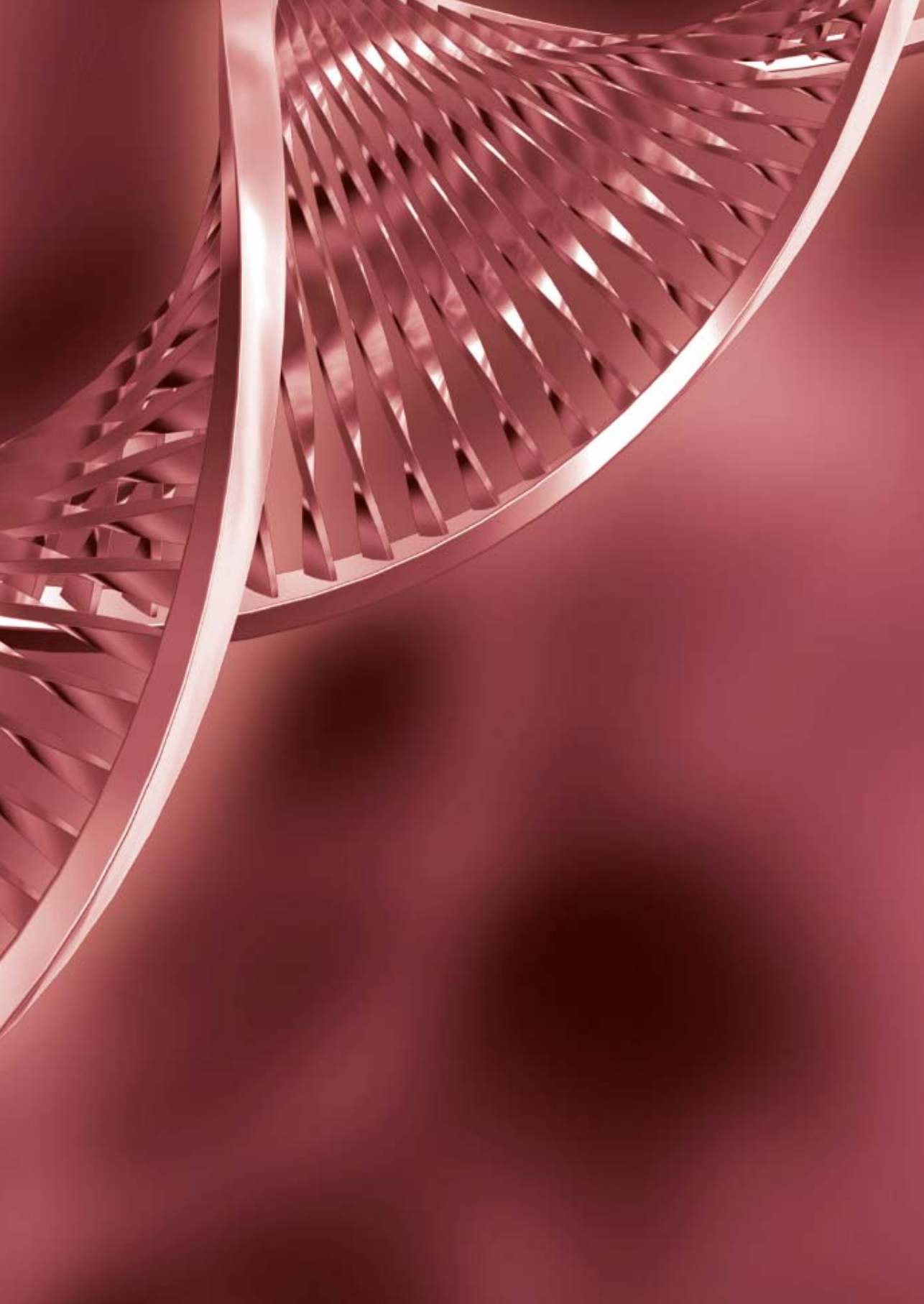
4th FEBS Advanced Lecture Course
FEBS – MPST 2013

Matrix Pathobiology, Signaling and Molecular Targets

Program & Abstracts

September 26–October 1, 2013
Kos, Greece



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Kos, September 26–October 1, 2013

Organizing Committee:

Nikos K. Karamanos (chairman)
Laboratory of Biochemistry, Department of Chemistry, University of Patras, 26110 Patras, Greece

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Laboratory of Biochemistry, Department of Chemistry, University of Patras, 26110 Patras, Greece

Conference venue:

Kipriotis Village Resort located at the Island of Kos-Greece

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

http://www.febs–mpst2013.upatras.gr
e–mail: febs–mpst2013@chemistry.upatras.gr

Course Secretariat:

C.P.R., Conferences – Public Relations • 11 Petmeza str. 26442, Patras Greece • tel: +30 2610 995698, fax: +30 2610 995691
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Preface

Dear Colleagues and Friends,

On behalf of the Organizing Committee, it is a pleasure to invite you to the **4th FEBS Advanced Lecture Course on Matrix Pathobiology, Signaling and Molecular Targets (4th FEBS-MPST 2013)**, which follows the previous successful FEBS-MPST 2011, 2009 and 2007 meetings.

Matrix Biology is a fast growing field with significant impact in all areas of Biosciences. Based on the great advances that have occurred during the last years in the field of matrix pathobiology and the increasing research interest on matrix organization and the matrix-mediated regulation of the various cell functions, the FEBS-MPST 2013 will offer oral sessions with **invited plenary lectures, general lectures and tutorials, selected talks** related to the topics of the presented abstracts, participant **poster presentations, panel discussions** and **speakers' corner**. These sessions will address both basic and applied science topics that appeal to the range of participants working in the fields of Proteoglycans, ECM in immune regulation, Cell surface receptors, Matrix organization, Interactions and functions of matrix macromolecules, Cell receptors signaling and ECM based nanotechnology, ECM and tumor microenvironment-cancer, Matrix pathobiology, Glycobiology and metabolic regulation of ECM molecules, Signaling and disease molecular targeting, Matrix regulation in health and disease.

The organizing committee has put together an outstanding group of internationally recognized speakers. The lectures and tutorials will provide you with an update of important new knowledge covering key areas of the field.

The most important goal of the **FEBS-MPST 2013** meeting is to bring together scientists from life sciences on an important and rapidly developing scientific field and to create the environment for a superb science, warm collegiality, and an all-around rewarding experience during this special time of year.

The course will be held at the Kipriotis Village Resort located at the Island of Kos, Greece. Kos Island has a long tradition of academic tourism, and every year it attracts a serious number of academic and research bodies/organizations. The Federation of European Biochemical societies (FEBS) is a major sponsor of this event, supporting both organization and the young scientists offering them young Travel Fellowships (YTF). We would also like to acknowledge the contribution of the University of Patras, the University of Patras Reasearch Committee, the Hellenic Society of Biochemistry and Molecular Biology and the other sponsors supporting the "FEBS–MPST 2013 Young Investigator Awards".

We are looking forward to welcoming you in Kos Island, Greece for an exciting and memorable scientific meeting. 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.

Nikos Karamanos
Chairman of the Organizing Committee

FEBS Advanced Lecture Course

Matrix Pathobiology, Signaling and Molecular Targets

Kos, 26 September - 1 October, 2013

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

Thursday, 26 September

14:30-16:00 Registration

Chairpersons: Vince Hascall and Nikos Karamanos

LECTURES / TUTORIALS - PROTEOGLYCANS (L1-L4)

16:00-16:20 J. Phillips *(University of California, USA)*
Proteoglycans in the brain tumor microenvironment

16:20-16:40 A. Theocharis *(University of Patras, Greece)*
Roles of serglycin in inflammation and cancer

16:40-17:10 S. Pals *(Academic Medical Center, Netherlands)*
Syndecan-1 is crucial for multiple myeloma growth and controls Bortezomib sensitivity and side-population size

17:10-17:30 S. Wilcox-Adelman *(University of Massachusetts Medical School, USA)*
Syndesmos-a partner in syndecan-4 mediated cell migration

17:30-18:00 Coffee break

SELECTED TALKS (ST1-ST6)

18:00-18:10 T. Szatmári (*Karolinska Institute, Sweden)*
Syndecan-1 overexpression inhibits SULF1 and modulates heparan sulfate chain composition in malignant mesothelioma cells

18:10-18:20 K. Pietraszek *(Université de Reims, France)*
Effect of Lumcorin, a lumican-derived peptide, on the melanoma cell growth and invasion

18:20-18:30 K. Herum *(Institute for Experimental Medical Research, Norway)*
Syndecan-4 promotes myocardial stiffness by regulating myofibroblast differentiation and extracellular matrix structure in response to pressure overload

18:30-18:40 G. Heidari-Hamedani *(Karolinska Institutet, Sweden)*
Syndecan-1 overexpression on mesotheliomal cells affects angiogenesis - related factors

18:40-18:50 A. Friedl *(University of Wisconsin -Madison, School of Medicine and Public Health)*
Stromal syndecan-1, matrix alignment and breast cancer invasion

18:50-19:00 F. Mundt *(Karolinska Institutet, Sweden)*
Syndecan-1 as a soluble biomarker for human malignancies

19:00-19:15 Welcome Addresses by the Chairman and members of the Organizing Committee
FEBS activities

OPENING LECTURE (L5)

19:15-20:00 J. Couchman *(University of Copenhagen, Denmark)*
Syndecan proteoglycans: specificity and redundancy in signaling to the cytoskeleton

20:30 Welcome Reception

Friday, 27 September

Chairperson/discussion leader: Evi Heldin

LECTURES / TUTORIALS - GLYCOBIOLOGY (L6-L8)

9:00-9:30 P. Heldin *(Ludwig Institute for Cancer Research, Uppsala, Sweden)*
HAS2 and CD44 signaling in breast cancer tumorigenesis

9:30-09:50 J. Turnbull *(University of Liverpool, UK)*
Exploiting the heparan sulphate interactome: from biology to synthetic chemistry to therapeutic applications

09:50-10:20 K. Sugahara *(University of Hokkaido, Japan)*
Mechanism of glycosaminoglycan-mediated tumor metastasis-Novel role for RAGE

SELECTED TALK (ST7)

10:20-10:30 K. Basu *(Ludwig Institute for Cancer Research, Uppsala, Sweden)*
Hyaluronan affects CD44-iASPP cooperation in mammary epithelial cells

10:30-11:00 Coffee break

Chairperson/discussion leader: Liliana Schaefer

LECTURES/TUTORIALS - ECM IN IMMUNE REGULATION (L9-L11)

11:00-11:20 A. Day *(The University of Manchester, UK)*
The role of ECM in immune regulation: new insights for Age-related Macular Degeneration

11:20-11:50 L. Schaefer *(Goethe-Univeristat, Germany)*
Small leucine-rich proteoglycans orchestrate receptor crosstalk during inflammation

11:50-12:10 S. Chakravarti *(Johns Hopkins School of Medicine, USA)*
Functions of lumican in immune response

SELECTED TALKS (ST8)

12:10-12:20 M-V. Nastase *(Goethe-University, Germany)*
The matrix component biglycan triggers the crosstalk between macrophages and podocytes during renal inflammation

12:20-14:00 Lunch

14:00-16:30 Poster session (I) **P1-P24**/ Discussion groups (I)

Chairperson/discussion leader: Donald Gullberg

LECTURES/TUTORIALS - CELL SURFACE RECEPTORS (L12-L14)

16:30-17:00 D. Gullberg *(University of Bergen, Norway)*
Novel aspects of collagen-binding integrins

17:00-17:20 J. Ivaska *(Turku Centre for Biotechnology, University of Turku, Finland)*
Integrin activity and filopodia regulate invasion and matrix remodeling in cancer

17:20-17:40 P. Bonaldo *(University of Padova, Italy)*
Collagen VI regulates muscle stem cell homeostasis and regeneration

SELECTED TALKS (ST9-ST10)

17:40-17:50 KV. Engebretsen *(Oslo University Hospital & University of Oslo, Norway)*
Inhibition of TGF-β signaling in left ventricular pressure overload leads to eccentric remodeling and reduced signs of heart failure, possibly due to reduced collagen content and LOX activity

17:50-18:00 I. Kaur *(University of Oulu, Finland)*
A possible role for Collagen XVIII N-terminal domain in the control of myeloid leukemia cells growth

18:00-18:20 Coffee Break

Chairperson/discussion leader: David Birk

LECTURES/TUTORIALS - MATRIX ORGANIZATION (L15-L16)

18:20-18:50 D. Birk *(University of South Florida, USA)*
Regulatory roles of small leucine-rich proteoglycans in matrix assembly

18:50-19:10 D. Vynios *(University of Patras, Greece)*
ADAMTSs in cancer

19:10-20:30 Session devoted to the memory Dick Heinegård (L17-L18)

19:10-19:40 V. Hascall *(Cleveland Clinic Foundation)*: Remembering Dick

19:40-20:30 K. Rubin & C. Ryden *(Lund University, Sweden)*:
The Matrisome its integration into pathologies

20:30 Dinner

Saturday, 28 September

Chairperson/discussion leader: Alberto Passi

INTERACTIONS AND FUNCTIONS OF MATRIX MACROMOLECULES (L19-L20)

9:00-9:30	V. Hascall *(Cleveland Clinic, USA)* Hyaluronan, Chondroitin Sulfate and Heparin: Dueling GAGs in hyperglycemic proliferating cells
9:30-10:00	A. Passi *(University of Insubria, Italy)* Hyaluronan synthesis and monocyte adhesion in human endothelial cells

SELECTED TALKS (ST11-ST13)

10:00-10:10	A. Ahamed *(University of Eastern Finland, Finland)* Rab10 mediated early endocytosis of HAS3 regulates hyaluronan synthesis and cell adhesion
10:10-10:20	D. Vigetti *(University of Insubria, Italy)* Role of natural antisense HAS2-AS1 RNA in the regulation of hyaluronan synthesis
10:20-10:30	R. Dawadi *(University of Tromsø, Norway)* Matrix Metalloprotease-9/Chondroitin Sulphate Proteoglycan Complexes
10:30-11:00	Coffee break

Chairperson/discussion leader: Mauro Pavão

CELL RECEPTORS SIGNALING AND ECM BASED NANOTECHNOLOGY (L21-L23)

11:00-11:30	P. Friedl *(Radbound Univ, Netherlands – Anderson Cancer Center, USA)* Tissue guidance of cancer invasion: mechanisms, limits and implications
11:30-12:00	A. Grodzinsky *(MIT Center for Biomedical Engineering, USA)* Nanomechanics of the Extracellular Matrix in Health and Disease
12:00-12:30	M. Pavaõ *(Universtiy of Rio de Janeiro, Brazil)* Effect of Heparin Analogs from Marine Invertebrates on Metastasis, Thrombus Formation and Inflammatory Bowel Disease
12:30-14:00	Lunch
14:00-17:00	Poster session (II) **P25-P50**/ Discussion groups (II)
16:30-17:00	Coffee break

Chairperson/discussion leader: Ralph Sanderson

ECM AND TUMOR MICROENVIRONMENT - CANCER (L24-L28)

17:00-17:30	R. Sanderson *(University of Alabama at Birmingham, USA)* Role of heparanase in regulating the tumor microenvironment

17:30-17:50 I. Vlodavsky *(Cancer and Vascular Biology Research Center, Israel)*
Rational design of heparanase inhibitors for cancer therapy

17:50-18:10 I. Kovalszky *(Semmelweis University, Hungary)*
Cancer associated fibroblast, the key player in matrix remodeling

18:10-18:30 M. Götte *(University of Münster, Germany)*
Modulation of matrix- and cytokine-dependent signaling by Syndecan-1 affects breast cancer metastasis, stemness, and resistance to radiotherapy

18:30-18:50 D. Ferning (University of Liverpool, UK)
The structure and functions of the cell microenvironment: from systems biology to single molecule imaging

SELECTED TALKS (ST14-ST17)

18:50-19:00 M. Gross-Cohen *(Cancer and Vascular Biology Research Center, Israel)*
Heparanase 2 expression is decreased in human cancer and attenuates lymphangiogenesis

19:00-19:10 Ch. Gialeli *(University of Patras, Greece)*
Dynamic interplay between normal endothelium and breast cancer cells via altered expression of matrix molecules

19:10-19:20 E. Lombardi *(The National Cancer Institute Aviano, Italy)*
NG2/CSPG4 control of cellular interactions under flow mimicking extravasation conditions

19:20-19:30 M. Pinto *(INEB- Institute for Biomedical Engineering, UPorto, Portugal)*
Decellularizing colorectal tumours: dissecting the role of macrophages and extracellular matrix for tumour progression

EXTRA TALK

19:30-19:50 T. Loukeris *(Institute of Molecular Biology and Biotechnology, Greece)*
Evidence for a protective role of the mosquito Matrix Metallo-Protease 1 (AgMMP1) during Plasmodium oocyst development

20:30 Dinner *(Kipriotis Panorama Hotel & Suites, Main Restaurant)*

Sunday 29, September

Chairperson/discussion leader: Renato Iozzo

MATRIX PATHOBIOLOGY (L29-L32)

9:00-9:30 R. Iozzo *(Thomas Jefferson University, USA)*
Proteoglycan regulation of cancer growth and angiogenesis

9:30-10:00 J. Esko *(University of California, USA)*
Proteoglycan-associated receptors

10:00-10:30 J. Filmus *(Sunnybrook Research Institute, Canada)*
The role of Glypicans in the regulation of Hedgehog signaling

10:30-11:00 C.-H. Heldin *(Ludwig Vancer Institute, Sweden)*
Signaling via receptors for PDGF and TGFβ –
possible targets in tumor treatment

SELECTED TALKS (ST18-ST20)

11:00-11:10 E. Åhrman *(Lund University, Sweden)*
Novel COMP neoepitopes identified from patients with joint diseases
by immune-affinity chromatography and mass spectrometry

11:10-11:20 U. Lindert *(University Children`s Hospital Zurich, Switzerland)*
Molecular consequences of defective SERPINH1
in Osteogenesis Imperfecta

11:20-11:30 A. Fullár *(Semmelweis University, Hungary)*
Hepatocarcinogenesis in matrilin-2 knock out mice

11:30-13:30 Speakers corner (I)

13:30-21:30 Lunch box - Excursion tour

Monday, 30 September

Chairperson/discussion leader: Lena Kjellen

GLYCOBIOLOGY AND METABOLIC REGULATION OF ECM MOLECULES (L33-L35)

9:00-9:30 L. Kjellen *(University of Uppsala, Sweden)*
Heparin and heparan sulfate biosynthesis - Heparan
sulfate and embryonic development

9:30-10:00 M. Maccarana *(University of Lund, Sweden)*
Biosynthesis and functions of dermatan sulfate

10:00-10:30 N. Karamanos *(University of Patras, Greece)*
Intracellular cross-talk in breast cancer and bone metastasis:
pharmacological targeting at the level of matrix molecules

SELECTED TALKS (ST21)

10:30-10:40 E. Karousou *(University of Insubria, Italy)*
Oxidized LDL affects hyaluronan synthesis
in human aortic smooth muscle cells

10:40-11:00 Coffee break

Chairperson/discussion leader: John Couchman

SIGNALING AND DISEASE MOLECULAR TARGETING I (L36-L37)

11:00-11:30 K. Dobra *(Karolinska Institute, Sweden)*
Signaling pathways regulated by syndecan-1
in malignant mesothelioma

11:30-12:00 D. Nikitovic *(University of Crete, Greece)*
Hormonal regulation of bone cell ECM organization-effects

on cell functions

SELECTED TALKS (ST22-ST24)

12:00-12:10 A. Kumar *(Münster University, Germany)*
Differential role for HS3ST2 in modulating breast cancer cell invasiveness: A molecular mechanism mediated by protease expression via MAP kinase and WNT pathways

12:10-12:20 M. Baghy *(Semmelweis University, Hungary)*
Decorin interferes with platelet-derived growth factor receptor signaling in experimental hepatocarcinogenesis

12:20-12:30 M. Farshchian *(University of Turku, Finland)*
EphB2 receptor modulates gene expression signature involved in migration and invasion of cutaneous squamous cell carcinoma

12:30-14:00 Lunch

14:00-16:00 Poster Session (III) **P51-P73** / Discussion groups (III)

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

Chairperson/discussion leader: Themis Kyriakides

SIGNALING AND DISEASE MOLECULAR TARGETING II (L38-L40)

17:00-17:30 T. Kyriakides *(Yale University, USA)*
Thrombospondin-2 and the eNOS/Akt axis in vascular remodeling

17:30-17:50 E. Papadimitriou *(University of Patras, Greece)*
Receptor protein tyrosine phosphatase beta/zeta is a regulator of VEGF induced endothelial cell migration

17:50-18:20 D. Kletsas *(NCSR "Demokritos", Greece)*
Cellular senescence, DNA damage and role in tissue homeostasis

IUBMB LECTURE (L41)

18:20-19:00 IUBMB Metal Award to be presented to Professor Thomas Wight by the IUBMB congress officer Dr. S. Gonos

19:00-19:45 T.N. Wight *(University of Washington, USA)*
Versican and the Control of Cell Phenotype in Disease

21:00 Dinner *(Traditional restaurant at the center Kos town)*

Tuesday, 1 October

Chairperson/discussion leader: Jan-Olof Winberg

MATRIX REGULATION IN HEALTH AND DISEASE (L42 – L47)

9:00-9:30	V.-M. Kähäri *(University of Turku, Finland)* Proteolytic control of cutaneous wound repair and cancer progression
9:30-10:00	J.-O. Winberg *(University of Tromsø, Norway)* Nature and properties of matrix metalloprotease-9/chondroitin sulphate proteoglycan complexes
10:00-10:30	B.Turk *(J. Stefan Institute, Slovenia)* Extracellular cysteine cathepsins: more than extracellular matrix degradation
10:30-10:50	N. Shworak *(Geisel School of Medicine at Dartmouth, USA)* What can a mouse teach us about cardiovascular disease risk
10:50-11:20	S. Gonos *(NHRF, Greece)* Proteasome activation as a novel anti-aging strategy
11:20-11:40	J.-P. Li *(University of Uppsala, Sweden)* Implications of heparan sulfate in amyloidosis
11:40-12:00	Closing remarks / Young Scientist Awards
12:00-13:00	Farewell / Departure

Invited Lectures/Tutorials

Proteoglycans in the brain tumor microenvironment

L 01

Anna Wade[1], Aaron Robinson[1], Joanna J. Phillips[1,2,3]

[1]Department of Neurological Surgery;
[2]Brain Tumor Research Center;
[3]Neuropathology, Department of Pathology; UCSF, San Francisco, CA

Glioblastoma (GBM), a malignant brain cancer, is characterized by abnormal activation of receptor tyrosine kinase signaling pathways and poor prognosis. Extracellular proteoglycans, including heparan sulfate and chondroitin sulfate, play critical roles in the regulation of cell signaling and migration via their interactions with morphogens, growth factors, extracellular matrix and growth factor receptors. In GBM, data from our laboratory and others suggest that proteoglycans regulate critical oncogenic signaling pathways. We have found that the expression of proteoglycans and their modifying enzymes is profoundly altered in GBM. Interestingly, many of these alterations are specific to molecularly defined subtypes of GBM characterized activation of different oncogenic signaling pathways. Thus, proteoglycan function may have subtype-specific roles in disease. In the laboratory we are interested in understanding the function of proteoglycans and their alterations in driving GBM and we hypothesize that they may represent novel therapeutic targets and biomarkers of disease.

Platelet-derived growth factor receptor A (PDGFRA) signaling is an important driver of oncogenesis in GBM. Heparan sulfate proteoglycans (HSPGs) interact with extracellular ligands and receptors to help regulate cell signaling. A major determinant of the affinity and specificity of HSPG-ligand interactions is the sulfation status of 6O-sulfate (6OS) on heparan sulfate (HS), which can be regulated post-synthetically by the extracellular sulfatases (SULFs). In recent studies, we identified a novel role for the extracellular sulfatase, SULF2, in regulating PDGFRA signaling in GBM. We demonstrated SULF2 expression in 50% of human GBM and knockdown or genetic ablation of SULF2 resulted in decreased PDGFRα phosphorylation and decreased tumor growth in vivo. While SULF regulates extracellular 6OS sulfation and could potentially alter PDGF binding to HS, its mechanism of action in GBM is unknown. Consistent with a role for SULF2 in PDGFRA signaling, we identified upregulation of SULF2 ($p=0.0253$) in PDGFRA-amplified GBM compared to non-amplified tumors ($n=40$ amplified, 332 non-amplified). In contrast, SULF2 was downregulated ($p=0.0006$) in EGFR-amplified GBM ($n=167$ amplified, 205 non-amplified). To determine how SULF2 alters HS binding or release of PDGF we performed HS-ELISAs in combination with cell-based assays and measured levels of HS-bound PDGFA or PDGFB, receptor localization on the cell membrane, and kinetics of receptor activation. These data will be reported. Interestingly, SULF2 knockdown and genetic ablation of SULF2 resulted in decreased expression of PDGFRA and PDGFB, suggesting SULF2 may modulate signaling via multiple mechanisms.

L
02

Roles of serglycin in inflammation and cancer

Achilleas D. Theocharis[1], Aggeliki Korpetinou[1], Spyros S. Skandalis[1], Vassiliki T. Labropoulou[2], Anastassios Minas[3], Argyrios Noulas[3], Nikos K. Karamanos[1]

[1]Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Division of Hematology, Department of Internal Medicine, University Hospital of Patras, Greece
[3]School of Health Professions, Department of Medical Laboratories, Technological Educational Institute of Larissa, Greece

Serglycin was initially characterized as an intracellular proteoglycan expressed by hematopoietic cells. All inflammatory cells highly synthesize serglycin stored in granules, where it interacts with various bioactive molecules, such as proteases, chemokines, cytokines and growth factors. Serglycin regulates the storage of inflammatory mediators within the granules, until their secretion and delivery to their molecular targets. During the last decade, numerous studies have demonstrated that serglycin is also synthesized by non-hematopoietic cell types. Recently, it has been shown that serglycin is highly expressed by tumor cells and promotes their aggressive phenotype, while it confers resistance against drugs and complement system attack. During tumor development, cancer cells activate the surrounding stroma and recruit inflammatory cells to support their growth and metastasis. Apart from its direct beneficial role in the tumor cells, serglycin may also participate in the inflammatory process and stroma activation via regulated secretion and delivery of functionally important molecules to their targets, thus promoting tumor cell growth, invasion and metastasis as well as angiogenesis. The involvement of serglycin in inflammation and tumor progression will be discussed.

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: ARCHIMEDES III. Investing in knowledge society through the European Social Fund.

Syndecan-1 is crucial for multiple myeloma growth and controls Bortezomib sensitivity and side-population size

L 03

Alexander de Bruin[1], Zemin Ren[1], Rogier M. Reijmers[1], Richard Groen[1,2], Annemieke Kuil[1], Anneke Kramer[1], Marcel Spaargaren[1], and Steven T. Pals[1]

[1]Department of Pathology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands;
[2]Department of Immunology, University Medical Center Utrecht, Lundlaan 6, 3584 EA Utrecht, The Netherlands; s.t.pals@amc.uva.nl

The uncontrolled growth of tumor cells is generally attributed to mutations in essential growth control genes, but tumor cells are also require signals from the environment. As targets for intervention, they may be equally important as mutated oncogenes. A potential key player in the interactions of multiple myeloma (MM) plasma cells with the bone marrow (BM) microenvironment is syndecan-1. This pleiotropic receptor can mediate adhesion to extracellular matrix components, while the heparan sulfate (HS) chains decorating syndecan-1 can accommodate soluable factors, promoting MM survival and growth. By employing inducible RNAi-mediated knockdown (kd) of EXT1, a co-polymerase essential for HS-chain synthesis, we recently demonstrated that the HS-chains decorating syndecan-1 are indeed crucial for the growth and survival of MM cells within the bone marrow environment. To test the effect the loss of HSPG on MM drug sensitivity, MM cell lines with and without EXT1 kd were cultured with increasing concentrations of the proteosome inhibitor Bortezomib, a key drug in the treatment MM patients. Interestingly, EXT1 kd markedly increases the sensitivity of MMs to this drug. Moreover, it strongly diminished the size of the fraction of cells able to efflux the fluorescent dye Vybrant DyeCycle; these so-called 'side population' (SP) cells exibit tumor initiating characteristics. Whereas Bortezomib treatment increased SP size in control cells, kd of EXT1 prevented this increase. These observations suggests that SP cells require expression of HSPGs and that HSPGs are involved in the expansion of this population upon Bortezomib exposure. How HSPGs regulate the size of the SP cell fraction is currently under investigation.

L
04

Syndesmos-a partner in syndecan-4 mediated cell migration

S. Wilcox-Adelman

Matrix Biology Institute, Edgewater, USA

The transmembrane heparan sulfate proteoglycan syndecan-4 acts as a co-receptor, with integrins, to regulate focal adhesion and actin stress fiber formation upon ligation to extracellular matrix proteins such as fibronectin. Syndecan-4 null mice exhibit impaired dermal wound repair due to delayed cell migration. The delay is partially due to impaired Rho-dependent focal adhesion kinase (FAK) phosphorylation. Syndesmos is a syndecan-4 binding protein that also interacts with the focal adhesion adaptor protein paxillin. Syndesmos-null mice have been generated and demonstrate an initial delay in wound healing although the overall time for wound healing was not affected. This does not fully recapitulate the syndecan-4 null phenotype. Fibroblasts isolated from syndesmos-null mice show enhanced focal adhesion size and actin stress fiber organization, suggesting that syndesmos may contribute to focal adhesion destabilization and syndecan-4 mediated cell migration.

Syndecan proteoglycans: specificity and redundancy in signaling to the cytoskeleton

L 05

John R. Couchman, Sandeep Gopal, Pernille Søgaard, Rafael Brandão, Csilla Pataki, Eva Tulin Christensen, Elena Okina, Betina Sèverine Fogh and Hinke A.B. Multhaupt

Department of Biomedical Sciences, University of Copenhagen, Biocenter, 2200 Copenhagen, Denmark

Syndecans in mammals are a four member family, with near ubiquitous expression in tissues. Many cell types have multiple members. In contrast, only one syndecan is present in all bilaterian invertebrates but their long evolutionary history points to important conserved roles. However, the phenotypes of syndecan-1, -3 and -4 knockout mice are mild. More obvious developmental defects are seen in lower vertebrates, where in the case of the Osteichthyes (bony fish), syndecan-1 has been secondarily lost. The mild phenotypes are in marked contrast to mutant mice lacking key transferases for heparan sulfate assembly, where embryonic lethality is commonly observed. This hints that syndecans have redundant functions, particularly through development.

Much work over the past decade has focussed on roles for syndecans in disease, with considerable data on their mis-expression in tumours, and relevance to vascular diseases. However, despite much work, syndecan signaling has remained a challenging area, with the exception of syndecan-4. Many years ago we described that the variable (V) region of the intracellular domain of this syndecan binds both phospholipid and protein kinase Cα. This combined with direct interactions with α-actinin facilitates roles in integrin-mediated adhesion to the extracellular matrix. In particular, focal adhesion assembly involves syndecan-4, where the heparan sulfate chains interact with ligands such as fibronectin, while signaling from PKCα leads downstream to Rho G proteins, Rho kinases and myosin II-mediated microfilament contraction.

So far as we know, however, this signaling pathway is unique to syndecan-4. All syndecans link to the actin cytoskeleton, so other conserved regions of the syndecan cytoplasmic domains are likely to be the source of any redundant function across the family. These C1 and C2 (constant) domain sequences unite the syndecan family and are clearly recognisable across the invertebrate members also. The syndecan phenotype in the nematode, *C.elegans*, is neural, but so far the genetics has not provided a molecular basis for its signaling function. We, therefore are undertaking further studies in vertebrate and invertebrate models to address how syndecans may possess specific, but also generic functions.

L 06

HAS2 and CD44 signaling in breast cancer tumorigenesis

Heldin Paraskevi, Bernert Berit, Porsch Helena, Kozlova Inna, Basu Kaustuv, Mehic Merima and Ruusala Aino

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

During tumor progression hyaluronan-CD44 interactions in the cellular local microenvironment affect the growth, migration and differentiation of cells.

We investigate the role of hyaluronan synthases, in particular HAS2, in breast cancer progression. Recent data indicate that TGFβ stimulates the production of hyaluronan in normal mammary breast epithelial cells by upregulation of HAS2 and that efficient TGFβ-induced epithelial to mesenchymal transition requires the expression of HAS2. Analysis of a panel of breast cancer cells, revealed that HAS2 is upregulated in most aggressive breast cancer cell lines including a clone selected for its ability to metastasize to bone. We observed that knockdown of HAS2 suppressed the invasive behavior of these cells most likely by the induction of the protease inhibitor TIMP-1 and inhibition of the EGF-mediated FAK/PI3K/Akt signaling pathway.

We also investigate the mechanisms through which hyaluronan signals via its receptor CD44 alone or in co-operation with the growth factor receptors for TGFβ and PDGF-BB. Using a proteomic approach we identified proteins interacting with the cytoplasmic part of CD44 such as IQGAP1 and iASPP. Studies on IQGAP1/CD44 interactions revealed that hyaluronan-induced activation of Rac1 is dependent on IQGAP1, whereas depletion of IQGAP1 enhances the activation of RhoA. CD44/iASPP complexes could identified in both fibroblasts and epithelial cells. The complexes were differently regulated in response to external stimuli, such as hyaluronan, TGFβ and PDGF-BB. The epitopes involed the interaction and the impact of the complex for cell proliferation or apoptosis are currently under investigation.

The above lines of research might provide new insights of how HAS2 and CD44 act during breast cancer progression.

Exploiting the heparan sulphate interactome: from biology to synthetic chemistry to therapeutic applications

L 07

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Heparan sulfates (HSs) are complex sulfated polysaccharides present on almost all cell surfaces in multicellular organisms. They have variant 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 that influences many cellular processes important in the development, regulation and repair of tissues. Understanding the chemical biology of these enigmatic molecules is now becoming possible through a variety of tools, reagents and approaches including saccharide libraries, microarray methods and novel sequencing approaches. We are developing and exploiting semi-synthetic and synthetic chemistry strategies to produce targeted libraries for activity screening 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 neural development, degeneration and repair, and cancer metastasis. This has led to a translational pathway of biomedical applications, including discovery of novel drug leads for Alzheimers disease and cancer metastasis, and potential interventions to improve neural cell transplantation for nerve repair.

L
08

Mechanism of glycosaminoglycan-mediated tumor metastasis —Novel role for RAGE—

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Altered expression of chondroitin sulfate (CS) and heparan sulfate (HS) at the surfaces of tumor cells plays a key role in malignant transformation and tumor metastasis. Previously we demonstrated that a Lewis lung carcinoma (LLC)-derived tumor cell line with high metastatic potential had a higher proportion of E-disaccharide units, GlcUA-GalNAc (4,6-*O*-disulfate), in CS chains as compared to low metastatic LLC cells and that such CS chains are involved in the metastatic process (1). The metastasis was markedly inhibited by the pre-administration of CS-E from squid cartilage rich in E units or by pre-incubation with a phage display antibody specific for CS-E (1). However, the molecular mechanism of the inhibition remained to be investigated.

In this study the receptor molecule for CS chains containing E-disaccharides expressed on LLC cells was revealed to be receptor for advanced glycation end products (RAGE)(2), which is a member of the immunoglobulin superfamily predominantly expressed in the lung. Interestingly, RAGE bound strongly to not only E-disaccharide, but also HS-expressing LLC cells. Furthermore, the colonization of the lungs by LLC cells was effectively inhibited by blocking of CS or HS chains at the tumor cell surface with an anti-RAGE antibody through intravenous injections in a dose-dependent manner. These results provide the clear evidence that RAGE is at least one of the critical receptors for CS and HS chains expressed at the tumor cell surface and involved in experimental lung metastasis and that CS/HS and RAGE are potential molecular targets in the treatment of pulmonary metastasis. Possible CS decasaccharide sequences, which may be involved in the binding to RAGE, will be discussed.

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The role of ECM in immune regulation: new insights for Age-related Macular Degeneration

L 09

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Complement factor H (CFH) regulates the innate immune system through its capacity to recognise glycosaminoglycans (GAGs) such as heparan sulphate (HS) within host tissues. Comprising 20 CCP domains, CFH binds GAGs *via* two distinct regions (CCPs6-8 and CCPs19-20). Recently, we have shown that the contribution from each of these GAG-binding sites depends on the context of the tissue, *i.e*. CCP19-20 plays a vital role in kidney glomeruli basement membrane, whereas CCP6-8 coordinates CFH binding to the Bruch's membrane, a multi-laminar ECM, in the eye (Clark et al. 2013 J Immunol 190;2049-57). This tissue specificity, which may correlate with differences in the GAG chain composition at each site, explains the organ-specific effects of mutations in CFH; e.g. the Y402H polymorphism in CCP7 is strongly associated with the ocular disease Age-related Macular Degeneration (AMD). Importantly, the Tyr to His coding change at amino acid 402 alters the specificity of CFH for HS (Clark et al. 2006 J Biol Chem 281;24713-20; Prosser et al. 2007 J Exp Med 204;2277-2283) and impairs its binding to Bruch's membrane (Clark et al. 2011 J Biol Chem 285;30192-202). However, while individuals are born with the disease-associated 402H allele they do not present with symptoms of AMD until their 50s.

Using post-mortem ocular tissue we have mapped the differential distribution of GAGs (Clark et al. 2011 Invest Ophthalmol Vis Sci 52;6511-21) and proteoglycan core proteins (Keenan et al. 2012 Invest Ophthalmol Vis Sci 53;7528-38) in the human eye. More recently we have found that there is an age-related reduction in the overall amount (and change in composition) of HS in human Bruch's membrane (the site of AMD pathology). Our data indicate that alterations in the levels of specific core proteins, as well as in HS biosynthesis/turnover, likely contribute to this pronounced (and significant) reduction.

These age-associated changes in ECM structure are likely to exacerbate the poor binding seen for the 402H form of CFH to the Bruch's membrane and could represent a 'tipping point' for loss of immune homeostasis in the eye. Thus our work may explain why the pathology of AMD does not usually develop until the 6th decade of life since it requires an age-related change in GAG structure in combination with an allotype of CFH that has a particular specificity for HS.

L 10

Small leucine-rich proteoglycans orchestrate receptor crosstalk during inflammation

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Inflammation is not only a defensive mechanism against microbial invasion, but also frequently represents a critical response to tissue injury under sterile conditions. It is now well established that tissue injury leads to the release of endogenous molecules of intra- and extracellular origin acting as damage-associated molecular patterns (DAMPs). SLRPs can act as powerful DAMPs following their proteolytical release from the extracellular matrix. Recent investigations of SLRP signaling networks revealed new levels of complexity, showing that SLRPs can cluster different types of receptors and orchestrate a host of downstream signaling events. This talk will summarize the evidence for the multifunctional proinflammatory signaling properties of the two archetypal SLRPs biglycan and decorin. These secreted proteoglycans link the innate to the adaptive immune response and operate in a broad biological context, encompassing microbial defense, tumor growth, and autoimmunity.

In Lupus Nephritis (LN), a prototypical autoimmune disease of the kidney, soluble biglycan triggers the expression of the B cell chemoattractant CXCL13 by signaling through TLR2 and TLR4 in interstitial macrophages and dendritic cells. Elevated tissue levels of CXCL13 then lead to the recruitment of CXCR5-positive B cells, preferentially the B1 subset, into the kidney. Furthermore, biglycan induces the synthesis of RANTES, MCP-1, and MIP-1α in macrophages, thereby attracting T cells and additional macrophages. Thus, by bridging the innate and adaptive immune systems endogenous soluble biglycan enhances the inflammatory response reaction and thereby aggravates the course of LN and other B cell-mediated inflammatory disorders as well (e. g. acute renal allograft rejection).

The mechanisms linking immunity and inflammation with tumor development are not well understood. We could show that the soluble form of decorin controls inflammation and tumor growth via Programmed cell death protein 4 (PDCD4) and miR-21 by a dual signaling mechanism. Firstly, decorin acts as an endogenous ligand of TLR2/4 stimulates the synthesis of proinflammatory PDCD4, TNFα, and IL-12 in macrophages. Secondly, decorin prevents translational repression of PDCD4 by attenuating the activity of TGFβ1 and the expression of oncogenic miR-21, a translational inhibitor of PDCD4. Moreover, enhanced PDCD4 downregulates anti-inflammatory IL-10, thereby further driving the cytokine profile towards a proinflammatory phenotype. Importantly, these mechanisms appear to operate in a broad biological context linking pathogen-mediated with sterile inflammation as shown here for sepsis and growth retardation of established tumor xenografts.

Functions of lumican in immune response

L 11

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The connective tissue extracellular matrix (ECM) is emerging as a modulator of innate and adaptive immunity to ultimately affect infections, inflammation and autoimmunity. Over the years our studies on lumican, a small leucine rich repeat proteoglycan of the ECM, demonstrated that it regulates toll-like receptor (TLR) 4 mediated innate immune response by binding CD14 on the surface of macrophages and neutrophils. The lumican-CD14 interactions have additional functions in phagocytosis and bacterial clearance; mice deficient in lumican show increased severity of *Pseudomonas aeruginosa* infections of the lungs and the cornea. By flow cytometry of cells extracted from the infected tissues of wild type and lumican deficient mice we detected key differences in the repertoire of infiltrating leukocytes and lymphocytes that suggest specific functions for lumican in immune cell migration and differentiation. Our studies are further investigating how lumican interactions with immune signals and its presentation as DAMPs are managed under homeostatic and injury-related conditions.

L
12

Novel aspects of collagen-binding integrins

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In the talk an overview of the mechanism whereby cells attach to fibrillar collagens will be given, including a discussion of direct integrin-mediated adhesion and indirect COLINBRI (collagen integrin bridging) mediated mechanisms. The remaining part of the talk will deal with the role of collagen-binding integrins, and in particular α11β1, on fibroblasts. The usefulness of heterospheroids for in vitro studies of tumor-stroma interactions in a 3D context will be discussed.

Integrin activity and filopodia regulate invasion and matrix remodeling in cancer

L 13

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Integrins are a family of cell adhesion receptors which critically regulate cell matrix interactions and cell motility. Integrin mediated cell adhesion is regulated by the conformational switching of integrins between active and inactive conformations. We have recently performed large-scale RNAi screens to indentify novel regulators of integrin activity in cancer cells. Both negative and positive regulators have been identified and our data demonstrate a correlation between increased integrin activity and cell invasiveness in vitro. In addition, we find that regulators of integrin activity influence cancer cell extravasation also in vivo.

Filopodia are finger-like plasma-membrane protrusions, extending from the leading edge of motile cells. Myosin-X (Myo10) is a motor protein which transports integrins to the tips of filopodia and stimulates filopodia formation. We have found that a set of filopodia related genes are upregulated in highly-invasive basal-like breast cancer cells. Interestingly, Myo10 silenced cells are less motile both *in vivo* and *in vitro*. The migration of the Myo10 silenced MDA-MB-231 breast cancer cells is remarkably reduced and less persistent on Matrigel. Also the Myo10 silenced cells invade less into Matrigel plugs and show reduced metastasis in vivo. Staining of Myo10 in clinical tissue samples reveals a clear correlation between high Myo10 expression and reduced survival among lymph node positive breast cancer patients. In conclusion, Myo10 is an important regulator of the directional movement and cell migration.

Taken together, our results indicate that regulators of integrin activity as well as filopodia inducing Myo10 are pro-invasive proteins and are most likely involved in cancer spreading.

L
14

Collagen VI regulates muscle stem cell homeostasis and regeneration

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Collagen VI (ColVI) is a major extracellular matrix (ECM) protein forming a distinctive microfilamentous network in various tissues. ColVI is particularly abundant in skeletal muscles, where it is localized in the endomysium surrounding myofibers. Studies with muscle-derived cultures proved that ColVI is produced by interstitial fibroblasts, but not by myogenic cells. Mutations of ColVI genes in humans cause several muscle diseases, including Bethlem myopathy and Ullrich congenital muscular dystrophy. A well-characterized animal model for ColVI disorders is the Col6a1 null mouse, which displays a myopathic phenotype with mitochondrial dysfunction, spontaneous apoptosis and failure of autophagosome formation in muscle fibers [1,2].

Satellite cells (SCs) represent the major stem cell population of adult skeletal muscles and play an essential role in muscle regeneration. The activity of SCs is strictly regulated by the biochemical and mechanical properties of their niche, which includes the ECM. Currently, the function of specific ECM molecules in regulating SCs and their niche is still unknown.

Given the pericellular localization and the critical functions exerted by ColVI in muscle, we investigated whether this protein plays any role for SCs. We found that ColVI is a key component of SC niche, required for proper SC activity and for the maintenance of muscle stiffness. In vivo studies revealed that ColVI is in close contact with quiescent and activated SC in normal and regenerating muscles. Lack of ColVI leads to impaired muscle regeneration and reduced self-renewal capability of SCs after damage, and to decreased muscle stiffness in Col6a1 null mice. These defects are recovered when ColVI deposition is reinstated by grafting with wild-type fibroblasts, a finding which opens new therapeutic venues for ColVI-related muscular dystrophies [3].

Thus, we identified for the first time a definite ECM molecule playing a critical role in the regulation of SC stemness and in the control of muscle mechanical properties.

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Regulatory Roles of Small Leucine-Rich Proteoglycans in Matrix Assembly

L 15

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Small leucine-rich proteoglycans (SLRPs) are involved in a variety of biological and pathological processes including critical regulatory roles in matrix assembly. Differential interactions of SLRPs with other molecules during development result in tissue-specific spatial and temporal distributions. The resulting changing expression patterns play key roles in the regulation of tissue-specific matrix assembly and therefore tissue function. SLRPs are involved in the regulation of collagen fibril growth, fibril organization and extracellular matrix assembly. In addition, they are involved in mediating cell–matrix interactions. SLRPs regulate assembly of the extracellular matrix, which defines the microenvironment, modulating both the extracellular matrix and cellular functions which impacts tissue function. Abnormal SLRP expression and/or structures result in dysfunctional extracellular matrices and pathophysiology. Altered expression of SLRPs has been found in many disease models, and structural deficiency also causes altered matrix assembly.

L
16

ADAMTSs in cancer

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Key features of malignant tumors are their abilities to invade surrounding tissues, to have access to the vascular and lymphatic systems, and to disseminate to distant organs by metastatic spreading. Cancer remains the second leading cause of death in Europe and the United States. Accumulating evidence demonstrates the crucial role of proteolytic enzymes such as matrix metalloproteinases (MMPs) and closely related ADAMs (a disintegrin and metalloproteinase) and ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) in cancer development and progression.

The family of ADAMTSs was first characterized in 1997 and has been associated with many physiological and pathological conditions. Nineteen members of this family have been described so far, ADAMTS 1-10 and 12-20. Certain members (ADAMTS-1, -4, -5, -8, -9 and -15) are called aggrecanases, because they can proteolytically process aggrecan within the interglobular domain by separating its globular G1 and G2 domains at a specific Glu373-Ala374 bond or at one or more sites within the more C-terminal glycosaminoglycan-bearing region.

Although information about functions of ADAMTSs in cancer is still limited, recent studies have provided evidence of dysregulation of various ADAMTSs in different types of cancer. Some of the ADAMTSs seem to have tumor suppressor activity due to the angioinhibitory capacities of their thrombospondin-1 domains. Moreover, in vivo studies using knock-out animals showed a direct relation with the expression of selected ADAMTS members with tumor growth and progression. In addition, overexpression of ADAMTSs in vivo seems to block tumor growth. Some members of the family, i.e. ADAMTS-12, may also exhibit specific antitumor properties.

Accumulated evidence suggests that ADAMTSs are epigenetically regulated in cancer, either through promoter hypermethylation or transcriptional silencing. Additional factors have also been shown to regulate ADAMTSs expression, and, among them hyaluronan seems to play a key role.

The Matrisome: its integration into pathologies

L 18

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Combinations of biochemical characterizations and bioinformatic approaches have allowed the definition of the Matrisome. The Matrisome, defined as the ensemble of extracellular matrix (ECM) and associated proteins, plays decisive roles in pathologies. During homeostasis the matrisomal units determine the physiologic properties of specific tissue types by variations in biochemical composition resulting in different biophysical properties. The build-up and properties of the matrisomal units are dynamic and modulated in response to environmental changes. Inflammatory reactions are common and central for the large disease groups autoimmune diseases, degradative diseases, carcinoma and infections. The inflammatory reactions and the Matrisome are reciprocally modulated in these pathologies resulting either in healing or, as in many cases a deterioration of the conditions.

L 19

Hyaluronan, Chondroitin Sulfate and Heparin: Dueling GAGs in hyperglycemic proliferating cells

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Our previous studies showed that mesangial cells dividing in hyperglycemic medium (15 mM or greater) induce hyaluronan (HA) synthesis in intracellular compartments beginning ~10 hours after initiating cell division (1,2). Deposition of the hyaluronan in these compartments (endoplasmic reticulum (ER), golgi, transport vesicles) induces ER stress, autophagy and cyclin D dependent extrusion of a monocyte-adhesive HA matrix at the end of cell division. Our hypothesis is that the hyperglycemia increases the substrates for HA synthesis, UDP-glcUA and UDP-glcNAc, in the cytosol, which creates the stress responses in dividing cells that drive PKC pathways that activate the HA synthase (HAS2) in the intracellular compartments. To test this, we used 4-methylumbelliferone (4MU) and 4-methylumbelliferyl-xyloside (4MU-xyl) to decrease the cytosolic substrates during cell division. Cells convert 4MU, a noxious agent, to a glucuronide, which uses UDP-glcUA. 4MU-xyl enters the golgi and greatly increases intra-golgi synthesis and secretion of chondrdoitin sulfate ~10 fold, which depletes both cytosolic substrates by antiporter transport of UDP-glcUA and UDP-galNAc (derived by epimerization of UDP-glcNAc) into the golgi. Both of these treatments prevented HA synthesis inside the cells, thereby allowing the cells to complete cell division without inducing the autophagy response. Previous studies showed that treatment of streptozotocin induced diabetic rats with daily IP of small amounts of heparin prevented the nephropathy and proteinurea over an 8 week period (3,4). Therefore, we treated hyperglycemic dividing mesangial cell cultures with 0.1 µg/ml heparin, which also prevented the intracellular HA and autophagy responses. However, surprisingly, at the end of cell division, the cells produced an extensive monocyte-adhesive HA matrix, 2-3 times more than hyperglycemic mesangial cells without heparin. We then treated diabetic rats daily with heparin, which prevented the nephropathy and proteinurea. In the untreated diabetic rats, HA in glomeruli increased steadily, and sections of glomeruli showed extensive HA, mesangial cell autophagy and influx of macrophages by 6 weeks. In contrast, the diabetic rats treated with heparin showed greatly increased glomerular HA at 1-2 weeks, which diminished to near control level by 6 weeks, at which time sections of glomeruli showed no mesangial autophagy, very little remaining HA and extensive numbers of macrophages. Our ongoing hypothesis is that mesangial cells in the diabetic rat that went through the autophagy recruit macrophages that are profibrotic with resulting kidney nephropathy and proteinurea while mesangial cells in the diabetic rat treated with heparin complete cell division without the autophagy, maintain their function while synthesizing the extensive HA matrix, and they recruit macrophages to enter the glomeruli and remove the HA matrix without inducing them to become profibrotic.

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Hyaluronan synthesis and monocyte adhesion in human endothelial cells

L 20

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Immune cells infiltrate into tissues driving inflammation which protects against infection and other pathological state. Chronic inflammation has now accepted to have a critical role in the onset of several diseases as well as in vascular pathology, where macrophages transformation in foam cells contributes in atherosclerotic plaque formation. Endothelial cells (EC) have a critical function in immune cells recruitment, in fact proinflammatory cytokines drive the specific expression of several adhesion proteins. During inflammatory responses several cells produce hyaluronan (HA) promoting monocyte/macrophage adhesion through interactions with the HA receptor CD44 present on the inflammatory cell surfaces. Human umbilical cord EC (HUVEC) cells are not able to produce HA in resting conditions, but when stimulated with inflammatory mediators start tp produce HA activating the enzyme HAS2 on the cell membrane. Interleukin 1β and tumour necrosis factors alpha and beta, but not transforming growth factor alpha and beta, strongly induced HA synthesis by NF-κB pathway as well as the CD44 expression. This signalling pathway is mediated mainly by hyaluronan synthase 2 (HAS2) mRNA expression without altering other glycosaminoglycans metabolism. Moreover, U937 monocyte adhesion on stimulated HUVEC cells strongly depends on HA, and transfection with short interference RNA of HAS2 abrogates HA synthesis revealing the critical role of HAS2 in this process. The over expression of mRNA of HAS2 is critically controlled by cells and other covalent modifications take place in this context, including phosphorylation, ubiquitination and O-GlcNAcylation. These modification are usually able to alter enzyme stability and therefore the enzymic activity. Taken together these findings indicate that beside a genetic control which needs hours to be carried out by the cells, exists another very fast control based on the protein covalent modifications which influence protein stability.

L
21

Tissue guidance of cancer invasion: mechanisms, limits and implications

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Different modes of cancer cell invasion contribute to local tissue invasion and initiation of metastasis, however the underlying mechanisms of each migration program, their limits and their relevance to metastasis remain unclear. In models for melanoma, sarcoma and breast cancer, within the same cancer lesion in vivo both single-cell and collective invasion mediate cell dissemination. Using intravital multiphoton microscopy, we here show the how tissue microniches impose diverse cancer invasion modes, either as barrier precluding migration, or as invasion-promoting tracks that enable either collective, single-cell or combined invasion modes. As main routes, non-destructive contact-guidance along preformed multi-interface perimuscular, vascular and –neural tracks of 1D, 2D and 3D topography were identified. Using in vitro analysis of engineered low- and high-density environments, the underlying physical and molecular limits of cancer cell invasion, showing nuclear deformability and ECM space as rate-limiting determinants and modulation by MMPs and mechanotransduction. Using in vivo targeting of beta1/beta3 integrins, unexpected plasticity of invasion, including de novo development of amoeboid dissemination, was associated with enhanced micrometastasis, implicating integrin-independent dissemination as major route to metastasis. In conclusion, cancer invasion and metastasis result from adaptive physicochemical programs that balance cell-intrinsic adhesion and mechanocoupling with encountered physical and molecular cues.

Nanomechanics of the Extracellular Matrix in Health and Disease

L 22

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The molecular structure and nanomechanical properties of aggrecan monomers extracted and purified from human articular cartilage from donors of different ages have recently been visualized and quantified via atomic force microscopy (AFM)-based imaging, force spectroscopy, and high bandwidth nano-rheology. AFM imaging enabled direct comparison of full length monomers at different ages. The demonstrably shorter glycosaminoglycan (GAG) chains observed in adult full length aggrecan monomers, one monomer at a time, compared to newborn monomers reflects markedly altered biosynthesis with age, consistent with previous biochemical studies reflected averages of large populations. Direct visualization of individual aggrecan monomers subjected to enzymatic treatments to remove chondroitin sulfate versus keratan sulfate GAG chains revealed conformational properties of aggrecan associated with these specific constituent GAGs. Additional studies have focused on the compressive stiffness of chemically end-attached layers of adult and newborn aggrecan, measured in various ionic strength aqueous solutions. Results suggest the importance of both electrostatic and non-electrostatic interactions in the nanomechanical stiffness of aggrecan. These results provide molecular-level evidence of the effects of age on the conformational and nanomechanical properties of aggrecan, with direct implications for the effects of aggrecan nanostructure on macro-level properties of cartilage tissue. Interactions of interstitial fluid flow within cartilage extracellular matrix, in particular the highly negatively charged aggrecan GAGs, have been hypothesized to regulate cartilage tissue-level properties, including transport, energy dissipation, and self-stiffening. We recently studied brush layers of aggrecan from these same different aged human donors, and utilizing a new high frequency AFM-based rheology system to quantify their dynamic deformational behavior in the 1Hz to 10 kHz frequency range. The magnitude and phase angle of the dynamic stiffness showed frequency-dependent trends remarkably similar to those of intact native cartilage tissue, also measured via AFM-based dynamic nanoindentation. These techniques have thereby enabled the measurement of the hydraulic permeability of aggrecan networks, and strongly suggest that aggrecan is the key molecule in determining the fluid-flow-dependent properties of healthy cartilaginous tissues.

L
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Effect of Heparin Analogs from Marine Invertebrates on Metastasis, Thrombus Formation and Inflammatory Bowel Disease

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Thrombosis, chronic inflammation and metastasis are processes strongly associated with cancer progression. P-selectin-dependent tumor cell interactions with platelets, leukocytes and endothelium facilitate metastasis. The anticoagulant heparin is known to attenuate metastasis and inflammation due to its ability to inhibit P- and/or L-selectin. Here we show that two ascidian dermatan sulfates (2,4- and 2,6- sulfated) contain P-selectin inhibitory activity. The 2,6-sulfated dermatan sulfate is devoid of anticoagulant activity, whereas the 2,4-sulfated dermatan sulfate is highly anticoagulant. The two ascidian dermatan sulfates effectively attenuate metastasis of both MC-38 colon carcinoma and B16-BL6 melanoma cells in a P-selectin-dependent manner, regardless of the anticoagulant activity. Furthermore, we show that the oversulfated dermatan sulfates reduce thrombosis in an arterial thrombosis model by inhibition of P-selectin. The occlusion time was prolonged also with dermatan sulfate containing minimal anticoagulant activity. Composition analysis of arterial thrombi revealed a markedly reduced platelet deposition upon dermatan sulfate treatment. Finally, nanoparticles of an ascidian heparin-like glycan remarkably attenuates the macroscopic and histologic inflammatory scores of colon sections from rats submitted to experimental colitis. Following oral administration of the ascidian heparin nanoparticles, cellular infiltration, collagen deposition, and leukocyte rolling reduced significantly. A drastic reduction in the levels of TNF-alpha and IL-1 beta was also observed in culture supernatants of animals treated with heparin nanoparticles. No hemorrhagic events were observed during the study. Collectively, these findings demonstrate that the marine invertebrate glycans represent potential therapeutic agents in the treatment of thrombosis, inflammation and metastasis.

L 24

Role of heparanase in regulating the tumor microenvironment

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The heparan sulfate degrading enzyme heparanase is upregulated in many types of cancer and associated with aggressive tumor growth and metastasis. Using myeloma as a model, we have uncovered multiple mechanisms whereby heparanase drives tumor progression. Upregulation of heparanase enhances expression of VEGF, HGF, MMP-9, RANKL and syndecan-1, all of which can contribute to a microenvironment that facilitates tumor progression. For example, the increase in VEGF expression coupled with enhanced syndecan-1 expression and shedding results in soluble VEGF/syndecan-1 complexes that stimulate angiogenesis. Similarly, soluble HGF/syndecan-1 complexes stimulate IL-11 secretion by osteoblasts resulting in enhanced RANKL secretion that drives myeloma bone disease. We recently discovered that heparanase also regulates tumor secreted exosomes, 30-100 nm microvesicles that can dock with recipient cells and deliver proteins and nucleic acids that regulate cell signaling and gene expression. Heparanase enhances exosome secretion by myeloma tumor cells and alters exosome protein composition. These exosomes are capable of stimulating tumor cell spreading and endothelial cell invasion, thus revealing a novel role of heparanase in tumor-host crosstalk. Recent studies also show that heparanase is upregulated in response to chemotherapy and that cells having high levels of heparanase are not as readily killed by drugs when compared to cells having low levels of heparanase expression. Thus, in addition to helping establish a microenvironment that supports tumor progression, heparanase may also play an important role in supporting tumor resistance to therapy, eventual relapse and poor patient outcome.

L
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Rational design of heparanase inhibitors for cancer therapy

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Heparan sulfate proteoglycans (HSPGs) are primary components at the interface between virtually every eukaryotic cell and its extracellular matrix (ECM). HSPGs not only provide a storage depot for heparin-binding molecules in the cell microenvironment, but also decisively regulate their accessibility, function and mode of action. As such, they are intimately involved in modulating cell invasion and signaling loops that are critical for tumor growth. In a series of studies performed since the cloning of the human heparanase gene we and others have demonstrated that heparanase, the sole heparan sulfate (HS) degrading endoglycosidase, is causally involved in cancer progression, inflammation, diabetes and kidney dysfunction, and hence is a valid target for drug development. We have generated a novel chemically modified non-anticoagulant heparin (termed *SST0001) that potently inhibits heparanase enzymatic activity and tumor growth and metastasis in xenograft cancer models (i.e., multiple myeloma, pancreatic carcinoma, Ewing's sarcoma, glioma). Compound SST0001 is being subjected to phase I/II clinical trial in myeloma patients. Building on the premise of this lead compound, we are currently developing second generation oligosaccharide-based heparanase-inhibiting compounds that exhibit therapeutic along with pharmacological properties that will further advance translation of this therapy to the clinic. Given the increasing significance of neutralizing monoclonal antibodies (mAb) in the treatment of human diseases, we have generated heparanase neutralizing mAb directed against the substrate binding domain of heparanase. These antibodies are being tested in preclinical cancer models. Newly resolved structural features of the heparanase protein provide a strong basis for an ongoing rational design of heparanase inhibitors, including small molecules.

* SST0001 is a property of Sigma-Tau Research Switzerland S.A.

Cancer associate fibroblast, the key player in matrix remodeling

L 26

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Besides tumor cells tumorous tissue contains stromal elements. For a long time this components of tumors have been considered as bystander in tumor growth and invasion.

Recently increasing amount of evidence put the tumorous stroma into the focus of cancer research. Research activity of last decade described quantitative and qualitative changes of tumorous extracellular matrix. The altered biological activity of tumor associated fibroblasts results in production of matrix proteins with altered structure or amount. Abnormal ECM can be responsible for disturbed regulation of cellular functions including impaired regulation, generation of mitogen signals, changes in adhesion, antiadhesion, as well as in homo and heterotypic interactions, etc, all together facilitating tumor growth. Furthermore these cells are implicated in the synthesis of regulatory factors responsible for the accelerated growth of tumor cells.

After the initiating observation revealing loss of syndecan-1 from the tumor cell surface, and detecting it on the tumor associated fibroblast it was decided to obtain more information about the phenotypic changes of stromal components of cervical cancer. To this end surgical specimens, as well as expression profile of normal and tumorous cervix derived fibroblast have been studied.

Increased immunostaining of fibronctin, laminin, vimentin, and smooth mucle actin indicated the presence of more activated fibroblasts with enhanced synthetic activity in the tumorous stroma of tumorous surgical specimens. Conversion of syndecan-1 expression could be witnessed, as well.

Fibroblasts explanted from cervical tissue supported the growth of primary as well as established cervical cancer cell lines. Microarray of fibroblasts from tumor free and tumorous tissue showed different expression profile where increased expression of fibroblast growth factor1, latent TGFB-binding protein 2, thrombospondin, and KISS-1 could be validated by PCR.

In the presence of tumor cells the serine protease inhibitor TFPI2 expression decreased both in normal and cancer associated fibroblasts. DNA methylation revealed complete methylation of this protein in CSCC7 cancer cells but not in fibroblast, suggesting different mechanism of inhibition in the latter. Inhibition of TFPI2 and increased production of MMP-1 and MMP-2 together with MMP-14, as well as decreases in TIMP-1m RNA and protein in tumor associate fibroblast indicates their central role in invasion. The increased expression of laminin and its receptor α6β4 on cancer cells and fibronectin and its receptors α5β1 on fibroblast suggests the regulatory effect of these matrix proteins on cancer cells and fibroblaststst. This results are supported by the fact that that migration of fibroblasts are facilitated by fibronectin, whereas tumor cells migrate only for matrigel. All these changes confer a proliferatory, migratory phenotype for the cervical cancer cells.

L 27

Modulation of matrix- and cytokine-dependent signaling by Syndecan-1 affects breast cancer metastasis, stemness and resistance to radiotherapy.

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Syndecan-1 (SDC1) is a cell surface heparan sulfate proteoglycan with various biological functions relevant to tumor progression and inflammation, including cell–cell adhesion, cell–matrix interaction, and cytokine signaling driving cell proliferation and motility. SDC-1 is a prognostic factor in breast cancer, and has a predicitive value for neodadjuvant chemotherapy. Here, we evaluated the potential role of SDC-1 in modulating matrix-dependent breast cancer cell migration in the presence of interleukin-6 (IL-6), and its potential involvement in resistance to irradiation in vitro. Moreover, we investigated the impact of SDC-1 on breast cancer stem cell properties. MDA-MB-231 and MCF-7 breast cancer cells were transiently transfected with SDC-1 siRNA or control reagents, followed by stimulation with IL-6 or irradiation. Cellular responses were monitored by adhesion, migration, flow cytometry, colony and sphere formation assays, as well as analysis of cell signaling. SDC-1 depletion increased cell adhesion to fibronectin. Increased migration on fibronectin was significantly suppressed by IL-6, and RGD peptides inhibited both adhesion and migration. IL-6-induced activation of focal adhesion kinase and reduction of constitutive nuclear factor kappaB signaling were decreased in SDC-1-deficient cells. Focal adhesion kinase hyperactivation in SDC-1-depleted cells was associated with dramatically reduced radiation sensitivity. Side population and ALDH1 activity measurement revealed that SDC-1 knockdown substantially reduces putative cancer stem cell pools by 60% and 27%, respectively. Moreover, the CD44(+)CD24(-/low) phenotype decreased by 6% upon siRNA-mediated SDC-1 depletion. IL-6, its receptor sIL-6R, and the chemokine CCL20, implicated in regulating stemness-associated pathways, were downregulated by >40% in SDC-1-silenced cells, which showed a dysregulated response to IL-6-induced epithelial-to-mesenchymal transition. Furthermore, activation of STAT-3 and NFkB transcription factors and expression of the Wnt coreceptor LRP6 were reduced by >45% in SDC1-depleted cells. At the functional level, SDC-1 siRNA reduced the formation of spheres and cysts in MCF-7 cells. We conclude that loss of SDC-1 leads to enhanced activation of beta-integrins and focal adhesion kinase, thus increasing breast cancer cell adhesion, migration, and resistance to irradiation. SDC-1 deficiency also attenuates the modulatory effect of the inflammatory microenvironment constituent interleukin-6 on cancer cell migration. Moreover, SDC-1 modulates the breast cancer stem cell phenotype via regulation of IL-6/STAT3 and Wnt signaling.

The structure and functions of the cell microenvironment: from systems biology to single molecule imaging

L 28

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The evolution of metazoans resulted in a transformation of the unit of natural selection, from the individual cell to the organism. This profound change required a deep integration of cellular function within the organism, achieved through a number of molecular innovations. One of these is the extracellular matrix.

Transport of effectors between cells is one means to integrate cellular function. Both development and, organism homeostasis depends on such transport of, e.g., growth factors, cytokines and chemokines from source to target cell; these effectors and their receptors are major targets for therapeutics. Such transport occurs in the extracellular and pericellular matrices that lie between cells where the heparan sulfate (HS) chains of proteoglycans (PG) are a dominant molecular species, due to their size (~40 nm to 160 nm long), amount and the number of proteins they bind to.

There are few insights into the structure of extracellular matrices and whether such structure impacts on molecular and cellular function. We have developed a network map of protein-HS interactions occurring in this space, which highlights the importance of HS binding proteins in cell regulation. To probe whether there is structure in the matrices of living cells, the dynamics of individual FGF molecules associated with the pericellular matrix has been measured. Finally, investigation of choanoflagellates, the sister clade of metazoans demonstrates that the last common unicellular ancestor of metazoans was likely to produce HS and CS.

The HS interactome of proteins of the pericellular matrix has been analysed by affinity proteomic analysis. Recent work of a disease model, acute pancreatitis, brings the number of extracellular heparin interacting proteins to over 600. Their interaction networks have some intriguing properties and demonstrate that this is a rich vein for biomarkers and novel drug targets.

To understand what actually occurs to soluble ligands when they interact with cell surface proteoglycans and receptors, novel nanoparticle probes have been developed, which enable the quantitative imaging of the dynamics of individual molecules in living cells ›10 min. These probes consist of gold nanoparticles and quantum dots (ø 5 nm to 10 nm), protected by a self-assembled monolayer of small ligands. This ligand shell is unique (no non-specific binding is detectable) and provides for specific and monovalent functionalisation e.g., 1 FGF:1 nanoparticle. Imaging of the dynamics of individual FGFs is possible by the application of advanced microscopy techniques (photothermal, RICS, *etc.*) and leads to a radical new model of this inter cellular compartment.

Taken together, these new insights provoke a re-think of strategies for the design of scaffolds for tissue engineering, for polymers for regenerative medicine and for therapies that aim to re-programme tissues, for example in inflammation.

L 29

Proteoglycan regulation of cancer growth and angiogenesis

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Strategies aimed at curbing tumor angiogenesis represent a viable therapeutic option to control cancer growth. The tumor stroma nourishes an angiogenic niche by providing instructive cues and signals in the form of processed autocrine and paracrine factors embedded within the extracellular matrix that act as either pro- or anti-angiogenic factors. Decorin is a powerful tumor repressor that acts in a paracrine fashion on the malignant cells enriched in receptor tyrosine kinases. We discovered that systemic delivery of decorin in an orthotopic breast carcinoma xenograft model upregulated a small group of genes, which were specifically induced in the tumor stroma of mouse origin, but not in the mammary carcinomas of human origin. One of the most upregulated genes was Peg3, a putative tumor suppressor gene frequently silenced by promoter methylation and/or loss of heterozygosity. We subsequently found that Peg3 was induced by decorin in both mouse microvascular and human umbilical vein endothelial cells, and that Peg3 redistributed from punctate cytoplasmic deposits into large vacuoles reminiscent of autophagosomes. This was confirmed by co-localization of Peg3 with two autophagy markers, Beclin 1 and LC3, and by co-immunoprecipitation of Peg3 with Beclin 1. The observation that decorin induced autophagy selectively in endothelial cells but not in tumor cells, led us to hypothesize that decorin could interact with an endothelial-specific receptor, i.e. VEGFR2. We discovered that decorin bound with high affinity to VEGFR2 ectodomain and that this binding could be efficiently displaced by VEGFA. Moreover, decorin induced mitochondrial fragmentation and loss of mitochondrial membrane potential in endothelial cells which could enhance its angiostatic function. Thus, we have unveiled a completely novel and unsuspected function for decorin in endothelial cells, that is, induction of autophagy and implication of Peg3 in this process. Decorin could be a novel antagonist of VEGFR2 by directly interfering with the VEGFA/VEGFR2 axis in tumor angiogenesis (Supported by NIH Grants RO1 CA39481, RO1 CA47282 and RO1 CA164462).

Proteoglycan-associated receptors

L 30

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Many cell surface receptors rely on heparan sulfate for activity, but depend on different modes of interaction. Fibroblast growth factor (FGF) receptors form 2:2:2 complexes with heparin and FGF ligands. Based on crystallization studies, the non-reducing end of each heparin chain docks with one FGF and a canyon defined by the two receptors. In contrast, vascular endothelial growth factor (VEGF) receptors depend on heparan sulfate for activity presumably due to interaction with a heparin-binding coreceptor, such as neuropilin-1, or for stabilization of receptor oligomers induced by ligand binding. In this system, interaction of the ligand with heparin is not required. Recently, we described a third model based on the receptor for advanced glycation end products (RAGE), which also requires heparan sulfate for activity. Here we show that RAGE undergoes a heparin-dependent, ligand-independent oligomerization, generating a stable hexamer of the RAGE extracellular domain. Site-directed mutagenesis mapped two separate heparan sulfate-binding sites in the Ig-like V and C1 domains of RAGE. A hydrophobic dimeric interface essential for the formation of hexamer was also identified in the V domain. Crystallization of RAGE V-C1 in the presence of a heparin dodecasaccharide revealed a hexameric structure in the crystal lattice, consistent with complex composed of a trimer of dimers. A solution structure consistent with a hexamer was also observed by small angle X-ray scattering. Mechanistically, the data suggests that heparan sulfate binds to a positively charged cleft created by two subunits of the dimer, stabilizing an otherwise weak hydrophobic dimeric interface and promoting dimer formation and assembly into a hexamer. A monoclonal antibody targeting the hydrophobic interface blocks receptor signaling, demonstrating that oligomerization is required for signaling. Thus, RAGE defines a third mode of interaction in which heparan sulfate is a subunit of the holoreceptor.

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The role of Glypicans in the regulation of Hedgehog signaling

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Glypicans are a family of proteoglycans that are bound to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor. The mammalian genome includes six glypicans (*GPC1* to *GPC6*), and ortholog genes have been identified across Metazoans, including two in *Drosophila* (Dally and Dlp). Glypicans do not display domains with obvious homology to characterized domains found in other proteins, suggesting that these proteoglycans have highly unique functions. Although the sequence identity between mammalian glypicans could be as low as 25 %, the three dimensional structure seems to be similar across the family. For example, the localization of 14 cysteine residues is highly conserved. Another feature shared by all glypicans is the position of the insertion sites for the GAG chains. These sites are located close to the C-terminus, suggesting that the GAG chains could mediate the interaction of these proteoglycans with other cell membrane proteins. The number of GAG insertion sites in each glypican, however, varies across the family (from 2 sites in GPC3, to 5 sites in GPC5). The functional implications of this variation are still not understood. In general, glypicans display heparan sulfate chains, but GPC5 produced by rhabdomyosarcoma (RMS) cells also exhibits chondroitin sulfate chains. Most glypicans can be secreted to the extracellular environment by a lipase called Notum, which cleaves the GPI anchor. In addition, glyicans can be cleaved into two subunits by a furin-like convertase. This cleavage generates two subunits that remain attached to each other by one or more disulfide linkages.Genetic and biochemical studies have shown that glypicans regulate the activity of the signaling pathways triggered by Hedgehogs, Wnts, Bone Morphogenetic Proteins, and Fibroblast growth factors.

The Hedgehog (Hh) signaling pathway plays a critical role in embryonic morphogenesis. In addition, hyperactivation of this pathway has been shown to promote the progression of various cancer types. In this lecture I will discussed several examples of human pathologies caused by deregulation of the Hh signaling pathway due to mutations or altered expression of glypicans. These pathologies include two genetic syndromes (Simpson-Golabi-Behmel and Recessive Omodysplasia), and one malignancy (rhabdomyosarcoma). The molecular mechanisms by which various glypicans regulate Hh signaling will also be discussed.

Signaling via receptors for PDGF and TGFβ – possible targets in tumor treatment

L 32

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Platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) affect cell growth, survival and migration, and have important roles during the embryonal development.

PDGF isoforms exert their cellular effects via two structurally similar tyrosine kinase receptors. We investigate the signal transduction mechanisms downstream of PDGF receptors which mediate PDGF′s effects, and the importance of receptor internalization and trafficking for signaling. Overactivity of the PDGF signaling pathway is associated with disease, e.g. certain malignancies. We have explored the use of PDGF antagonists in tumor treatment, and found significant inhibition of tumor growth in animal models of tumors driven by autocrine PDGF production. In addition, we have observed that inhibition of paracrine stimulation of stromal fibroblasts and vessel pericytes affects tumor interstitial fluid pressure and tumor angiogenesis. The latter effects are probably linked to the observation that PDGF antagonists act synergistically with chemotherapy in the treatment of several types of solid tumors in mice. We also noted an additive anti-angiogenic effect by combining VEGF receptor and PDGF receptor kinase inhibitors.

TGF-β has a more complicated role in cancer; first it is a tumor suppressor through its ability to inhibit growth and to promote apoptosis of tumor cells. At later stages, when tumor cells become resistant to the cytostatic effects of TGF-β, it has tumor promoter effects through stimulation of epithelial-mesenchymal transition of tumor cells, as well as stimulation of angiogenesis and suppression of the immune system. We are currently delineating the signaling pathways involved in the cytostatic and apoptotic effects of TGF-β, as well as in its effects on epithelial-mesenchymal transition which is linked to increased invasiveness. Our aim is to explore the possible use of TGF-beta antagonists in tumor treatment, and to develop selective TGF-β antagonists which inhibit the tumor promoting effects of TGF-β while leaving the tumor suppressive effects unperturbed.

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Heparin and heparan sulfate biosynthesis – Heparan sulfate and embryonic development

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Heparan sulfate structure varies greatly during embryonic development and differs also when heparan sulfate isolated from different tissues and cell types of an adult animal are compared. Heparin is the highly sulfated variant of heparan sulfate synthesized by connective tissue type mast cells. Biosynthesis takes place in the Golgi compartment and relies on the action of a multitude of enzymes. Our main goals are to find out how the cell decides on a particular heparan sulfate design and to characterize the molecular machinery responsible for its biosynthesis. Our model systems are mouse and zebrafish where we study study biological effects of mutations in biosynthesis enzymes. Embryonic stem cells and embryonic fibroblasts derived from mutant mice as well as mammalian cell-lines overexpressing or lacking selected biosynthesis enzymes are important tools.

Biosynthesis and functions of dermatan sulfate

L 34

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Dermatan sulfate (DS) differs from chondroitin sulfate (CS) for the presence of iduronic acid which can quantitatively vary from being few in each chain or the major component. Indeed all chains are a mixed of dermatan and chondroitin sulfate (CS/DS chains). Functions of iduronic acid-containing motifs in CS/DS span from representing cellular receptor or co-receptor of a variety of proteins like growth factors (i.e. FGFs, hepatocyte growth factor, neurotropic growth factors) or cell surface proteins of pathogens (i.e. Borrelia) to modulate collagen fibrilr formation in the extracellular matrix. DS is also an anticoagulant drug, although of rare use, due to its ability to increase the binding of thrombin to its inhibitor heparin cofactor II (HCII).

Dermatan sulfate epimerase 1, 2 (DS-epi1 and 2), together with dermatan 4-O-sulfotransferase 1 are the only dermatan sulfate specific enzymes. We have previously cloned dermatan sulfate epimerase 1 (DS-epi1) and DS-epi2, both of them converting glucuronic acid into iduronic acid. Since then the constitutive deficient mouse models were obtained. From our data it is apparent that DS-epi1 is the major epimerase in the body, but not in brain, where DS-epi2 is the main epimerase, and in kidney. *In vivo* the synthesis of the iduronic acid blocks, long extensions of adjacent iduronic acid, is carried out by and large by DS-epi1 while the synthesis of isolated iduronic acid is carried out by both enzymes. DS-epi1 KO mice in a mixed genetic background are vital and fertile and show defects in collagen fibril maturation and in basement membranes. On the opposite, most of the DS-epi1 deficient mice of pure genetic background, either C59BL6 or NFR, are perinatally lethal. The embryos show epidermis thickening due to faulty differentiation of keratinocytes. Some of the embryos show gastroschisis, an embryological defect, present also in humans, where the abdominal wall does not close properly and therefore the intestines are out. DS-epi2 KO mice have no anatomical, histological or morphological abnormalities. Despite a moderate reduction in brain iduronic acid, adult DS-epi2-/- brain showed normal extracellular matrix features by immunohistological stainings. Finally, DS-epi1 and 2 double KO were obtained. This is indeed a model of iduronic acid-free, or DS-free, animals. In a mixed genetic background most of the mice die perinatally. The few survivors are dwarf, weighing approx. 40% of their control littermates. Some of these mice present with intracellular deposition in bronchiolar epithelial cells of YM1, a poorly studied protein thought to be involved in modulation of innate immunity. DS-epi1 and 2 were downregulated also in Xenopus laevis. Several interesting phenotypes were apparent, among which the impaired migration of neural crest cells which led for instance to loss of the dorsal fin and decreased body pigmentation.

Effects of iduronic acid-containing structures on cellular migration, due to CS/DS proteoglycan(s) sitting in the cellular membrane, were also described in three cellular models: the squamous cell carcinoma TE-1, mouse embryonic fibroblasts, and aorta smooth muscle cells. In the latter model it has been showed that iduronic acid affects the directional migration of the cells and the structure and number of the focal adhesion sites.

In summary, our in vivo observations are pointing to multiple critical functions of DS in the modulation of extracellular matrix-cell communication.

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Intracellular cross-talk in breast cancer and bone metastasis: pharmacological targeting at the level of matrix molecules

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Studies throughout the last decades turned (or established) the present perception concerning the role of extracellular matrix (ECM) from an inactive network of macromolecules to a functional network essential for structural support, cell migration, adhesion and signaling. Cancer cells participate in several interactions with the tumor microenvironment constituents, including the various ECM macromolecules, growth factors and cytokines, as well as the surrounding cells (endothelial cells, fibroblasts, macrophages, mast cells, neutrophils, pericytes and adipocytes). ECM components, such as proteoglycans (PGs), mettaloproteinases (MMPs) and integrins contribute to these dynamic interactions, affecting the growth as well as cell migration, invasion and metastatic potential of cancer cells. Our laboratory in recent studies is dealing with novel approaches in respect to the pharmacological targeting at the ECM level in cancer. ECM molecules and especially proteoglycans (PGs) as well as glycosaminoglycans (GAGs) seems to interact with growth factors and receptors in tumor microenvironment regulating cancer homeostasis. Notably, estrogens represent the most important factors implicated in the progression of hormone-dependent breast cancer. The action of estrogens, regulated via estrogen receptors (ER), 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 expression profiles and properties in respect to their phenotype and invasion potential. In this presentation the importance of the cross talk of ERs with tyrosine kinase receptors EGFR and IGFR in the expression of proteoglycans, matrix proteolytic effectors and on the functional properties of breast cancer cells will be discussed.

Recent and useful reference: Tsonis et al., FEBS J, 2013; 280 (10): 2248–2259

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Signaling pathways regulated by syndecan-1 in malignant mesothelioma

L 36

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Syndecan-1 is a cell surface proteoglycan (PG) important for the differentiation of mesothelial and epithelial cells. Dedifferentiated tumor components and mesenchymal tumours gradually loose their syndecan-1 expression. In mesothelioma the expression of syndecan-1 correlates to favorable prognosis, epithelioid morphology and inhibition of growth and migration.

Syndecan-1 exerts its effect partly at the level of the cell membrane through growth factor (GFs) – growth factor receptor complexes. We have, however, shown that syndecan-1 also translocates to the nucleus in a regulated manner by a tubulin mediated transport mechanism. Similar nuclear transport of growth factors and their receptors indicates a possible co-regulation with syndecan-1 and heparanase. The RMKKK motif at the cytoplasmic tail of syndecan-1 is the minimal sufficient sequence for this nuclear translocation. The molecular basis and function of the nuclear translocation of syndecan-1 in malignant mesothelioma cells are addressed by both over expression and silencing of syndecan-1 gene, and functional assays downstream of syndecan-1. Deletion of the RMKKK sequence allows us to separately analyze the cellular functions related to cell surface and nuclear syndecan-1.

Syndecan-1 over-expression had profound effects on genes involved in regulation of cell growth, cell cycle progression, adhesion, migration and extracellular matrix organization as evidenced by microarray data. In particular, expression of several growth factors, interleukins, and enzymes of importance for heparan-sulfate sulphation pattern, extracellular matrix proteins and proteoglycans were significantly altered. 14 genes showed response to both up- and down-regulation of syndecan-1. The “cytokine – cytokine-receptor interaction”, the TGF-β, EGF, VEGF and ERK/MAPK pathways were enriched in both experimental settings. Most strikingly, nearly all analyzed pathways related to cell cycle were enriched after syndecan-1 silencing and depleted after syndecan-1 over-expression.

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Hormonal regulation of bone cell ECM organization-effects on cell functions

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Contacts between the cells of the osteoblast lineage and surrounding extracellular matrix (ECM) play a key role in the mechanisms that dictate cellular behaviour. Parathyroid hormone (PTH) is an 84-amino acid polypeptide hormone produced by the parathyroid gland that regulates calcium homeostasis and bone remodelling. The anabolic effect of intermittent administration yielding a transient peak blood level and the catabolic effect of continuous administration of PTH on bone formation have been demonstrated in human and animal models. Importantly, PTH has been reported to regulate extracellular matrix (ECM) component production in both normal and carcinogenic cells of the osteoblastic lineage. The effects of PTH in the regulation of osteoblastic cells' ECM and consecutive modulations of these cells' motility will be discussed with a focus on the hyaluronan metabolism and the fibroblast growth factor-proteoglycan signaling axis.

Thrombospondin-2 and the eNOS/Akt axis in vascular remodeling

L 38

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Angiogenesis is essential for tissue repair and is regulated by the balance of anti- and pro-angiogenic factors angiogenic factors

activation of Akt and its substrate eNOS, respectively. eNOS knockout (K) and Akt1 KO mice have similar phenotypes that are opposite of TSP2 KO mice in dermal wound healing and hindlimb ischemia. In addition, eNOS KO and Akt1 KO mice display elevated TSP2 expression in both injury models. Previously, we demonstrated that genetic deletion of TSP2 in the eNOS KO mice rescued the angiogenic phenotype and showed that nitric oxide repressed the production of TSP2 at the transcriptional level. To investigate the contribution of increased TSP2 to the compromised phenotype of these mice, we generated Akt1/TSP2 double KO (DKO) mice and analyzed their responses to injury. DKO mice healed at an accelerated rate and displayed improved blood flow recovery concomitant with improved tissue salvage following hindlimb ischemia. To determine the cellular and molecular basis for these observations we isolated and analyzed the function of Akt1 KO mouse Lung Endothelial Cells (MLECs) and dermal fibroblasts. Akt1 KO MLEC expressed increased TSP2 and were defective in migration. These findings were consistent with the previous demonstration that eNOS represses TSP2 expression. However, we also observed increased TSP2 in Akt1 KO dermal fibroblasts, which do not have eNOS activity, suggesting that TSP2 could be regulated in an eNOS-independent manner. Akt1 KO fibroblasts displayed defects in migration and adhesion associated with altered morphology, which were rescued in Akt1/TSP2 DKO dermal fibroblasts. Moreover, shRNA knockdown of TSP2 in Akt1 KO fibroblasts rescued their adhesive defect. Taken together, these observations suggest Akt1 represses TSP2 expression and the loss of this function in Akt1 KO mice and cells contributes to a number of defects that compromise repair processes.

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Receptor protein tyrosine phosphatase beta/zeta is a regulator of vascular endothelial growth factor-induced endothelial cell migration

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Receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ) is a transmembrane protein-tyrosine-phosphatase, characterized by the presence of an N-terminal carbonic anhydrase-like domain, a fibronectin type III domain and a serine, glycine-rich domain for chondroitin sulfate attachment in the extracellular region. It was initially found in the nervous system as a cell surface receptor that binds to a number of extracellular matrix and neuronal cell adhesion molecules from the Ig superfamily. More recently, RPTPβ/ζ was identified as a receptor for the soluble ligands midkine and pleiotrophin (PTN), which constitute a distinct family of heparin binding growth factors. RPTPβ/ζ is the best characterized PTN receptor up to date and is present on human endothelial and tumour cells. Upon binding of PTN, RPTPβ/ζ leads to c-Src dephosphorylation at Tyr530, β_3 integrin Tyr773 phosphorylation, cell surface nucleolin (NCL) localization and stimulation of cell migration.

Vascular endothelial growth factor 165 ($VEGF_{165}$) is known to activate several functions of endothelial cells through its receptor type 2 (VEGFR-2). c-Src-mediated phosphorylation of tyrosine residues 773 and 785 within the β_3 cytoplasmic tail seems to be essential for VEGFR-2-β_3 integrin association and subsequent signaling, although it remains unclear how c-Src is activated. NCL cell surface translocation is also observed upon $VEGF_{165}$ stimulation of endothelial cells, playing a significant role in $VEGF_{165}$-induced cell migration. Interestingly, RPTPβ/ζ-mediated c-Src-dependent β_3 Tyr773 phosphorylation and the downstream activation of PI3K were found to be required for $VEGF_{165}$-induced NCL cell surface localization. By using immunoprecipitation, immunofluorescence and proximity ligation assays, $VEGF_{165}$ was found to directly interact with RPTPβ/ζ, and this interaction was interrupted by PTN. RPTPβ/ζ was not found to interact with VEGFR-2 but was required for $VEGF_{165}$-induced interaction of VEGFR-2 with $\alpha_v\beta_3$ through $VEGF_{165}$-mediated c-Src activation and β_3 Tyr773 phosphorylation. Finally, as shown by down-regulation of RPTPβ/ζ expression by siRNA, RPTPβ/ζ is required for $VEGF_{165}$-induced cell migration.

Collectively, RPTPβ/ζ is a receptor for $VEGF_{165}$ that is involved in c-Src-mediated β_3 Tyr773 phosphorylation and interaction with VEGFR-2, NCL cell surface localization and endothelial cell migration. These data support the notion that RPTPβ/ζ is potentially an alternative therapeutic target for cancer or other angiogenesis-related pathologies.

Acknowledgments

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Cellular senescence, DNA damage and role in tissue homeostasis

L 40

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DNA damage response, either due to telomere shortening or to repeated subcytotoxic genotoxic stresses is a common molecular mechanism leading replicative- or stress-induced senescence. Cellular senescence is considered a potent anticancer mechanism. On the other hand, due to their proinflammatory phenotype, it is believed that senescent cells may affect local tissue homeostasis and probably to contribute to the development of age-related degenerative diseases, including cancer. In this vein, we have shown that subcytotoxic therapeutic doses of ionizing radiation provoke premature senescence in stromal human fibroblasts and these cells enhance the growth of cancer cells in vitro and in immunocompromised mice in vivo. Ionizing radiation-mediated premature senescence impairs also the ability of several types of cells towards different lines of differentiation. Low back pain represents a common age-related pathology and it is strongly associated with intervertebral disc (IVD) degeneration. We have studied the effect of various stresses IVD cells are continuously exposed to during daily activities and have found that hyperosmolality provokes modifications in the nuclear structure, provokes DNA damage, activates the ATM-Chk2-p53-p21WAF1-pRb axis and thus it regulates their proliferation. It also enhances DNA repair capacity in these cells. On the other hand, it also provokes premature senescence in IVD cells, probably contributing in the degeneration of this tissue. Finally, we present evidence from another cell system, i.e. periodontal ligament fibroblasts, showing that replicative- and stress-induced senescence impair their ability for an osteoblastic differentiation and alters their gene expression profile towards a catabolic phenotype.

L 41

Versican and the Control of Cell Phenotype in Disease

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Versican is a large proteoglycan that contains chondroitin sulfate glycosaminoglycan chains and is found in most soft tissues where it interacts with a variety of other extracellular matrix (ECM) molecules to partially regulate the biomechanical properties of tissues. In addition, versican interacts with cells to influence such events as cell adhesion, proliferation, migration and ECM remodeling While present in low amounts in most tissues, this matrix molecule dramatically increases in a number of diseases, most notable in vascular disease and in several types of cancers. In vascular disease, versican accumulates in early developing atherosclerotic lesions where it supports intimal hyperplasia as well as the accumulation of lipid and macrophages as the lesions become more severe. Growth factors such as TGFβ and platelet-derived growth factor (PDGF) stimulate the synthesis of versican by arterial smooth muscle cells (ASMCs) and interference with versican synthesis by these cells blocks their proliferative and migratory response to these growth factors. Furthermore, blocking the interaction of versican with lipoproteins in a mouse model of atherosclerosis blocks the development of lipid-filled lesions and the development of atherosclerotic plaques in this animal model. Versican also influences the binding and retention of leukocytes as part of the inflammatory response that characterizes vascular lesion formation and other diseases as well. Versican interacts with hyaluronan to form ECM fibrils that serve as attachment sites for leukocytes, including eosinophils, monocytes, and T-lymphocytes and this interaction can be blocked by blocking antibodies to versican. Furthermore, treatment of versican with chondroitin ABC lyase or inhibiting versican synthesis by siRNA interferes with monocyte binding to ECM generated by ASMCs. To further test the importance of the chondroitin sulfate chains of versican in the inflammatory response, ASMCs were transduced with the gene that codes for the versican isoform that lacks chondroitin sulfate chains, V3—the isoform we like to call versican't! Overexpression of V3 produces an ECM enriched in elastic fibers and depleted of versican, involving several distinct signaling pathways. This ECM resists monocyte adhesion and when the ASMCs expressing this isoform are seeded into injured carotid arteries in rabbits placed on a high fat diet, expression of V3 inhibited lipid and macrophage accumulation and significantly reduced vascular inflammation in this animal model. Collectively, these studies highlight how a specific component of the ECM can impact several key events in the pathogenesis of vascular disease and may represent a unique target to treat this disease and others in the future.

Proteolytic control of cutaneous wound repair and cancer progression

L 42

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Wound healing is a fundamental process that reconstitutes structure and function of tissue after injury. Wound repair involves several cell types orchestrated by growth factors and cytokines, as well as by interactions with extracellular matrix (ECM) molecules. Proteolysis is an essential feature in wound healing. 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, strictly controlled proteolysis is important for normal wound healing, and alterations in proteolytic activity are associated with aberrant wound closure and scar formation. The principal proteinases in healing wound include serine proteinases plasmin and plasminogen activators (PAs), and a variety of metalloproteinases. The roles of plasmin and matrix metalloproteinases (MMPs) in wound repair have been extensively examined but increasing 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 wound healing.

Cutaneous squamous cell carcinoma (cSCC) is the second most common malignant tumor of skin, and its incidence is increasing globally. Chronic ulceration is a well recognized risk factor for cSCC and ulceration is a typical clinical feature during progression of UV-induced cSCC from intraepithelial early lesion (actinic keratosis) to invasive and metastatic SCC. Therefore, many characteristics of normal wound healing, including proliferation of epidermal keratinocytes, inflammation, and angiogenesis, are also typical features in cSCCs.

Here, the roles of plasmin, MMPs, ADAMs and ADAM-TS proteinases in cutaneous wound healing and in progression of cSCC will be discussed.

L 43

Nature and Properties of Matrix Metalloprotease-9/Chondroitin Sulphate Proteoglycan Complexes

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Proteolytic processing of extracellular matrix proteins, cell surface receptors, growth factors, cytokines, chemokines, enzymes and inhibitors are important biological processes in normal as well as pathological conditions. Among the proteases contributing to peptide processing is matrix metalloproteinase-9 (MMP-9) which belongs to a family of metalloproteases called matrix metalloproteases (MMPs). The enzymatic activity of proteases including MMPs is regulated at various levels. One type of regulation involves complex formation between the proteases and other macromolecules where the location, activity as well as the specificity of the enzyme is altered.

MMPs are built up of different domains, moduls and motifs. Most MMPs including MMP-9 contain a pro-, a catalytic and a hemopexin-like (HPX) domain. 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. The FnII module facilitates the localization of MMP-9 to connective tissue matrices as well as the degradation of various biological substrates. Regions outside the catalytic cleft that are important for the enzyme activity against specific biological substrates are called exosites, and these are important in the development of new specific MMP inhibitors.

In addition to its momomeric form, MMP-9 is also found as a homodimer or as a heterodimer. Some of these MMP-9 dimers are not dissociated by SDS, but are reduction sensitive, suggesting that these proteins are either covalently linked to each other through disulfide bonds or through a very strong reversible interaction where intramolecular disulfide bonds are essential. Among the biological macromolecules to which MMP-9 binds and form reduction sensitive complexes are chondroitin sulphate proteoglycan (CSPG) core proteins. Serglycin and versican are two CSPGs which form complexes with MMP-9. *In vitro* reconstitution of the proMMP-9/CSPG complexes reveals the presence of both SDS-soluble and SDS-stable complexes, where the latter complexes are reduction sensitive. MMP-9 and its various complexes have unique biochemical and enzymatic properties. A part of the lecture will focus on the *in vivo* and *in vitro* formed MMP-9/CSPG complexes, type of interactions involved, motifs in the MMP-9 and CSPG that is involved in the complex formation, biochemical properties of the enzyme in these complexes and possible consequences this will have for the biological function of the enzyme.

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Extracellular cysteine cathepsins: more than extracellular matrix degradation

L 44

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Cysteine cathepsins are a group of papain-like cysteine proteases, normally confined to the endolysosomal system, where they have a major role in intracellular protein turnover and in antigen presentation. However, 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. There is, however, increasing evidence that the cathepsins are, through the cleavage of different extracellular or membrane proteins, involved in the regulation of many other processes leading to disease progression. Identification of their physiological substrates is therefore of major importance for understanding their signaling pathways linked with disease progression and provides a major opportunity for identification of novel cathepsin-dependent biomarkers and will be further discussed.

L 45

What can a mouse teach us about cardiovascular disease risk?

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Vascular endothelial cells produce HS^{AT+}, heparan sulfate with a specific pentasaccharide motif that binds the plasma protein antithrombin (AT). AT is the most potent physiologic inhibitor of blood clotting, so it has long be thought that vascular HS^{AT+} may convey an antithrombotic tone to the blood vessel wall. Indeed, *in vitro* studies show that HS^{AT+} can catalyze AT neutralization of activated coagulation proteases. Yet, *in vivo* role for vascular HS^{AT+} in preventing blood clotting has never been conclusively demonstrated. To elucidate the *in vivo* role of HS^{AT+}, we first discovered that endothelial production of HS^{AT+} is governed by the rate limiting action of heparan sulfate 3-*O*-sulfotransferase (3-OST-1), which is encoded by the H*s3st1* gene. Consequently, we generated *Hs3st1*$^{-/-}$ knockout mice which are devoid of 3-OST-1 activity and exhibit large reductions in HS^{AT+}. Surprisingly, the coagulation status of *Hs3st1*$^{-/-}$ mice was completely normal under a variety of conditions including base line tissue fibrin accumulation, systemic prothrombotic challenge with hypoxia (which upregulates tissue factor expression) and macrovascular injury which induces occlusive thrombosis. Thus, vascular HS^{AT+} is not essential for normal hemostasis. However, AT is a multifunctional molecule that also exhibits anti-inflammatory and pro-inflammatory activities that are mechanistically distinct from its anticoagulant activity. We are now testing the hypothesis that the *in vivo* role of vascular HS^{AT+} is to mediate the anti-inflammatory activity of AT. Such a role might be important in human cardiovascular disease, which is strongly driven by inflammatory processes. Potential clinical implications of this model will be discussed.

Proteasome activation as a novel anti-aging strategy

L 46

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Aging and longevity are two multifactorial biological phenomena whose knowledge at molecular level is still limited. We have studied proteasome function in replicative senescence and cell survival (Mol Aspects Med, in press, 2013). We have observed reduced levels of proteasome content and activities in senescent cells due to the down-regulation of the catalytic subunits of the 20S complex (J Biol Chem 278, 28026-28037, 2003). In support, partial inhibition of proteasomes in young cells by specific inhibitors induces premature senescence which is p53 dependent (Aging Cell 7, 717-732, 2008). Stable over-expression of catalytic subunits or POMP resulted in enhanced proteasome assembly and activities and increased cell survival following treatments with various oxidants. Importantly, the developed "proteasome activated" human fibroblasts cell lines exhibit a delay of senescence by approximately 15% (J Biol Chem 280, 11840-11850, 2005; J Biol Chem 284, 30076-30086, 2009). Our current work proposes that proteasome activation is an evolutionary conserved mechanism, as it can delay aging in various in vivo systems. Moreover, additional findings indicate that the recorded proteasome activation by many inducers is Nrf2-dependent (J Biol Chem 285, 8171-8184, 2010). Finally, we have studied the proteolysis processes of various age-related proteins and we have identified that CHIP is a major p53 E3 ligase in senescent fibroblasts (Free Rad Biol Med 50, 157-165, 2011).

L
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Implications of heparan sulfate in amyloidosis

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Amyloidosis is a generic term for a group of diseases characterized by abnormal deposition of insoluble materials (amyloid) in the ECM of different organs. So far, more than 30 different endogenous proteins are identified to be able to form amyloid. Apart from the tissue specific amyloid formation peptide, heparan sulfate proteoglycan (HSPG) and its free heparan sulfate (HS) polysaccharide chains are commonly found in most amyloid deposits regardless of the species of amyloid, suggesting that HS/HSPG may be functionally involved in the pathogenesis of amyloidosis. *In vitro* experiment has revealed that HS and HSPG interacts with majority of the identified amyloid peptides, displaying a promoting effect on amyloid fibrilization and aggregation. Although little is known about the *in vivo* mechanisms regarding the co-deposition of HS with amyloid in different amyloid diseases, experiments carried out in animal models, especially in transgenic mouse model where HS molecular structure is modified, support an active role for HS in amyloidogenesis. Further experimental evidence is required to strengthen these *in vivo* findings. Animal models that express mutant forms of HS due to knockout of the enzymes involved in HS biosynthesis and degradation are expected to provide valuable tools for studying the implications of HS, as well as other glycosaminoglycans, in amyloid disorders. This lecture will present the experimental results regarding the potential functional roles of HS in three different types of amyloidosis.

Evidence for a protective role of the mosquito Matrix Metallo-Protease 1 (AgMMP1) during Plasmodium oocyst development

Extra Talk

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The group of Matrix Metalloproteases (MMPs; metzincin clan of metallopeptidases) includes Zn-dependent endopeptidases identified by a common structural architecture. MMPs are involved in a wide range of developmental and physiological processes, including epithelial restructuring and host defenses. In the annotated genome of the malaria vector *Anopheles gambiae*, the MMP family is represented by three members (AgMMP1, AgMMP2, and AgMMP3) in addition to a single gene encoding for a potential homologue of the selective MMP inhibitor, Tissue Inhibitor of Metalloproteases (TIMP).

By combining cell biology and reverse genetics approaches we studied the gene function of *AgMMP1* and *AgTIMP*, particularly during the processes of blood meal digestion, midgut epithelium invasion by *Plasmodium* ookinetes and Plasmodium oocyst differentiation. AgMMP1 was chosen over the other two *A. gambiae* MMPs since is most prominently expressed in the mosquito midgut. We found that AgMMP1 in adult mosquitoes exists in two alternative tissue restricted isoforms resulting from alternative splicing; one potentially secreted (S-MMP1) localized in the peripheral hemocytes, and one membrane-associated (M-MMP1), enriched in the cell attachment sites of the midgut and follicular epithelium. AgTIMP was found abundantly present in the midgut epithelial cells, while it was barely detected in hemolymph extracts and was absent in peripheral hemocytes.

RNAi-mediated silencing of *AgMMP1* drastically reduced the numbers of oocysts growing in contact to the basal lamina of the midgut, without affecting the ookinete midgut invasion process *per se*, advocating for a likely post-invasion protective function of AgMMP1 over the young developing oocysts; a hypothesis that is further supported by the masking of a fraction of young oocysts with MMP1 immunoreactive material. In contrast to *AgMMP1*, RNAi silencing of *AgTIMP* had no apparent effect on mosquito midgut invasion and/or oocyst growth.

This is the first study reported for a member of this multifunctional group of proteases in *Anopheles* that consist an ideal model to study the complex molecular aspects of MMP regulation in diverse developmental and physiological processes.

Selected Talks/ Abstracts

Syndecan-1 overexpression inhibits SULF1 and modulates heparan sulfate chain composition in malignant mesothelioma cells

ST 01/P1

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Malignant pleural mesothelioma is a highly malignant tumour, originating from mesothelial cells lining the pleural cavities. In this tumor syndecan-1 (SDC1) is downregulated, and its expression correlates with epitheloid morphology. We have previously shown that SDC1 alters malignant mesothelioma cell adhesion, migration and proliferation by modulating several critical pathways. SDC1 exerts its functions by binding different growth factors, morphogens and cytokines through its heparan sulfate (HS) chains. The sulfation pattern of HS chains is pivotal in these interactions and is determined by sulfotransferases which add sulfate groups to the HS precursors, and extracellular sulfatases which selectively remove the 6-O-sulfate groups from the mature HS chains. Our previous studies also revealed that SDC1 might alter the expression of enzymes responsible for HS biosynthesis and sulfation.

The aim of this study is to elucidate if SDC1 overexpression affects the structure of HS chains and sulfation pattern of proteoglycans and how this affects proliferation.

We overexpressed SDC1 in a human mesothelioma cell line and analyzed the expression of enzymes involved in HS chain biosynthesis and sulfation. To determine the alterations of the fine structure of HS chains, we used a panel of phage-display antibodies, specific for differently sulfated HS sequences. To assess the activity of sulfatase-1 (SULF1), the alterations in HS epitopes in SDC1 overexpressing cells were evaluated in the presence or absence of exogenously added SULF1. Cell proliferation was measured in SDC1 overexpressing cells in the presence and absence of SULF1.

Expression of SULF1 was 52-fold downregulated in SDC1 overexpressing cells; 6-O-sulfotransferase-1 was 3,5 fold downregulated, while expression of 2-O-sulfotransferase-1 was slightly enhanced. Cell surface expression of RB4EA12 epitope, which is specific for 6-O-sulfation increased in SDC1 overexpressing cells. This difference disappeared with addition of SULF1. Exogenously added SULF1 reverted the effect of SDC1 overexpression on proliferation.

Conclusion: Syndecan-1 increases HS 6-O sulfation in mesothelioma cells by inhibiting the expression of SULF1. We can assume that SDC1 not only influences the proliferation of malignant mesothelioma cells by being a co-receptor for growth factors, but it can also affect the binding capacity of other HS proteoglycans by modulating the HS sulfation pattern through SULF1.

ST 02/P2

Effect of Lumcorin, a lumican-derived peptide, on the melanoma cell growth and invasion

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Lumican, a small leucine-rich proteoglycan of the extracellular matrix, presents potent antitumour properties. Previous works from our group showed that lumican was able to inhibit melanoma cell migration and tumor growth in vitro and in vivo. Melanoma cells are capable to adhere to lumican, resulting in a remodeling of their actin cytoskeleton and preventing their migration. In parallel, an inhibition of the phosphorylation of focal adhesion kinase (FAK) was observed. In addition, we identified a sequence of 17 amino acids (aa) within the lumican core protein, named lumcorin, which was able to inhibit cell chemotaxis and reproduce anti-migratory effect of lumican in vitro (Zeltz et al., 2009). The aim of the present study was to characterize the antitumor mechanism of action of lumcorin.

Lumcorin significantly decreased the growth in soft agar of colonies of two melanoma cell lines - B16F1 cells (mice melanoma cell line) and SkMel-28 (human melanoma cell line) in comparison to control. Addition of 100µM lumcorin to serum free medium significantly inhibited B16F1 and SK-MEL28 cell migration. To characterize the mechanisms involved in the inhibition of cell migration by lumcorin, the status of the phosphorylation/dephosphorylation of proteins was examined. Lumcorin inhibited FAK phosphorylation in B16F1 cells. Since cancer cells have been shown to migrate and to invade by mechanisms that involve matrix metalloproteinases (MMPs), the expression and activity of MMPs in B16F1 cells were analyzed. The presence of lumcorin induced an accumulation of an intermediate form of MMP-14 (~59kDa), and inhibited MMP-14 activity. Altogether, these results suggest that lumcorin inhibits melanoma cell migration by involvement of two simultaneous mechanisms: inhibition of phosphorylation of specific proteins and decrease of MMP14 activity.

Syndecan-4 promotes myocardial stiffness by regulating myofibroblast differentiation and extracellular matrix structure in response to pressure overload

ST 03/P3

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Pressure overload of the heart leads to remodeling of the left ventricle involving excessive production of extracellular matrix (ECM) by activated cardiac fibroblasts that differentiate into contractile myofibroblasts. This compromises heart function by increasing myocardial stiffness. The molecular mechanisms underlying stress-induced myofibroblast differentiation and the role of this process in regulating cardiac stiffness are poorly defined. We recently identified the focal adhesion proteoglycan syndecan-4 as important for myofibroblast differentiation in response to mechanical stress. Here we investigate the effect of syndecan-4 deletion on the mechanical properties of the left ventricle following pressure overload.

Passive tension was reduced in muscle fiber bundles from left ventricles of syndecan-4-/- (syn4-/-) mice compared to wild-type (WT) mice and increased in both genotypes following aortic banding, albeit to a lower degree in syn4-/- mice. Salt extraction of myosin and actin filaments was performed to eliminate the effect of titin, a cardiac protein which is central in determining passive tension. Passive tension after salt extraction was affected equally in muscle fiber bundles from WT and syn4-/- mice, indicating that the reduced passive tension in mice lacking syndecan-4 was due to alterations in the ECM and not changes in titin. Initial effects of 24 hrs aortic banding included a ~6-fold increase in collagen I and III mRNA in the left ventricle of WT mice. Remarkably, this response was completely absent in mice lacking syndecan-4. Consistent with this, quantification of ECM, fibroblasts and blood vessels by electron microscopy, revealed increased number of fibroblasts in left ventricles of WT mice and reduced amount of ECM in syn4-/- mice. mRNA levels of the collagen cross-linking enzyme lysyl oxidase (LOX) were also dramatically increased in WT left ventricles, whereas this response was blunted in syn4-/- mice, suggesting impaired collagen cross-linking following mechanical stress in mice lacking syndecan-4. Supporting these findings, LOX activity was reduced in left ventricles of syn4-/- mice. Furthermore, cardiac fibroblasts from syn4-/- mice had lower expression of collagen I and III and LOX when plated on fibronectin in vitro, a model previously shown to induce myofibroblast differentiation.

In conclusion, we demonstrate reduced passive tension in left ventricular tissue of syn4-/- mice possibly due to inhibited differentiation of fibroblasts into myofibroblasts, reduced ECM production and attenuated collagen cross-linking.

ST 04/P4

Syndecan-1 overexpression on mesotheliomal cells affects angiogenesis-related factors

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Malignant mesothelioma is an aggressive tumor of serosal membrane. The most common form involves the pleura (Malignant Pleural Mesothelioma, MPM) with poor prognosis. Data suggest that angiogenesis is crucial in MPM progression and anti-angiogenic therapies do not show significant improvement in patients' survival. Syndecan-1 is a transmembrane heparan sulfate proteoglycan that acts as a regulatory co-receptor in different cellular processes including angiogenesis. The angiogenesis regulatory mechanism of syndecan-1 is known to be through binding VEGF and also via activation of integrins and insulin like growth factor-1 receptor (IGF-1R). In addition there is a unique sequence on syndecan-1 extracellular domain known as synstatin that regulates the signaling pathways involved in angiogenesis. The expression of syndecan-1 is low on MPM cells and it has been shown that decrease of syndecan-1 deteriorates the prognosis.

To understand the function of syndecan-1 in relation to angiogenesis of MPM cells and to elucidate how modulation of syndecan-1 influences endothelial cell functions.

Syndecan-1 was overexpressed in a human MPM cell line and transcriptomic responses were assessed by microarray analysis. Conditioned mediums from syndecan-1 overexpressing cells were collected and expression of soluble angiogenic factors was measured using Proteome Profiler Array. In order to see if proteins modulated by syndecan-1 overexpression affect endothelial cell proliferation, HUV-EC-C cells were treated with conditioned mediums from syndecan-1 overexpressing cells. Cell proliferation was assessed using WST1 proliferation assay.

Overexpression of syndecan-1 in MPM cells affects a range of growth factors and interleukins of importance in angiogenesis at transcriptomic levels. Gene expression of several receptor tyrosin kinases and their ligands were altered in syndecan-1 overexpressing MPM cells (EGF/EGFR, PDGF/PDGFR and FGF/FGFR and VEGF). Further, a number of angiogenesis related proteins were altered by syndecan-1, Including both pro-angiogenic (as FGF-4, HGF, NRG-1, IL-8, Ang-1) and anti-angiogenic proteins (as ES, TSP-1, serpinE-1). Endothelial cells proliferation was affected by adding condition medium from cells overexpressing syndecan-1.

Stromal Syndecan-1, Matrix Alignment and Breast Cancer Invasion

ST 05/P5

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Breast cancer progression is governed by dynamic and reciprocal interactions between carcinoma cells and stromal constituents. Several groups including ours have reported that expression of the heparan sulfate proteoglycan syndecan-1 (Sdc1) is induced in stromal fibroblasts of some but not all breast carcinomas. In vitro and in vivo, Sdc1 expressing fibroblasts produce an extracellular matrix (ECM) with an aligned fiber organization, which promotes directional migration and invasion of breast carcinoma cells. The goal of this study was to determine the mechanism by which Sdc1 regulates ECM fiber alignment.

With domain deletion and substitution experiments in human mammary fibroblasts (HMF), we determined that amino acids 88-252 of the extracellular domain and the heparan sulfate chains are required for ECM fiber alignment, whereas the cytoplasmic and transmembrane domains are dispensable. These findings point to an integrin interaction as potential mechanism of action. HMF express α4, α5, αv, β1 and β3 integrin subunits, while β4, β5 and β6 are undetectable. This implicates αvβ3 integrin as partner in Sdc1-induced ECM fiber alignment, since this integrin heterodimer is known to be involved in ECM assembly and to interact with Sdc1. RNAi knockdown of β3 subunit expression or treatment with αvβ3-blocking antibody diminishes ECM fiber alignment in Sdc1 expressing HMF. Forced activation of αvβ3 with clasp region peptides partially bypassed the requirement for Sdc1 for ECM fiber alignment.

We hypothesize that Sdc1-mediated activation of the αvβ3 integrin in stromal fibroblasts is at least partially responsible for ECM fiber alignment and thus the creation of a migration and invasion-permissive tumor microenvironment.

ST 06/P6

Syndecan-1 as a soluble biomarker for human malignancies

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Syndecan-1 is well known to play a role in several human diseases including cancer. Studies link the expression and post-translational modification of syndecan-1 to cancer progression and patient survival. For example, in several epithelial malignancies, such as lung and breast cancer, the distribution of syndecan-1 provides information about the tumours invasiveness and angiogenic potential. Furthermore, syndecan-1 is the main marker of plasma cells (CD138) and is used to diagnose the haematological malignancy caused by these cells. Regarding mesenchymal tumours, our group has earlier shown a link between syndecan-1 expression and proliferation in malignant mesothelioma. This tumour originates in the pleura and is an asbestos induced cancer with a very high mortality rate.

In this study we describe soluble syndecan-1 levels from several metastatic carcinomas, malignant mesothelioma and benign reactive conditions.

We have measured the levels of syndecan-1 in 190 pleural effusions and 231 serum samples using enzyme-linked immunosorbent assays. Overall, syndecan-1 is upregulated in fluids from patients with epithelial cancers compared to malignant mesothelioma and benign reactive conditions. In pleural effusions syndecan-1 differentiated metastatic carcinomas from benign reactive conditions with an area under the receiver operator characteristic curve (AUROC) of 0.79 (95%CI = 0.71 to 0.87). Syndecan-1 showed a significant odds ratio of 1.34 (95%CI = 1.03-1.77) between metastatic carcinomas and malignant mesothelioma. When comparing malignant mesothelioma against metastatic carcinomas in serum, syndecan-1 had an AUROC of 0.63 (95%CI = 0.55 to 0.70). Additionally, we identified syndecan-1 in an unbiased proteomic screening of pleural effusions from patients with either metastatic lung adenocarcinoma or malignant mesothelioma. Network analysis of this proteome profiling identified several syndecan-1 interactors in these patient fluids.

Herein we describe that elevated levels of syndecan-1 in pleural effusions and serum from patients are associated with epithelial cancers.

ST 07/P7

Hyaluronan affects CD44-iASPP cooperation in mammary epithelial cells

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The hyaluronan receptor and breast cancer stem cell marker CD44 interacts with intracellular key regulatory molecules and affects cell growth and proliferation. Our research group has demonstrated a novel interaction between CD44 and the iASPP oncoprotein, a conserved inhibitor of p53-mediated cell death. We have demonstrated that iASPP binds to the cytoplasmic tail of CD44, and the complexes are regulated differently in epithelial and mesenchymal cells. To characterize the iASPP epitopes involved in the interaction with CD44, we transfected various deletion mutants of iASPP followed by co-immunoprecipitation in vitro. Our studies indicate that the ankyrin repeat domain of iASPP most likely interact with CD44. Experiments are currently in progress to characterize the epitopes of the cytoplasmic part of CD44 that interact with iASPP, by using deletion and functional serine residue mutants.

In addition, we are trying to elucidate the effect of CD44-iASPP complexes on cell growth and apoptosis. FACS analysis did not show significant differences in the cell cycle progression between cells expressing CD44 or not. But in hyaluronan-activated and CD44-depleted mammary epithelial cells, we could observe increased phosphorylation of Akt, ERK and c-Jun indicating increased proliferation. A down-regulation of pNFkB, a regulatory molecule of iASPP, was observed under CD44 depletion that was not evident in untreated cells. The growth-inhibitory role of CD44 was further corroborated with 3H-thymidine incorporation data.

ST 08/P8

The matrix component biglycan triggers the crosstalk between macrophages and podocytes during renal inflammation

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The extracellular matrix component biglycan acts in its soluble form as an endogenous ligand for innate immunity Toll-like receptors-2 and -4 (TLR2/TLR4) in macrophages. By clustering TLR2/TLR4 with the P2X7 purinergic receptor, biglycan activates the NLRP3 inflammasome and caspase-1 resulting in the secretion of mature IL-1β in a reactive oxygen species (ROS) dependent manner. Biglycan was reported as an initial factor contributing to the pathogenesis of different renal glomerular disease conditions. However, the downstream signaling pathway of biglycan-mediated maturation of IL-1β in macrophages as well as the mechanisms through which it determines renal damage remain still unclear.

It is tempting to speculate that biglycan might have an important role in the interplay between innate immune cells, macrophages, and the renal resident cells, podocytes, in aggravating inflammatory renal conditions.

Objectives 1) to investigate the mechanisms and sources of the biglycan-mediated generation of ROS involved in the maturation of IL-1β in macrophages; 2) to elucidate the signaling mechanism of biglycan in podocytes which could lead to podocyte injury.

Methods Primary murine peritoneal macrophages from WT, knockout mice of inflammasome components and NADPH oxidase (NOX) subunits in the presence of pharmacological inhibitors ROS production; human podocytes and primary mouse podocytes stimulated with IL-1β or biglycan.

Results In macrophages, biglycan triggered ROS production in a TLR2/4-dependent manner. Pharmacological inhibition of NOX or H2O2 showed that biglycan-induced synthesis and maturation of pro-IL-1β are ROS-dependent. Moreover, by using NOX-deficient macrophages, we found that both biglycan-triggered secretion of mature IL-1β and activation of caspase-1 are NOX2- and NOX4-mediated.

In vivo enhanced levels of soluble biglycan were associated with the upregulation of Nox2, Nox4, Il-1β, and higher levels of active caspase-1 and mature IL-1β in the kidney.

On the other hand, IL-1β is able to induce synthesis of biglycan mRNA in podocytes. In turn, biglycan signals in podocytes by generating ROS in a TLR2/4-dependent manner. This is correlated with the biglycan-induced actin cytoskeleton rearrangement and apoptosis of podocytes.

In conclusion, here we show a novel role for the matrix component biglycan as a signaling molecule creating a crosstalk between IL-1β production in macrophages and podocyte injury in a NOX/ROS-dependent manner.

Inhibition of TGF-β signaling in left ventricular pressure overload leads to eccentric remodeling and reduced signs of heart failure, possibly due to reduced collagen content and LOX activity.

ST 09/P9

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We have investigated the effects of inhibiting the main signaling pathway of TGF-β in cardiac pressure overload using SM16, a small molecular inhibitor that prevents phosphorylation of SMAD2/3 (Kim S et al. Cancer Res 2008).

One week after of aortic banding (AB), mice with velocity ›3m/sec over the banded site were randomized to get standard mouse chow (STD) or chow with 0.45g SM16/kg (SM16). Sham operated mice served as controls. Four weeks after AB, mice were characterized by echocardiography and MRI before sacrifice. Hearts and lungs were weighted and then snap frozen in nitrogen or fixated in formalin for molecular and histological analyses. Paraffin embedded hearts were sectioned at a thickness of 4 µm, stained with picrosirius red and analyzed using a polarizing microscope. The effect of SM16 was further investigated in cultures of cardiac fibroblasts.

Echocardiography revealed reduced left ventricular (LV) wall thickness and increased LV internal diameter in ABSM16 compared to ABSTD. This was confirmed by MRI demonstrating increased LV end diastolic and systolic volumes and by impaired expression of the hypertrophic biomarkers ACTA1 and β-MHC in ABSM16 compared to ABSTD. There were no differences in heart weight between ABSM16 and ABSTD; however the 1.7-fold increased lung weight of ABSTD was attenuated in ABSM16 and expression of the cardiac biomarkers of heart failure, ANP and BNP was reduced in ABSM16 compared to ABSTD. The increased mRNA expression of the stiff collagen type I and the collagen cross-linking enzyme LOX seen in ABSTD was attenuated in ABSM16. In line with this, picrosirius red quantification demonstrated 28- and 11-fold increase of collagen content in ABSTD and ABSM16 compared to respective sham with significant lower collagen content in ABSM16 compared to ABSTD. Smooth muscle actin (SMA) and SM22 mRNAs, both associated with fibroblast activation, were decreased in ABSM16 compared to ABSTD. An in vitro model of cyclic mechanical stretch simulating in vivo mechanical stress of cardiac fibroblasts demonstrated increased SMA and SM22 expression that were abolished by SM16.

Inhibition of TGF-β/SMAD signaling by SM16 in pressure overload, leads to eccentric LV remodeling and reduced signs of heart failure. Reduced collagen content, LOX mRNA and fibroblast activation by SM16 might contribute to his finding.

ST 10/P10

A possible role for Collagen XVIII N-terminal domain in the control of myeloid leukemia cells growth

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Collagen XVIII (Col18), characterized by the antiangiogenic/tumor-suppressive endostatin domain at its C-terminus, occurs as three N-terminal variants. The corresponding gene has two promoters, promoter 1 encoding the shortest variant (only containing a thrombospondin-like domain called TSP-1) and promoter 2 the middle (where TSP-1 domain is flanked by a domain of unknown function DUF) and longest variants. In addition to the DUF/TSP-1 structure of the middle isoform, the longest variant is characterized by a frizzled-like domain (FZC18), known to be shed from full-length Col18, and shown to act as a soluble inhibitor of the Wnt3a/í-catenin pathway. In view of the known importance of Wnt3a in the control of myeloid cells growth and leukemia development, and the fact that Col18 is expressed in the bone marrow, we decided to investigate the effects of different N-terminal domains of Col18 on the growth of murine myeloid leukemic cells.

Our results show that a full-length N-terminal Col18 fragment (consisting of DUF, TSP-1 and FZC18) determines a significant reduction of leukemic cells growth, an effect which was also partially seen in a protein fragment containing the DUF and FZC18 domains and Tsp-1. Conversely, a fragment containing the DUF alone domain was not able to determine significant reduction in myeloid leukemic cells growth.

These data thus provide evidence of a novel role for Col18 and its FZC18 variant, capable of binding Wnt molecules, as endogenous controllers of myeloid development being also endowed with anti-leukemic properties.

Rab10 mediated early endocytosis of HAS3 regulates hyaluronan synthesis and cell adhesion

ST 11/P11

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Hyaluronan synthase 3 (Has3) is one among the three hyaluronan synthases family (Has1-3), involved in the synthesis of an extracellular polysaccharide, hyaluronan (HA). In this study, we have identified Rab10 and Rab35 as novel Has3 trafficking associated proteins. Rab10 and Rab35 are found to be associated with Has3 in endosomes and plasma membrane respectively, as shown with coimmunoprecipitation and colocalization analysis. Using MCF7-EGFP-Has3 stable cells and gene knockdown approach with siRNAs, we have studied the role of Rab10 and Rab35 in Has3 traffic. Silencing Rab10 and Rab35 by siRNAs induced a significant increase in hyaluronan secretion. While only Rab10 silencing but not Rab35 silencing induced a significant increase in hyaluronan surface coat formation. The plasma membrane retention of Has3 is increased with both Rab10 and Rab35 silencing with Rab35 having an even more significant effect. We are now studying the kinetics of Has3 traffic, following Rab10 and Rab35 silencing, with a technique called photoactivation and tracking in live cell imaging. We are also studying subcellular distribution of Has3 with the transient over-expression of Rab10 and Rab35 GTPase mutants (i.e. dominant positive and dominant negative). Based on our current understanding, we are proposing that Rab10 regulates Has3 endocytosis but the role of Rab35 still remains unclear.

ST 12/P12

Role of natural antisense HAS2-AS1 RNA in the regulation of hyaluronan synthesis

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Changes in the microenvironment organization within vascular wall are critical events in the pathogenesis of vascular pathologies including atherosclerosis, restenosis and diabetic macro- and microangiopathies. As the accumulation of HA into arteries wall supports vessel thickening and is involved in many vascular diseases, we studied how cells regulates HA synthesis and, in this work, we assayed whether its synthetic enzymes could be controlled by nutrients availability.

Literature suggests that the excess of glucose could enter in the hexosamine biosynthetic pathway (HBP) that could increase the concentration of the HA precursor UDP-N-acetylglucosamine (UDP-GlcNac). Besides to glycoconjugates, UDP-GlcNac can be the donor of GlcNac for O-GlcNacylation, a type of nuclear/cytoplasmic protein O-glycosylation by which the monosaccharide GlcNac attaches to ser/thr residues via an O-linked glycosidic bond.

Different compounds were used to stimulate or reduce the flux through the HBP in human primary aortic SMCs (AoSMCs) as previously described finding that the inhibition of O-GlcNacylation strongly reduced HA production whereas treatments that induced protein O-GlcNacylation increased HA secretion. Now, we report the studies on the gene expression of HA synthases (HASes) and found that specifically HAS2 mRNA was the most sensible to O-GlcNacylation and accumulated after its induction with glucosamine. Interestingly, we found that transcription factors as SP1 and YY1 (that are previously known to regulate HAS2 transcription were not involved in HAS2 hyperexpression and O-glcNAcylation did not increased HAS2 promoter activity although actinomycin D treatment (which inhibited RNA transcription) prevented HAS2 mRNA increment. Therefore, we hypothesized the presence of a more complex epigenetic control of HAS2 transcription probably involving HAS2-AS1, the long noncoding RNA with complementarity with exon 1 of HAS2.

As it has been recently shown that histones can be modifiable by O-GlcNAc , we studied the chromatin conformation around the promoter of HAS2 and HAS2-AS1 (the long noncoding RNA with complementarity with exon 1 of HAS2). Surprisingly we found that chromatin were in an open state after O-GlcNAcylation in both the promoters favouring the accessibility of transcription machinery. Interestingly, we found that the abrogation of HAS2-AS1 completely block the accumulation of HAS2 transcript after glucosamine treatment confirming the new positive role of long noncoding RNA HAS2-AS1 favouring HAS2 mRNA transcription/stability.

Our results clearly indicate that HAS2 transcription is finely regulated not only simply by binding transcription factors to the promoter, but also by modulating chromatin accessibility by epigenetic modifications. Our findings on the regulation of HA metabolism could represent new strategies to develop anti-atherosclerotic and vaso-protective drugs.

Matrix Metalloprotease-9/Chondroitin Sulphate Proteoglycan Complexes

ST 13/P13

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Matrix metalloprotease-9 (MMP-9) can process extracellular matrix proteins, cell surface receptors, growth factors, cytokines, chemokines, enzymes and inhibitors, and are important in normal as well as in pathological processes.

MMPs are built up of different domains, moduls and motifs. MMP-9 like most other MMPs contains 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. The FnII module facilitates the localization of MMP-9 to connective tissue matrices as well as the degradation of various biological substrates.

The leukemic monocyte cell line THP-1 produces the matrix metalloproteinase-9 (MMP-9) as a monomer, homodimer and a heterodimer. In the latter, MMP-9 is strongly linked to one or several chondroitin sulphate proteoglycan (CSPG) core proteins which affects the activation of the enzyme and it's binding to gelatin and collagen. PMA stimulation of these cells resulted in a large increase in the synthesis of MMP-9 and the MMP-9/CSPG complex, but did not affect the total synthesis of CSPGs. In contrast to this, neither the PMA stimulated nor the untreated leukemic monocytes U-937 and MonoMac produced significant amounts of proMMP-9/CSPG complexes in spite of their synthesis of proMMP-9 and various types of PGs. However, when purified proMMP-9 was mixed and incubated with isolated PGs from the three monocyte cell lines, proMMP-9/CSPG complexes were formed. This in vitro reconstitution revealed that both SDS-stable and SDS-soluble complexes were formed. This was also the case when proMMP-9 was mixed with the PGs serglycin and versican. Both the HPX domain and the FnII module in proMMP-9 were involved in the complex formation. Formation of the SDS-stable and the SDS-soluble complexes involve different regions in the HPX domain of MMP-9. Motifs in MMP-9 and the serglycin core protein that is involved in the complex formation will also be presented.

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ST 14/P14

Heparanase 2 expression is decreased in human cancer and attenuates lymphangiogenesis

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Heparanase is an endo-β-D-glucuronidase capable of cleaving heparan sulfate (HS) side chains of proteoglycans at a limited number of sites. Heparanase activity is considered a prerequisite for cellular invasion associated with tumor metastasis, inflammation, and angiogenesis, a consequence of HS cleavage and remodeling of the sub-endothelial and sub-epithelial basement membrane and extracellular matrix. The clinical significance of heparanase activity critically emerges from numerous recent publications describing induced heparanase expression in human hematological and solid tumors, and its inverse correlation with post operative patients' survival, encouraging the development of heparanase inhibitors. Based on amino acid sequence, cloning of heparanase homolog termed heparanase 2 (Hpa2) was reported. Detailed characterization of Hpa2 at the biochemical, cellular, and clinical levels has not been so far reported and its role in normal physiology and pathological disorders is obscure. Although Hpa2 lacks HS-degrading activity typical of heparanase, it exhibits even higher affinity for heparin and HS compared to heparanase, thus competing for HS binding and inhibiting heparanase enzymatic activity. In head & neck carcinoma, Hpa2 expression was associated with reduced lymph nodes metastasis and prolonged follow-up time, possibly a consequence of heparanase inhibition. Here, we examined Hpa2 expression in bladder and breast carcinomas by immunohistochemistry. Hpa2 staining was readily observed in normal breast epithelium and appeared gradually decreasing in ductal carcinoma in situ and invasive ductal carcinoma. A similar trend was found in bladder cancer where strong staining for Hpa2 was observed in normal bladder urothelium which was markedly decreased in bladder cancer. In order to explore the function of Hpa2 in human cancer we infected cancer cell lines with Hpa2 gene constructs. Hpa2 over expression by FaDu laryngeal carcinoma cells was associated with 5- and 2-fold decrease in tumor volume and weight, respectively. Notably, Hpa2 over expression resulted in reduced VEGF-C levels and consequently decreased lymphatic vessel density. These results imply that Hpa2 functions as a tumor suppressor, attenuating tumor progression and metastasis.

Dynamic interplay between normal endothelium and breast cancer cells via altered expression of matrix molecules

ST 15/P15

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Metastasis involves the dissemination of cancer cells from the primary tumor into the extracellular matrix, invasion through connective tissue, adhesion to the endothelium of blood/lymph vessels, transmigration through the endothelium, and subsequently formation of a secondary tumor in a distant targeted organ. The aim of the present study was to determine the cellular responses that the breast cancer cells(BCC) (MDA-MB-231 & MCF-7), elicit in the human umbilical vein endothelial cells (HUVEC). For this purpose two models were utilized; one involves HUVEC culture in the presence of BCC-derived conditioned media (CM) and the other co-culture of both cell populations in a Transwell system. We found that CM from cancer cells decreases the cell migration of HUVEC cells. It is worth noticing that the adhesion of cancer cells is favored by the presence of HUVEC cell monolayer in comparison with plastic surface, effect that is further induced by the presence HUVEC-secreted matrix effectors. Tumor cells may take advantage of hyaluronan-rich matrices to invade more easily into the surrounding tissues. Real-Time PCR analysis showed that gene expressions of HA receptor CD44 and HA synthase HAS2 in HUVEC are substantially up-regulated in both culture models. Moreover, HA levels are significantly upregulated in both models. ICAM-1 and VCAM-1 play a key role not only in trafficking of cancer cells across endothelial and epithelial barriers. Gene expression levels of the adhesion molecules by HUVEC are also highly upregulated in the above experimental set. Notably, the expression of the membrane metalloproteinase MT1-MMP, MMP-2 by HUVEC is significantly downregulated in both culture systems, where MMP-9 is up-regulated. On the other hand, ubiquitin proteasome system is the central protein degradation mechanism, involved in the regulation of protein quality control, intracellular signaling pathways and cell cycle. Alteration of its activity represents a promising strategy for the treatment of cancer. Proteasome consists of one 20S protein subunit and two 19S regulatory cap subunits. The β1, β2, and β5 subunits of 20S are catalytic, representing distinct substrate specificities chymotrypsin-like, trypsin-like, and peptidyl-glutamyl peptide-hydrolyzing, respectively. Proteasome inhibition in the vascular system are controversial, ranging from beneficial anti-inflammatory and anti-oxidative effects to potentiation of inflammation and oxidative stress. The gene expressions of all proteasome subunits are upregulated, especially by the action of MDA-MB-231 cells on HUVEC. Fact that is depicted also in the activity of β5 subunit. Conclusively, these data suggest that factors secreted by BCC regulate the expression of matrix macromolecules, implicated in endothelial functional properties as well as proteasomal protein degradation.

ST 16/P16

NG2/CSPG4 control of cellular interactions under flow mimicking extravasation conditions

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To accomplish the metastatic process, disseminating tumour cells enter haematic and lymphatic conduits to reach distant sites where they egress the circulation by penetrating the vessel wall – a phenomenon denoted extravasation and thought to involve complex interactions between the tumour cells, the endothelium and the underlying ECM. More recently, experimental data suggest that pericytes may also play a key role in this process, while a plephora of cell surface components present on all these cell types are believed to act as mediators. Cell surface proteoglycans (PGs) are among the components believed to play an active role in this context, although it is not known how, and in particular NG2/CSPG4 seems to be a good candidate regulator of the cellular interactions ensuing during extravasation. We have devised a flow-based experimental paradigm for assaying the importance of both cell-cell contact and cell-ECM interactions in shear force conditions mimicking the rheology of the haematic circuits. In parallel we have re-adapted a protocol for the in vitro isolation of native, cell-free perivascular matrices and are gradually characterizing matrices isolated from the different cellular elements structuring the vascular wall structurally and compositionally. Sarcoma and melanoma cells immunosorted for their expression of NG2/CSPG4 (NG2+) bind more effectively to human endothelial cells than their counterpart NG2- cell subsets or cells with siRNA-abrogated NG2/CSPG4 and this binding is partly mediated by the chondroitin sulphate chains of the PG. Expression of NG2/CSPG4 differentially affects ECM binding to matrices from pericytes/mural cells at different maturation degree and propriety antibodies against different isoforms of NG2/CSPG4 profoundly modulate, in a differentiated manner, these binding phenomena. Global phospho-proteomic analyses are exploited to generate phospho-portraits of the signalling events induced by NG2/CSPG4 upon cell/ECM binding, such as to delineate the involved pathways, while CAM assays are utilized to explore the function of the PG in intra- and extravasation settings in vivo. To identify endothelial and pericyte cell surface components responsible for the NG2/CSPG4-mediated cellular interactions we are using a combined cross-linking, immunoprecipitation and mass spectrometric approach. The findings contribute to our understanding of the prometastatic role of NG2/CSPG4 seen in animal models and support tyhe consistently observed, enhanced expression of the PG in metastatic lesions of different tumour types.

Decellularizing colorectal tumours: dissecting the role of macrophages and extracellular matrix for tumour progression

ST 17/P17

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Tumours are highly complex microecosystems composed of cancer cells, extracellular matrix (ECM) components and other cell types. The molecular crosstalks established between cancer cells and the surrounding environment are crucial for tumour progression. Macrophages have been described as key elements in this process, preventing the establishment and spreading of cancer cells – M1 macrophages– or supporting tumour growth and progression – M2 macrophages. In colorectal cancer (CRC) little is known about the effect of tumour cells and ECM components on macrophage polarization. In this work, we are particularly interested to elucidate how the ECM and tumour cells contribute to macrophage differentiation and polarization. Therefore, we are profiling macrophage population and ECM composition along tumour progression on human colorectal cancer specimens. In parallel, we are creating a 3D-organotypic culture model to evaluate macrophage differentiation, by decellularizing human CRC tissue fragments and by repopulating them with monocytes and or/tumour cells, mimicking more closely the natural tumour microecosystem.

To achieve this goal, we started by characterizing, through immunohistochemistry analysis, CD68, CD163 and HLA-DR expression profile of macrophage populations present in 27 colorectal cancer cases from Centro Hospitalar de Syo Joyo (CHSJ) Tumour Bank. Additionally, the decellularization protocol, for both normal and tumour colorectal fragments, was optimized. DNA quantification and DAPI staining confirmed the efficiency of the decellularization method. SEM analysis allowed the visualization of the ECM fiber meshwork, without any visible cells. Staining with Hematoxylin Eosin and Masson's Trichrome revealed that decellularized fragments retain the histological features of the tissues. Decellularization reduced significantly the glycosaminoglycans (GAGS) content in normal and tumours but other ECM components, such as laminin or fibronectin, are retained. These matrices were repopulated with freshly isolated monocytes and allowed to differentiate for 7 or 14 days. Preliminary results clearly evidenced that monocytes are able to colonize decellularized matrices and to differentiate into macrophages within the fiber network. We are currently evaluating macrophage differentiation profile through immunohistochemistry and RNA expression analysis. Additionally we are evaluating, through ELISA, the conditioned media obtained from these repopulated matrices, regarding the expression of relevant cytokines/chemokines.

At completion of this project we expect to have elucidated the role of tumour cells and of ECM components, derived from the tumour microecosystem, on macrophage differentiation and polarization, contributing to the design of novel therapeutic strategies targeting macrophages.

ST 18/P18

Novel COMP neoepitopes identified from patients with joint diseases by immune-affinity chromatography and mass spectrometry

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COMP (cartilage oligomeric matrix protein) is a molecule primarily found in cartilage. It is a pentameric protein of 434 kDa composed of five identical subunits that are joined together by a coiled-coil domain in the N-terminal. The C-terminal globular domain is involved in interactions with other proteins in the extracellular matrix. Each subunit can bind a collagen molecule and thereby catalyzes collagen fibril assembly. COMP is cleaved and released from the tissue into synovial fluid in both osteoarthritis (OA) and rheumatoid arthritis (RA). Currently available COMP assays for synovial fluid/serum have provided important information but do not discriminate between e.g. normal and pathologic turnover or release due to different pathological processes such as OA or RA.

To identify patients at risk for progressive joint damage there is a need for early diagnostic tools to detect molecular events leading to cartilage destruction. Isolation and characterization of distinct COMP fragments derived from cartilage and released into synovial fluid will allow discrimination between different pathological conditions, monitoring of disease progression and severity and treatment evaluation. Early detection of disease and processes in the tissue as well as an understanding of the pathologic mechanisms will also open for novel treatment principles.

Disease specific COMP fragments were isolated by immune-affinity chromatography using mouse monoclonal antibodies directed towards three different domains in COMP. Synovial fluid from patients with RA, OA or acute trauma were used. Enriched COMP fragments were separated by SDS-PAGE followed by in-gel digestion and the peptides were identified and characterized by mass spectrometry. By using the enzymes trypsin, chymotrypsin and Asp-N for the in-gel digests an extensive analysis of the enriched fragments could be accomplished.

By using these techniques we found 12 novel neoepitopes in COMP which showed clear differences between patients with different diagnoses. Polyclonal antibodies have been raised towards these neoepitopes and immunoassays are being set up. Based on the specificity, selectivity and sensitivity of each neoepitope a new generation of molecular markers for cartilage involvement in joint disease will be developed.

Molecular consequences of defective SERPINH1 in Osteogenesis Imperfecta

ST 19/P19

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Osteogenesis imperfecta (OI) is a heritable connective tissue disease characterized by bone fragility and increased risk to fractures. Up to now mutations in at least fourteen genes have been associated with dominant, respectively recessive forms of OI, and have been implicated in altered posttranslational processing of procollagens, or altered bone homeostasis. Among those, SERPINH1 (HSP47) was identified to cause a severe form of OI in Dachshund (Leu326Pro), as well as in humans (1 single case with a Leu78Pro mutation). The gene product, heat-shock factor 47 (HSP47) acts as a chaperon in procollagen folding in the ER, and it is speculated to prevent premature lateral aggregation, and to provide a quality control mechanism in collagen biosynthesis.

To elucidate the disease mechanism underlying OI in the Dachshund model, we applied a set of biochemical assays, as follows: SDS-PAGE analysis of in vitro produced procollagens of an OI-Dachshund and a control dog, tandem mass spectrometry of bone collagens, and transmission electron microscopy of cultured fibroblasts. SDS-PAGE analysis of in vitro produced procollagens, showed in the Dachshund decreased migration of type I and type V collagens and slightly delayed secretion of collagens compared to control dogs, thus suggesting a defect in procollagen processing and/or folding. In line with the migration shift detected on SDS-PAGE, tandem mass spectrometry from bone of the OI-Dachshund and control dogs showed alterations in collagen cross-linking and hydroxylation levels. Interestingly, transmission electron microscopy of cultured fibroblasts of the Dachshund showed enlarged ER cisterns.

Biochemical studies in the human case have shown slightly delayed secretion of procollagen I trimers and increased sensitivity of collagens type I and type III to proteases (Christiansen et al., 2010). However, in contrast to our findings on the Dachshund, they have shown a normal migration of collagens on SDS-PAGE gels . Furthermore, the mutated HSP47 protein was shown to be unstable, providing a hint at the disease mechanism, namely HSP47 deficiency. Surprisingly, in our in vitro experiments on Dachshund fibroblasts the mutant HSP47 protein could be clearly detected by western blot, as well as by immunofluorescent staining. While the pathomechanism in the human case seems to be a deficiency of HSP47, in the Duchshund we speculate that the SERPINH1 mutation might lead to an alteration of the binding specificity of HSP47 for collagen.

Taken together our findings suggest that the SERPINH1 mutation in the Dachshund may lead to delayed folding and secretion of collagens, to the accumulation of slow-folding, or misfolded collagens in the ER cisterns, and consequently to the activation of an ER-stress response. In the future the contribution of the collagen binding efficiency of mutant SerpinH1 and the role of ER-stress to the pathomechanism will be investigated in more detail.

ST 20/P20

Hepatocarcinogenesis in matrilin-2 knock out mice

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Matrilin-2 (Matn2) is a multidomain adaptor protein which plays a role in the assembly of extracellular matrix (ECM). It is produced by oval cells during stem cell-driven liver regeneration. In our study, the impact of Matn2 on hepatocarcinogenesis was investigated in Matn2-/- mice comparing them with wild-type (WT) mice in a diethylnitrosamine (DEN) model. The liver tissue was analyzed macroscopically, histologically and immunohistochemically, at protein level by macroarray and Western blot analysis. Matn2-/- mice exhibited higher susceptibility to hepatocarcinogenesis compared to wild-type mice. In the liver of Matn2-/- mice, spontaneous microscopic tumor foci were detected without DEN treatment. After 15 μg/g body weight DEN treatment, the liver of Matn2-/- mice contained macroscopic tumors of both larger number and size than the WT liver. In contrast with the WT liver, spontaneous phosphorylation of EGFR, Erk1/2 GSK-3α/β and retinoblastoma protein (p-Rb), decrease in p21/CIP1 level, and increase in β-Catenin protein expression were detected in Matn2-/- livers. Focal Ki-67 positivity of these samples provided additional support to our presumption that the lack of Matn2 drives the liver into a pro-proliferatory state, making it prone to tumor development. This enhanced proliferative capacity was further increased in the tumor nodules of DEN-treated Matn2-/- livers. Our study suggests that Matn2 functions as a tumor suppressor in hepatocarcinogenesis, and in this process activation of EGFR together with that of Erk1/2, as well as inactivation of GSK-3β, play strategic roles.

Oxidized LDL affects hyaluronan synthesis in human aortic smooth muscle cells

ST 21/P21

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Human native LDL retention at the subendothelial level is one of the first events triggering atherosclerosis, with the concurrent involvement of smooth muscle cells for thickening of the vessel due to hyaluronan (HA) deposition in the neointima. LDL trapped within the arterial wall undergoes modifications like oxidation (oxLDL) that may affect both lipid and protein moieties of the particles and therefore a series of biologically active species including peroxides, aldehydes and oxysterols may be produced. Intracellular and cell membrane cholesterol contents are finely regulated by a feedback system involving: regulated nLDL uptake by LDL receptor; a fine balance between biosynthesis and efflux; cholesterol acylation/deacylation; and cholesterol intracellular trafficking between organelle membranes (mainly ER, lysosomes and plasma membrane). Nevertheless, modified LDL can escape the regulated uptake and enter the cell via a series of unregulated scavenger receptors (e.g. LOX-1). The aim of our study is to elucidate the link between internalization of oxLDL and HA production in vitro, using human Aortic Smooth Muscle Cells (AoSMC). LDLs were used at effective protein concentration of 20-50μg/mL, which allowed 80% cell viability. HA content in medium of untreated cells was 28.9±3.7 nmol HA-disaccharide/cell and increased after oxLDL treatment to 53.9±5.6. OxLDL treatments enhanced the transcription of HA synthases, HAS2 and HAS3, at 1.9 and 3.8 folds, respectively. Accumulated HA stimulated AoSMC migration and monocyte adhesiveness to ECM. These effects were inhibited by blocking LOX-1 scavenger receptor with a specific antibody (10μg/mL). HA accumulation was not affected by the cholesterol moiety of LDL since free cholesterol (20μg/mL) delivered to AoSMC did not induce HA synthesis or HAS expression. Moreover, HA deposition was associated with higher expression of endoplasmic reticulum stress markers (CHOP and GRP78). Concerning ER stress activation, SREBPs can be candidates for the link with the HAS2 regulation of expression. In fact, HAS2 promoter has been proposed to have potential SREBP-2 binding sites, which can up-regulate the HAS2. However, no clear evidence in support of such a hypothesis has been presented so far, and recent analysis of SREBP with ChIPseq in the human HAS2 locus did not highlight specific peaks within the gene promoter. Other pathways involved in the regulation of cholesterol homeostasis, such as the oxysterols receptor LXR (liver X receptor), could be responsible for the HAS2 activation.

ST 22/P22

Differential role for HS3ST2 in modulating breast cancer cell invasiveness: A molecular mechanism mediated by protease expression via MAP kinase and WNT pathways

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Heparan sulfate proteoglycans (HSPGs) which are ubiquitously present virtually in all cells, have been shown to be involved in the process of breast cancer metastasis partially due to their heterogeneous sulfation patterns, which allow for specific binding of a multitude of ligands relevant to tumor progression[1]. HS3ST2, one of the enzymes involved in the 3-O-sulfation modification of the HSPGs, is known to be silenced by hypermethylation in breast cancer[2]. The aim of this study was to elucidate the role of HS3ST2 in breast cancer cell behaviour using an ectopic overexpression approach by stably transfecting HS3ST2 expressing plasmid in human breast cancer cell lines MDA-MB-231 and MCF-7. To address the potential role of this type of modification we reintroduced, we investigated the phenotypical changes in vitro. HS3ST2 transfected MCF-7 cells became less invasive while MDA-MB-231 cells showed a highly significant increase in invasiveness and motility is accompanied by significantly increased expression of several matrix metalloproteinases (MMPs), including MMP9 and MMP13, CEACAM-1 as well cadherin 11, and E-cadherin. Treatment of MDA-MB-231 cells with TIMP-1, a protease inhibitor, hampered invasion, suggesting a role of MMP's in increased invasiveness. In addition, both the cell lines became sensitive to chemotherapeutic drugs which is due to the dysregulated ion transporters and also significantly increased cytosolic acidification was observed in HS3ST2 expressing MDA-MB-231 cells. HS3ST2 overexpression in MDA-MB-231 lead to increased basal and FGF-specific signalling through the p44/42 MAPK pathway, which depend on the presence of heparan sulfate. Increased MAPK activation was accompanied by a significantly increased expression of the transcription factor TCF4 and í-catenin. MAPK inhibition with a MEK1/2 inhibitor downregulated the expression of TCF-4 and also reduced invasion in MDA-MB-231 cells, providing a clue that increased MAPK signalling also plays a role in the invasion having a cross talk with Wnt pathway.

This study provides the first in vitro evidence of the involvement of HS3ST2 in breast cancer cell invasion. Increased activation of the p44/42 MAPK signaling pathway and of TCF4 in the presence of HS3ST2-specific sulfation patterns emerge as novel mechanistic aspects leading to increased expression of proinvasive gene products. These results highlight that increased invasion in HS3ST2 overexpressing MDA-MB-231 cells is due to increased MAPK and Wnt signaling corresponding to increased expression of proteases.

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Decorin interferes with platelet-derived growth factor receptor signaling in experimental hepatocarcinogenesis

ST 23/P23

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Decorin, a secreted small leucine-rich proteoglycan, acts as a tumor repressor in a variety of cancers, mainly by blocking the action of several receptor tyrosine kinases such the receptors for hepatocyte, epidermal and insulin-like growth factors. In the present study we investigated the effects of decorin in an experimental model of thioacetamide-induced hepatocarcinogenesis, and its potential role in modulating the signaling of platelet-derived growth factor receptor-α (PDGFRα). Genetic ablation of decorin led to enhanced tumor prevalence and higher tumor count as compared to wild-type animals. These findings correlated with decreased levels of the cyclin-dependent kinase inhibitor p21WAF1/CIP1 and concurrent activation (phosphorylation) of PDGFRα in the hepatocellular carcinomas generated in the decorin-null vis-ű-vis wild-type mice. Notably, in normal liver PDGFRα localized primarily to the membrane of non-parenchymal cells, whereas in the malignant counterpart PDGFRα was expressed by the malignant cells at their cell surfaces. This process was facilitated by a genetic background lacking endogenous decorin. Double immunostaining of the proteoglycan and the receptor revealed only minor colocalization leading to the hypothesis that decorin would bind to the natural ligand PDGF rather than the receptor itself. Indeed, we found that decorin binds to PDGF using purified proteins and immune blot assays. Collectively, our findings support the idea that decorin acts as a secreted tumor repressor during hepatocarcinogenesis by hindering the action of another receptor tyrosine kinase such as the PDGFRα, and could be a novel therapeutic agent in the battle against liver cancer.

ST 24/P24

EphB2 receptor modulates gene expression signature involved in migration and invasion of cutaneous squamous cell carcinoma

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Cutaneous SCC (cSCC) is the second most common cutaneous malignancy in white population. We have studied the role of Eph receptors, the largest family of receptor tyrosine kinases in cSCC. Cutaneous SCC cell lines and tumors were obtained with consent from Turku University Hospital. Gene expression analysis was performed with oligonucleotide-based microarray, SOLiD™ whole transcriptome analysis, and real-time PCR. Western blot (WB) was used to study protein levels. Immunohistochemistry of human tissue microarrays and chemically induced mouse cSCC were performed using specific antibody against EphB2. Proliferation, migration and invasion assays were performed following EphB2 knock-down. Specific upregulation of EphB2 was noted in cSCC cell lines (n=8) and tumors (n=6) compared with normal keratinocytes (n=5) and healthy skin (n=7) using Affymetrix-based profiling, SOLiD™ analysis, quantitative RT-PCR, WB and immunofluorescence staining. Immunohistochemistry revealed tumor cell-specific overexpression of EphB2 in cSCC in situ (n=56) and cSCC (n=68) compared with actinic keratoses (n=69) and normal skin (n=12) ($p<0.001$). Moreover, upregulation of EphB2 expression was noted in DMBA-TPA-induced mouse cSCCs (n=19) compared with normal skin (n=13) ($p<0.001$). EphB2 knockdown showed inhibition of proliferation, migration and invasion of cSCC cells. Microarray analysis identified 2460 differentially expressed genes ($P<0.05$) in cSCC cell lines (n=3) following EphB2 knockdown. Gene expression profile after EphB2 knockdown was subjected to Ingenuity Pathway Analysis (FC (log2) >0.75 and $P<0.05$). Over 11% of downregulated genes belonged to peptidases classification. *Invasion of tumor cells* (z-score= -2.099, $p<0.001$) and *migration of tumor cells* (z-score= -2.358, $p<0.001$) were among the top biofunctions significantly decreased after EphB2 knockdown. These findings provide evidence for the role of EphB2 in progression of cSCC and particularly in invasion and migration of SCC cells, suggesting it as a therapeutic target in these invasive and metastatic tumors.

Posters/ Abstracts

P 25

Cross-talk of ERs with growth factors receptors affects the expression and distribution of breast cancer-associated heparan sulfate proteoglycans

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Heparan sulfate proteglycans (HSPGs) play a diverse role in tumor biology by mediating cell adhesion and migration but also regulating cellular responses through mitogenic and angiogenetic growth factors. Syndecans constitute one major category of cell-transmembrane HSPGs, which regulate cell-cell and cell-extracellular matrix (ECM) adhesion, migration and growth factor activity. Recent studies have revealed new insights into ER action in breast cancer, highlighting the role of an intimate cross-talk between the ER, 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. The aim of this study is to elucidate the role of EGFR and IGFR pathways in gene and protein expression of SND-2 and SND-4 in the presence and the absence of E2. Furthermore, we examine the localization of SND-2 and SND-4 in same system under correlation with ERs distribution. For this purpose, two breast carcinoma cell lines MCF-7 (ERα+) and MDA-MB-231 (ERβ+) were cultured in the presence and the absence of E2 after pre-treatment with EGFR and IGFR inhibitors. As a result, the use of inhibitors show that the expression of SND-2 and SND-4 are mediated through both signaling pathways independently of the cells' ER status. Moreover, we show that either EGFR or IGFR has the main importance in signaling pathways that regulate SND-2 and SND-4 expression in both cell lines, but in some cases both pathways are principal for HSPGs expression. Furthermore, the use of E2 seems to affect the localization of SNDs and ERs. Finally, our study highlights that the use of EGFR and IGFR inhibitors in combination or separately may be a promising therapeutic tool for HSPGs-mediated metastatic potential in cancer microenvironment.

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Serglycin expressed by aggressive testicular germ cell tumors regulates the expression of matrix remodeling enzymes

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Serglycin is expressed by aggressive tumor cells and promotes their proliferation, adhesion, migration and invasion, while it confers resistance against drugs and complement system attack. We examined the expression and distribution of serglycin in testicular germ cell tumors by immunohistochemical analysis of tissues from patients and biochemical analyses in tumor cell lines. We found that serglycin is accumulated in tissues from patients and is synthesized by tumor cells showing a cytoplasmic distribution. Analysis of tumor cell lines revealed that serglycin is highly expressed by aggressive tumor cell lines of teratocarcinoma (NCCIT) and embryonal carcinoma (NTERA-2/D1) cells compared to less invasive seminoma cells (JKT-1). Over-expression of serglycin in all cell lines differentially regulated the expression of enzymes involved in hyaluronan metabolism as well as that of matrix metalloproteinases (MMPs) and their endogenous inhibitors (TIMPs). In JKT-1 seminoma cells serglycin over-expression affected only the expression of enzymes involved in hyaluronan metabolism. Serglycin up-regulated the expression of HAS-3, Hyal-2, Hyal-3, whereas decreased the expression of PH-20. In invasive NCCIT and NTERA-2/D1 cells serglycin over-expression significantly increased the expression of MMP-2 and MMP-9 whereas decreased the levels of TIMPs. Furthermore, serglycin increased the expression of HAS-2 and HAS-3 as well as that of Hyals1-3 and PH-20. Our data suggest that serglycin is involved in matrix remodeling occurred during cancer progression. This is a novel function of serglycin and may contribute to disease development.

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Regulation of complement system by serglycin secreted in malignancies

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Serglycin is the major proteoglycan that is synthesized from hematopoietic cells and participates in immune responses. The role of serglycin in malignancies has been studied in multiple myeloma. Serglycin is constitutively secreted by myeloma cells and inhibits both bone mineralization and complement system activation. We demonstrated that serglycin is constitutively secreted by aggressive cancer cells and promotes cancer cell migration and invasion. The aim of our study was to examine the role of serglycin secreted by aggressive cancer cells in the regulation of complement system and to compare with the properties of serglycin synthesized from myeloma cells. We found that serglycin secreted by cancer cells carries chondroitin sulfate and inhibited both the classical and lectin pathways of complement without influencing alternative pathway activity using hemolytic assays and deposition of complement components from serum. The inhibitory effect of serglycin was mediated in the early steps of both the classical and the lectin pathways of complement by binding to C1q and mannose-binding lectin. Solid phase microtiter binding assays revealed that serglycin isolated from breast cancer cells interacts with C1q and mannose-binding lectin with similar affinity to that of serglycin isolated from myeloma cells. Free chondroitin sulfate chains (CS-A and CS-C) did not affect the activity of the classical and lectin pathway and was not capable for binding to C1q and mannose-binding lectin. Serglycin was found in elevated amounts in the serum of patients with malignancy. These patients did not exhibit significant differences in the activity of the classical and alternative pathway activity in the serum. The activity of the classical pathway showed a trend to be reduced in the serum of patients with elevated amounts of serglycin. Our data suggest that serglycin secreted in malignancies is a regulator of complement system activity and may contribute in disease progression.

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Syndecan-4 shedding in the hypertrophic heart is likely mediated by the MMP9 and ADAMTS4 enzymes, a process involved in cardiac inflammation and failure progression

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Inflammation is central during cardiac remodeling and progression to heart failure. We have linked the transmembrane proteoglycan syndecan-4 to the inflammatory response of pressure-overloaded hearts, showing that in parallel to impaired hypertrophic remodeling and premature failure, mice lacking syndecan-4 have reduced recruitment of T-cells in response to aortic banding (AB). The ectodomain of syndecans can be shed from cells, a process believed to modulate inflammation. Importantly, we found that syndecan-4 shedding from cardiac cells is induced by innate immune signaling, and that syndecan-4 shedding is increased in failing human hearts. Here we investigated molecular mechanisms regulating cardiac syndecan-4 shedding.

Syndecan-4 mRNA was increased in the myocardium of mice following 24h and 1 week of AB. Acutely (AB24h), and during hypertrophic remodeling (AB1w and AB3w) as determined by echocardiography and organ weights, cardiac levels of a ~15kDa extracellular syndecan-4 fragment was increased 2.7-, 24.3- and 2.7-fold, respectively, measured by immunoblotting after heparitinase treatment of myocardial lysates. Moreover, full-length syndecan-4 was decreased by 16.6% at AB24h and 55.7% at AB1w with no difference at AB3w, suggesting that the initially increased syndecan-4 mRNA is followed by subsequent shedding of the extracellular part during hypertrophic remodeling. Cyclic mechanical stretch of cardiomyocytes, an in vitro model of pressure overload, increased syndecan-4 shedding 1.3-fold, suggesting pressure overload per se as a stimulus for shedding. In cultured cardiac cells, pro-inflammatory TNFα, IL-1β and LPS induce syndecan-4 mRNA and protein shedding. Enzymes proposed to mediate shedding of syndecans were measured in cardiac cells following TNFα, IL-1β and LPS treatment. Parallel mRNA upregulation of MMP2 (1.3-2.5-fold), MMP9 (7.9-71.0-fold), Adam17 (1.8-2.6-fold) and Adamts4 (1.5-7.6-fold) indicated these as candidate regulators of cardiac syndecan-4 shedding. After AB, cardiac mRNA of all four were increased, however interestingly, syndecan-4 levels correlated positively to MMP9 (R^2=0.69) and Adamts4 (R^2=0.79), suggesting they might regulate in vivo syndecan-4 shedding. Our preliminary results show that constitutive and TNFα-induced syndecan-4 shedding could be reduced by MMPs and Adamts blockers, GM6001 and epigallocathechin gallate (EGCG), respectively, suggesting both enzyme families to be important. We are currently co-expressing MMP9 and Adamts4 with syndecan-4 in cells to investigate their specific roles in syndecan-4 shedding.

In conclusion, increased syndecan-4 expression was followed by subsequent shedding of the ectodomain during hypertrophic remodeling of the pressure-overloaded heart. Although requiring further research, we suggest that cardiac shedding of syndecan-4 is mediated by MMP9 and Adamts4, and that targeting these could represent a strategy to modulate cardiac inflammation and hypertrophic remodeling.

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Defective proteoglycan synthesis in Desbuquois dysplasia is caused by mutations in CANT1 gene

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Desbuquois dysplasia (DD) is an autosomal recessive chondrodysplasia characterized by antenatal and postnatal growth retardation, multiple dislocations and advanced carpal ossification. Two forms of DD have been described on the basis of the presence (type 1) or absence (type 2) of characteristic hand deformities. Studying DD type 1 families, several mutations in the Calcium-Activated Nucleotidase 1 gene (CANT1) have been identified (Huber C et al. Am J Med Genet 2009, 85:706-710; Nizon M et al. Hum Mutat 2012, 33:1261-6).

CANT1 is a calcium activated nucleotidase that preferentially hydrolyzes UDP followed by GDP, CDP and ADP, two different enzyme forms have been characterized: i) a membrane bound form in the endoplasmic reticulum and Golgi, that can be involved in protein glycosylation and protein quality control; ii) a soluble secreted form that can take part in pyrimidinergic signalling by modulating the availability of extracellular UDP to P2Y receptors.

Since DD shares phenotypic features with other chondrodysplasias characterized by defects in cartilage proteoglycan metabolism, we hypothesized that CANT1 may play a role in proteoglycan synthesis. To test this hypothesis, fibroblasts from two DD patients homozygous for p.R300H and p.P245RfsX3 mutations respectively, and four controls were double labelled with [35S]sulfate and [3H]glucosamine. In patient fibroblasts glycosaminoglycan (GAG) synthesis was almost normal under basal conditions, but significantly reduced GAGs synthesis was observed in presence of

β-D-xyloside, a compound which enhances synthesis and secretion of chondroitin and dermatan sulfate chains acting as a chain initiator. In addition, gel filtration chromatography on Superose 6 of GAGs released from newly synthesized proteoglycans after beta-elimination demonstrated that GAG chains were shorter compared to the controls. Interestingly hyaluronic acid synthesis, which occurs in the plasma membrane, was within normal values in patients cells, confirming the involvement of CANT1 in the ER/Golgi compartment.

These data suggest that CANT1 is involved in proteoglycan and GAGs metabolism and demonstrate the key role of a nucleotidase in the endochondral ossification process. In order to clarify the physiological function of CANT1 and its role in the etiology of DD we are generating a CANT1 knock-in mouse reproducing a mutation already detected at the homozygous state in DD type 1 patients.

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

P 30

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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. 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. Integrins are divalent cation-dependent molecules, whereas magnesium (Mg) and manganese (Mn) promote integrin activation. Zinc (Zn) promotes integrin inactivation by dislocation of Mg. Mn, on the other hand, cannot be dislocated by Zn due to higher binding affinity. These observations lead to the hypothesis that the equilibrium between syndecan expression and Mg/Mn availability influence tumor cell EMT and migration to new metastatic sites and manipulation of these elements may alter tumoral development and outcome. Our preliminary results show that when Lewis lung carcinoma (LLC) cells are exposed in vitro to Mg, the mesenchymal population doubles in comparison to control cells, an indication that Mg promotes EMT in our in vitro model. In addition, elemental analyses were carried out for an in vivo model of spontaneous metastasis. These analyses indicate the presence of the element Mn exclusively in the metastatic sites, while primary tumors do not present this element. Zn was detected in all tissues, an indication that, in order to migrate, tumor cell integrins must overcome Mg-Zn dislocation by using Mn to activate integrins. Our perspectives include analyses on tumor-derived syndecan ability to bind Mg and Mn, and the combined treatment of tumor cells with HS analogues and Zn with further evaluation of the metastatic potential.

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Differential expresion of serglycin proteoglycan in cancer cell lines and malignant tissues

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Serglycin is the major proteoglycan of haematopoietic origin cells and it is highly expressed and constitutively secreted by multiple myeloma and endothelial cells. Although human serglycin gene consists of three exons, studies in normal neutrophils revealed the presence of an alternatively spliced transcript missing exon2. In the present study, the expression of human serglycin gene was detected in various lung, breast, colon, and prostate cancer as well as glioma cell lines by RT-PCR analysis. Several cell lines were positive for the expression of both transcripts as identified by sequencing analysis. Semi-quantitative RT-PCR was performed to evaluate the expression levels of serglycin normalized to the housekeeping β-actin gene. Higher levels of expression were detected in cell lines of lung, prostate and breast cancer that reveal a more metastatic type in contrast to the lower expression levels of less metastatic colon, lung and breast cancer cell lines. Additionally, high expression levels of serglycin were detected in invasive glioma cell lines. In order to examine the secretion pattern of serglycin in cancer cell lines, culture medium was obtained. Core proteins of serglycin were identified by SDS-PAGE and Western blotting analysis and the relative levels of serglycin were quantified. We further examined the cellular expression and distribution of serglycin in two tissue microarrays; the first contained 6 neuroglial tumors of all grades, as well as normal brain tissue. The second contained 40 carcinomas of different grade and TNM stage, origination from colon, breast, lung and prostate, as well as normal epithelial tissues. As regards the brain tumors, our immunohistochemical analysis revealed that the distribution of serglycin was almost exclusively cytoplasmic. The cellular levels of serglycin seemed to be elevated in the tumors of higher grades (G3 and G4). Notably, normal astrocytes and oligodenrocytes, as well as endothelial cells were devoid of serglycin immunoreactivity; on the contrary neuron and their processes were positive for this protein. As regards the epithelial tumors the cellular distribution of serglycin was similar to the brain neoplasms. Indeed, the immunostain was diffuse and almost exclusively cytoplasmic in all the carcinomas evaluated. Notably, in some cases (lung large cell and breast carcinomas) serglycin also had membrane localization. Serglycin was also expressed in normal colon, breast, prostate and lung epithelia, as well as in plasmatocytes, endothelial, smooth muscle and stromal cells. The expression of serglycin in all malignant cells and the correlation of serglycin levels with the metastatic potential of the cells indicate its participation in the development of malignancies.

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Specificity of interaction of FGFs and HS

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Heparan sulfate (HS) is a key regulator that controls the functions of a large number (›435) of extracellular regulatory proteins. Fibroblast growth factors (FGFs) are heparin-binding proteins and interactions with HS determine their transport between cells and are required for the assembly of high affinity signalling complexes with cognate FGF receptors (FGFRs). Some specificity is apparent, particularly for FGFR preferences. However, the specificity of the interaction of FGFs with HS is still debated. One school, drawing on the high specificity of the interaction of antithrombin III with a pentasaccharide in heparin, contends that there are rare and unusual sequences of saccharides in heparin/HS responsible for high affinity binding of the proteins. Another school contends that largely non-specific ion-exchange interactions underpin protein binding by the sugar [1. 2].

The FGF family of growth factors has expanded to clearly defined subfamilies through a series of genome duplications. We are using the FGF family as a defined system, subjected to natural selection, to determine the specificities of interactions of FGFs with the polysaccharide. Our existing work with six FGFs from five subfamilies suggests that there is specificity in FGF: heparin interactions and this reflects the evolution of the FGF family members [3], which parallels the specificity of FGF ligands for FGFRs. To determine if this hypothesis is correct, we have now produced further FGF proteins to provide more comprehensive coverage of the different subfamilies. The specificity of their interaction with heparin has been determined at different levels. The preference of FGFs for particular sugar structure has been explored using differential scanning fluorimetry (DSF) and a library of chemically modified heparins, heparin derived oligosaccharides and model glycosaminoglycans. This work supports the hypothesis e.g. FGF-3 and FGF10 prefer sugar structure closer to those preferred by FGF-7 compared to FGFs in other subfamilies. Protect and label mapping of the sugar binding site in the FGFs is will map the conservation of primary and secondary heparin binding sites.

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Hyaluronan/RHAMM receptor signaling in fibrosarcoma cell proliferation

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Hyaluronan (HA) is a high molecular weight glycosaminoglycan and a basic ECM component supporting tissue homeostasis. HA interacts with its specific receptors CD44 and RHAMM (hyaluronan mediated motility receptor), regulating key cellular functions including cell cycle, proliferation, migration, adhesion, and invasion. RHAMM overexpression has been correlated to tumor development and a prognostic significance of its expression has also been indicated in several tumor types. In our previous study we showed that HT1080 cells express three different RHAMM isoforms in an HA-dependent manner and that RHAMM exhibits a key role in fibrosarcoma cell adhesion. In the present study, we investigated the effect of HA / RHAMM signaling on the proliferation of HT1080 fibrosarcoma cells. An in vitro proliferation assay showed that both low molecular weight HA (LMWHA) as well as high molecular weight HA (HMWHA) significantly increased ($p \leq 0.05$) the proliferation of HT1080 cells. In order to study the direct role of RHAMM on the HA-induced HT1080 proliferation, RHAMM expression was inhibited using RNA interference. HT1080 cells transfected with siRHAMM showed a significant down-regulation in their basal ($p \leq 0.05$) and LMWHA induced ($p \leq 0.01$) growth rate demonstrating a crucial role of RHAMM on these cells growth. Moreover, we investigated possible candidate molecules involved in the HA / RHAMM downstream signaling pathway, such us cell cycle regulators known to be affected by RHAMM, including p53, β-catenin and cyclin-D1. RHAMM-deficient cells showed a significant decrease in p53 ($p \leq 0.01$), in β-catenin ($p \leq 0.01$), and cyclin D1 ($p \leq 0.001$) expression. Concluding, RHAMM is a potential regulator of fibrosarcoma cell proliferation.

Unexpected sulfation of chondroitin in *C. elegans*

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The nematode Caenorhabditis elegans is a well-known model organism in many fields of research and has become interesting for the study of glycosaminoglycans (GAGs) during recent years. 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 C. elegans 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 chondroitin (C4S) using RPIP-HPLC analysis of C. elegans GAGs following a modified purification protocol. The presence of sulfated chondroitin at larval and adult stages of the worm was further confirmed by immunofluorescent staining using the CS-56 antibody. C4S disaccharides were only detected after chondroitinase digestion and animals lacking 2-O and 6-O sulfation of HS contained increased amounts of C4S compared to wild type worms. We assume that the large amount of non-sulfated chondroitin masked these structures in earlier studies and we could only detect them because we had modified the purification protocol accordingly. Nevertheless, total amounts of C4S and HS were in a similar range in wild type animals, indicating a biological significance for the sulfated chondroitin in C. elegans. Our aim is now to identify and characterize the responsible sulfotransferase(s) in vivo.

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p53/iASPP growth-promoting function is affected by CD44 expression

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During tumor progression hyaluronan-CD44 interactions in the cellular local microenvironment affect the growth and differentiation of cells. A large body of studies implicates CD44 in both tumor promotion and suppression. This dual nature of CD44 is not well understood. Using a proteomic approach we recently demonstrated that CD44 interacts with iASPP which represses the p53-mediated cellular apoptosis both in vitro and in vivo.

Endogenous CD44-iASPP complexes have been detected in both mammary epithelial cells and dermal fibroblasts, and preferentially in the cytosolic fraction compared to nucleus. Currently experiments are going on to eluciadate the functional significance of CD44-iASPP complexes and in relation to iASPP-p53 complexes in non-malignant and malignant epithelial as well as mesenchymal cells. Notably, CD44, iASPP and p53 are found in the same complex and experiments are currently in progress to investigate how these molecules anchor each other. Importantly, iASPP interacts only with the standard form of CD44 and not with its variant forms. We also investigated how external stimuli, such as hyaluronan and TGF-β affected the levels of endogenous CD44-iASPP complex formation. The analysis revealed that these external stimuli increased the formation of such complexes in fibroblasts but had less pronounced effect in epithelial cultures, indicating different regulatory roles of the complexes in mesenchymal versus epithelial cells.

Most interestingly, the presence of CD44 is required for p53-iASPP complex formation, since its silencing with specific siRNA suppressed these complexes in fibroblast cultures. However, the formation of iASPP-p53 complexes dramatically increased in epithelial cells depleted of CD44 compared to those expressing CD44. Thus, the interaction between iASPP and CD44 differentially affects epithelial and mesenchymal cellular growth and survival by modulating iASPP-p53 complexes.

Effects of radioiodine therapy on protease-antiprotease balance and serum levels of soluble tumor necrosis factor receptors in papillary thyroid carcinomas with/ without autoimmune thyroid diseases

P 36

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Under physiological conditions the expression of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) is highly coordinated at the level of gene expression and this balanced expression guarantees normal tissue structure and organ function and prevents both excessive ECM deposition and increased ECM degradation. In papillary thyroid carcinomas (PTC) with/without autoimmune thyroid diseases (AITD), the balance between expression of MMPs and TIMPs is broken. After radioiodine (I-131) therapy, tumor necrosis factor-alpha (TNF-α) precursor is cleaved to release soluble TNF-α to increase apoptosis. TNF-α can be slowly cleaved from the cell surface by MMPs, producing a bioactive cytokine, soluble TNF-α, which may increase apoptosis through binding to the TNF receptor type I (TNFRI).

We aimed to evaluate the effects of therapeutic irradiation with I-131 on serum levels of MMP-9, TIMP-1, TNF-α, soluble TNFRI (sTNFRI) and ----TNFRII (sTNFRII), in PTC and PTC associated with AITD (PTC+AITD) patients.

Methods and patients: We selected 54 patients with PTC (8M/46F) and 41 with PTC+AITD (3M/38F). PTC+AITD patients had positive titers of anti-Tg autoantibodies (TgAb). Peripheric blood samples were collected before and at 96 hours after I-131 administration. All patients received the same dose of I-131 (3.7 GBq, first dosage after thyroidectomy). The serum levels of TgAb, TNF-α, sTNFRI, sTNFRII, MMP-9 and TIMP-1 were measured by ELISA.

I-131 therapy of PTC+AITD patients was associated with an increase with 18% in TgAb level ($P=0.001$), 5% in MMP-9/TIMP-1 ratio ($P=0.003$) and a decrease with 29% in TNF-α/sTNFRI ratio and 31% in TNF-α/sTNFRII ratio ($P<0.001$). TgAb titers are positively related to MMP-9/TIMP-1 ratio ($r=0.52$, $P<0.01$). In PTC group, the beneficial effect of I-131 was illustrated by a significant reduction of MMP-9/TIMP-1 ratio with 44% ($P=0.003$) and an increase with 66% in TNF-α/sTNFRI ratio and 61% in TNF-α/sTNFRII ratio ($P<0.001$). The reduction of MMP-9/TIMP-1 ratio is positively correlated with sTNFRI level at follow-up ($r=0.67$, $P=0.009$).

Elevated TNF-α/sTNFRI, TNF-α/sTNFRII ratios indicate a decline in disease activity after I-131 therapy more pronounced in PTC than in PTC+AITD, suggesting that suppression of sTNFRI, sTNFRII or increased production of TNF-α is required to initiate remission of cancer. In PTC patients, I-131 therapy has almost halved the imbalance between MMP-9 and TIMP-1 and this decrease may reduce tumor cell viability and migratory potential. In PTC+AITD patients, increased TgAb titers partially block the beneficial effect of I-131.

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Complete deficiency for CREB3L1, encoding the ER-stress transducer OASIS, causes severe autosomal recessive Osteogenesis Imperfecta in humans

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Osteogenesis imperfecta (OI) is a heritable brittle bone disorder which characterized by highly variable clinical severity, ranging from mild to numerous (in utero) fractures with perinatal lethality. The overwhelming majority of OI patients (>90%) harbour heterozygous mutations in either one of the type I collagen genes (COL1A1 or COL1A2), thereby causing decreased type I collagen production or the generation of structurally abnormal type I collagen molecules (autosomal dominant OI). Autosomal recessive OI is caused by biallelic mutations in genes which encode proteins involved in type I procollagen processing or chaperoning. Although the list of genes involved in OI is growing, still some OI cases remain molecularly unexplained.

We performed a candidate gene approach in a family with autosomal recessive inheritance of the OI phenotype, in whom previously all known OI genes were excluded. The CREB3L1 gene, encoding the endoplasmic reticulum (ER)-stress transducer OASIS, was selected since OASIS-/- mice present with an OI phenotype with multiple fractures. PCR amplification of the entire CREB3L1 gene failed for the affected foetus of the presented family, which suggested a whole gene deletion. ArrayCGH analysis confirmed the presence of a homozygous genomic deletion of the entire CREB3L1 gene in the affected foetus. Biochemical collagen analysis revealed a normal appearance of type I collagen. This is in accordance with the observation that type I collagen is normal in dermal fibroblasts of the OASIS-/- mice, while a decreased expression of type I collagen is seen in OASIS-/- osteoblasts. RT-qPCR experiments confirmed the total absence of CREB3L1 expression in dermal fibroblasts of the affected foetus. The expression of the transcription factor Runx2, involved in osteoblast differentiation, and of the ER-stress markers BiP, CHOP and the spliced form of XBP1 was comparable to control samples, which is similar to what is observed in OASIS-/- mice.

In summary, the identification of OASIS as a cause of human autosomal recessive OI links for the first time an ER-stress transducer to human OI. This finding expands the OI gene and underscores the importance of ER-stress not only in human OI pathogenesis but also in human bone development.

Effects of interleukin-6 and its soluble receptor on the expression of matrix metalloproteinases and their inhibitors in nasal polyps fibroblasts

P 38

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Nasal polyposis is a chronic inflammatory disease of the upper airways, featuring inflammatory cell infiltration, and tissue remodeling including basement membrane thickening, gland modifications, extracellular matrix (ECM) accumulation and oedema. Interleukin-6 (IL-6) is a multifunctional cytokine that is involved in various inflammatory conditions and implicated in several fibrotic lesions. It has been reported that it stimulates fibroblast proliferation, increases collagen deposition, and induces the expression of TIMP-1. The soluble IL-6 receptor (sIL-6R) forms an active complex with IL-6 and gp 130 membrane molecule, acting as an agonist of IL-6 functions.

The aim of this work was to study the effects of IL-6 and sIL-6R on the expression of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs), factors which are implicated in nasal polyposis pathogenesis.

RT-PCR and western blot analysis revealed the high expression of IL-6 and the presence of sIL-6R in polyps and polyps tissue extracts, respectively. When polyps fibroblasts were cultured in the presence of IL-6 and (or) sIL-6R a stimulation of TIMP-1 was observed, which was significantly suppressed in the presence of MAP kinases and PI-3 kinase inhibitors. The sIL-6R alone caused significant enhancement of MMP-1 and MMP-3 expression, which was suppressed in the presence of IL-6. IL-6 suppressed also the constitutive expression of MMP-1 and MMP-3. In contrast, IL-6 and (or) sIL-6R enhanced the expression of MMP-14. It was also found that sIL-6R enhanced the TNF-α-induced expression of MMP-1, MMP-3 and MMP-14, while it did not affect the TNF-α-, IGF-I- and TGF-β1-induced expression of TIMP-1.

It has been proposed that one from the main stages of polyp formation is epithelial rupture. The extracellular matrix degradation by MMPs may be the key point for epithelial rupture and polyp formation. On the other hand, TIMP-1 is implicated in tissue remodeling of ECM through of its ability to inhibit the action of activated MMPs. High expression of TIMP-1 has been associated with ECM accumulation and fibrosis, which are both characteristics of nasal polyposis. In this study it was shown that IL-6 and sIL-6R, up-regulate the expression of factors associated with both ECM accumulation and ECM degradation, which are distinct features of nasal polyposis. Thus, the exact role of IL-6 and sIL-6R in nasal polyposis pathogenesis remains to be elucidated.

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Trypanosoma cruzi infection disorganizes TGF-β receptor type II costameric distribution in cardiomyocytes and affects host cell TGF-β response

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Chagas disease, caused by the protozoan Trypanosoma cruzi, is a major cause of cardiomyopathy in Latin America. Transforming growth factor β (TGF-β) is involved in its establishment and progression, participating in T. cruzi host cell invasion, intracellular parasite cycle, regulation of immune response and heart remodeling. Since previous reports demonstrated that T. cruzi can modulate host cell receptors, we were analyzed the TGF-β receptor type II (TβRII) expression and distribution during T. cruzi – cardiomyocyte interaction. TβRII staining by indirect immunofluorescence revealed an unexpected striated organization of in cardiomyocytes, which was enhanced (38%) after TGF-β treatment. Double labeling with anti-vinculin and anti-TβRII antibodies showed a co-localization of TβRII with costameres of vinculin by confocal microscopy. The association of TβRII with the cytoskeleton was also demonstrated by cytochalasin D treatment, which resulted in a decrease of 45.3% in the ratio of cardiomyocytes presenting TβRII striations. This association of TβRII with the cytoskeleton may be involved in triggering TGF-β signaling, since western blot analysis showed that cytochalasin D significantly inhibited Smad 2 phosphorylation and fibronectin stimulation after TGF-β treatment in cardiomyocytes. T. cruzi infection elicited a decrease of 79.8% in the frequency of cardiomyocytes presenting TβRII striations. The treatment of T. cruzi-infected cultures with TGF-β did not provoke any significant alteration in the frequency of TβRII striations, still showing low TβRII striation percentage, in contrast with the raise observed in control cultures. In addition, T. cruzi infected cardiomyocytes present a lower response to exogenous TGF-β, showing a reduction of phosphorylated smad 2 after TGF-β treatment. Together, these results suggest that the co-localization of TβRII with costameres are important to activate TGF-β signaling cascade, and T. cruzi derived cytoskeleton disorganization could result in altered or low TGF-β response in infected cardiomyocytes.

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The collagen binding protein fibromodulin contributes to atherosclerotic plaque inflammation and cerebrovascular events

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We have recently shown that mice lacking the collagen binding protein fibromodulin develop atherosclerotic plaques with altered collagen fibril structures. These plaques were also smaller in size and contained less lipids in comparison to plaques from mice expressing fibromodulin. The results suggest that fibromodulin has a significant impact on the development of atherosclerotic lesions. The aim of the present study was to analyze the importance of fibromodulin in human atherosclerotic plaques.

The amount of fibromodulin, lipids and smooth muscle cells in carotid plaques (152 patients) was analyzed on stained sections which were scanned and digitalized using an Aperio ScanScope digital slide scanner. Positively stained areas were quantified using BioPix iQ software. Cytokines and vascular endothelial growth factor were analyzed in homogenates (multiplex immunoassay). A total of 75 plaques were symptomatic, e.g. they were obtained from patients that had experienced a cerebrovascular event prior to surgery. Fibromodulin expression was higher in symptomatic plaques ($p=0.0001$). Fifty-one plaques were from diabetic patients and these had also higher levels of fibromodulin than plaques from non-diabetics ($p=0.001$). A high plaque content of fibromodulin correlated with increased lipids ($r=0.18$; $p=0.033$) and with low content of smooth muscle cells ($r=-0.17$; $p=0.048$). Fibromodulin expression in plaques was also associated with increased concentration of CD40L ($r=0.19$;$p=0.044$), macrophage inflammatory protein-1b ($r=0.20$; $p=0.032$) and vascular endothelial growth factor ($r=0.22$; $p=0.018$) and with low concentrations of the anti-inflammatory cytokine interleukin-10 ($r=-0.29$; $p=0.002$).

Our observations provide clinical support for previous experimental findings of a role of fibromodulin in atherosclerosis and suggest that fibromodulin is associated with plaque inflammation and cerebrovascular events.

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Elastin content of carotid plaques predicts risk for future stroke

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Myocardial infarction and stroke are currently two of the major causes of mortality and morbidity. They are often caused by the rupture of an atherosclerotic plaque, leading to thrombosis or embolization. Atherosclerotic plaques with a low content of connective tissue proteins are believed to have an increased risk of rupture and to give rise to clinical events. The aim of the present study was to investigate if the content of elastin and collagen in plaques removed at surgery are predictive of the risk of future cardiovascular events.

The intraoperative specimens of 221 patients undergoing carotid endarterectomy were analyzed and their elastin and collagen contents measured biochemically were related to the incidence of future cardiovascular events.

Elastin and collagen contents correlated positively ($r = 0.554$, $P<0.0001$). The collagen and elastin contents were lower in males and in patients with diabetes. Elastin was higher in asymptomatic plaques and correlated inversely with age ($r=-0.16$, $P<0.05$). After a mean follow-up time of 39.6 ± 16.4 months, 33% of patients had suffered one or multiple post-operative events. Patients in the lowest tertile of plaque elastin had increased post-operative all-cause mortality and incidence of ipsilateral stroke (P for trend ‹0.05 and 0.009 using Log Rank Chi-square test). The increased risk for ipsilateral stroke remained significant when controlling for age, gender, hypertension, diabetes, smoking and pre-operative symptoms in a Cox Proportional Hazard model (hazard ratio 7.38, 95% C.I. 1.50-36.31), while the increase in all-cause mortality did not demonstrate independent significance.

These observations support the concept that extracellular matrix proteins are important for plaque stability and suggest that patients with a low plaque content of elastin should undergo a stricter follow up.

Gelatinases and Hyaluronidases in serum of colorectal cancer patients

P 42

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Gelatinases and Hyaluronidases are enzymes acting predominantly extracellularly. They are present in normal tissues, but usually their amounts increased in pathologic states. Gelatinases (MMP-2 and MMP-9) are metalloproteinases secreted from cells in latent forms being activated under well-regulated conditions, and thereafter their activity is regulated by TIMPs. Hyaluronidases are enzymes acting mainly on hyaluronan, however they degrade chondroitin sulphate. Four different hyaluronidases are well studied, hyal-1, hyal-2, hyal-3 and PH-20. The best characterized isoform, hyal-1, is a lysosomal enzyme with a pH optimum of 3.7. MMP-2, MMP-9 and hyal-1 are present in human serum and their activity is altered in various diseases. Especially in serum of cancer patients, accumulated evidence suggests that the activity of all three enzymes is increased and in some cases they have been proposed as diagnostic markers. In the present study, semi-quantitative zymographic procedures have been applied for the detection and quantification of MMP-2, MMP-9 and hyal-1 in human serum obtained from healthy donors and colorectal cancer patients, before surgery and after surgery, in three months interval for up to one year. The results indicated the presence of only latent gelatinases' forms and a hyal-1 band migrating at about 65 kDa, in both cancer and healthy serum samples. The presence of these enzymes was verified by Western blotting. The levels of both proMMP-2 and proMMP-9 were higher in serum of cancer patients before surgery compared to that of healthy donors. On the other hand, hyal-1 activity did not show significant differences. The enzymes behaved differently during the follow-up period. ProMMP-2 decreased a week post-operatively and thereafter increased until 12 months, where a decline was observed with a tendency to reach healthy values. ProMMP-9 increased a week post-operatively and thereafter decreased until 12 months, where an increase was observed with a tendency to reach healthy values. Hyal-1 decreased in patients a week post-operatively and thereafter gradually increased to reach the pro-surgery levels. In some cases, for proMMP-9 and hyal-1, the post-surgery levels overcame the pro-surgery ones without an indication of an abnormal condition for the patients or recurrence of cancer. It should also be noted that the results were site- and stage-related. The obtained results suggested that proMMP-9 in serum of colorectal cancer patients might be examined as a biochemical marker for cancer recurrence or metastasis, as it has been proposed for other cancers, but studies utilizing prolonged time serum collection are required.

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Nodules origin in Hyaline Fibromatosis Syndrome

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Hyaline Fibromatosis Syndrome (HFS) is a rare autosomal recessive disorder caused by loss-of-function mutations in Anthrax Toxin Receptor 2, also known as Capillary Morphogenesis Gene 2 (CMG2),. Patients develop subcutaneous nodules, joint contracture and for the severe form of the disease, diarrhea and increased sensitivity to pulmonary infections. Little is known about the function of CMG2, but the disease symptoms point toward the regulation of the extracellular matrix (ECM) homeostasis. Indeed, multiple case reports and our laboratory observed that the subcutaneous nodules, mostly acellular, are the result of an accumulation of ECM proteins. Considering the positioning of the nodules, it has been hypothesized that mild and repeated mechanical stresses could be the initiating events. The aim of this study is to understand better the nodule biogenesis. We confirmed by histology and immunochemistry the fibrillar and cellular composition of the nodules and observed mainly spindle-shaped cells and blood vessels. Gene profiling analysis comparing nodule-derived fibroblasts to non-affected tissue derived fibroblasts highlighted a increased level of alpha smooth muscle actin (αSMA) and TGF-Beta 2 mRNA in the nodule-derived cells. αSMA is a well described marker of myofibroblast differentiation, a process mainly regulated by the TGF-Beta pathway. We showed that all nodule-derived cells showed an elevated level of αSMA mRNA and protein compared to cells derived from patient non-affected tissues. Moreover, enlarged focal adhesions, another myofibroblast marker, were observed by microscopy in nodule-derived cells. Finally, we observed by immunohistochemistry the presence of αSMA positive cells in the nodules. These cells are known to produce large amount of collagen and ECM proteins and are induced during wound healing. We hypothesized that they could play a role in the nodule biogenesis, and that their regulation may involve CMG2.

Anticoagulant Heparan Sulfate in the human endometrium : role in permissiveness to invasion and angiogenesis

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Anticoagulant heparan sulfate (aHS) is present in blood vessels, where its anti-protease activity can limit tissue remodeling and confer stability to the vessels. Preliminary evidence suggests that aHS are downregulated during angiogenesis. In human uterus, the endometrium undergoes constant remodeling during the estrous cycle.

Vascular permeability is increased by angiogenic factors like VEGF, with fibrin deposition in vessel walls, forming a provisional matrix for developping vessels. Endothelial aHS limits fibrin formation and downregulation of aHS could promote angiogenesis.

Endometrial carcinoma is a frequent cancer with variable clinical outcome and available markers have limited predictive value of tumor aggressivity. aHS could serve as marker of tissue stability and the aHS present in follicular fluid could modulate tissue invasion in the endometrium.

Determine aHS distribution in normal endometrium and in endometriood cancer to reveal modulations of aHS expression during the cycle and in cancer.

We study human endometrium during the estrous cycle and endometriood carcinoma grade G1, with approval of the local ethics committee.

Endometrium sections stained with Alexa488-coupled probes, Antithrombin for detection of aHS and endothelial markers CD-31 and D2-40 for blood and lymphatic vessels. Vessel numbers are evaluated in the endometrium.

During menstrual cycle, aHS is present in blood and lymphatic vessels and in glandular epithelium. Vascular aHS are constant during the cycle and vessel numbers remain stable in the endometrium. In contrast, epithelial aHS is downregulated in the functional layers of the endometrium during the secretory phase.

In endometriood carcinoma, aHS is suppressed to undetectable levels in tumoral tissue and in the vasculature.

During regeneration in the proliferative phase, angiogenesis occurs by elongation of existing vessels involving minimal changes no alteration of aHS. In the secretory phase, aHS disappears from the epithelium during the implantation window. In endometriood cancer, aHS downregulation aHS indicates active tissue plasticity.

These data support our hypothesis that aHS is expressed in stable vascular and epithelial walls and is downregulated during tissue remodelling, angiogenesis and tumor invasion. Further experiments are underways to correlate the mechanisms of implantation and cancer invasion with the disappearance of aHS.

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Identification of the "Uncharacterized protein C10orf118" in breast cancer cells and its role on the hyaluronan metabolism

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The association of hyaluronan (HA) with tumourigenesis has been known for a long time. HA-rich matrices around tumours favour the cancer cells migration and infiltration of newly formed blood vessels (Savani et al. 2001). In fact high level of HA is often associated with malignant progression in many cancers, such as breast cancer, colorectal cancer, and glioma (Itano et al. 2004). At all stages of tumourigenesis, stromal cells become "activated" and release growth factors and cytokines that further increase HA synthesis by both stromal and tumour cell.

Recently in our laboratory in co-cultures (Transwell system) of human dermal fibroblasts with the breast cancer cell line BC 8701, we identified at the conditioned media the presence of a protein named "Uncharacterized protein C10orf118" or "CTCL-tumour associated antigen" whose accession number is Q7z3e2. Using the Real-time PCR, we observed that this protein was highly expressed in BC8701 cells and less in fibroblasts. Therefore, the aim of the present project is to explore the functional properties of the Q7z3e2 protein and the role it may have in the control of HA synthesis and in cross-talk between the two breast cancer cell lines: MCF-7 (ERα positive, low metastatic) and MDA-MB-231 (ERα negative, high metastatic) and stromal cells.

The Q7z3e2 protein and gene expressions were higher in MCF-7 than MDA-MB-231 cells and mainly localized around or within the nucleus, even though the protein was identified also in the conditioned media. Western blot analysis of cell cultures showed higher amount of protein in both tumor cells respect to fibroblasts. Fibroblasts co-cultured with each tumour cell line using the Transwell system showed that MCF-7 cells induced the HAS2, HYAL2 and Q7z3e2 expression in fibroblast, demonstrating a cross-talk between these cells. However, silencing of Q7z3e2 in breast cancer cells and co-culture with fibroblasts demonstrated that suppression of HAS2 and HYAL2 was observed only with MDA-MB-231. These data suggest that Q7z3e2 is possibly able to trigger some particular signal pathways inside the cells to regulate the expression of different genes involved in the metabolism of hyaluronan.

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Heparanase as a new player in renal fibrosis: analysis of its contribution in the epithelial to mesenchymal transition process and its validation as pharmacological target.

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Diabetic nephropathy (DN) is a life-threatening complication of both type 1 and 2 diabetes and is considered the leading cause of end-stage renal failure. DN was originally considered to be primarily dependent from glomerular alterations but it is nowadays recognized that the disease severity is better associated with the degree of tubulo-interstitial fibrosis. The tubular epithelial-to-mesenchymal transition (EMT) is a central event of the fibrosis development. Recent evidence prove the involvement of heparanase (HPSE) in the pathogenesis of several proteinuric disease including DN. Actually, HPSE-KO mice are resistant to streptozotocin-induced diabetes.

HPSE is an endo-glycosidase that cleaves the heparan sulfate (HS) chains and participates in ECM remodeling as well as in the release from ECM of many HS-bonded molecules. An increased heparanase activity has also been detected in plasma and urine of diabetic patients suggesting that the heparanase activity in body fluids could be a diagnostic marker for the detection and/or progression of renal disease.

In a recent report we showed that in tubular cells HPSE is a target gene of DN mediators and that its up-regulation gives rise to the HS reduction through its endo-glycosidase activity and the ability to regulate syndecan-1.

Recently we provided evidence that HPSE is specifically involved in the establishment of tubular fibrosis, being necessary for the epithelial-mesenchymal transition of tubular cells induced by FGF-2. In summary, the increased levels of HPSE creates a milieu that favors fibrosis and thus the inhibition of HPSE could control fibrosis progression. Most of the HPSE inhibitors are heparins or modified heparin derivatives. Interestingly heparin derivatives have been previously shown to favorably affect experimental diabetic nephropathy.

Despite a great deal of studies, there are no specific treatments to target renal fibrosis so far. Therefore understanding those mechanisms which regulate the development of renal fibrosis is crucial for future identification and development of specific therapeutic strategies which may alleviate this damage.

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Control of Transport of FGFs in Cell-Cell Communication

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Cell communication in metazoa is regulated by the extracellular matrix (ECM) and in particular by the glycosaminoglycan heparan sulfate (HS). HS exerts its regulatory functions by controlling the movement and receptor binding functions of its more than 400 protein partners. The effector proteins (growth factors, cytokines, etc.) possess different HS binding kinetics and selectivity for binding structures in HS. However, it is still unknown how the matrix is organised and structured. A recent study has suggested that the movement of fibroblast growth factor 2 (FGF2) in ECM is controlled by a combination of its binding kinetics for HS and by the spatial arrangement of its binding sites in the polysaccharide[1]. To test whether this is general property of ECM and HS binding effectors, a series of FGFs have been produced. Some (FGF1 and FGF10) have been conjugated to nanoparticles (NPs) in the same way as FGF2 previously[1]. Initial analysis of the movement of FGF1-NP and FGF10-NP by photothermal microscopy indicates that FGF1-NP is extremely mobile, far more so than FGF2. In contrast, FGF10-NP appears to be very immobile. These results are coherent with measurements of the binding kinetics of FGF1 to cellular HS2. They also suggest a key functional difference between FGF1 and FGF2, as the former will have a much greater range and the latter will produce steeper gradients. FGF10 may require external force to move, either as heparanase[3] or driven by mass action. New methods of conjugation of NPs to FGFs have also been explored. Of these, Halo-Tag technology looks to be exceptionally promising.

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Effects and interplay of legumain and cystatin E/M on migration and invasion of macrophages

P 48

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Legumain is a lysosomal protease repeatedly shown to be associated with several types of cancer. Although the localisation of the active enzyme is primarily lysosomal, the proform can be secreted and could thus take part in degradation of extracellular matrix (ECM), a process required for invasive growth and metastasis. In this regard, legumain has been shown to activate pro-MMP2, process cathepsins and degrade fibronectin. Activation of prolegumain takes place in acidic environments and can be facilitated by components in ECM (for example the glycosaminoglycan chondroitin sulphate). The activity of legumain is regulated by endogenous inhibitors like cystatin E/M and C. Growth of malignant tumours involves interplay between transformed cells and invading inflammatory cells. Both cancer cells and tumour associated macrophages contain increased levels of legumain but the role of this enzyme in cancer is still unclear.

To explore this, mouse macrophages (RAW 264.7) were labeled with [methyl-3H] thymidine, cultured in transwell permeable plate inserts coated with or without Matrigel, to study cell invasion and migration, respectively. In the bottom wells transfected HEK293 cells or colorectal cell lines (HCT116, SW620) were grown to study how secretions from these cells affected invasion and/or migration of macrophages from the inserts above. Stably transfected HEK293 over-expressing either legumain (M38L cells) or cystain E/M (M4C cells) were compared to untransfected HEK293 (control).

As expected M38L cells expressed high levels of legumain both intracellularly and secreted to the conditioned medium as proform. Also, these cells had much higher legumain activity when compared with control. M4C cells over-expressed cystatin E/M which was both secreted to the medium and found in the cell lysates. These cells had reduced legumain activity compared to control. Secretion of cystatin E/M was also observed from HCT116 but not from SW620. The HCT116 cell lysates contained both the active 36 kDa legumain form and the proform (56 kDa), whereas SW620 lysates contained only prolegumain, as observed by immunoblotting. Also, legumain activity was higher in HCT116 than in SW620.

In co-culture, the M4C cells over-expressing cystatin E/M significantly reduced both migration and invasion of macrophages. The two colorectal cancer cell lines were also found to affect migration and invasion of macrophages. However, M38L cells over-expressing legumain seemed not to affect either macrophage invasion or migration. The interplay between legumain and cystatin E/M in cancer cells and in relation to macrophage migration and invasion will be further studied and presented.

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The Role Of Hyperglycaemia In Pancreatic Fibrosis

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Diabetes mellitus is a major public health challenge and has been linked to a number of cancer types including pancreatic cancer (PaC). Activated pancreatic stellate cells (PSC) have an essential role in PaC development and growth. We hypothesize that PSC can be stimulated by increased glucose concentration corresponding to a poorly controlled diabetic individual.

We examined the effect of higher glucose concentration in PSC activation compared to the effect of the well known cell activator TGFβ. We investigated if type-1, type-3 collagen and αSMA levels were elevated in cell culture supernatants and cells. To certify the glucose uptake, we identificated glucose transporters in our cells. We looked for other altered target genes that can have a role in the effects of PSC activation.

We cultured a human immortalised PSC cell line in normal (5,5 mmol/l) and in parallel in elevated (15,3 mmol/l) glucose concentration for three weeks. To assess its effect on cell activation, the known PSC activator TGFβ (5 ng/ml) was added to the cell clones for the last 48 hours. We used ELISA, dot blot and RT-PCR to measure alterations in type-1 and type-3 collagen expression in cell culture supernatants and cells. We examined αSMA and type-1 collagen synthesis, and the presence of glucose transporters (GLUT-1, -2, -3, -4) by immunocytochemistry and Western blot. Microarray analysis was performed from isolated mRNA; genes with altered expression were selected and validated by RT-PCR, ELISA and Western blot analyses.

High glucose concentration alone enhanced production of collagens and αSMA. This was further increased after TGFβ exposure. All types of glucose transporters were identified on our cells. On the basis of microarray analysis and validation, a few target genes had been chosen for further investigation. High glucose concentration caused elevated cFOS, ID-1, LTBP-2, CXCL12, TXNIP, IGFBP-2, Col5a1, THBS1, CTGF and decreased PPARγ, RND-3, MMP-1, FGF-2, KLF-6, DUSP10 mRNA levels in our examinations.

Higher glucose concentration activates pancreatic stellate cells and probably promotes the development of pancreatic fibrosis, thus, also plays a role in the formation of pancreatic carcinoma.

Interactions between extracellular matrix, cell surfaces and the novel growth factor tartrate resistant acid phosphatase 5a

P 50

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Tartrate resistant acid phosphatase (TRAP), secreted by macrophages has recently been shown to have a growth factor effect. The growth factor effect of the low phosphatase activity isoform, TRAP 5a, was observed when mice overexpressing this form developed obesity, by increased proliferation and differentiation of pre-adipocytes. The current projects aim on elucidating the routes through which TRAP 5a acts as a growth factor upon secretion to the ECM and identifying potential partners of TRAP in the ECM and cell surface of pre-adipocytes. TRAP has been shown to exhibit, as many known growth factors, an affinity towards heparan sulfate and heparin, suggesting the possibility of a binding to heparan sulfate proteoglycan co-receptor on cells. In the search for potential binding partners a pull down assay followed by mass spectrometry was performed on the 3T3-L1 pre-adipocytic cell line. The extracellular matrix protein nidogen-2 was identified as candidate for interaction and a pull down assay for nidogen-2 and TRAP recombinant proteins suggested a direct interaction between the proteins in vitro. In vivo, nidogen-2 and TRAP are partly colocalized in bone tissue as shown by immunohistochemistry, suggesting that TRAP could potentially be accumulated and stored in the ECM by binding to nidogen-2. Several known growth factors, exhibit the ability to bind to the cell membrane of cells and the ECM through proteoglycans carrying heparan or chondroitin sulfate. The heparan sulfate proteoglycan glypican-4 has been earlier suggested to interact with TRAP in bone. We investigated the co-localization between glypican-4 and TRAP in 3T3-L1 pre-adipocytes when cells were treated with TRAP. Immunocytochemistry showed co-localization between the two proteins, indicating that glypican-4 could be a potential binding partner for TRAP in pre-adipocytes. These findings suggest that TRAP 5a, similar to other known growth factors, can possibly upon secretion be stored in the ECM by binding to nidogen-2 until release and interaction with the cells. On the cell surface, an interaction of TRAP with glypican-4 could facilitate the signaling and uptake of TRAP leading to signaling for enhancement of proliferation and differentiation.

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Heparanase internalization and processing is mediated by syndecan 1 cytoplasmic domain and involves syntenin and α actinin

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Heparanase is the only functional endoglycosidase capable of cleaving heparan sulfate (HS) in mammals, activity that is highly implicated in cell dissemination associated with tumor metastasis, inflammation and angiogenesis. Heparanase uptake is considered a pre-requisite for the delivery of latent 65 kDa heparanase to lysosomes and its subsequent proteolytic processing and activation into 8 and 50 kDa protein subunits by cathepsin L. Efficient uptake of heparanase was evident also by GPI-deficient cells (i.e., lack cell surface glypicans), suggesting preferential involvement of syndecans in this process. The molecular mechanism underlying heparanase uptake is still largely obscure. Here, we examined the necessity of syndecan 1 cytoplasmic domain for heparanase internalization and processing. To this end, we transfected cells with full length mouse syndecan 1 or deletion constructs lacking the entire cytoplasmic domain (delta), the conserved (C1 or C2) or variable (V) regions. Heparanase binding, internalization and processing were then evaluated by immunofluorescent staining and immunoblotting. Heparanase uptake was markedly increased following syndecan 1 over expression, thus challenging the notion that HS coat of the cell membrane is at saturation and does not limit ligand binding. In contrast, heparanase was retained at the cell membrane and its processing was impaired in cells over expressing syndecan 1 deleted for the entire cytoplasmic tail. We have next identified that conserved domain 2 (C2) and variable (V) regions of syndecan 1 cytoplasmic tail mediate heparanase internalization and processing. Furthermore, we found that syntenin, known to interact with syndecan C2 domain and α actinin, mediates heparanase uptake. These results further illustrate the tight regulation of heparanase uptake and bioavailability.

Heparanase co-operate with RAS to drive breast and skin tumorigenesis

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While heparanase up-regulation and its pro-malignant features are well documented, the timing of its induction in the course of tumor development is less investigated and understood. In order to explore heparanase function in the early phases of tumor initiation and progression, we have utilized non-transformed human mammary epithelial cell line (breast MCF 10A) and genetic (Hpa-tg mice) approaches. Once plated on and overlaid with reconstituted extracellular matrix (Matrigel), MCF10 cells produced acinar structures that displayed many of the properties of normal mammary structures in vivo. Notably, over-expression of heparanase or its C-terminal domain (8C) resulted in significantly bigger and asymmetrical acinar structures, indicating increased cell proliferation and decreased organization. These features are far more dramatic when heparanase variants were over-expressed in MCF10AT1 cells that acquired the ability for xenograft growth after transfection with T24 H-Ras. Once inoculated into the mammary fat pad of immunodeficient mice, these cells produced lesions depicting mild-atypical hyperplasia and ductal carcinoma in situ. Over- expression of heparanase or its 8C variant resulted in far greater amount of highly cellular lesions. These lesions exhibited a high cell proliferation index, decreased recruitment of myoepithelial cells, and decreased cell-cell contact (E-cadherin staining), together indicative of progressive disease. Indeed, lesions formed by heparanase- or 8C- over expressing MCF10AT1 cells were diagnosed as invasive carcinoma. In order to substantiate co-operation between Ras and heparanase, we exposed Hpa-tg and control mice to two-steps DMBA/TPA skin carcinogenesis model because more than 90% of skin cancer initiated by DMBA contained Ha-Ras activating mutations. Hpa-tg mice were far more sensitive to DMBA/TPA treatment, exhibiting a 10-fold increase in the number and size of tumor lesions compared with control mice. Remarkably, tumor lesions were not developed in heparanase KO mice subjected to DMBA/TPA treatment, clearly depicting a critical role of heparanase in skin tumorigenesis.

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The role of extracellular galectin-3 in canonical Wnt signaling pathway activation in human breast cancer cell lines

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Evidences of galectin-3 (gal-3) deregulation in multiple cancers lead to a broad range of works investigating the role of gal-3 in tumor cell biology. Through specific interactions with a variety of intra and extracellular glycoproteins gal-3 affects several biological processes. Recently some works pointed out that not only the gal-3 expressed by tumor cells seems to be important for tumor progression, reinforcing the pivotal role of microenvironment in disease. Mesenchymal stromal cells (MSC) are a well-known component of breast tumor associated stroma and secrets gal-3. Nevertheless, if gal-3 secreted by MSC could interfere in breast cancer cells (BCC) behavior remains elusive. We used multicellular spheroids composed of bone marrow MSC from wild-type or gal-3 knock-out mice to test invasive capacity of BCC. We observed through histological and flow cytometry analyses that the absence of gal-3 significantly diminished the invasive potential of BCC. This brings into question how the gal-3 present in the microenvironment can affect BCC invasive behavior. Newly it was showed that the binding of gal-3 to the cell surface glycoprotein N-cadherin can modulate the stability of the junctional complex (JC) formed by this molecule. β-catenin, the active transcriptional factor of canonical Wnt signaling, also composes this JC. Furthermore, gal-3 has been implicated as regulator in the Wnt/β-catenin pathway. Based on this evidences we speculate that gal-3 secreted by MSC in tumor microenvironment could favor the activation of Wnt signaling through β-catenin release from JC after cadherin destabilization. To test this hypothesis we stable silenced gal-3 expression in 3 different subtypes of human breast cancer cell lines. Afterward, we transfected control and silenced cells with a plasmid that enables a precisely Wnt/β-catenin signaling evaluation. At moment we are comparing the Wnt signaling status in control and gal-3 silenced cells. To precisely investigate the role of extracellular gal-3 in Wnt/β-catenin signaling, silenced cells will be treated with an exogenous gal-3. Next steps of this initially work include also evaluation of migration and invasion of gal-3 silenced cells treated or not with exogenous gal-3.

Overexpression of soluble biglycan aggravates renal damage via the MyD88 and TRIF signaling pathways

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There is growing evidence that components of the extracellular matrix such as the proteoglycan biglycan, beside their structural role, act as signaling molecules. Soluble biglycan binds to Toll-like receptor (TLR) 2 and 4, triggering the secretion of TNF-α. Additionally, intact biglycan can mediate the clustering of TLR2/TLR4 with the purinergic P2X4/P2X7 receptors and induce the assembly of the NLRP3 inflammasome, followed by activation of caspase-1 and secretion of IL-1β. Several reports have emphasized the role of biglycan in different inflammatory renal conditions, but the detailed mechanisms of its effects have not been fully elucidated.

Objectives: 1) To investigate the pro-inflammatory effects of soluble Biglycan in vivo and in vitro. 2) To unravel the mechanisms by which soluble biglycan triggers the production of chemoattractants and induces infiltration of mononuclear cells in the kidney. 3) To study the role of soluble biglycan in the ischemia/reperfusion mouse model.

Methods: 1) In vitro: Primary murine peritoneal macrophages from WT, Tlr2-/-, Tlr4-/-, Tlr2-/-Tlr4-m, TRIF-m, MyD88-/- and MyD88-/-TRIF-/- mice stimulated with soluble biglycan 2) In vivo: WT, TRIF-m, MyD88-/- and MyD88-/-TRIF-/- mice injected with a pLIVE mouse expression vector, containing the human biglycan gene (pLIVE-hBGN); WT, biglycan-deficient and overexpressing mice subjected to ischemia/reperfusion of the kidney.

Here we show that, following pLIVE-hBGN injection of WT mice, soluble biglycan is released into the bloodstream and distributed to various organs, including the kidney. Consequently, this leads to the production of chemoattractants CXCL1, CXCL2 and CCL2 in a TLR2/TLR4/MyD88 dependent manner, while the production of CCL5 is TLR4/TRIF dependent. This induces neutrophil, macrophage and T-cell infiltrations in kidneys in a TLR2/TLR4/MyD88-dependent and a TLR4/TRIF-dependent manner, respectively. Furthermore, mice overexpressing biglycan had significantly higher levels of infiltrating mononuclear cells in the kidney after ischemic reperfusion, while biglycan-deficient mice had less infiltration. Correspondingly, the chemoattractant levels were increased in mice overerexpressing soluble biglycan, while mice lacking biglycan showed lower chemoattractant levels vs. WT animals.

Transient overexpression of soluble biglycan revealed that biglycan triggers the secretion of chemoattractants and infiltration of leucocytes in a TLR2/TLR4/MyD88- and TLR4/TRIF-dependent manner. This study enabled to elucidate the mechanisms by which biglycan aggravates acute renal injury with the model of ischemia/reperfusion of the kidney.

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The effect of human recombinant decorin on liver cancer cell line Hep3B

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Liver cancer is one of the most frequent tumors in developing countries, but the number of cases increases in Central Europe as well. Hepatocellular carcinoma (HCC), usually formed in a cirrhotic background, is closely related to the tumor micro-environment and its constituent factors, like many other malignancies. Its growth, motility and metastatic ability all depend on the state of the micro-environment.

Decorin, a small leucine-rich proteoglycan binds to collagens, therefore it is responsible for assembling and maintaining the integrity of the extracellular matrix. In addition, decorin is capable to bind and interfere the action of cell surface receptors (e.g. EGFR, Met, IGF-1R) influencing certain signal transduction pathways initiated on receptor tyrosine kinases. These events finally induce cell cycle arrest in G1 phase via p21WAF1/CIP1, and blockage of tumor growth.

Our aim was to explore whether decorin exerts any effect on the proliferation and behavior of the hepatoma cell line Hep3B originated from HBV-induced HCC. Hep3B harbors deleterious mutation of retinoblastoma protein, and inactived p53 tumor suppressor gene, thus inhibition of cell cycle in G1 phase is not possible in this model system.

Our results indicate that treatment with human recombinant decorin inhibited the proliferation of Hep3B cells. In parallel, induction of p21WAF1/CIP1 and a decrease in c-myc expression were observed. We detected lower level of proliferating cell nuclear antigen (PCNA) upon decorin treatment vs. control cells, suggesting that PCNA might be the interaction partner of p21WAF1/CIP1. These molecular events caused cell cycle arrest in S phase as proved by FACS analysis.

Decorin had an impact not only on cell proliferation but on apoptosis as well. There was a marked increase in the number of apoptotic cells upon decorin treatment. Moreover, the proteoglycan increased the relative gene expression of proapoptotic bax as well as the level of activate caspase-3.

Based on our results decorin might be a candidate therapeutic agent in the battle against liver cancer, as it blocks tumor cell proliferation and favors apoptosis, but several questions still need to be answered. What is certain that decorin is capable to exert its tumor suppressor effect on such tumor cells, which are derived from a highly aggressive and often late diagnosed disease.

Effect of prostate cancer cells and normal fibroblasts co-culture on proteoglycan expression in these cells

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Over time, study of malignant tumors was concentrated on cancer cells only. Now, however, an important role of cancer cell microenvironment and cell-extracellular matrix (ECM) interactions is becoming evident. Tumor cells affect ECM, modifying normal fibroblasts to cancer-associated fibroblasts (CAFs) and creating favorable conditions for the growth and development of tumors. One of the main components of ECM is proteoglycans - complex glycosylated molecules consisting of core protein and one or more carbohydrate chains of glycosaminoglycans. Proteoglycans are expressed on the cell surface and in extracellular matrix for all mammalian cells and tissues, playing an important role in cell-cell and cell-matrix interactions and signaling. The aim of our work was to study an interference of prostate cancer cells (PC3, LNCaP, DU145) and normal fibroblasts in cells co-culture model in vitro in terms of proteoglycans expression and localization. Expression pattern of main proteoglycans (syndecan-1, glypican-1, perlecan, aggrecan, versican, decorin, lumican, NG2, brevican) was determined in fibroblasts, normal prostate epithelial cells (PNT2) and prostate cancer cell lines (PC3, LNCaP, DU145) before and after co-culture using RT-PCR analysis and immunocytochemical staining for both protein cores and glycosaminoglycan chains. It was shown that fibroblasts express mainly glypican-1, syndecan, versican, NG2, decorin, lumican; PNT2 cells - glypican-1, syndecan-1, lumican, NG2; LNCaP and DU145 cells - syndecan-1, lumican, NG2; PC3 cells - syndecan-1, lumican, NG2, glypican-1, decorin, versican. The determined proteoglycans expression patterns were different both between normal and prostate cancer cells, and between different prostate cancer cell lines. After co-culture and cell types' separation, changes in individual proteoglycans expression and overall proteoglycan patterns were detected both in prostate cancer cells and fibroblasts. Significant down-regulation of glypican-1 expression and distortion of versican and NG2 expressions in cancer-cells-exposed fibroblasts were the main molecular events, reflecting, possibly, a fibroblasts transformation to CAFs.

Collectively, the obtained data show mutual influence of prostate cancer cells and fibroblasts to each other in cell culture model in vitro, supporting an important role of extracellular matrix in tumor development and suggesting proteoglycans as novel molecular markers of cancer-associated fibroblasts or targets for microenvironment-targeted gene-therapy.

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Unraveling extracellular matrix regulation by tumor- associated macrophages in colorectal tumors

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The tumor microenvironment plays an active role in cancer initiation, progression and dissemination. This complex microenvironment consists of cells, such as neoplastic, stromal and infiltrating immune cells. This cellular eco-system is crucially supported by a-cellular components such as the extracellular matrix (ECM), which is a collection of proteins, glycoproteins, proteoglycans and polysaccharides. Accumulated evidences show that abnormal ECM may influence cellular behavior such as differentiation, proliferation and migration and is a hallmark of many cancers. Tumor associated macrophages (TAMs) constitute up to 50% of the tumor mass and are considered pivotal players in suppressing the immune response and mounting pro-tumoral functions. Among others, TAMs secrete enzymes capable of shaping the ECM tumoral landscape including serine proteases, cathepsins and matrix-metalloproteases. Yet, their effect on tumor development and invasion through ECM remodeling remains largely unknown. We hypothesize that TAMs-mediated deregulation of ECM composition and topography favorably influences cancer progression via various patho-physiological mechanisms. Here, we utilized a novel murine model of colorectal cancer (CRC) based on the direct orthotopic implantation of MC38 CRC cells into the colonic lamina propria of C57BL/6 WT mice or Ccr2-/- mice, the later lacking TAMs due to deficiency in monocyte recruitment. Scanning electron microscopy analysis of cell-free ECM scaffolds revealed that ECM-derived from WT tumor differs significantly from that of the Ccr2-/- mice, manifested by increased linearization of collagen fibers. Moreover, mass spectrometry analysis revealed differences in ECM protein composition, namely in the ratio between collagen types composing this microenvironment. Specifically, collagen type I is more abundant in tumors from WT than from Ccr2-/- mice, whereas collagen type VI comprises a larger portion of the microenvironment of tumors from Ccr2-/- mice then tumors from WT mice. Importantly, using Affymetrix microarray and complementary RT-PCR approaches we defined the specific panel of ECM effector molecules expressed by sorted CRC-TAM subsets in comparison with their counterparts from steady state and inflamed colons. Overall, our integrated biophysical-immunologic analysis hold the promise to reveal key molecular mechanisms associated with the role of TAMs derived ECM remodeling in CRC.

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Phosphorylation of bacterial effector CagA may be required for the induction of molecules involved in extracellular matrix remodeling in Helicobacter pylori experimental in vitro infection of gastric epithelial cells.

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Following adhesion of H. pylori to gastric epithelial cells, the bacterial oncoprotein CagA is translocated intracellularly and deregulates cellular polarity, inducing the appearance of a scattering phenotype that resembles to the epithelial to mesenchymal transition. Hierarchic phosphorylation of CagA by Src and Abl kinases, on repetitive tyrosine phosphorylation peptide sequences (EPIYA), located at the carboxyl-terminus of the protein plays a pivotal role in this transition. In clinical isolates the type of EPIYA motifs has been shown to vary depending on the surrounding sequence namely, EPIYA-A:EPIYAKVNK, EPIYA-B:EPIYAQVAKK and EPIYA-C:EPIYATIDDLG and the number of EPIYA-C repeats has been correlated to oncogenic potential. In this study, we investigated the potential involvement of CagA protein in the activation of matrix metalloproteinase-9 (MMP-9) and its activator MMP-3 in H. pylori-infected gastric epithelial cells. We utilized isogenic H. pylori mutants, based on the reference P12 strain, expressing CagA protein with variable numbers (n=0-3) of functional EPIYA-C and phosphorylation-deficient EPIFA-C motifs, as well as the corresponding P12 cagA- and cagE-knock out strains. These strains were used to infect gastric epithelial cells (AGS) in vitro and MMP-specific transcriptional activation was measured by Reverse Transcriptase quantitative Real Time PCR, at several time points. MMP expression in total cell lysates and cell culture supernatants was also determined by western blot analysis at 24h post-infection. A nearly 100-fold increase in MMP-3 and 80-fold increase in MMP-9 was observed in the presence of CagA protein and proportionally to the number of EPIYA-C terminal motifs. On the contrary, infection with CagA phosphorylation-deficient H. pylori mutants induced only background levels of MMP transcriptional activation, equal to those observed for the cagA- and cagE-knock out mutants. CagA-dependent increase in gelatinase and caseinolytic activity was detected in H. pylori-infected supernatants, utilizing zymography techniques. Phosphorylation of the bacterial effector CagA may be required for induction and secretion of MMP-3 and MMP-9 in experimental H. pylori infection.

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Cardiac ECM-analysis for engineering heart muscle models

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The heart is a complex organ which is composed of a diverse set of muscle and non-muscle cells embedded in a connective tissue matrix, the extracellular matrix (ECM). The cardiac ECM plays an important role in the contractility of the heart by forming an aligned or anisotropic network which provides cues for proliferation, differentiation, alignment and coupling of cell with each other and their environment, thereby providing contractile function. During pathogenesis of the heart, the composition and architecture of the ECM alters. The aligned ECM changes into a chaotic or isotropic organization which alters cardiac structure and function. Current therapies for heart disease focus on attenuating cardiac function although often with only a temporary beneficial effect, possibly caused by the chaotic organization of the ECM.

More insights in the changes of the cellular microenvironment and how this affects cardiac function in disease might lead to the development of new therapies. The use of engineered cardiac tissue models becomes increasingly important for understanding healthy and disease cardiac physiology. Here we describe the characterization of the cardiac microenvironment in heart disease which can be implemented in 3D engineered cardiac tissue. Pressure overload was produced by constricting the transverse aorta of mice and after 9 weeks the hearts were subjected to histological and biochemical analysis. The composition and architecture of the ECM combined with mechanical and cellular interactions were elucidated in these hearts. TAC hearts were characterized by induction of fibrosis and change in ECM organization into a more isotropic organization.

To study the effect of the change in ECM organization on the mechanical performance of cardiac cells, microtissues on an array of polydimethylssiloxane (PDMS) microposts allow real-time characterization of cells and their environment. The 3D tissues consists cardiac muscle cells and non-muscle cells in a matrix with an aligned (anisotropic) and chaotic (isotropic) organization which represent healthy and diseased ECM, respectively. Spontaneous contraction of the microtissues was followed for 7 days by video recordings and forces can be quantified from micropost displacement. The beating frequency of both healthy and diseased microenvironments decreased over time.

By implementing the effect of healthy vs diseased cellular microenvironment of the functionality of cardiomyocytes, more insight into heart pathobiology will be generated and facilitate in the optimalization of new therapies for cardiac disease.

The role of heparin and nano-heparin derivatives in cell functions and proteasome activity regulation in breast cancer.

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There is accumulating evidence that heparin and its derivatives, apart from their anticoagulant activity exhibit significant anticancer activity. Even though, a multitude of studies suggest that cancer progression can be prevented by administration of unfractionated heparin (UFH), therapeutic approaches based on UFH are limited due to the relative low uptake of UFH in the intestine. For this reason, the therapeutic use of low molecular heparin (LMWH) with a significantly longer half life, but with similar effects to those of UFH, is preferred. In the present study, we examined the effects on functional properties such as cell proliferation and cell motility, as well as on proteasome activity of LMWH, UFH and two heparin nano-derivatives, one extracted from a marine invertebrate Ascidia (Styela) and a second one self weight porcine heparin, in two breast cancer cell lines with different metastatic potential [MDA-MB-231 (high metastatic potential) & MCF-7 (low metastatic potential)]. For optimal dispersion, the two heparin nano-forms were sonicated in PBS solution.

A dose-dependent effect was observed on cell viability as regards all heparins, while cell migration was induced after treatment with nano-heparins and heparins, with the exception of self weight porcine heparin where an inhibitory effect was observed in MCF-7, whereas in MDA-MB-231 this effect was present only in low concentrations. Furthermore, RT-PCR analysis of proteasomal catalytic subunits (β5, β1, β2) on MCF-7 and MDA-MB-231 breast cancer cells showed an upregulation and a slight downregulation, respectively, both treated with different types of heparin. However, proteasome activity levels were not affected with all heparin derivatives, with an exception in the case of heparin extracted from Ascidia, which significantly reduced proteasome activity in MDA-MB-231 cells. As it seems, there is a correlation between mRNA levels of catalytic proteasome subunits and proteasome activity only after treatment with Ascidia in MDA-MB-231 cells. To conclude, it is of great importance that heparin and its nano-derivatives have the ability to regulate breast cancer cells' functions and proteasomal activity at certain concentrations. These primary results indicate that heparin and its nano-forms except from anticoagulant activity may have a potential anticancer action in breast cancer cells.

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Toxicological assessment of pristine multiwall-carbon nanotubes in terms of extracellular matrix expression pattern and functional properties in normal lung fibroblasts

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The evolution of nanotechnology provides an increasing number of applications and products, containing or using materials at nanoscale dimensions not only in laboratory tests but also for industrial use. Carbon nanotubes (CNTs) are among these nanomaterials which are used in a wide range of applications. Their unique physicochemical properties, involving nano-size (shape, length) and surface area, diverse surface chemistry as well as increased surface area to size ratio, raise several toxicological concerns. It is already reported that CNTs have the ability to penetrate cell membranes, locate in the cytoplasm, sub-cell organelles and even in the nucleus, interacting with important biomolecules. Cells exposed to CNTs are reported to undergo oxidative stress, inflammation and cytotoxicity. Thus, the understanding of the toxicological impact of CNTs is a critical issue for the future application of these emerging nanomaterials. In the present study, we performed a systematic investigation in order to examine the toxic effect of the pristine multiwall-CNTs (MWCNTs). For optimal dispersion, CNTs were tip sonicated in 1% w/v surfactant Pluoronic-127 (PF-127) solution. A dose-dependent effect of MWCNTs was observed on cell viability and cell migration is also affected at high concentrations. As shown through the RT-PCR analysis, Nrf2 gene expression, indicator of oxidative stress, is slightly upregulated compared to 1% PF-127. Furthermore, treatment with MWCNTs seems to affect important signaling, adhesion and migration macromolecules of ECM, such as syndecans, integrins and CD44, in terms of gene expression. Moreover, MWCNTs didn't exhibit any substantial haemolytic effect in red blood cells. To conclude, it is of great importance to further assess the potential health and safety concerns raised.

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Evaluation of the intracellular cross-talk of estrogen receptors with growth factor receptors on both extracellular proteolytic events by MMPs and intracellular proteolysis by the proteasome in breast cancer cells

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Estrogen receptors (ERs) play an important role in the clinical care of breast cancer patients, both as a prognostic factor and as a therapeutic target. Accumulating evidence suggests that the cross-talk between ERs, epidermal growth factor receptor (EGFR) and/or insulin-like growth factor receptor (IGFR) is critical for the observed-resistance to endocrine therapies.

Breast cancer is characterized by significant quantitative changes of extracellular network components. Previous studies in our laboratory have shown that gene and protein expressions of major matrix macromolecules, such as heparan sulphate proteoglycans, are critically affected by the interplay between ERs with EGFR/IGFR [1]. In this study, we evaluated the role of EGFR-IGFR signaling on constitutive expression and E2-mediated expression of matrix metalloproteinases (MMPs) in two epithelial breast cancer cell lines, with different metastatic capacity and different ER expression profile (MCF-7/ERα+ and MDA-MB-231/ERβ+) using specific intracellular inhibitors of EGFR and IGFR.

Preliminary data revealed that the expression of certain MMPs (MMP-1, MMP-7 and MT1-MMP/MMP-14) as well as that of the MMP inhibitor TIMP-1 are regulated by the tyrosine kinase receptors EGFR and IGFR, in coordination with the action of E2-ERs. Moreover, the expression of the proteasome (as assessed by the expression of x, y, z proteasome subunits) and its activity are also critically affected by the coordinated actions of ERs with EGFR/IGFR. This finding provides a link between extracellular proteolytic events with intracellular proteolysis in breast cancer cells, since the proteasome pathway emerges as an elegant molecular regulator of specific matrix macromolecules (including specific MMPs and TIMPs) [2].

[1] Tsonis et al., *FEBS J,* 2013
[2] Skandalis et al., *Curr Mol Med,* 2012

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Regulation of expression and activity of the catalytic proteasomal subunits by EGFR and HER2 receptors in colon cancer cells

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Colon cancer is the third, most common type of cancer worldwide. Epidermal growth factor receptor (EGFR) plays a crucial role in the preservation and the promotion of the disease. Another very important ErbB receptor in cancer is also HER2. In colon cancer, dual EGFR/HER2 inhibition increases the anticancer impact of EGFR. KRas and BRaf mutations are associated with the promotion of the disease and the anti-EGFR resistance. The Ubiquitin-Proteasome system plays a very important role in cancer, modulating important cellular processes and is responsible for the activation of various transcription factors, such NRF2. A variety of studies in many types of cancer, including colon, have demonstrated that, the proteasomal inhibition leads cancer cells in apoptosis. Furthermore, in many cases that inhibition has the ability to affect the action and the protein levels of EGFR. Nevertheless, there is no evidence if the reversed option is possible. The aim of this study was, therefore to investigate the impact of EGFR and HER2 on gene expression and the activity of the catalytic proteasomal subunits, as well as to evaluate whether NRF2 plays a role in that process. Three colon cancer cell lines were used: Caco-2 (wild-type KRas and BRaf), DLD-1 (KRas mutated), HT-29 (BRaf mutated). Our results suggest that, EGFR can significantly regulate the expression and the activity of the proteasome, a process in which EGF significantly contributes. In addition, the dual EGFR/HER2 inhibition reduces both mRNA levels and the activity of the catalytic proteasomal subunits, even in cases of KRas and BRaf mutations. Finally, in some cases inhibition of EGFR increases NRF2 mRNA levels, possibly as a response to EGFR- mediated proteasomal inhibition.

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EGFR and HER2 signaling in regulation of functional properties and matrix macromolecules expression in Caco-2 colon cancer cells

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Colon cancer is one of the most common types of cancer with high morbidity and mortality. Epidermal growth factor receptor (EGFR) is widely expressed in multiple solid tumors including colorectal cancer by promoting cancer cell growth and proliferation. Coexpression of EGFR and ErbB2 is found to a subset of colon cancer and may cooperatively promote cell survival, as heterodimerization is known to provide for diversification of signal transduction. The aim of this study was to evaluate the influence of EGFR inhibition on HER2 expression and on the other hand the inhibition of ErbB2 on EGFR mRNA levels. Moreover we investigated EGFR/HER2 signaling pathways which may regulate the expression of extracellular matrix molecules. Such molecules are the membrane-type metalloproteinases which involve in tumor migration, metastasis and invasion. According to our results, in human colon adenocarcinoma Caco-2 cells, EGFR is the major regulator of HER2 expression and its own gene,too. In addition, EGFR inhibition downpregulates the mRNA levels of MT1-MMP, but uparegulates the expression of MT2-MMP. HER2 seems to be the crucial mediator for the regulation of MMP-15. Moreover, the expression of EGFR as well as MT1-MMP seems to be mediated through the mitogen-activated protein kinase (MAPK) pathway. Finally, the inhibition of several pathways downstream of EGFR and HER2 downregulates the migration of Caco-2 cells may be through the modification of MT-MMPs. Conclusively, the inhibition of EFGR as well as HER2 activity may establish a clinical strategy of colon cancer therapy.

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: Heracleitus II. Investing in knowledge society through the European Social Fund.

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Fulvestrant and tamoxifen modify breast cancer cells' migration differently

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The prognosis of breast cancer patients is tightly correlated with the degree of spread beyond the primary tumor. The mechanism by which epithelial tumor cells escape from the primary tumor and colonize to a distant site is not entirely understood. Although hormonal therapy is followed with increased benefits for patients with breast cancer, current evidence suggests that estrogen receptor (ER) blockage using anti-estrogens is associated with a small induction of invasiveness in vitro. This study investigates the effect of two selective antagonists of the ER, Fulvestrant (Fulv) and Tamoxifen (Tam), on the metastatic ability of breast cancer cells.

Materials and Methods: In the current study, two ER+ breast cancer cell lines, MCF-7 and T47D were used. Cells were stimulated by 17β-estradiol (E2) and then treated with Fulv, Tam, endoxifen (End) and 4-OH-tamoxifen (4OHT). We studied the migration following the treatment with E2 and the combination of E2 with the agents using boyden chamber and wound healing assays. The invasiveness was evaluated using the matrigel assay. Matrix metalloproteinases' (MMPs) expression was examined using gelatin zymography assay and the proteins Snail and E-cadherin by Western blot analysis. Using immunofluoresence, we studied the expression and localization of phospho-FAK, as well as its correlation with F-actin.

E2 demonstrated a protective role regarding cell migration and invasion. Fulv did not alter this effect while Tam stimulated active cell migration according to an increase in Snail and a decrease in E-cadherin protein expression. Furthermore, both tested agents increased expression of MMPs and enhanced invasive potential of breast cancer cells. These changes were in line with FAK rearrangement.

The anti-estrogens counteracted the protective role of E2 concerning migration and invasion since their effect was not limited to anti-proliferative events. Although Fulv caused a less aggressive result compared to Tam, the benefits of hormonal therapy concerning invasion and metastasis yet remain to be investigated.

Impaired Endo180 function during photoaging correlates with accumulation of extracellular collagen fragments

P 66

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Collagen is the most abundant protein in the skin and gives the skin its resilience. The process of collagen turnover is very slow and its regulation is only partly known. In particular during photoaging, collagen homeostasis is out of balance leading to a continuous loss of intact collagen and the observed signs of aged skin like diminished tensile strength and wrinkle development. Here, we investigated the role of different collagen receptors for cellular uptake of collagen concerning collagen homeostasis during photoaging. Others have identified the mannose receptor Endo180 and integrin α2β1 being responsible for collagen uptake in mesenchymal cells. We show that Endo180 and integrin α2β1 are downregulated in photoaged skin and after acute UV stress in vivo and in vitro. Knockdown experiments revealed that Endo180 is essential for cellular uptake of collagen by dermal fibroblasts, whereas integrin α2β1 is important for initial binding of collagen facilitating its uptake. UV irradiation decreases collagen endocytosis which correlates with reduced Endo180 gene expression. We could show that during photoaging the cellular uptake of collagen is impaired leading to the accumulation of pericellular collagen fragments. We suggest that the altered pericellular niche of fibroblasts in photoaged skin has an impact on collagen homeostasis.

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Green tea and glycine improve the repair of the extracellular matrix of Achilles tendon with tendinitis

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Tendinopathy in Achilles tendon is a clinical problem that motivates the scientific community to search alternative treatments that assist in restoring their functional properties. Glycine has broad biological effects, acting as a modulator in the inflammatory cascade, having benefits also on the microcirculation. This amino acid is predominant in collagen molecules. The implication of glycine in the diet has been surprising, as 5% glycine in diet has provided beneficial effects against liver toxicity and inflammation. Green tea (Camellia sinensis) has anti-inflammatory properties and has been used for many people for therapeutic purposes. The pharmacological properties of green tea are attributed to its high content of polyphenols, especially epigallocatechin-3-gallate. Considering that there are no studies on the effect of green tea and glycine-rich diet in animals with tendinitis, and also considering the low cost and low side effect of these products, we investigated the effect of these diets in rats with tendinits induced by collagenase (10mg/ml). The experimental groups were divided into: C (control group), G1 (tendinitis group), G2 (green tea group), G3 (glycine group), G4 (glycine and green tea group). The rats were treated for seven days after tendon lesion and the concentrations of hydroxyproline, non-collagenous proteins and glycosaminoglycans were analyzed. It was performed analysis to the activity of MMP-2 and 9 by zymography and western blotting to the type I collagen. The organization of collagen fibers was detected by polarizing microscope. All the results were based in comparison between the treated groups and the tendinitis group. A higher concentration of non-collagenous proteins and type I collagen were observed in the G3 group and e high concentration of the hydroxyproline was noted in G2. A higher activity of the active isoform of MMP-2 was detected in the G3 and G4. All treated groups showed increase in the activity of MMP-9. However, in G3 was greater than G2 and G3. The measure of the birefringence images showed better organization of collagen fibers in all treated groups, whereas G4 showed an organization similar to control group. Our results suggest that glycine increases the concentration of important elements of the extracellular matrix of Achilles tendon. Treatment with both glycine and green tea after induced inflammation of tendons stimulates the activity of MMP-2 and 9 and improves the organization of collagen fibers. Concluding, this study could be a guide to further studies including glycine and green tea for the treatment of the inflammations in tendons.

Paracrine anti-fibrotic activities of neonatal cells and living cell constructs on human skin fibroblasts persist at senescence

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Increased numbers of senescent cells have been observed in chronic wounds, such as pressure and venous ulcers. Senescent cells may affect wound healing either due to their inability to proliferate, or by altering the wound environment. Living cell constructs (LCCs) containing fibroblasts and/or keratinocytes have been used as therapeutic approaches against chronic wounds. Accordingly, aim of the present work was to examine the effects of factors secreted by early passage neonatal fibroblasts and LCCs – in the form of a conditioned medium (CM) – on senescent vs. early passage adult skin fibroblasts.

Target cells were fibroblasts senescent either due to subsequent divisions (replicative senescence; RS) or due to an exogenous stress (stress-induced premature senescence; SIPS). Fibroblast functions related to the healing process, i.e. cell proliferation, alpha-smooth muscle actin (α-SMA) and metalloprotease expression, as well as, collagen synthesis were studied following CM treatment.

All CMs were observed to induce early passage fibroblast proliferation, while having no effect on the proliferation of senescent fibroblasts, as expected. All CMs were found to inhibit overall collagen synthesis both in early passage and in senescent fibroblasts. The LCC-derived CM was found to be more potent than fibroblast-derived CMs and, furthermore, to inhibit α-SMA expression.

In conclusion, the above results may indicate anti-contractile and anti-fibrotic activities of factor(s) secreted by neonatal skin fibroblasts, and more intensely by LCCs. Regarding these activities senescent fibroblasts exhibited similar responses to CMs as early passage fibroblasts, at least qualitatively. This may indicate that, although the senescent cells that are present in the chronic wound environment possess a catabolic phenotype, they are able to respond to paracrine factors secreted by early passage cells present in fibroblast-populated constructs.

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Tartrate resistant acid phosphatase (TRAP/ACP5) as a regulator of cancer cell proliferation and invasion

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TRAP/ACP5 is a glycosylated metalloenzyme which was early recognized as a marker for osteoclasts1. By dephosphorylation of osteopontin (OPN), an extracellular protein of the bone matrix, with impairment of the adhesive capacity, it increases migration of osteoclasts2. In a previous study from our group TRAP/ACP5 expression and enzymatic activity at metastatic sites from different primary tumors was substantiated2. It revealed a novel monomeric TRAP/ACP5 variant in metastatic lesions and demonstrates the presence of the TRAP/ACP5 isoform 5a in cancer cells and isoform 5b in the surrounding stromal cells (e.g. macrophages) in metastatic bone disease. TRAP/ACP5 expression was furthermore detected in the cancer cells of several primary malignant tumors, including breast, ovary and melanoma, whereas benign tumors from these sites were negative3. Scott et al were able to prove TRAP/ACP5s pivotal role in early stage melanoma to become metastatic4. Very recently it was shown in hepatocellular carcinoma (HCC) that increased TRAP/ACP5 expression correlates with maximal tumor size, microvascular invasion, poor tumor differentiation and a higher TNM-stage. Upregulation of TRAP/ACP5 expression further indicated poor prognosis and a lower survival in HCC patients5. We could show so far that augmented TRAP/ACP5 expression in the breast cancer cell line MDA-MB-231 resulted in increased migration on the phosphorylated matrix protein OPN as well as in the wound scratch healing system. Our interest focuses subsequently if the phosphatase activity of TRAP/ACP5 is the crucial feature. Moreover we are interested if overexpression of TRAP/ACP5 confers an increased proliferative and invasive phenotype upon tumor cells. By cell counting and combined Propidium Iodide (PI) and Annexin-staining TRAP/ACP5 transfected breast cancer cells displayed increased proliferation but no changed apoptosis level. Preliminary results from cell cycle analysis by FACS-measurement using combined BrDU- and PI- staining further confirm our hypothesis. Comparing different cancerous tissue we also want to look in detail into the regulation and signaling of TRAP/ACP5 in cancer cells during the metastatic process. Collectively, these data suggest a role for TRAP/ACP5 in the pathogenesis of cancer and in particular in growth and migration/invasion of cancer cells, which is a prerequisite for cancer metastasis.

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Renal fibronectin accumulation is associated with altered lysosomal cystein proteinase activity in streptozotocin treated rats

P 70

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The fundamental lesion of diabetic nephropathy (DN) is characterized by hypertrophy of the renal cells, thickening of glomerular and tubular basement membranes and accumulation of extracellular matrix (ECM) proteins within the nephrons. The progressive increase of ECM proteins concentration is caused by an imbalance between synthetic and degradative pathways. While the role of the different matrix metallo-proteinases in the impaired ECM degradation has been studied in detail, the function of lysosomal cysteine proteinases remains to be elucidated. The aim of this study was to investigate a potential relationship between the accumulated ECM protein fibronectin (FN), and cathepsin B activity in isolated glomeruli of diabetic and healthy rats. Twenty male Wistar rats were included: 10 healthy and 10 with streptozotocin-induced diabetes. After 6 weeks, the experiments were terminated. In the homogenates of isolated glomeruli, FN content and cathepsin B activity were measured by ELISA or spectrofluorimetry. FN was also analyzed by immunohistochemistry. Diabetic rats showed a significant rise of systolic blood pressure, impaired renal function and an enhanced urinary excretion of albumin, FN and cathepsin B comparing to the control group. In the homogenates of the glomeruli isolated from DN rats the ratios of FN/protein and FN/DNA showed a trend to higher values, while the ratios of cathepsin B/protein and cathepin B/DNA were reduced in comparison to the untreated animals. The strong positive association between intraglomerular FN content and cathepsin B activity of in both groups suggests that this cysteine proteinase plays role in the degradation of ECM protein FN. The obtained results suggest that inadequate cathepsin B activity can contribute to the insufficient FN degradation during diabetic nephropathy.

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Inflammatory stimuli up-regulate all four members of the syndecan family of transmembrane proteoglycans during hypertrophic remodeling of the heart

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Cardiac hypertrophy is the response of the heart to diverse pathological stimuli, including those arising from pressure overload and genetic mutations, and when sustained, leads to heart failure. Heart failure remains the major cause of mortality in the Western world, reflecting that disease mechanisms are poorly understood. We recently found that the transmembrane proteoglycan syndecan-4 is up-regulated in the pressure-overloaded hypertrophic myocardium of aortic stenosis patients and aortic-banded (AB) mice. Importantly, using syndecan-4 knock-out mice, we found that syndecan-4 was essential for cardiac hypertrophy. The aim of this study was to investigate regulation of all four members of the syndecan family in clinical and experimental cardiac hypertrophy.

Syndecan-1-4 mRNA were measured in the myocardium of hypertrophic obstructive cardiomyopathy (HOCM, n=15) patients and compared to dilated mycardium from end-stage heart failure patients (n=20) and controls (n=10); in mice following AB (n=88); and in neonatal rat cardiac fibroblasts and myocytes. Cardiac mRNA of all four syndecans were elevated in HOCM patients; syndecan-1 and -3 ≈3.5-fold and syndecan-2 and -4 ≈2-fold, with minor changes in dilated human hearts. Consistently, all four syndecans were up-regulated during hypertrophic remodeling after AB (AB24hrs, AB1 and 3 weeks), but not during end-stage failure with dilatation (AB16w and 18 weeks), assessed by echocardiography and organ weights. Thus, these data suggested the syndecan family to participate in hypertrophic remodeling. In contrast to syndecan-4 having equal expression levels in fibroblasts and myocytes, syndecan-1, -2 and -3 mRNAs were ≈2-, 4.5- and 3-fold higher in fibroblasts. Confirming our previous findings, syndecan-4 mRNA was up-regulated by TNFα, IL-1β and LPS (≈2-3-fold) in cardiac myocytes and fibroblasts. Syndecan-1 mRNA was also regulated by these three (≈1.5-2.5-fold), while in contrast, syndecan-2 and -3 were induced by TGFβ (≈2- and 2.5-fold). Consistently, cardiac syndecan-1 and -4 mRNA, but not -2 and -3, correlated positively to TNFα (R2=0.31 and 0.47) and IL-1β (R2=0.53 and 0.93) mRNA in mouse hearts after AB. They all correlated positively to TGFβ mRNA (R2=0.34-0.88). Finally, cardiac TNFα and TGFβ mRNA were increased in HOCM (≈1.6 and 1.7-fold), while IL-1β was down-regulated, suggesting TNFα and TGFβ to induce syndecan-1-4 expression in HOCM hearts. In mice, TNFα, IL-1β and TGFβ mRNA were up-regulated at AB24hrs, AB1w and AB3w, i.e. in parallel with up-regulation of syndecan-1-4, suggesting they all induce the syndecans after AB.

In conclusion, up-regulation of syndecan-1, -2, -3 and -4 by inflammatory mediators in the myocardium of HOCM patients and aortic-banded mice suggests a role for all four members of the transmembrane syndecan proteoglycan family in hypertrophic remodeling of the heart. In contrast to syndecan-4, syndecan-1-3 might have more prominent functions in fibroblasts than cardiomyocytes.

The role of extracellular galectin-3 in canonical Wnt signaling pathway activation in human breast cancer cell lines

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Anneliese Costa-Fortuna[1], Araci Rondon[1], Camila Longo-Machado[2], Renato S. Carvalho[3], Marcelo A. Carvalho[3], Martin Bonamino[4], Roger Chammas[2], Maria Isabel Rossi[5] and Mauro Pavão[1].

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Evidences of galectin-3 (gal-3) deregulation in multiple cancers lead to a broad range of works investigating the role of gal-3 in tumor cell biology. Through specific interactions with a variety of intra and extracellular glycoproteins gal-3 affects several biological processes. Recently some works pointed out that not only the gal-3 expressed by tumor cells seems to be important for tumor progression, reinforcing the pivotal role of microenvironment in disease. Mesenchymal stromal cells (MSC) are a well-known component of breast tumor associated stroma and secrets gal-3. Nevertheless, if gal-3 secreted by MSC could interfere in breast cancer cells (BCC) behavior remains elusive. We used multicellular spheroids composed of bone marrow MSC from wild-type or gal-3 knock-out mice to test invasive capacity of BCC. We observed through histological and flow cytometry analyses that the absence of gal-3 significantly diminished the invasive potential of BCC. This brings into question how the gal-3 present in the microenvironment can affect BCC invasive behavior. Newly it was showed that the binding of gal-3 to the cell surface glycoprotein N-cadherin can modulate the stability of the junctional complex (JC) formed by this molecule. β-catenin, the active transcriptional factor of canonical Wnt signaling, also composes this JC. Furthermore, gal-3 has been implicated as regulator in the Wnt/β-catenin pathway. Based on this evidences we speculate that gal-3 secreted by MSC in tumor microenvironment could favor the activation of Wnt signaling through β-catenin release from JC after cadherin destabilization. To test this hypothesis we stable silenced gal-3 expression in 3 different subtypes of human breast cancer cell lines. Afterward, we transfected control and silenced cells with a plasmid that enables a precisely Wnt/β-catenin signaling evaluation. At moment we are comparing the Wnt signaling status in control and gal-3 silenced cells. To precisely investigate the role of extracellular gal-3 in Wnt/β-catenin signaling, silenced cells will be treated with an exogenous gal-3. Next steps of this initially work include also evaluation of migration and invasion of gal-3 silenced cells treated or not with exogenous gal-3.

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Functional interplay of Syndecan-1 and heparanase in colon cancer pathogenesis

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The heparan sulphate proteoglycan Syndecan-1 (Sdc1) modulates signaling processes governing cell proliferation, adhesion, motility, proteolysis and angiogenesis. Sdc1 is highly expressed in by normal epithelial cells, whereas loss of expression has been associated with aberrant epithelial-mesenchymal transition during development and tumorigenesis. Heparanase is an endo-í-D-glucuronidase that cleaves the heparan sulphate chains of Sdc1, thereby participating in extracellular matrix remodeling and degradation. It has been postulated that an increase in heparanase expression and the associated decrease in Sdc1 expression promote colonic inflammation and associated tumor development, however, the underlying molecular mechanisms are unclear. The aim of the present project was to elucidate the functional interplay of Sdc1 and heparanase in colon cancer progression using a siRNA knockdown approach in human colon carcinoma cell lines. Employing this approach, we could demonstrate that Sdc1 regulates heparanase expression at the promoter level. At the functional level, we were able to demonstrate an increased invasiveness of Sdc-1 depleted CaCo2 cells in matrigel invasion assays. The role of heparanase upregulation in the Sdc1-dependent invasion process was confirmed using the heparanase inhibitor SST0001, a glycol-split heparin. In contrast to heparanase, matrix metalloproteinase expression and activity were not substantially altered. Noteably, we could demonstrate increased activation of focal adhesion kinase in Sdc1-depleted colon carcinoma cells. Sdc1 siRNA-treated cells showed increased proliferation and increased adhesion to fibronectin, whereas confocal immunofluorescence microscopy revealed altered organization of the cytoskeleton, suggestive of altered integrin activation. Furthermore, E-cadherin was downregulated upon Sdc1-depletion, thus contributing to the proinvasive phenotype. In summary, Sdc1 and heparanase act at multiple levels of tumor progression: Downregulation of Sdc1 is associated with an upregulation of heparanase expression, resulting in increased invasiveness. In addition, increased activation of integrin-dependent signalling upon Sdc1-depletion promotes tumor cell proliferation, and possibly cell motility. Sdc1 and heparanase emerge as novel functionally interrelated players in colon carcinogenesis, with potential implications for future therapeutic approaches.

The role of extracellular galectin-3 in canonical Wnt signaling pathway activation in human breast cancer cell lines

P 74

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