5th FEBS Advanced Lecture Course
FEBS – MPST 2015

Matrix Pathobiology, Signaling and Molecular Targets

Program & Abstracts

September 24– September 29, 2015
Rhodes, Greece

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Organizing Committee:

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Mari E Strand (University of Oslo, Norway)

Conference venue:

Rhodes Palace located at the Island of Rhodes-Greece

Website and e–mail address of the FEBS Advanced Lecture Course:

http://www.febs–mpst2015.upatras.gr
e–mail: febs–mpst2015@chemistry.upatras.gr

Course Secretariat:

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Preface

Dear Colleagues and Friends,

On behalf of the Organizing Committee, it is a pleasure to invite you to the **5th FEBS Advanced Lecture Course on Matrix Pathobiology, Signaling and Molecular Targets (5th FEBS-MPST 2015)**, which follows the previous successful FEBS-MPST 2013, 2011, 2009 and 2007 meetings.

Matrix Biology is a fast growing field with significant impact in all areas of Biosciences. The 6-days FEBS-MPST 2015 offers oral sessions with i**nvited plenary lectures, talks by confirmed speakers, general lectures and tutorials, selected talks** related to the topics of the presented abstracts**, poster presentations, panel discussions and speakers' corner/meet the expert.** These sessions will address both basic and applied science topics that appeal to the range of participants working in the fields of Matrix Biology, Biochemistry, Cell & Molecular Biology, Glycobiology, Structural Biology, Pharmacology, Biotechnology and Medicine.

The organizing committee has put together an outstanding group of internationally recognized experts as invited speakers. Moreover there are several talks that include selected short oral presentations and lectures from confirmed registered speakers as well. This year **a young investigators committee** has been established as to deal with general lectures/tutorials of the first days of the meeting providing in this way the necessary knowledge for those entering the field of Matrix Pathobiology. The lectures and tutorials will provide you with an update of important new knowledge covering key areas of the field.

The Federation of European Biochemical societies (FEBS) is the major sponsor of this event, supporting both organization and young scientists offering them Youth Travel Funds (YTF). We would also like to acknowledge the contribution of the University of Patras Research Committee, the International Society of Matrix Biology (ISMB), the Mizutani Foundation, the International Union of Biochemistry and Molecular Biology (IUBMB) and the Hellenic Society of Biochemistry and Molecular Biology (HSBMB) and the other sponsors supporting the organization and "FEBS–MPST 2015 Young Investigator Awards".

The course will be held at the Rhodos Palace Hotel near the seaside. Rhodes is the capital of the Dodecanese and offers wonderful views of bright green hills, rich green valleys, uninterrupted line of golden beaches and it combines special blend of cosmopolitan and traditional, and numerous cultural and archaeological sites. Moreover Rhodes Island has a long tradition of academic tourism, and every year attracts a serious number of academic and research bodies/organizations.

We hope that this course will bring together scientists with different expertise in order to pursue fundamental and applied themes in matrix pathobiology and create an environment for superb science, and warm collegiality. We are looking forward to welcoming you in Rhodes Island, Greece for an exciting and memorable scientific meeting.

Nikos Karamanos
Chairman of the Organizing Committee

5th FEBS Advanced Lecture Course

Matrix Pathobiology, Signaling and Molecular Targets
Rhodes, September 24–September 29, 2015

Program: Lectures (L), Selected Talks (ST), Posters (P)

Thursday, 24 September

13:45-15:45 Registration

Chairperson/discussion leader: John Couchman
GENERAL LECTURES / TUTORIALS (L1-L6)

15:45-16:05 **A. Theocharis** *(University of Patras, Greece)*
Overview of extracellular matrix: structure and functional properties

16:05-16:10 Discussion

16:10-16:30 **J. Couchman** *(University of Copenhagen, Denmark)*
Proteoglycans: Structure, pathobiology, and signaling

16:30-16:35 Discussion

16:35-16:55 **P. Heldin** *(University of Uppsala, Sweden)*
Insights into the function of glycans

16:55-17:00 Discussion

17:00-17:30 Coffee break

17:30-17:50 **J. -O. Winberg** *(University of Tromsø, Norway)*
Matrix Metalloproteinases: biological significance in health and disease

17:50-17:55 Discussion

17.55-18.15 **M. Franci** *(University of Bologna, Italy)*
Collagen and functional implications

18:15-18:20 Discussion

18.20-18.40 **D. Gullberg** *(University of Bergen, Norway)*
ECM cell surface receptors

18:40-18:45 Discussion

Chairpersons: Nikos Karamanos & Renato Iozzo

18.45-19:00 Welcome Addresses by
- Chairman and members of the Organizing Committee
- Rector of the University of Patras
- Chair of the FEBS Advanced Courses Committee
- President of Hellenic Society of Biochemistry and Molecular Biology

IUBMB LECTURE (L7)

19:00-20:00	**H. Nagase** *(University of Oxford, UK)* The endocytic receptor LRP1 is a master regulator of extracellular levels of ECM-degrading metalloproteinases Honorory Medal Award conferred to Professor Hideaki Nagase by the Rector of the University of Patras
20:00	Welcome Reception

Friday, 25 September

Chairperson/discussion leader: Peter Friedl

LECTURES / TUTORIALS - MATRIX PATHOBIOLOGY (L8-L10)

9:00-9:20	**M. Paulsson** *(University of Cologne, Germany)* How perifibrillar proteins influence collagen secretion and fibril interactions
9:20-9:25	Discussion
9:25-9:45	**M. Elkin** *(Hadassah-Hebrew University Medical Center, Jerusalem, Israel)* Tumor-host interactions in pancreatic carcinoma: role of heparanase enzyme
9:45-9:50	Discussion
9:50-10:10	**P. Friedl** *(Radbound University, Netherlands)* Tissue niches for collective cancer cell invasion and therapy resistance
10:10-10:15	Discussion

SELECTED TALK (ST1)

10:15-10:25	**M. Onisto** *(University of Padova, Italy)* Novel molecular pathways potentially involved in renal fibrosis: role of HIPK2 and Heparanase
10:25-10:30	Discussion
10:30-11:00	Coffee break

Chairperson/discussion leader: Martin Götte

LECTURES / TUTORIALS - MATRIX PATHOBIOLOGY (L11-L13)

11:00-11:20	**T. Wight** *(University of Washington, USA)* Versican: An enigmatic matrix component and the control of disease cell phenotype
11:20-11:25	Discussion
11:25-11:45	**M. Götte** *(Muenster University, Germany)* Interplay of syndecan-1 and heparanase in cancer stem cell function
11:45-11:50	Discussion

11:50-12:10 **K. Dobra** *(Karolinska University, Stockholm, Sweden)*
The role of syndecan-1 in mesenchymal tumors

12:10-12:15 Discussion

SELECTED TALKS (ST2-ST5)

12.15-12:25 **E. Regős** *(Semmelweis University, Hungary)*
Syndecan-1 in liver fibrosis and regeneration

12:25-12:30 Discussion

12:30-12:40 **P. Bouris** *(University of Patras, Greece)*
Tumorigenic role of serglycin in breast cancer cells

12:40-12:45 Discussion

12:45-12:55 **D. Barbouri** *(University of Patras, Greece)*
Syndecans as key partners in the interaction between breast cancer cells and endothelium

12:55-13:00 Discussion

13:00-13:10 **M. Farshchian** *(University of Turku, Finland)*
AIM2 promotes progression of cutaneous squamous cell carcinoma

13:10-13:15 Discussion

13:15-14:45 Lunch

14:45-16:30 Poster session (I) (P1-P26)/ Discussion groups (I)

Chairperson/discussion leader: Renato Iozzo
LECTURES/TUTORIALS - MATRIX PATHOBIOLOGY (L14-L16)

16:30-16:50 **R. Iozzo** *(Thomas Jefferson University, USA)*
Novel proteoglycan roles in evoking autophagy independent of nutrient deprivation

16:50-16:55 Discussion

16:55-17:15 **I. Kovalszky** *(Semmelweis University, Hungary)*
Tumor-stroma interaction of hepatomas with slow or fast proliferation rate

17:15-17:20 Discussion

17:20-17:40 **K. Forsberg-Nilsson** *(University of Uppsala, Sweden)*
Modeling glioma and targeting the glioma niche

17:40-17:45 Discussion

SELECTED TALKS (ST6-ST8)

17:45-17:55 **T. Neill** *(Thomas Jefferson University, USA)*
A new mechanistic link between decorin-evoked Peg3 and TFEB for autophagic induction and angiostasis

17:55-18:00 Discussion

18:00-18:10	**K. Baghy** *(Semmelweis University, Hungary)* Decorin expression in human hepatocellular carcinoma
18:10-18:15	Discussion
18:15-18:25	**D. Avery** *(University of Pennsylvania, USA)* The role of fibroblast activation protein (FAP) in lung tumorigenesis
18:25-18:30	Discussion
18:30-19:00	Break

Chairpersons: Nikos Karamanos & Dimitris Kletsas
HONORARY PLENARY LECTURE (L17)

19:00-20:00	**R. Sanderson** *(University of Alabama, USA)* Targeting heparanase for myeloma therapy Honorary Medal Award conferred to Prof. R. Sanderson by the Rector of the University of Patras
20:00	Dinner

Saturday, 26 September

Chairperson/discussion leader: Paraskevi Heldin
LECTURES/TUTORIALS - GLYCOBIOLOGY AND METABOLIC REGULATION OF ECM MOLECULES (L18-L19)

9:00-9:20	**V. Hascall** *(Cleveland Clinic Foundation, USA)* How does heparin prevent intracellular synthesis of hyaluronan in hyperglycemic dividing cells?
9:20-9:25	Discussion
9:25-9:45	**P. Heldin** *(University of Uppsala, Sweden)* Role of hyaluronan-CD44 interactions during cancer progression
9:45-9:50	Discussion

SELECTED TALKS (ST9-ST11)

9:50-10:00	**M. Mehić** *(Uppsala University, Sweden)* The deubiquitinating enzymes USP4 and USP17 target hyaluronan synthase 2 and affect its function
10:00-10:05	Discussion
10:05-10:15	**D. Vigetti** *(University of Insubria, Italy)* The long non-coding RNA HAS2-AS1 is a new regulator of hyaluronan synthesis and its involved in human vascular diseases
10:15-10:20	Discussion

10:20-10:30 **A. -J. Deen** *(University of Eastern Finland, Finland)*
The UDP-sugar substrates of hyaluronan synthase 3 (HAS3) regulate its intracellular traffic and extracellular shedding, controlling hyaluronan synthesis and correlating with phenotypic changes in early stages of melanoma

10:30-10:35 Discussion

10:35-11:05 Coffee break

Chairperson/discussion leader: Alberto Passi

LECTURES/TUTORIALS - I GLYCOBIOLOGY AND METABOLIC REGULATION OF ECM MOLECULES (L20-L22)

11:05-11:25 **H. Watanabe** *(Aichi Medical University, Japan)*
Role of versican, and chondroitin sulfate in development and diseases

11:25-11:30 Discussion

11:30-11:50 **A. Passi** *(University of Insubria, Italy)*
Hyaluronan amount and size are critical in human pathology

11:50-11:55 Discussion

11:55-12:15 **L. Kjellen** *(University of Uppsala, Sweden)*
Cellular design of heparan sulfate

12:15-12:20 Discussion

SELECTED TALKS (ST12-ST15)

12:20-12:30 **T. Dierker** *(Uppsala University, Sweden)*
Detection of hitherto unknown chondroitin core proteins together with unexpected sulfation of chondroitin in the nematode C. elegans

12:30-12:35 Discussion

12:35-12:45 **F. Malfait** *(Ghent University, Belgium)*
Zebrafish modeling of β3GalT6 and β4GalT7-deficient types of Ehlers-Danlos syndrome stresses the importance of glycosaminoglycans in development

12:45-12:50 Discussion

12:50-13.00 **S. -L. Taylor** *(University of Liverpool, UK)*
The purification and characterization of heparin by-products for applications in wound healing

13:00-13:05 Discussion

13:05-13:15 **A. Abbadi** *(Cleveland Clinic Foundation, USA)*
Heparin inhibits the differentiation of M1 and promotes M2 macrophages under hyperglycemic stress

13:15-13:20 Discussion

13:20-14:15 Speakers corner (I)

14:15-21:30 Lunch box - Excursion tour

Sunday, 27 September

Chairperson/discussion leader: Donald Gullberg

LECTURES/TUTORIALS- INTERACTIONS AND FUNCTIONS OF MATRIX MACROMOLECULES (L23-L24)

9:00-9:20	**M. Franci** *(University of Bologna, Italy)* Collagen in tendon and ligament
9:20-9:25	Discussion
9:25-9:45	**D. Gullberg** *(University of Bergen, Norway)* Collagen receptors- static contacts or dynamic modulators?
9:45-9:50	Discussion

SELECTED TALKS (ST16-ST18)

9:50-10:00	**D. Gavriilidou** *(Imperial College London, UK)* Interactions of discoidin domain receptors with fibrillar and non-fibrillar collagen forms: ligand binding versus receptor activation
10:00-10:05	Discussion
10:05-10:15	**B. Pilecki** *(University of Southern Denmark, Denmark)* MFAP4 is a novel regulator of elastic fiber assembly and integrin-dependent cellular signaling
10:15-10:20	Discussion
10:20-10:30	**S. Molon** *(University of Padova, Italy)* Role of collagen VI in intestine homeostasis
10:30-10:35	Discussion
10:35-11:05	Coffee break

Chairperson/discussion leader: François-Xavier Maquart

LECTURES/TUTORIALS- INTERACTIONS AND FUNCTIONS OF MATRIX MACROMOLECULES (L25-L26)

11:00-11:20	**F. -X. Maquart** *(University of Reims, France)* Basement membrane collagens in the control of tumor cell invasion
11:20-11:25	Discussion
11:25-11:45	**A. Blom** *(Lund University, Malmo, Sweden)* Cartilage oligomeric matrix protein (COMP) contributes to the development and metastasis of breast cancer
11:45-11:50	Discussion

SELECTED TALKS (ST19-ST20)

11:50-12:00	**M. Keremidarska** *(Bulgarian Academy of Sciences, Bulgaria)* Mesenchymal stem cells adhesive behavior to different extracellular matrix proteins alters during osteogenic differentiation

12:00-12:05 Discussion

12:05-12:15 **A. -C. Silva** *(Universidade do Porto, Porto, Portugal)*
The versatility of fetal and adult ECM-derived microenvironments: The impact of ontogeny on cardiac cells

12:15-12:20 Discussion

12:30-14:15 Lunch

14:15-16:15 Poster session (II) P27-P53/ Discussion groups (II)

16:15-17:00 **B. -G. Vertessy** *(Budapest University of Technology and Economics, Hungary)*
Career Planning

Chairperson/discussion leader: Liliana Schaefer

CELL RECEPTORS SIGNALING AND ECM BASED NANOTECHNOLOGY (L27-L30)

17:00-17:20 **L. Schaefer** *(Goethe-Universität Frankfurt am Main, Germany)*
SLRP signaling in inflammation

17:20-17:25 Discussion

17:25-17:45 **J. Couchman** *(University of Copenhagen, Denmark)*
Syndecans: transmembrane proteoglycans controlling the cell adhesion phenotype

17:45-17:50 Discussion

17:50-18:10 **M. Pavao** *(University of Rio de Janeiro, Brasil)*
Inhibition of manganese-mediated tumor cell migration by heparin analogs

18:10-18:15 Discussion

18:15-18:45 Break

18:45-19:05 **R. -C. Savani** *(University of Texas Southwestern Medical Center, USA)*
Molecular mechanism of RHAMM activation of Src kinase and the signaling pathway for nitric oxide production in the endothelium

19:05-19:10 Discussion

SELECTED TALKS (ST21-ST23)

19:10-19:20 **F. -C. -O. -B. Teixeira** *(Universidade Federal do Rio de Janeiro, Brasil)*
Sulfated fucans and sulfated galactans from sea urchins as potent inhibitors of selectin-dependent hematogenous metastasis. Are there any structural requirements?

19:20-19:25 Discussion

19:25-19:35 **A. -O. Melleby** *(University of Oslo, Norway)*
Glypican-6 is increased in experimental and clinical heart failure and might play a role in cardiac fibrosis through BMP4 signaling

19:35-19:40 Discussion

19:40-19:50	**H. Pratsinis** *(NCSR "Demokritos", Greece)* Three-dimensional culture systems for the study of growth factors on intervertebral disc cells
19:50-19:55	Discussion
20:00	Dinner

Monday, 28 September

Chairperson/discussion leader: Carl-Henrik Heldin

SIGNALING AND DISEASE MOLECULAR TARGETING (L31-L33)

9:00-9:20	**C. -H. Heldin** *(Ludwig Cancer Institute, Sweden)* Smad and non-Smad signaling via TGF-β receptors – possible targets in tumor therapy
9:20-9:25	Discussion
9:25-9:45	**N. Karamanos** *(University of Patras, Greece)* The regulatory roles of estrogen receptors and syndecans in breast cancer cell properties and functions
9:45-9:50	Discussion
9:50-10:10	**D. Nikitovic** *(University of Crete, Greece)* IGF-I/ EGF signaling affect breast cancer cell adhesion through cytoskeleton reorganization
10:10-10:15	Discussion

SELECTED TALKS (ST24-ST28)

10:15-10:25	**N. Afratis** *(University of Patras, Greece)* Syndecan-4 as a switch of epithelial to mesenchymal transition in breast cancer cells
10:25-10:30	Discussion
10:30-10:40	**S. -K. Jha** *(University of Helsinki, Finland)* CCBE1 enhances lymphangiogenesis by regulating VEGF-C activation
10:40-10:45	Discussion
10:45-11:15	Coffee break
11:15-11:25	**J. Nuechel** *(University of Cologne, Germany)* TGF-β release by fibroblasts requires regulated secretion via autophagosomal intermediates
11:25-11:30	Discussion
11:30-11:40	**C. Kolliopoulos** *(University of Uppsala, Sweden)* HMGA2 regulates HAS2 and its natural antisense transcript during TGF-β-mediated EMT
11:40-11:45	Discussion

11:45-11:55 **Z. Piperigkou** *(University of Patras, Greece)*
The role of ERβ in regulation of functional properties and gene expression of matrix macromolecules in aggressive breast cancer cells

11:55-12:00 Discussion

Chairperson/discussion leader: Jerry Turnbull

SIGNALING AND DISEASE MOLECULAR TARGETING (L34-L35)

12:00-12:20 **J. Turnbull** *(University of Liverpool, UK)*
Proteoglycans as drug development targets: chemical biology routes to new therapeutics

12:20-12:25 Discussion

12:25-12:45 **D. Kletsas** *(NCSR "Demokritos", Greece)*
Senescence of stromal fibroblasts triggered by anticancer treatments: implications in tumor progression

12:45-12:50 Discussion

SELECTED TALK (ST29)

12:50-13:00 **T. Brown** *(Monash University, Australia)*
Development of hyaluronan as a CD44-targeted drug delivery vehicle in the treatment of cancer

13:00-13:05 Discussion

13:05-14:30 Lunch

14:30-16:00 Poster Session (III) P54-P79 / Discussion groups (III)

16:00-17:00 Speakers corner (II)

Chairperson/discussion leader: Jan-Olof Winberg
MATRIX REGULATION IN HEALTH AND DISEASE (L36-L38)

17:00-17:20 **J. -O. Winberg** *(University of Tromsø, Norway)*
The core protein of serglycin is a matrix metalloprotease-9 substrate

17:20-17:25 Discussion

17:25-17:45 **S. Brezillion** *(University of Reims, France)*
Lumican: A new inhibitor of matrix metalloproteinase-14 activity

17:45-17:50 Discussion

17:50-18:10 **S. Heymans** *(KU Leuven, Belgium)*
Osteoglycin (mimecan) protects against ischemic heart disease but increases adverse cardiac inflammation in viral myocarditis

18:10-18:15 Discussion

18:15-18:45 Break

SELECTED TALKS (ST30-ST33)

18:45-18:55	**E. Karousou** *(University of Insubria, Italy)* Cross talk between breast cancer cells and fibroblasts: effect of the uncharacterized protein q7z3e2 on hyaluronan synthesis
18:55-19:00	Discussion
19:00-19:10	**B. Deschrevel** *(University of Rouen, France)* An innovative biomaterial for tridimensional eukaryotic cell development: applications in tissue engineering and in tumor engineering
19:10-19:15	Discussion
19:15-19:25	**X. Stachtea** *(Lund University, Sweden)* Loss of dermatan sulfate results in neonatal lethality in mice despite normal lymphoid and non-lymphoid organogenesis
19:25-19:30	Discussion
19:30-19:40	**M. -E. Strand** *(University of Oslo, Norway)* Shedding of syndecan-4 promotes immune cell recruitment and preserves heart function after lipopolysaccharide challenge
19:40-19:45	Discussion
20:00	Dinner

Tuesday, 29 September

Chairperson/discussion leader: Boris Turk

MATRIX REGULATION IN HEALTH AND DISEASE (L39 – L41)

9:00-9:20	**B. Turk** *(J. Stefan Institute, Slovenia)* Extracellular cysteine cathepsins: from signalling to matrix degradation
9:20-9:25	Discussion
9:25-9:45	**V. -M. Kähäri** *(University of Turku, Finland)* Proteolytic control of skin cancer progression
9:45-9:50	Discussion
9:50-10:10	**D. Vynios** *(University of Patras, Greece)* ADAMTS proteinases in health and disease
10:10-10:15	Discussion

SELECTED TALKS (ST34-ST37)

10:15-10:25	**K. Andenæs** *(University of Oslo, Norway)* The extracellular matrix proteoglycan fibromodulin is up-regulated in experimental and clinical heart failure, and might attenuate development of myocardial fibrosis by interacting with TGF-β signaling

10:25-10:30	Discussion
10:30-10:40	**B. Bluhm** *(University of Cologne, Germany)* MicroRNAs: posttranscriptional modulators of cellular and extracellular cartilage compartments
10:40-10:45	Discussion
10:45-10:55	**L. Monti** *(University of Pavia, Italy)* Animal models of desbuquois dysplasia type 1 to study the role of CANT1 in proteoglycan metabolism
10:55-11:00	Discussion
11:00-11:10	**A.-V. Suhovskih** *(Novosibirsk State University, Russia)* Prostate cancer cells stably change proteoglycans expression in normal fibroblasts in cell culture model *in vitro*
11:10-11:15	Discussion
11:15-11:40	Closing remarks / Young Scientist Awards
11:40-13:15	Farewell / Departure

Invited Lectures/Tutorials

L
01

Overview of extracellular matrix: structure and functional properties

Achilleas D. Theocharis

Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece

Extracellular matrix (ECM) is a complex and dynamic structure that provides the scaffold wherein cells reside in all tissues and organs. ECM is a non-cellular network composed of collagens, proteoglycans / glycosaminoglycans, elastin, fibronectin, laminins, and several other glycoproteins. Matrix components encompass multiple, independently folded domains as well as specific glycosylation that provide them with specific sites for interaction. ECM components contain various interacting sites with different specificities to matrix components and cell surface receptors, which make them capable to bind with each other forming a complex three-dimensional network associated with resident cells. ECM provides signals to cells regulating their behavior and can trigger multiple biological activities that are essential for normal organ development and tissue homeostasis. Loss-of-function mutations and modifications in ECM molecules are associated with various pathologies. ECM undergoes an enduring and controlled remodeling normally by several matrix proteases and other degrading enzymes, such as heparanase. Although this fine-tuned process is normally under control, it progresses unrestrained in pathological conditions resulting in the formation of a favorable microenvironment for disease development and progression. All recent evidence supports the notion that abnormalities in ECM structure and composition and establishment of a modified cross-talk between abnormal ECM and cells are crucial for the development and progression of diseases.

Syndecans: transmembrane proteoglycans controlling the cell adhesion phenotype

L 2/28

John R. Couchman

Department of Biomedical Sciences and Biotech Research & Innovation Center, University of Copenhagen, Biocenter, 2200 Copenhagen N, Denmark

The syndecans are a small family of transmembrane proteoglycans. There are four syndecan genes in mammals while all invertebrate bilaterians have one. This long evolutionary history suggests conserved and important functions, but these have been elusive at a molecular level. All syndecans have the same structural organization, glycosaminoglycan chains towards the N-terminus, a single transmembrane domain and a small C-terminal cytoplasmic domain that nevertheless has highly conserved sequences suggestive of functional roles. Over the years, we and other groups have established that all syndecans link to the actin cytoskeleton and contribute to its organization, with impact on adhesion and migration. It is now also clear that syndecans can work alongside other receptors, such as integrins or tyrosine kinase growth factor receptors, but also signal independently.

Of the four mammalian syndecans, most is understood regarding syndecan-4 signaling. It can interact directly with α- actinin and protein kinase Cα, with downstream phosphorylation of several proteins, mostly connected to microfilament regulation. However, while these specific effects of syndecan-4 are quite well understood, a variety of evidence suggests that syndecans as a family possess a common signaling pathway that became our goal to elucidate. We now have considerable data suggesting a key role of syndecans is to regulate intracellular calcium metabolism. It has its basis in the regulation of a conserved subfamily of calcium channels, and our recent data show that this function of syndecans can be demonstrated in mammalian cells as well as the invertebrate genetic model, *Caenorhabditis elegans*. We therefore propose that a key function of all syndecans is to regulate calcium metabolism that in turn sets the actin cytoskeletal phenotype for adhesion, junction formation and motility.

L 3/19

Role of hyaluronan-CD44 interactions during cancer progression

P. Heldin, C. Kolliopoulos, M. Mehic and A. Ruusala

Ludwig Institute for Cancer Research, Science for life Laboratory, Uppsala University, Biomedical Center, Box 595, SE-75124 Uppsala, Sweden.

Clinical and experimental studies have revealed that hyaluronan and its receptor CD44 accelerate breast cancer progression. Our aim is to elucidate the mechanism of regulation of hyaluronan and the importance of its interaction with CD44 in progression of breast cancer.

We investigate the role of hyaluronan synthases, in particular HAS2 which is upregulated in most aggressive breast cancer cells. Our data demonstrated that TGF-β stimulates the production of hyaluronan in normal mammary breast epithelial cells by upregulation of HAS2 and that efficient TGFβ-induced epithelial to mesenchymal transition (EMT) requires the expression of HAS2. To elucidate further the molecular mechanisms by which TGF-β induces HAS2 at the transcriptional level, we investigated the possible involvement of a natural antisense transcript of HAS2 (HAS-AS), which has been shown to be required for efficient transcription of HAS2. We found that HAS2-AS expression is regulated in a similar manner as HAS2 upon stimulation with TGF-β. Interestingly, by microarray analysis and RT-PCR, we have demonstrated that HAS2 mRNA is regulated by the high mobility group A2 (HMGA2) protein, which has been shown also to be induced by TGF-β; conversely, the co-silencing of HAS2 and HAS-AS significantly reduced HMGA2 mRNA levels. We recently found that HAS2 promotes invasion of breast cancer cells metastasizing to bone by negative regulation of the expression of the protease inhibitor TIMP-1. Furthermore, the interaction between hyaluronan and CD44 is required for microvascular endothelial cell tubulogenesis by affecting the expression of cytokines and their receptors, as well as dissemination of breast cancer cells. We have shown that the activity and stability of HAS2 are regulated by mono- and poly-ubiquitination, respectively. Experiments are currently in progress to explore the regulation of monoubiquitination as well as different types of polyubiquitination of HAS2 by different de-ubiquitinases, and their role in breast cancer.

We also investigate the mechanisms through which hyaluronan signals via its receptor CD44 alone or in co-operation with the growth factor receptors for TGF-β and PDGF-BB. Using a proteomic approach we identified proteins interacting with the cytoplasmic part of CD44 such as IQGAP1 and iASPP. Our data demonstrate a role of IQGAP1/CD44 interactions in fibroblast migration. With regards to CD44/iASPP complexes, hyaluronan-activated CD44 binds iASPP leading to a decreased interaction between iASPP and p53 in hTERT BJ fibroblasts. Most interestingly, the CD44 expression levels affected the cellular localization and the function of the p53-iASPP complexes.

Matrix Metalloproteases

L
04

Jan-Olof Winberg

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Matrixines or matrix metalloproteases (MMPs) are zinc and calcium dependent secreted and membrane anchored neutral endopeptidases belonging to the metzincin clan. They have a broad and overlapping substrate specificity that together can degrade almost all proteins in the extracellular matrix (ECM). MMPs also process a large number of non-matrix proteins such as cell surface receptors, growth factors, cytokines, chemokines, enzymes and inhibitors in addition to a large number of intracellular proteins. The different MMPs are tightly regulated at various levels and one involves their localization and interaction with other ECM components which might affect their ability to be activated, regulated by endogen inhibitors and degrade specific substrates. MMP mediated protein processing is important in many physiological processes including embryonic development and homeostasis. A dysregulation of MMPs occurs in many pathological conditions such as inflammation, cancer, cardiovascular diseases, rheumatoid arthritis, osteoarthritis, pulmonary diseases, chronic wounds, fibrosis and neuronal disorders. The role of MMPs in the various disorders appears to be dual where it either promotes or protects against the disease. The outcome appears to be dictated of the timing of the MMP expression and the nature of the substrate cleaved. In many cases cleavage of a substrate requires that it interacts with sites outside the active site (exosite) in order to be presented to the active site. Exosites appear to be specific for a given substrate and the processing enzyme and are now regarded as targets for new specific MMP inhibitors. This is some of the themes that will be discussed.

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L 5/23

Collagen and functional implications

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ECM is essentially a complex fiber-composite material in which collagen fibrils are the major component. Collagen is the most abundant protein in mammals. It is widespread throughout the human body and forms the structure of the tissues in the musculoskeletal system, especially bones and joints, and the protective system such as skin and fasciae. The term 'collagen' defines a large family of related proteins fulfilling different functional roles in connective tissues. Vertebrates contains at least 28 different collagens of which types I, II, III, V, XI, XXIV and XXVII belong to the fibril-forming subgroup. Collagens plays a role for tissue load-bearing scaffold, tissue and organ protection, cell adhesion, cell migration. cancer, angiogenesis, tissue morphogenesis and tissue repair. Tissue load-bearing scaffold function of collagen is stricktly related to the mechanical tensional strength of the connective tissues. Collagen fibrils are comprised of many individual collagen molecules which arrange in a hierarchical order. Collagen consists in three polypeptide left handed (α) chains which are held together by interchain hydrogen bonds and assemble forming a unique triple right-handed tropocollagen supercoil. Five tropocollagens are twisted in a left-handed helix forming a single microfibril. Then microfibrils arrange in a slight rightward helix to form a single collagen fibril. This alternated hierarchical handedness (left-right-left-right) from a molecular to a supramolecular level increases the fibril's resistance to elongation, as collagen fibrils are the major source of mechanical tensional strength of connective tissues. In fact the collagen fibrils may be compared to right ropes because when tendon is stretched they reduce their diameter and show interfibril compression. Fibrils are arranged in parallel bundles (tendons and ligaments), orthogonal lattices (cornea) and concentric lamellae (bone). Both fibril diameter and array are expression of the tissue mechanical functions. In different connective tissue collagen fibrils show a different microfibrillar array. Two type of fibrils have been described: a) fibrils with almost straight microfibrils (5°), which show a large and heterogeneous diameter and mainly consist of type I collagen (tendon, bone); b) fibrils with a spiral microfibrillar arrangement (17°) which exhibit a homogeneous small diameter and mainly consist of type III collagen (skin). The first fibrils are stiffer and stressed under unidirectional loads, while the second ones are flexible and submitted to multidirectional forces (skin). In tendon/ligament the stiff large fibrils show knots called fibrillar crimps when they change their course. Morphological studies show that in the fibrillar crimp region each fibril twists on the left and then sharply bend changing its plane of run. Fibrillar crimps act as a shock absorber system during tendon stretching. and as a recoiling system after muscle relaxation.

Collagen receptors- static contacts or dynamic modulators?

L 6/24

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Cells interact with collagen directly via collagen-binding integrins and indirectly in contacts mediated via RGD-binding integrins linked to fibrillar collagens via COLINBRIs (Collagen-integrin-bridging molecules)[1]. The relative importance of these contacts for connective tissue homeostasis will be discussed.

Previous wound healing studies have failed to define a role for either α1β1 or α2β1 integrin in fibroblast-mediated wound contraction, suggesting the involvement of another collagen receptor in this process. Therefore, the repair of excisional wounds in mice with a global ablation of α11 integrin subunit was investigated. Analyses of wounds demonstrated that α11β1 deficiency results in reduced granulation tissue formation and impaired wound contraction, independently of the presence of α2β1.

Our combined *in vivo* and *in vitro* data further demonstrate that dermal fibroblasts lacking α11β1 are unable to efficiently convert to myofibroblasts, resulting in scar tissue with compromised tensile strength. Moreover, we suggest that the reduced stability of the scar is a consequence of poor collagen remodeling in α11-/- wounds associated with defective TGF--β-dependent JNK signaling[2].

[1.] Zeltz, C., Orgel, J. & Gullberg, D. Molecular composition and function of integrin-based collagen glues-introducing COLINBRIs. Biochim. Biophys. Acta 1840, 2533-2548 (2014),
[2.] Schulz, J.N. et al. Reduced Granulation Tissue and Wound Strength in the Absence of alpha11beta1 Integrin. J. Invest. Dermatol. 135, 1435-1444 (2015).

L
07

The endocytic receptor LRP1 is a master regulator of extracellular levels of ECM-degrading metalloproteinases

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Matrix metalloproteinases (MMPs) and adamalysin-like metalloproteinases with thrombospondin motifs (ADAMTSs) are secreted proteinases that play major roles in extracellular matrix (ECM) turnover, but their activities are not readily detected in normal steady state of tissues. Many of these metalloproteinase activities are regulated at several levels, such as gene transcription, activation of zymogens, inhibition by endogenous inhibitors during development, tissue remodelling and in diseases such as cancer, arthritis, cardiovascular diseases and pulmonary diseases. Among them, ADAMTS-5, a major aggrecan-degrading proteinase considered to be responsible for the development of osteoarthritis, is interesting as its mRNA levels are not significantly elevated in osteoarthritic cartilage. We have recently found that in normal healthy cartilage ADAMTS-5 is constitutively expressed, but its activity is minimally detected due to its rapid internalization and degradation, which is mediated by the endocytic receptor, low-density lipoprotein receptor-related protein 1 (LRP1). We have also found this endocytic process applies to ADAMTS-4, MMP-13 and their inhibitor TIMP-3 in the normal cartilage. However, the LRP1-mediated endocytic pathway is impaired in osteoarthritic cartilage due to an increased shedding of LRP1. This shifts the trafficking of these matrix-degrading proteinases and TIMP-3 to the extracellular space. Using cultured chondrocytes, we found that shedding of LRP1 was enhanced when the cells were treated with interleukin 1 or tumour necrosis factor a, primarily due to increased activity of MMP-14 and ADAM17. Shed LRP-1 (sLRP-1) forms complexes with ADAMTS-4 and -5, MMP-13 and TIMP-3, and the bound enzymes and inhibitor retain their activities. We propose that LRP1 is a master regulator of extracellular trafficking of matrix-degrading metalloproteinases, and that shedding of LRP1 in the local tissues under inflammatory or chronic pathological conditions dysregulates normal turnover of the ECM molecules and cellular homeostasis.

How perifibrillar proteins influence collagen secretion and fibril interactions

L 08

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Matrilins and COMP are non-collagenous proteins in the cartilage extracellular matrix and have been implicated as mediators of interactions between cartilage collagen fibrils and other matrix components. While the matrilins form a unique four-member protein family, COMP is a thrombospondin and is also referred to as TSP5. In biochemical studies strong interactions have been demonstrated between matrilins and COMP and between these proteins and collagen IX, which is present at the surface of the cartilage collagen fibril. It was therefore surprising that mice in which COMP or each of the matrilins have been ablated do not show any pronounced skeletal phenotype, a fact that is probably due to a functional redundancy between the proteins found at the collagen fibril surface. To analyse the function of these peri-fibrillar proteins as a group, we have created mice that lack expression of more than one of the genes and also turned to genetic analysis in the zebrafish system. In both kinds of experiments distinct skeletal changes are seen that we now analyse to determine the biological importance of this interacting set of proteins at the collagen fibril surface. A surprising first finding was that both COMP and matrilin 1 are required for efficient collagen secretion

Patient mutations in matrilin-3, COMP and collagen IX have been shown to cause several forms of chondrodysplasia and, in case of matrilin-3, also to predispose for osteoarthritis. The common view has been that such mutations cause a protein misfolding, leading to intracellular protein accumulation, an ER stress response and chondrocyte death. We have analysed a set of COMP and matrilin-3 mutations by biochemical, cell biological and genetic means and found a considerable heterogeneity in the molecular and cellular mechanisms by which they cause disease. While some mutations indeed induce intracellular retention and ER stress, proteins carrying other mutations are readily secreted, but still cause disease. Mechanistic investigation of the mutations that have extracellular effects show a dominant negative influence on collagen fibril formation, a process that is not strongly affected by the lack of the same protein.

L 09

Tumor-host interactions in pancreatic carcinoma: Role of heparanase enzyme

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It is now becoming increasingly clear that enzymatic remodeling of heparan sulfate proteoglycans profoundly affects a variety of patho-physiological processes, including chronic inflammation, diabetic/metabolic disorders, and tumor development. Heparanase is the sole mammalian endoglycosidase that cleaves heparan sulfate (HS) at the cell surface and in extracellular matrix (ECM) of a wide range of tissues. Of particular significance are studies showing correlation between enhanced heparanase expression and shorter postoperative survival of cancer (including pancreatic carcinoma) patients.

Recently, applying histological specimens from pancreatic carcinoma patients, mouse model of pancreatic cancer, ex vivo and *in vitro* experimental systems, we described a novel mechanism of heparanase-driven polarization of tumor-associated macrophages (TAM) toward tumor-promoting action in the setting of pancreatic carcinoma-associated "sterile" inflammation. Overexpression of heparanase was correlated with increased infiltration of TAM in both experimental and human pancreatic cancer. Moreover, macrophages residing in heparanase-rich tumors [which grew faster in the mouse host], displayed pronounced pro-cancerous phenotype. Furthermore, heparanase enzyme conferred to macrophages, stimulated by necrotic cells (which were invariably present in human and experimental pancreatic tumors) cancer-promoting activity, characterized by augmented production of key cytokines participating in pancreatic tumorigenesis, as well as by ability to induce epithelial STAT3 signaling [a cornerstone of inflammation-related pancreatic tumor progression] and to augment proliferation of pancreatic carcinoma cell *in vitro*. In further agreement with the *in vitro* studies, activation of STAT3 was observed in experimental and clinical specimens of pancreatic cancer that overexpressed heparanase. In addition, we provided molecular explanation for preferential expression of heparanase in pancreatic carcinoma.

Collectively, our findings describe what we believe to be a novel role of heparanase in a molecular "decision making" process that guides inflammation-associated tumor progression, highly relevant to pathogenesis of pancreatic carcinoma and additional cancer types associated with aseptic inflammation.

Tissue niches for collective cancer cell invasion and therapy resistance

L 10

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The tumor microenvironment supports both cancer cell invasion and growth/survival programs, with impact on response to therapy and prognosis. Using intravital near-infrared/infrared multiphoton microscopy, we have established preclinical sarcoma and melanoma models for spontaneous cancer cell invasion and distant metastasis to lymph nodes and lungs. Using multi-parameter 3D detection with subcellular resolution, we identify the tissue niches enabling growth and invasion, as well as therapy resistance. Interstitial dissemination away from the primary lesion occurred along blood vessels, myofibers, nerves, adipocytes and collagen bundles, as guided migration along preformed multi-interface conduits. Once populated by cancer cells, these invasion niches enabled tumor cell survival and relapse of disease even after high-dose hypofractionated radiotherapy. This invasion-associated resistance was sensitive to combined anti-β1/β3 integrin and radiation therapy, which resulted in complete eradication and relapse-free survival. Despite its radiation-resistance, the invasion niche was particularly sensitive to adoptive CTL-based therapy, as consequence of particularly high local CTL accumulation, robust CTL-mediated apoptosis induction in tumor cells, and eradication of invasion strands. This establishes tumor-stroma interaction niches of overlapping invasion and resistance programs which can be exploited by combining conventional with integrin- and CTL-targeted therapy.

L
11

Versican: An enigmatic matrix component and the control of disease cell phenotype

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Versican is a chondroitin sulfate (CS) proteoglycan that is present in the extracellular matrix (ECM) surrounding cells and in the interstitial matrix between cells in most tissues. It is synthesized by a variety of cells, including both stromal and myeloid cells, and is important in regulating the differentiated state of these cells. In normal tissues, levels of versican are low, but in disease, versican increases dramatically, as is seen in diseases of the vascular system, eye, lung, and in many different cancers. Accumulation results from shifts in the balance of increased synthesis and decreased degradation. Versican exists in at least four different isoforms generated by alternative splicing of the two large exons that code for the two glycosaminoglycan attachment domains in the core protein. The larger forms, V0, V1, and V2 contain CS, while the smallest form, V3, lacks glycosaminoglycan chains. All the isoforms are differentially regulated and interact with cells to alter specific signaling pathways generating different cell phenotypes. Attention will be given to mechanism(s) by which different isoforms of versican influence cell behavior and/or phenotype either through direct or indirect pathways. Controlling versican accumulation is critical in the engineering of tissues and examples will be highlighted, such as the tissue engineering of blood vessels and skin. Studies demonstrating that the different versican isoforms and associated molecules, such as hyaluronan, impact immune and inflammatory events in diseases such as atherosclerosis, leiomyosarcoma, and type 1 diabetes will be discussed. Finally, targeting versican is offered as a therapeutic strategy in the prevention and treatment of a variety of diseases.

Interplay of syndecan-1 and heparanase in cancer stem cell function

L 12

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The heparan sulfate proteoglycan syndecan-1 binds growth factors, morphogens, chemokines and extracellular matrix components, thereby regulating cancer cell proliferation, motility and invasiveness. In colon cancer pathogenesis, complex changes occur in the expression pattern of syndecan-1 during progression from well-differentiated to undifferentiated tumors. Loss of syndecan-1 is associated with a change in phenotypic plasticity with an increase in invasiveness, metastasis and dedifferentiation. Empirical evidence shows that this change in phenotypic plastiticty allows cells to dynamically enter into a stem-cell-like-state. In this study, we investigated the role of syndecan-1 in a potential colon cancer stem cell-function employing an siRNA approach in the human colon cancer cell lines Caco2 and HT-29.

We demonstrate thas siRNA-depletion of syndecan-1 increased the stem cell phenotype based on sphere formation assays, xenografting studies and phenotypic marker analysis (Side-population, ALDH activity, CD133 FACS). Moreover, syndecan-1 depletion increased invasiveness of Caco2 cells in a matrigel invasion chamber assay. Mechanistically, decreased expression of syndecan-1 was associated in an upregulation of the activated form of beta-integrin, and to increased activation of focal adhesion kinase (FAK), suggesting that syndecan-1 was implicated in integrin-mediated actin remodeling. Importantly, Wnt signaling was increased upon syndecan-1 siRNA delpetion as revealed by TOP Flash assays and qPCR, resulting in upregulation of the transcription factor TCF4 and a strenghtening of the FAK:WNT signaling axis. Notably, we also observed an upregulation of the heparan sulfate-degrading enzyme heparanase (HPSE) in syndecan-1 depleted cells. Increased HPSE-expression could be linked to an upregulation of the transcription factor Egr1, which regulated HPSE at the promoter level. The enhanced stem cell phenotype in syndecan-1-depleted cells could be blocked by pharmacological inhibitors of FAK and HPSE, suggesting a functional interplay of these molecular pathways.

We conclude that reduced syndecan-1 expression cooperatively enhances activation of integrins and FAK, which then generates signals for increased invasiveness and cancer stem cell properties.

Our findings may provide a novel concept to target a stemness-associated signaling axis as a therapeutic strategy to reduce metastatic spread and cancer recurrence.

L 13

The role of syndecan-1 in mesenchymal tumors

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Cell-membrane expression of syndecan-1 is associated to favorable prognosis, epithelioid morphology and inhibition of growth and migration in mesenchymal tumors such as malignant mesothelioma. However, recent evidence points also toward possible nuclear functions.

Expression of syndecan-1 was modulated followed by downstream functional assays and simultaneous monitoring of transcriptomic and proteomic responses. Transfection with a construct where the nuclear localization signal was deleted allowed us to separately analyze the cellular functions related to cell surface and nuclear syndecan-1.

Syndecan-1 over-expression had profound inhibitory effects on angiogenesis, regulation of cell growth and cell cycle progression paralleled by alteration of EGF, PDGF, VEGF and TGF-β related signaling pathways. Downstream of MAPK pathways Ras, Myc and Fos were inhibited. Most strikingly, nearly all analyzed pathways related to cell cycle were enriched after syndecan-1 silencing and depleted after syndecan-1 over-expression.

Syndecan-1 orchestrates different growth factors converging to downstream phosphor-kinase pathways. Deletion of the RMKKK nuclear localization signal resulted in diminished nuclear translocation and showed a distinctive gene expression profile. A better understanding of the complex role of syndecan-1 and its molecular interactions may provide possibilities in the future to control the growth of this highly aggressive mesenchymal tumor.

Novel proteoglycan roles in evoking autophagy independent of nutrient deprivation

L 14

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Autophagy is a highly-conserved, fundamental catabolic process that non-specifically reclaims nutrients for maintaining proper cellular and tissue homeostasis. Canonically, autophagy is regulated via nutrient-sensitive signaling pathways for restoring proper energy balance and metabolic function. Decorin, a prototypical member of the class I family of small leucine-rich proteoglycans, attenuates several oncogenic signaling networks, particularly those operating within the tumor microenvironment, via receptor tyrosine kinase antagonism. Suppression of pro-angiogenic factors (HIF-1a/VEGFA) and a concomitant induction of angiogenic inhibitors (thrombospondin-1 and TIMP3) culminate in mitigating tumor vascularization and unchecked tumorigenic growth. Recently, we discovered that soluble decorin evokes autophagy in endothelial cells downstream of VEGFR2 and in a Peg3-dependent manner. Peg3 is a decorin inducible, genomically imprinted tumor suppressor gene that is frequently silenced in many solid tumors. We found that Peg3 is necessary for the competent expression of the pro-autophagic genes *BECN*1 and *MAP1LC3A* in response to decorin. Mechanistically, the high affinity interactions of decorin and VEGFR2 allows for robust AMPK activation, which subsequently inhibits anti-autophagic mTOR signaling and permits autophagic progression. Endothelial cells represent a major cell type of the tumor stroma and are directly involved in tumor neovascularization. Thus, this novel function of a secreted proteoglycan suggests that some tumoricidal properties of decorin may be attributable to protracted autophagic stromal induction. Moreover, we found that autophagy is regulated by soluble matrix components in a receptor dependent fashion that is seemingly independent of prevailing nutrient conditions. Therefore, our research elaborates a new role for decorin as an innovative therapeutic modality for combatting tumorigenesis and angiogenesis via the engagement of excessive autophagy.

L
15

Tumor-stroma interaction of hepatomas with slow or fast proliferation rate.

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Tumor phenotype if greatly influenced by the synthesized tumor microenvironment. This non-tumorous component includes inflammatory cells, tumor associated fibroblasts, blood vessels, and the macromolecules of extracellular matrix.

In the presented research we addressed the question if fibrogenic response of Ito cells for the presence of hepatoma cells is homogenous, or it is influenced by the phenotype of hepatocellular carcinomas?

Hepatoma cell lines were received from the Pathology Department of Heidelberg University. A fast growing dedifferentiated, (HLE) and a slowly growing, more differentiated (HUH7), cell line was selected for our experiments. Immortalized LX2 Ito cell line was a kind gift of Scxott Friedman (USA).
The increased invasivity of HLE cells were proved by their increased proliferation rate, and their faster migration in would healing assay, as well as in Boyden chamber for Matrigel compared to HuH7 cells.

Both cell lines express vimentin an intermedier filament described in hepatomas with poor prognosis. They also express syndecan-1 and CXCR4 chemokin receptors.

When hepatoma cells were growing with Ito cells in coculture we witnessed changes in their matrix protein synthesis. However, the proteins synthesized by the two cocultures differed from each other.

The coculture of LX2 with the more aggressive HLE cells produced more laminin b1, TIMP1, type IV collagen, whereas there were fibronectin, thrombospondin1 and type IV collagen in the coculture with HuH7 cells. Syndecan-1 shedding increased in both cocultures.
Our results indicate, that the response of stromal cells are determined by the phenotypic characteristics of cancer cells.

Modeling glioma and targeting the glioma niche

L 16

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Glioblastoma (GBM) is the most frequent and malignant form of primary brain tumor. GBM is essentially incurable and its resistance to therapy attributed a subpopulation of cells called glioma stem cells (GSCs). To meet the present shortage of relevant GBM cell (GC) lines have we developed a library of annotated and validated cell lines derived from surgical samples of GBM patients, maintained under conditions to preserve GSC characteristics (Xie et al, 2015). We demonstrated that these cell lines are tumorigenic, harbor genomic lesions characteristic of GBMs, and represent all four transcriptional subtypes. This resource can be used to advance GBM biology, drug screens and drug discovery (Kitambi et al, 2014).

Neural progenitors and GCSs have several common traits, such as sustained proliferation and a highly efficient migratory capacity in the brain. There are also similarities between the neurogenic niche where adult neural stem cells reside, and the tumorigenic niche. Heparan sulfate (HS) proteoglycans are main components of the ECM where they interact with a large number of physiologically important macromolecules, thereby influencing biological processes. HS modulate growth factor activities, and we have shown a vital role for HS biosynthetic enzymes in the formation of the neural lineage (Forsberg et al, 2012). The major enzymatic activity degrading HS is Heparanase (HPSE). The activity of this HS-editing enzyme is thus an important regulator of ECM remodeling, and has also been suggested to promote cancer growth by generating a supportive microenvironment. This prompted us to investigate the role of HPSE in glioma.

HPSE was highly expressed in glioma and our GC lines, compared to normal brain, and high HSPE levels correlated with shorter patient survival. Down-regulation of HSPE reduced GC proliferation *in vitro*, while exogenous HPSE stimulated cell growth and efficiently activated ERK and AKT signaling. Using a syngenic ortothopic mouse model of glioma, we demonstrated that tumor development *in vivo* was positively correlated with HPSE levels in the host brain. HPSE also modified the glioma microenvironment, influencing the amount of reactive astrocytes, microglia/monocytes and recruitment of blood vessels in the peritumoral area. Furthermore, we demonstrated an efficient reduction of glioma growth by an inhibitor of HPSE. We thus provide proof-of-concept for anti-HPSE treatment of malignant glioma, and novel insights into HPSE as targets and/or biomarkers of this devastating disease.

Reference:

Xie et al., The human glioblastoma cell culture (HGCC) resource: validated cell models representing all molecular subtypes, EBioMedicine in press DOI 10.1016/j.ebiom.2015.08.026

Kitambi, et al., Vulnerability of Glioblastoma Cells to Catastrophic Vacuolization and Death Induced by a Small Molecule. Cell, 2014, 157(2): 313-28

Forsberg, M., et al., Under-sulfation of heparan sulfate restricts the differentiation potential of mouse embryonic stem cells. J Biol Chem, 2012. 287(14):10853-10862

L 17

Targeting heparanase for myeloma therapy

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Heparanase is an endoglucuronidase that cleaves the heparan sulfate chains of proteoglycans, releasing biologically active fragments of heparan sulfate, and leaving shortened (trimmed) chains on the proteoglycan core protein. There is mounting evidence that this enzymatic action of heparanase impacts cell signaling and promotes tissue remodeling that drives tumor growth, invasion, metastasis and angiogenesis. Using *in vitro* and *in vivo* models of multiple myeloma, we have discovered that mechanistically, heparanase regulates tumor progression by: i) upregulating expression of a number of growth factors (*e.g.*, VEGF, HGF), ii) enhancing the shedding of syndecan-1 from the surface of tumor cells and iii) elevating the secretion and altering the composition of tumor-derived exosomes. Together, these findings indicate that heparanase is a viable target for anti-cancer therapy. In addition, its potential as a therapeutic target is enhanced by the fact that there is a single enzymatically active heparanase in humans and the finding that heparanase knockout mice appear to be healthy. Thus, therapeutic neutralization of heparanase activity would likely have limited negative side effects.

SST0001 (proposed International Nonproprietary Name (INN): Roneparstat) is a rationally developed heparanase inhibitor produced by the modification of porcine mucosal heparin, the final product being 100% N-acetylated and 25% glycol split. These modifications endow SST0001 with high anti-heparanase activity (IC_{50} = 3 nM) while retaining only negligible anti-coagulant activity. Preclinical studies of SST0001 in animal models of multiple myeloma indicated anti-tumor efficacy and pharmacodynamic effects consistent with its anti-heparanase activity *in vivo* (*e.g.*, reduced angiogenesis and diminished expression of HGF, VEGF and MMP-9). This led to an ongoing Phase I study of SST0001 in advanced refractory multiple myeloma patients. In recent studies we have discovered that treatment of tumor cells with anti-myeloma drugs such as bortezomib and melphalan significantly upregulated expression and secretion of heparanase by myeloma tumor cells and that tumor cells with high levels of heparanase expression have enhanced resistance to killing by these drugs. This indicates a key role for heparanase in promoting myeloma chemoresistance and provides strong rationale for testing the heparanase inhibitor SST0001 in combination with anti-myeloma drugs. Using a SCID mouse model of disseminated myeloma we found that SST0001 in combination with either bortezomib or melphalan dramatically decreased both the number of animals with detectable tumor and the tumor burden when compared with animals treated with either of these drugs alone. Together these findings support further clinical testing of SST0001 in myeloma patients and indicate that anti-heparanase therapy increases the efficacy of cytotoxic drugs used to treat myeloma.

How does herapin prevent intracellular synthesis of hayaluronan in hyperglycemic dividing cells?

L 18

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Hyperglycemic dividing cells activate hyaluronan synthases in cytosolic membranes, which induces synthesis of hyaluronan into intracellular compartments (ER, Golgi, transport vesicles). This initiates autophagy and promotes inflammatory mechanisms that compromise functions *in vivo* in diabetic pathologies, including kidney failure.[1] Heparin prevents the intracellular hyaluronan synthesis, prevents the autophagy and addresses the sustained hyperglycemia after completing division by synthesizing an extensive extracellular monocyte-adhesive hyaluronan matrix, and *in vivo*, glomerular mesangial cells sustain kidney function while the influx of macrophages remove the hyaluronan matrix.[1] The mechanism involves a cell surface receptor that interacts with the highly sulfated non-reducing terminal oligosaccharide of heparin, which is exposed by heparanase from cryptic binding motifs in serglycin heparin chains and in the highly sulfated regions of heparan sulfate. Interaction of the oligosaccharide motif with the receptor: 1) internalizes the heparin to ER, Golgi, nuclei compartments within 3 hours; 2) inhibits the PKC pathway that activates the hyaluronan synthase in these compartments; 3) inhibits glucose uptake and elevation of cytosolic UDP-glucose concentration; and 4) reprograms the cell to synthesize the monocyte adhesive extracellular matrix to address the continued hyperglycemic stress after cell division. We propose that elevation of the cytosolic concentrations of glucose initiate the PKC pathway that activates intracellular hyaluronan synthesis and that heparin binding to its receptor maintains UDP-GlcNAc cytosolic concentration within an acceptable range by preventing elevation of cytosolic glucose levels during cell division in hyperglycemia.

Reference: Wang et al. J. Biol. Chem. 289:9418-29, 2014.

L 20

Role of versican, and chondroitin sulfate in development and diseases

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Versican (Vcan) is a large chondroitin sulfate (CS) proteoglycan of the extracellular matrix (ECM). Its core protein consists of two globular domains at both N- and C-terminal ends and two CS binding domains CSa and CSb. The N-terminal G1 domain binds to HA and link protein, and the C-terminal G3 domain binds to fibrillins, tenascins, and fibulins, forming a supramolecular complex. During development, Vcan is transiently expressed at high levels in heart, mesenchymal condensation areas of cartilage primordium, dermis and hair follicles. We have been investigating *in vivo* function of Vcan, using Vcan (Δ3/Δ3) mice, which express Vcan without the A subdomain of the G1 domain, and different conditional knockout (cKO) mice. Here, we demonstrate how Vcan affects in cardiac and skeletal development, and tumor growth. Vcan (Δ3/Δ3) mice with C57Bl/6 background are small with dilated heart, and die at E9.5~10.5, similar to gene-trapped Vcan-null hdf mice. Their heart exhibits dilated atria and ventricles, and the wall is thin without cardiac jelly. Vcan (Δ3/Δ3) mice with mixed background of C57Bl/6 and Balb/c survive longer, exhibiting cardiac dilatation and ventricular septal defect, due to impairment of myocardial cell differentiation with reduced BMP2/4 signaling, and altered distribution of HA and link protein. Prx1-cre: Vcan‹flox/flox› mice lack Vcan expression in mesenchymal condensation areas of cartilage primordium. Though viable and fertile, these mice display distorted digits, due to impaired joint formation and slightly delayed endochondral ossification. In the joint interzone and the condensation areas, TGF-β -signaling is diminished, concomitant with the absence of Vcan. QRsP11 fibrosarcoma cells are implantable to mice with B6 background and lack Vcan expression. When implanted subcutaneously together with adenovirus expressing Cre (Ad-Cre) into Vcan‹flox/flox› mice, the tumor grows larger with increased levels of angiogenesis, and decreased collagen fibers. These results indicate that Vcan plays a pivotal role when provisional matrix is formed and matrix structure is reconstituted, by affecting matrix structure and regulating BMP and TGF β-signaling.

Hyaluronan amount and size are critical in human pathology

L 21

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An emerging body of scientific evidence suggests the cell responses are hyaluronan (HA) molecular weight dependent. HA fragments, generated by multiple mechanisms including inflammation, free radicals production and hyaluronidase, elicit cellular responses distinct from intact HA. The HA fragments are usually defined oligosaccharides and it is commonly accepted that HA chain with molecular weight less than 500 kDa is a fragmented oligosaccharide. Nevertheless, the biological activities of oligosaccharides of about 500 kDa are quite different to smaller fragments as 50 kDa or less, ranging from NFkB and MMP activation (4-6-mers) to and cytokines and chemokines synthesis (6-32 mers) and COX2 and iNOS activation (40-800 kDa). All these activities are triggered by HA receptors as CD44 and RHAMM. Recently the involvement of innate immunity is also triggered by the oligosaccharides throughout TLR activity. The specific action of single size HA fragments are still not completely understood, so we designed experiments using different human primary cells in order to identify the activity of specific size HA preparation commercially available. The human fibroblasts were incubated with 100 ug/ml of different preparations and increased the proliferation at 5 days ranging from 50% to 10% compared to controls. The expression of specific genes has been assessed by qRT-PCR. In particular collagen type I and III were almost unaltered by treatments, whereas significantly decreased by very small fragments. The gene expression of IL8, hyaluronan synthase 2 and 3 were influenced by treatments. The apoptosis CHOP expression was down regulated by all HA preparations whereas MT1-MMP, prolyl-hydroxylase, TIMP 1 and TIMP 2 have been up regulated by all HA preparation. Alpha actin has been up regulated by HA1-4 being the HA4 the most effective. Cell migration test based on the use of scratch injury demonstrated that HA1 and 2 improved the cell migration whereas HA3 markedly reduced cell migration.

HA synthesis is regulated by several factors including covalent modification of the synthetic enzymes HAS2. We described the role of AMPK in the phosphorylation of HAS2 which blocks the HA synthesis. In human smooth muscle cell (AoSMC) model, we show that the hexosamine biosynthetic pathway (HBP) may increase the concentration of UDP-N-acetyl-glucosamine leading to an increase of HA synthesis, and this is remarkable in diabetic patients. It was reported that proteins can be O-glycosylated by specific transferase OGT, and we found that the inhibition of O-GlcNacylation reduced HA production whereas opposite effect was obtained inducing protein O-GlcNacylation altering the enzyme life span. We found that the natural antisense transcript (NAT) of HAS2 (HAS2-AS1) was absolutely necessary to induce the transcription of the HAS2 gene. From ChIP data we were able to identify the protein involved in this mechanism: p65 from NFkB signaling pathway. This protein can be O-GlcNacylated and in this form triggers the expression of NAT transcript as well as the stabilization of HAS2 promoter. In a specific pattern of experiments the over expression of p300 increased the HA production whereas the activity of HDAC1 reduced the polymer synthesis. Similarly, the HAS2-AS1 increased during p300 transfection and diminished in HDAC1 transfected cells, indicating that the acetylation is also involved in HAS2-AS1 expression. From our data emerge that not only the size but also the amount of hyaluronan are finely tuned by several complex machineries and have different biological activities in human tissues.

L
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Cellular design of heparan sulfate

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Heparan sulfate (HS) proteoglycans are important constituents of the cell surface and extracellular matrix of both vertebrate and invertebrate cells. While HS is synthesized by most animal cells, heparin is synthesized exclusively by connective tissue-type mast cells where the polysaccharide chains occur attached to the serglycin core protein. The two glycosaminoglycans have the same polysaccharide backbone, but differ in their degree of modification, heparin being more heavily sulfated than HS. The HS and heparin sulfation patterns determine the ability of the glycosaminoglycans to interact with proteins and thereby affect biological processes. The patterns are created during biosynthesis, where sulfotransferases in the Golgi compartment add sulfate groups while the chains are being polymerized. The sulfation patterns may also be altered post-synthetically by specific sulfatases. In the presentation, different ways to regulate biosynthesis is discussed, from transcriptional and translational control of enzyme expression to control of concentration of the sulfate donor PAPS.

Basement membrane collagens in the control of tumor cell invasion

L 25

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Basement membranes are highly specialized forms of extracellular matrix that represent a barrier between the epithelium and the underlying connective tissue. They consist of type IV collagen and associated collagens (collagens XV, XVIII and XIX), structural glycoproteins (mainly laminin and entactin,) and proteoglycans, as perlecan. The interactions of the various basement membrane components with the cells, via surface receptors as integrins, may regulate many biological activities such as cell migration, proliferation or differentiation.

Upon the influence of hypoxia, tumor cells secrete cytokines that activate stromal cells to produce proteases and angiogenic factors. In addition to stromal ECM breakdown, proteases exert various pro- or anti-tumorigenic functions and participate in the release of various ECM fragments, named matrikines or matricryptins, capable to act as endogenous angiogenesis inhibitors and to limit tumor progression.

Several basement membrane collagen-derived matrikines have been shown to decrease tumor growth in various cancer models. These matrikines exert their anti-tumor effects through anti-angiogenic and/or anti-tumor activities at different levels. As an example, we'll focus on NC1(XIX), an anti-tumor matrikine derived from type XIX collagen, that was recently characterized in our laboratory

Type XIX collagen is a minor collagen that localizes to basement membrane zones, together with type IV, type XV and type XVIII collagens. Since several NC1, C-terminal, domains of other chains from basement membrane collagens were reported to exhibit anti-tumor activity, we decided to study the effects of the NC1 domain of collagen XIX [NC1(XIX)] on tumor progression, using an experimental *in vivo* model of mouse melanoma. We observed a 70% reduction in tumor volume in NC1(XIX)-treated mice, compared to the corresponding controls. Histological examination of the tumors showed a strong decrease in tumor vascularization in treated mice. *In vitro*, NC1(XIX) inhibited the capacity of tumor cells to invade Matrigel®. It also strongly inhibited the migratory capacities of melanoma cells in the scratch wound model and in Ibidi® devices. Similar results were obtained with UACC 903 human melanoma cells. NC1(XIX) also inhibited the capacity of Human Microvascular Endothelial Cells to form pseudo-tubes in Matrigel®. This effect was accompanied by a strong inhibition of MT1-MMP (MMP-14) and VEGF expression. We also demonstrated that plasmin, one of the most important enzyme involved in tumor invasion, was able to release a fragment of NC1(XIX), which retained all the anti-tumor activity. Molecular modeling studies showed that NC1(XIX) and the anti-tumor fragment released by plasmin adopted locally the same type I β-turn conformation, suggesting that the anti-tumor effect was conformation-dependent.

Further studies are in progress in our laboratory to characterize the receptor(s) for NC1(XIX) on human melanoma cells and the intracellular transduction pathway involved in its anti-tumor effects. Our works demonstrate that collagen XIX is a novel proteolytic substrate for plasmin and that its cleavage by plasmin may release a new anti-tumor matrikine. Release of anti-tumor matrikines may constitute a defense of the organism against tumor invasion.

L 26

Cartilage Oligomeric Matrix Protein (COMP) contributes to the development and metastasis of breast cancer

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Cartilage Oligomeric Matrix Protein (COMP) is a large soluble protein consisting of five identical subunits that are held together at the N-termini by disulphide bridges. COMP is expressed in cartilage where it is involved in the organization of collagen in the extracellular matrix and has been used as biomarker for cartilage destruction. Data collected from mRNA expression arrays (Oncomine) indicated that COMP is overexpressed in breast cancer. To validate these data by determining protein expression, we immunostained tissue microarrays derived from two independent cohorts of patients with breast cancer (n = 122 and n = 408). The staining revealed varying amounts of COMP, both in the tumor cells and surrounding stroma, and the intensity of the staining was scored. High levels of COMP in tumor cells correlated independently with poor survival and faster recurrence (metastases). To understand why COMP expression aggravates breast cancer, we created stable human breast cancer cell lines (MDA-MB-231 and BT20) expressing COMP. The MDA-MB-231 cell line was used for *in vivo* experiments where we injected the cells orthotopically into the mammary fat pad of SCID mice. Tumors expressing COMP grew to a significantly larger volume and had increased metastasis as compared to control tumors. A particularly strong COMP staining was observed at the invasive margin of the tumors. *In vitro* experiments indicated that COMP expression increased the invasiveness of both cell lines by upregulation of metalloproteases. Further, microarray analyses of gene expression in tumors formed *in vivo* showed that expression of COMP increased expression of proteins protecting from endoplasmatic reticulum stress. This observation was confirmed *in vitro* since COMP expressing cells showed better survival than mock when treated with brefeldin, which leads to accumulation of proteins in endoplasmatic reticulum. In conclusion, COMP is a novel biomarker in breast cancer and contributes to the severity of the disease.

SLRP Signaling in inflammation

L 27

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Inflammatory processes in the body can be triggered not only in response to external signals, but also in response to the release of endogenous molecules called damage-associated molecular patterns. One such molecule, the small leucine-rich proteoglycan biglycan, is ubiquitously distributed in the body and can be proteolytically released from the extracellular matrix following tissue injury. In its soluble form, biglycan acts as a ligand of Toll-like receptors (TLR)-2 and -4, leading to the activation of NF-κB and subsequently the transcription and secretion of several pro-inflammatory cytokines and chemokines, including TNF-α, CCL2, CCL5, CXCL 1, CXCL2 or CXCL13. Notably, we discovered that biglycan plays a role in both the secretion and the maturation of pro-IL-1β. Murine primary macrophages exposed to nanomolar concentrations of recombinant biglycan overexpressed Il-1δ mRNA in a process dependent on TLR-2 and -4. At the same time, biglycan triggered the activation of caspase-1 in a process dependent on the NLRP3 inflammasome, even in the absence of ATP, which is normally required for triggering NLRP3 assembly. Interestingly, enhanced reactive oxygen species (ROS) production was found in biglycan-stimulated mouse primary macrophages, while addition of ROS inhibitors could partially reduce biglycan-dependent IL-1β and CXCL13 production. However, it remains unclear what the sources for biglycan induced ROS are and their involvement in the mechanism of biglycan-triggered cytokines release. One of the main sources of ROS in the cells is represented by NADPH oxidase (NOX) proteins, which transfer electrons across biological membrane to reduce oxygen to superoxide. Thus, we have uncovered a novel role for the NADPH oxidases in distinctly mediating biglycan-dependent cytokines production, both *in vitro* in macrophages and *in vivo* in a mouse model of renal ischemia reperfusion injury, a major cause of intrinsic acute renal failure.

L 29

Inhibition of manganese-mediated tumor cell migration by heparin analogs

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The metastatic disease accounts for over 90% of the deaths in cancer patients. Tumor progression is very complex, involving several molecular and cellular phenomena that occur at the primary tumor, inside blood vessel, at pre-metastatic niche and secondary tumor site. In all of them, dynamic interactions among tumor cells and the microenvironment are observed. Our hypothesis on tumor cell migration involves cell surface integrin and heparan sulfate proteoglycan forming a manganese-dependent macromolecular complex with extracellular fibronectin, leading to migration. In the presence of manganese, integrin undergoes a conformational change. Our data indicates that this change allows its interaction with heparan sulfate proteoglycans at the tumor cell surface, resulting in enhanced tumor cell migration. Heparin analogues are able to drastically attenuate this effect, both *in vitro* and *in vivo*. Circular dichroism and X ray fluorescence analyses suggest that manganese can be sequestered by heparin analogs, preventing it from binding to cell surface integrin. Additionally, an initial retrospective study on colon adenocarcinoma patients, carried out at Serviço de Patologia and Instituto de Pesquisas Biomédicas (Hospital Naval Marcílio Dias, Rio de Janeiro, Brazil), indicates that manganese is detected at the primary tumor, metastatic site and tumor-bearing lymph nodes, while it is not within detection range in tumor-free sites or lymph nodes.

Molecular mechanism of RHAMM activation of Src kinase and the signaling pathway for nitric oxide production in the endothelium

L 30

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RHAMM is necessary for endothelial cell (EC) migration in vitro and angiogenesis *in vivo*, but the mechanisms are incompletely understood. Nitric oxide (NO) production by endothelial nitric oxide synthase (eNOS) controls diverse physiological and pathological processes, involves a signaling cascade through Src kinase and Akt, and results in eNOS phosphorylation. A number of diverse agonists, including VEGF, HDL, estradiol, acetylcholine and insulin, activate eNOS to produce NO. To determine the molecular mechanism by which RHAMM regulates eNOS activation, we used cultured EC and RHAMM KO mice.

RHAMM siRNA knockdown in HUVEC inhibited the increased cell migration and eNOS activity observed with treatment with all EC agonists, and the exogenous NO donor NONOate rescued this inhibition, suggesting that RHAMM is universally required for eNOS activation and NO production. RHAMM siRNA-mediated decrease in cell migration and eNOS activity correlated with decreased Akt and eNOS phosphorylation but not Erk phosphorylation. In RHAMM KO mice, normal alveolarization and re-endothelialization after arterial injury, processes that require NO, were delayed. RHAMM KO also did not respond to exogenous VEGF, but treatment with either inhaled NO (iNO) or Molsidomine normalized lung alveolarization and restored re-endothelialization after arterial injury.

Since activation of Src is a proximal step in the signaling cascade, we examined the effect of RHAMM knockdown on Src activation. RHAMM siRNA treatment of EC was associated with hyperphosphorylation of the inhibitory site of Src (Y527) and an inhibition of the phosphorylation of the activation site of Src (Y416). The phosphatase Shp2 is critical in the dephosphorylation of Y527 and Src activation, and associates with VEGFR2 in this process. Interestingly, Shp2 and Src both co-immunoprecipitated with RHAMM *in vivo* as well as in vitro. Compared to EC treated with scrambled siRNA, co-immunoprecipitation of Shp2 and VEGFR2 with VEGF stimulation was significantly reduced in RHAMM siRNA-treated EC. These data suggest that the RHAMM-Shp2 complex facilitates Shp2 tethering to VEGFR2, which is necessary for the dephosphorylation of Y527 in Src and the downstream activation of the signaling pathway for NO production in endothelial cells.

L 31

Smad and non Smad signaling via TGF-β receptors – possible targets in tumor therapy

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Transforming growth factor β (TGF-β) affects growth, survival and differentiation of most cell types. Following ligand-induced hetero-tetramerization of type I and type II serine/threonine TGF-β receptors, Smad2 and 3 are phosphorylated whereafter they form complexes with Smad4, which are translocated to the nucleus where they regulate the transcription of certain genes. Smad signaling leads to growth arrest as well as epithelial-to-mesenchymal transition of epithelial tumor cells (Heldin et al. 2012. FEBS Lett 586: 1959-1970). It is carefully regulated by posttranslational modifications, including phosphorylation, ubiquitination, acetylation, sumoylation and PAR ylation (Lönn et al. 2010. Mol Cell 40: 521-532). TGF-β also activates non Smad signaling pathways. One example is the activation of p38 MAP kinase, which is of importance for TGF-β-induced apoptosis. P38 is activated by its upstream kinases MKK3/6 and TAK1; TAK1 is activated by poly-ubiquitination via Lys63 linked chains exerted by the E3 ligase TRAF6 (Sorrentino et al. 2008. Nature Cell Biology 10: 1199-1207). Interestingly, this mechanism is independent of the kinase activities of the TGF-β receptors. TRAF6 also mediates another non Smad signaling pathway, i.e. cleavage of the type I receptor by the metalloprotease TACE, in a PKCz dependent manner (Mu et al. 2011. Nature Communications 2: 330; Gudey et al. 2014. Sci Signal 7: ra2). The released intracellular receptor domain is translocated to the nucleus where it induces several genes coding for proteins involved in invasion of cancer cells, e.g. snail and MMP1. Thus, this pathway, which is not activated in normal cells, contributes to the TGF-β-induced invasiveness of advanced cancers. EMT is an important part of the tumor promoting effects of TGF-β. We found important roles of the DNA architectural protein HMGA2, and the transcription factors Snail and Twist in Smad induced EMT (Thuault et al. 2006. J Cell Biol 174: 175-183). We are now attempting to find ways of selectively inhibiting TGF-β-induced EMT to develop treatment regimens for patients with advanced cancers.

The regulatory roles of estrogen receptors and syndecans in breast cancer cell properties and functions

L 32

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The role of extracellular matrix (ECM) the last decades is converted from an inactive network of macromolecules to a functional network essential for structural support, cell migration, adhesion and signaling. Our laboratory has elucidated the significant role of ECM in hormone-dependent breast cancer. ECM molecules, especially proteoglycans (PGs), glycosaminoglycans (GAGs) and matrix metalloproteinases (MMPs), seem to interact with growth factors and receptors in tumor microenvironment by regulating cancer homeostasis. In recent studies is dealing with novel approaches in respect to the pharmacological targeting at the ECM level in cancer.

Estrogens represent the most important factors implicated in the progression of hormone-dependent breast cancer. The action of estrogens, regulated via estrogen receptor α and β (ERα/β), promotes different roles in tumor initiation and progression. We have recently shown that estradiol (E2) via estrogen receptors (ERs) can regulate the expression of structural and functional extracellular matrix (ECM) macromolecules leading cancer cells to alter their phenotype.

Taking into account the new insights into ER action in breast cancer, we have established two stable cell lines MCF-7 SP10+ and MDA-MB-231shERβ, which have been created after down-regulation of ERα and ERβ, respectively. Further investigation on those two cell lines has indicated the significant role of ERs in breast cancer cells homeostasis, via regulation of the expression levels of ECM macromolecules and breast cancer cells functional approaches.

Furthermore, we examined the cross-talk between the ERs, epidermal growth factor receptor (EGFR) and insulin growth factor receptor (IGFR) signaling pathways in the development of resistance to endocrine therapies against the ER pathway, and the involvement of important ECM macromolecules. The different expression of these molecules by tumor cells per se as well as through paracrine interactions with stromal cells, lead to their establishment as potential biomarkers of the metastatic potential and as well as possible targets for inhibiting growth, neoangiogenesis and invasiveness of tumor cells. These data indicated that cross-talk of ER, EGFR or IGFR is of crucial importance for the expression of breast cancer cell ECM molecules and might provide a potential target for the design of a more advanced treatment of breast cancer therapies.

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L 33

IGF-I/ EGF signaling affect breast cancer cell adhesion through cytoskeleton reorganization

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Breast cancer is a multifactorial neoplasia, common among women worldwide. Insulin-Like Growth Factor- I (IGF) and Epidermal Growth Factor (EGF) signaling pathways and their crosstalk are very critical for breast cancer cell functions. IGF-I and EGF have anti apoptotic effects and their actions affect the attachment of breast cancer cells on different extracellular matrix components. The growth factor downstream regulatory cascade involves several signaling pathways such as PI-3kinase and Mitogen- Activated Protein Kinase (MAPK). It is well established that Focal Adhesion Kinase (FAK) is a critical mediator for cell adhesion; specifically, the phosphorylation of FAK results in increased cell adhesion. Furthermore, IGF-I signaling affects the organization of cytoskeleton, which is directly linked with the ability of cells to adhere different substrates. The aim of this study was to examine the effect of IGF and EGF on the activation of FAK and on the organization of the actin cytoskeleton. . MCF-7 cells were used as a biological model. Our results indicate that IGF and EGF activate FAK in a MAPK (ERK1/2)-dependent manner. Furthermore, immunofluorescence microscopy demonstrated that IGF and EGF induce an adhesive phenotype, with well formed actin filaments in breast cancer cells. In parallel, the inhibition of ERK disrupts this IGF/EGF- induced organization of actin filaments. The immunofluorescence results are in accordance with the adhesion assay, indicating that the IGF/ EGF induced adhesion and cytoskeleton changes are ERK dependent. In conclusion, the results of our study suggest that IGF-I/ EGF signaling affect breast cancer cell adhesion, leading to cytoskeleton reorganization, via the activation of FAK and a downstream participation of ERK.

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Thales. Investing in knowledge society through the European Social Fund.

Proteoglycans as drug development targets: Chemical biology routes to new therapeutics

L 34

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Heparan sulfate proteoglycans are complex sulfated glycoproteins present on almost all cell surfaces in multicellular organisms. They have variant HS chain structures which represent a molecular code that is a subset of the glycome called the 'heparanome'. These sulfation patterns confer the ability to interact selectively with a wide interactome of proteins, especially growth factors and matrix proteins, that influence many cellular processes important in cell development and regulation. Importantly, they act as "master regulators" which coordinate many cellular processes relevant to disease processes. Chemical biology tools and glycomics approaches are being used to decode the molecular basis of the functional diversity of HS. This has yielded new insights into this code in a variety of biological contexts, including neurodegeneration (AD), cancer metastasis and nerve repair mechanisms. We are now undertaking a translational pathway of biomedical applications, including discovery of novel drug leads for Alzheimers disease and cancer, and potential interventions to improve neural cell transplantation for nerve repair.

L 35

Senescence of stromal fibroblasts triggered by anti-cancer treatments: implications in tumor progression

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Although cancer research is traditionally focused on the neoplastic cell per se, numerous studies have revealed that stroma plays an important role in tumor development and progression. Stromal fibroblasts, the most abundant cell type of tumor microenvironment, synthesize extracellular matrix (ECM) molecules and soluble mediators, highlighting their importance on paracrine interactions between them and tumor cells. On the other hand, stromal cells can become senescent after extensive proliferation or after repeatedly exposed to non-cytotoxic doses of genotoxic agents, due to activation of a DNA damage response pathway, i.e. the stimulation of the ATM-Chk2-p53-p21WAF1-pRb axis, leading to a permanent growth arrest. Senescent fibroblasts express a specific catabolic and inflammatory phenotype, which affects locally tissue homeostasis. Interestingly, genotoxic anticancer treatments, beyond cancer cells, they also affect stromal cells, thus possibly provoking premature senescence. We found that repeated non-cytotoxic doses of ionizing radiation provoke senescence of stromal lung fibroblasts in a p53-dependent mode. These cells express an inflammatory phenotype and they further enhance significantly the growth of cancer cells in co-cultures *in vitro* and in immunocompromised mice *in vivo*. This effect seems to be largely due to the overproduction of MMPs by senescent fibroblasts, as an MMP inhibitor significantly reduced tumor growth. Interestingly, a non-classical genotoxic agent, i.e. taxol, also provokes premature senescence and enhances angiogenesis and the subsequent growth of adjacent tumor cells. As radiotherapy is often used in breast cancer treatment, we also studied the effect of ionizing radiation on breast stroma fibroblasts. As in lung cells, irradiation provokes premature senescence and the appearance of a catabolic and inflammatory phenotype. Interestingly, senescent cells overexpress considerably syndecan-1 a marker of a reactive stroma in breast cancer. This overexpression seems to be independent of the activation of two major pathways in senescence, i.e. those regulated by p53 and p38 MAPK. Finally, we found that aggressive breast cancer cells enhance further syndecan-1 expression in stromal cells showing a positive feedback loop in tumor progression. These data indicate a putative side-effect of anticancer therapies in tissue homeostasis.

The core protein of serglycin is a matrix metalloprotease-9 substrate

L 36

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ProMatrix metalloproteinase-9 form strong complexes with the core protein of the chondroitin sulphate proteoglycan Serglycin. *In vitro* reconstitution of the MMP-9/Serglycin complexes requires the presence of an intact proenzyme as HPX and FnII deleted MMP-9 variants do not form these stable complexes. Trypsin activated MMP-9 (83 kDa) lacks the prodomain and form only small amounts of complex, while the presence of the metalloprotease inhibitor EDTA results in complex formation similar to the proenzyme. These along with other previous results suggest that the Serglycin core protein is a substrate for MMP-9. APMA activated recombinant human MMP-9 produced in insect cells (sf9) cleaves the Serglycin core protein at several places and generates peptides of different size. To which extent this cleavage involves the sites in MMP-9 that is involved in the formation of the proMMP-9/Serglycin complexes will be discussed.

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Lumican: A new inhibitor of matrix metalloproteinase -14 activity

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We previously showed that lumican regulates MMP-14 expression. The aim of this study was to compare the effect of lumican and decorin on MMP-14 activity. In contrast to decorin, the glycosylated form of lumican was able to significantly decrease MMP-14 activity in B16F1 melanoma cells. Our results suggest that a direct interaction occurs between lumican and MMP-14. Lumican behaves as a competitive inhibitor which leads to a complete blocking of the activity of MMP-14. It binds to the catalytic domain of MMP-14 with moderate affinity (KD _ 275 nM). Lumican may protect collagen against MMP-14 proteolysis, thus influencing cell–matrix interaction in tumor progression.
Keywords: Decorin; Lumican; MMP-14; Small leucine-rich proteoglycan

Pietraszek et al, FEBS Lett. 2014, 588(23):4319-24.

Osteoglycin (mimecan) protects against ischemic heart disease but increases adverse cardiac inflammation in viral myocarditis

L 38

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Osteoglycin (OGN, also called mimecan) was first described in 2009 as a marker of cardiac hypertrophy yet its biological role in cardiac function and morphology after myocardial infarction is unknown. Through its leucin-rich repearts, this secreted proteoglycan, in particular its 34kDa isoform appears to be critical for proper collagen assembly in the human infarcted heart, and its absence in mice leads to increased cardiac rupture and ventricular dilatation in the mouse. Conversely, its administration by adenoviral gene overexpression enhances collagen crosslinking in the scar and improves left ventricular function following myocardial infarction. In human patients with myocardial infarction, blood levels predict the progression of cardiac dysfunction and overall outcome.

Next, in viral myocarditis, we discovered a different glycosylation variant of OGN in myocarditis. Whereas the 34kDa dermatan sulfate-OGN increases in infarcted hearts -responsible for collagen cross-linking-, a previously unidentified 72kDa chondroitin sulfate-OGN predominates in viral myocarditis. This 72kDa OGN variant is expressed on circulating and resident cardiac innate immune cells, in both mouse and human. Here, it interacts with toll-like receptor-4 (TLR4) leading to its enhanced activation upon liposaccharide (LPS) stimulation as a result of increased phosphorylation of NFκB/ c jun and subsequent cytokines secretion. Accordingly, the absence of OGN in KO mice significantly decreased cardiac inflammation in CVB3-induced myocarditis and reduced the production of pro-inflammatory cytokines in LPS induced endotoxemia. Finally, FACS-analysis in human peripheral OGN positive leukocytes revealed a strong pro-inflammatory phenotype of these cells, emphasizing the importance of OGN in innate immune activation.

Together, osteoglycin is a novel diagnostic and therapeutic target regulating cardiac inflammation and fibrosis in human heart disease.

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Extracellular cysteine cathepsins: from signalling to matrix degradation

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Endolysosomal system contains over 50 hydrolases, including a number of proteases, which have a major role in numerous processes, in addition to intracellular protein turnover. Among these proteases, the most abundant are cysteine cathepsins. In human genome there are 11 cysteine cathepsins, and although structurally similar, they do not have the same roles. Several of them are also associated with MHC II-mediated antigen presentation, prohormone processing and bone resorption. In a number of inflammation-associated diseases, including cancer, arthritis and atherosclerosis, they have been found to be secreted in the extracellular milieu. Early *in vitro* work suggested that their primary extracellular role is the degradation of the extracellular matrix. Several of cathepsins were suggested to be involved, but the major roles seem to have cathepsins B, K, S and L. Moreover, in arthritis, a number of proteins are citrulinated, which may also affect their hydrolysis. In addition, there is increasing evidence that the cathepsins are, through the cleavage of different extracellular or membrane proteins, including various CAMs, CD44, EGFR and plexins involved in the regulation of many other processes, such as regulation of Ras GTPase activity. Identification of their physiological substrates is therefore of major importance for understanding their signaling pathways linked with disease progression and will be further discussed.

Proteolytic control of skin cancer progression

L 40

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Cutaneous squamous cell carcinoma (cSCC) is the most common metastatic cancer of skin, and its incidence is increasing globally. Chronic ulceration is a well recognized risk factor for cSCC and ulceration is also a typical clinical feature during progression of UV-induced cSCC from intraepithelial early lesion (actinic keratosis) to invasive and metastatic cSCC. Progression of skin cancer involves several cell types orchestrated by growth factors and cytokines, as well as by interactions with extracellular (ECM) molecules. Proteolysis is an essential feature in cancer progression. In addition to degrading ECM barriers for migrating cells, proteinases coordinate cellular functions by regulating the availability and activity of various bioactive molecules in wound tissue. Moreover, proteinases regulate cell motility and proliferation by modifying cell-cell and cell-ECM contacts. In general, proteolysis is an important part of cancer progression, and alterations in proteolytic activity are associated with invasion and metastasis of cancer. The principal proteinases in skin cancer include serine proteinases plasmin and plasminogen activators (PAs), and a variety of metalloproteinases. The roles of matrix metalloproteinases (MMPs) in cancer progression have been extensively examined but data are also emerging about the roles of a disintegrin and metalloproteinases (ADAMs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAM-TS) proteinases in cancer progression. Here, the roles of plasmin, MMPs, ADAMs and ADAM-TS proteinases in in progression of skin cancer will be discussed.

L
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ADAMTS proteinases in health and disease

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Normal turnover rate of ECM components is of great importance for tissue homeostasis. ECM remodeling under normal and pathologic conditions proceeds through the activity of ECM proteases, MMPs, ADAMs and ADAMTSs. They belong to the same superfamily since they share structural and functional domains.

The term ADAMTS (ADAM with thrombospondin motifs) is derived from the similarity of their catalytic site and disintegrin-like domain with the respective of type I membrane proteins (ADAMs – a disintegrin and metalloproteinase), and of snake venom metalloproteinases (adamalysins). In human, 19 members of ADAMTS family have been identified so far and 7 ADMTS-like counterparts. Unlike membrane anchored ADAMs, ADAMTSs are extracellular and they may bind to ECM through their C-terminal region. ADAMTS are characterized by the presence of one or more thrombospondin motif (Thrombospondin Type 1 repeat-TSR). Their general structural organization, from N-terminal, is: (i) signal peptide (secretion), (ii) propeptide (latent form, except ADAMTS9 and ADAMTS13), (iii) catalytic region (where Zn2+ is bound), (iv) disintegrin-like domain, (v) central TSR motif, (vi) Cys-rich region, (vii) spacer and (viii) variable number of C-terminal TSR repeats. All ADAMTSs are produced by the cells in their latent form and they are activated after propeptide excision mediated by proprotein convertases, such as furin. All members, except ADAMTS-10 and -12, contain at least one region susceptible to furin, before the catalytic region.

ECM metalloproteinases and their tissue inhibitors are key molecules in ECM remodeling during many physiological processes, like ovulation, fertilization, morphogenesis, etc. Differential expression of specific ADAMTS members is required in embryogenesis, as well as in tissue homeostasis in adult. Some members, like ADAMTS-2, -3, -10 and -17 are implicated in polymerization of ECM proteins. Other members, like ADAMTS-1, -4, -5, -8, -9, -12, -15 and -20 are responsible for degradation of ECM proteoglycans. In addition, ADAMTS-2, -3 and -14 are implicated in fibrillar procollagen maturation, ADAMTS-13 in von Willebrand factor proteolysis and hemostasis, and ADAMTS-3 in VEGF-C activation during lymphangiogenesis.

It is important to note that ADAMTSs substrate specificity is largely influenced by their ancillary domains, i.e., Cys-rich region and TSR motif(s). Moreover, some ADAMTSs undergo fragmentation, with potentially new functions of the derivative fragments.

According to their functions, their expression regulation, and their fragmentation, ADAMTSs are implicated in many pathologic conditions, well-established in osteoarthritis and thrombotic thrombocytopenic purpura. The last years, accumulated evidence suggests their implication in many types of cancer.

Selected Talks/ Abstracts

ST/P 1

Novel molecular pathways potentially involved in renal fibrosis: role of HIPK2 and Heparanase.

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Kidney fibrosis is an unavoidable outcome of a broad range of kidney disease and despite a great deal of intense study, there are no specific treatments to target fibrosis in the clinic. On this basis, to understand which mechanisms regulate the development of fibrosis is critical to future identification and development of specific therapeutic strategies which may alleviate this damage. Homeodomain-interacting protein kinase 2 (HIPK2) is a conserved serine/threonine nuclear kinase that regulates gene expression by phosphorylating transcription factors and accessory components of the transcription machinery. The dysregulation of HIPK2 can result in p53 dysfunction and augmented proliferation of cell population as it occurs in cancer and fibrosis. Recently, it has been shown that HIPK2 is a master regulator of kidney fibrosis in experimental models of chronic kidney diseases (CKD) [1].

Several experimental data also support the involvement of heparanase (HPSE) in the pathogenesis of kidney fibrosis. HPSE is an endoglycosidase that cleaves heparan sulfate (HS) chains and participates in ECM remodeling and degradation as well as in the regulation of the release from ECM storages of HS-bonded molecules. Recently we provided evidence that HPSE is specifically involved in the establishment of tubular fibrosis, being necessary for the epithelial-mesenchymal transition (EMT) of tubular cells induced by FGF-2 and TGF-β [2,3]. Starting from here and also from data introducing HIPK2 as a potential key regulator of kidney fibrosis, we started to characterize the role of HIPK2 as fibrosis regulatory molecule and the possible link between HIPK2 and heparanase in pro-fibrotic condition. In particular we aim to analyze the effect of HIPK2 depletion in the expression of EMT and fibrosis markers in human fibroblast (hFB) and kidney tubular epithelial (HK2) cell lines. Preliminary RT-PCR analysis performed in hFB HIPK2-depleted cells showed the induction of HPSE and EMT markers such as vimentin, N-cadherin and αSMA, while the e-cadherin was down-regulated.

The results of this research project, that could identify the interplay between HIPK2 and HPSE as a novel therapeutic target against the development of fibrosis, will be discussed.

[1]. Jin Y, Ratnam K et al. Nat Med 18(4):580-8, 2012.
[2]. Masola V, Gambaro G et al. J Biol Chem 287(2):1478-88, 2012.
[3]. Masola V, Zaza G et al. Biochim Biophys Acta 1843(9):2122-8, 2014

Syndecan-1 in liver fibrosis and regeneration

ST/P 2

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Aims: Syndecan-1 is a transmembrane proteoglycan, participating in various pathological conditions including inflammation, carcinogenesis and regeneration. Physiologically syndecan-1 appears on the basolateral surface of hepatocytes. As syndecan-1 expression increases during fibrosis, we aimed to understand its role by inducing liver fibrosis and regeneration in wild type (WT) and trangenic mice, overexpressing human syndecan-1 (SynTG) in their liver.

Materials and Methods: We examined the overexpression and localization of human syndecan-1 by immunohistochemistry. The amount of proteoglycans and mouse syndecan-1 were obsereved by Western blot and gradient gel electrophosresis. Plasma level of shedded syndecan-1 was measured by ELISA. Liver fibrosis was induced by thioacetamid (TA). The percentage of accumulated connective tissue was determined by morphometrical analysis. Activated myofibroblasts were detected using αSMA Western blot. Connective tissue degradation was measured by gelatinase and caseinase assays. We detected TIMP-1 gene expression using qRT-PCR, and by Western blot TIMP-1 amount was measured. Signaling pathways were examined also by Western-blot. Binding of syndecan-1 to TGF-β, the main mediator of fibrosis was tested by dot-blot. Expression of TIEG, the early response gene of TGF-β, was analyzed by qRT-PCR.

Results: Production of the recombinant protein was stable in SynTG animals. Without experimental challenge, overexpression of syndecan-1 did not cause any pathological condition. When exposed to TA, development of liver fibrosis was delayed in SynTG mice compared to WT. However, after TA withdrawal, transgenic animals exhibited slower regeneration than that of wild type. The amount of mouse syndecan-1 elevated due to TA administration. Higher total proteoglycan level was detected in fibrotic livers of WT animals compared to control samples. Contrary, in SynTG mice decrease of proteoglycans was observed upon TA exposure. In the latter, TA provokes shedding of human syndecan-1 measured in mouse sera by ELISA method.

Gelatinase assay proved attenuated activation of MMP-2 and 9 in SynTG animals interfering with connective tissue degradation. Various signaling pathways were found activated in fibrosis, most of them with lower activity in transgenic animals. By dot-blot analysis we proved that the shedded syndecan-1 directly binds TGF-β1.

Conclusion: Syndecan-1 overexpression modifies the accumulation and degradation of connective tissue in liver fibrosis and regeneration. Shedded syndecan-1 directly binds to TGF-β inhibiting the downstream signaling pathways, which was indicated by the decreased TIEG expression.

ST/P 3

Tumorogenic role of serglycin in breast cancer cells

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Serglycin is the only characterized intracellular proteoglycan mainly expressed in hematopoietic cells. It is stored in granules and vesicles interacting with several macromolecules including growth factors and cytokines. Serglycin regulates the storage of inflammatory mediators in the granules until their secretion, affects their bioavailability as well as signal transduction. It has been shown that serglycin is also overexpressed in many types of cancer, including breast cancer, promoting cell proliferation and migration. In the present study, we have established stably transfected MCF-7 breast cancer cells overexpressing serglycin (MCF-7VSG) as well as MDA-MB-231 breast cancer cells with suppressed expression levels of serglycin (MDA-MB-231SGsh). The aim of this study was to investigate whether serglycin affects crucial cell functions and the expression of ECM molecules and which signaling pathways are involved in such regulations. Our data demonstrated that the overexpression of serglycin enhanced cell proliferation, migration and invasion of MCF-7VSG cells. Furthermore, MCF-7VSG cells exhibited elevated expression of MMPs and interleukins, while the suppression of serglycin caused the opposite effects in MDA-MB-231SGsh cells. Notably, serglycin overexpression enhanced breast cancer cell aggressiveness through activation of IL-8/CXCR2, PI3K and Src signaling as shown by the use of specific inhibitors. Finally, the overexpression of serglycin enhanced chemoresistance of MCF-7VSG cells against commercially available anticancer drugs. Conclusively, this study reveals a regulatory role of serglycin in breast cancer, acting as a tumorogenic factor in several cellular aspects.

Acknowledgments:

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Syndecans as key partners in the interaction between breast cancer cells and endothelium

ST/P 4

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Cancer cells recruit normal stromal cells, such as endothelial, to achieve tumor progression, making tumor microenvironment particularly important. Key process is metastasis that leads to migration of tumor cells through the endothelium into the bloodstream and in the final invasion of the tissue where metastasis will be established. The transmembrane syndecans (SDCs) - proteoglycans containing heparan sulfate chains (HS) - seem to play a primary role in these interactions (Barbouri et al., 2014). Syndecans are actively involved in cell-cell interactions, cell-matrix interactions, and signaling by growth factors (Kousidou et al., 2008). In order to evaluate the paracrine interactions between tumor and endothelial cells, the effect of endothelial cells-derived conditioned media (CM) in cancer cells was evaluated. Specifically, two breast cancer cell lines with different metastatic potential and ER status were used, a highly metastatic MDA-MB-231 ERβ positive and a low-metastatic MCF-7 ERα positive, as well as normal human endothelial cells from umbilical vein (HUVEC). Furthermore in order to examine if the different ER status of the breast cancer cell lines is a mediator of this interplay focusing on ERα stably transfected MCF-7 cells by knocking down the ERα gene (MCF-7/SP10 + cells) were used (Bouris et al., 2015). To evaluate the role of the syndecans HS chains and their contribution to the action of the CM, cancer cell monolayers were pre-incubated with heparinase III enzyme, which degrades specifically HS chains. The gene expression of SDC-1, SDC-2, SDC-4 and heparinase assessed by Real Time PCR revealed changes in the expression levels of all molecules under the different incubations, while altered expression levels were detected in other ECM molecules (Matrix metalloproteinases: MT1-MMP,MMP-2,MMP-9). The highly invasive MDA-MB-231 cells migration is upregulated upon CM incubation, effect partly mediated by HS chains of SDCs. Especially, MDA-MB-231 cells have distinct morphology with star like filamentous extensions of tubulin. Moreover, changes in the localization and expression pattern of SDCs were identified by immunofluorescence. SDC-1 & SDC-4 were spotted intracellular inside large size vesicles upon CM incubation and also the ERα status seems to have an impact in their localization judging from the contrasting motive that SDCs have in MCF7 and MCF-7/SP10 + cells. The role of HS chains of SDCs, and the effect of CM in the activity of metalloproteinases, key enzymes in the metastatic potential, was also evaluated through zymography. The modified gene expression and localization profiles of SDCs in combination with the changes in the functional properties of the cells indicate the importance of SDCs and their HS chains in the interplay of cancer and endothelial cells. It is suggested that the tumor microenvironment is providing at some level the cancer cells with necessary "fuel" in order to proceed with their migration and finally metastasize considering always the bidirectional character of this process.

ST/P 5

AIM2 promotes progression of cutaneous squamous cell carcinoma

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Cutaneous squamous cell carcinoma (cSCC) is the most common metastatic skin cancer. Chronic inflammation is associated with progression of cSCC and patients with chronic inflammatory diseases have higher risk of developing cSCC. The human IFN-inducible genes comprise absent in melanoma 2 (AIM2), IFN-inducible protein X (IFIX), IFI16, and myeloid nuclear differentiation antigen (MNDA). We have studied the role of AIM2 in the progression of cSCC. Analysis of the expression of human IFN-inducible genes in cSCC cells (n=8) and normal epidermal keratinocytes (n=5) revealed overexpression of AIM2 mRNA in primary (n=5) and metastatic (n=3) cSCC cell lines and cSCC tumors (n=6) compared with controls. Strong and moderate tumor cell specific cytoplasmic labeling of AIM2 was noted in human sporadic cSCCs (n=81), whereas the expression of AIM2 was mainly moderate in cSCC in situ (cSCCIS) (n=60) and low or absent in actinic keratosis (AK) (n=71) and normal skin (n=15). Analysis of the staining intensity revealed significantly stronger AIM2 staining in cSCC compared with cSCCIS, AK and normal skin ($P<0.05$). AIM2 knockdown inhibited proliferation, and invasion of cSCC cells and production of invasion proteinases MMP13 and MMP1. Analysis of the gene expression profile following AIM2 knockdown revealed downregulation of cell cycle related genes CDK1, cyclin A and B. Knockdown of AIM2 potently delayed growth and inhibited vascularization of cSCC xenografts *in vivo*. Together, these findings provide evidence for the role of AIM2, an important inflammasome component, in the progression of cSCC and identify AIM2 as an attractive therapeutic target in these invasive tumors especially metastatic and unresectable cSCC.

A new mechanistic link between decorin-evoked Peg3 and TFEB for autophagic induction and angiostasis

ST/P 6

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Autophagy is an evolutionarily conserved and fundamental catabolic process that eradicates damaged and aging macromolecules and organelles in eukaryotic cells for proper cellular homeostasis. Decorin, a prototypical member of the small leucine-rich proteoglycan family, activates a protracted autophagic program within endothelial cells that strictly requires Peg3, and is downstream of VEGFR2. We have discovered a novel mechanistic link between Peg3 and TFEB, a master transcription factor of lysosomal biogenesis, for decorin-evoked endothelial cell autophagy. We found a physical association of Peg3 protein with TFEB mRNA and a functional dependency of Peg3 for TFEB induction and nuclear translocation, both *in vitro* and *in vivo* systems. Moreover, inhibiting AMPK, a major energy sensor kinase activated by soluble decorin downstream of VEGFR2, prevented decorin-evoked TFEB induction. Further, we investigated whether decorin suppresses angiogenesis by downregulating pro-angiogenic factors in endothelial cells as a biological consequence of endothelial cell autophagy. As such, decorin required the newly-defined AMPK/Peg3/TFEB axis for competent VEGFA suppression in endothelial cells. Silencing of either autophagic regulator abrogated decorin-mediated angiostasis. Serendipitously, we discovered a novel mechanism for decorin mediated angiokine clearance by rapid incorporation into autophagosomes via a strict Peg3 and TFEB dependent manner. VEGFA and VEGFB strongly colocalized with canonical autophagic markers including Peg3, Beclin-1, and LC3 under a variety of autophagic stimuli. Thus, we have discovered a noncanonical mechanism underlying the potent angiostatic properties of decorin, via Peg3 and TFEB mediated autophagy.

ST/P 7

Decorin expression in human hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) represents one of the most rapidly spreading cancers in the world. In the majority of cases, an inflammation-driven fibrosis or cirrhosis precedes the development of the tumor. During malignant transformation, the tumor microenvironment undergoes qualitative and quantitative changes that modulate the behavior of the malignant cells. A key constituent of the hepatic microenvironment is the small leucine-rich proteoglycan decorin (DCN), known to interfere with cellular events of tumorigenesis mainly by blocking various receptor tyrosine kinases (RTK) and inducing p21WAF1/CIP1. In this study, we aimed to examine the expression of decorin in HCC using in silico approaches as well as FFPE tissue samples of HCC with or without cirrhosis. To avoid distortion of results by the different number of decorin producing myofibroblasts (MFs), decorin expressions both at mRNA and protein level were normalized to smooth muscle actin (SMA) content. Generally, HCC tumor samples have decreased or blocked decorin expression compared to non-tumorous adjacent tissues (NAT), which was not due to changes in MF number. In our sample collection 52% of HCCs were decorin negative, 33% exhibited low, and 15% high decorin levels. Negativity and low expression was more emphasized in HCCs without cirrhosis. In addition, SMA corrected DCN amount negatively correlates with EGFR level, known to be downregulated by decorin. The fact that MFs reduce their decorin production in the tumor stroma compared to non-tumorous area of the same liver raises the possibility of epigenetic alterations such as promoter methylation. In silico mRNA expression analysis revealed that DCN/SMA content distinguishes between normal and tumorous samples, and even characteristic for very early stage HCC. The most prominent blockade of decorin was seen in tumors with b-catenin mutations, and marked decrease was seen in TP53 mutated tumors as well. Normalized DCN expression did not correlate with pRPS6, pAkt immunopositivity, with age or with gender. However, a certain DCN/SMA ratio (0.7) seemed to divide tumor samples into two well defined clusters regardless to the feature examined. Tumors with ‹0.7 DCN/SMA ratio were characterized by b-catenin mutations and low level of inflammation, whereas those with ›0.7 DCN/SMA b-catenin mutations were less frequent and inflammation was a typical feature. By comparing ‹0.7 tumors with ›0.7 ones, EFEMP1, ASPN and THBS2 were found to show the strongest correlation with DCN expression. Our results indicate that decorin may act as a tumor suppressor in liver cancer as its expression is reduced or completely blocked in HCC.

The role of Fibroblast Activation Protein (FAP) in lung tumorigenesis

ST/P 8

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In virtually all epithelial-derived tumors, carcinoma-associated stromal cells (CASCs) selectively express FAP, a cell surface serine protease that promotes tumorigenesis. We investigated the mechanisms that govern FAP expression as well as the mechanisms by which FAP regulates the intratumoral desmoplastic reaction and, consequently, lung tumor progression.

We found that the inducible expression of FAP in lung fibroblasts depended on ECM components and revealed differential requirements for induction of FAP and αSMA (a canonical marker of myofibroblasts). Unlike TGF-β-mediated induction of αSMA expression, TGF-β-mediated induction of FAP expression depended on a collagen-rich substratum. These results underscore the role of ECM in fibroblast differentiation.

We further delineated bidirectional regulatory mechanisms between activated fibroblasts and ECM by studying FAP-dependent remodeling of ECM. Using two-photon second harmonic generation (2P-SHG) microscopy, we found that activated FAP-/- lung fibroblasts deposited ECMs with an increase in fibrillar collagen accumulation compared to WT fibroblast-derived ECMs. In addition, we found that FAP negatively regulates fibroblast-mediated contraction of type I collagen. Collectively, these experiments highlight the importance of FAP in orchestrating fibroblast-dependent remodeling of intratumoral ECM composition and architecture.

Lastly, we found that FAP promotes tumor growth in inducible, spontaneous, and xenograft models of lung adenocarcinoma. By analyzing tumor burden in the spontaneous KrasG12D latent allele model, we found that FAP-/- mice had smaller lung tumors than WT mice. However, the multiplicity of lung tumors did not differ between WT and FAP-/- mice. These results indicate that FAP promotes progression, rather than initiation, of lung tumorigenesis. Current analyses are studying differences in intratumoral ECM in this model and how these changes to lung CASC-derived ECM influence tumor cell proliferation. In summary, we have delineated bidirectional regulatory mechanisms between FAP and ECM as well as FAP-dependent regulation of the desmoplastic reaction and lung tumor progression. These studies further clarify the mechanisms by which the microenvironment promotes tumorigenesis and validate FAP as a potential therapeutic target in the treatment of cancer.

ST/P 9

Deubiquitinating enzymes USP4 and USP17 target hyaluronan synthase 2 and affect its function

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Hyaluronan is a ubiquitous glycosaminoglycan which is important for tissue homeostasis and is synthesized by hyaluronan synthases (HAS1, 2, 3) and degraded by hyaluronidases (Hyal 1, 2, 3 and PH20). Hyaluronan accumulates in rapidly remodeling tissues, such as breast cancer, due to deregulated expression of the HAS2 gene and/or alterations of HAS2 activity. The activity of HAS2 is regulated by post-translational modifications, including mono- and polyubiquitination; lysine residue 190 (Lys190) of HAS2 is monoubiquitinated and is needed for activity, whereas polyubiquitination destabilizes HAS2. Ubiquitination is reversed by deubiquitinating enzymes (DUBs), proteases which keep the balance in ubiquitin-signaling networks and regulate key cellular processes such as cell cycle progression, proliferation and apoptosis. Thus they have emerged as promising therapeutic targets for many diseases.

In order to identify deubiquitinating enzymes (DUBs) involved in deubiquitination of HAS2, a cDNA library of 69 Flag-tagged human DUBs cloned into retroviral vectors was screened in HEK293T cells for the ability to deubiquitinate myc-tagged HAS2. Several DUBs were found to decrease the ubiquitination of HAS2, among the most efficient ones were USP4 and USP17. Co-immunoprecipitation studies revealed interactions between USP4 or USP17 and HAS2 in membrane fractions of HEK293T cells. Silencing of USP4 increased the monoubiquitination of HAS2 and the hyaluronan production in aggressive breast cancer cells, suggesting a role for USP4 in removing Lys190-linked monoubiquitin moieties from HAS2. USP17 was found to remove both mono-and polyubiquitin chains, and to stabilize HAS2 protein levels; namely, overexpression of Flag-USP17 significantly increased the half-life of 6myc-HAS2 from ~ 5h to ~ 30h after cycloheximide treatment. A catalytically inactive mutant of USP17, in which cysteine in the catalytic core was mutated to serine, was not able to deubiquitinate HAS2, indicating that the effect was direct and mediated through the catalytic activity of USP17. Furthermore, knock-down of USP17 in aggressive breast cancer cells abolished their hyaluronan production and decreased endogenous HAS2 protein levels. Interestingly, in breast cancer cells in which HAS2 was stably knocked-down, the endogenous expression of USP17 was decreased.

In conclusion, we have identified two DUBs, USP4 and USP17, which appear to have opposite effects on HAS2 function and activity. Further studies will aim at revealing the importance of the cooperation between HAS2 and these DUBs in the proliferation and invasion of breast cancer cells.

The long non-coding RNA HAS2-AS1 is a new regulator of hyaluronan synthesis and its involved in human vascular diseases

ST/P 10

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The role of hyaluronan (HA) is critical in the pathogenesis of vascular pathologies including atherosclerosis, restenosis and diabetic angiopathies. HA accumulation into arteries wall supports vessel thickening and modulate inflammatory responses, therefore, our laboratory is studying how cells regulate HA synthesis.

HA synthase 2 (HAS2), the main responsible enzyme for HA synthesis, is strictly regulated at transcriptional and post-translational levels [1]. Recent work from our laboratory suggests that the long non-coding RNA HAS2-AS1, which belongs to the natural antisense transcript subfamily, is able to modulate HAS2 transcription modulating the accessibility of chromatin around HAS2 proximal promoter in human aortic smooth muscle cells (AoSMCs) [2].

The expression of such long non-coding RNA is verified in human atherectomy specimen at different stages of severity and in mice fed with a high fat diet that develop atherosclerosis after 4 weeks of treatment.

The molecular mechanisms through which long non-coding RNAs modulate gene expression are complex and involve several epigenetic modifications. Acetylation is one of the main epigenetic modification that alters histone tails permitting the aperture of nucleosomes chain and favouring transcription. Although several proteins possess a histone acetyltransferase activity, we studied the role of the transcriptional coactivator p300 in the regulation of HA metabolism.

In AoSMCs, the transfection of a vector coding for p300 induces a significant accumulation of HA in the conditioned culture medium. On the other hand, the overexpression of histone deacetylase 1 (HADC1) reduced HA secretion. After p300 transfection, the expression of HAS2 and HAS2-AS1 increased whereas HDAC1 reduced HAS2 and HAS2-AS1 RNA level. As HAS2-AS1 shares a complementarity region on HAS2 exon 1, we found that exon 1 is necessary to improve the activation of luciferase reporter assay after HAS2-AS1 overexpression.

These results lead to the hypothesis the HAS2-AS1 could drive p300 in the proximity of HAS2 promoter permitting chromatin remodelling and favouring transcription.

1.Vigetti D, et al. (2014) Metabolic control of hyaluronan synthases. *Matrix Biol.* 35:8-13.
2.Vigetti D, et al. (2014) Natural antisense transcript for hyaluronan synthase 2 (HAS2-AS1) induces transcription of HAS2 via protein O-GlcNAcylation. *J Biol Chem.* 289:28816-26.
3.Rinn, J. L., and Chang, H. Y. (2012) Genome regulation by long noncoding RNAs. *Annu Rev Biochem* 81:145-166

ST/P 11

The UDP-sugar substrates of hyaluronan synthase 3 (HAS3) regulate its intracellular traffic and extracellular shedding, controlling hyaluronan synthesis and correlating with phenotypic changes in early stages of melanoma

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Hyaluronan is synthesized by hyaluronan synthases (HAS1-3) at plasma membrane (PM). Using photo-convertible Dendra2-HAS3, we show that unless engaged in hyaluronan synthesis, the HAS3 half-life in the PM of cultured MV3 melanoma cells is in the range of minutes. When cellular UDP-N-Acetylglucosamine (UDP-GlcNAc) content was increased by treatment with 1-2 mM glucosamine, uptake of HAS3 from PM to early endosomes was inhibited, and hyaluronan synthesis increased. In contrast, shortage of the HAS substrates (UDP-GlcNAc) or UDP-glucuronic acid (UDP-GlcUA) limited the time HAS3 remained in PM, and inhibited hyaluronan synthesis. HAS3 depletion from PM was also enhanced by hyaluronidase digestion. HAS3 was post-translationally modified by O-GlcNAc, the level of which correlated with the cellular concentration of UDP-GlcNAc. Increased O-GlcNAcylation, even without changes in UDP-GlcNAc content, also blocked HAS3 endocytosis and increased hyaluronan synthesis. Thus, as substrates, both UDP-GlcNAc and UDP-GlcUA control HAS3 endocytosis, while UDP-GlcNAc also supports PM retention and hyaluronan synthesis through HAS3 O-GlcNAcylation. UDP-GlcNAc surplus and O-GlcNAc modification also blocked the lysosomal decay of HAS3. The amount of Dendra2-HAS3 in intracellular storage vesicles corresponding to early endosomes increased and decreased with low and high levels of UDP-sugars, respectively, suggesting a role in regulation of HAS3 recycling in cell surface. Indeed cell surface biotinylation and TIRF imaging of EGFP-HAS3 vesicles showed that UDP-sugars control HAS3 recycling in PM. Mediated by the changes in hyaluronan synthesis and O-GlcNAc signaling, the contents of UDP-sugar levels exerted major regulatory functions, including secretion of extracellular vesicles, adhesion and proliferation of the cells. In human melanoma tissue samples, GFAT1 protein levels – representing cellular UDP-GlcNAc levels showed respectively increased and decreased staining in early and deep melanoma (› 4mm), which is correlative to tissue staining for hyaluronan content. Our data demonstrates the vital importance of the glucose metabolites, UDP-GlcUA and UDP-GlcNAc in hyaluronan synthesis and early stages of melanomagenesis.

Detection of hitherto unknown chondroitin core proteins together with unexpected sulfation of chondroitin in the Nematode C. elegans

ST/P 12

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The nematode Caenorhabditis elegans is a well-known model organism that during recent years also has been used for the study of glycosaminoglycans (GAGs). Worms express heparan sulfate (HS) that is similar to mammalian HS with regard to both pattern and level of sulfation and modification. However, only non-sulfated chondroitin has so far been detected in GAG preparations from C. elegans. Both HS and chondroitin are essential for development of these animals and removal of enzymes from the biosynthetic pathway for either of these GAGs affects multiple processes, ranging from cell division during embryonic development to axon guidance and vulval formation.
In contrast to earlier reports, we were able to detect 4-O-sulfated and 6-O-sulfated chondroitin disaccharides (D0a4, D0a6) using RPIP-HPLC and LC/ESI-MS2 analysis of C. elegans GAGs. The presence of sulfated chondroitin at embryonal, larval and adult stages of the worm was further confirmed by immunofluorescent staining using the CS-56 antibody. We assume that the large amount of non-sulfated chondroitin masked these structures in earlier studies and we could only detect them due to our modified purification protocol. Nevertheless, total amounts of CS and HS were in a similar range, indicating a biological significance for the sulfated chondroitin in C. elegans. By using a glycoproteomics approach we were also able to identify two novel chondroitin sulfate (CS) core proteins, in addition to the CS core proteins reported earlier.

Furthermore, preliminary data indicate that the amount of CS and HS varies during C. elegans development, with HS levels increasing during early developmental stages and CS synthesis increasing in the adult stages. We are currently confirming these results and have also started to correlate GAG levels and structure to the expression of the corresponding GAG-synthesizing enzymes, which will provide useful insights into the differing structures and functions of GAGs during animal development.

ST/P 13

Zebrafish modeling of β3GalT6 and β4GalT7-deficient types of Ehlers-Danlos syndrome stresses the importance of glycosaminoglycans in development

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Proteoglycans are important components of cell plasma membranes and extracellular matrices. They are composed of glycosaminoglycan (GAG) chains attached to a core protein through a tetrasaccharide linker region. The addition of the second and third residue in this linker is catalysed by galactosyltransferase I (β4GalT7) and II (β3GalT6), encoded by B4GALT7 and B3GALT6. Biallelic mutations in *B4GALT7* cause the progeroid type of the Ehlers-Danlos syndrome (EDS). The main features of this disorder are short stature with bone deformities, and an aged facial appearance, in addition to the classical signs of EDS. We previously identified biallelic mutations in B3GALT6 in several families with a severe pleiotropic EDS-like disorder characterized by soft connective tissue fragility, early-onset bone fractures, spondyloepimethaphyseal dysplasia (SEMD) and intellectual disability. B3GALT6 mutations were simultaneously identified in patients with disproportionate short stature and SEMD but lacking the other features.

To characterize the function of β4GalT7 and β3GalT6 *in vivo* we developed a zebrafish model. A morpholino-based approach was used to characterize the developmental effects of b4galt7 and b3galt6 knockdown in zebrafish embryos. Complete knockdown of these genes is lethal, but partial knockdown results in an abnormal pharyngeal cartilage phenotype and most notably a reduced head and eye size and a compression of the jaw region in the anterior-posterior axis. Structural analysis of injected *Tg(flia1a:EGFP)* larvae shows the inability of chondrocytes to intercalate and form elongated cartilage elements. These morphological changes are accompanied by a significant reduction in HS and CS and in the total amount of sulfated GAG chains in general. Knockdown also results in abnormal muscle function with a delayed or abnormal touch-evoked escape response, and results in severe abnormalities in muscle structure in the tails and heads of zebrafish embryos. In conclusion, our results emphasize a crucial role for these enzymes in multiple major developmental and pathophysiological processes, both *in vitro* and *in vivo*.

The purification and characterisation of heparin by-products for applications in wound healing

ST/P 14

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Heparin is a widely used anticoagulant drug harvested from animal tissue, predominantly pig intestines. During the production of pharmaceutical grade heparin there are by-products removed at each stage of the purification process that contain useful GAGs such as heparan sulphate. As such they represent a waste material that can provide an economical and novel resource for bioactive heparan sulphate saccharides for research and biotechnology applications. Our investigation has led to the analysis of 10 heparinoid samples from a commercial manufacturer, which have all been harvested from different stages of heparin production/purification. We hypothesised that these by-products would contain HS with different sulphation levels useful for the activation of growth factors essential for wound healing. Analytical processes such as MonoQ and compositional analysis highlighted fractions of interest for further preparative analysis. Size exclusion chromatography and strong anion exchange chromatography along with cell assays have been used to produce a library of bioactive HS saccharides for further refinement of aspects of wound healing, such as angiogenesis, fibroblast and endothelial cell migration and proliferation.

ST/P 15

Heparin inhibits the differentiation of M1 and promotes M2 macrophages under hyperglycemic stress

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After the onset of diabetes, elevated blood glucose induces kidney glomerular mesangial cells to divide and initiates abnormal hyaluronan (HA) synthesis in intracellular compartments. This initiates a large stress response (autophagy) that makes unique HA structures outside the cell that recruit inflammatory cells (a monocyte-adhesive HA matrix) [1,2]. A chronic inflammation is then developed within the kidney glomeruli, which initiates tissue damage, fibrosis and loss of filtration function. Dialogue between macrophages recruited into the glomeruli and the damaged rat mesangial cells (RMCs) leads to DN, fibrosis and proteinurea, and at the 6 week stage the glomeruli contain extensive HA matrix, macrophages and autophagic RMCs. Our studies [2] with STZ-diabetic rats provide evidence: 1) that heparin regulates the metabolic responses in dividing hyperglycemic kidney mesangial cells and the phenotypic activation of the monocyte/macrophages; 2) that the M1 macrophages in the uncontrolled diabetic glomeruli initiate inflammatory and fibrotic responses that are ineffective in removing the HA matrix and the autophagic RMCs cannot maintain their function; and 3) that the M2 macrophages in the glomeruli of diabetic rats treated with heparin effectively remove the HA matrix without initiating the inflammatory and fibrotic responses, which allows the RMCs to maintain glomerular function while still synthesizing a monocyte-adhesive HA matrix in response to the hyperglycemia. To better understand this behavior, we investigated the effect of heparin on monocyte to macrophage differentiation under hyperglycemic stress. Our results show that heparin inhibits the gene expression of an M1 marker, inducible nitric oxide synthase (iNOS), and induces an M2 marker, arginase, in murine monocytes grown in hyperglycemic medium. Furthermore, heparin inhibits CD80, an M1 surface marker, and induces CD163, an M2 surface marker, in U937 cells (a human monocytic cell line) when they are stimulated to a macrophage phenotype. These results indicate that heparin inhibits hyperglycemic monocyte to M1 macrophage differentiation that promotes a pro-inflammatory phenotype while promoting M2 macrophage differentiation that is an anti-inflammatory phenotype. This appears to be critical for the resolution of inflammation that is caused in this case by hyperglycemic stress and provides a possible explanation for the sustained glomerular function in diabetic rats treated with heparin.

References:

1. Wang et al. FEBS J, 2011. 278: 1412-18.
2. Wang et al. J. Biol. Chem. 289:9418-29, 2014

Interactions of discoidin domain receptors with fibrillar and non-fibrillar collagen forms: ligand binding versus receptor activation

ST/P 16

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The Discoidin Domain Receptors (DDRs) are proteins of the Receptor Tyrosine Kinase (RTK) family. The two DDR members, DDR1 and DDR2 are unique RTKs because they are activated by a component of the extracellular matrix, collagen, whereas typical RTKs are activated by small soluble peptide-like growth factors. The DDRs regulate many cellular processes such as cell migration and differentiation and they have important functions during development. For example, DDR1 is essential for mammary gland development whereas DDR2 is crucial for long bone growth. Additionally, dysregulation of both DDRs promotes a number of human diseases. While it is known that different types of collagen function as DDR ligands, the details of DDR interactions with tissue forms of collagens are not clear. Previous literature has shown that monomeric collagen (i.e. collagen as single triple helices) can bind to and activate the DDRs. However, in tissues, many collagens are found as fibrils and fibres, and it is not clear whether collagen in fibrillar form can promote DDR activation. In this project, we are testing DDR binding and activation by *in vitro* reconstituted collagen fibrils made up of collagen type I or of cartilage collagens II/IX/XI. Our data show that both DDR1 and DDR2 bind to fibrils of collagens II/IX/XI and that both DDRs can interact with the complex of collagen II/IX/II but this complex can induce only DDR1 activation, not DDR2 activation. In addition to using collagen fibrils produced *in vitro*, we also aim to test whether the DDRs are activated by native collagen fibrils produced by fibroblasts cultures. To this end we are currently adapting protocols for generating cell-derived matrices.

A widely expressed collagen that has not been tested for its interactions with the DDRs is collagen VI. Collagen VI is initially secreted to the extracellular matrix as tetramers, where it forms its final structure, microfibrils. We tested DDR binding and activation using collagen VI from two different sources, skin and cornea. Our data show that both tetramers and microfibrils of collagen VI bind to both DDRs, but can promote only DDR1 activation. We are currently testing whether binding and activation of DDR1 is promoted by the triple-helical region of collagen VI. In conclusion, it seems that even though both DDRs can bind to the same collagens, they have different collagen requirements for receptor activation.

ST/P 17

MFAP4 is a novel regulator of elastic fiber assembly and integrin-dependent cellular signaling

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Microfibrillar-associated protein 4 (MFAP4) is an extracellular matrix (ECM) protein that co-localizes with elastic fibers in tissues such as the lung. MFAP4 contains an RGD sequence and has been shown to interact with cell surface integrins. Moreover, we have recently generated MFAP4-deficient mice and showed that they exhibit age-dependent emphysema-like phenotype, suggesting its direct involvement in elastic fiber biology.

In the current study, we aimed to characterize molecular interactions between MFAP4 and elastic fiber components, and to investigate the potential role of MFAP4 in integrin-dependent cellular signaling.

We detected strong, calcium-dependent direct binding between MFAP4 and tropoelastin by both solid phase binding assays and surface plasmon resonance. Using site-directed mutagenesis, we produced MFAP4 variants mutated in the putative ligand-binding site and identified residues Ser203 and Phe241 as important for full binding capacity. Furthermore, MFAP4 actively promoted tropoelastin self-assembly.

We also used *in vitro* culture of primary asthmatic bronchial smooth muscle cells (BSMCs) and identified integrin αVβ5 as a main MFAP4 recognition partner on BSMC surface. MFAP4 stimulation induced focal adhesion kinase activation, suggesting initiation of downstream integrin signaling. Indeed, MFAP4 stimulation caused BSMC proliferation and CCL11 up-regulation, which could be inhibited by pharmacological inhibitors of PI3 kinase as well as anti-integrin αVβ5 antibody.

In summary, our results characterize MFAP4 as a tropoelastin-binding protein involved in proper elastic fiber assembly. In addition, we showed that MFAP4 interaction with integrin αVβ5 results in PI3K-BSMC proliferation and CCL11 release, thus identifying a novel pathway possibly involved in airway smooth muscle biology.

Role of collagen VI in intestine homeostasis

ST/P 18

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Collagen VI (ColVI) is an extracellular matrix (ECM) protein made of three different chains, α1(VI), α2(VI) and α 3(VI), encoded by distinct genes. ColVI forms a microfilamentous network that is present in various tissues and particularly abundant in skeletal muscles. In intestine, this ECM molecule is widely expressed along the villus/crypt axis, in the smooth muscular layer, and in close contact with myenteric ganglia and nerve fibers. Histological analysis of the intestine of ColVI null (Col6a1-/-) mice did not reveal any overt morphological defect under physiological conditions . With the aim to investigate the intestinal functionality, we performed *in vivo* motility studies by providing fluoresce in isothiocyanate-dextran to 3-month-old mice. Interestingly, the gastrointestinal transit resulted faster in Col6a1-/- mice when compared to wild-type. Moreover, *in vitro* contractility experiments revealed that Col6a1-/- intestine display a higher spontaneous contractility when compared to wild-type intestine. We next used a well-established model of acute colitis to study mucosal injury and repair processes, by administering 3% dextran sodium sulphate (DSS) dissolved in drinking water for 5 days. After 5 days DSS treatment, Col6a1-/- mice displayed a lower decrease of body weight ratio when compared to wild-type animals. Histological analysis of colon sections from DSS-treated mice showed that wild-type animals had extensive areas of inflammation and ulceration with disappearance of crypts, whereas Col6a1-/- mice had few and more circumscribed inflammation areas. RT-qPCR analysis of pro-inflammatory cytokines (IL-1beta, TNF-alpha, IL-6) confirmed reduced inflammation in DSS-treated Col6a1-/- mice. In addition, RT-qPCR for MCP-1 and F4/80 revealed a decreased recruitment and activation of macrophages, the main cell type involved in DSS-induced colitis, in Col6a1-/- mice. With the aim to investigate the repair process after DSS injury, we treated mice for 4 days with 3% DSS followed by 10 days of recovery. Wild-type mice died after 3 days from the beginning of the recovery. At the end of the experiment, Col6a1-/- mice were unable to recover the body weight loss displayed during the acute phase of colitis and histological analysis revealed that inflammation areas were still present, as confirmed by RT-qPCR. Altogether, these data suggest that the absence of ColVI in Col6a1-/- mice delays the inflammatory process of DSS-induced colitis.

ST/P 19

Mesenchymal stem cells adhesive behavior to different extracellular matrix proteins alters during osteogenic differentiation

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Most of the skeletal diseases, such as ostegenesis imperfecta, osteoporosis and rickets, are characterized with alteration in density, composition and organization of bone matrix. Recently many efforts have been applied to develop strategies for recovering damaged or diseased bone tissue. Among them tissue engineering, combining cells, biomaterial scaffolds and growth factors, is very promising. Mesenchymal stem cells (MSCs) play a key role in bone regenerative strategies because of their self-renewal capacity and capability to differentiate into multiple cell types, including osteoblasts-the cells that produce and mineralize the bone matrix. During osteogenic differentiation MSCs alter their adhesive phenotype in response to the alteration in structure and composition of bone matrix. Bone extracellular matrix is composed of collagen and non-collagen proteins which are believed to play a key role in different steps of differentiation and activity of bone cells and in the maturation and mineralization of bone matrix. Cell adhesion is the first event that happens under contact of cells with the substratum and precedes further proliferation, functions and differentiation. Therefore the aim of the present study was to investigate how the adhesive behavior of rat MSCs to different matrix proteins (fibronectin, vitronectin, laminin, collagen I and collagen IV) changes for 1, 7, 14 and 21 days after plating. Cell adhesion was studied in respect to the number of attached cells, cell spreading area and overall cell morphology. In addition, we have studied expression of beta1 integrin and fibronectin matrix synthesis and organization. The results showed that at the day 1 after seeding adhesion affinity of MSCs was higher to laminin and collagen IV-the basal lamina proteins, but with osteogenic differentiation their adhesive potential to the other proteins increased. In the osteogenic progress fibronectin matrix deposition decreased. No significant differences was detected in beta 1 integrin expression at the certain time points which suggested that cells utilized other receptor molecules to exchange information with the extracellular matrix. The results indicated dynamically changing adhesive properties of MSCs during osteogenic commitment and are valuable for better understanding the mechanisms of stem cell differentiation which are of interest for the needs of regenerative medicine.

The versatility of fetal and adult ECM-Derived microenvironments: The impact of ontogeny on cardiac cells

ST/P 20

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The adult mammalian heart following injury undergoes intense remodelling characterized by substantial loss of cardiomyocytes and formation of a non-functional fibrotic scar. Despite, observation of cardiomyocytes cell cycle reactivation, the number of new cardiomyocytes is insufficient to promote evident neomyogenesis and functional improvement. In contrast, neonatal heart appears to be capable of, at least partial, histo-functional recovery in response to damage. Owing to that the extracellular matrix (ECM) is a dynamic entity, fundamental for proper heart morphogenesis and growth, we hypothesize that tissue specific ECM provides ontogenic-stage dependent cues that govern cell phenotype and thus impact on tissue remodelling and functional restoration in an injury setting. Recent reports highlighted the potential of ECM as potent modulator of cellular response in 2-D systems, however, a full understanding is still out to reach. Our work unveils how fetal (E18) and adult cardiac tissue as 3-D systems modulate cardiac cells behaviour. We developed a protocol for parallel decellularization of whole-fetal hearts and adult heart explants that enables reliable comparison between fetal and adult-derived ECM. Fetal and adult native ECM display a complex network arrangement, preserved ECM components and evident biochemical and structural differences. Contrarily to the adult bioscaffolds, fetal-derived ECM was shown to be a more amenable microenvironment for cardiac neonatal cardiomyocyte and adult Sca-1+ progenitors demonstrated by the higher number of cells that migrate to the centre of the scaffold. A first sketch of the mechanism(s) that control this differential potential will be presented.

ST/P 21

Sulfated fucans and sulfated galactans from sea urchins as potent inhibitors of selectin-dependent hematogenous metastasis. Are there any structural requirements?

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Metastasis is responsible for the majority of cancer-associated deaths. However, only a small part of tumor cells efficiently complete all the steps of this process. One limiting point is their survival in the bloodstream, which depends on the interaction with platelets to reach the secondary site of colonization. This interaction is mediated by the platelets' glycoprotein P-selectin, which is capable of interacting with its ligand on the membrane of tumor cells. Inhibition of this interaction can prevent metastasis. Heparin has a potent anti-selectin effect, preventing the formation of platelet-tumor cell emboli and attenuating metastasis. Likewise, other sulfated polysaccharides extracted from marine invertebrates showed ability in inhibiting P-selectin. In this work we investigate anti-selectin activity of sulfated polysaccharides extracted from sea urchins – Fucan from Strongylocentrotus franciscanus (Fucan 1); Fucan II of Strongylocentrotus droebachiensis (Fucan 2); and Galactan from Echinometra lucunter. On *in vitro* assays we observed that Fucan 1 has low inhibitory potency (IC50 = 170 µg/mL), compared to heparin (IC50 = 24.5 µg/mL). On the other hand, Fucan 2 and Galactan have a more significant inhibitory potency (IC50 = 11 µg/mL and 9.6 µg/mL, respectively). On *in vivo* experiments, control animals showed 69% of tumor cells associated with platelets, similarly to Fucan 1-treated animals (61 %). The animals treated with Fucan 2 and Galactan present only 27% and 24% of platelet-tumor cells complexes. Corroborating this result, only control animals or those treated with Fucan 1 form metastasis, while Galactan and Fucan 2 were able to prevent metastatic nodules formation. These results suggest that despite being very similar molecules, these compounds have different anti-selectin effects and demonstrate potential for future treatments. Further structure-activity relationship studies are being made to determine specific determinant features that can explain these results.

Glypican-6 is increased in experimental and clinical heart failure and might play a role in cardiac fibrosis through BMP4 signalling

ST/P 22

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Pressure overload is a leading cause of heart failure, a disease affecting millions of people worldwide. Proteoglycans, despite playing important roles in connective tissues and wound healing, are poorly understood in the heart. We have investigated the six-membered glypican (GPC1-6) family, evolutionary ancient heparan sulphate proteoglycans anchored to the extracellular part of cell membrane, in pressure overload in mice *in vivo*, in cardiac biopsies from patients and in cultured fibroblasts.

Our results show that mice subjected to pressure overload by aortic banding (AB; n=88) had elevated left ventricular (LV) GPC6 mRNA during hypertrophic remodelling (1 and 3 weeks of AB; 2.8- and 1.9-fold) and dilated, end-stage heart failure (16 and 18 weeks of AB; 2.0- and 2.4-fold). Immunoblotting confirmed GPC6 protein upregulation. There were minor alterations in GPC1-4 mRNA, while GPC5 was not expressed. GPC6 mRNA was 1.8-fold higher in LV tissue from explanted hearts of patients with end-stage, dilated heart failure (NYHAIII-IV, n=18) than in controls (n=7). A negative correlation with LV ejection fraction in patients and positive correlations with lung weight and LV weight after AB in mice suggested that increased GPC6 levels are associated with heart failure progression. In primary cell cultures from neonatal rat hearts, GPC6 mRNA was 3.8-fold higher in fibroblasts than cardiomyocytes, indicating fibroblasts as the main source of GPC6 in the heart. Interestingly, after AB in mice, GPC6 mRNA correlated positively with collagen I and III mRNA, suggesting a role in fibrosis. Consistently, GPC6 was upregulated by bone morphogenetic protein 4 (BMP4), a known mechanosensitive, pro-fibrotic growth factor, in the fibroblast cell line NIH3T3. BMP4 mRNA was elevated at 3-18 weeks of AB, similar to GPC6. Finally, HEK293 cells overexpressing GPC6 displayed enhanced ERK1/2 activation upon BMP4 stimulation, indicating that GPC6 modulates pro-fibrotic BMP4-dependent signalling.

In conclusion, our data suggest that GPC6 is involved in experimental and clinical heart failure progression through potentiation of pro-fibrotic BMP4 signalling.

5th FEBS Advanced Lecture Course

ST/P 23

Three-dimensional culture systems for the study of growth factors on intervertebral disc cells

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Low back pain has been reported to be the leading cause of disability worldwide. This condition is strongly associated with intervertebral disc (IVD) degeneration. Therapeutic options against IVD degeneration are currently limited to surgical interventions, such as discectomy or spinal fusion, which however are not always successful and may have detrimental complications. Hence, research efforts are focusing on tissue engineering and the use of autologous or stem cell therapy. For an effective design of such approaches, an in depth understanding of the unique environment of the IVD, as well as, of its physiology is required. Accordingly, we reported previously on the proliferative response of IVD cells *in vitro* to growth factors and on the signaling pathways involved.

In the present study, we examined the proliferative responses of IVD cells to Platelet-Derived Growth Factor (PDGF), basic Fibroblast Growth Factor (bFGF) and Insulin-like Growth Factor-I (IGF-I) in various three-dimensional (3D) culture systems, designed to mimick the natural environment of the disc *in vivo*. Among them, the popular — in the field of chondrocyte biology — system of alginate beads was used for the culture of nucleus pulposus (NP) cells. Moreover, two systems more relevant to the extracellular matrix composition of IVD were employed: collagen type-I gels for the culture of annulus fibrosus (AF) cells, and collagen gels supplemented with chondroitin sulfate for the culture of NP cells.

In all three 3D culture systems IVD cells responded to all three growth factors with higher proliferation rates compared to their responses in the conventional monolayer cultures. Furthermore, two pivotal signaling pathways — MEK/ERK and PI-3K/Akt — were observed to be activated by all three growth factors. The use of pharmacologic inhibitors blocking these two signaling pathways — PD98059 for MEK/ERK and wortmannin for PI-3K/Akt — indicated that both pathways contribute to the proliferative effect of the growth factors, however not to the extent that is observed in monolayer cultures.

In conclusion, IVD cells cultured in a 3D environment show a more intense proliferative response to PDGF, bFGF, and IGF-I compared to the conventional monolayer cultures. This could be associated with the probable engagement of more signaling pathways in the case of the 3D culture systems. These data may contribute to the design of the optimal cell-support scaffolds for the future delivery of cell therapies to patients suffering from IVD degeneration.

Syndecan-4 as a switch of epithelial to mesenchymal transition in breast cancer cells

ST/P 24

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Heparan sulfate proteglycans (HSPGs) play diverse roles in tumor biology by mediating cell adhesion, migration and also by regulating cellular responses to a wide array of growth factors, cytokines and inflammatory mediators. Syndecans constitute a major category of transmembrane HSPGs that are capable of independent signaling or co-operation with, for example, growth factor receptors. Syndecan expression levels are regulated in part by estrogen receptor α (ERα) in ERα+ breast cancer cells. Recent studies have revealed new insights into ER action in breast cancer, highlighting its role in cell behavior. Suppression of ERα causes epithelial-to-mesenchymal transition (EMT) in MCF-7 cells and at the same time causes the loss of cell surface syndecan-1 and syndecan-4 and alters the profile of epidermal- and insulin-like- growth factor receptors. The aim of this project was to investigate the role of syndecans in the transition of breast cancer cell morphology. For this purpose a stable cell line with suppressed ERα, MCF-7 SP10+, was established, which has been characterized for its striking changes in several EMT markers. Re-establishing expression of syndecans, particularly syndecan-4, through full-length cDNAs, converted the mesenchymal phenotype back to epithelial (MET), to resemble wild-type MCF-7 cells. During the transition of breast cancer cells, there have been observed differences on intracellular calcium levels. These changes of intracellular calcium seem to be regulated by the interaction of syndecans-4 with transient receptor potential channels. The calcium levels differences contribute significantly to cell cytoskeleton rearrangement. The ongoing study characterizes syndecans as key regulators of cell phenotype, highlighting their involvement in EMT and/or MET behavior of cancer cells, and potential regulatory role in breast cancer progression.

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CCBE1 enhances lymphangiogenesis by regulating VEGF-C activation

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Lymphangiogenesis is the growth of lymphatic vessels from pre-existing vessels. Vascular endothelial growth factor C (VEGF-C), the major lymphangiogenic growth factor, is produced as an inactive precursor and needs to be proteolytically processed in order to activate its receptors VEGFR-3 and VEGFR-2. Hennekam lymphangiectasia-lymphedema syndrome (OMIM 235510) is an autosomal recessive disease and, in a subset of patients with this syndrome, associated with mutations in the collagen- and calcium-binding EGF domains 1 (CCBE1) gene. Both CCBE1 and VEGF-C are essential for the early stages of lymphatic development that require budding, sprouting and migration of endothelial cells from the cardinal vein (CV), followed by assembly of endothelial cells into lymph sacs. Vegf-c deletion results in failure of lymphatic endothelial cells (LECs) emigration from the CV and Ccbe1 deletion results in abnormal LECs emigration, both resulting in a lack of the lymphatic vasculature. Because of the similar phenotypic features of Vegf-c and Ccbe1 gene deletion studies and because of the lymphangiogenic potential of CCBE1, we studied potential links between CCBE1 and VEGF-C.

Combined application of VEGF-C and CCBE1 in cell culture and in-vivo resulted in enhanced activity of VEGF-C. We found that CCBE1 itself has no effect on VEGF-C activation but regulates VEGF-C activation by A disintegrin and metalloproteinase thrombospondin type 1 motif, 3 (ADAMTS3) cleavage. The processed VEGF-C increased VEGFR-2 and VEGFR-3 signaling as observed by proliferation of Ba/F3 cells stably expressing VEGFR-3/EpoR or VEGFR-2/EpoR chimeras. We also found that both pro-VEGF-C and mature VEGF-C can bind to VEGFR-3 but only mature VEGF-C is able to induce VEGFR-3 mediated signaling as observed in VEGFR-3 phosphorylation study.

This mechanism suggests a possible role for CCBE1 as a therapeutic means to treat diseases that involve the lymphatic system, such as lymphedema or lymphatic metastasis.

TGF - b release by fibroblasts requires regulated secretion via autophagosomal intermediates

ST/P 26

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Myofibroblasts are essential effector cells in fibrosis depositing the vast amounts of extracellular matrix that lead to tissue stiffness. Their formation depends on perception of mechanical forces and presence of TGF. We showed previously that ILK (integrin-linked kinase)-null fibroblasts release severely reduced TGF amounts. Mice with specific ablation of ILK in fibroblasts demonstrated marked reduction of skin fibrosis upon bleomycin injection and failed to form myofibroblasts, suggesting that fibroblast-generated TGF is crucial for their formation. We showed that TGF transcription/translation was normal but secretion was blocked. TGF is released as latent growth factor complex. Activation is achieved extracellularly by integrin-mediated forces opening the latent complex or by proteolysis.

Using gene ablation and silencing in murine and human fibroblasts, we show that release of latent TGF1 crucially depends on low RhoA activity, which is controlled by GRAF-1. GRAF-1 localizes to focal adhesions and its GAP activity is strongly enhanced by direct interaction with ILK. Accordingly, fibroblasts with low ILK or GRAF-1 levels display elevated RhoA-GTP levels and ROCK activity resulting in severely impaired TGF secretion.

TGF secretion is also virtually abrogated upon suppression of autophagy by ablating Atg5 in fibroblasts, indicating a link to regulated secretion by an autophagosomal intermediate. Indeed, silencing of Rab8, required for this pathway, also impairs TGF release. However, autophagy is strongly impaired in the absence of ILK as well as in the absence of GRAF1 in a ROCK-dependent manner.

Together, our results demonstrate that in fibroblasts, latent TGF is almost exclusively exported via regulated secretion. We have deciphered the molecular mechanism, which is linked to integrins via ILK that is essential for balancing RhoA/ROCK signaling, which in turn regulates autophagosome maturation. Thus, besides their well documented role in TGF activation in the extracellular space, integrins are essential for controlling intracellular TGF trafficking regulating myofibroblast differentiation and fibrosis.

ST/P 27

HMGA2 regulates HAS2 and its natural antisense transcript during TGF-β-mediated EMT

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Hyaluronan accumulates in conjunction with rapid tissue remodeling during embryogenesis, as well as in inflammatory conditions and cancer. Among the three hyaluronan synthases, HAS2 has been shown to be overexpressed in breast cancer cells and to promote transforming growth factor β (TGF-β)-induced epithelial-mesenchymal transition (EMT). Since HAS2 antisense RNA (HAS2-AS) regulates the expression of HAS2, we investigated its involvement in the transcription of HAS2 during TGF-β-mediated EMT. Both the short and long transcripts of HAS2-AS were induced after stimulation by TGF-β in mammary epithelial cells. The induction promoted HAS2 transcription, since knockdown of HAS2-AS diminished the mRNA expression of HAS2 after TGF-β stimulation. High mobility group protein A2 (HMGA2) is a non-histone chromatin architectural protein which is required to elicit EMT in mammary epithelial cells by driving the expression of key EMT transcription factors, like Snail and Twist. Microarray data and qPCR suggested that the expression of HAS2 and HAS-AS is regulated by HMGA2. This was also reflected in elevated levels of secreted hyaluronan in cells stably over-expressing HMGA2 in comparison to parental cells. Moreover, silencing of HMGA2 significantly decreased the levels of HAS2 after stimulation with TGF-β. The HAS2 promoter has binding sites for both Smads and HMGA2. Binding of HMGA2 to the HAS2 gene locus proximal to its promoter was demonstrated by a chromatin immunoprecipitation (ChIP) assay. Thus, HMGA2 regulates HAS2 and the HAS2-AS transcript during TGF-β-induced EMT.

The role of ERβ in regulation of functional properties and gene expression of matrix macromolecules in aggressive breast cancer cells

ST/P 28

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Estrogen receptors (ERs) are implicated in the growth and progression of hormone-dependent breast cancer. There are two main subtypes of nuclear ERs (ERα, ERβ) which are both expressed in mammary tissue. Specifically, the role of ERα in breast cancer initiation, promotion and progression has been well established and current studies indicate its critical role in morphological and transcriptional status of breast cancer cells. On the other hand, the role of ERβ in breast cancer has not been completely clarified. In the present study, we highlight the critical impact of ERβ on gene expression of major extracellular matrix (ECM) macromolecules as well as on breast cancer cell properties. Transcriptional reprogramming but also induction of alterations in essential cell functions of ERβ-knocked down breast cancer cells [MDA-MB-231(ERβ+)] was investigated. For stable inhibition of ERβ expression, MDA-MB-231 cells were transduced with either ERβ or scramble (control) shRNA lentiviral particles. Interestingly, ERβ silencing in MDA-MB-231 breast cancer cells significantly reduced cell viability and invasion. Moreover, the suppressed breast cancer cells appear significantly reduced spreading capacity, followed by inhibited cell migration. These functional modulations are accompanied by gene expression changes in extracellular network components, such as metalloproteinases (MMP2, MMP7, MMP9), their endogenous tissue inhibitors (TIMP1, TIMP2) and the plasminogen activation system (uPA, tPA) as well as in EMT markers, such as E-cadherin and Vimentin. These alterations are also supported by disorganized cell cytoskeleton and also differentiated protein levels of critical breast cancer biomolecules, through immunofluorescence imaging. Moreover, it is worth noticing that the ERβ suppressed MDA-MB-231 cells exhibit differentiated gene expression motifs for specific ECM molecules, among them the proteoglycans serglycin and syndecans as well as the cell surface receptors EGFR and IGF-IR, being involved in fundamental biological processes, such as cell–cell and cell–matrix interactions and the control of cell proliferation, differentiation and the initiation of metastasis. These data suggest the pivotal role of ERβ in modulation of cell behavior and tumor microenvironment of MDA-MB-231 breast cancer cells.

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ST/P 29

Development of hyaluronan as a CD44-Targeted drug delivery vehicle in the treatment of cancer

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Despite advances in chemotherapeutic regimens, the treatment of metastatic cancer remains a challenge. A key problem with chemotherapy drugs is nonspecific drug distribution, resulting in low tumour concentrations and systemic toxicity. The holy grail of clinical cancer research has been to establish more specific ways of directing therapeutics to tumours, whether through more targeted anti-cancer agents or via the method of delivery. Many tumour cells have an up-regulated expression of receptors for the polysaccharide hyaluronan (HA), resulting in HA having a high affinity for tumours. The primary tumour–related HA receptor is CD44 which is activated and over-expressed on greater than 95% of solid cancers. These observations have led to the clinical development of HA-derivatised cytotoxic drugs that use HA as the tumour recognition moiety. The targeted drug delivery platform utilizes the large volumetric domain of HA to entrain small chemotherapeutic drugs within the 3-dimensional milieu resulting in the formation of 120nm nanoparticles. After intravenous administration the resultant HA/drug nanoparticles accumulate in the microvascular of the tumour, forming a microembolism that increases drug retention at the tumour site and allows for rapid intracellular uptake of the nanomedicine via the activated CD44. Clinical development of three anti-cancer drugs has been undertaken and has demonstrated that such formulations are safe and efficacious. This presentation follows the preclinical and clinical development of this technology where mechanisms associated with; i) the mode of molecular interaction between HA and the chemotherapeutic drugs, ii) ability of HA to act as a targeted transport vehicle for anti-cancer agents, iii) effect of HA on the therapeutic index of chemotherapeutic drugs iv) the clinical safety and efficacy of these HA-based anti-cancer agents and vi) the regulatory approach which has enabled an accelerated development pathway are discussed.

Cross talk between breast cancer cells and fibroblastast: effect of the uncharacterized protein q7z3e2 On hyaluronan synthesis

ST/P 30

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The influence of the extracellular matrix (ECM) components on breast cancer progression has been exceedingly well described. Hyaluronan (HA) and the enzymes implicated in its metabolism are closely related to the progression of breast cancer. The role of HA in tumour cell functions depends on its molar mass which is regulated by the enzymes that synthesize HA, i.e. hyaluronan synthases (HAS), and the degrading enzymes, called hyaluronidases. Low invasive breast cancer cells express lower amounts of HASes, respect to the high invasive tumour cells. Moreover, HAS2 gene expression is induced in the stromal cells surrounding breast tumour, resulting to an increased HA secretion in the tumour microenvironment which, in turn, stimulates metastasis and angiogenesis.

Recently, in our laboratory using proteomic techniques we found a new protein called "Uncharacterized protein of c10orf118 or Q7z3e2" in the extracellular matrix of the co-culture of the low invasive breast tumour cell line BC8701 and the normal human dermal fibroblasts (NHDF). It was demonstrated that the synthesis and secretion of Q7z3e2 protein is derived from BC8701 cells. For a deepened study regarding the synthesis and role of this protein, the two well-known breast cancer cell lines MCF-7 (estrogen receptor-ER positive) and MDA-MB231 (triple negative) were included. Interestingly, MCF-7 showed a higher amount of this protein in both cell lysates and conditioned media respect to MDA-MB231. In order to study the role of Q7z3e2 in tumour cells, the protein was stably knocked-down in MCF-7 and migration and proliferation were examined. Results showed that there was no difference in tumour cell functions. As reported previously, breast cancer cells promote HA synthesis in stromal cells. In order to explore a possible role of Q7z3e2 in the increment of HA, MCF-7 cells were stably transfected with pCMV6-Q7z3e2 and direct co-cultured with NHDF. Immunostaining experiments demonstrated an increased staining of HA on both MCF-7 and NHDF cells and a co-localization with Q7z3e2 on the contact area of tumour and stromal cells. Moreover, increased Q7z3e2 levels in MCF-7 cells were accompanied to an induction of HAS2 in both MCF-7 and NHDF cells. The effect of Q7z3e2 on the increased gene expression of HAS2 was further verified by treating NHDF with a recombinant protein of Q7z3e2 (part of protein sequence 1-211 a.a.) or with the conditioned medium of MCF-7 which was previously treated with anti-Q7z3e2 that blocks this protein.

Thus, we hypothesize that this novel protein has an important role in cellular mechanisms and its secretion is implicated in a cross-talk of breast tumour and stromal cells.

ST/P 31

An innovative biomaterial for tridimensional eukaryotic cell development: applications in tissue engineering and in tumor engineering

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We designed an innovative biomaterial for tridimensional eukaryotic cell development with the idea to combined structural, mechanical and biological properties. Our biomaterial consists of a tridimensional porous scaffold made of biosourced and biocompatible polymers which display an interconnected network of macro- and micropores and whose surface is functionalized with a nanolayer of biomolecules [1]. Our biomaterial is highly tunable and scalable and it may be used for a great diversity of applications *in vitro* as well as *in vivo*. For a large part of our studies, we used porous asymmetric films made of poly(lactic acid), a FDA approved bioresorbable polymer, and surface was functionalized with hyaluronan (HA). Here we show the potentialities of these biomaterials in cartilage tissue engineering and in tumor engineering.

Cartilage tissue engineering using mesenchymal stem cells (MSC) is one of the most promising approaches for treatment of osteoarthritic damages. We showed that our biomaterial, in the presence of TGF-β3, enables a high MSC proliferation level associated with a great cell viability. Flow cytometry analysis showed change in phenotypic profile which clearly revealed MSC differentiation into chondrocytes. This was associated to a sulfated glycosaminoglycans and collagens enriched-MEC deposition. In addition, expression of SOX-9 and collagen I, indicating the chondrogenesis condensation phase, was followed by that of collagen II which revealed the maturation phase with deposition of a hyaline cartilaginous matrix.

Biological functions of HA are known to depend on its chain size. Since i) HA is involved in cancer, ii) MSC are recruited to tumor sites and iii) functionalization of our scaffold can be performed efficiently whatever the HA chain size, we used our biomaterial to study effects of HA chain size on interactions between MSC and HT-29 colorectal cancer cells. Co-cultures of the two cell types showed that while there was no physical interaction between the two cell types with high molecular weight HA (HMW-HA), MSC came to surround HT-29 spheroids in the presence of low molecular weight HA (LMW-HA). Moreover, with HMW-HA the microenvironement was found to be proinflammatory, while an increased secretion of proangiogenic cytokines were observed with LMW-HA.

[1] Al Tawil E *et al.*, Patent, FR014 51478 (2014), PCT/FR2015/050441 (2015)

Loss of dermatan sulfate results in neonatal lethality in mice despite normal lymphoid and non-lymphoid organogenesis

ST/P 32

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Introduction: Glycosaminoglycans (GAGs) belong to a heterogeneous family of polysaccharides and consist of specific disaccharide repeats. In Chondroitin/Dermatan sulfate (CS/DS) GAGs, CS is composed of alternating N-acetyl-D-galactosamine (GalNAc) and D-glucuronic acid (GlcA) and DS derives from CS by enzymatic transformation of GlcA into L-iduronic acid (IdoA). The epimerization of CS to DS can be performed by dermatan sulfate epimerase-1 (Dse) or -2 (Dsel), two enzymes expressed in the Golgi. Dse is the major epimerase in most tissues except from the brain, where Dsel is predominant. Dse and Dsel knockout mouse models have previously been characterized in our lab. Dse deficient mice revealed a fragile skin phenotype and tail malformation with no other obvious alterations while Dsel deficient mice appeared normal. These results suggest that although tissue-specific expression of Dse and Dsel is observed, redundancy cannot be ruled out. This is particularly interesting, as lymphoid and non-lymphoid organs, including spleen, lung and kidney, exhibit very high DS epimerase activity. For this reason, we generated double-deficient mice that are lacking both Dse and Dsel expression and therefore are a DS-free *in vivo* model. Aim: Investigation of the role of DS in prenatal development and organogenesis. Methods: In total 183 embryos from mid- and late-embryonic stages were analyzed. Hematoxylin and Eosin staining was used for histological studies. Bone and cartilage of whole 18.5 dpc embryos were stained with Alcian Blue/Alizarin Red. Sections of 18.5 dpc lungs were stained with anti-pro-SPC. CXCL13 binding assay in mouse embryonic fibroblasts (MEFs) was performed. Lymph nodes of 19.5 dpc embryos were digested and stained with antibodies for CD45/CD4/CD3 and lymphoid tissue inducer (LTi) cells were identified with flow cytometry. Results: Loss of DS in mice resulted in perinatal death. Macroscopic and histological evaluation of whole 19.5, 16.5 and 13.5 dpc DS-free embryos revealed abdominal wall and neural tube defects of variable penetrance but otherwise normal development and Mendelian ratio. Immunofluorescent staining of pro-SPC showed no difference in lungs from 18.5 dpc DS-free embryos compared to littermate controls. Despite CXCL13 binding impairment in DS-free MEFs, lymphoid organ development and localization and LTi cell subpopulations were normal in 19 dpc DS-free embryos compared to littermate controls. Conclusions: Loss of dermatan sulfate results in neonatal lethality in mice despite normal lymphoid and non-lymphoid organogenesis.

ST/P 33

Shedding of syndecan-4 promotes immune cell recruitment and preserves heart function after lipopolysaccharide challenge

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The innate immune system is activated in response to pathological stimuli and plays a role in remodeling, dysfunction and failure of the heart. Heart failure remains the leading cause of morbidity and mortality in the Western world, reflecting the need for improved understanding of the underlying mechanisms. We have previously shown that cardiac expression of the transmembrane heparan sulfate (HS) proteoglycan syndecan-4 is increased in experimental and clinical heart failure. Interestingly, we found that subsequent to its upregulation by innate immune signaling, syndecan-4 is shed from the surface of cardiac cells. Circulating levels of shed syndecan-4 are elevated in heart failure patients, however, the pathophysiological and molecular processes associated with shedding of syndecan-4 in the heart remain poorly understood. In this study we used LPS challenge *in vivo* and *in vitro* to investigate regulation and effects of syndecan-4 shedding in the heart.

LPS challenge in wild-type mice (10 mg/kg/, 9h, n=8-10) and cultured neonatal rat cardiomyocytes and fibroblasts upregulated cardiac syndecan-4 mRNA up to 10-fold without altering full-length protein. Elevated levels of shedding fragments in the myocardium (30-fold) and in blood collected from the heart (3-fold) confirmed that the majority of LPS-induced syndecan-4 was shed *in vivo*. Cardiac dysfunction was exacerbated in mice unable to shed syndecan-4 (syn-4 KO) after LPS challenge; echocardiography revealed reduced ejection fraction and diastolic tissue velocities compared with WT. The reduced heart function was accompanied by attenuated expression of the immune cell markers CD8, CD11a and F4/80 and the adhesion molecules Icam1 and Vcam1 in syn-4 KO hearts, suggesting that syndecan-4 shedding promotes immune cell recruitment to LPS-challenged hearts. Overexpression of syndecan-4 in HEK293 cells induced constitutive shedding and high levels of shed syndecan-4 ectodomains in the conditioned medium. When this medium was applied to cardiac myocytes and fibroblasts, increased expression of Icam1 (4- and 5-fold) and Vcam1 (7- and 115-fold) and activation the innate immunity NF-κB signaling cascade was induced, in line with a direct regulation of immune cell recruitment by shed syndecan-4. Moreover, syndecan-4 ectodomains increased levels of collagen cross-linking enzyme lysyl oxidase (LOX) mRNA 1.7-fold in cardiac fibroblasts, indicating that shedding of syndecan-4 affects collagen matrix quality. Interestingly, shed ectodomains of syndecan-4 with mutated HS chain attachment sites failed to induce signaling in cardiac cells, indicating that this effect was dependent on the presence of HS chains. Finally, elevated levels of shed syndecan-4 in blood from the coronary sinus compared to the radial artery of open heart surgery patients (n=30) demonstrated that syndecan-4 is shed from the human heart, suggesting a clinical relevance for this post-translational modification of syndecan-4 in cardiac diease.

In conclusion, shedding of syndecan-4 ectodomains is part of the cardiac innate immune response, promoting immune cell recruitment and preserving heart function in response to LPS.

ST/P 34

The extracellular matrix proteoglycan fibromodulin is up-regulated in experimental and clinical heart failure, and might attenuate development of myocardial fibrosis by interacting with TGF-β signaling

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Cardiac remodeling is a key event in development of heart failure (HF), a disease with high morbidity and mortality affecting millions of people worldwide. Cardiac remodeling involves extracellular matrix (ECM) alterations, hypertrophy, fibrosis, and tissue inflammation. Fibromodulin (FMOD) is a small leucine rich proteoglycan, known to bind collagens and inhibit fibrillogenesis in vitro. Increased FMOD expression has been detected in inflammatory- and fibrosis-like conditions. In scar tissue, FMOD expression has an inverse relationship to TGF-β activity, and FMOD has been suggested to regulate fibrotic responses in kidney, lung and skin by interacting with TGF-β. Despite these intriguing effects of FMOD on scarring and fibrosis in other tissues, the role of FMOD in the heart has not been characterized. We hypothesized that FMOD is involved in cardiac remodeling and attenuates development of cardiac fibrosis, interacting with the key pro-fibrotic molecule TGF-β.

We examined FMOD expression in left ventricular (LV) tissue from both HF patients and mice with progressing HF development, due to pressure overload induced by aortic banding (AB). We also investigated FMOD expression and effects of increased FMOD levels, in neonatal rat cultures of cardiomyocytes (CM) and cardiac fibroblasts (CFB) in vitro.

LV expression of FMOD was increased after 1 week (8-fold), 3 (10-fold), 16 (6-fold) and 18 (8-fold) weeks of AB, but showed no difference after 24 hours of AB, compared to sham-operated mice. Echocardiography and organ weights confirmed hypertrophic remodeling at 1-3 weeks of AB and end-stage, dilated HF at 16-18 weeks. Compared to expression of other SLRPs (i.e. lumican, decorin and biglycan), FMOD was more robustly increased after AB. Positive correlation between FMOD and both LV weight, and lung weight indicated a relationship between levels of cardiac FMOD and severity of remodeling and HF. Similarly, patients with end-stage HF had increased cardiac FMOD expression (3-fold), compared to controls. In accordance with our hypothesis, a positive correlation between FMOD and TGF-β1 expression suggested an interaction between these molecules in the failing human heart. Interestingly, when treating both cultured CM and CFB with TGF-β1 for 24 hours, FMOD expression decreased by 50%. This decrease was attenuated by co-stimulation with an inhibitor (SM16 or SB431542) of SMAD2/3, a main downstream signaling pathway of TGF-β.

In conclusion cardiac FMOD expression was robustly increased in experimental and clinical HF. Our data suggesting an interaction between FMOD and the key-fibrotic molecule TGF-β in the heart calls for further investigation of the role of FMOD in cardiac remodeling and HF.

5th FEBS Advanced Lecture Course

ST/P 35

MicroRNAs: posttranscriptional modulators of cellular and extracellular cartilage compartments

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MicroRNAs (miRNAs) are key components of transcriptional gene regulation. Recently, cartilage specific ablation of Dicer in mice revealed, that miRNAs regulate chondrocyte maturation within the growth plate leading to severe growth defects and premature death. Hence, miRNAs are essential for skeletal development. In order to determine essential miRNAs for growth plate cartilage development we redetermined the miRNA expression profile within the distinct differentiation zones of growth plate cartilage using microarray analysis. We were able to identify two miRNAs, miR-26a and miR-181a, which are differentially expressed during maturation of chondrocytes within the growth plate.

We hypothesized that those miRNAs modulate the expression of several genes important for growth plate development and extracellular matrix (ECM) production.

Here we could show that miR-26a was specifically downregulated in hypertrophic chondrocytes whereas miR-181a was upregulated in prehypertrophic to hypertrophic chondrocytes. Bioinformatic target analysis indicated that miR-26a may bind to the 3'UTR of genes normally expressed in hypertrophic growth plate chondrocytes as well as to genes of the extracellular matrix. In contrast, miR-181a may target several genes of the insulin-receptor pathway needed for chondrocytes proliferation and survival. Luciferase interaction studies and immunoblot analysis revealed that

miR-26a directly inhibits the expression of collagen IX and X. Reduction of both proteins results in the decrease of the interaction partners matrilin 3 and COMP to cause a destabilization of the ECM in cultured chondrocytes. Transfection of synthetic miR-181a mimics induced cell death and reduction of cell proliferation due to changes in the activation of the MAPK-signaling pathway, but the direct targets of the miRNA are not yet identified.

Hence, miRNA-26a mainly inhibits matrix gene expression and miR-181a dampens insulin-receptor pathway activation to induce terminal differentiation of chondrocytes within the growth plate. Both miRNAs are therefore essential posttranscriptional regulators of growth plate cartilage homeostasis.

Animal models of desbuquois dysplasia type 1 to study the role of CANT1 in proteoglycan metabolism

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Desbuquois dysplasia (DBQD) is a rare recessive chondrodysplasia, characterized by growth retardation, multiple dislocations and advanced carpal ossification. Two forms of DBQD have been described on the basis of the presence (type 1) or absence (type 2) of characteristic hand anomalies. DBQD type 1 is caused by mutations in the *Calcium-Activated Nucleotidase 1* gene (CANT1), while mutations in the *xylosiltransferase* 1 (XYLT1) gene have been demonstrated in DBQD type 2.

CANT1 is a nucleotidase of the ER/Golgi that preferentially hydrolyzes UDP, suggesting its involvement in protein glycosylation; for this reason its role in PG metabolism has been hypothesized.

To better characterize CANT1 role in the etiology of DBQD, we generated a Cant1 knock-in mouse carrying the R302H substitution reproducing the R300H mutation detected in patients. Morphometric analyses demonstrated that mutant mice are smaller with shorter and thinner tibiae, femurs and ilia compared to wild-type animals. Limb extremities of KI mice reproduced the hand anomalies described in patients: additional carpal ossification centers, bone dislocations and the delta phalanx. Thus the KI mouse develops a skeletal phenotype reminiscent of DBQD type 1. To better investigate CANT1 role in PG synthesis we generated a Cant1 knock out mouse by excision of exon 3 and 4. The KO mouse showed the same growth defects and hand anomalies of patients already observed in the KI mouse. To study PG synthesis, rib chondrocytes were metabolically labeled with 35S-sulfate and the amount of newly synthesized PGs was evaluated. KO cells showed reduced PG synthesis compared to wild types both in presence and in absence of β-D-xyloside, an enhancer of GAG synthesis. Gel filtration chromatography of GAGs released from newly synthesized PGs after β-elimination demonstrated that the hydrodynamic size of GAG chains was reduced in KO chondrocytes compared to the controls. Ultrastructural analysis of KO and wt chondrocytes by TEM demonstrated the presence of dilated RER cisternae containing electrondense proteinaceus material suggesting a role of CANT1 in protein secretion. In fact pulse-chase labeling of cells with 35S-sulfate demonstrated reduced PG secretion in mutant cells compared to the controls.

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

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Prostate cancer cells stably change proteoglycans expression in normal fibroblasts in cell culture model in vitro

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Cellular microenvironment plays an important role in the interactions of cells with each other and with the extracellular matrix (ECM). Fibroblasts are the main cellular components of microenvironment and synthesise the extracellular matrix components. However, tumour cells affect normal fibroblasts and reprogram them in cancer-associated fibroblasts (CAFs), which produce a cancer-associated matrix thereby creating favorable conditions for the growth and development of tumours. One of the main components of ECM is proteoglycans which are expressed on the cell surface and in ECM and play an important role in cell-cell and cell-matrix interactions and signaling. We have previously shown that the expression of main proteoglycans was changed in fibroblasts after co-culturing with prostate cancer cell lines (LNCaP, PC3, DU145). Significant down-regulation of glypican-1 expression and distortion of versican and NG2 expressions resulted in different expression pattern for proteoglycans in cancer cells-exposed fibroblasts, suggesting proteoglycans involvement in transformation of normal fibroblasts to CAFs.

The aim of the work was to investigate a stability of these changes in proteoglycans expression in fibroblasts. To study this problem, prostate cells-exposed fibroblasts were returned in culture after cells separation and were cultivated during two weeks. Expression pattern of main proteoglycans (syndecan-1, glypican-1, perlecan, aggrecan, versican, decorin, lumican, NG2, brevican) was determined in fibroblasts using RT-PCR analysis. It was shown that changes in proteoglycans expression were very stable and thus may be associated with reprogramming of normal fibroblasts to CAFs. Collectively, obtained results show that prostate cancer cells affect fibroblasts in cell culture model in vitro and change transcriptional activity of proteoglycan-coding genes in cancer cells-exposed fibroblasts. This data support a possible contribution of proteoglycans in stable reprogramming of normal fibroblasts to cancer-associated fibroblasts, and normal microenvironment in cancer-associated matrix.

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Posters/ Abstracts

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Mass spectrometric characterization of the human lung matrisome in health and in disease

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Common to many lung diseases is an altered extracellular matrix (ECM) that distorts the interplay of the cells and contributing to the malfunction of the tissue. To understand the factors driving pathology a comprehensive and quantitative analysis of all the ECM components is needed. Mass spectrometry (MS) based proteomics has developed rapidly the past years where it is now possible to quantify thousands of proteins with high degree of quantitative accuracy in cells, tissues and body fluids.

We are currently developing and applying MS-based proteomics methods for in-depth proteomic profiling of lung tissue. To investigating the role of the ECM we specifically target all protein sequences associated with the matrisome using mass spectrometry to identify and quantify the matrisome proteins in healthy and diseased human lung tissue. The method relies on a three-step protein extraction protocol for mass spectrometric protein analysis of lung tissue. In step one the tissue is homogenized and soluble proteins are collected. In step two the tissue pellet is boiled in 2% SDS and released proteins are collected. Finally the remaining tissue pellet is incubated with urea followed by in-solution digestion using the proteases Lys-C and trypsin. The released proteins from the soluble and first pellet extraction were further fractionated by SDS-PAGE prior to in-gel digestion. The samples were analyzed on a Q-Exactive Plus mass spectrometer (Thermo Scientific), using RP-HPLC with a 2h linear gradient in shotgun MS-mode. The resulting mass spectra were searched with a combinatorial search engine approach and statistical analysis using the iPortal framework. Categorization into matrisome groups was performed using DDB.

In distal lung tissue from a late stage chronic obstructive pulmonary disease (COPD) patient, the matrisome represented 5,9% of the total lung proteome, whereas the matrisome represented 5,2% of the lung proteome in distal lung tissue from a patient with idiopathic pulmonary fibrosis (IPF). The quantitative differences of these matrisomes are now being evaluated. We are currently extending our analyses to also include distal lung tissue from five healthy subjects, seven late stage COPD patients and seven IPF patients with different degree of fibrosis. The outlined set-up for protein extraction, identification and classification into matrisome groups forms the platform for how we analyze the protein composition of healthy and diseased lung tissue.

Synthesis of a hexasaccharide partial sequence of hyaluronan for click chemistry and more

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The extracellular matrix (ECM) consists of different molecular components. Besides proteins such as collagen and elastin the ECM's major constituents are glycosaminoglycans (GAGs).[1] Hyaluronan (HA) as a GAG and major component of the ECM serves various functions in organism. It acts as water storage and it affects cell proliferation, migration and differentiation.[2] In addition, HA is a specific ligand for numerous receptors, thus playing a significant role in various physiological and pathological processes. HA is also important in tissue injury and repair. Furthermore, during the different stages of wound healing HA conduces to manifold tasks.[3]

In the frame of a research project aiming the investigation of protein-GAG-binding a convergent synthesis of a HA hexamer with a suitably modified aglycone that can be easily attached to polymer matrices as well as functionalized surfaces or covalently attached to peptides was developed. First, glycosylation precursors were generated starting from D glucose or D glucosamine derivatives, respectively. Subsequently, the monosaccharide building blocks were linked to form disaccharide subunits which were furthermore used in glycosylation reactions to yield the fully protected hexasaccharide. The next step was the modification of the aglycone by introduction of an azido group. Finally, global cleavage of the protective groups furnished the desired hexasaccharide 1. This can now be employed in NMR spectroscopic investigations or be used for biophysical studies (e.g. through attachment on surfaces by means of Click chemistry).

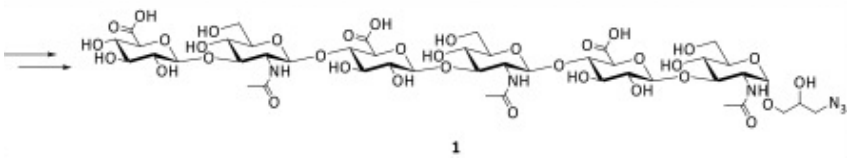

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Fig. I: Hyaluronan hexamer 1 with modified aglycone.

References:

[1] Giannis *et al.*, *Bioorg. Med. Chem.* 2013, 21, 733–741.
[2] Ghatak *et al.*, *FEBS Journal* 2011, 278, 1429–1443.
[3] Gandhi *et al.*, *Chem. Biol. Drug Des.* 2008, 72, 455–482.

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The UVB radiation suppresses the proteasome expression in human eye pterygium fibroblasts

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Backround: Pterygium is a condition characterized by epithelial overgrowth of the cornea, inflammatory cell infiltration and an abnormal extracellular matrix accumulation. Chronic UV exposure is considered as a pathogenetic factor of this disease. Proteasome is an intracellular multi-subunit protease complex that degrades intracellular proteins. It possesses multiple endopeptidase activities including chymotrypsin (subunit β5), trypsin (subunit β2) and caspase (subunit β1)-like activities. In most of cases its subunits expression is mediated by Nrf2-ARE pathway. The aim of this study was to investigate the implication of proteasome in pterygium pathogenesis.

Materials and Methods: Normal conjunctival specimens were obtained from healthy individuals undergoing cataract surgery. Pterygium specimens were obtained from patients after the surgical removal of primary tissue. Fibroblasts were isolated from pterygium tissue by treatment with collagenase. The expression of proteasome subunits and Nrf2 was ascertained by RT-PCR analysis. The cells survival was confirmed by MTT method. Proteasome activity was determined fluorometrically.

Results: RT-PCR analysis showed that the expression of proteasome subunits as well as of Nrf2 was lower in pterygium than in normal conjunctiva. When pterygium fibroblasts were cultured in the presence of the Nrf2 activator, Oltipraz, an enhancement of proteasome subunit β5 expression was observed, suggesting that its expression is mediated by Nrf2-ARE pathway. Upon UVB exposure of pterygium fibroblasts at radiation doses 0-50 mJ/cm2, a radiation dose-dependent suppression of Nrf2 and proteasome subunit β5 expression was observed. On the contrary, UVB radiation did not affect the chymotrypsin-like activity as well as the expression of subunits β1 and β2. When the UVB exposure took place in the presence of src kinases inhibitor PP2, the expression of subunit β5 was restored almost at control levels, although the expression of Nrf2 remained un-changed. UVB radiation had very low deleterious effect on fibroblasts survival.

Conclusion: In pterygium fibroblasts the UVB exposure leads to suppression of Nrf2 expression as well as, possibly, to its nuclear exclusion, since of its phosphorylation from a src kinase as previously described, and consequently in down-regulation of ARE-mediated proteasome subunit β5 gene expression. Known that proteasome is involved in the regulation of expression of various factors, which may contribute in pterygium formation, such as IL-6, it would be concluded that the implication of UVB radiation in pterygium pathogenesis is partially mediated by the suppression of proteasome expression.

Epitopes involved in formation of the matrix metalloprotease-9/serglycin complexes

P 41

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Matrix metalloprotease-9 (MMP-9) can process a large number of extracellular matrix proteins such as cell surface receptors, growth factors, cytokines, chemokines, enzymes and inhibitors, and this protease is important in normal as well as in pathological processes.

MMPs are built up of different domains, modules and motifs, involving a pro-, a catalytic and a hemopexin-like (HPX) domain. In addition, MMP-9 also contains a unique fibronectin II-like module (FnII) inserted in the catalytic region, as well as a special long and glycosylated hinge region that connects the catalytic and the HPX domains. Both the HPX domain and the FnII module facilitate the localization of MMP-9 to connective tissue matrices as well as the degradation of various biological substrates.

ProMMP-9 form strong complexes with the core proteins of various CSPG proteoglycans such as Serglycin and Versican. These strong complexes are of two types, one is SDS-stable and reduction sensitive while the other is SDS soluble. These complexes has been found in CSPGs isolated from THP-1 cells and could also be reconstructed in vitro, suggesting that they can be formed in the extracellular environment. Both the HPX domain and the FnII module are involved in the complex formation, while the prodomain is not involved. The different regions and amino acids in the HPX domain, the FnII module and the Serglycin core protein involved in the complex formation has been studied by peptide arrays and mutated peptide arrays. Soluble peptides were used as inhibitors in the in vitro reconstitution experiments to distinguish the parts involved in the SDS-stable from those involved in the SDS-soluble complex formation.

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CRISPR-Cas9 technology in zebrafish proteoglycan studies

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Unique proteins with covalently linked linear, sulfated and therefore negatively charged glycosaminoglycan (GAG) chains are defined as proteoglycans (PGs). Depending on their composition the sulfated GAGs can be further divided into heparan sulfate (HS), heparin, chondroitin sulfate (CS), dermatan sulfate (DS) and keratan sulfate (KS). PGs are present on the cell surface, in the extracellular matrix, but also in the storage granules of e.g.mast cells. GAG chains have characteric sulfation "finger prints" essential for their interactions with signaling molecules, cell surface and matrix proteins.

Glucosaminyl N-deacetylase N-sulfotransferase (NDST) is the first enzyme modifying the heparan sulfate GAG chain during biosynthesis by removing acetyl groups and then adding sulfate groups to the previously deacetylated glucosamine residues. The NDSTs play a crucial role during the HS modification process since most of the sulfate groups added by O-sulfotransferases will be located to the N-sulfated regions of HS chain where also epimerization occurs. Action of the various GAG-sulfotransferases, including NDSTs is dependent on the availability of the general sulfate donor 3'-phosphoadenosine 5'-phosphosulfate (PAPS) (Venkatachalam 2003).

Taking advantage of the CRISPR-Cas9 technology allowing targeted mutagenesis of virtually any zebrafish gene we are aiming to create a collection of zebrafish lines carrying null mutation for genes involved in GAG biosynthesis. My main interest within this project is focused on knocking-out the five zebrafish Ndst enzymes and two zebrafish orthologues of PAPSS2. By now, we have generated and identified at least one adult mutant carrier for all of these seven genes. Our further work will include phenotypical characterization of each of the mutants, as well as studies of combination of the different mutants to more efficiently alter GAGs sulfation patterns.

We have previously reported on the essential role of the ndst1b gene in craniofacial cartilage development, based on studies of zebrafish morphant embryos and are now interested to verify these results under stable knock-out conditions (Filipek-Gorniok, Carlsson et al. 2015). Moreover, knock-out of the single zebrafish ndst3 gene, represented by two genes in the mouse and human genomes (NDST3 and NDST4), could reveal new information about the function of these genes. Additionally, a recent study has linked PAPSS2 loss-of-function with human brachyolmia (heterogenous skeletal dysplasia) manifesting itself by short stature combined with spinal and joint deformation (Iida, Simsek-Kiper et al. 2013). Generation of zebrafish mutants for genes involved in human diseases will give us tools to reveal how altered GAG sulfation may underlie the pathological changes seen in these disorders.

The effect of polarized macrophages on the tissue remodeling process in fibrosis

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Background/Aims: Liver fibrosis is the outcome of chronic liver injury and a major cause of liver related morbidity and mortality. The cellular and molecular mechanisms of liver fibrosis are not fully elucidated. In this study we evaluated the effects of M1 and M2 polarized macrophages, cultured in presence of fibrotic precision-cut liver slices (PCLS), on the tissue remodeling process in fibrosis.
Methods: M1 and M2 macrophages were obtained from the murine macrophage cell line RAW 264.7 by polarizing the cells for 24h with IFN-γ/LPS and IL-4/IL-13 respectively. After the polarization, the cells were incubated with PCLS obtained from 8-10 week old MDR2-/- mice, which spontaneously develop liver fibrosis. The co-incubation was performed for 24 or 48h, and viability was assessed by measuring ATP content of the PCLS. Fibrosis was quantified using Sirius Red staining; furthermore RT-PCR was performed for fibrosis and inflammation markers (TNF-α, IL-1β, IL-6, α-SMA, Procollagen-1, Fibronectin, Synaptophysin, HSP47, CTGF).
Preliminary results: The co-incubations of M1, M2 and untreated raw cells showed distinctive effects on fibrotic liver slices. M1 macrophages induced a higher gene expression of fibrosis and inflammation markers in comparison to the M2 and untreated raw cells. After fibrosis quantification of the liver slices, those incubated with M2 macrophages showed a reduction in parenchymal fibrosis in comparison to the M1 group.
Conclusions: Our results show that M1 macrophages are profibrotic and M2 macrophages are antifibrotic, which is in line with *in vivo* data. Fibrotic PCLS might represent a valuable tool to study tissue remodeling in fibrosis.

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CRISPR/Cas9 generated loss of function alleles for CS/DS biosynthesis enzymes - A systematic approach to investigate the role of glycosaminoglycans

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Chondroitin/Dermatan Sulfate (CS/DS) proteoglycans consist of sulfated polysaccharides attached to core proteins. CS/DS is produced by virtually all vertebrate cells and has multiple functions during animal development. The biosynthesis of CS/DS is a complex process with a large number of enzymes involved. It produces chains varying in length, charge density and sulfation pattern, which affect physical docking to extracellular matrix molecules, such as growth factors. However, the poorly understood non-template derived regulation of CS/DS biosynthesis makes studies of the specific roles of complex CS/DS structures difficult. In the present study we aim to identify the roles of CS/DS modifications in zebrafish by creating loss of function alleles for all biosynthesis enzymes by using high throughput CRISPR/Cas9 technology. We are characterizing phenotypes of each mutant, as well as combining null alleles to generate a collection of zebrafish lines with altered CS/DS structures ranging from subtle differences to complete absence. A first cohort of adult zebrafish with homozygous loss of function alleles in selected genes has been generated. The CS/DS glycosyltransferase chsy1 has in several previous studies using the morpholino methodology been suggested to be essential for embryo development. We find however that chsy1 mutants develop to adulthood without obvious morphological phenotypes. The CS/DS glycosyltransferase chpfa also lacks an apparent adult phenotype. Csgalnact enzymes are critical for CS/DS attachment to core proteins. csgalnact1 mutants survive to adulthood, but develop distinct craniofacial abnormalities. csgalnact2 adult mutants do not show any obvious phenotype, presumably due to the presence of functional csgalnact1. To further reduce CS/DS production we are combining these alleles into double mutants, as well as investigating the effect of the acana null allele, the most extensively CS/DS decorated proteoglycan. Embryos lacking CS/DS sulfation by CS/DS sulfotransferases chst3a and ust develop normally and we are currently studying loss of function alleles of chst3b and chst7, as well as identifying null alleles for the remaining CS/DS biosynthesis enzymes. We conclude that removing single CS/DS biosynthesis enzymes typically does not result in gross morphological phenotypes. CS/DS biosynthesis enzymes are highly redundant and combinations of mutant alleles will be necessary to shed light on the regulating functions of CS/DS structures.

Osteonectin (SPARC) is essential for the maintenance of the endothelial barrier. Its absence increases cardiac inflammation and overall mortality in viral myocarditis.

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Viral myocarditis consists of exaggerated cardiac inflammation in response to viral infection, which can result in sudden death or heart failure. Viral presence triggers extensive leukocyte recruitment to the heart and pathological remodelling processes that lead to ventricular dysfunction. Extracellular non-structural matrix proteins, such as SPARC, are critical regulators of fibrosis but their role in modulating inflammation is not well established.

Here we show a novel function for extracellular matrix protein SPARC in a crucial role for SPARC in regulating cardiac inflammation during viral myocarditis by regulating endothelial function. In the murine model of myocarditis, mice lacking SPARC had increased cardiac inflammation and mortality, which was reversible with an adenoviral vector overexpressing SPARC. Using intra-vital cremaster microscopy we observed that the absence of SPARC resulted perturbed leukocyte-endothelial behaviour; increased leukocyte adhesion efficacy and decreased leukocyte velocity, as well as enhanced microvascular permeability. Electron microscopic imaging revealed an impaired microvascular endothelial glycocalyx in SPARC null mice as compared to wild type mice. Finally, to demonstrate the importance of the endothelial barrier during viral myocarditis, we enzymatic digested the main glycocalyx components using hyaluronidase and observed comparable increases in cardiac inflammation. Hyaluronidase treatment also resulted in increased leukocyte velocity and microvascular leakiness. Systemic administration of recombinant SPARC restored the phenotypic changes in leukocyte velocity and vascular leakiness induced by the enzymatic treatment.

In conclusion, we identify SPARC as a novel component of the endothelial glycocalyx where it contributes to the regulation of cardiac inflammation through reducing leukocyte adhesion and transmigration. Besides its protective role in ischemic heart disease, it also appears crucial to prevent exaggerated inflammation upon viral infection of the heart.

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The effect of human recombinant decorin on various hepatoma cell lines

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Hepatocellular carcinoma (HCC) is the third most common tumor and the sixth most frequent death-related malignancy over the world. Most cases occur in Sub-Saharan Africa and East Asia, however, the incidence of HCC is increasing in developed countries as well including UK, France, USA and Japan. HCC usually develops in a cirrhotic background, considered as a pathologically altered tumor micro-environment. Thus the growth, motility and metastatic ability of tumor cells will be driven by this impaired milieu. According to epidemiologic data detailed understanding the mechanism of HCC progression on molecular level and uncovering new possibilities for therapy is an urgent requirement.

Decorin, a small leucine-rich proteoglycan binds to collagens, therefore it is responsible for the assembly and integrity of the extracellular matrix. In addition, decorin found to be capable to bind and interfere the action of cell surface receptors (e.g. EGFR, Met, IGF-1R) influencing signal transduction pathways initiated on them. These events finally induce cell cycle arrest in G1 phase via $p21^{WAF1/CIP1}$, and inhibition of tumor growth.

Our aim was to explore whether decorin can influence the proliferation and behavior of 4 different hepatoma cell lines (HepG2, Hep3B, HuH7 and HLE) in an in vitro cell culture model. Each cell line has different molecular characteristics, therefore we focused our attention on their tyrosine kinase signaling pathways expected to be altered among the cell types.

Our results proved the inhibitory potential of decorin on all hepatoma cell lines. Induction of $p21^{WAF1/CIP1}$, increase in c-myc and beta-catenin inactivating phosphorylation and decrease of EGFR, GSK3í and ERK1/2 phosphorylation were observed in HepG2 cells what are well-known proliferation inhibitory effects of decorin. However, other cell lines showed alternative molecular mechanisms; while HuH7 and HLE had no change or decreased level of activated Akt (PKB), we found intensive activation of the latter in Hep3B cells together with increased phosphorylation of IGF1-receptor.

While HLE and Hep3B showed no considerable change in ERK1/2 phosphorylation, HuH7 presented a significant concentration dependent elevation in ERK activation.

Decorin might play candidate role in outcome of HCC. However, some of its underlying molecular mechanisms are not fully clarified so far, therefore deciphering them may lead us closer to exploit decorin's potential in therapy.

Urine bikunin/Urinary trypsin inhibitor (UTI) quantitation and structural characterization in patients affected by type 1 and type 2 diabetes

P 47

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Bikunin is a small plasma proteoglycan (PG) with Ser-proteinase inhibitory activity, consisting of a small polypeptide of 147 amino acid residues, which carries a N-linked oligosaccharide at Asn45 and a O-linked low-charge chondroitin sulfate (CS) chain, at Ser10. Synthesized by hepatocytes, bikunin circulates primarily as a subunit of Inter-α-Inhibitor (IαI) family molecules, covalently linked, via the CS chain, to one or two polypeptides, called the heavy chains (HCs). After an inflammatory stimulus, IαI leaves the circulation and, in extravascular sites, the HCs are transferred from CS chain to the locally synthesized hyaluronic acid (HA) to form the serum-derived hyaluronan-associated-protein- (SHAP-) HA complex. This complex plays important roles in stabilizing extracellular matrices and it is often associated with inflammatory conditions. When bound to IαI, bikunin lacks some of its known activities, and there is evidence that its release, by partial proteolytic degradation, may function as a regulatory mechanism. Free bikunin is rapidly cleared from circulation by both tissue uptake and renal excretion and it is found in urine as the urinary trypsin inhibitor (UTI). UTI levels are usually low in healthy individuals but they increase up to 10 fold in both acute and chronic inflammatory diseases.
The aim of the present work was to perform direct UTI quantitation and structural characterization in patients with type 1 and 2 diabetes.
UTI purification was performed by two steps. Firstly, an UTI enriched fraction was obtained by anion exchange chromatography, exploiting the O-linked CS negative charge. Part of this fraction was subjected to chondroitin sulfate lyase treatment and disaccharide fluoro-tagging followed by fluorophore-assisted carbohydrate electrophoresis. The remaining was resolved, according to its molecular weight, by SDS-PAGE followed by image analysis and mass spectrometry (MS).
Quantitative analysis evidenced higher levels of UTI in both type 1 or type 2 diabetic patients respect to controls ($p < 0.01$), whereas no differences between the two groups of patients were evidenced ($p = 0.309$). Accordingly, CS levels were significantly higher in type 1 and type 2 diabetes patients with respect to controls, being about 1.65 times greater ($P = 0.037$). Furthermore, sulfation degree of CS chains was lower in both classes of patients with respect to controls ($P = 0.002$ and $P = 0.018$, for type 1 and 2 diabetes, respectively). Finally, by MS analysis, it was possible to identify some post-translational UTI modifications potentially able to affect UTI localization, function and pathophysiological activity.
These preliminary results suggest that both UTI levels and structure could be modified in type 1 and 2 diabetes, possibly because of the chronic inflammatory condition. The effects of such quantitative and structural alterations on UTI localization, function and pathophysiological activities deserve further studies.

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The UDP-sugar substrates of hyaluronan synthase 3 (HAS3) regulate its intracellular traffic and extracellular shedding, controlling hyaluronan synthesis and correlating with phenotypic changes in early stages of melanoma

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Hyaluronan is synthesized by hyaluronan synthases (HAS1-3) at plasma membrane (PM). Using photo-convertible Dendra2-HAS3, we show that unless engaged in hyaluronan synthesis, the HAS3 half-life in the PM of cultured MV3 melanoma cells is in the range of minutes. When cellular UDP-N-Acetylglucosamine (UDP-GlcNAc) content was increased by treatment with 1-2 mM glucosamine, uptake of HAS3 from PM to early endosomes was inhibited, and hyaluronan synthesis increased. In contrast, shortage of the HAS substrates (UDP-GlcNAc) or UDP-glucuronic acid (UDP-GlcUA) limited the time HAS3 remained in PM, and inhibited hyaluronan synthesis. HAS3 depletion from PM was also enhanced by hyaluronidase digestion. HAS3 was post-translationally modified by O-GlcNAc, the level of which correlated with the cellular concentration of UDP-GlcNAc. Increased O-GlcNAcylation, even without changes in UDP-GlcNAc content, also blocked HAS3 endocytosis and increased hyaluronan synthesis. Thus, as substrates, both UDP-GlcNAc and UDP-GlcUA control HAS3 endocytosis, while UDP-GlcNAc also supports PM retention and hyaluronan synthesis through HAS3 O-GlcNAcylation. UDP-GlcNAc surplus and O-GlcNAc modification also blocked the lysosomal decay of HAS3. The amount of Dendra2-HAS3 in intracellular storage vesicles corresponding to early endosomes increased and decreased with low and high levels of UDP-sugars, respectively, suggesting a role in regulation of HAS3 recycling in cell surface. Indeed cell surface biotinylation and TIRF imaging of EGFP-HAS3 vesicles showed that UDP-sugars control HAS3 recycling in PM. Mediated by the changes in hyaluronan synthesis and O-GlcNAc signaling, the contents of UDP-sugar levels exerted major regulatory functions, including secretion of extracellular vesicles, adhesion and proliferation of the cells. In human melanoma tissue samples, GFAT1 protein levels – representing cellular UDP-GlcNAc levels showed respectively increased and decreased staining in early and deep melanoma (› 4mm), which is correlative to tissue staining for hyaluronan content. Our data demonstrates the vital importance of the glucose metabolites, UDP-GlcUA and UDP-GlcNAc in hyaluronan synthesis and early stages of melanomagenesis.

CCBE1 enhances lymphangiogenesis by regulating VEGF-C activation

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Lymphangiogenesis is the growth of lymphatic vessels from pre-existing vessels. Vascular endothelial growth factor C (VEGF-C), the major lymphangiogenic growth factor, is produced as an inactive precursor and needs to be proteolytically processed in order to activate its receptors VEGFR-3 and VEGFR-2. Hennekam lymphangiectasia-lymphedema syndrome (OMIM 235510) is an autosomal recessive disease and, in a subset of patients with this syndrome, associated with mutations in the collagen- and calcium-binding EGF domains 1 (CCBE1) gene. Both CCBE1 and VEGF-C are essential for the early stages of lymphatic development that require budding, sprouting and migration of endothelial cells from the cardinal vein (CV), followed by assembly of endothelial cells into lymph sacs. Vegf-c deletion results in failure of lymphatic endothelial cells (LECs) emigration from the CV and Ccbe1 deletion results in abnormal LECs emigration, both resulting in a lack of the lymphatic vasculature. Because of the similar phenotypic features of Vegf-c and Ccbe1 gene deletion studies and because of the lymphangiogenic potential of CCBE1, we studied potential links between CCBE1 and VEGF-C.

Combined application of VEGF-C and CCBE1 in cell culture and in-vivo resulted in enhanced activity of VEGF-C. We found that CCBE1 itself has no effect on VEGF-C activation but regulates VEGF-C activation by A disintegrin and metalloproteinase thrombospondin type 1 motif, 3 (ADAMTS3) cleavage. The processed VEGF-C increased VEGFR-2 and VEGFR-3 signaling as observed by proliferation of Ba/F3 cells stably expressing VEGFR-3/EpoR or VEGFR-2/EpoR chimeras. We also found that both pro-VEGF-C and mature VEGF-C can bind to VEGFR-3 but only mature VEGF-C is able to induce VEGFR-3 mediated signaling as observed in VEGFR-3 phosphorylation study.

This mechanism suggests a possible role for CCBE1 as a therapeutic means to treat diseases that involve the lymphatic system, such as lymphedema or lymphatic metastasis.

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The Syndecan-3 interactome in myogenesis

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Muscle satellite cells are quiescent muscle progenitors residing within the muscle tissue which are required for skeletal muscle maintenance and regeneration. The signalling networks that regulate satellite cell fate decisions are not fully understood. Particularly it is still unclear how satellite cells are maintained quiescent, but it is thought that proteoglycans found within the satellite cells niche play a critical role. Previous studies have shown that genetic ablation of the proteoglycan syndecan-3 in mice dramatically improves muscle health and regenerative potential in ageing and in pathological conditions such as muscular dystrophy. Syndecan-3 is a transmembrane proteoglycan expressed in satellite cells. Syndecan-3 consists of a small intracellular domain, a transmembrane domain, and a large ectodomain containing heparan and chondroitin sulphate attachment sites. Syndecans are thought to interact with their binding partners through their heparan sulphate chains. However, little is known about syndecan-3 interactions and whether they primarily involve the glycosaminoglycan chains or the core protein. Emerging concepts suggest that syndecan-3 core ectodomain contains biological activity independent of its glycosaminoglycan chains. We hypothesise that Syndecan-3 interacts with several proteins during the course of myogenic differentiation both via its core proteins and via its glycosaminoglycan chains. We are using a proteomics approach to identify syndecan-3 binding partners in proliferating and differentiating muscle stem cells using recombinant and endogenous syndecan-3 proteins. We will then use bioinformatics to infer syndecan-3-regulated signaling pathways based on syndecan-3 interactors and cell biology to validate binding partners and signaling pathways. This project will further our understanding of satellite cell homeostasis and syndecan-3 function in myogenesis, and possibly indicate novel therapeutic targets to boost muscle regeneration in ageing and disease.

EGF, IGF and E2 crosstalk regulates breast cancer cell adhesion

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Breast cancer is by far the most common cancer diagnosed in women worldwide. Breast cancer cell functions, such as proliferation, adhesion and migration are regulated by growth factor signaling pathways. Specifically, IGF-I (Insulin-Like Growth Factor- I), EGF (Epidermal Growth Factor) and the steroid hormone Estradiol (E2) are significant regulators of breast cancer cell progression. IGF-I, EGF and E2 have mitogenic and anti-apoptotic actions mediated by their receptors. Furthermore, IGF-I/EGF activation leads to subsequent activation of several signaling pathways such as PI-3kinase and Mitogen- Activated Protein Kinase (MAPK). The crosstalk between IGF-I/ EGF and E2 signaling pathways is critical for breast cancer cell functions. The aim of this study was to investigate the role of Insulin-Like Growth Factor –I Receptor (IGF-IR), and the possible participation of ERK1/2 in the IGF-I, EGF and E2 signaling pathways-dependent breast cancer cell adhesion. MCF-7 cells were used as a biological model. The effect of IGF-I on IGF-IR expression and activation was examined, where it was duly confirmed that IGF activates the IGF-IR receptor in ($p<0,001$) in MCF-7 cells. The adhesion ability of breast cancer cells onto fibronectin was strongly stimulated by IGF-I ($p<0,01$), EGF ($p<0,01$) as well as E2 ($p<0,01$). The attenuation of IGF-IR signaling, decreased the basal ($p<0,05$) and significantly inhibited IGF-I-, E2- and EGF-dependent cell adhesion of MCF-7 cells ($p<0,05$); ($p<0,001$) and ($p<0,001$); respectively. The effects of IGF, EGF and E2 on breast cancer cell adhesion were ERK1/2 dependent. Furthermore, the effects of IGF, EGF and E2 on the activation of ERK1/2 is IGF-IR dependent. Our results suggest that the IGF-IR is a key molecule in breast cancer cell adhesion through IGF-I, EGF and E2-dependent mechanisms. A crosstalk among these factors on breast cancer cell adhesion is suggested with a downstream participation of ERK1/2.

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Trophic factors delivered by cell-based device promote wound healing

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Cell-based therapy is emerging as the next pillar of therapeutic intervention as it mimics natural regenerative processes with reduced systemic side effects in comparison to conventional therapy. Elderly, diabetics, patients with venous leg and pressure ulcers can develop chronic wounds. Stem cells are emerging as one of the most promising approaches for treatment of chronic wounds. Stem cells have the ability to differentiate into different cell types that could replace the damaged tissue, but perhaps their ability to secrete trophic factors promoting regeneration is even more important. The aim of our study is to test the wound healing capacity of a therapeutic device based on cells secreting bioactive molecules, confined within the sealed semipermeable container.

We identified growth factors and other bioactive molecules secreted by stem cells that have a positive impact on wound healing. Murine cell lines NIH3T3 stably expressing bioactive molecules were prepared. We demonstrated that a combination of six bioactive molecules had the greatest effect on the gap closure in fibroblast culture in vitro. Cells confined to the semi-permeable container remained viable and functional for at least 20 days in vitro and *in vivo*. The wound healing effect of engineered cells was confirmed *in vivo* using dorsal splinted excisional wound model on C57BL/6J mice. Reepithelization of wound with implanted therapeutic cell device was significantly faster compared to control with carrier cells. In the presence of soluble factors wound area was filled with granulation tissue that restored skin barrier in only 7 days. Studies evaluating the safety of this cell-based device are underway.

Similar cell-based devices could be used in treatment of a variety of diseases, including neurodegenerative diseases and vascular pathologies.

Evaluation of lumican effects in breast cancer functional properties, gene expression and activity of matrix effectors

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Lumican is a small leucine-rich proteoglycan that has been shown to contribute to several physiological processes, but also to exert anticancer activity and to compromise the activity of membrane type matrix metalloproteinase (MMP-14) [1,2]. On the other hand, it has been recently shown that suppression of the estrogen receptor-α (ERα) significantly alters the functional properties of breast cancer cells in terms of growth, migration, proliferation as well as the gene expression profile of matrix effectors related to cancers progression and cell morphology [2]. Searching for novel pharmacological agents, we therefore found of importance to evaluate the effects of lumican in two breast cancer cell lines [MCF-7 (ERα+) and MDA-MB-231 (ERβ+)] before and after suppression of the estogen receptors-α and β, respectively. It is worth noticing that lumican significantly affects the functional properties (migration, proliferation and invasion) of all cell lines as well as the activity of MMP-14. The effect of lumican was found to be related to the type of breast cancer cells and the estrogen receptor type. These data open a new area for fine pharmacological evaluation of lumican as a potent anticancer agent in solid tumors including invasive breast cancer.

Keywords: Lumican; breast cancer; matrix metalloproteinases; proliferation; migration; invasion; pharmacological targeting;

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A dual inhibition of epidermal growth factor receptor and notch pathway in non-small cell lung cancer cells. Is there a benefit?

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Introduction: The contribution of Epidermal Growth Factor Receptor (EGFR) signaling in lung cancer development is well established. Monoclonal antibodies and inhibitors of tyrosine kinase targeting EGFR are widely used in non-small cell lung cancer (NSCLC) therapy. Despite the promising action of new drugs, it is known in advance that all patients are going to develop drug resistance, underlying the great importance to identify novel and effective combinations of agents based on different molecular mechanisms. Notch may behave both as an oncogene or a tumor suppressor gene in lung cancer cells. Notch receptor undergoes cleavage by enzymes, including γ-secretase, generating the active Notch intracellular domain (NICD), which interacts with specific transcription factors in the nucleus. The aim of the present study was to investigate the effect of both EGFR and Notch blockage in NSCLC cells, using the irreversible ErbB family blocker afatinib and the γ-secretase inhibitor DAPT.

Material and Method: In vitro experiments were conducted in H661 and H23 human NSCLC cell lines, expressing different EGFR and NICD protein levels. Collective cell migration and single cell migration were determined by using the wound-healing assay and the boyden chamber assay, respectively. Secretion of MMP-9 and MMP-2 levels was detected using gelatin zymography. Cell adhesion and spreading dynamic were determined. Immunofluorescence assay was used to illustrate the actin cytoskeleton localization.

Results and discussion: The combination of DAPT and afatinib inhibited both collective and single cell migration in H661 cells, expressing both low EGFR and NICD protein levels while it had no effect on H23 cells that express both high EGFR and NICD protein levels. These results were in accordance with changes in MMP-9 and MMP-2 secretion. In addition, H661 cells did not present any intense spreading dynamic after agents' combination while this effect was not observed in H23 cells. This result was supported by studying the actin cytoskeleton distribution. However, as far as cell adhesion's experiments are concerned, the agents' combination had no significant impact on both cell lines.

Conclusion: The current study presents indications that a dual inhibition of EGFR and Notch pathway might be promising in NSCLC cells that express low EGFR and NICD protein levels. However, these findings merit further investigation.

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Prematurely senescent human breast stromal fibroblasts after exposure to ionizing irradiation: Production of extracellular matrix components and interplay with breast cancer cells

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The importance of stroma in cancer development and progression is recently largely appreciated. Stromal fibroblasts, the most abundant cell type of tumor microenvironment, are responsible for the formation and remodeling of extracellular matrix (ECM), thus they are highly important for the paracrine interplay between tumor and stromal cells. Ionizing radiation represents a powerful tool for the control of tumor growth. However, besides cancer cells, radiotherapy affects also stromal cells and can induce a state of premature senescence. Yet, the role of senescent breast stromal fibroblasts in the formation of tumor microenvironment has not been widely investigated. Accordingly, we developed primary cultures of human breast stromal fibroblasts which were rendered senescent after exposure to ionizing radiation. Senescent fibroblasts express a catabolic phenotype, characterized by an increased expression and activity of matrix metalloproteinases (MMPs), decreased collagen expression and accumulation, down-regulation of aggrecan, as well as up-regulation of ADAMTS-5. In addition, we found an intense up-regulated expression of syndecan-1 (Sdc1) in senescent fibroblasts, at the mRNA and protein level, and this increase is p53-, p38 MAPK- and NF-κB independent. This is of particular interest as it is known that Sdc1 expression is increased in stromal fibroblasts of breast carcinomas and that it promotes breast tumor growth *in vivo*. Moreover, we found that the aggressive epithelial breast cancer cells (MDA-MB-231) increase even more, while non-aggressive MCF-7 decrease, the expression of syndecan-1 in young and senescent fibroblasts, in a paracrine manner. These alterations indicate that ionizing radiation-induced senescence in human breast stromal fibroblasts, as well as stroma-tumor interactions, provoke alterations in the accumulation and organization of ECM components, thus creating a permissive environment for tumor development.

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Suppression of serglycin affects cell behaviour and gene expression of glioblastoma cell lines

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Glioblastoma (GBM), the most common primary malignant brain tumor in human, is characterized by rapid cell proliferation and high infiltration to the surrounding normal brain tissues through degradation of the components of the extracellular matrix (ECM). Serglycin is an intracellular proteoglycan which is overexpressed and be secreted by aggressive tumor cells and plays critical roles in several cellular processes such as proliferation, migration and invasion. Recently, it has been shown that the overexpression of serglycin increases the aggressiveness and the metastatic ability of different kind of tumors, including breast and nasopharyngeal cancer as well as multiple myeloma. As shown by immunohistochemical analysis, GBM tumors express elevated levels of serglycin in contrast to normal brain tissue, a fact that possibly contributes to the aggressiveness of the GBM cells. Considering the above, our goal was the investigation of the role of serglycin in two GBM cell lines, LN18 and U87, which express high levels of serglycin. For this reason, we suppressed the expression levels of serglycin in both LN18 and U87 cells establishing stably transfected cell lines and then we examined crucial functional properties as well as the expression of molecules related with them including various ECM molecules. Our data revealed alterations at some cellular properties of the LN18 and U87 transfected cells such as cell migration and proliferation, as well as at the expression levels of ECM molecules including MMPs. These results imply that serglycin may conduce to the aggressive potential of GBM cells, strengthening the view of the regulatory role of serglycin in several malignancies.

Nanoscale control of growth factors for the enhancement of osteogenic differentiation

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The bone extracellular matrix is composed of a network of proteins and Glycosaminoglycans (GAGs) to which the Bone Morphogenetic Proteins (BMPs) binds and and is released from for cellular activity. Currently, recombinantly expressed BMP-2 is used in clinic to promote the healing of bone fractures. However, the absence of control over the growth factor surface density and cell adhesion induces side effects such as ectopic bone formation, inflammation and cancer. The objective of this project is to develop biomimetic surfaces which present immobilized BMP-2 and BMP-2 bound to Heparan Sulphate (HS), to (i) control the osteogenic differentiation of human mesenchymal stem cells (hMSCs) and (ii) clarify the role of HS in this process.

To characterize the binding between BMP-2 and HS, surface sensitive techniques were used. Thus, Quartz Crystal Microbalance and Atomic Force Microscopy were used to characterize the formation of the biomolecular assembling. In particular BMP-2 was immobilized either on a Streptavidin (SAv) monolayer or on HS bound to SAv monolayer on gold surfaces. Moreover, hexagonal gold nanoparticle arrays, produced by block-copolymer micellar nanolithography, were used to bind BMP-2 to single SAv on each nanoparticle. With this approach it is possible to control and vary the spatial arrangement of BMP-2 bound to SAv-coated gold nanoparticles.

To determine the impact of growth factor internalization on osteogenic differentiation, BMP-2 alone or bound to HS will be presented to human mesenchymal stem cells (hMSCs). Additionally, the surfaced that present both growth factors and adhesive motifs of the ECM will be also developed and used in cell studies.

The biomimetic surfaces proposed here are designed stepwise and with increasing complexity, by binding each component in a controlled manner, in terms of orientation, surface density and spatial arrangement. As such, they offer several advantages and breakthroughs (i) reduced amount of BMP-2: when bound to the surface, the cellular internalization of the growth factors is hindered, resulting in sustained presentation of the molecule to cells; (ii) possibility to enhance the osteogenic differentiation by surface co-presentation of BMPs or/and of ECM adhesive motifs.

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Human serum albumin Cys^{34} oxidative modifications following infiltration in the carotid atherosclerotic plaque

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Human serum albumin (HSA) is the most abundant multifunctional plasma protein. It is a small globular protein that accounts for both antioxidant functions such as ROS/RNS scavenging, extracellular redox balance, redox active transition metal ion binding and transport functions for many molecules such as fatty acids, nitric oxide, hemins and drugs. Albumin has one redox active free cysteine residue (Cys^{34}), responsible for many of these functions. HSA is present primarily in the reduced form (mercaptalbumin), although about 30-40% could be variably oxidized Albumin S-thiolation by low molecular weight (LMW) thiols, such as cysteine (Cys), homocysteine (Hcy), cysteinylglycine (Cys-Gly), glutamylcysteine (Glu-Cys) and glutathione (GSH), is the most common Cys^{34} oxidative modification. Even though high plasma levels of homocysteine are known to be an important risk factor in arterial disease, the pathophysiological processes leading to arterial injury have not been fully understood yet.
The aim of this work was to evaluate if the pro-oxidant environment present in atherosclerotic plaque could oxidatively modify the filtered albumin. In particular we analysed fluoresceine-5-maleimide labelled plasma and plaque extracts from 27 patients who had undergone carotid endarterectomy by non-reducing SDS-PAGE for Cys^{34} oxidation. Furthermore, degree and pattern of S-thiolation in both circulating and filtered albumin were assayed by capillary electrophoresis analysis.
Albumin filtered in the atherosclerotic plaque showed higher levels of Cys^{34} oxidative modification than the corresponding circulating form as well as different patterns of S-thiolation. In this respect, a significant reduction of Cys-Gly (~7 folds) and Hcy (~2 folds) as well as an increase of GSH (~2.8 folds) in plaque-filtered HSA compared to the circulating form was evidenced. Overall, results on Cys34 thiolation show that, once filtered into the plaque environment, HSA releases 15.8 ± 10.9 and 32.4 ± 24.9 pmol / nmol HSA of HCy and Cys-Gly, respectively (corresponding to 16.2 ± 11.2 and 32.8 ± 23.9 nmol/g extracted proteins, respectively), which is noteworthy considering the high HSA levels in plaque extracts (971.7 ± 536.9 nmol/g extracted proteins).
By comparing circulating and plaque-filtered HSA, we evidenced that the pro-oxidant environment present in atherosclerotic plaque could modify filtered proteins also by protein-SH group oxidation, probably contributing to plaque progression. Moreover, the results showed patterns of HSA thiolation specific for the filtered form and demonstrated, for the first time, that albumin is a Hcy and Cys-Gly carrier inside the plaque environment. In this respect, the contribution of GSH to the intra-plaque protein-bound LMW thiols equilibrium seems to be of particular importance. For the first time, such modifications in a plasma protein largely filtered in the subendothelial extracellular matrix of carotid plaque have been described.

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Novel heparan-based drugs for wound healing

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Introduction: Heparan sulfate (HS) glycosaminoglycans are highly complex polysaccharides with information encoded within their chains that imparts the ability to regulate an enormous range of proteins, particularly growth factors [1]. They are constituted of repeating disaccharide units onto which are superimposed specific modification patterns, most notably sulfate groups.

We at A*STAR Singapore's Glycotherapeutics-Stem Cell and Tissue Repair Laboratory within the Institute of Medical Biology have been examining the role of HS during tissue repair. Building on our strong record with HS3, a purified biologic fraction that controls the activity of the powerful bone regeneration factor BMP2 [3], it is our conviction that HS fractions that control the actions of an extensive range growth factors, particularly those that have great clinical potential, including the VEGFs, the PDGFs, the FGFs, the BMPs, the interleukins and the Wnts can be isolated, purified and their disaccharide codes sequenced.

Materials and Methods: HS is synthesized as a homogeneous polymer of glucuronic acid linked to N-acetyl glucosamine, which is then modified by a series of complex sulfation and epimerization reactions [2]. There is thus an ordered structure to an HS chain, in which short protein-binding domains with differing types and density of modifications are spaced apart along the molecule, like "pearls in a necklace". These modifications essentially create a "code". The Carbohydrate Synthesis Group at A*STAR Singapore has recently devised new and unprecedented ways to synthesise very precise HS/heparin sequences at scale to clinical grade.

Results and discussion: We report here on the creation of a completely new class of synthetic therapeutic carbohydrates of the heparan sulfate (HS) class aimed at promoting a wide range of wound-healing capabilities, including accelerated healing in long bone, spine, cartilage, blood vessels and skin. Thus, conceptually, we are deciphering the key sequence "codes" within our HS biologic pipeline so that we can formulate them as fully defined synthetics.

With our experience in both deciphering structure-function relationships in the parent biologics and in fabricating synthetic HS oligosaccharides, we will begin to apply this accumulated know-how to the rest of our HS therapeutic pipeline, which currently numbers over 20 compounds. In so doing, we intend to open a whole new avenue for therapeutic development. We have exemplified sugar-triggered wound healing in mouse, rat, rabbit, sheep, pig and monkey and have just received permission to file our HS3 candidate with the FDA, preparatory to human clinical trials.

Conclusions: Our expertise with these sugars, particularly their structure-function relationships, and our newly acquired synthetic capabilities also allows us to design completely novel forms of these sugars for niche clinical application.

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References:

1. Ori A, Wilkinson MC, Fernig DG (2011) J Biol Chem. 286:19892-904
2. Ori A, et al. (2009) Mol Cell Proteomics 8:2256-65.
3. Murali, S. et al. (2013) Biomaterials 34(22):5594-605

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N-acetylcysteine treatment ameliorates the skeletal phenotype in a mouse model of Diastrophic Dysplasia

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Diastrophic Dysplasia (DTD) is an autosomal recessive chondrodysplasia caused by mutations in the SLC26A2 gene encoding for a sulfate/chloride antiporter of the cell membrane. Nowadays patients are treated only by physiotherapy or orthopedic procedures and no pharmacological therapies are available.

Functional impairment of the sulfate transporter causes reduced sulfate uptake leading to cartilage proteoglycan (PG) undersulfation. In normal conditions intracellular sulfate is mainly dependent on extracellular uptake, but a small fraction comes from the catabolism of sulfur-containing amino acids. In this work using a mouse model of diastrophic dysplasia (dtd mouse) we evaluated N-acetyl-L-cysteine (NAC) as an intracellular sulfate source to ameliorate cartilage PG sulfation and thus the clinical phenotype.

Since the diastrophic phenotype already develops in the fetal period we administered NAC in the drinking water of pregnant mice for the whole pregnancy. By HPLC analysis of fetus homogenates we demonstrated transplacental delivery of the drug to fetuses.

To evaluate NAC treatment cartilage PG sulfation was studied in wild-type and mutant newborns from females treated with NAC or the placebo by chondroitin sulfate HPLC disaccharide analysis: a marked increase of PG sulfation was observed in newborn mutant mice from females treated with NAC respect to mutant newborns from untreated females (78% and 63% respectively of sulfated disaccharides (ΔDi-4S + ΔDi-6S) relative to the total amount of disaccharides (ΔDi-0S + ΔDi-4S + ΔDi-6S)). Differential staining with alcian blue and alizarin red demonstrated skeletal phenotype amelioration towards the normal bone morphology in dtd mice born from treated females compared to mutant mice from untreated females. These observation was supported by morphometric studies: femurs and ilia of dtd treated mice were longer and thinner and tibias were straighter than untreated ones. Finally, μCT analysis demonstrated no mineralization defects. Moreover, by PCNA immunohistochemistry we demonstrated a marked increase of proliferating chondrocytes in tibia epiphyses of dtd treated newborns compared to dtd untreated mice approaching wild-type values.

In conclusion we demonstrated that NAC ameliorates PG sulfation and the skeletal phenotype of dtd mice, suggesting a potential pharmacological treatment of diastrophic dysplasia.

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Structure and antiproliferative activity of xyloside-primed glycosaminoglycans from cancer cells and normal cells *in vitro*

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D-xylopyranosides, or more commonly, xylosides, are a group of compounds consisting of the carbohydrate xylose linked to an aglycon. They can compete with endogenous xylosylated proteoglycan core proteins as substrates for glycosaminoglycan (GAG) chain synthesis. We have previously shown that 2-(6-hydroxynaphthyl) -D-xylopyranoside (XylNapOH), in contrast to 2-naphthyl -D-xylopyranoside (XylNap) and most other xylosides, selectively inhibits tumor cell growth. Since there are indications that the XylNapOH-primed GAG chains could be involved in this process, we isolated large amounts of XylNapOH- and XylNap-primed GAG chains from normal and cancer cell cultures. These were used to characterize their antiproliferative activity in cancer cells and normal cells *in vitro*, and to study their structure.

The results showed that both the XylNapOH- and XylNap-primed GAG chains from the cancer cells had an antiproliferative effect on the cells, although the effect of the XylNap-primed GAG chains was much less pronounced. In contrast, neither of the xyloside-primed GAG chains from the normal cells had any effect on cell proliferation.

Structural analysis of the xyloside-primed GAG chains showed that the majority of the GAG chains consisted of chondroitin sulfate/dermatan sulfate (CS/DS). The CS/DS disaccharide composition of the xyloside-primed GAG chains from the cancer cells was almost identical irrespective of whether they were primed on XylNapOH or XylNap, but differed completely from that of the normal cells. Interestingly, investigations of cell proliferation using enzymatically degraded xyloside-primed GAG chains from the cancer cells indicated that the CS/DS GAG chains were responsible for the antiproliferative activity. There was less heparan sulfate present in the XylNapOH-primed GAG chains from the cancer cells than in the XylNap-primed ones, which could explain why the former had a more pronounced antiproliferative effect.

Taken together, our findings suggest that certain CS/DS structures induced by xylosides in cancer cells have an antiproliferative activity. Further studies will provide us with more detailed structural data and help us elucidate the mechanism of action of the antiproliferative GAG structures.

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Dermatan sulfate is a binding partner for transglutaminase 2

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Transglutaminase 2 (TG2) is a multifunctional protein which is widespread in human tissues where it also exists in extracellular space. In this localization TG2 forms multi-molecular complexes with its binding partners including fibronectin, some integrin subunits and/or syndecan-4 heparan sulfate (HS) chains. These interactions are engaged in cell adhesion to extracellular matrix (ECM). In addition, TG2 has also Ca^{2+}-dependent transglutaminase activity which leads to the formation of cross-links between ECM proteins thereby modifying biomechanical properties of these molecules and their susceptibility to proteolysis. The extracellular expression of TG2 and its activity are especially increased in wound healing or tissue fibrosis. In these processes an enhanced deposition of dermatan sulfate (DS) bearing proteoglycans (decorin but especially biglycan) in ECM is also observed. However, it is unknown whether DS may bind TG2 or influence its activity. The ability of DS prepared from fibrosis affected human fascia to bind TG2 was measured by plasmon surface resonance (SPR) method. *In vivo* relevance of the interaction was verified by an evaluation of TG2 extractability from fascia after enzymatic degradation of the tissue DS. The DS effect on TG2 mediated transamidation activity was estimated by incorporation of cadaverin to collagen or artificial substrate. Our investigation showed that both the total DS from fibrous fascia and DS chains of decorin and especially biglycan derived from this tissue are strong binding partners of TG2 as judged from low values of equilibrium dissociation constant characterizing these interactions. The interaction has some requirements as to DS structure since DS from porcine intestinal mucosa and especially chondroitin-6-sulfate (C-6-S) from shark cartilage bound TG2 with low affinity. DS occupies the same binding site(s) on TG2 molecule as HS, as results from our finding that heparin effectively competes with DS for TG2 binding. In contrast to strong TG2 binding to DS measured by SPR, enzymatic elimination of DS from fascia samples surprisingly yielded marked reduction in TG2 extraction as compared to buffer treated samples. Substantial increase in TG2 extractability was only observed when DS degradation was performed in the presence of anti-collagen type I antibody. In contrast to strong binding, DS from fibrous fascia was unable to affect TG2 transamidation activity at any of used concentrations. In turn, porcine DS and shark C-6-S which have longer chains than fascia DS, only at their highest concentration exerted inhibitory effect on TG2 activity. Our results show that DS regulates TG2 anchoring in ECM and may affect this enzyme activity most probably via control of substrate availability.

Syndecan-4 regulates profibrotic α11β1 integrin expression in the pressure-overloaded heart

P 63

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Fibrotic cardiac disease is a major hallmark of heart failure, categorized by an accumulation of extracellular matrix (ECM) which culminates in a loss of organ function. Cardiac fibroblasts are the main cell type responsible for the maintenance of the cardiac ECM, however upon activation these cells differentiate into myofibroblasts that produce excessive amounts of ECM. The activation of fibroblasts is mediated by fibrogenic cytokines and proteins including transforming growth factor (TGF)β, circulating hormones and mechanical stimulation. The syndecan (SDC) and integrin families are the key mechanosensing receptors in the heart. SDC4 deficient mice (SDC4-/-) develop left ventricular dilatation and dysfunction following pressure overload. The cooperation between SDC4 and integrins has been partly categorized in the context of synergistic control of cell adhesion; however their functional contributions to fibrosis remain obscure.

In vitro stimulation of NIH/3T3 fibroblasts with TGF-β1 or mechanical stretch induced ablation of SDC4 surface levels; however only in combination did these stimulations induce a significant upregulation in SDC4 protein expression ($P < 0.01$). Conversely, α11 integrin cell surface expression was upregulated by either stimulation and further enhanced by combined stimulation ($p < 0.05$). WT mice underwent aortic banding (AB) and the relative mRNA levels of the receptors was measured from between 1 hour and 16 weeks following AB. SDC4 mRNA levels peaked within 6 hours of AB and returned to basal level after 1 week whilst α11 integrin mRNA expression peaked at 1wk and remained higher than sham operated mice at 16 weeks ($p < 0.05$). α11 integrin overexpressing transgenic (α11TG) mice developed cardiac fibrosis within a year of life (unpublished data, Gullberg group). We show without intervention these mice also develop left ventricular wall thickening by 6 months ($p < 0.05$). SDC4-/- mice also had elevated α11 integrin protein expression 3 weeks following AB compared to WT AB mice ($p < 0.05$) whilst no significant difference was observed between sham operated mice, indicative of a compensatory upregulation of α11 integrin when SDC4 is deleted.

We show SDC4 and α11β1 integrin respond to combinations of profibrotic stimulations and together mediate fibrosis in the pressure overloaded heart. SDC4 is an early responder to increased mechanical stress; whilst α11 integrin perpetuates the long term profibrotic response in the pressure overloaded heart.

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N-glycosylation in Tusc3 deficient fibroblasts

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TUSC3 is a subunit of the oligosaccharyltransferase (OST) complex – an integral membrane protein that catalyzes N-linked glycosylation of proteins in the endoplasmic reticulum. N-glycosylation is a post-translational modification occurring to the majority of eukaryotic proteins and plays a pivotal role in the regulation of transport, sorting and the biological function of proteins and consequently in processes like intercellular and cell-matrix interactions. Mutations of TUSC3 have been identified in patients with non-syndromic autosomal mental retardation. This study aimed to investigate the glycosylation level of proteins expressed by fibroblasts isolated from a patient with TUSC3 mutation in comparison to lines of control fibroblasts. The cells were labeled with 3H-mannose and 35S-cysteine/methionine and the incorporation of radioactivity was analyzed in the cell lysates in relation to total protein amount. The results showed increased levels of mannose incorporation in patient cells, which is in contrast to previous observations. This observation, however, was dependent on the cell confluency with a significantly higher glycosylation level at high but not medium confluency.

The growth-promoting function of p53/iASPP is affected by CD44

P 65

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During tumor progression interactions between hyaluronan and its cell surface receptor CD44 affect the growth and differentiation of cells. Using a proteomic approach we recently demonstrated that CD44 interacts with iASPP (Skandalis et al., IUBMB Life, 62:833, 2010), an evolutionary specific inhibitor of p53, which represses the p53-mediated cellular apoptosis both in vitro and *in vivo*.

We have demonstrated endogenous CD44-iASPP complexes in human dermal fibroblast cultures, hepatoma and normal mammary epithelial cells, by performing co-immunoprecipitation experiments as well as a proximity ligation assay. Most interestingly, we found that hyaluronan treatment increases iASPP/CD44 complexes in mesenchymal but not in epithelial cells, indicating different regulatory roles of the complexes in mesenchymal versus epithelial cells. Notably, CD44, iASPP and p53 were found in the same complex. Experiments are currently in progress to investigate how these molecules anchor each other and affect the cellular behavior of fibroblasts. In order to characterize the epitopes of the cytoplasmic part of CD44 that interacts with iASPP, we have generated CD44 mutants: 1) Ser325 have been mutated to Ala (mutation of Ser325 modulates CD44-mediated migration); 2) Mutation of the nuclear localization signal sequence (prevents the translocation of CD44 to the nucleus); 3) $CD44_{D320\text{-}361}$ mutant (prevents CD44 taking part in transcriptional regulation). Transfection of CD44wt and its mutants followed by co-IP with anti-iASPP revealed a stronger interaction between iASPP and each of the CD44 mutants than wt CD44. Thus, most likely cytosolic interactions between CD44 and iASPP determine a non-migratory quiescent fibroblastic fate. Notably, silencing of iASPP specifically inhibited the hyaluronan-induced cell migration in a CD44-dependent manner; Hermes1 antibodies, that specifically block the binding of hyaluronan to CD44, inhibited hyaluronan-mediated migration. Furthermore, preliminary data indicate that the expression of CD44 modulates the propensity of p53 to form complexes with iASPP in the nucleus and inhibition of cell death. This was further confirmed by 3H-thymidine proliferation assay, where the suppression of CD44 resulted in the stimulation of cell proliferation.

Our studies may shed light onto how CD44/iASPP interactions affect the growth-promoting function of p53/iASPP complexes and may lead to identification of strategies to prevent or suppress cancer progression.

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Inhibition of LOXL2: Pharmaxis' small molecule approach to treat fibrosis

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Background

Fibrosis is characterised by excessive accumulation of tough, fibrous scar tissue that results from the ongoing inflammation, excessive extracellular matrix deposition and cell death which is associated with most types of chronic diseases. Currently, transplantation is the only viable therapeutic option for severe cases. As treatment options for fibrosis are very limited research on therapeutic agents aimed at reducing or ideally, reversing fibrosis is of upmost importance. We have developed anti-fibrotic compounds aimed at inhibiting the excessive deposition of extracellular matrix proteins as well as preventing crosslinking of elastin and collagen.

Pharmaxis' approach

Amine Oxidases.

Collagen and elastin fibrils are arranged in different combinations and concentrations in various tissues to provide varying properties such as tensile strength, flexibility and elasticity. This arrangement is in part due to the intermolecular crosslinks, the formation of which is almost exclusively mediated by the LOX family of secreted amine oxidases (lysyl oxidase and lysyl oxidase-like 1-4). These enzymes catalyse the oxidation of primary amines (e.g. lysine residues present on collagen and elastin chains) to aldehydes. These aldehydes are highly reactive, and undergo spontaneous reactions with other lysyl oxidase-derived aldehyde residues or unmodified lysine residues. This leads to the formation of intermolecular crosslinks of collagen and elastin, which are essential for the stabilization of collagen fibrils and for the integrity and elasticity of mature elastin.

Mature crosslinked collagen and elastin are highly expressed in fibrotic disease. Lysyl oxidases are also over-expressed in fibrosis and can be detected in the sera of patients with liver cirrhosis and idiopathic pulmonary fibrosis. In lung fibrosis, LOX (and family) expression is also associated with activated fibroblasts, reactive pneumocytes and vasculature in fibrotic foci. Inhibition of LOXL2 has shown reduced levels of activated fibroblasts and TGF-β pathway signalling in human fibroblasts and bleomycin-treated mice.

Pharmaxis has recently developed PXS-4728A, an orally bioavailable small molecule inhibitor of semicarbazide-sensitive amine oxidase (SSAO), an enzyme which belongs to the same class of copper-dependent amine oxidases as LOXL2. Pharmaxis' in-depth knowledge of structure-activity relationships of amine oxidases has subsequently been successfully applied to the LOX family.

Mannose-6-phosphate analogues.

Mannose-6-phosphate (M6P) is a strong inhibitor of fibrosis and its administration has been associated with beneficial effects in tendon repair surgery as well as nerve repair after injury. Given this promising therapeutic approach we sought to develop an improved analogue of M6P, namely PXS64. PXS64 inhibited extracellular matrix deposition and influenced tissue remodelling by inhibiting TGF-β1 signalling in NHLF and HF19 cell lines, as well as in IPF patient fibroblasts.

The role of EXT-proteins in tumor development

P 67

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Heparan sulfates (HSs), abundant at cell surfaces and in the extracellular matrix, are complex polysaccharides that play dynamic functional roles in a diverse number of biological events related to intracellular signaling and tissue morphogenesis and several aspects of cancer biology including tumorigenesis, tumor progression and metastasis. HS executes its function by the binding to a variety of molecules including growth factors, morphogens and extracellular matrix proteins. By binding diverse growth factors HS may act as tumor promoter or as suppressor. Structural alterations of HS are observed in various cell models of transformation, lung cancer and certain human liver cancers. Tumors may also arise due to defective HS biosynthesis. HS is synthesized in a multi-step process involving a large repertoire of enzymes. Chain elongation in HS synthesis is carried out by a hetero-complex of EXT1 and EXT2. Mutational defects in either EXT1 or EXT2 causes multiple osteochondromas, a human autosomal dominant disorder characterized by cartilage capped bony outgrowth known as osteochondromas or exostoses, at the ends of the long bones. Transcriptional silencing of EXTI is common in human leukemia leading to loss of HS synthesis in the process of tumor onset and progression. Additional gene family members not yet associated with disease include three EXT-like genes (EXTL1, EXTL2 and EXTL3).

We have previously shown that Ext1 mutated embryonic fibroblasts produce short sulfated HS chains with relatively intact sulfation pattern (1). The mutated fibroblasts have a reduced proliferation rate, reduced FGF2 signaling response and reduced ability to adhere to and to contract collagen gels (2). Furthermore, stromal fibroblasts Ext1 levels strongly influences tumor cell behavior and the interstitial fluid pressure in multicellular spheroid mini tumors (3).

The present project focuses on EXT1 and EXT2, glycosyltransferases involved in HS chain elongation and how structural changes in EXT1 and EXT2 influences HS structure and function in cancer cells.

References:

1. Yamada, S., Busse, M., Ueno, M., Kelly, O. G., Skarnes, W. C., Sugahara, K., and Kusche-Gullberg, M. (2004) *J Biol Chem*279, 32134-32141

2. Osterholm, C., Barczyk, M. M., Busse, M., Gronning, M., Reed, R. K., and Kusche-Gullberg, M. (2009) *J Biol Chem*284, 34935-34943

3. Osterholm, C., Lu, N., Liden, A., Karlsen, T. V., Gullberg, D., Reed, R. K., and Kusche-Gullberg, M. (2012) *PLoS One*7, e41334

P 68

Role of syndecan-1 on cancer epithelial-to-mesenchymal transition and metastasis via integrin activation by Manganese

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Events leading to the metastatic disease depend on the acquisition of an invasive phenotype by cells in the primary tumor, a process called epithelial-to-mesenchymal transition (EMT). During EMT, cancer cells acquire a mesenchymal phenotype with migratory activity via cell surface and extracellular matrix molecules. Heparan sulfate proteoglycans (HSPGs) have been implicated in cancer development due to their ability to bind key growth factors and regulate cell adhesion and migration. Recent work has also shown integrin activation via the HSPG syndecan in tumoral and non-tumoral migrating cells in vitro. Integrins are divalent cation-dependent molecules, and manganese (Mn) promotes integrin activation by high affinity binding. These observations lead to the hypothesis that the equilibrium between syndecan-1 expression and Mn availability influence tumor cell EMT and migration to new metastatic sites and manipulation of these elements may alter tumor development and outcome. Our results show that when murine Lewis lung carcinoma (LLC) cells are submitted to a scratch assay in vitro, migration is accelerated in the presence of Mn. Stimulation with TGF-β1 enhances this aspect, as expected due to its EMT trigger properties. Also, cell surface syndecan-1 levels were upregulated in LLC cells in the presence of Mn. In order to investigate if heparan sulfate analogues, such as the ascidian *Styela plicata* dermatan sulfate (ascDS), could compete with endogenous HSPGs, we challenged Mn-treated cells with ascDS, verifying that migration levels return to control. In addition, syndecan-1 expression and Mn content were analyzed in experimental model of spontaneous metastasis. Primary tumors and metastatic livers cryosections were mapped by X-ray fluorescence and we observed close proximity of Mn "hotspots" and high syndecan-1 cell clusters and also by X-ray fluorescence, we found a decreased concentration of blood Mn and high concentration of this cation on liver as the disease progresses suggesting a displacement of blood Mn to metastatic organs. These analyses point to an intimate relationship between Mn and syndecan-1 in the successive events leading to metastasis.

The inhibition of IL-8 pathway reduces the aggressiveness of ERα suppressed MCF-7 breast cancer cells

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Estrogen receptor alpha (ERα) and its signaling pathway are of high significance in hormone-dependent breast cancer since about 70% of breast cancers are detected as ERα positive. It has been shown that ERα suppression induces EMT thus enhancing the aggressive behavior of breast cancer cells, as well as significantly alters the expression profile of a variety of molecules related with the aggressiveness of breast cancer cells. Inflammatory mediators such as chemokines and their receptors regulate crucial cell functions of breast cancer cells, such as migration and invasion. The aim of our study was therefore to evaluate the contribution of chemokines and their signaling pathways on the enhanced aggressive phenotype and behavior of ERα suppressed MCF-7 breast cancer cells. For this reason we investigated the constitutive expression levels of chemokines CXCL-1, IL-6, IL-8 and their receptors, as well as the effect of their signaling on several cell functions and cell morphology of ERα suppressed MCF-7 cells, referred as SP10+. Our data showed that, ERα suppression strongly increased both gene and protein levels of chemokines, especially that of IL-8. The inhibition of IL-8/CXCR2 signaling axis rapidly rearranges cell spreading and morphology and significantly reduces cell proliferation, migration and invasion. The above data suggest a regulatory role for IL-8, thus enhancing the perspective of its inhibition as a potent anticancer approach in aggressive breast cancers.

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Heparanase in pediatric brain tumors

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Heparanase (HPSE) is an endo-β-D-glucuronidase which has the capacity to cleave heparan sulfate side chains at a specific number of sites, thus yielding fragments of still appreciable size 5-7 kDa (1). Overexpression of HPSE has been detected in a wide cohort of tumors (2). However, there are not studies investigating the role of HPSE in pediatric brain tumors. Hence, the Forsberg-Nilsson lab has recent data of its involvement in glioma, as well as pediatric Medulloblastoma (MB) and supratentorial PNET brain tumors. Robust phosphorylation of Erk, Akt and Src was shown after addition of recombinant HPSE (rHPSE), suggesting the important role of HPSE on the tumor cell lines. Consequently, it becomes increasingly clear that HPSE has many functions besides its enzymatic activity. By using lentiviral transduction and introducing shRNA, we have successfully reduced the levels of HPSE in tumor cell lines and we further investigated proliferation, invasion and migration of these cell lines. Based on our findings, addition of rHPSE leads to higher rate of cell proliferation while reduced levels of HPSE attenuated the invasion capacity of the cells in collagen matrix, but also the cell proliferation and migration in specific cell lines.

Our research has been expanded on further investigating the role of this unique enzyme in the pediatric brain tumors by using a small-molecule HPSE inhibitor (HPSEinh). This small molecule which has the capacity to block the activity of HPSE reduced tumor cell proliferation, migration but also invasion in a 2D and 3D collagen matrix. Moreover, we have shown that the HPSE inhibitor affects the activation of signaling pathways (Erk, Akt, FAK) related to proliferation, survival and invasion. Most recently, we found a significant reduction of the growth and weight of subcutaneous sPNET and MB tumors *in vivo* after treatment with the HPSEinh. These intriguing results encourage us to investigate *in vivo* invasion, migration of the cells but also angiogenesis, tumor vascularization, immune cell infiltration, metastasis and last but not least differentiation of the tumors cells.

Heparanase has been associated to increased cancer metastasis, increased angiogenesis and significantly reduced post-operation survival of patients, providing thus a reliable diagnostic biomarker in a specific tissue for the detection of malignancy and an intriguing target for drug development against brain tumors.

Transforming growth factor-beta 1 is a major mediator of tissue responses to radioactive iodine therapy

P 71

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Introduction: The pleiotropic cytokine TGF-β is involved in the regulation of numerous aspects of the immune response, including T cell homeostasis and plays a crucial role in the response to ionizing radiation. TGF-β can exhibit strong immune suppression but has also been shown to promote T-cell growth. Previous studies have reported that ablative radiotherapy dramatically increases T-cell priming in draining lymphoid tissues, leading to reduction/eradication of the primary tumor or distant metastasis in a CD8+T cell–dependent fashion.

Purpose: We aimed to investigate the differential effect of TGF-β1 on CD8+ T-cells and MMP-9 during tissue remodeling in response to radioactive iodine (I-131) therapy in patients with papillary thyroid cancer (PTC) and papillary thyroid cancer associated with Hashimoto's thyroiditis (PTC+HT).

Methods and patients: Peripheral blood samples were collected from patients with PTC (8M/46F) and PTC+HT (3M/38F) before and at 96 hours after I-131 (3.7 GBq). The lymphocyte subpopulations were measured by flow cytometry and the serum levels of TgAb, TGF-β1, MMP-9, TIMP-1 by ELISA.

Results: In PTC group, I-131 therapy resulted in a reduction of TGF-β1 with 15% (P=0.001), MMP-9/TIMP-1 ratio with 44% (P=0.003) and an increase with 11% of CD8+ T-cells count number. The reduction of MMP-9/TIMP-1 ratio was negatively correlated with TGF-β1 level at follow-up ($r = -0.73$, $P<0.001$). I-131 therapy of PTC+HT patients lead to a rise with 18% in TgAb level (P=0.001), 9% in CD19+ B-lymphocytes (P=0.03), 27% in TGF-β1 (P=0.001), 5% in MMP-9/TIMP-1 ratio (P=0.003) and a reduction with 8% in CD8+ T-cells count number (P=0.02). TgAb titers were positively related to MMP-9/TIMP-1 ratio ($r = 0.51$, $P<0.001$) and negatively to TGF-β1 ($r = -0.56$, $P<0.001$).

Conclusions: TGF-β1 is a major mediator of tissue responses to I-131 therapy. In PTC patients TGF-β1 inhibition enhances antitumor immunity mediated by CD8+ T-cells and decreases the imbalance between MMP-9 and TIMP-1. In PTC+HT patients, elevated TgAb titers are associated with increased TGF-β1 concentrations and decreased tumor-specific CD8+ T-cell response. TGF-β1 activation decreases the antitumor efficacy of I-131 therapy in patients with coexisting papillary thyroid cancer and Hashimoto's thyroiditis.

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Extracellular vesicles as carriers of hyaluronan and their role as novel biomarkers

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Hyaluronan (HA) is the most abundant and essential polysaccharide of the extracellular matrix for maintenance of normal tissues, which also promotes cancer progression by creating a favorable microenvironment for growth of tumor cells. In many pathological states like cancer and inflammation the amount of HA around the cells increases. HA is synthesized by specific plasma membrane-bound enzymes, hyaluronan synthases (HASs). The HASs activate growth of extremely long filopodia coated with hyaluronan which induces shedding of extracellular vesicles (EVs) originating from the tips of filopodia (Rilla et al., 2013). This plasma membrane derived EVs of different size secreted by many cell types are thought to carry and transfer cytosolic components, proteins, RNA, ribosomes and selected plasma membrane proteins. Interestingly, EVs are suggested to interact with their target cells by utilizing receptors on the vesicular surface, such as CD44, which is the most common receptor for hyaluronan. These CD44-hyaluronan interactions may act as universal mechanism facilitating cellular binding and uptake of EVs.

The quantification of the hyaluronan-coated EVs secreted by GFP-HAS3 overexpressed cells using techniques like immunoblotting and qPCR showed the presence of GFP HAS3 (both as protein and mRNA). Moreover by using live cell confocal microscopy and correlative light and electron microscopy, attachment of GFP-HAS3 containing EVs (i.e. HAS3-EVs) on target cell membrane showed cell morphological changes and also increased pericellular hyaluronan coat. The HA secretion of target cells analyzed by ELISA-like assay showed increased levels of HA indicating possible induction by HAS3-EVs. Furthermore, the binding of HAS3-EVs were blocked using CD44 blocking antibody which decreased the vesicle binding to the target cell, thereby showing that HAS3-EVs interacted with CD44 receptor for binding to target cells.

HA-induced EVs act as carriers for hyaluronan on their surface and are potential vehicles in preparing the premetastatic niches. Accumulation of hyaluronan acts as a poor prognostic factor in many epithelial cancers, and non-invasive methods to detect hyaluronan levels in cancer patients would be valuable in diagnostics. Furthermore, hyaluronan-induced EVs could be utilized as diagnostic tools and targets of therapy.

Novel types of autosomal recessive cutis laxa caused by mutations in the vacuolar ATPase complex

P 73

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Acidification of vesicular compartments is crucial for the function of intracellular trafficking pathways. Defects in this process caused by mutations in genes encoding one of the subunits of the vacuolar-type H+-ATPase (V-ATPase) cause autosomal recessive cutis laxa (ATP6V0A2), osteopetrosis (ATP6V0A3) and distal renal tubular acidosis (ATP6V0A4, ATP6V1B1). We now identified mutations in two other components of the V-ATPase complex using whole exome sequencing. We found a homozygous missense mutation in ATP6V1E1 in an Iranian consanguineous pedigree with severe and generalized cutis laxa, arthrogryposis, congenital hip dysplasia, and severe aortic root dilatation and biventricular hypertrophy. In addition, we found biallelic mutations in the ATP6V1A gene in a German boy with severe cardiomyopathy, abnormal fat distribution, cataract, white matter abnormalities, and cutis laxa that improved with age. Brefeldin A treatment of the patients' dermal fibroblast cultures significantly delayed the retrograde translocation of Golgi membranes to the endoplasmic reticulum, similar to observations in cell cultures from patients with ATP6V0A2 related cutis laxa. Ongoing experiments are focusing on the impact of these defects on vesicular trafficking and their consequences for the secretion of extracellular matrix components such as elastin and collagen.

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Salivary gelatinases as a useful tool for head & neck cancer diagnosis

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Matrix metalloproteinases (MMPs) are a major class of enzymes implicated in cancer. In head & neck tumors, various MMPs are found to be involved in cancer growth and progression, depending to the anatomic site. Among these, gelatinases (MMP-2 and -9) seems to play a key role. Since a simple and non-invasive assay for cancer head & neck diagnosis is not described so far, the presence work focused in the analysis of gelatinases in saliva of head & neck cancer patients.

Saliva of 36 patients suffering of head & neck cancer were obtained and applied to gelatinases' zymography and western blotting. The patients were divided to two groups, one of the laryngeal cancer patients (23) and the other of the oral cancer patients (13). Saliva was also obtained from healthy volunteers (12).

Total gelatinolytic activity was increased in saliva of cancer patients, as compared with the healthy subjects. The increase was more than 100% in oral cancer and very low in laryngeal cancer. The gelatinolytic bands present differed between the various groups. Healthy saliva contained five main bands, oral cancer saliva contained four main bands and laryngeal cancer saliva contained seven main bands. Healthy saliva contained only the latent form of MMP-9, whereas in cancer the active form was also detected. The most characteristic feature in saliva gelatinases of laryngeal cancer patients was the presence of both latent and active MMP-2, being absent in other saliva samples.

The obtained data suggested that gelatinases' analysis could be applied for head & neck cancer differential diagnosis. More data, however, are required and especially of early cancer patients to verify the applicability of the assay.

Profile of metalloproteinases of the pulmonary extracellular matrix in obesity-associated asthma

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Asthma is a complex chronic inflammatory disorder. The relative magnitude, type of inflammatory cells and site of inflammatory infiltrate may differ among patients. In the immune and inflammatory responses to allergens in asthma, T cells, mast cells, neutrophils and eosinophils are present. The increase in the prevalence of both asthma and obesity has prompted researchers to suggest that obesity may have an important role in the development of asthma, or even worsen the pre-existing asthma. We showed recently that obese mice fed with a high-fat diet for 10-12 weeks become insulin resistant, and exhibit exacerbation of pulmonary eosinophilic infiltrate and marked bone marrow eosinophil accumulation (Calixto et al., 2010, 2013). Recent advances in the pathogenesis of asthma have revealed that remodeling of the airways is due, at least in part, to deposition of excess of extracellular matrix (ECM) in the airway walls, which may play a significant role in airway obstruction. The metalloproteinases of extracellular matrix (MMPs) are key proteolytic enzymes responsible for the remodeling of the airways and / or severity of asthma. Obesity and asthma individually elevate the levels of TNF-α, that lead to induction of metalloproteinases. Based on these findings, we find relevant to study the imbalance of MMPs and consequently the elements of MEC in obese-asthmatics. Thus, in this study we proposed to elucidate the pathophysiological profile of the lung ECM of obese animals challenged with the antigen ovalbumin (OVA), with particular attention in the activity of MMP-2, 8, 9, 13, as well as the amount of fibronectin, collagen type I and IV found in the lung. We divided the experimental groups into: non-sensitized lean (NSL), non-sensitized obese (NSO), sensitized lean (SL) and sensitized obese (SO). The activity of MMP-2 and proMMP-2 was high in both asthmatics groups; however, low activity of MMP-2 was detected in SO group. Pro and active isoforms of MMP-9 showed a high activity in SO in comparison with SL group. Besides, MMP-8 exhibited a decrease in SO. Both sensitized groups showed a low presence in pro and active isoform of MMP-13. It was found high amount of fibronectin in sensitized compared with non-sensitized group. Between the sensitized groups, SO showed a high concentration of fibronectin. Collagen IV showed low amount in SO. There were no differences in sensitized groups in relation to amount of collagen type I. However, NSO showed a high quantity of the collagen type I in comparison with NSL. It was noted an unbalance of the MMPs in the lung of lean and obese animals. MMP-9 is present in chronic inflammation, and in elevated levels were noted in SO group. In addition, in SO group there was a decrease in MMP-2 and -8 activity. Both enzymes are important to degradation and remodeling process that can be a favorable during the asthma. In view of the complexity of the MMPs, it is necessary to study its inhibitors aiming an effective remodeling process and a decrement of the asthma in obese.

References:

Calixto MC, Lintomen L, Schenka A, Saad MJ, Zanesco A, Antunes E. Obesity enhances eosinophilic inflammation in a murine model of allergic asthma. Br J Pharmacol. 2010; 159(3):617-625.

Calixto MC, Lintomen L, Andrı DM, Leiria LO, Ferreira D, Lellis-Santos C, Anhê GF, Bordin S, Landgraf RG, Antunes E. Metformin attenuates the exacerbation of the allergic eosinophilic inflammation in high fat-diet-induced obesity in mice. PLoS One. 2013; 8(10):e76786.

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Helicobacter pylori CagA protein induces factors involved in the Epithelial-to–Mesenchymal-Transition (EMT) in infected gastric epithelial cells in an EPIYA - phosphorylation-dependent manner

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After adhesion of H. pylori to gastric epithelial cells, the bacterial effector CagA is translocated intracellularly and following its hierarchic phosphorylation in repeating EPIYA phosphorylation motifs, has been demonstrated to deregulate cellular polarity, inducing the appearance of an elongation and scattering phenotype that resembles epithelial to mesenchymal transition (EMT). In epithelial cells, stromelysin-1 (matrix metalloproteinase 3, MMP3) has been reported to induce a sequence of molecular alterations leading to stable EMT conversion and carcinogenesis. In this study, we investigated the putative role of phosphorylated CagA protein, in the activation of MMP3 as well as, other factors involved in mesenchymal transition and stemness ability. We utilized isogenic mutants of the P12 H. pylori reference strain, expressing CagA protein with variable number of functional terminal EPIYA and corresponding phosphorylation deficient EPIFA motifs. Included in the study were also the cagA- and cagE-gene knock-out strains, the latter being defective in its type IV secretion system. Following in vitro infection of AGS and MKN-45 gastric epithelial cell lines, increased transcriptional activation of MMP3 gene was determined by Real Time quantitative PCR. This was closely associated to the expression and functional translocation of CagA, as well as, the number of repeating terminal EPIYA phosphorylation motifs. MMP3 protein expression in total cell lysates of infected cells was also found to be associated to CagA expression, translocation and intracellular phosphorylation ability. Similarly, levels of secreted MMP3 protein in cell culture supernatants, appeared to be CagA phosphorylation-dependent. Corresponding increase in gelatinase and caseinolytic activity was detected in culture supernatants, utilizing zymography. Interestingly, in total cell lysates of H. pylori-infected AGS cells, expression of stromelysin-2 (MMP-10), which shares an 82% homology in amino acid sequence with MMP3, was observed to be infection-independent. Significant increase in the transcriptional activation of mesenchymal markers Snail, Vimentin and ZEB1 as well as, stem cell marker CD44, was observed in a CagA EPIYA phosphorylation-dependent manner. Collectively, our results suggest that following H. pylori infection, CagA can induce a variety of factors involved in epithelial to mesenchymal transition in an EPIYA phosphorylation-dependent manner, thereby contributing to carcinogenesis and metastasis in the gastric mucosa.

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Antiproliferative and anti-metastatic profile for glioblastoma multiform of a novel tumor homing compound

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Introduction: Glioblastoma Multiform (GBM), is one of the most fatal types of cancer with a median patient survival of 12 to 15 months. Current therapies include radiotherapy concurrent with temozolamide. Bevacizumab has been approved recently, as adjuvant therapy for patients with progressive GBM. Another therapeutic perspective involves tyrosine kinase inhibitors (TKIs), investigating single or multiple targeting concomitantly. Unfortunately, the majority of therapeutic approaches are so far disappointing, emerging crucially the need for novel, more effective treatment agents. In the current project we developed a tumor homing peptide able to selectively kill glioblastoma multiform cells without affecting normal cells.

Materials and Methods: Synthesis of the peptide was performed using classical Fmoc-a-aminoacid based synthesis. The novel agent was tested for its effect in both GBM cells (U87, LN18 and M059K) and non cancer cells (L-929). Cell number was estimated using MTT assay. Furthermore, we evaluated the novel compound's effect on 3D culture in soft agar. In addition, several types of cell death were determined including apoptosis and necrosis as well as cell cycle arrest. Annexin V and dead cell assay kit were used for apoptosis and necrosis and a cell cycle assay kit for the distribution of cell cycle phases. Wound healing and boyden chamber assay were used for evaluating the ability of cells to migrate and zymography was performed to estimate secretion of MMP-2.

Results and discussion: The novel compound exerted an antiproliferative effect in all GBM cell lines in 2D and 3D cell cultures. However, it did not affect L929 cells, a normal fibroblast cell line that was used as control. The anti-proliferative effect was associated with a specific type of cell death. This effect was in accordance with reduced cell migration and secretion of MMP-2

Conclusions: Herein, we have developed a novel tumor homing peptide that selectively targets glioblastoma cells and decreases both cell proliferation and migration. The novel compound is able to be used as a carrier of biological cargoes and/or cytotoxic agents, too. In addition, the novel compound could be used as a theranostic agent if combined with an appropriate fluorophore.

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Resistance to hormone therapy in breast cancer cells favors an aggressive phenotype, regarding migration and mechanical properties

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Introduction: Breast cancer is considered to be, the most frequent malignancy in women worldwide. The majority of breast tumors (75%), are estrogen receptor positive (ER+) and their growth is stimulated by estrogens. Therapies based on anti-estrogens, represent the mainstay treatment in clinical practice using Fulvestrant (Fulv) and Tamoxifen (Tam) as the key drugs for the manipulation of ER+ breast cancer patients. However, in many patients resistance has been observed, either acquired or de novo that must be addressed. Our aim is to study the profile of breast cancer cells with acquired hormone resistance regarding cell motility and mechanical properties.

Materials and Methods: We developed clones of breast cancer cell line MCF-7, resistant to Fulv and Tam named hereafter as MCF-7/Fulv, MCF-7/Tam. Scanning electron microscopy (SEM) was applied to observe cell morphology and 3D cell culture in soft agar was performed to estimate cells' growth. Furthermore, scratch-wound assay and modified Boyden chamber assay were applied for studying cell migration and invasion, respectively. Micropipette method was used to determine the mechanical properties of cells prior and after resistance to hormone therapy. The evaluated parameters were elastic shear modulus (G) and viscosity (n) that indicate cell stiffness and rheology, respectively. Real Time PCR was performed in order to evaluate gene expression of MMPs, uPA and uPAR.

Results and Discussion: Resistance to endocrine therapy stimulated both clones' growth in 3D soft agar. In addition, the resistant cells exerted increased ability to migrate and invade. More specifically, MCF-7/Fulv were more aggressive regarding migration compared to MCF-7/Tam, whereas MCF-7/Tam found to be more invasive compared to MCF-7/Fulv. Regarding the mechanical parameters of cells, elastic shear modulus was found to be elevated in both resistant clones compared to untreated cells, in accordance with the migration pattern. Furthermore, the viscosity of clones was decreased in MCF-7/Fulv and increased in MCF-7/Tam. SEM analysis was in line with the changes in cell viscosity whereas MCF-7/Fulv demonstrated monolayers while MCF-7/Tam formatted multilayers. Gene expression of MMPs, uPA and uPAR was in line with the aggressive phenotype of cells.

Conclusions: Our data show that the resistance of MCF-7 cells to hormone therapy provoked a more aggressive phenotype and altered their mechanical behavior in a way that favors their metastatic progression.

Evaluating the pro-fibrotic effects of IL-17A using precision-cut tissue slices

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Background: Interleukin (IL)-17A is a pro-inflammatory cytokine that has been implicated in fibrosis in various tissues; therefore, it has a potential utility for antifibrotic drug targeting. Recently, we developed ex vivo precision-cut tissue slices (PCTS) culture technique to model the early onset of fibrosis.

Objective: Our objective was to investigate the effect of IL-17A on viability and expression of fibrosis markers in murine intestinal and liver PCTS

Methods: Murine PCTS (jejunum and liver) were prepared by slicing tissue cores in ice-cold Krebs-Henseleit buffer using Krumdieck tissue slicer. The slices with a wet weight of 3-4 mg had an estimated thickness of 300-400 µm. PCTS were then incubated in multi-well plates for 48 h in Williams Medium E with L-glutamine supplemented with glucose and gentamycin (and additionally fungizone for intestinal PCTS) in the presence of IL-17A (50 ng/mL) or vehicle (water). During the incubation plates with slices were kept at 370C and 80% O2/5% CO2 with continuous shaking. The viability of tissue slices was assessed by measuring the ATP content (n=6) of the slices. Gene expression of the fibrosis markers, such as pro-collagen 1a1 (Col1α1), alpha smooth muscle actin (αSMA), heat shock protein 47 (Hsp47), and fibronectin (Fn2), was determined by real-time PCR (n=6).

Results: Murine intestinal and liver PCTS remained viable up to 48 h of incubation in the presence of 50 ng/mL IL-17A. Intestinal PCTS treated with vehicle (water) for 48 h showed statistically significant increase in the expression of HSP47 (4.4 fold) and Fn2 (3.4 fold) and decrease in Col1α1 (0.58 fold) and αSMA (0.07 fold) gene expression compared to 0 h control. In similar conditions, liver PCTS showed significant increase in Col1α1 (13.3 fold), HSP47 (3.9 fold) and Fn2 (44.7 fold) gene expression. IL-17A further induced significant up-regulation of fibrosis markers in both intestinal PCTS (Col1α1, 1.3 fold; Fn2, 1.6 fold) and liver PCTS (Col1α1, 2.1 fold; Hsp47, 1.8 fold and Fn2, 1.75 fold).

Conclusions: The pro-inflammatory cytokine IL-17A showed clear pro-fibrotic effect on gene expression of most fibrosis markers in murine intestinal and liver PCTS, confirming its crucial role in development of tissue fibrosis. On the other hand, IL-17A has no effect on αSMA gene expression, indicating that this cytokine is unlikely involved in the regulation of alpha-smooth muscle actin. The investigation of the effects of IL-17A in human PCTS is currently ongoing. PCTS technique is a promising model to assess the mechanisms and key readouts for fibrosis in rodent, as well as in human, tissues.

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