed by stressful events, which can lead to organ dysfunction and failure [1]. This is particularly true for the heart, where injury resulting from myocardial infarction can result in the loss of functional myocardial tissue and ultimately heart failure. Unlike the adult mammalian heart, the fetal heart has the innate ability for self-repair following injury [2]. This regenerative capacity has been linked to the extracellular matrix (ECM) of the fetal heart [3]. Here we aim to investigate if we can harness the ability of the fetal ECM to guide cardiac repair, thereby setting the stage for next generation regenerative strategies.
Methods: We utilized transcriptomics to map the ECM composition of the developing murine heart [4]. Candidate fetal ECM components were screened for their ability to induce cardiomyocyte proliferation in vitro. Finally, we bio-engineered a synthetic hydrogel harboring the most promising fetal ECM component and determined its feasibility as a delivery system for future translational application.
Results: We identified 13 cardiac fetal ECM genes, including the well characterized Agrin [5]. As a proof-of-concept, Agrin was incorporated into a variant of the synthetic ureido-pyrimidinone (UPy) hydrogel6. Cardiomyocytes cultured on the UPy hydrogel demonstrated increased proliferation as compared to Matrigel coating. Similar results were observed when Glypican-3, a novel fetal cardiac ECM component, was incorporated within the synthetic UPy hydrogel.
Discussion / Conclusion: We demonstrate that it is feasible to incorporate fetal ECM components within a synthetic hydrogel and that this hybrid hydrogel induces cardiomyocyte proliferation in vitro, thereby making an important step towards a novel injectable material for therapeutic implementation.

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P148

Role of β1 integrin and mTOR/AKT signalling in tendon matrix adaptation to mechanical stimuli

Rouhollah Mousavizadeh[1], Payman Hojabrpour[2], Felipe Eltit Guersetti[3], Paul C. McDonald[4], Shoukat Dedhar[4,5], Robert G. McCormack[6], Vincent Duronio[1], Seyed Mehdi Jafarnejad[7], Alex Scott[8]
[1]Department of Physical Therapy, [2]Department of Medicine, [3]Department of Materials Engineering, [5]Department of Biochemistry and Molecular Biology, [6]Department of Orthopaedics, [9]Department of Physical Therapy, University of British Columbia, Vancouver, Canada, [7]Centre for Cancer Research and Cell Biology, Queen's University, Belfast, Canada; [4]Department of Integrative Oncology, BC Cancer Research Centre, Canada

Purpose: To understand the mechanotransduction controlling tendon matrix adaptation to mechanical stretching.
Methods: 3D cultures of bio-artificial tendons and 2D cultures of human tendon cells were exposed to cyclic tension by Flexcell Tension Systems. Also, magnetic beads conjugated with peptides and antibodies were used to mechanically stimulate targeted integrins through a controlled oscillatory magnetic field. Peptides, siRNA and chemical inhibitors were used against integrins and their interactors to evaluate the role of mechanical stretching in phosphorylation of AKT/mTOR components and collagen synthesis.
Results: We discovered that mechanical stimulation of integrin β1 leads to the phosphorylation of Akt, an event which required the presence of integrin-linked kinase (ILK). Repetitive stretching of tendon fibroblasts activates the Akt and mTOR pathways, which in turn regulate mRNA translation and collagen expression.
Discussion: β1 integrin and ILK are the interface between mechanical stimulation and mTOR/AKT signalling pathways to reguate tendon matrix adaptation. β1 integrin and ILK sense mechanical signals from collagen type I matrices to regulate AKT phosphorylation and cell survival in fibroblast cells [1]. Mechanical stimulation has been shown to trigger a cascade of AKT/mTOR signaling which regulates the rate of mRNA translation [2] thereby increasing cellular protein production. IGF-1 is also anabolic for bone, but whether the mTOR pathway plays a role in the rate of bone matrix protein production by osteoblasts is unknown. We hypothesized that anabolic stimuli such as mechanical loading and IGF-1 stimulate protein synthesis in osteoblasts via activation of the AKT-mTOR pathway. MC3T3-E1 osteoblasts were either or not subjected for 1?h to mechanical loading by pulsating fluid flow (PFF Our study strongly suggests a role of mTOR/AKT signalling in collagen synthesis, and the results are in keeping with previous studies that have examined this regulatory mechanism in other cell types [3-7] cell-cycle progression, and cell proliferation, and has recently been implicated in collagen regulation. The aim of this study was to determine the role of Akt in collagen deposition by normal dermal fibroblasts, and to determine the sensitivity of cultured systemic sclerosis (SSc)
Conclusion: These results support a model in which integrins are an upstream component of the mechanosensory cellular apparatus, regulating fundamental tendon cell functions relevant to exercise-induced adaptation.

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P149
Physiological and molecular exploration of skin microvessel defects in murine model of classical-like Ehlers-Danlos syndrome

Laura Prigent[1], Perrine Mercier-Gouy[1], Audrey Josset-Lamaugarny[1], Félicia Yang[1], Elise Lambert[1], Simone Bovio[2,3], Bérengère Fromy[1], Ulrich Valcourt[1]
[1]Laboratoire de Biologie Tissulaire et Ingénierie Thérapeutique, Lyon, France; 2Laboratoire Reproduction et Développement des Plantes, Rhône, Lyon, France; 3LYMIC-PLATIM imaging and microscopy core facility, Rhône, Lyon, France

Purpose: The classical-like Ehlers-Danlos Syndrome (clEDS) is a rare inheritable disease caused by a complete absence of Tenascin-X (TNX), an extracellular matrix (ECM) glycoprotein. One of the main clinical symptoms is easy bruising with 86% of clEDS patients suffering from spontaneous ecchymoses. As the aetiology of this manifestation remains unknown, we investigated functional and architectural defects of skin microvessels in TNX-deficient mice.
Methods: Skin vascular functional capacities were compared between wild-type (WT) and TNX-deficient (KO) mice using a laser Doppler flowmeter in response to different stimuli such as vasoactive drugs, local heating or mechanical stresses. Molecular composition of dermal matrix was determined using RT-qPCR and immunohistochemistry analyses. Finally, we developed a new atomic force microscopy (AFM) protocol to compare the elastic modulus between WT and KO dermis.
Results: Pressure-Induced Vasodilation (PIV) -one of the low pressure-response mechanisms maintaining skin homeostasis-was fully abolished in KO mice, compared to WT ones. In contrast, the other vascular and neurovascular functional capacities of the skin were preserved, as microvessel vasodilation was similarly observed between both genotypes in response to vasoactive molecules and local heating. We therefore assumed that PIV defect was due to a disorganization of microvessel matrix environment, which could have an impact on its protective role in response to a low pressure. To test this hypothesis, AFM analyses were performed. We observed a lower elastic modulus in KO dermis compared to WT one suggesting that KO mouse skins were more deformable. However, we found no significant difference in the levels of the main structural components between WT and KO dermis.
Discussion: We are currently performing a comparative matrisome analysis, using a quantitative mass spectrometry approach, in order to deeper investigate the molecular composition of the dermis in the absence of TNX.
Conclusion: Astonishingly, main vascular and neurovascular functions of skin are not altered in TNX KO mice, except for the PIV. However, ECM organization of the dermis seems to be impacted by the loss of TNX at the supramolecular level, as we found a significative difference in the elastic modulus of the skin between WT and KO mice.

P150
Therapeutic added-value of vectorized photodynamic therapy in human bladder cancer models of increasing complexity

Maxime Labroy[1,2,3], Gouarderes Sara[1], Stéphane Chabaud[2], Stéphane Bolduc[2], François Bordeleau[3], Laure Gibot[1]
[1]IMRCP, CNRS UMR5623, Toulouse, France; [2]Centre de Recherche en Organogénèse Expérimentale/LOEX, CHU de Québec-Université Laval Research Center, Quebec City, Canada; [3]CHU de Québec-Université Laval Research Center, Université Laval Cancer Research Center, Quebec City, Canada

Purpose: Photodynamic therapy (PDT) is an anti-cancer therapeutic approach based on the selective activation by light of photosensitive molecules called "photosensitizers". A main drawback of photosensitizers is their low aqueous solubility which lower their PDT efficacy. We aimed at quantifying how encapsulation of photosensitizer improves its efficiency.
Methods: We used Pheophorbide a as photosensitizer, encapsulated or not within self-assembled polymer micelles based on poly(ethylene oxide)-block-poly(ε-caprolactone) PEO-PCL [1]. Efficacy of PDT was tested on 4 human bladder tumour cell lines, grown under increasing complexity: 2D monolayer, 3D tumour spheroids and human cancerous bladder substitutes produced by tissue engineering using the self-assembly approach, rich in endogenous extracellular matrix [2].
Results: The encapsulation of pheo in PEO-PCL micelles resulted in improvement of PDT efficiency, yielding a 10-fold improvement of the therapeutic index, mainly due to increased cell and tissue penetration (Fig. 1). Experiments in human 3D bladder cancer substitutes are still ongoing and will help to better describe tumour cell response to PDT within a complex microenvironment.

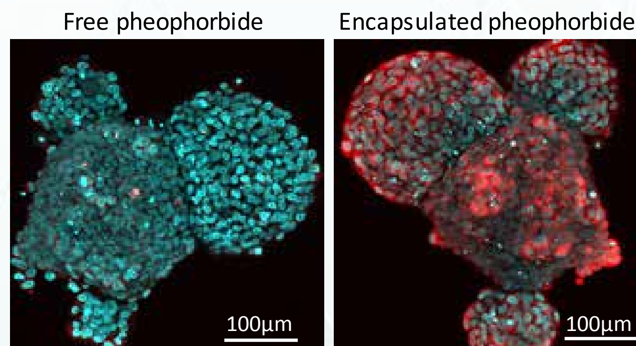

Figure 1. Encapsulation favors pheo penetration (red) in 3D bladder tumor spheroids (two-photon microscopy).

Discussion: Vectorization of hydrophobic photosensitizers increases its diffusion capacity within tumour tissue, ensuring a better therapeutic efficacy when stimulated with light during photodynamic therapy.
Conclusion: PDT with encapsulated photosensitizer could became a safe, efficient and easy to handle treatment against human bladder cancers due to the accessibility (instillation and light irradiation) of the bladder cavity.

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P151
Chemical modification of hyaluronan oligosaccharides to differentially modulate protein binding

Rebecca Dodd[1,2], Thomas A. Jowitt[1,2], Caroline M. Milner[2,3], Charles D. Blundell[4], Anthony Day[1,2,3]
[1]Wellcome Centre for Cell Matrix Research, [3]Division of Cell Matrix Biology and Regenerative Medicine, University of Manchester, Manchester, United Kingdom; [2]Faculty of Biology, Medicine and Health, [4]C4X Discovery, Manchester, United Kingdom

Purpose: The Link module superfamily of hyaluronan-binding proteins (HABPs) is diverse, both in terms of protein function and interaction with hyaluronan (HA) [1]. Building on known biochemical differences, we have chemically modified HA oligosaccharides to determine whether it is feasible to differentially modulate interactions with HABPs. The well-characterised HA-binding domains of TSG-6 (Link_TSG6)2,3 and CD44 (CD44_HABD)4 have been used as initial targets.
Methods: Various chemical groups were conjugated to the reducing terminus of HAnAN oligosaccharides. Interactions of the modified species with Link_TSG6 and CD44_HABD were studied using several biophysical techniques. An in vitro assay was used to investigate the modified oligosaccharides as substrates for TSG-6-catalysed transfer of heavy chains (HC) from inter-alpha-inhibitor (IαI).
Results: Hexasaccharides modified with 2- or 3-aminobenzoic acid or 2-amino-4-methoxybenzoic acid (HA6-2AA, HA6-3AA, HA6-2A4MBA) had increased affinities for Link_TSG6 compared to HA6AN. The same modifications did not improve the affinity of the oligosaccharide for CD44_HABD20-169. Modelling of HA6-2AA with Link_TSG6 indicated the 2AA-carboxyl moiety is orientated towards the R81 residue of the protein, suggestive of a salt bridge interaction. Several modifications to HA4AN and HA6AN oligosaccharides were found to convert the oligosaccharide into a substrate for HC-transfer (whereas unmodified HA4AN and HA6AN are not).
Discussion: Here we show that modified HA oligosaccharides have differentially altered affinities for Link_TSG6 and CD44_HABD.
Conclusion: Our data provide proof of concept that specific chemical modifications of HA oligosaccharides can differentially modulate binding to HABDs. Chemically modified HA oligosaccharides with altered substrate activity for HC-transfer have potential as research tools to further understand HA biology.

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P152
Pulmonary symptoms in congenital myasthenic syndrome type 19 patients most likely derive from respiratory muscle myasthenia

Oula Norman[1], Jarkko Koivunen[1], Emmi Thomson[1], Joni Mäki[1], Antti Salo[1], Riitta Kaarteenaho[2], Johanna Myllyharju[1], Anne Heikkinen[1], Taina Pihlajaniemi[1]
[1]Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland; [2]Research unit of Internal Medicine and Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland

Purpose: Collagen XIII (ColXIII) is a transmembrane collagen associated with neuromuscular junction development, and in humans its deficiency results in congenital myasthenic syndrome type 19 (CMS19), manifesting as muscle weakness and leading to breathing difficulties, in some cases progressing fatally. ColXIII expression in the lung is marked, but function still unclear. We strove to find out ColXIII's location and function in the lung to understand the origin of pulmonary symptoms in human patients.
Methods: We used immunohistochemistry (IHC) and correlative light and electron microscopy (CLEM) to investigate the localization of ColXIII. Lung function of wild-type and ColXIII knockout mice was measured with flexiVent. Pulmonary fibrosis was induced with bleomycin and response assessed with lung function measurements, histological scoring and 4-hydroxyproline analysis.
Results: IHC of human lung showed ColXIII expression at the alveolar septum. CLEM pinpointed ColXIII expression to the septal lipofibroblasts between capillaries and alveolar epithelium. Lung function measurements revealed increased lung volume and elevated static compliance of lung and chest wall in ColXIII-deficient mice. No difference was observed in the degree of bleomycin-induced pulmonary fibrosis between wild-type and ColXIII-deficient mice.
Discussion: Based on these experiments, lack of collagen XIII does not seem to affect the development of pulmonary fibrosis. Changes in lung function appear to represent the myasthenic manifestation of ColXIII deficiency.
Conclusions: We suggest that respiratory muscle myasthenia is the primary cause for breathing problems and recurrent pulmonary infections suffered by CMS19 patients.

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P153
Collagen XIII as the neuromuscular junction organizer

Aishwarya Prasannan[1], Oula Norman[1], Anne Heikkinen[1], Taina Pihlajaniemi[1]
[1]University of Oulu, Faculty of Biochemistry and Molecular Medicine, Oulu, Finland

Purpose: Collagen XIII (ColXIII) is a postsynaptic membrane protein of the neuromuscular junction (NMJ) (1). In humans, COL13A1 mutations lead to congenital myasthenic syndrome type 19 (CMS19) where typical symptoms are muscle weakness and fatigability (2). Collagen XIII is important for NMJ maturation and function (1,3), but little is known about its role in neuromuscular development. Here we sought to identify origin of the disease and clarify underlying mechanisms.
Methods: To follow developmental events, immunofluorescent stainings for various NMJ markers were performed as whole mount on the diaphragm muscle of wild-type and ColXIII mutant mice. Bulk RNAseq was carried out on the adult slow twitch muscle soleus, proved to exhibit morphological changes along disease progression (3).
Results: In the absence of collagen XIII, the phrenic motor axons often fail to stop at the developing acetylcholine receptor (AChR) clusters and the NMJs stabilize only postnatally when sprouting gradually declines. The endplate band gets widened and together these results suggest either delayed or compromised establishment of the motor synapse in the lack of ColXIII. RNAseq confirms extensive changes in the adult knock-out muscle transcriptome and reveals interesting potential interactors of ColXIII at the NMJ.
Discussion: Together, these results show that ColXIII acts as a stop signal for motor axon growth; directly as a transsynaptic adhesion molecule or through organization of the developing AChR clusters.
Conclusion: Through NMJ, collagen XIII markedly contributes to the muscle integrity and the data on differentially expressed genes will enable identifying ColXIII-dependent interactomes and help resolving the underlying CMS19 disease mechanism.

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P154
Characterization of lipids cargos in VLDL, LDL and HDL plasma fractions from atherosclerotic patients with advanced carotid lesions

Antonio Junior Lepedda[1], Silvia Rocchiccioli[2], Nicoletta Di Giorgi[2], Elena Michelucci[2], Giovanni Signore[3], Gabriele Nieddu[1], Cristina Ciampelli[1], Gabriele Obino[1], Marilena Formato[1]
[1]Department of Biomedical Sciences, University of Sassari, Sassari, Italy;
[2]Institute of Clinical Physiology, National Research Council, Pisa, Italy;
[3]Department of Biology, University of Pisa, Pisa, Italy

Purpose: Carotid atherosclerosis represents a relevant healthcare problem since unstable plaques are responsible for approximately 15% of neurologic events, namely transient ischemic attack, and stroke [1]. This study aimed at characterizing the specific lipidomic patterns of plasma VLDL, LDL and HDL fractions from atherosclerotic patients, associated with features of carotid plaque instability.
Methods: Twenty-eight patients undergoing carotid endarterectomy, having either a hard (n. 12) or a soft plaque (n. 16), were enrolled. Lipoproteins fractions were isolated from fasting plasma samples by isopycnic salt gradient ultracentrifugation as previously reported [2], and lipids were extracted by using the Folch procedure. Targeted lipidomic analyses were performed using a selected reaction monitoring (SRM)-based HPLC-MS/MS method. Differential analysis was performed using lipidr package (version 2.8.0) of R software [3].
Results: 130 lipid species encompassing different lipid (sub) classes (cholesterol ester [CE], ceramide [Cer], phosphatidylcholine [PC], phosphatidylethanolamine [PE], lysophosphatidylcholine [LPC], lysophosphatidylethanolamine [LPE], sphingomyelin [SM], triacylglycerol [TG], diacylglycerol [DG]) were quantified. Several lipids were found differently abundant (p value < 0.01) between patients with hard or soft plaque, including some CE, SM and DG species in HDL, CE, PE, SM and DG species in LDL, and PE, SM species in VLDL.
Discussion: The obtained lipids profile could represent a circulatory fingerprint of plaque erosion/instability with potential clinical relevance as a source of putative diagnostic and prognostic biomarkers, or therapeutic targets [4].
Conclusion: MS/MS-based technologies are making a significant contribution to unravelling both lipid and protein signatures specific for patients with hard or soft plaque. Although in its infancy, lipoprotein lipidomics is showing promising results that deserve further research.

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