3rd FEBS Advanced Lecture Course

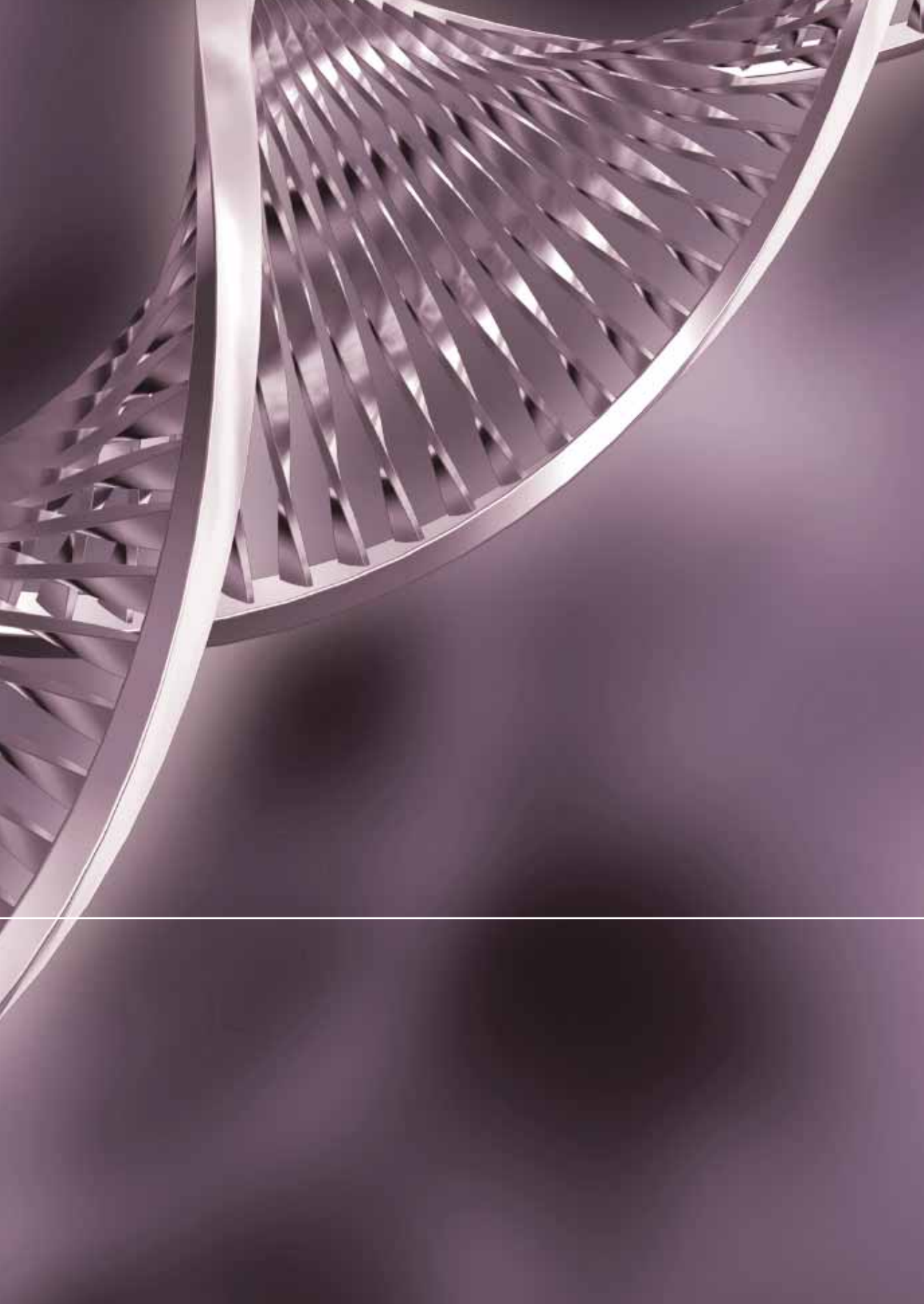
FEBS – MPST 2011

Matrix Pathobiology, Signaling and Molecular Targets

Spetses Hotel and
"Anargyrios and Korgialenios School of Spetses"

Program & Abstracts

September 2–7, 2011
Spetses, Greece



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Matrix Pathobiology, Signaling and Molecular Targets

Spetses, September 2–7, 2011

Organizing Committee:

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

Renato V. Iozzo
Kimmel Cancer Center, Thomas Jefferson University, 1020 Locust Street , Room 249 JAH Philadelphia, PA 19107, 969

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

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

Conference venue:

Spetses Hotel and “Anargyrios and Korgialenios School of Spetses” located at the Island of Spetses-Greece

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

http://www.febs–mpst2011.upatras.gr
e–mail: febs–mpst2011@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 3rd FEBS–MPST Advanced Lecture Course, which follows the previous successful FEBS–MPST 2009 and 2007 meetings.

Based on the great advances 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 2011 will offer oral sessions with invited plenary lectures, general lectures/tutorials, selected short 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 matrix pathobiology and pathobiochemistry, structural biology and cell and molecular biology.

The organizing committee has put together an outstanding group of internationally recognized speakers. The lectures and tutorials will provide you with an update important new knowledge covering key areas of the field. The most important goal of this course is to bring together scientists from biochemistry, life sciences and molecular cell biology 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 Spetses Hotel and "Anargyrios and Korgialenios School of Spetses" located at the Island of Spetses, Greece. Spetses 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 and TransYTF). We would also like to acknowledge the contribution of the University of Patras Reasearch Committee, the Mizutani Foundation for Glycoscience, the Hellenic Society of Biochemistry and Molecular Biology and the other sponsors supporting the "FEBS–MPST 2011 Young Investigator Awards".

We are looking forward to welcoming you in Spetses 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

Program

Friday, 2 September

16:00–18:00 Registration

Chairpersons: Renato Iozzo and Nikos Karamanos

18:00–18:30 Welcome Addresses by the Chairman and members of the Organizing Committee
S. Gonos, *Director of Research, Natl Hellenic Res Fnd, Athens, Greece*
Member of FEBS Advanced Course Committee

OPENING LECTURES / TUTORIALS (L1–L2)

18:30–19:15 **J. Esko** *(University of California, San Diego, USA)*
Syndecan–1 shedding reduces clearance of triglyceride–rich lipoproteins byhuman hepatocytes and causes hypertriglyceridemia

19:15–20:00 **L. Schaefer** *(Goethe–Univeristat, Frankfurt, Germany)*
SLRPs signaling in innate and adaptive immunity

20:00–20:30 Honorary Medal Awards to Prof. **J. Esko** and Prof. **L. Schaefer** granted by the Rector of the University of Patras

20:30 Welcome Reception

Saturday, 3 September

Chairperson/discussion leader: Hideaki Nagase

LECTURES / TUTORIALS – METALLOPROTEINASES (L3–L4)

9:00–9:30 **J. O. Winberg** *(University of Tromsø, Norway)*
Functions and properties of matrix metalloprotease complexes

9:30–10:00 **H. Nagase** *(Imperial College, London, UK)*
Fibrillar collagen degradation and cell migration

10:00–10:30 Coffee break

LECTURES / TUTORIALS – PROTEOGLYCANS (L5–L7)

Chairperson/discussion leader: John Couchman

10:30–11:00	**J. Couchman** *(University of Copenhagen, Denmark)* Syndecans, proteoglycan regulators of cell adhesion
11:00–11:30	**J. Whitelock** *(University of New South Wales, Australia)* The complexity of proteoglycans – different cores, different gly cosaminoglycans in different cells and tissues at different times
11:30–12:00	**A. Theocharis** *(University of Patras, Greece)* Novel roles of serglycin in malignancy
12:00–14:00	Lunch
14:00–16:00	Poster session (I) / Discussion groups (I)

Chairperson/discussion leader: Kristofer Rubin

LECTURES / TUTORIALS – INTEGRINS (L8–L9)

16:00–16:30	**K. Rubin** *(Uppsala University, Sweden)* Role of loose connective tissues in microvascular fluid exchange and drug uptake in carcinoma and localized infections
16:30–17:00	**J. Pouwels** *(University of Turku, Finland)* Negative regulation of integrin Activity

LECTURES / TUTORIALS – ECM REMODELLING IN CANCER (L10–L11)

17:00–17:30	**I. Kovalsky** *(Semmelweis University, Hungary)* Extracellular matrix remodelling in cervical cancer
17:30–18:00	**D. Vynios** *(University of Patras, Greece)* Reorganization of extracellular matrix in cancer
21:00	Dinner

Sunday, 4 September

Chairperson/discussion leader: Renato Iozzo

MATRIX PATHOBIOLOGY (L12–L14)

9:00–9:30	**R. Iozzo** *(Thomas Jefferson University, USA)* Perlecan interacts with both the α2β1 integrin and VEGF receptor 2 via its C–terminal angiostatic fragment endorepellin: A novel concept of dual receptor antagonism
9:30–10:00	**S. Menashi** *(University Paris–Est, CNRS, France)* EMMPRIN: key regulator in the dynamic interplay between tumor cells and their microenvironment
10:00–10:30	**I. Vlodavsky** *(Cancer and Vascular Biology Research Center, Haifa, Israel)* Involvement of heparanase in cancer progression, chronic inflammation and diabetic nephropathy
10:30–11:00	Coffee break

SELECTED TALKS (ST1–ST4)

11:00–11:15	**E. Karousou** *(University of Insubria, Italy)* Extracellular matrix perturbation in human cells and tissue affected with Down syndrome
11:15–11:30	**C. Bui** *(Newcastle University, UK)* DNA methylation: a possible implication in MMP–13 deregulation in osteoarthritis?
11:30–11:45	**S. Swaidani** *(Cleveland Clinic, USA)* Endogenous TSG–6 is crucial for the development of antigen induced pulmonary hyaluronan deposition, eosinophilia, and air way hyperresponsiveness
11:45–12:00	**K. Kouvidi** *(University of Crete, Greece)* Hyaluronan signaling through RHAMM in fibrosarcoma cell adhesion
12:00–12:30	Light lunch
12:30–14:30	Speakers' corner (I)
14:30–22:30	Excursion tour and dinner

Monday, 5 September

Chairperson/discussion leader: Vincent Hascall

INTERACTIONS AND FUNCTIONS OF MATRIX MACROMOLECULES (L15–L18)

9:00–9:30	**V. Hascall** *(Cleaveland Clinic Foundation, USA)* Bone marrow stem cells that divide in hyperglycemic glucose initiate intracellular hyaluronan synthesis, autophagy and pathological adipogenesis
9:30–10:00	**A. Passi** *(University of Insubria, Italy)* Aspects of regulation of hyaluronan synthesis
10:00–10:30	Coffee break
10:30–11:00	**P. Heldin** *(Ludwig Institute for Cancer research, Uppsala, Sweden)* The role of hyaluronan–CD44 interactions in tumor progression
11:00–11:30	**S. Misra** *(Medical University of South Carolina, USA)* Hyaluronan/CD44v6 signaling, motility, angiogenesis and tumorigenesis in colon cancer

SELECTED TALKS (ST5–ST7)

11:30–11:45	**C. Rydén** *(Uppsala University, Sweden)* Interaction between Staphylococcus aureus and extracellular matrix protein bone sialoprotein induces an immunological response in patients
11:45–12:00	**I. Sørensen** *(University of Bergen, Norway)* Generation of transgenic mouse strains overexpressing integrin α11 in muscle tissues
12:00–12:15	**A. Korpetinou** *(University of Patras, Greece)* Serglycin is secreted by aggressive breast cancer cells and inhibits the complement system
12:15–14:00	Lunch
14:00–16:00	Poster Session (II) / Discussion groups (II)

C*hairperson/discussion leader: Paraskevi Heldin*

MATRIX–MEDIATED SIGNALING AND EMT (L19)

16:00–16:30 **M. Pavão** *(Universtiy of Rio de Janeiro, Brasil)*
Dual effects of unique oversulfated dermatan sulfates on tumor invasion: inhibition of epithelial–mesenchymal transition and hematogeneous metastasis

SELECTED TALKS (ST8–ST15)

16:30–16:45 **S. Magnussen** *(University of Tromso, Norway)*
Characterization of the murine oral squamous cell carcinoma cell line AT84 stably overexpressing uPAR

16:45–17:00 **H. Porsh** *(Ludwig Institute for Cancer Research, Sweden)*
Role of Hyaluronan synthase 2 (HAS2) in TGFβ–induced epithelial–to–mesenchymal transition (EMT) of NMuMG normal mammary epithelial cells

17:00–17:15 **M.V. Nastase** *(Goethe–University, Germany)*
The matrix component biglycan induces generation of reactive oxygen species by signaling through TLR2/TLR4 receptors and the inflammasome

17:15–17:30 **Ch. Gialeli** *(University of Patras, Greece)*
Targeting epidermal growth factor receptor in colon cancer

17:30–17:45 **C. Jarosz** *(University Paris–Est, CNRS, France)*
EMMPRIN modulates TGFβ signalling pathway: implications in tumour stroma activation

17:45–18:00 **H. C. Lim** *(University of Copenhagen, Denmark)*
Interplay between syndecans and cadherins in breast carcinoma

18:00–18:15 **A. Evangelatov** *(Sofia University, Bulgaria)*
Src, but not FAK, regulates cellular migration in three dimensional environment

18:15–18:30 **E. Milia–Argeiti** *(University of Patras, Greece)*
Imbalance of MMP–2 and MMP–9 expression versus TIMP–1 and TIMP–2 reflects increased invasiveness of human testicular germ tumors

18:30–19:30 Speakers' corner (II)

21:00 Dinner

Tuesday, 6 September

Chairperson/discussion leader: Lena Kjellen

GLYCOBIOLOGY AND METABOLIC REGULATION OF ECM MOLECULES (L20–L21)

9:00–9:30 **K. Sugahara** *(University of Hokkaido, Japan)*
Involvement of chondroitin sulphate E–type structure in the experimental metastasis of the Lewis lung carcinoma cell line

9:30–10:00 **L. Kjellen** *(University of Uppsala, Sweden)*
Regulation of heparan sulfate biosynthesis

10:00–10:30 Coffee break

Chairperson/discussion leader: Carl–Henrik Heldin

SIGNALING AND DISEASE MOLECULAR TARGETING –1 (L22–L25)

10:30–11:00 **C.H. Heldin** *(Ludwig Institute for Cancer research, Sweden)*
Signaling via receptors for TGFβ via Smad and non–Smad pathways

11:00–11:30 **N. Karamanos** *(University of Patras, Greece)*
Cellular models to evaluate ECM as pharmacological target in breast cancer bone metastasis

SELECTED TALKS (ST16–ST18)

11:30–11:45 **S.S. Skandalis** *(Ludwig Institute for Cancer research, Sweden)*
Proteomic identification of CD44 interacting proteins: the CD44–IASPP–p53 axis

11:45–12:00 **G.P. Botta** *(Drexel University College of Medicine, USA)*
K–Ras activation of ERK2 in3–D human pancreatic cells regulates invasionand proliferation via induction of matrix metalloproteinase–1 and tissue inhibitor of matrix metalloproteinase–1

12:00–12:15 **M. Mongiat** *(Experimental Oncology Division, Italy)*
The ECM protein MULTIMERIN2 impairs tumor angiogenesis growth by interfering with VEGFR2 signaling

12:15–14:00 Lunch

14:00–14:30 **D. Nikitovic–Tzanakakis** *(University of Crete, Greece)*
Glycosaminoglycan / proteoglycans–mediated–signaling in cancer growth

14:30–15:00 **D. Kletsas** *(NCSR "Demokritos", Greece)*
Cellular senescence: Molecular mechanisms and implications in tissue homeostasis

15:00–15:30 Coffee break

Chairperson/discussion leader: Dimitris Kletsas

SIGNALING AND DISEASE MOLECULAR TARGETING – 2 (L26–L29)

15:30–16:00 **R. Tenni** *(University of Pavia, Italy)*
Interaction of collagen 1 with three small leucine–rich proteoglycans

16:00–16:30 **A. Giannis** *(Univeristy of Leipzig, Germany)*
Synthesis and development of bioactive natural products

16:30–17:00 **M. Zaman** *(Boston University, USA)*
Quantitative approaches to probe Integrin cluster diffusion and downstream signaling in native like 3D environments

17:00–17:30 **S. Ghatak** *(University of South Carolina, USA)*
Differentiation of heart valve progenitor cells is dependent on communication between periostin–integrin and hyaluronan–induced signalling

SELECTED TALKS (ST19–ST22)

17:30–17:45 **Elin Hadler–Olsen** *(University of Tromsø, Norway)*
Stromal impact on tumor invasiveness and gelatinolytic activity at the invasive front

17:45–18:00 **C.L.R. Belmiro** *(Universtiy of Rio de Janeiro, Brasil)*
Nanoparticles of heparins of marine invertebrates: anti–inflammatory effect on model of inflammatory bowel disease

18:15–18:30 **K. Moreth** *(University of Frankfurt, Germany)*
Biglycan aggravates lupus nephritis through TLR2/4 and regulation of the B cell chemoattractant CXCL13

18:30–18:45 **V. Tillgren** *(Lund University, Sweden)*
Extracellular matrix proteins with short clusters of basic amino acids show tight binding to glycosaminoglycan chains of cell surface proteoglycans leading to a unique targeting and activation of specific cell types

18:45–19:15	Extra talk by **M. McDowall** *(Waters MS Technology Centre, Manchester, United Kingdom)* A Ion Mobility Assisted Data Independent Approach Towards the-Qualitative and Quantitative Profiling of Biomarkers in Complex Protein Mixtures
21:00	Dinner

Wednesday, 7 September

Chairperson/discussion leader: Dick Heinegård

MATRIX REGULATION IN HEALTH AND DISEASE (L30–L32)

09:00–09:30	**G. Murphy** *(Cancer Research Institute, Cambridge, UK)* Metalloproteinases and their roles in the tumour microenvironment
09:30–10:00	**K. Dobra** *(Karoliska Institutet, Stockholm, Sweden)* Transdifferentiation of mesenchymal tissues; with special focus on syndecan–1 cell membrane nucleus
10:00–10:30	**D. Heinegård** *(University of Lund, Sweden)* ECM derived molecular fragments with roles in the innate immune response

SELECTED TALKS (ST23–ST26)

10:30–10:45	**Megan Lord** *(The University of New South Wales, Australia)* Perlecan, a multifunctional proteoglycan in the vasculature
10:45–11:00	**T. Neill** *(Thomas Jefferson University, USA)* Decorin negatively regulates the tumor proangiosome
11:00–11:15	**H. Siiskonen** *(University of Eastern Finland, Finland)* Intracellular hyaluronan localization and effect of microinjected oligosaccharides on hyaluronan coat in HAS3 transfected MCF–7 cells
11:15–11:30	**C.E. de Andrea** *(Leiden University Medical Center, Netherlands)* Growth plate regulation and osteochondroma formation: insights from tracing proteoglycans in zebrafish models and human cartilage
11:30–12:00	Closing remarks: D. Heinegård, V. Hascall, R. Iozzo and N. Karamanos
12:00–13:00	Farewell Party
Afternoon	Departure

Curriculum Vitae's of Invited speakers/ tutors

Renato Iozzo, MD, Ph.D. is a Professor of Pathology and Cell Biology at Thomas Jefferson University. He is also a Professor of Biochemistry and Molecular Biology at the same institution. In 2008 Dr. Iozzo received an Honorary Professorship at the School of Life Sciences, University of Manchester, UK. Dr. Iozzo has published more that 260 peer–reviewed papers in the proteoglycan field and is the Past–President of both the American Society for Matrix Biology and the International Society for Matrix Biology. He will discuss latest developments in the proteoglycan field focusing on the role various proteoglycans play in controlling cancer growth, tumor microenvironment and angiogenesis.

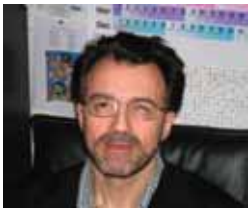
Vincent C. Hascall, Ph.D. Dr. Hascall is a Staff member of the Departments of Biomedical Engineering and Orthopaedic Surgery at the Cleveland Clinic and a Professor of Biological Chemistry at Case Western Reserve University in Cleveland, Ohio. He is currently an Associate Editor for the Journal of Biological Chemistry. His current research interests include connective tissue biology and the role of hyaluronan matrices in inflammations. He earned his Ph.D. at the Rockefeller University in New York followed by a faculty appointment at the University of Michigan, Ann Arbor, Michigan, and then as a Section Chief in the National Institute of Dental Research, Bethesda, Maryland before his present position. He is a member of several professional societies, including the American Society of Biological Chemists and the Glycobiology Society, and is a co–founder of the International Society for Hyaluronan Sciences. He has received honorary degrees at the Universities of Lund, Sweden and Kuopio, Finland.

Hideaki Nagase, M.S.c Ph.D. is Professor and Head of Matrix Biology Department at the Kennedy Institute of Rheumatology Division, Imperial College London, London, UK. He is investigating the structure and function of matrix metalloproteinases and tissue inhibitors of metalloproteinases (TIMPs) and their roles in cartilage matrix destruction during the progression of arthritis. He holds a B.Sc. in Pharmacy from Tokyo College of Pharmacy, a M.Sc. degree in Physiological Chemistry from Science University of Tokyo in Japan and a Ph.D. degree in Biochemistry from the University of Miami, USA. He received his postdoctoral training at Strangeways Research Laboratory, Cambridge, UK and Dartmouth Medical School, USA. He was elected as an Honorary Fellow of The Royal College of Physicians in 2004. He elucidated the stepwise mechanism of activation of matrix metalloproteinases and the inhibition mechanism of TIMPs, the latter in collaboration with Professor Wolfram Bode and Professor Keith Brew.

John Robert Couchman is a Danish National Research Foundation Professor, Department of Biomedical Sciences and Biotech Research & Innovation Centre (BRIC) University of Copenhagen. His research areas include: Cell adhesion to extracellular matrix, signalling from adhesion receptors, potential involvement of cell surface proteoglycans and their ligands in tumour progression, heparan sulphate biosynthesis and regulation. Dr. Couchman has authored 168 publications in peer review journals and is the current Editor–in–Chief of the Journal of Histochemistry & Cytochemistry and Editorial Board member of the Journal of Biological Chemistry.
For more information visit the websites: http://www.bric.ku.dk/staff/couchman_group/; www.momed.dk

Israel Vlodavsky, PhD., received his Ph.D., in Life Sciences from the Weizmann Institute in rehovot in 1975. He was post–doctoral researcher in the Department of Biochemistry in the University of California at Los Angeles in 1975–1976 and in the Cancer Research Center in the University of California at San Francisco in 1976–1979. He was Visiting Professor in Vascular Biology in Harvard medical School at Boston in 1985–1986. He was appointed as Associate Professor in Experimental Oncology in Hadassah–Hebrew University in Jerusalem in 1985 and as Professor and Head of the Tumor Biology Research Unit in 1990–2002. From 2002 he is Professor and Head in the Cancer and Cascular Biology Research Center in technion in Haifa.

His scientific work has focused on the mammalian heparanase and its involvement in tumor metastasis and angiogenesis in inflammation, autoimmunity and diabetic nephropathy. He is also working on the role of heparan sulfate proteoglycans, heparin binding growth factors and heparin–mimicking compounds. His research interests include the control of cell proliferation and differentiation by the extracellular matrix and the role of vascular endothelial and smooth muscle cells in neo–vascularization and restenosis. Israel Vlodavsky received the Judith Segal Prize in 1980 and the Elkeles Prize in 1997. He received the Teva Prize – Distinction in Cancer Research in 2002 and The Henry Taub Prize in 2005. He was also honored with the Landau Prize in 2006 and the Professorship Award from the Israel Cancer research Fund in 2007. He has authored or co–authored more than 380 research articles, reviews and book chapters.



Dimitris KLETSAS, PhD. is a research director in the Laboratory of Cell Proliferation & Ageing at the Institute of Biology NCSR "Demokritos", Athens. He was a post–doctoral fellow in EMBL, Heidelberg, on the expression of specific cell cycle marker genes and in Imperial College, London, on cellular immortalization. Since 1999, is the Head of the Laboratory of Cell Proliferation & Ageing. He published over 100 papers and 10 book chapters, He coordinated or participated in national, European and other international research grants and he was a national expert for the FP6 of EU. Currently he is the Secretary General of the Hellenic Society for Biochemisty and Molecular Biology. His research interests include: the role of growth factors on the proliferation of several cell types and on extracellular matrix homeostasis, as well as the signaling pathways involved; alternative mechanisms of cell proliferation and differentiation, such as cell–matrix interactions, exogenous stress and the effect of mechanical forces; the mechanism of cellular senescence and the role of the senescent cell on the development of age–related diseases, including cancer; evaluation of natural products and new synthetic compounds with putative cytostatic/cytotoxic, as well as anti–ageing and wound healing action, and their mode of action.

Jan–Olof WINBERG, Dr. philos., received his Cand. real. in 1982 and Dr. philos. in 1990 from Biochemical Institute, Faculty of Science, University of Oslo, Norway. From 1983–1986 he was a research scholar at the Department of Genetics, The Norwegian Radium Hospital, Oslo, Norway. Between 1987 and 1993 he was a senior researcher at the Department of Medical Genetics, University Hospital of Northern Norway, Tromsψ and since 1994 he is a Professor at the Institute of Medical Biology, Faculty of Medicine, University of Tromso, Norway.

He is member of the Editorial Advisory Board of the Biochemical Journal and consulted as a reviewer for various journals. He has authored or co–authored 58 peer–reviewed full–length papers including 5 reviews and contributed to more than 100 oral and poster presentations at national and international conferences. His research interests include biochemical and kinetic characterization of enzymes belonging to the short–chain dehydrogenase / reductase (SDR) superfamily and the matrix metalloproteinase (MMP) family, the role of individual enzymes from these two families in diseases like cancer, the role of extracellular matrix on cells expression of these enzymes and on activation of MMPs.

Liliana Schaefer, M.D. has been trained in Internal Medicine and Nephrology at the University of Poznan, School of Medicine, Poland. She graduated as a Medical Doctor from the University of Wuerzburg, Germany. After working as a Postdoc at the University of Wuerzburg, she became a principal investigator in the Department of Medicine at the University of Muenster, Germany. Since 2006 she is a Professor of Nephropharmacology at the Institute of Pharmacology and Toxicology, University of Frankfurt/Main with extensive expertise in matrix biology. Her laboratory is addressing the role(s) of the two TGF–beta–binding, small leucine–rich repeat proteoglycans decorin and biglycan in inflammation and fibrosis. Her work gave rise to the novel concept that, under certain conditions, matrix components may act as endogenous "danger" signals, which can be recognized by innate immunity receptors, and which are capable to trigger inflammatory response reactions. Currently, Dr. Schaefer is president of the German Society for Matrix Biology.

Jeroen Pouwels: is a Postdoctoral researcher in the research group of Prof. Johanna Ivaska with an Academy of Finland Postdoc Grant. He joined VTT Biotechnology in Turku (Finland) with Excellence Grant from the Marie Curie Foundation, as postdoctoral researcher, during 2004-2008. He obtained his PhD at the Laboratory for Molecular Biology of Wageningen University dealing with Molecular characterization of the movement protein of Cowpea mosaic virus. He worked during 1999 as part of an internship at the department of molecular pathology at the Dutch Cancer Institute (NKI) in Amsterdam studying the estrogen receptor β expression in human breast cancer. He has also two master degrees concerning activity and stability of hyperthermophilic enzymes as well as engineering and characterization of their substrate specificity. His current research interests involve cell surface receptors integrins and their activity regulation.

Achilleas Theocharis, Ph.D., received his B.S. in 1994 and his Ph.D. in 2000 from the Department Chemistry, University of Patras. He joined the Department of Experimental Medical Sciences, Lund University as visiting scientist, during his Ph.D. He was postdoctoral fellow at the Division of Pathology, Department of Laboratory Medicine, Karolinska Institute during 2002–2003. In 2003, he was appointed as Lecturer in the Laboratory of Biochemistry, Department of Chemistry in the University of Patras and as Assistant Professor in 2008. He joined Matrix Biology Group in Ludwig Institute for Cancer Re-

search at Uppsala University in 2008 as visiting scientist. He has authored over 55 peer–reviewed papers and book chapters / reviews and 70 oral and poster presentations in National and International Conferences. His research interests focus in the structure and function of proteoglycans / glycosaminoglycans in the development of diseases such as atherosclerosis and cancer.

Nikos Karamanos, Ph.D., is a Professor of Biochemistry at the University of Patras and collaborating member of ICETH/FORTH. He obtained his diploma (Chemistry) in 1984 and Ph.D. (Biochemistry) in 1988 from the University of Patras. He has carried out pre– and post-doctoral research work (annual periods, total three years) at Karolinska Institute (School of Medicine, Stockholm. His main area of research involve matrix pathobiology, cell signaling and gene expression, pharmacological targeting, preclinical evaluation studies at cell level, development of cell culture models that mimic in vivo conditions and development of highly sensitive bioanalytical assays for structure analysis and diagnostic purposes. Studies are focused on the implication of biological matrix effectors (Proteoglycans, glycosaminoglycans, metalloproteinases and glycoproteins) in tissue organization and the pathogenesis and progression of various disorders, such as cancer growth, invasiveness and metastatic potential.

He is a member of the editorial boards of several peer–review journals [The journal of Biological Chemistry and Current Medicinal Chemistry, etc]. He is a board member of the Hellenic Society of Biochemistry and Molecular Biology, coordinator of the matrix biology research society and was an expert in FP7 "Health" (2007–2009). He is the current Head of the Department of Chemistry, University of Patras and contact person of the Federation of European Connective Tissue Societies. Dr karamanos has authored more than 200 original publications in peer review journals and book chapters and his work is cited more than 2500 times (H–index=25).

For more detail see the following websites:

Departmental & Personal website: www.chem.upatras.gr/faculty/karamanos

List of Publications: www.publicationslist.org/n.k.karamanos
Publications and citation metrics: www.researcherid.com/rid/A–3616–2008
Research Network coordinator: www.biotargeting.upatras.gr
Scientific Societies/Board member: www.eebmb.gr

Paraskevi Heldin, PhD., received her Ph.D., in Medical and Physiological Chemistry from the Faculty of Medicine, Uppsala University in 1987. During 1987–1990, she was a postdoctoral researcher at the Department of Medical and Physiological Chemistry, Uppsala University under the supervision of Professor T.C. Laurent and during 1991–1997, a research assistant at the same university. Since 2001, she is Associate Investigator and Head of the Matrix Biology Group at the Ludwig Institute for Cancer Research in Uppsala University. She has authured or co–authored more than 60 research articles and over 10 invited reviews and book chapters. Dr P. Heldin's interests are focused on understanding how the stromal microenvironment influences cellular behavior. She aims to dissect the signaling pathways by which cells sense microenviromental cues. In different research approaches she investigates hyaluronan–CD44 complexes, as molecular switches, that in a co–receptor fashion crosstalk with growth factor receptors including PDGF– and TGF í–receptor and convey signals into the cells.

Jeffrey D. Esko, Ph.D., M.D. (h.c) is a Professor of Cellular and Molecular Medicine (cmm.ucsd.edu) and Co–Director of the Glycobiology Research and Training Center (grtc.ucsd.edu) at the University of California, San Diego. Dr. Esko received his Ph.D. in Biochemistry at the University of Wisconsin in Madison. After an independent fellowship at the Molecular Biology Institute at the University of California, Los Angeles, he moved to the University of Alabama at Birmingham and then to Department of Cellular and Molecular Medicine at the University of California, San Diego in 1996 to help build a program in glycobiology. Work in his laboratory focuses on the structure, biosynthesis, and function of proteoglycans, including structural studies of heparan sulfate by mass spectrometry, application of genome–wide methods to identify genes involved in heparan sulfate assembly, analysis of guanidinylated glycosides that act as molecular transporters, studies of proteoglycans in lipoprotein metabolism, and analysis of proteoglycans in modulating vascular permeability (eskolab.ucsd.edu). His work is supported by grants from the National Institutes of Health and the private sector. Dr. Esko has served on the numerous editorial boards and scientific boards and serves as Associate Editor for Glycobiology. He was past President of the Society for Glycobiology, past Director of the Biomedical Sciences Graduate Program at UCSD (biomedsci.ucsd.edu), and he cofounded Zacharon Pharmaceuticals, Inc **(www.zacharon.com).**

Dick Heinegård, M.D., Ph.D., received his training in medicine and in science largely at the Lund University; for a year he was a visiting scientist at the Kennedy Institute of Rheumatology in London and at the NIH in Bethesda. In 1974, he received his Ph.D. from Lund University and appointed as professor of medical and physiological chemistry at the Department of Experimental Medical Science, Lund University, Sweden, in 1983. During 1985 and 1994, he was chairman of the Department and during 1998 and 2004 he was director of the national program on research on inflammation. Dick Heinegɛrd has received several scientific awards including the Steindler award presented by the Orthopedic Research Society, the EULAR (European league against rheumatism) award, the basic science award by the Osteoarthritis Research Society International, as well as number of awards in Scandinavia including the Jahre prize for medical research for his work. A major research interest of Dick Heinegɛrd, evolved over the years, is to understand the biology and pathology of diseases involving the musculo–skeletal system, particularly with regard to cartilage (including the intervertebral disc), bone and tendon. In this work he has identified, purified, characterized and determined primary sequences for a number of extracellular matrix proteins, including those interacting specifically with cell surface receptors. In subsequent work posttranslational modifications and their roles in functions of proteins have been characterized. A major focus currently is to understand and identify functional domains of the proteins and their roles in matrix assembly, in modulating cellular activities and how and by which enzymes they are targeted in matrix breakdown, both in normal turnover and in pathology. Recent work includes the use of fragments of matrix molecules as molecular markers of disease processes, particularly in joint disease and also how such fragments activate innate immune responses. He has published some 290 peer–reviewed primary papers in science and 50 book chapters/reviews. He was the president of the International Society for Matrix Biology during 1999–2000. Dick Heinegɛrd has served or serves as an editor as well as on editorial boards for several journals in general biochemistry as well as in the field of connective tissue biology and pathology.

Alberto Passi, MD PhD, Full professor in biochemistry at the Medical School University of Insubria (Varese – Italy), dean of the Faculty of Exercise and Sport Sciences of University of Insubria, director of biochemistry laboratories at Department of Biomedical Experimental and Clinical Sciences (DSBSC) of University of Insubria. Since his PhD he focused his scientific interest in extracellular matrix metabolism. Cell cultures, lung and vascular tissues were initially approached studying proteoglycan metabolism including the effect of free radicals and proteases on extracellular matrix macromolecules. After a sabbatical period in Hascall's lab in Cleveland (OH – USA), the Passi's interests moved toward the hyaluronan and in particular studies were carried out on hyaluronan metabolism and its control. The role of UDP sugars precursors in the glycosaminoglycan synthesis and the covalent modification of en-

zymes involved in glycosaminoglycan metabolism are at the moment the main interest in Passi's lab.

Carl–Henrik Heldin, M.D., PhD., received his M.D. and B.S.in Natural Sciences from the Uppsala University in 1975 and 1981, respectively and his Ph.D., in Medical and Physiological Chemistry from the Faculty of Medicine, Uppsala University in 1980. Since 1986, he is Director of the Ludwig Institute for Cancer Research in Uppsala. He was apointed as Professor of Molecular Cell Biology at the Medical Faculty of the University of Uppsala in 1992. The research interests of Carl–Henrik Heldin are related to the mechanisms of signal transduction by growth regulatory factors, as well as their normal function and role in disease. Carl–Henrik Heldin has received several scientific awards including the Anders Jahre's Medical Prize for Younger Scientists, the K. Fernstrφm's Medical Prize for Young Swedish Scientists, the EMBO Medal, the K. Fernstrφm's Large Medical Prize for Nordic Scientists, the Meyenburg Prize from the German Cancer Center in Heidelberg, the Pezcoller–AACR Award from the Pezcoller Foundation and the American Association for Cancer Research. He has authored or co–authored more than 500 research articles, reviews and book chapters. Carl–Henrik Heldin has served or serves as editor as well as on editorial boards for several journals in Biochemistry and Molecular Biology.

Suzanne Menashi, Ph.D., is the leader of the research group "Epithelial/Stromal interaction and Matrix Metalloproteinases (MMPs)" at the Laboratoire CRRET, CNRS (French Institute for Scientific Research) at Universitι Paris –Est. Her primary research interest focuses on MMP regulation and their role in different biological models, including wound healing, cancer, and dental development and pathology. Recently, Dr. Menashi's research focused on EMMPRIN, a membrane protein known for its ability to induce MMP expression (hence its name: Extracellular Matrix MetalloProteinase Inducer), and which is increasingly gaining attention for its role in tumor progression. Dr. Menashi demonstrated that EMMPRIN is also responsible for MMP induction in pathological wound healing, including corneal ulceration, following basement membrane rupture and direct epithelial–stromal interactions. She also discovered novel biological functions of EMMPRIN including its ability to regulate VEGF and VEGF receptor signaling and myofibroblast differentiation, and their role in angiogenesis, cancer, and fibrosis. In addition, Dr. Menashi reported new developmental defects in EMMPRIN knockout mice consistent with its role in protease regulation and matrix turnover. Current research at Dr. Menashi's laboratory is focus on EMMRPIN structure /function relationship studies with the goal of utilizing this knowledge for the development of pharmacological inhibitors for the treatment of cancer and fibrotic diseases.

Dr Dragana Nikitovic is a faculty member at the Laboratory of Histology–Embryology, School of Medicine, University of Crete in Heraklion She graduated from the Faculty of Natural Sciences, University of Belgrade, obtaining the title of B.Sc in Biochemistry. Dr. Nikitovic has carried out postgraduate research at the Department of Biochemistry, School of Medicine, Queen Mary and Westfield College, London, and Department of Biochemistry and Biophysics, Karolinska Institute, Stockholm where she obtained the Half–Time PhD Diploma. She earned a Doctor of Medical Sciences degree from the Department of Morphology, Medical School, University of Crete. Her scientific interests are focused on the study of proteoglycans (PGs) and glycosaminoglycans (GAGs) structure and functions as well as on their role in cancer pathogenesis. Dr. Nikitovic is co–author of 35 original publications in peer reviewed international journals.

Prof. Lena Kjellen has a PhD from Uppsala University in medical chemistry. Her area of research is heparan sulfate proteoglycans where she has studied both their biosynthesis and more functional aspects. During recent years she has generated and utilised mice deficient in heparan sulfate biosynthesis enzymes for her studies. Since 2001 she is professor in medical glycobiology at Uppsala University where she moved from the Swedish University of Agricultural Sciences.

Homepage: http://www.imbim.uu.se/forskning/kjellenresearch.html

Kazuyuki Sugahara, M.Sc., Ph.D., obtained his BS in Pharmaceutical Sciences in 1971, his M.Sc. in Biochemistry in 1973 and his Ph.D. in Biochemistry from the University of Kyoto, Japan. Kazuyaki Sugahara was research associate at the Department of Pediatrics and Biochemistry, University of Chicago (1976–1982) and associate professor at the Department of Physiological Chemistry at Kobe Women's College of Pharmacy (1990–1993). Since 2006, he is Professor at the Laboratory of Proteoglycan Signaling and Therapeutics, Frontier Research Center for Post–genomic Life Science and Technology, Faculty of Advanced Life Science, Hokkaido University Graduate School of Life Science. Kazuyuki Sugahara has been honored several times; he has received the Human Frontier Science Program Award, Strasbourg, France (2000, 2005) and Honorary doctor in Medicine from Uppsala University, Sweden (2002). Prof. Sugahara has published 175 original articles in peer–reviewed international scientific journals and about 20 review articles. His research is focused on the structure, function and biosynthesis of sulfated glycosaminoglycans. The biological phenomena Prof. Sugahara has been dealing with are cell

proliferation and cell differentiation such as neurite outgrowth and angiogenesis, which are regulated at least in part by sulfated glycosaminoglycans. His group studies involve analysis of molecular interactions between glycans and growth factors/cytokines as well as molecular biology of glycosyl– and sulfotransferases involved in the biosynthesis of these classes of glycan chains.

Katalin Dobra, MD, PhD., obtianed her MD, Summa Cum Laude, from Semmelweis University Budapest, Hungary, 1994 and her PhD in Experimental Pathology, from Karolinska Institutet in 2002 (Dissertation title: Malignant mesothelioma, an experimental study with emphasis on proteoglycans in mesothelial cell growth and differentiation). Dr. Dobra is an Associate Professor at Karoliska Institute, Sweden since 2010, She is a Specialist in Clinical Pathology and Cytology since 2008 and her Current Position involve Clinical Research 50% (Swedish Cancer Funds) and consulting in Clinical Pathology and Cytology at Karolinska University Hospital 50%, Dr. Dobra is the Group leader and principal investigator in the Mesothelioma Research Group in Karolinska Institute and she is presently supervising 2 post docs and 3 PhD students.

Suniti Misra, Ph.D. Dr. Misra is an Assistant Professor of the Departments of Regenerative Medicine and Cell Biology at the Medical University of South Carolina (MUSC), Charleston, South Carolina (SC), USA. She earned her Ph.D. at the Calcutta University in India followed by a faculty appointment at the University of Calcutta, Visiting Scientist in Harvard School of Public Health at the Harvard University, MA, USA, and then Associate Professor in Bose Research Institute, Calcutta, India, and is currently an Assistant Professor in MUSC, SC, USA. Her current research interests include understandingof the potential mechanism to block colon/intestine/pancreas/prostate tumor growth and invasiveness by specific delivery of novel CD44variant–shRNA/nanoparticles that inhibit hyaluronan/ CD44variant–induced signaling pathways in these tumor cells both in vitro and in vivo. She is a member of professional societies, including the American Society of Biological Chemists, International Society for Hyaluronan Sciences, and Hollings Cancer Society. She is Editorial Board Member of World J of Biological Chemistry and Guest reviewer of British Journal of Cancer, PLOS ONE, BioMed Central, and IUBMB Life. She has two patents: one is US. Application Nos. 60/392,905 dated 07/1/2002: "Methods and compositions for inhibition of multi–drug resistance by hyaluronan oligomers", and the other is: US. Provisional Application Nos. 61/308,140 dated 2/25/2010 "Delivery of CD44shRNA/nanoparticles within cancer cells: perturbation of hyaluronan/CD44v6 interactions and reduction in adenoma growth in Apc Min/+ mice".

Kristofer Rubin is since 1995 professor in Connective Tissue Biochemistry at Uppsala University, Sweden, and presently chairman at the Dept. of Medical Biochemistry and Microbiology. He has authored and co–authored some 120 original scientific articles and, in addition, reviews and book–chapters. His main research area concerns fluid balance in loose connective tissues. These tissues form a large body compartment and are present in all organs. In particular the group studies the interplay between integrins, growth factors and cytokines in the regulation of tissue tension. Furthermore, studies on the role of this tension for inflammatory reactions including edema formation and for the physiologic properties of the stroma in carcinoma are conducted. Finally, in more recent experiments the group aims to delineate the relevance of the collagen network architecture in carcinoma for transport phenomena.

Gillian Murphy, Ph.D., F. Acad. Med. Sci. received her Ph.D. from the University of Birmingham, UK (1971). During 1972–1974 she received a NATO Science Fellow at MPI, Martinsried, Germany. During 1997–2002 was a Professor at University of East Anglia (UEA), Norwich and from 2002 is a Professor at the University of Cambridge, Dept of Oncology, Cancer Research UK Cambridge Research Institute. Gill Murphy has been involved in research into the function of metalloproteinases, including their structure–function relationships and extracellular regulation, including the function of the TIMP inhibitors. Her Group's research is currently focused on cell surface associated forms of the zinc–dependent proteinases, notably the membrane type matrix metalloproteinases (MT MMPs) and the disintegrin–type metalloproteinases (ADAMs), and the natural inhibitors that control them, the tissue inhibitors of metalloproteinases, TIMPs. They aim to elucidate how these metalloproteinases and inhibitors function and what their importance is as effectors of both physiological processes and pathologies, such as cancer. They hope to develop novel reagents for disease therapy and diagnosis from the fundamental data accrued.

Athanassios Giannis is a chemist and physician. He was born in Greece in 1954 and studied chemistry (1980) and medicine (1987) at the University of Bonn / Germany. He completed his PhD in 1986 with Konrad Sandhoff and habilitiated in 1992 at the University of Bonn in organic chemistry and biochemistry. From 1998 to 2002 he was an associate professor at the University of Karlsruhe. Since 2002 he has been full professor for organic chemistry and natural products chemistry at the University of Leipzig. His area of research is biological and medicinal–oriented organic chemistry.

Research interests: Synthesis of bioactive natural products and their analogues like alka-

loids, peptidomimetics, ATP analogues and sphingolipids. In particular he is interested in the rational design and synthesis of inhibitors of angiogenesis, modulators of glycoconjugate biosynthesis and in the development of inhibitors of sphingomyelinase and ceramide formation. Furthermore, the development of inhibitors of histone modifying enzymes and of kinesines as well as modulators of the hedgehog signaling pathway are part of his research interests.

Home page: http://www.uni–leipzig.de/~organik/giannis/index.htm

Dr. Zaman is Kern Assistant Professor of Biomedical Engineering and Medicine at Boston University. At BU he is the director of Laboratory of Engineering Education and Development and PI of Cellular and Molecular Dynamics Lab. Dr. Zaman got his PhD in Physical Chemistry from the University of Chicago in 2003 where he was Burroughs–Wellcome Graduate Fellow in Interdisciplinary Sciences. After his Ph.D. he was Herman and Margaret Post–Doctoral Fellow at MIT from 2003–2006. He was Assistant Professor of Biomedical Engineering at UT Austin from 2006–2009 and moved to BU in Fall 2009. His lab focuses on multi–scale modeling and quantitative approaches to cell matrix interactions in 3D tumor microenvironments.

He has received numerous teaching and research awards including the Young Investigator Award of the FEBS Advanced Lecture Course MPST–2007, CIMIT Research Award, American Society for Engineering Education Teaching Award, UT Austin College of Engineering Outstanding Teaching by Assistant Professor Award and the Highest Teaching Award at the UT System "The Regents Teaching Award". He serves on the editorial board of numerous journals including Cellular and Molecular Bioengineering, Journal of Non–Equilibrium Thermodynamics, Cell Adhesion and Migration and Molecular and Cellular Biomechanics.

Mauro S.G Pavão, Ph.D. is an Associate Professor of Biochemistry and Chair of the Program of Glycobiology of the Institute of Medical Biochemistry at the Rio de Janeiro Federal University, Rio de Janeiro, Brazil. He received his Ph.D. in Molecular Biology at the Syo Paulo School of Medicine in Syo Paulo. In 1996 he received a Post–Doctoral Fellowship from the PEW–Latin American Fellows Program to work in the Laboratory of Douglas Tollefsen at the Division of Hematology–Oncology, Washington University–Saint Louis, USA. After returning to Brazil in 1998, he was appointed Head of the Laboratory of Connective Tissue 3 in the University Hospital at Federal University of Rio de Janeiro, where he has been working on the structure and pharmacological effects of unique sulfated glycosaminoglycans using experimental models of thrombosis, inflammation and metastasis. More recently, he became interested on the role of cell surface heparan sulfate proteoglycans in modulating growth factor signalling during the initial steps of tumor progression, named Epithelial to

Mesenquimal Transition, and the possible inhibitory effect of heparin–like glycans on this complex phenomenon.

Ruggero Tenni. Full professor at the Faculty of Medicine and Surgery, University of Pavia, Italy. Current teaching duties for chemistry and biochemistry courses are in the Harvey curriculum (in English) of the medical school. His scientific interests of the last fifteen years concern collagen properties, structural features and interactions with other components of the ECM. Studies on stability and interaction have been performed also with purified and characterized fragments (CNBr peptides) of collagens 1 and 2, in particular with the peptides able to trimerize, and with peptides and whole collagen trimeric molecules after derivatization in mild conditions. Concerning interaction studies, it turned out that collagen 1 and 2 have multiple binding sites for decorin, fibromulin and biglycan (three small leucine–rich proteoglycans). The use of collagen peptides was one of the useful techniques for the determination of collagen sites for binding to SPARC(BM–40).

Shibnath GHATAK, Ph.D., received his Ph.D. (Biochemistry) from the University of Calcutta, India on carbohydrate transport in Leishmania donovani. He then continued his research on biochemical studies in leishmaniasis. After finishing Ph.D. he joined Organon Research Center in Calcutta as a Senior Scientist–in–charge of Parasitology Division. In 1985 he moved to Tufts University, Boston to work on molecular basis of systemic lupus erythromatosus and hormonal basis of dysplasia in the rat prostate. In 2003 he joined as an Assistant Professor in the Department Cell Biology and Anatomy at the Medical University of South Carolina, Charleston, South Carolina. He has coauthored 30 peer–reviewed papers, one book chapter, and one review in an international journal.

His research interest is centered on hyaluronan regulation of growth of various cancer cells via its interaction with the isoforms of main receptor CD44 and inhibition of growth by interrupting the interaction. Currently he is working on a novel therapeutic approach by tissue specific targeting of CD44 variant in vitro and in vivo in cancer and cardiovascular system.

Invited Lectures/Tutorials

Syndecan–1 shedding reduces clearance of triglyceride–rich lipoproteins by human hepatocytes and causes hypertriglyceridemia

L 01

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The heparan sulfate proteoglycan syndecan–1 mediates hepatic clearance of triglyceride–rich lipoproteins in mice based on systemic deletion of Sdc1 and hepatocyte–specific inactivation of heparan sulfate biosynthesis (MacArthur et al. (2007) *J. Clin. Invest.* 117:153–164; Stanford et al. (2009) *J. Clin. Invest.* 119:3236–3245; Stanford et al. (2010) *J. Biol. Chem.* 285:286–294). Here we show that syndecan–1 is expressed on primary human hepatocytes and Hep3B human hepatoma cells and can mediate binding and uptake of VLDL, based on heparin lyase inhibition and siRNA directed against SDC1. We also show that syndecan–1 is spontaneously shed from primary human and murine hepatocytes and Hep3B cells. In human cells, syndecan–1 shedding was induced with phorbol myristic acid (PMA), resulting in 70–75% reduction of syndecan–1 expression on the cell surface and accumulation of syndecan–1 ectodomains in the medium. Shedding occurred through a protein kinase C–dependent activation of ADAM–17 (A Disintegrin and Metalloproteinase–17) based on pharmacological inhibition studies and siRNA–mediated silencing. PMA–stimulation significantly decreased diD–VLDL binding. Furthermore, the shed syndecan–1 ectodomains bound to VLDL based on an assay in which association of VLDL with [^{35}S]ectodomains caused a decrease in the buoyant density of the proteoglycan. Induction of Sdc1 shedding in mice by injection of lipopolysaccharide resulted in loss of hepatic Sdc1, accumulation of ectodomains in the plasma, and hypertriglyceridemia. Thus, shedding of syndecan–1 provides a mechanism that might explain hypertriglyceridemia in patients with sepsis or in patients undergoing therapeutic regimens that result in idiopathic hyperlipidemia.

L 02

SLRPs signaling in innate and adaptive immunity

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Research over the last ten years has provided new evidence that small leucine–rich proteoglycans (SLRPs) of the ECM, commonly thought to function exclusively as structural elements, could act as signaling molecules. With the identification of ECM/SLRP–derived endogenous ligands of Toll–like and NOD–like receptors of innate immunity, this field has recently gained notable relevance. It provoked a general question about the mechanisms of SLRP–derived signaling in pathogen–mediated inflammation. Moreover, it provided a fascinating concept of SLRPs as autonomous triggers of sterile inflammatory processes. Accordingly, some soluble SLRPs may act as fundamental danger signals which signify tissue injury and elicit a robust proinflammatory response by the innate immune system. 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. Finally, this will give rise to the formation of lymphoid follicle–like clusters of B cells, T cells, and macrophages in the kidney. Importantly, biglycan is markedly elevated in the plasma and kidneys from patients with LN and from lupus–prone (MRL/*lpr*) mice. Genetic elimination of biglycan in MRL/*lpr* mice improved systemic and renal outcomes by lowering autoantibodies, reducing enlargement of the spleen and lymph nodes, and preventing renal damage and albuminuria. This was associated with reduced plasma and renal levels of CXCL13, RANTES, MCP–1, and MIP–1α as well as lower numbers of macrophages and B and T cells in the kidney. Collectively, transient overexpression of soluble biglycan provided the first direct *in vivo* proof for the involvement of TLR2/4 in biglycan–mediated signaling in the kidney. Enhanced levels of circulating biglycan triggered CXCL13 expression and B cell infiltration both in healthy and in lupus kidneys in association with worsening of albuminuria and organ damage. 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).

Functions and Properties of Matrix Metalloprotease Complexes

L 03

J. O. Winberg

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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 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.

In addition to the pro–, catalytic and hemopexin–like (HPX) domain, MMP–2 and MMP–9 contains a unique module inserted in their catalytic region. This module contains three fibronectin II–like repeats (FnII) which is similar but not identical in the two gelatinases. This module facilitates the localization of these enzymes to connective tissue matrices as well as the degradation of various biological substrates. Furthermore, there are also other regions outside the catalytic cleft that are important for the enzyme activity against various biological substrates, such as motifs in the HPX domain. MMP–9 exist in addition to its momomeric form as either 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. *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, biochemical properties of the enzyme in these complexes and possible consequences this will have for the biological function of the enzyme.

L
04

Fibrillar collagen degradation and cell migration

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Triple helical fibrillar collagens I, II and III are major structural proteins of connective tissues such as bone, cartilage, tendons, skin and blood vessels. By forming the extracellular matrix network interacting with other extracellular matrix components and cell surface receptors, they function not only to hold the tissues and organs together by providing the scaffolding and tensile strength but also to guide the cell to migrate, proliferate and differentiate. Therefore, timely degradation of fibrillar collagens is an integral part of many biological events such as embryonic development, organ morphogenesis, tissue remodelling and repair. Aberrant collagenolysis may result in diseases such as arthritis, cancer, atherosclerosis, aneurysm and fibrosis. In vertebrates, collagen degradation is initiated by collagenases that are members of the matrix metalloproteinase (MMP) family. I first discuss the mode of collanase–collagen interaction which provides insights into how collagenases may unwind triple helical collagens before they cleave the peptide bonds. I then discuss how collagen fibrils may be degraded in the tissues. Collagen fibrils are more resistant to collagenolysis. This is partly due to that the collagenase–cleavage site in collagen fibrils are mainly covered by the C–telopeptide of an adjacent collagen molecule. The degradation of fibrillar collagen may be therefore initiated by binding of a collagenase to damaged collagen fibres or by cleavage of the C–telopeptide, which exposes the collagenase cleavage site. The removal of the C–terminal 1/4 fragment containing the C–telopeptide by the collagenase subsequently reveals another collagenase–cleavage site in the adjacent collagen that is located to the C–terminal of the previously cleaved site. Such scenario may explain directional cell movement through pericellular degradation of collagen fibrils by cell surface–anchored MMP–14.

Syndecans, proteoglycan regulators of cell adhesion

L 05

J.R. Couchman, E. Okina, S. Gopal, H.C. Lim, M. Lendorf, C. Pataki, B.S. Fogh, T. Manon–Jensen, A.Oneda, H.A. Multhaupt

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All four mammalian transmembrane syndecans have a conserved cytoplasmic domain that interacts with the actin cytoskeleton. Direct and indirect associations with integrin receptors have also been reported. While syndecans have often been referred to as "co–receptors", it is clear that they have an independent ability to signal. Syndecan–4 is perhaps best understood, both from structural and signaling viewpoints. Fibroblasts derived from knock–out mice have an altered cytoskeleton; they possess fine microfilament bundles that contain β–actin, but lack α–actin, unlike their wild–type counterparts. Microfilament bundles terminate at specialised adhesion and signaling organelles known as focal adhesions, or focal contacts. These are smaller in knock–out cells, and appear lacking in α–actinin. Transfection of wild type syndecan–4 cDNA in null cells can restore a normal phenotype, but truncated forms that lack the C–terminal half of the cytoplasmic domain cannot do so. There appears to be at least two elements in the signaling through syndecan–4. First, interactions with protein kinase C lead to a phosphorylation cascade that culminates in Rho family GTPase activation. GTP–Rho is required for microfilament bundle formation. Second, syndecan–4 directly interacts with α–actinin and both interactions may be required for normal cytoskeletal organisation.

However, recent work in MDA–MB231 mammary carcinoma cells indicates that syndecan–4 has different roles. Here the proteoglycan is also present in adhesion structures, in this case invadopodia, and experimental alteration of surface levels of the proteoglycan influences both invasion and collagen gel degradation. However, in these cases, the cytoplasmic domain of the proteoglycan appears to be dispensable. Its heparan sulphate chains are not, and recent work indicates that heparan sulphate and heparin can strongly influence actin cytoskeletal organisation of these tumour cells, although the mechanism is still under investigation. Collagen gel degradation by MDA–MB231 cells is largely dependent on MMP14 (MT1–MMP), so it may be relevant that syndecan–4 can be cleaved by this proteinase, and thereby shed from the cell surface where it can function in the pericellular environment.

In summary, syndecans are widespread heparan sulphate proteoglycans in vertebrate cells with roles in cell adhesion. Many different ligands may bind their heparan sulphate chains, while linkage to, and regulation of, the actin cytoskeleton appears to be a common theme. However, much remains to be established regarding the molecular basis of their action.

L 06

The complexity of proteoglycans – different cores, different glycosaminoglycans in different cells and tissues at different times

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Proteoglycans (PG) are a group of molecules that exist on the cell surface and in the extracellular space. They are characterized by their post–translational modifications known as glycosaminoglycans (GAGs), which are linear alternating co–polymers of hexuronic acid and hexoxasamine. These are differentially modified with sulfate that provides charge heterogeneity, which together with the size polydispersity of the GAG chain length makes these molecules some of the most complex in the proteome. Versican is an extracellular matrix molecule that via alternative splicing events may either be expressed as a protein or a PG decorated with chondroitin suflate (CS). Lubricin is also a CSPG that has been shown to undergo alternative splicing of a section of the gene where the CS decoration occurs. We have recently shown that a sub–popualtion of lubricin present in synovial fluid is a PG, while the remainder is a glycoprotein, highlighting the heterogeneity with respect to glycosylation within tissues. A third CS PG, bikunin, has been shown to be important in the cross–linking of hyaluronan into the extracellular matrix. We have shown that the CS sulfation pattern is different amongst individuals and this variablity in structure affects the ability of the CS PG to perform its biological role.

We have also demonstrated the complexity of these GAG structures on another proteoglycan known as perlecan, which is a heparan sulfate PG that can sometimes be decorated with CS and keratan sulfate. These changes have profound effects on the ability of perlecan to bind and modulate the signaling of growth signaling molecules such as the FGF family. We have commenced a series of experiments to ascertain whether this proteoglycan also undergoes alternative splicing of the protein core, which would add further complexity to that seen already due to the GAG side chains. Our preliminary results support our hypothesis and suggest that the structure of PGs is inherently more complex than we could have initially thought and that this complexity changes dynamically in response to environmental and cell signals.

Novel Roles of Serglycin in Malignancy

L 07

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Serglycin is a unique proteoglycan containing a small peptide core rich in serine/glycine repeats onto which up to eight glycosaminoglycan chains are attached. Serglycin was initially characterized as intracellular proteoglycan that was mainly synthesized by hematopoietic cells playing important roles in packaging of proteins into secretory granules and/or directing the secretion of these molecules. Recent data indicate that serglycin is expressed and constitutively secreted by tumor cells in various malignancies. Although serglycin does not contain a transmembrane domain, it is found at the cell membrane of tumor cells, where associates through its glycosaminoglycan chains. Serglycin secreted by tumor cells carries chondroitin sulphate side chains mainly substituted with 4–sulfated disaccharides. It is capable to interact with complement system components C1q and MBL in a glycosaminoglycan–dependent manner and inhibits both the classical and lectin pathway of the complement system without influencing alternative pathway. We found that serglycin secreted in the microenvironment of the tumor cells or/and serglycin present at the cell surface protects tumor cells from complement activation induced by treatment with specific antibodies during immunotherapy. Serglycin is over–expressed and secreted by more aggressive tumor cells and serves as an independent prognostic indicator of metastasis–free survival and disease–free survival in nasopharyngeal carcinoma patients. Over–expression of serglycin in low metastatic tumor cells increased the gene expression of several proteolytic enzymes indicating a regulatory role of serglycin in their biosynthesis. Tumor cells are also capable to adhere to matrix molecules such as collagen type I through cell membrane–associated serglycin. The increased adhesion of these cells in turn, up–regulates the expression of collagen–degrading enzymes promoting the breakdown of the microenvironment of tumor cells. These data revel that serglycin is seriously implicated in tumor cell biology and may protect tumor cell by immune system attack or/and promotes their metastatic potential.

L 08

Role of loose connective tissues in microvascular fluid exchange and drug uptake in carcinoma and localized infections

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Extracellular matrix (ECM) elements are intrinsic to all organs. The ECM provides structural support and also governs physiology. An abundant ECM characterizes the connective tissues of which loose connective tissue constitutes a major compartment amounting to some 15% of the body volume. The extracellular (interstitial) fluid volume is essentially contained within the ECM present in the loose connective tissues. All peripheral blood and lymph vessels involved in exchange with tissues are embedded in this compartment, which makes up the route of transport for nutrients and waste materials between endothelial and parenchymal cells of any tissue. Furthermore, inflammatory processes, infections and carcinoma invasion all occur in, or involve, this body compartment.

Interstitial fluid pressure (IFP) is one of the Starling forces that determine capillary–to–interstitium fluid transport. IFP is close to zero in normal loose connective tissues. However, during anaphylaxis, inflammation, and burn injuries IFP is rapidly lowered leading to edema formation. We have established a mechanistic model for the control of the IFP in which connective tissue cells apply tensile forces on ECM–fibers that in turn restrain the under–hydrated ground substance from taking up fluid and swell. A decrease in cellular tension applied to the ECM fibers allows the ground substance to swell, *i.e.* form edema. The tensile forces are mediated by β_1–integrins in normal tissues and can be pharmacologically modulated. Dermal IFP lowered after anaphylaxis can be normalized by instilments of PDGF–BB or insulin by a mechanism dependent on integrin $\alpha_V\beta_3$ instead of β_1–integrins.

Carcinomas are characterized by a pathologically high IFP. This characteristic leads to a disturbed physiology and reflects stroma properties that result in an impaired uptake of anti–cancer drugs into the carcinoma. Agents that lower IFP in experimental carcinoma increase uptake and efficacy of small molecular weight chemotherapeutic agents. Work in our group has identified collagen fibril diameter and density of the collagen network to correlate with the formation of a stroma with high IFP and decreased extracellular fluid volume.

Studies of physiological characteristics in solid infectious lesions are scarce. The inflammatory response and changes in connective tissue in which the infection takes place are affected not only by innate immune reactions but also by bacterial proteins that interact with host proteins. Species of pathogenic staphylococci and streptococci express several cell wall–bound or released proteins that specifically interact with host ECM proteins. We investigate the possibility that such proteins can modulate edema formation and tension in tissues. Such effects could influence the uptake of *e.g.* antibiotics.

Negative Regulation of Integrin Activity

L 09

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β1–integrins constitute a large group of widely distributed adhesion receptors, which regulate the ability of cells to interact with their surroundings. Regulation of the expression and activity of integrins is critical for tissue homeostasis and development and contributes to inflammation and cancer. Protein interactions with the β1–integrin cytoplasmic tail are known to influence integrin affinity for extracellular ligands, but regulating binding partners for the α–subunit cytoplasmic tails have remained elusive. Furthermore, studies investigating cellular components which specifically regulate the activity of distinct integrin α/β1 heterodimers are not known. We have employed yeast–two–hybrid analysis of integrin α–subunit binding proteins as well as RNA interference screens to uncover genes involved in the regulation of integrin activity. Our recent data reveal two novel integrin α–tail interacting proteins as negative regulators of integrin activity. Furthermore, a combination of RNAi screening with proximity–ligation technique has allowed us to analyse heterodimer specific changes in integrin activity using cell spot microarray technology in cancer cell lines. Cell biological analysis of the identified β1–integrin regulatory genes revealed that modulation of integrin activity can influence cell invasion in three–dimensional matrix, cancer cell extravasation in vivo and correlate with poor clinical outcome in breast cancer.

L 10

Extracellular matrix remodeling in cervical cancer

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Besides tumor cells, tumorous tissue contains stromal elements. For a long time these components have been considered as bystanders in tumor growth and invasion. In the last decade, increasing amount of evidence put the tumorous stroma into the focus of cancer research. The altered biological activity of tumor associated fibroblasts results in production of matrix proteins with changes in their structure or amount. Abnormal ECM can be responsible for disturbed cellular functions including impaired regulation, generation of mitogen signals, changes in adhesion, as well as in homo and heterotypic interactions, all together facilitating tumor growth and invasion. Furthermore these cells are implicated in the synthesis of regulatory factors responsible for the accelerated proliferation of tumor cells.

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

Increased immunostaining of fibronectin, laminin, vimentin, and smooth muscle actin indicated fibroblasts with enhanced synthetic activity in the tumorous stroma of surgical specimens. Conversion of syndecan–1 expression from epithelial to mesenchymal cells could be witnessed, as well. Fibroblasts outgrown from cervix cancer supported the proliferation of primary as well as established tumor cells *in vitro*.

Decreased expression of matrilin–2, decorin, syndecan–4, Smad3 and increased expression of fibroblast growth factor1, latent TGFβ–binding protein 2, thrombospondin, and KISS–1 was observed in fibroblasts derived from tumorous tissues compared to normal ones by microarray analysis validated by qRT–PCR. When cultivated in coculture with cervical cancer cells PCR array showed further alterations both in normal and tumor derived fibroblast expression pattern. The latter produce more TGFβ1 and CTGF, proteins with antiadhesive properties, and matrix metalloproteases. Upregulation of α6 and β4 integrins indicates that signals from the remodeled matrix are mediated via this matrix receptor. All these changes confer a proliferatory migratory phenotype for tumor cells.

Our results indicate that there is a vivid crosstalk between tumor cells and stromal fibroblasts. This interaction may require direct cell–cell contact, but a considerable proportion is mediated by humoral factors. Interestingly the majority of proteins with altered mRNA level are residents of the extracellular matrix or cell surface, which underlines the role of ECM in tumor invasion.

Reorganization of extracellular matrix in cancer

L 11

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Proteoglycans (PGs) are significant components of the extracellular matrix (ECM). They are complex macromolecules made up of a protein core onto which variable mumber of glycosaminoglycan (GAG) chains, a specific class of heteropolysaccharides, are attached. Chondroitn/dermatan sulfate is the main GAG bound to proteoglycans, whereas hyaluronan is free of protein. Chondroitn/dermatan sulfate is synthesized in the Golgi apparatus by the action of specific synthases, CHSY1, CHSY2 and CHSY3, which possess both glucuronyltransferase and N–acetyl–galactosaminyltransferase activity. The action of 5–epimerase together with the action of the sulfotransferases (C4ST1, D4ST1 and CHST3) direct the biosynthesis of chondroitin–4–sulfate or –6–sulfate and dermatan sulfate. Hyaluronan is synthesized in the plasma membrane by the action of either of HAS–1, –2 or –3, depending mainly to the state of the tissue or the cell, which add alternatively glucuronyl– and N–acetyl–glucosaminyl–residues to the growing chain. Degradation of PGs in the ECM is possible through the action of MMPs and ADAMTs at specific peptide bonds of the protein core and of the GAG chains via the action of hyaluronidases. At least two MMPs and two ADAMTs have been found so far to possess the ability to degrade PGs, and four of the six different hyaluronidase genes identified are widely expressed in body cells.

Degradation and/or alternative splicing of PGs and alteration of GAG structure occur in almost all types of cancer. However, the pattern is different, depending to the tissue/organ affected and therefore detailed examination regarding the expression of the enzymes involved in both biosynthetic and catabolic processes should be performed to identify the key points related to tumor progression and thus possible targets for drug design and treatment.

L
12

Perlecan interacts with both the α2β1 integrin and VEGF receptor 2 via its C–terminal angiostatic fragment endorepellin: A novel concept of dual receptor antagonism

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Endorepellin, the C–terminal module of perlecan, negatively regulates angiogenesis counter to its proangiogenic parental molecule. Endorepellin binds α2β1 integrin on endothelial cells and triggers a signaling cascade that leads to disruption of the actin cytoskeleton. Here we show that both perlecan and endorepellin bind directly and with high affinity to both VEGF receptor 1 and 2, in a region that differs from VEGFA–binding site. In both human and porcine endothelial cells, this interaction evokes a physical downregulation of both the α2β1 integrin and VEGFR2, with concurrent activation of the tyrosine phosphatase SHP–1 and downstream attenuation of *VEGFA* transcription. We demonstrate that endorepellin requires both the α2β1 integrin and VEGFR2 for its angiostatic activity. Endothelial cells that express α2β1 integrin but lack VEGFR2, do not respond to endorepellin treatment. Thus, we provide a new paradigm for the activity of an antiangiogenic protein and mechanistically explain the specificity of endorepellin for endothelial cells, the only cells that simultaneously express both receptors. We hypothesize that a mechanism such as dual receptor antagonism could operate for other angiostatic fragments of extracellular matrix proteins which specifically act on endothelial cell homeostasis (Supported in part by NIH grant RO1 CA47282).

EMMPRIN: key regulator in the dynamic interplay between tumor cells and their microenvironment

L 13

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Tumor microenvironment undergoes extensive changes during cancer development which involve the activation of fibroblasts and the resultant alteration of the extracellular matrix (ECM), enhanced angiogenesis and infiltration of inflammatory cells. These alterations in the peri–tumoral environment which are triggered by the cross–talk between the tumor cells and the host stromal cells are thought to benefit the cancer cell and to promote its progression. Understanding the nature of the interaction of the tumor with the surrounding stroma would be important in generating new therapeutic targets. EMMPRIN, a membrane glycoprotein highly enriched on tumor cells has been suggested to be an important mediator in tumor–stroma interaction. EMMPRIN was first identified in the late eighties by the group of Biswas as a tumor cell factor able to induce in fibroblasts the production of several matrix metalloproteinases (MMPs) and therefore to facilitate tumor invasion. Other malignant properties of EMMPRIN associated with cancer, such as invasiveness, metabolism, survival and anchorage–independent growth have been since described. High levels of EMMPRIN are detected in numerous malignant tumors which often correlate with tumor progression and poor prognosis.

Accumulating evidence including those of from our laboratory suggests that EMMPRIN has a prominent role in the alteration of tumor microenvironment. In addition to signaling fibroblasts to increase matrix proteases production which facilitate tumor invasion, we have shown that EMMPRIN promotes fibroblasts differentiation to myofibroblasts, identified by their expression of alpha smooth muscle actin (αSMA). Myofibroblasts are present in large numbers in the stroma of invasive cancer and are considered as central actors of tumorigenesis. We furthermore demonstrated that EMMPRIN stimulates angiogenesis through the upregulation of VEGF soluble isoforms (the most angiogenic forms) and of its receptor VEGFR–2 in neighboring endothelial cells. This effect of EMMPRIN on angiogenesis was shown to involve HIF–2alpha. Thus, EMMPRIN increased endothelial cell migration, cell survival and capillary–like formation. EMMPRIN may also be implicated in the inflammatory cells infiltration often seen in the tumor stroma, as it was shown to be required for leukocytes activation and maturation and to stimulate their chemotaxis by serving as a signaling receptor for cyclophilins. The accumulated evidence therefore suggests that EMMPRIN, enriched on tumor cell surface, is a central mediator in the cross–talk with host stromal cells, recruiting them for the tumor's benefit.

L
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Involvement of heparanase in cancer progression, chronic inflammation and diabetic nephropathy

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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, inflammation and kidney function. 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 and diabetic nephropathy (DN) and hence is a valid target for drug development. Importantly, heparanase over–expression correlates with increased tumor vascularity and poor postoperative survival of cancer patients. Moreover, heparanase levels in the urine and plasma of cancer patients often correlate with the severity of the disease and response to anti–cancer treatments. The feasibility of targeting heparanase for therapy is supported by the fact that there is a single enzymatically active heparanase in humans, its expression is rare in normal tissues and heparanase knock–out mice are viable and exhibit no visible disorders, supporting the notion that drugs designed to block heparanase function *in vivo* will inhibit disease progression with minimal side effects. Heparanase is causally involved in inflammation and accelerates colon tumorigenesis associated with inflammatory bowel disease. Briefly, heparanase stimulates macrophage activation, while macrophages induce production and activation of latent heparanase contributed by the colon epithelium, together generating a vicious cycle that powers colitis and the associated tumorigenesis. Heparanase also plays a decisive role in the pathogenesis of diabetic nephropathy, degrading HS in the glomerular basement membrane and ultimately leading to proteinuria and kidney dysfunction. The heparanase structure delineates a TIM–barrel fold harboring the enzyme' active site and a C–terminus domain that is critical for heparanase secretion and signaling function. Heparanase–inhibiting compounds directed against these domains are being developed aiming at halting tumor growth, metastasis, angiogenesis, inflammation and diabetic nephropathy. We have generated a novel chemically modified non–anticoagulant heparin (termed SST0001) that binds tightly to the heparanase active site and potently inhibit its enzymatic activity. This compound yielded impressive results in preclinical models of cancer progression (i.e., multiple myeloma) and diabetic nephropathy (DN) and is being subjected to phase I clinical trial.

Bone marrow stem cells that divide in hyperglycemic glucose initiate intracellular hyaluronan synthesis, autophagy and pathological adipogenesis

L 15

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Isolated rat bone marrow stromal cells, cultured in osteogenic medium, in which the normal glucose concentration (5.6 mM) is changed to hyperglycemic glucose (25.6 mM): 1) dramatically increase lipid accumulation between 21 to 31 days after induction, which is associated with a decrease in mineral deposition within the same time frame; 2) upregulate expression of cyclin D3 and two adipogenic markers (C/EBPα and PPARγ) within 5 days of culture; 3) increase neutral and polar lipid synthesis within 5 days of culture; and 4) form an extensive monocyte–adhesive hyaluronan matrix through an endoplasmic reticulum (ER) stress driven autophagic mechanism (1,2). Further, within 4 weeks after the onset of diabetes in the streptozotocin (STZ)–induced diabetic rat model, there is: 1) a large loss of trabecular bone mineral density without proportional changes in underlying collagen matrices; 2) a large accumulation of a hyaluronan matrix within the trabecular bone marrow spaces; 3) a large number of adipocytes that underwent the autophagic mechanism embedded in this hyaluronan matrix; and 4) extensive numbers of mononuclear, CD44 positive cells, likely monocytes/macrophages, embedded in this hyaluronan matrix. These results support the hypothesis that hyperglycemia in bone marrow diverts dividing osteoblastic precursor cells (bone marrow stromal cells, mesenchymal stem cells) to a pathological adipogenic pathway, which induces synthesis of an abnormal hyaluronan matrix that recruits inflammatory cells. We hypothesize that this process establishes a chronic inflammatory process that leads to demineralization of trabecular bone thereby explaining the underlying mechanism of diabetic osteopenia.

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L
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Aspects of Regulation of Hyaluronan Synthesis

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Regulation of hyaluronan (HA) synthesis shows several aspects of remarkable interest. In mammals, HA is synthesized by three homologues HA synthases on the cell membrane, identified as HAS1, HAS2 and HAS3, which polymerize HA chain using UDP–glucuronic acid and UDP–N–Acetyl glucosamine as precursors. The availability of sugars precursors plays a role in the HA synthesis and depends on the different pathways involved in UDP sugars synthesis. Moreover, the protein sorting process of HASes shows an intriguing mechanisms. In order to shed light over it, we developed a non–radioactive assay for HASes activity in eukaryotic cells, addressing in the same time the question about HASes activity during protein intracellular trafficking. Active proteins from three fractions: plasma membrane, microsomal cytosol (containing membrane proteins mainly from endoplasmic reticulum (ER) and Golgi) and nuclei were obtained (1–4). After the incubation with UDP–precursors, we quantify new synthesised HA by electrophoretic approach (FACE) and HPLC. This new method is able to measure HASes activity in plasma membrane fraction as well as in cytosolic membranes. This new technique was used to evaluate the effect of 4–methylumbelliferone, phorbol 12–myristate 13 acetate (PMA), IL–1β, PDGF–BB and tunicamycin on human mammalian cells and we found that the HASes activity can be modulated by post–translational modification such as phosphorylation and glycosylation. Interestingly, we were able to detect a significant increase in HASes activity in plasma membrane and cytosolic fractions after tunicamycin treatment. which induces ER stress by inhibiting N–glycosilation and to promote particular HA structures called "HA cables" (1,2), effect confirmed by PNGase treatment. The phosphorilation is also critical for HAS activity, in fact the use of phosphorilase modulated the activity of the enzymes in the membrane preparation showing that phosphorilaton is critical for enzyme activity. Using the AMPK kinase and mutated protein we were able to develop a model for this covalent modification. O–glycosdamination is also important for HAS activity, showing an opposite effect respect the phosphorilation. Mutants HASes were produced in order to identify the specific residue and the effect of O–GlcNAcation on enzyme activity. These results indicate that a covalent modifications of HASes may occur in the cells affecting the production of HA.

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The role of hyaluronan–CD44 interactions in tumor progression

L 17

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The aim of our project is to gain new insights into how the microenvironment influences tumorigenesis by exploring signaling pathways through which the cells sense extracellular stimuli. The hyaluronan – CD44 complexes are of particular interest for these studies, since they regulate cellular growth or growth inhibition as well as differentiation and migration. Currently experiments are in progress where we investigate:

1) The molecular mechanisms involved in the control of the endogenous production of hyaluronan in order tounravel its essential roles in tissue homeostasis and cellular functions. For that we investigate the regulation of HAS gene expression and the functional events that regulate their activities. Recent data indicate that HAS2 activity is regulated through mono–ubiquitination at K190 and oligomerization. Transfection of breast cancer cell line Hs578T with K190R mutant HAS2 suppressed their hyaluronan synthesizing capacity.

2) The downstream signaling events of the hyaluronan receptor CD44, and mechanisms whereby hyaluronan–activated CD44 modulates the activation and specificity of growth factor receptors, including the receptors for platelet–derived growth factor–β (PDGF) and transforming growth factor– β (TGF–β). For that, using Maldi–TOF–MS analysis we have identified, from pull down approaches, CD44–associated proteins that play key roles in the dynamic reorganization of the actin cytoskeleton and cell proliferation. Furthermore, preliminary data indicate the formation of a ternary complex between TGFβRI, PDGFRβ and CD44, and cooperation between TGFβ–mediated EMT and hyaluronan synthesis.

3) The significance of hyaluronan–CD44 interaction for breast cancer cell invasion and adhesion to endothelium. These studies revealed that silencing of HAS2 using shRNA–expressing RNAi decreases the malignant phenotype of bone–seeking breast cancer cells through the induction of tissue inhibitor of metalloproteinases type I (TIMP1). Additionally, our data demonstrate that peritumoral hyaluronan mediates an adhesion prevalence of bone–seeking breast cancer cells for vascular endothelium.

L 18

Hyaluronan/CD44v6 signaling, motility, angiogenesis and tumorigenesis in colon cancer

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The glycosaminoglycan hyaluronan (HA) is expressed at elevated levels in colon rectal cancer (CRC) matrices. CD44 is a primary cell surface receptor for HA, and CD44–dependent cell migration involves CD44 cleavage and shedding by matrix metalloproteinases (MMPs). Alternative splicing of CD44 RNA leads to numerous variants of CD44. CD44v6 is significantly higher in various cancers, including CRC. Ras signaling promotes CD44v6 RNA splicing, and CD44v6 expression sustains late Ras signaling. CD44v6 is essential for: 1) hepatocyte growth factor (HGF)–induced activation of its receptor, c–MET, 2) VEGFR2 activation in human endothelial cells (ECs), angiogenesis, and metastasis[3].

Our previous studies indicated that constitutive HA/tumor–CD44 interaction enhanced cell survival pathways in CRC, and in prostate tumors, and that perturbation of this interaction suppresses cell survival pathways. Importantly, we developed a CRC–specific CD44v6shRNA that suppresses HA/CD44v6 activation, reduces tumor growth, decreases cell motility and prevents CRC development in the Apc Min/+ mouse in vivo.

We now report that: 1) CD44v6shRNA inhibit xenograft tumor growth using HT29M cells. 2) Further, primary CRC cultures express CD44v6 on 60% of the cells, and treatment with CD44v6shRNA reduced their clonogenic ability by ›80%. 3) Stromal HGF stimulates CD44v6 expression in the murine pre–neoplastic Apc10.1 cells, and in human CRC cells and endothelial cells and inflammaatory monocytes/lymphocytes. 4) HA/CD44v6 interaction on tumor cells induces cell motility, increases angiogenesis, and alters cytokines (IL10, IL12, TNF–α and TGF–β) in the colon to be tumor–permissive milieu. Thus, we conclude that the HA/CD44v6 signaling appears very important in CRC growth, motility, and angiogenesis.

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Dual effects of unique oversulfated dermatan sulfates on tumor invasion: inhibition of epithelial–mesenchymal transition and hematogeneous metastasis

L 19

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Metastatic disease is responsible for most cancer–associated deaths and epithelial–mesenchymal transition (EMT) along with hematogeneous metastasis are critical steps in cancer progression. Therefore, inhibition of these events could be an effective approach to reduce the metastatic disease. Heparin has been show to modulate EMT–associated growth factors and also to inhibit P–selectin, leading to attenuation of metastasis. Previously, we showed that unique dermatan sulfates (DS) from Styela plicata and Pallusia nigra, composed by 2,4–O–sulfated and 2,6–O–sulfated disaccharide units, respectively, bind with high affinity (KD of ~ 33nM) to the EMT–related growth factor, hepatocyte growth factor (HGF), modulating its MET–dependent intracellular signaling. In the present work, the effect of the ascidian DSs on EMT–mediated cell migration and P–selectin–mediated metastasis in experiments in vitro and in vivo were evaluated. To investigate migration, confluent human mammary cells were scraped with a pipette tip and cultured for 3 days in the presence of the ascidian DSs. The migration of human mammary cells in the wound–healing cell migration assay was drastically inhibited by the glycans. The anti–selectin activity was evaluated by the binding of LS180 cells to immobilized P–selectin in the presence of the ascidian DSs. The ascidian DSs inhibited the binding of tumor cells to P–selectin with IC_{50} values of 13.51 µg/mL (2,4–DS) and 12.19 µg/mL (2,6–DS) and were better inhibitors than unfractionated heparin (IC_{50} of 24.51 µg/mL). DS from porcine skin had no effect. To investigate the effect of ascidian DSs in vivo, mice were injected with LS180 cells 10 minutes after treatment with PBS, UFH (1 mg/mouse) or ascidian DSs (100 µg/mouse) and the presence of platelets–tumor cells aggregates in the lung microvasculature was evaluated. The ascidian DSs inhibited the adhesion of platelets to tumor cells in vivo in a concentration 10–fold lower than heparin. The inhibitory effect of glycans could be observed as early as 30 minutes upon cells injection or 3 hours later. Metastasis mice in vivo was investigated by injecting 100 µg of each ascidian DSs or PBS 10 minutes prior injection of MC–38 cells. Twenty–eight days later, lungs were harvested for macroscopic evaluation of metastasis. Both ascidian DSs drastically reduced metastasis of MC–38 cells. PBS had no effect. Metastasis was drastically reduced in P–sel–/– mice and neither 2,4– nor 2,6–DS had any additional effect. In conclusion, ascidian DSs inhibit cell migration and reduce hematogeneous metastasis by a EMT– and P–selectin mediated events and could be used therapeutically to prevent tumor invasion and metastasis.

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L 20

Involvement of the chondroitin sulfate E–type structure in the experimental metastasis of the Lewis lung carcinoma cell line

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Altered expression of chondroitin sulfate (CS) and dermatan sulfate (DS) at cell surface of cancer cells plays a key role in malignant transformation and tumor metastasis. However, the molecular mechanism of the involvement of CS/DS chains in the metastatic process has not been well understood. Immunocytochemical analysis of two cell lines derived from mouse Lewis lung carcinoma using a CS–E–specific phage display antibody showed a stronger expression of the CS–E epitope for the highly metastatic rather than the low metastatic cells. Disaccharide analysis of the CS/DS chains showed a higher proportion (5.2%) of ΔHexUA–GalNAc(4, 6–O–disulfate) generated from E–units [GlcUA–GalNAc(4, 6–*O*–disulfate)] in a highly metastatic than in a low metastatic cell line (0.1%), although the total amount of CS/DS expressed by the former is much less (10%) than that of the latter. These findings prompted us to study the role of CS–E–type structures in experimental metastasis. Experimental metastasis was performed by injecting the tumor cells from a tail vein under various conditions, and the metastasis was assessed 3 weeks after the injection of the tumor cells. The metastasis of the high metastatic cells to mouse lungs was efficiently inhibited by enzymatic removal of CS from the tumor cell surface or by pre–administration of CS–E, which is derived from squid cartilage and rich in E–units, in a dose–dependent manner (ref. 1). Notably, a CS–E decasaccharide fraction, the minimal structure recognized by the phage display antibody, strongly inhibited the metastasis of the carcinoma cells. Similar results were obtained using a mouse osteosarcoma cell line, which metastasizes to mouse livers (ref. 2). Disaccharide analysis of the osteosarcoma cells showed a significant proportion (12%) of E–units. A pre–administration of CS–E or a pretreatment of the osteosarcoma cell line with the CS–E–specific antibody strongly inhibited the metastasis to the liver. These results altogether suggest that the E–unit–containing epitopes may be involved in the metastatic process of this cell line also. To characterize CS–E–binding proteins in a mouse lung, an extract of a lung homogenate was analyzed by affinity chromatography using a CS–E–immobilized column, which revealed a few protein bands by SDS–PAGE followed by silver staining (S. Mizumoto, J. Takahashi, and K. Sugahara, unpublished results). Characterization of these putative receptors for the lung metastasis of Lewis lung carcinoma cells is in progress. The results appear to suggest that the E–unit–containing epitopes of CS/DS at the tumor cell surface are recognized by the putative receptors in the lung, and are promising targets for diagnosis and therapy of malignant tumors. CS–E at the tumor cell surface and their receptors in the lung will be discussed in the context of the molecular mechanism of the lung metastasis.

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Regulation of heparan sulfate biosynthesis

L 21

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Heparan sulfate proteoglycans influence embryonic development as well as adult physiology through interactions with protein ligands. The interactions depend on heparan sulfate structure, which is determined largely during biosynthesis by Golgi enzymes. The biosynthesis enzymes are subject to regulation on both transcriptional, translational and post-translational levels. In addition to the enzymes, other regulatory proteins and the concentration of the sulfate donor PAPS may also greatly influence the final structure of the heparan sulfate chains produced. Heparan sulfate biosynthesis cab be studied in vitro using recombinant enzymes, in cells manipulated to overexpress or to lack components of the biosynthesis machinery, or in model organisms such as mice, zebrafish, *Drosophila melanogaster* or *C. elegans*.

L
22

Signaling via receptors for TGFβ via Smad and non–Smad pathways

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Transforming growth factor–β is a pluripotent cytokine which affects growth and differentiation of most cell types. Following ligand–induced heterodimerization of Type I and Type II TGFb receptors, several intracellular pathways are induced. Important mediators are members of the Smad family; Smad2 and Smad3 are phosphorylated by the Type I receptors, form complexes with Smad4, which are translocated into the nucleus where the transcription of specific genes are affected. Smad signaling is carefully controlled by posttranslational modifications, such as phosphorylation, ubiquitination and acetylation. In addition, we recently found that Smad signaling is negatively modulated by poly–ADP–ribosylation by PARP1 (1). In addition to Smad pathways, TGFβ also induces non–Smad pathways, including Erk, JNK and p38 MAP kinase pathways. We have reported on the mechanism whereby TGFβ activates p38, which is important for TGFβ–induced apoptosis. After ligand–induced receptor oligomerization, the ubiquitin ligase TRAF6, which is constitutively bound to a motif in the Type I receptor, is activated and ubiquitinates the serine/threonine kinase TAK1. Thereby, TAK1 is activated and can phosphorylate MKK3/6, which in turn phosphorylates and activates p38 (2). This process is facilitated by Smad7 which acts as a scaffolding protein. Recently, we have obtained evidence for yet another TGFb–induced signaling pathway. After TGFβ binding to its receptor, the metalloproteinase ADAM17 is activated and cleaves the Type I receptor. The intracellular part of the receptor is then translocated into the nucleus where it actives an invasiveness program (3). Interestingly, this pathway occurs in cancer cells but not in normal cells, and can contribute to the pro–tumorigenic properties of TGFβ.

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Cellular models to evaluate ECM as pharmacological target in breast cancer bone metastasis

L 23

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Cancer cells participate in several interactions with the tumor microenvironment constituents, including the various extracellular matrix (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. In order to mimic tumor microenvironment and to evaluate various pharmacological agents, several cellular mimetic models have been examined and established in our lab.

At the level of breast cancer bone metastasis, where an increased bone resorption by osteoclasts takes place, we evaluated the effects of bisphosphonates (BPs) on key ECM molecules that promote cancer cell growth and metastasis. Their effects in the activation of osteoclasts co–cultured with breast cancer cells on bone particles in the presence of appropriate stimuli were also evaluated. Gene expression studies (PGs, MMPs, integrins), *in vitro* invasion, migration and adhesion of cancer cells demonstrated that BPs may be powerful therapeutic agent in preventing the growth of breast cancer cells and their metastatic potential in bone. These results highlight the importance of cell models that mimic tumor microenvironment and may help to a deeper understanding of the role of ECM macromolecules on cancer development, progression as well as in their pharmacological targeting.

Recent and useful references

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L
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Glycosaminoglycan / proteoglycans–mediated–signaling in cancer growth

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The consecutive steps of tumor growth, local invasion, intravasation, extravasation and invasion of anatomically distant sites are obligatorily perpetrated through specific interactions of the tumor cells with their microenvironment. Free glycosaminoglycans (GAGs) and proteoglycan (PG)–containing GAGs, key effectors of cell surface, pericellular and extracellular microenvironments, perform multiple functions in cancer by virtue of their coded structure and their ability to interact with both ligands and receptors that regulate cancer growth and dissemination. Cell type and tissue specific alterations in fine GAG structure, which are strictly predetermined, allow these molecules to modulate different cellular processes with high specificity. Cell–associated and tumor microenvironment GAG content and distribution is markedly altered during tumor pathogenesis and progression. Consecutively, GAGs have been proposed as potential prognostic / diagnostic markers for cancer type or differentiation stage. The specific involvement of GAGs/PGs in the regulation of key tumor cell functions will be discussed.

Cellular Senescence: Molecular Mechanisms and Implications in Tissue Homeostasis

L 25

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Normal cells after serial duplication in vitro or after exposure to various genotoxic stresses enter a state called senescence, characterized by their inability to proliferate. Several studies have indicated that a DNA damage response is a major motif for the induction of replicative or premature senescence. Senescent cells are expressing an inflammatory phenotype, characterized by the overexpression of matrix metalloproteases (MMPs), inflammatory cytokines and growth factors, as well as other inflammatory molecules, thus possibly affecting local tissue homeostasis. In accordance, they have been found to accumulate in several age–related pathologies, e.g. atherosclerosis, intervertebral disc degeneration, chronic wounds, etc. Interestingly, it is proposed that senescence represents an anti–cancer barrier. However, senescent cells when formed seem to enhance tumor growth. In this vein, we have shown that ionizing radiation provokes premature senescence in stromal fibroblasts, which in turn promote the growth of cancer cells in vitro and in immunocompromised cells in vivo. This phenomenon is partly mediated by the overexpression of MMPs by senescent cells.

Cellular senescence represents a problem in cell replacement therapies, as in the latter several duplications of the cell population is needed as to acquire a considerable number of cells to be transplanted. However, this leads inevitably to an increased percentage of senescent cells. On the other hand, mesenchymal stem cells have been proposed as an alternative cell source for these therapies. Interestingly, mesenchymal stem cells have a short in vitro lifespan, and when senescent express inflammatory markers and have a decreased ability for differentiation.

According to the above, the development of strategies for the inhibition of the senescence process and/or the reversal of the inflammatory phenotype of senescent cells is of obvious importance. The use of various approaches based on young cells or on novel pharmacological compounds will be discussed.

L 26

Interaction of collagen 1 with three small leucine–rich proteoglycans

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Collagen 1 and 2 interact with decorin, fibromodulin and biglycan, all small–leucine rich proteoglycans (SLRPs). We showed in the past that several cyanogen bromide peptides from collagen 1 and 2 are able to interact with decorin (both with the whole proteoglycan and its core only) (ref. 1), fibromodulin (ref. 2) and biglycan (unpublished results), thus demonstrating that collagen 1 has multiple binding sites for the three SLRPs. Most mutations in the α chains of collagen 1 cause Osteogenesis Imperfecta (OI), where mainly bone is involved with a phenotype spanning from mild to extremely severe.

We recently demonstrated that some mutations in the α2(I) chain of collagen 1 influence the ability of this collagen to bind SPARC (ref. 3). Here we report the influence of the same mutated collagen samples on the binding to the three above cited SLRPs. The results show that only a very low ability to bind the three SLPRs remains for some mutated collagen samples (work in collaboration with J.C. Marini and S. Leikin, NIH, Bethesda, MD, USA). This residual ability is much lower that 50% of the normal control, at variance with the value of 50% expected for mutations in the α2(I) chain of collagen 1.

Any possible explanation of the results must take into account several facets of bone pathophysiology. The following aspects will be discussed: any gross structural abnormality in the mutated samples; the presence of overmodifications (mainly overglycosylation of the mutated collagen 1 trimers); the results obtained on null mice for decorin, fibromodulin and biglycan and eventually double–null mice; recent papers on bone physiology under stress (*e.g.*, ref. 4); recent papers demonstrating that the mineral phase of bone is able to interact with some glycosamino–glycans (*e.g.*, ref. 5).

As an over–simplification, we can postulate that the complex "collagen 1 – a SLRP – hydroxyapatite crystals" constitutes a module of bone physiology. The loss of binding ability of the protein core of the SLRPs to collagen fibrils uncouples the mineral phase from the fibrils and can contribute to cause a bone pathology, such as a OI.

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Synthesis and Development of Bioactive Natural Products

L 27

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Throughout the ages humans have relied on nature for their basic needs for the production of medicines. Recently a study using US–based prescription data from 1993, demonstrated that natural products still play a major role in drug treatment, as over 50% of the most–prescribed drugs in the US had a natural product either as the drug, or as a 'forebear' in the synthesis or design of the agent.[1] Natural products (i.e. secondary metabolites, naturally occurring small organic molecules) continue to play a pivotal role in modern drug discovery. Several drugs like antibiotics, antimalarials, lipid control agents, muscle relaxants, antidepressants, immunesuppressants, anti–inflammatory agents and anticancer drugs are of natural origin. They changed medicine and improved life expectancy as well as the quality of life of patients.

In my talk I will present some examples of synthesis of "hot" bioactive natural products and their analogues as well as their transformation into useful drugs. Biological aspects and medical perspectives will be discussed. [2]

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L
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Quantitative approaches to probe Integrin cluster diffusion and downstream signaling in native like 3D environments

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Cell–matrix interactions play a central role in maintaining homeostasis or regulating disease progression. In this regard, the role of integrin receptors in mediating adhesion, migration and differentiation is particularly important. However, most studies to date have looked at the complex mechano–chemical interactions between integrins and their extracellular ligands in artificial 2D environments. Our current work aims to address this critical void in our understanding by developing quantitative computational and experimental tools to study integrin clustering, clustered diffusion on membranes and consequent downstream signaling in native like 3D environments. Our results suggest a possible mechanism for clustered integrin diffusion as a function of membrane and matrix properties. We also observe a balance between structure, mechanics and porosity in maximizing kinase and MMP responses in 3D. Overall, our results not only emphasize the need for studying fundamental molecular mechanics in 3D but also highlight new mechanisms observed in integrin–ligand interactions in 3D.

Differentiation of heart valve progenitor cells is dependent on communication between periostin–integrin and hyaluronan–induced signaling

L 29

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Studies have shown heart valves and septa are derived from cushion tissues that are formed by an endocardial transformation to mesenchyme or EMT in atrioventricular (AV) junction and the ventricular outflow track. The post–EMT development processese e.g. proliferation, migration, differentiation and compaction – that serve to remodel the primitive, mesenchymalized prevalvular and preseptal cushions into fibrogenic tissues are much less understood.

We propose to identify extrinsic and intrinsic signaling mechanisms that regulate the post–EMT prevalvular cushion development that may explain human cardiac malformations. Accordingly, we have focused on effector–genes like periostin (PN) that is a member of matricellular proteins and is a proven downstream target whose early lethality has suggested roles in valvuloseptal development. This study demonstrates that: (1) that PN increases HA secretion via PN/integrin/PI3K signaling in embryonic valve cells, (2) PN–induced signaling communicates with HA/CD44 interacted signaling, (3) PN interacts with integrin β3 mediated FAK/MAPK pathway linked to, PI3K mediated cell survival to stimulate adhesion/growth of AV cell, (3) selective kinase inhibitors can delineate the signaling pathways involved in PI3K– induced PN, and HA synthesis, (4) PN/integrin–β1 interaction activate cytoskeletal associated filamin A (FLNA), and (5) finally we demonstrate that PN and HA are co–expressed in wild type E16.5 mouse valve sections, whereas HA expression is suppressed in E16.5 PN null mouse valve sections. The significance of our findings would be that it addresses morphogenetic processes which have clinical relevance to a spectrum of human malformations that include atrial septal defects (ASDs), atrioventricular septal defects (AVSDs).

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L 30

Metalloproteinases and their roles in the tumour microenvironment

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Epithelial tumours evolve in a multi–step manner, involving both inflammatory and mesenchymal cells. Although intrinsic factors drive malignant progression, the micro–environment of neoplastic cells is a major feature of tumorigenesis. Extracellular proteinases, notably the Metzincin clan of metalloproteinases (MPs), including the matrix metalloproteinases (MMPs), the disintegrin metalloproteinases (ADAMs) and the disintegrin metalloproteinases with thrombospondins (ADAMTSs) play important roles in the cellular modulation of the microenvironment of tumours. They degrade or modify the extracellular matrix as well as many cell surface or membrane associated adhesion and signalling proteins, such as growth factors and their receptors. Events as diverse as cell proliferation, invasion and apoptosis, gene transcription and cellular metabolism are ultimately under extracellular proteolytic control. Hence, proteolytic modification is a key effector of events associated with both normal developmental processes and in disease. Apart from overt degradation, molecules can be **clip**ped to activate /inactivate, **cut** to generate neo epitopes with different activities or they can be **shed** from the cell surface to modify their spectrum of activity. In this way very rapid changes in cellular behaviour can be effected and stringent levels of control are therefore required. Regulation of MPs at the transcriptional level is common and is backed up by intracellular trafficking, focussed localisation at the cell surface or on the pericellular matrix, and eventually endocytosis. Activation of the MPs by proteolysis means interaction with other proteolytic systems, leading to a complex web of activities. The major natural inhibitors of the MPs are the tissue inhibitors of MPs, TIMPs which are also subject to many cellular regulatory mechanisms. To fully understand MPs therefore is a demanding process of elucidating specific functions and regulatory processes. This is essential for the development of inhibitors as therapeutic agents. A further problem for such endeavours is the basic similarity of the active site structure of the Metzincins which presents considerable challenges to attempts to design selective synthetic inhibitors. An alternative strategy is the consideration of the role of the extra–catalytic domains that are determinants of MP specificity at a variety of levels. Dissecting the relationships between structure and function of these interaction sites is allowing the development of new approaches to the inhibition of enzyme activity.

Transdifferentiation of mesenchymal tissues; with special focus on Syndecan–1 in cell membrane and nucleus

L 31

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Mesothelial progenitor cells and mesenchymal cells are able to transdifferentiate between different cell phenotypes depending on the local environment and depending on their proteoglycan profile. 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. The expression of syndecans is strictly regulated in a tissue dependent manner, but little is known about how they influence the malignant behaviour of mesenchymal tumours. Therefore we aim to gain further insight in the structure–function relationship of syndecan–1 in the transdiffererentiation and proliferation of mesenchymal tissues and malignant mesenchymal tumors.

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. This was the first evidence for the nuclear translocation of the syndecan–1 molecule, and the concept have recently got broad international acceptance. 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 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. Experimental settings targeting crucial cellular functions such as tumor cell proliferation, adhesion and migration have high therapeutic potential and are addressed in this project.

L
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ECM derived molecular fragments with roles in the innate immune response

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The extracellular matrix has an overall similar composition between tissues where also the detailed organization of tissue elements is similar although their gross organization can be very different. Major tissue elements are large very polyanionic proteoglycans providing a fixed charge density important for water retention and fibrillar networks of collagen fibrillar structures to provide tensile properties, in tissues experiencing compressive load important for maintaining overall volume and distributing load and in tissues experiencing tensile load taking up this load. The collagen fibrils contain a number of molecules with major function in crossbridging to other matrix molecules including other fibers, important for tissue properties and function.

The setting up and disassembly of the extracellular matrix are organized by the cells in the tissue that has a number of receptors recognizing matrix molecules and providing different signals depending on combinations of receptors involved. Examples show that different matrix proteins contain domains that will differently affect cells such that modulation will be different between fragments containing one of the domains and again different to the intact protein.

In pathology proteins in the extracellular matrix are fragmented proteolytically and bioactive fragments are released that can modulate the activity of the cells in the tissue, but also importantly activate various pathways of an inflammatory response. Examples in the innate immune response are different fragments of matrix proteins as activators of the classical and alternative pathways in complement as well as direct binding to cellular receptors of inflammatory cells. The ensuing inflammation is likely to enhance and/or perpetuate tissue degradation.

Selected Talks/ Abstracts

Extracellular matrix perturbation in human cells and tissue affected with Down syndrome

ST 01/P1

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Down syndrome (DS) is a common birth defect characterized by the trisomy of chromosome 21. Studies have shown that this extra copy of chromosome causes alterations in proteins whose genes are located in chromosome 21 and this phenomenon is correlated to specific phenotypes. Type VI collagen (COLVI) is formed from three chains, the α1(VI) and α2(VI) whose genes are located in chromosome 21 and the α3(VI) whose gene is located in chromosome 2. In this study, we sought to explore the different amount and localization of COLVI, the gene expression of the three chains and the hyaluronan (HA) production in human umbilical cords of euploid and DS fetuses. We then used human skin fibroblast cell lines obtained from healthy and DS individuals in order to elucidate the correlation between COLVI and hyaluronan. We also focused our attention in ER stress.

Using the technique of immunofluorescence we revealed that umbilical cords of DS were richer in COLVI and distributed in the subamniotic zone and the vessels. In addition, confocal microscopy using antibody against α1 and α2 chains showed that they are mainly localized in the cytoplasm of both normal and DS fibroblasts, whereas western blot analysis shows a clear higher amount of these two chains in DS cells.

The gene expression of α1 and α2 chains is found to be higher in DS fibroblasts and umbilical cord, respect to normal cells and tissue, confirming the hypothesis that the extra copy of chromosome results to a higher expression of genes in this chromosome. Interestingly, DS fibroblasts showed also to produce more HA than normal cells and to express more HAS2 and less HYAL2 genes. Increase amounts of COLVI did not show significant changes in ER stress.

In order to investigate if the increase amount of COLVI provokes alteration in HA production, we silenced the α2 chain of COLVI in both normal and DS fibroblasts. Although in normal cells the HAS2 and HYAL2 did not change significantly, in DS fibroblasts HAS2 decreased 80% and HYAL2 62%, showing that overexpression of COLVI is closely related to the HA metabolism.

These results may be an explanation for the alteration of the matrix composition and the morphology of Down syndrome umbilical cord.

ST 02/P2

DNA methylation: a possible implication in MMP–13 deregulation in osteoarthritis?

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Osteoarthritis (OA) is a degenerative joint disease characterized by a progressive and irreversible loss of the cartilage that covers the bone ends. A breakdown in the normal homeostasis of cartilage extracellular matrix contributes to the pathology onset by enhancing the degradative process. The matrix metalloproteinases (MMPs) are involved in cartilage degradation and are significantly upregulated in end–stage OA [1]. Prevention of cartilage degradation using MMP inhibitor still remains a challenge notably because of their lack of specificity and the associated adverse effects [2]. Recent studies have underlined the possible implication of DNA methylation in the regulation of the MMP–3, MMP–9, MMP–13 and ADAMTS–4 [3]. Our group is particularly interested in the MMP–13 which is considered to be the most potent type II collagen–degrading enzyme and thus represents an attractive pharmacological target in joint diseases. Herein, we have investigated DNA methylation as a possible mechanism responsible for MMP–13 deregulation in OA. We determined by pyrosequencing the methylation status of the *MMP–13* promoter region in control (NOF – neck of femur fracture) and OA cartilage and showed a significant decrease of methylation in a confined region of the *MMP–13* promoter encompassing three CpG sites (–136, –115 and –110; relative to the transcription starting site). Interestingly, this region appears to be also demethylated in human articular chondrocytes (HACs) treated with a demethylating agent 5–aza–2'–deoxycytidine (Aza) *versus* untreated cells, which correlates with an upregulation MMP–13. Together, these data suggest that DNA methylation may play a role in the regulation of MMP–13 in OA. To confirm our hypothesis, we generated *MMP–13* promoter constructs where the cytosine of each the three CpG were individually mutated to a thymine. We next analysed the effect of the mutation and the impact of DNA methylation on the *MMP–13* promoter activity (after *in vitro* methylation) using luciferase reporter assays. We also carried electrophoretic mobility shift assays and identified a new transcription factor whose binding on the *MMP–13* promoter is sensitive to the methylation status of one single CpG site (–110). Our results may be of particular relevance in understanding the pathways involved in regulation of MMP–13 expression in joint diseases and contribute to the development of new therapeutic strategies in order to prevent cartilage destruction.

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Endogenous TSG–6 is Crucial for the Development of Antigen Induced Pulmonary Hyaluronan deposition, Eosinophilia, and Airway Hyperresponsiveness

ST 03/P3

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Hyaluronan (HA) deposition is often correlated with mucosal inflammatory responses where HA mediates both protective as well as pathological responses. By modifying the HA scaffolding, tumor necrosis factor–α (TNFα)–induced protein–6 (*Tnfaip6*; also known as TNFα–stimulated gene–6 (*Tsg6*)) is thought to potentiate anti–inflammatory and anti–plasmin effects that are inhibitory to leukocyte extravasation. We have recently reported the temporal association between pulmonary HA deposition and pulmonary eosinophilia during the induction and propagation of acute antigen induced pulmonary eosinophilia and airway hyperresponsiveness (AHR). In this study, we examine the role of endogenous TSG–6 in the pathophysiological responses associated with acute pulmonary eosinophilia. When compared to wild–type littermate controls, *Tsg6–/–* mice exhibited attenuated inflammation marked by a significant decrease of pulmonary HA concentrations measured in the brochoalveolar lavage (BAL) and lung tissue. Interestingly, despite the equivalent induction of both humoral and cellular T helper type 2 (Th2) immunity, and the comparable levels of cytokines and chemokines typically associated with eosinophilic pulmonary inflammation, airway eosinophilia was significantly decreased in *Tsg6–/– mice*. Most importantly, contrary to their counterpart wildtype littermates, *Tsg6–/– mice* were resistant to the induction of AHR and manifested improved lung mechanics in response to methacholine challenge. In summary, our study demonstrates that endogenous TSG–6 is dispensable for the induction of Th2 immunity, nevertheless TSG–6 is essential for the robust increase of pulmonary HA deposition and propagation of acute eosinophilic pulmonary inflammation, and the development of AHR. Thus, TSG–6 is implicated in experimental murine models of allergic pulmonary inflammation and is likely to contribute to the pathogenesis of asthma.

ST 04/P4

Hyaluronan signaling through RHAMM in fibrosarcoma cell adhesion

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Hyaluronan (HA), a nonsulfated high molecular weight glycosaminoglycan, is one of the major ECM components that provide tissue homeostasis. HA signaling, through its specific receptors CD44 and RHAMM has been linked to the promotion of cell motility, adhesion, migration and metastasis. Previous studies have suggested overexpression of RHAMM in tumour development and a prognostic significance of its expression several tumour types. In the present study we investigated the effect HA signalling, through its specific receptor RHAMM, on the capacity of a fibrosarcoma cell line (HT1080) to adhere onto fibronectin. An *in vitro* adhesion assay showed that low molecular weight HA (LMWHA) significantly increased ($p \leq 0.05$) the adhesion capacity of HT1080 cells, whereas exogenous addition of high molecular weight HA (HMWHA) inhibited cell adhesion. HT1080 cells were found to express RHAMM at both protein and mRNA level using Western blot and real time PCR analysis, respectively. Three different RHAMM isoforms were detected at 95, 73 and 45 kDa. Treatment with LMWHA increased the protein expression of the 95 and 73 kDa (53% and 37% respectively) as well as RHAMM transcript levels ($p \leq 0.05$) in HT1080 cells. In order to study the role of RHAMM on the HA–induced effects on HT1080 adhesion capacity, RHAMM expression was inhibited using RNA interference. The ability of HT1080 RHAMM– deficient cells to adhere was significantly decreased ($p \leq 0.001$) compared to control cells. In addition, their adhesion capacity did not change with the presence of LMWHA. Furthermore, LMWHA–dependent adhesion was diminished when an ERK inhibitor was added suggesting a possible role of ERK signaling in the regulation of HT1080 cell adhesion. Importantly, RHAMM inhibition also reduced ERK1/2 and FAK phosphorylation. In conclusion, RHAMM is suggested to mediate LMWHA induced fibrosarcoma cell adhesion through the activation of FAK and ERK1/2 signaling pathways.

Interaction between Staphylococcus aureus and Extracellular matrix protein bone sialoprotein induces an immunological response in patients

ST 05/P5

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Staphylococcus aureus bone sialoprotein binding protein, Bbp, has been a field of research for the group since two decades. The interaction with the host extracellular matrix glycoprotein, bone sialoprotein, BSP, of bone tissue was characterized, and the Bbp of *S. aureus* O24 was purified. Bbp is a cell surface protein in the MSCRAMM type of adhesive protein family, and a member of the Sdr family of cell wall anchored staphylococcal proteins. Bbp was shown to specifically interact with BSP, an interaction found preferentially in clinical *S. aureus* isolates from osteitis and septic arthritis. In patients with infection affecting bone tissue it induces an immunological response, with increased IgG antibody levels compared to pateints with staphylococcal soft tissue infection only. Thus we believe that antibodies to bone sialoprotein binding protein indicate infectious osteomyelitis. (Persson L., Johansson C., and C. Rydın. Clin.Vacc. Immun. (2009) 16(6):949–52). Antibody levels against Bbp in children suffering from staphylococcal infection as well as the influence of nasal carriage of *S. aureus* on antibody levels in children have been studied. A mutant devoid of Bbp Δ*bbp* was obtained by allelic replacement of *S. aureus* O24, and compared with the wild type in a murine skin abscess model of infection. We found that Δ*bbp* induced sepsis in mice and caused significantly higher mortality than the wild type strain. Our hypothesis that the immobilization of a Bbp expressing *S. aureus* strain cleared bacteria from the circulation and thus renderd the bacteria less fatal.

ST 06/P6

Generation of transgenic mouse strains overexpressing integrin α11 in muscle tissues

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Integrin α11β1 is a collagen–binding integrin normally expressed in fibroblasts at sites characterized by high microenvironment mechanical stress, and which in mice is uniquely needed in periodontal ligament fibroblasts during incisor eruption. Little is known about α11 in pathological situations, except that α11 is up regulated in the tumor stroma of non–small cell lung cancer.

To learn more about α11 function in the cellular and tissue context, we have generated transgenic mice misexpressing α11, governed by the chick β–actin promoter. Four strains (actin–α11 TG1 – 4), all over–expressing α11 in muscle tissues, were partially characterized, and the strain with the best breeding characteristics (actin α11–TG1) was studied in more detail. As judged by immunohistochemistry of mouse embryos, α11–TG was over–expressed at the highest level in adult muscle cells in heart, skeletal muscle and lung cells not normally expressing α11. Western blotting and immunohistochemistry of selected tissues confirmed that the high transgenic expression was retained in adult muscle tissues.

The transgenic strain actin α11–TG1 will next be analyzed for potential phenotypic defects in muscle tissues. Isolation of primary cells from the transgenic mouse strain will be used to investigate the function of α11 in the context of primary cells generating high internal mechanical stress (i.e. muscle cells).

Serglycin is secreted by aggressive breast cancer cells and inhibits the complement system

ST 07/P7

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The major proteoglycan that is synthesized from hematopoietic cells is serglycin. Although its participation in immune responses is well defined, the role of serglycin in malignancies is a new field of interest. In previous studies, the constitutive secretion of serglycin by multiple myeloma cells has been shown to inhibit both bone mineralization and complement system activation. The aim of our study was to examine the role of serglycin in breast cancer biology. The expression of serglycin at transcriptional levels in cancer cell lines showing different metastatic potential was examined by RT–PCR analysis. The high expression of serglycin in the most aggressive breast cancer cells MDA–MB–231 was revealed compared to the low expression in MDA–MB–468 and MCF–7 cells, which show low metastatic potential. Immunohistochemistry in tissues from breast cancer patients confirmed the expression of serglycin by breast cancer cells. The localization of serglycin was shown to be mostly cytoplasmic and less on the cell membrane in MDA–MB–231 and tumor cells in breast cancer biopsies. Serglycin was constitutively secreted in detectable levels only in aggressive MDA–MB–231 cells and was isolated from the culture medium by combined anion–exchange and gel–permeation chromatographies. Isolated serglycin carries CS–side chains as demonstrated by SDS–PAGE and capillary electrophoresis before and after treatment with specific enzymes. Solid phase microtiter binding assays revealed the interaction of serglycin isolated from MDA–MB–231 cells with C1q with similar affinity to that of serglycin isolated from myeloma cells. Hemolytic assays confirmed the inhibition of the classical pathway of complement system by the isolated serglycin. Our results show for the first time that serglycin is highly expressed and constitutively secreted from the aggressive breast cancer cell line MDA–MB–231. Moreover, it carries CS–side chains, binds C1q and inhibits the classical pathway of complement activation implying a role of serglycin in the protection of cancer cells against immune system attack.

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ST 08/P8

Characterization of the murine oral squamous cell carcinoma cell line AT84 stably overexpressing uPAR

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Cancers of the oral cavity are associated with poor prognosis, and almost half of the patients diagnosed with oral squamous cell carcinoma (SCC) will die from the disease. Compared to its counterpart in skin, one main differing feature is the ability of oral SCC to metastasize. A prerequisite for metastasis is the capability to invade the surrounding tissue and penetrate into the nearest blood– or lymphatic vessel. This initial step in metastasis requires several extracellular matrix degrading enzymes. One such enzyme is uPA (urokinase–type plasminogen activator), involved in pericellular proteolysis, where high levels are correlated with poor prognosis in several cancers. Upon binding of pro–uPA to its extracellular receptor uPAR, the enzyme is cleaved and activated. uPA further activates matrix metalloproteases (MMP's), also needed for matrix degradation. In addition, ample evidence shows that binding of uPA to uPAR triggers receptor–mediated cellular signaling. uPAR lacks a membrane spanning domain hence signal transduction has to be mediated through one or several membrane partners. uPARs role in the regulation of cell adhesion, migration and proliferation has been linked to its interplay with membrane partners such as integrins, EGFR, caveolin and G–protein coupled receptors (e.g. FPRL1). To better understand the role of uPAR in the invasive growth and metastasis of oral SCCs, the mouse oral SCC cell line, AT84 [1, 2], was stably transfected with uPAR. Overexpression of uPAR resulted in down regulation of epithelial marker E–cadherin and the cells display a more mesenchymal appearance, indicating epithelial–mesenchymal transition (EMT). In addition, elevation of uPAR expression increased migration in a wound healing assay. In contrast to previous reports, an increase in MMP–9 activity was not observed in these cells, although an increase in overall proteolytic activity could be found using gelatin as substrate. The cells were reintroduced into the C3H mouse to observe the role of uPAR in cancer progression in vivo. Results from the analysis of the host–tumor interactions within this syngenous mouse model will be presented.

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Role of Hyaluronan synthase 2 (HAS2) in TGFβ–induced epithelial–to–mesenchymal transition (EMT) of NMuMG normal mammary epithelial cells

ST 09/P9

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Epithelial to mesenchymal transition (EMT) is a cellular program that renders epithelial cells contact–independent and migratory. Although EMT is crucial during embryonic development, when activated in tumour cells the EMT program tends to render cells metastatic. Transforming growth factor β (TGFβ) is a potent inducer of EMT in many cells, including NMuMG mouse mammary epithelial cells. Lately, over–expression of the hyaluronan synthase HAS2 has also been implicated in EMT in mesothelioma and mammary epithelial cells[1,2]. It is however not yet established whether or not this effect is independent of the TGFβ–pathway.

Here we show cross–talk between the signaling pathways of hyaluronan and TGFβ in NMuMG cells. Using immunoprecipitation, we have demonstrated that the hyaluronan receptor CD44 and the TGFβ–receptor I form a complex in NMuMG cells. Furthermore, stimulation of NMuMG cells with TGFβ induced hyaluronan synthesis by an 8–fold induction of HAS2 mRNA expression. This effect was dependent on the activation of both the Smad– and the p38 pathways. The hyaluronan synthesis was specific for HAS2 since HAS1 and HAS3 mRNA expression levels were not affected. The up–regulation of HAS2 was independent of the expression or hyaluronan–binding activity of CD44.

To elucidate the role of hyaluronan in EMT, we compared the effect of TGFβ alone to that of TGFβ in combination with HAS2 siRNA and found that TGFβ failed to induce EMT in the absence of HAS2. This inhibition was not achieved by removal of the hyaluronan by *Streptomyces* hyaluronidase or by blocking binding of hyaluronan to CD44. Experiments are currently going on to establish the role of HAS2 in TGFβ–induced EMT.

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ST 10/P10

The matrix component biglycan induces generation of reactive oxygen species by signaling through TLR2/TLR4 receptors and the inflammasome

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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). 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. However, the downstream signaling mechanisms of biglycan–mediated maturation of IL–1β remain still unclear. Reactive oxygen species (ROS) are generated by TLR agonists and stimulate maturation of IL–1β due to uncertain molecular mechanisms. Therefore, it is tempting to speculate that biglycan might induce the generation of ROS, which in turn might induce inflammasome activation and maturation of IL–1β.

The objectives of this study were threefold: 1) to investigate biglycan–mediated generation of ROS (mechanisms and sources); 2) to characterize the influence of biglycan on ROS half–life; 3) to elucidate how ROS induced by biglycan might influence the maturation of IL–1β.

Methods 1) *In vitro*: Primary murine peritoneal macrophages from WT, biglycan–deficient and –overexpressing mice, knockout mice of inflammasome components and NOX subunits in the presence of pharmacological inhibitors of NOX– and mitochondria–derived ROS generation; 2) *In vivo*: WT and MRL/*lpr* lupus mice deficient for or overexpressing biglycan.

Results In macrophages biglycan triggered ROS production in a TLR2/4–dependent manner. Pharmacological inhibition of NOX or H_2O_2 showed that biglycan–induced synthesis of pro–IL–β and IL–1β maturation are both 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. Preliminary data suggest additional involvement of mitochondria in biglycan–dependent ROS generation. *In vivo* in MRL/*lpr* lupus mice 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. Furthermore, by generating MRL/*lpr* mice, either lacking or overexpressing biglycan, we provided *in vivo* proof that biglycan regulates both *Nox2* and *pro–Il–1β* expression and is also involved in IL–1β maturation. In addition, the superoxide dismutases SOD1 and SOD2 are regulated in macrophages and in MRL/*lpr* mice in a biglycan–dependent manner.

In conclusion, here we show a novel role for the matrix component biglycan as a trigger of mitochondrial and NOX2/4–dependent ROS generation upstream of the synthesis of pro–Il–1β and the inflammasome/caspase–1–mediated maturation of IL–1β.

Targeting epidermal growth factor receptor in colon cancer

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The epidermal growth factor receptor (EGFR) is a member of the HER family receptors. The binding of EGF enables the EGFR signalling. Receptor antagonists, targeting EGFR, have been of high interest, as overexpression of EGFR is associated with colorectal neoplasms. Genetic alterations of the intracellular effectors involved in the EGFR–related signalling pathways can play a critical role in the effectiveness of anti–EGFR therapies.

The aim of the present study was 1) to determine the cellular responses mediated by the activation of EGFR in colon cancer cells (HCT–8, wild type KRAS) and 2) evaluate the effect of the fully human monoclonal antibody Panitumumab (Pmab), specific for targeting the extracellular domain of EGFR, on cell proliferation, intracellular signaling, gene expression of matrix molecules implicated in cancer progression as well as its effect on functional properties of colon cancer cells.

First of all, the EGFR phosphorylation is significantly highly induced by EGF (30 fold) and this activation is almost completely blocked by Pamb at the levels of control. Cell proliferation showed that the effect of PmAb depends on the time of exposure at cell culture. Following, we found that EGF significantly induces the proliferation of HCT–8 cells; this stimulatory effect is blocked by Pmab, indicating a significant inhibitory action of Pmab on the EGF–induced proliferation of colon cancer cells. At the functional level, we assayed the effect of Pmab on colon cancer migration, invasion and intracellular signaling. Notably, directional cell migration induced by EGF is significantly inhibited by Pmab. In consistency with cell migration, tyrosine phosphorylation of Focal adhesion kinase (FAK) is increased in the presence of the Pmab as compared with the dephosphorylation of FAK by EGF. In addition, the invasive potential of HCT–8 cells mediated by EGFR–EGF is suppressed, when EGFR is blocked by Pmab. RT–PCR analysis showed that EGF affects the expression pattern of certain biomacromolecules of the extracellular matrix of important in cancer progression, such as metalloproteinases and proteoglycans, attributing to the observed cellular properties.

Conclusively, EGFR is a determining factor for colon cancer cell behavior. Pmab is a potent blocker of EGFR activation and its signaling pathway even after exposure to EGF, resulting in a significant inhibition of cancer cell proliferation in early stages of growth, migration and invasiveness of colon cancer cells.

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ST 12/P12

EMMPRIN modulates TGFβ signalling pathway: implications in tumour stroma activation

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Cancer development is not only the consequence of somatic mutations of cancer cells but also of tumour stroma activities. Cancer cells–stroma interactions lead to the activation of quiescent fibroblast and their differentiation into myofibroblasts, also called Cancer Associated Fibroblasts (CAF), characterized by the expression of specific markers, such as alpha smooth muscle actin (αSMA). In parallel, CAF also produce a large amount of proteinases such as MMPs. Extracellular matrix metalloproteinase inducer (EMMPRIN), a cell surface glycoprotein enriched on cancer cells, is known to induce MMPs within fibroblasts following tumour–stroma interactions. EMMPRIN is also involved in fibroblast differentiation by its capacity to induce αSMA expression. As TGFβ, a well–known growth factor implicated in tumour metastasis, induces also fibroblasts differentiation and regulates MMPs expression, this study was aimed at defining if TGFβ effects on tumour stroma formation can be mediated by EMMPRIN. Using fibroblasts co–transfected with control or EMMPRIN siRNA and the (CAGA)9–lux reporter construct, our results show that TGFβ–Smad2/Smad3 dependent transcription of luciferase reporter gene is decreased in fibroblasts inhibited for EMMPRIN. Furthermore our results suggest that EMMPRIN inhibition reduces Smad2 phosphorylation. We also demonstrate a co–localization of EMMPRIN and TGFβ receptors which suggest that EMMPRIN effects on Smad signalling may occur through receptor interactions. Stimulation of collagen lattice contraction by TGFβ was inhibited when lattices were populated with fibroblasts derived from EMMPRIN KO mice. In parallel, MMP–2 and αSMA inductions by TGFβ also are inhibited in EMMPRIN KO fibroblasts. Taken together, our results suggest that EMMPRIN expression in fibroblasts is important for the formation and the functions of tumour stroma. Targeting EMMPRIN within tumour stroma may represent a novel strategy to inhibit cancer cells invasion and metastasis.

Interplay between syndecans and cadherins in breast carcinoma

ST 13/P13

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Alteration in cell–cell adhesion is important for tumor cells to acquire invasive and metastatic characteristics. Syndecans and cadherins are transmembrane glycoproteins, which have profound roles in regulating cell adhesion and invasion. Aberrant expression of syndecans and cadherins has been shown implicated in tumorigenesis and metastasis of breast cancer. Elevated expression of syndecan–4 is associated with estrogen, progesterone receptor positive status and therefore good prognosis in breast cancer. However, the mechanism that underlies the interplay between syndecans and cadherins and its roles during malignant transformation is still poorly understood. In this study, we found that syndecan–4 overexpression as well as exogenous expression of heparan sulphate and heparin in MDA–MB 231 human mammary carcinoma cells impaired cell invasion into type I collagen in vitro. Moreover, in the presence of exogenous heparan sulphate and heparin, these cancer cells increase spreading morphology with organized actin stress fibers. Strikingly, expression of OB–cadherin at cell junctions is increased following exogenous expression of heparan sulphate and heparin. These data suggest that syndecans and cadherins could be attractive target in breast cancer.

ST 14/P14

Src, but not FAK, regulates cellular migration in three dimensional environment

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We developed a three–dimensional (3D) cell culturing model, using the GD25β1 cell line that can proliferate past 100% confluence. Major advantage of this system is that the three dimensional environment is formed by natural extracellular matrix (ECM), synthesized and organized by the fibroblasts unlike the commercially available, artificial 3D model systems formed by collagen gels, Matrigel, etc.

Investigating the cell migration in 3D we employed a novel in vitro wounding assay. Cells grown in three–dimensional conditions showed a faster migration rate compared to 2D conditions. Our results demonstrate no change in the activation of the focal adhesion kinase (FAK) in 3D compared to 2D environment and no significant change after experimental wounding. At the same time the Src kinase showed no significant change in its activity compared to monolayer cultures and a two fold increase in Tyr418 phosphorilation after wounding. Immunoflourescent labeling of actin after experimental wounding revealed thinner stress fibers and increased filopodia formation. It is known that Src regulates the activity of the small GTPases Rho and Rac – key players in the actin cytoskeleton organization – via the guanine nucleotide exchange factor (GEF) p190RhoGAP. To investigate our hypothesis that Src, but not FAK, regulates the cell migration in 3D environment we examined the state of activation of the small GTPases RhoA and Rac1 in our cell culturing model. A two fold increase of the activity of the RhoA GTPase compared to conventional monolayer cell cultures was revealed, as well as redistribution of both GTPases in a distinct type of membrane fractions – lipid rafts.

Our aim for the future is to develop this tissue–like culturing system as an advanced model for in vitro studies of the processes of wound healing and scar formation.

Imbalance of MMP–2 and MMP–9 expression versus TIMP–1 and TIMP–2 reflects increased invasiveness of human testicular germ tumors

ST 15/P15

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Testicular germ cell tumors (TGCTs) are histologically divided into seminoma and non–seminoma type tumors of different invasion rates and clinical outcome. Little is known of their cellular characterization and the mechanisms mediating their invasion potential. Metalloproteinases–2 and–9 (MMP–2 and MMP–9) have been associated with the invasive phenotype and poor prognosis in several malignancies. Here we investigated MMP–2 and MMP–9 expression in both testicular tumor tissues and testicular germ cell lines of seminoma and nonseminoma origin. Immunohistochemistry and zymographic analysis showed that tumoral tissues express significantly higher levels of both MMP–2 and MMP–9 as compared to normal testis. Active forms of these enzymes were detected only in the tumor tissues. Three cell lines representative of the different tumor types, JKT–1 seminoma, NCCIT teratocarcinoma and NTERA2–D1 embryonal carcinoma cells were also evaluated for the expression of MMPs at both the mRNA and protein levels. The more invasive non–seminomatous teratocarcinoma and embryonal cells express considerably higher levels of MMP–2 and MMP–9 compared to seminoma cells exhibiting lower invasiveness. Inverse relation was observed between the expression of their endogenous tissue inhibitors (TIMP–1 and TIMP–2) and invasiveness, with the highest levels of both TIMPs expressed in the less invasive seminoma cells. Marimastat, a specific MMPs inhibitor inhibited invasion through Matrigel in all cell lines. Notably, the highest inhibition was observed in the more invasive NTERA2/D1 and NCCIT cells presenting the highest ratio of MMP–2 and MMP–9 versus TIMP–1 and TIMP–2. These results highlight the importance of MMP–2 and MMP–9 in the invasiveness of testicular cancer cells and suggest that their levels, versus those of TIMP–1 and TIMP–2, may represent potential biomarkers for testicular cancer diagnosis.

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ST 16/P16

Proteomic Identification of CD44 Interacting Proteins: the CD44–iASPP–p53 axis

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The adhesion/homing molecule CD44, the major cell surface receptor for the glycosaminoglycan hyaluronan, affects cell adhesion, proliferation and migration, and has been implicated in chronic inflammation and tumorigenesis. The extracellular and the cytoplasmic domains of CD44 can associate with a large array of molecules. The cytoplasmic tail and its association with partner molecules are crucial for the accessory functions of CD44 in signal transduction and its subdomain localization during cell migration.

The aim of the present study was to identify new candidate molecules that interact with the cytoplasmic tail of CD44 and may be important for the multiple effects of CD44 on diverse cellular functions. Nonphosphorylated or phosphorylated peptides from the intracellular CD44 C–terminus, were immobilized and used as baits. Interacting proteins were subjected to SDS–gel electrophoresis and were identified by MALDI–TOF mass spectrometry. Several interaction partners were identified, including proteins involved in cytoskeletal reorganization, transcription, endocytosis, and intracellular transport.

NFkappaB–interacting protein 1 or iASPP [inhibitor of Apoptosis Stimulating Protein of p53] was among the newly identified CD44 interacting proteins. iASPP inhibits the p53–mediated apoptosis in mammalian cells and could allow cancer cells to bypass the tumor–suppressor functions of p53 and the ASPP proteins. Co–Immunoprecipitation studies confirmed the *in vivo* CD44–iASPP interaction in human normal and telomerase–immortalized fibroblasts as well as in the carcinoma cell line, HepG2. The epitopes involved in CD44–iASPP interaction were characterized using truncated forms of iASPP containing specific domains. The analysis revealed that the ankyrin regions of iASPP interact with the cytoplasmic domain of CD44. The findings of this study indicate a new possible regulatory role of CD44 on the apoptotic functions of p53 through iASPP. Notably, endogenous CD44/iASPP complexes were hyaluronan– and PDGF–BB–dependent.

The further characterization of the extracellular and intracellular signals that regulate the temporal and spatial organization of CD44 cytoplasmatic tail–based interactions, and the functional roles of the newly identified CD44 interacting proteins, may help to elucidate the molecular mechanisms of CD44 multiple actions.

K–Ras Activation of ERK2 in 3–D Human Pancreatic Cells Regulates Invasion and Proliferation via Induction of Matrix Metalloproteinase–1 and Tissue Inhibitor of Matrix Metalloproteinase–1

ST 17/P17

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Constitutively active, oncogenic mutations in K–Ras are found in 90% of all patient pancreatic adenocarcinomas (PDAC) and, along with invasion, appear early in the pathologic sequence of transformation. In addition, histological and genetic analysis of the stiff tumor microenvironment (TME) surrounding PDAC shows increased expression of pro–metastatic matrix metalloproteinase–1 (MMP–1) and tissue inhibitor of matrix metalloproteinase–1 (TIMP–1), both indicators of poor prognosis. However, the relationship between K–Ras, expression of these metalloproteinases, and cellular invasion & proliferation in human pancreatic ductal epithelial cells (PDECs) has yet to be established.

Primary human PDECs overexpressing oncogenic K–RasG12D reveal increased invadopodial extensions when cultured in 3–D basement membrane conditions. Increased invasiveness of K–Ras cells through the extracellular matrix is associated with MMP–1/3/10 & TIMP–1 mRNA induction, assessed by microarray and qRT–PCR, and coincides with MMP–1 & TIMP–1 protein increases by ELISA and zymography. FRET proteolysis assays imply correlated increases in MMP–1 activity within transformed PDECs. Specific Ras activation of ERK2 is a necessary intracellular signaling event for invasiveness and metalloproteinase induction, since pharmacological (U0126, PD98059, AEMT) or shRNA inhibition of MAPK/ERK2 reduces invasion and MMP–1 & TIMP–1 levels. Exogenous TIMP–1 addition causes an induction of hyperproliferation in PDECs, exponentially increasing the K–Ras proliferation of the mutated line over that of non–mutated. Lastly, we find that tension of the tumor microenvironment adds to the K–Ras mutation signaling via Fak, causing increases in invasion morphology. Taken together, these results uncover for the first time, a K–Ras directed ERK2–specific pathway capable of regulating pro–invasive MMP–1 and hyperproliferative TIMP–1. In addition, the tension of the TME synergizes with the K–Ras mutation to induce an invasive phenotype. As these proteins are critical for the invasion of human PDECs, our data suggests that the specific K–Ras–ERK2 pathway may be a potential therapeutic target for specifically inhibiting the perpetually active Ras–controlled PDAC invasion and metastasis within the local tumor microenvironment.

ST 18/P18

The ECM Protein MULTIMERIN2 impairs tumor angiogenesis and growth by interfering with VEGFR2 signaling

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MULTIMERIN2 (MMRN2) is an extracellular matrix glycoprotein whose function has so far remained elusive. Given its specific localization in tight association with the endothelium we have hypothesized that this protein could be involved in the regulation of angiogenesis. By multiple *in vitro* and *in vivo* assays we have demonstrated that MMRN2 significantly impairs endothelial cell migration and the organization of a functional vessel network. We have found that the interaction of endothelial cells (ECs) with MMRN2 is accompanied by a striking impairment of VEGFR1 and VEGFR2 activation. Given that VEGFR2 is the chief regulator of EC behaviour and angiogenesis we have studied the activation of this receptor in relation with MMRN2 expression in more detail. Our data demonstrate that MMRN2 interferes with the VEGF/VEGFR2 axis through a direct binding to VEGF–A. The interaction has been corroborated in a number of tests and the calculated Kd is ~ 50 nM. Transfection of HT1080 cells with MMRN2 did not lead to changes in their proliferative or apoptotic rate. On the contrary and likely accounting for the anti–angiogenic properties of this molecule, the MMRN2–positive cells failed to efficiently grow and form tumors in nude mice. Analysis of tumor sections has revealed a significant decrease of the blood vessel density in these tumors. Similar results were obtained following treatment of established tumors with a MMRN2 adenoviral construct. Our results suggest that VEGF may be sequestered by MMRN2 and thus be less available for the interaction with its receptor. Taken together these results put forward a novel player of the tumor microenvironment demonstrating a crucial role of MMRN2 in the regulation of EC function, angiogenesis and tumor growth. We hypothesize that an intact MMRN2 layer may function as homeostatic barrier halting the sprouting novel vessels, and suggest that these studies may embody the potential for the development of novel tools for cancer treatment.

Stromal Impact on Tumor Invasiveness and Gelatinolytic Activity at the Invasive Front

ST 19/P19

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Proteolytic enzymes play a complex role in tumor growth and invasion.The aim of this study was to explore the impact of tumor stroma on invasiveness and expression of gelatinases in a xenograft model of skin and oral carcinoma.

Xenograft tumors of human carcinoma cells were established either in the skin or the tongue of BALB/c Nude mice. Cell lines originating from skin and oral SCCs were used, as well as a cell line foreign to both organs. Gelatinolytic activity in the tumors was examined by a novel in situ zymography technique that enables high image resolution. *In vivo* and *in vitro* expression of various proteolytic enzymes were analyzed at transcriptional and protein level using RT–qPCR, immunohistochemistry and SDS–PAGE substrate zymography.

Histological analyzes revealed that tongue tumours had an invasive growth pattern, associated with reduced E–cadherin expression. In contrast, the skin tumours established from the same cell lines were non–invasive. Tongue tumors of all cell lines showed strong gelatinolytic activity, especially at the invasive front. In contrast, the skin tumors showed only weak gelatinolytic activity. At the mRNA level all cell lines were found to express MMP–2, –7, –14 and uPA. In addition, two out of three cell lines expressed MMP–9.

Our results suggest that invasiveness as well as activity of proteolytic enzymes in the tumors is more dependent on the tumor microenvironment than on inherent properties of the cancer cells.

ST 20/P20

Nanoparticles of heparins of marine invertebrates: anti–inflammatory effect on model of inflammatory bowel disease.

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Heparin is a polysaccharide of animal origin, mainly used as an anticoagulant in the prevention and treatment of deep vein thrombosis. Recent pre–clinical studies have also shown a potent antiinflammatory effect of this glycosaminoglycan. Despite highly used in clinical practice, heparin has a high–risk of bleeding and its pharmacological effect is only observed after parenteral administration. Therefore, the search for heparin analogues with lower side effects and better oral activity it is extremely important. In this context, several studies have shown promising results. For example, several works indicate the presence in marine invertebrates of heparin–like glycans containing low anticoagulant activity, potent anti–inflammatory effect and no bleeding tendency. It has been shown recently that nanoparticles increase the oral bioavailability of macromolecular drugs, including heparin. The present work describes the effect of nanoparticles of heparin analogues from marine invertebrates in an experimental model of inflammatory bowel disease (IBD) in rats. Preliminary results showed efficient formation of nanoparticles of heparin, with a good range of distribution and size. Performance and efficiency assays of encapsulated heparin were satisfactory. In IBD, increased production of TNF–α is associated with tissue damage mediated by immune response of the cellular infiltrate. Rectal administration of TNBS induced significant increase in the levels of TNF–α in inflamed colon. The evaluation of the anti–inflammatory action of the heparin nanoparticles in animals revealed a great reduction in cellular infiltrate and other pro–inflammatory parameters, in addition to a drastic reduction in the levels of TNF–α. These results indicate that heparin nanoparticles possess anti–inflammatory effect in an animal model of IBD.

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Biglycan aggravates lupus nephritis through TLR2/4 and regulation of the B cell chemoattractant CXCL13

ST 21/P21

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Objective: The role of endogenous inducers of inflammation is poorly understood.Lupus nephritis (LN), a renal manifestation of SLE, is characterized by systemic autoimmunity due to generation of autoantibodies against nuclear antigens and local inflammatory tissue response to immune injury. B lymphocytes, apart from their role in antibody formation, play an important role in causing local renal damage in LN. CXCL13 is a key B cell chemoattractant and marker of disease activity in patients with SLE. In this context, the objective of this study was to examine the role of biglycan in autoimmune disease Lupus nephritis.

Results: Here, we have shown that the proteoglycan biglycan triggers CXCL13 expression via TLR2/4 in macrophages and dendritic cells. In vivo, levels of biglycan were markedly elevated in the plasma and kidneys of human SLE patients and lupus–prone (MRL/*lpr*) mice. Overexpression of soluble biglycan in MRL/*lpr* mice raised plasma and renal levels of CXCL13 and caused accumulation of B cells with an enhanced B1/B cell ratio in the kidney, worsening of organ damage, and albuminuria. Importantly, biglycan also triggered CXCL13 expression and B cell infiltration in the healthy kidney. Conversely, biglycan deficiency improved systemic and renal outcome in lupus–prone mice, with lower levels of autoantibodies, less enlargement of the spleen and lymph nodes, and reduction in renal damage and albuminuria. This correlated with a marked decline in circulating and renal CXCL13 and a reduction in the number of B cells in the kidney.

Conclusion: Collectively, soluble biglycan by activating the TLR signaling pathway triggers the synthesis of CXCL13, a B cell chemoattractant thereby critically determining the systemic and renal outcome of lupus erythematosus by modulating both innate and adaptive immunity. Our results describe a novel mechanism for the regulation of CXCL13 by biglycan, a host–derived ligand for TLR2/4. Blocking biglycan–TLR2/4 interactions might be a promising strategy for the management of SLE and other B cell–mediated inflammatory disease entities.

ST 22/P22

Extracellular matrix proteins with short clusters of basic amino acids show tight binding to glycosaminoglycan chains of cell surface proteoglycans leading to a unique targeting and activation of specific cell types.

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The C–terminal nine amino acids of chondroadherin a member of the extracellular matrix small leucine rich–repeat proteins, have recently been shown to bind tightly to heparin (1). We have further investigated differences between domains with different clusters of basic amino acids, exemplified by the N–terminal domain of PRELP and the heparin–binding C–terminal of oncostatin M in binding to cell surface proteoglycans. Synthesized peptides corresponding to the basic domains of these three proteins, profoundly stimulated cell adhesion and spreading. An increased activity in intracellular signal pathways was observed with tyrosine phosphorylation of ERK as the read–out when cells bind to the chondroadherin peptide. Using the CHOK1 cells and the PGSA cells in FACS analysis glycosaminoglycan containing proteins was identified as the cell surface receptor involved in the binding to all of the three peptides. By inhibition experiments in FACS analysis with the different peptides it appears that these have affinity to specific stretches of glycosaminoglycans with dissimilar structure recognized.

To identify the cell surface proteoglycan involved in the binding to the chondroadherin peptide, affinity purification experiments in the presence of 6 M Urea, to focus on ionic interactions were performed using a human chondrosarcoma cells (105kc cells) and mouse osteoblasts (MC3T3–E1). The 105ck cells express all of the four members of the syndecan family confirmed by FACS analysis and western blot, while the MC3T3–E1 cells express only syndecan 1 and 4, identified by specific antibodies. All membrane bound proteins were isolated from the cells before the affinity purification by a novel method to disrupt existing lipid RAFTs.

The cell surface proteoglycan syndecan 4 from the MC3T3–E1 cell extract was absorbed to the immobilized chondroadherin peptide identified by western blot with specific antibodies. At the same time by using the 105kc cell in affinity chromatography to the same peptide another member of the syndecan family, differently from the syndecan 4 molecule, was identified as the receptor, by a pan–syndecan antibody. Thus, there appears to exist structural and functional differences between the glycosaminoglycans chain of the cell surface proteoglycans both within a cell and between cells of different types.

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Perlecan, a multifunctional proteoglycan in the vasculature

ST 23/P23

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Perlecan is a heparan sulfate (HS) proteoglycan produced by cells and deposited into the extracellular matrix (ECM). The HS chains attached to the N–terminal domain have been shown to be important in the binding of various heparin binding growth factors, while the C–terminal domain has been shown to be important for cell adhesion. Proteolytic fragments containing this domain, known as endorepellin, have been shown to modulate angiogenesis. Mice deficient in perlecan show major abnormalities in vasculogenesis suggesting an important role for perlecan in modulating vascular cell proliferation and migration. The molecular mechanisms of endothelial cell adhesion and proliferation as well as platelet adhesion and activation were investigated using purified endothelial cell derived perlecan.

Endothelial perlecan was able to support endothelial cell adhesion and growth, but not platelet adhesion. We have also shown this *in vivo* using an end–to–end anastomosis graft model where perlecan coated grafts supported endothelialisation of the graft over a period of 6 weeks, but not platelet adhesion. When the heparan sulfate chains were removed from perlecan by treatment with heparanase, which is a major product released by platelets upon activation, both platelets and endothelial cells attached quite effectively to the perlecan protein core. Platelets adhered to the perlecan protein core and this involved the α2β1 receptor, which has been known up until now as the major cell integrin receptor for collagen. Antibodies specific for domain V of perlecan, which is known to contain the α2β1 binding site, inhibited the binding as did antibodies that bound to the laminin–like domain III. Interestingly, the addition of anti–perlecan domain III and V antibodies also slowed endothelial cell migration in an *in vitro* "scratch" assay.

We also have preliminary data suggesting that the endothelial cell matrix is less adhesive for smooth muscle cells than the smooth muscle cell matrix, supporting a role of the endothelial cell matrix in modulating smooth muscle cell proliferation. These novel results suggest for the first time that the major perlecan receptor of platelets and endothelial cells is the previously well–characterised collagen receptor and that the heparan sulfate has a differential inhibitory activity on the adhesion of platelets suggesting that there may be cell / platelet selectivity residing in the basement membrane proteoglycan.

ST 24/P24

Decorin negatively regulates the Tumor Proangiosome

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A complex communication exists between tumor cells and their surrounding microenvironment, orchestrated by putative extracellular matrix molecules. Decorin, a prototypical member of the small leucine–rich proteoglycan gene family, is known to negatively regulate tumor growth through the concerted downregulation of key receptor tyrosine kinases, including EGFR and Met. In the present study, we investigated the anti–tumorigenic effects of exogenous decorin as it relates to angiogenic signaling under normoxia. Utilizing a PCR Profiler Angiogenesis Array, we discovered that key pro–angiogenic signaling genes were transcriptionally antagonized by decorin. Notably, hypoxia inducible factor 1 α (*HIF1A*) was among the most severely inhibited. Decorin evoked a downregulation of both HIF–1α transcript and protein in triple–negative MDA–231 breast carcinoma cells expressing a constitutively–active HIF–1α unresponsive to oxygen. Congruent with the above findings, decorin evoked a significant downregulation of VEGFA as determined by a combination of slot blot, Western blot, and immunofluorescence analyses. Importantly, suppression of Met in the presence of decorin or utilization of a specific siRNA, evoked a similar downregulation of VEGFA protein and transcript by concurrently attenuating downstream δ2–catenin signaling. These data establish a non–canonical Wnt role for b–catenin in the regulation of VEGFA. We also found that $p19^{ARF}$, a CDK inhibitor and regulator VEGFA mRNA stability, was upregulated in contrast to MMP–9 and MMP–2 protein and transcript levels. Gel zymography and ELISA revealed an appreciable decrease in the enzymatic activity for both MMP2 and MMP9. Finally, our findings were substantiated by an *in vivo* angiogenic assay and further confirmed by immunohistochemistry of VEGFA in a tumor xenograft mouse model. Collectively, our data establish a novel role for decorin as a suppressor of pro–angiogenic signaling pathways under normoxia to achieve antagonism of tumor angiogenesis.

Intracellular Hyaluronan Localization and effect on Microinjected Oligosaccharides on Hyaluronan coat in HAS3 transfected MCF– 7 cells

ST 25/P25

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Except its regular location on cell surface and extracellular matrix, hyaluronan (HA) is frequently seen within cells, mostly in vesicular structures like endosomes. However, it has also been suggested that HA exists in the nucleus, in the cytosol during synthesis, or associated with microtubuli, or interacting with cytosolic proteins. It has been difficult to confirm the cytosolic location of HA or the functions suggested for cytosolic HA. We addressed these questions by checking how HA brought into cells by microinjection is distributed between subcellular compartments and whether it influences HA coat.

MCF–7 cells were transfected with GFP–HAS3 to produce a cell surface coat from HAS–associated, newly synthesized HA, visualized by a fluorescent HA binding probe (fHABC). HA with chain lengths of 4, 6, 8, 10, 14, 28 and 120 monosaccharides (HA4–HA120) end–labeled with fluorophores, or fHABC were injected and subsequently located in different subcellular compartments. Following injections with unlabeled HA10, glucose, or mannose, HA coat was measured 2h , 4h and 8h later by image analysis, and compared with cells receiving buffer only.

MCF–7 cells injected with fluorescent HA oligosaccharides showed an evenly distributed signal in the cytosol and nucleus with no specific localization. Cells injected with fluorescent HA120 (25kDa) showed an even staining of the cytosol with no signal in the nucleus. HA120, unlike the shorter oligosaccharides, was apparently too large to enter the nucleus. Injected fHABC was seen in vesicular structures in some cells.

Microinjection of glucose caused a significant decrease in the mean HA coat intensity at 2h, while at 4h postinjection the values were close to buffer–injected controls. HA10 or mannose did not have statistically significant effect on mean HA coat intensity or area at the time points investigated.

It is concluded that small fragments of HA are freely mobile within a cell and do not interfere with HA synthesis e.g. by competing for binding sites on proteins involved in synthesis and translocation through plasma membrane. Larger HA is excluded from nucleus but shows no enrichment in cytoplasmic organelles or cytoskeletal structures. Interestingly, high cellular glucose content appears to inhibit HA synthesis or enhance its detachment from cell surface.

ST 26/P26

Growth Plate Regulation and Osteochondroma Formation: Insights from Tracing Proteoglycans in Zebrafish Models and Human Cartilage

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Proteoglycans are secreted into the extracellular matrix of virtually all cell types and function in several cellular processes. They consist of a core protein onto which glycosaminoglycans (e.g., heparan or chondroitin sulphates) are attached. Proteoglycans are important modulators of gradient formation and signal transduction. Impaired biosynthesis of heparan sulphate glycosaminoglycans causes osteochondroma, the most common bone tumour to occur during adolescence. Cytochemical staining with positively charged dyes (e.g., polyethyleneimine—PEI) allows visualisation of proteoglycans and provides a detailed description of how proteoglycans are distributed throughout the cartilage matrix. PEI staining was studied by electron and reflection contrast microscopy in human growth plates, osteochondromas, and five different proteoglycan–deficient zebrafish mutants displaying one of the following skeletal phenotypes: *dackel (dak/ext2)*, lacking heparan sulphate and identified as a model for human multiple osteochondromas; *hi307 (β3gat3)*, deficient for most glycosaminoglycans; pinscher *(pic/slc35b2)*, presenting with defective sulphation of glycosaminoglycans; *hi954 (uxs1)*, lacking most glycosaminoglycans; and *knypek (kny/gpc4)*, missing the protein core of the glypican–4 proteoglycan. The panel of genetically well–characterised proteoglycan–deficient zebrafish mutants serves as a convincing and comprehensive study model to investigate proteoglycan distribution and the relation of this distribution to the model mutation status. They also provide insight into the distributions and gradients that can be expected in the human homologue. Human growth plate, wild–type zebrafish and fish mutants with mild proteoglycan defects *(hi307 and kny)* displayed proteoglycans distributed in a gradient throughout the matrix. Although the mutants *pic* and *hi954* that had severely impaired proteoglycan biosynthesis showed no PEI staining, *dak* mutants demonstrated reduced PEI staining and no gradient formation. Most of the chondrocytes from human osteochondromas showed normal PEI staining. However, approximately 10% of tumour chondrocytes were similar to those found in the *dak* mutant (e.g., lack of PEI gradients). The cells in the reduced PEI–stained areas are likely associated with loss–of–function mutations in the *EXT* genes, and they might contribute to tumour initiation by disrupting the gradients.

Posters/ Abstracts

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Importance of Extracellular pH in Cartilage Tissue Engineering

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In cartilage tissue engineering there is much interest in the role of environmental signals in regulating cell proliferation and extracellular matrix (ECM) accumulation; attention has focused in particular on optimizing mechanical stress applications and oxygen tension. By contrast, the role of extracellular pH has been virtually ignored even though chondrocytes respire almost entirely by glycolysis, producing large quantities of lactic acid, which can rapidly acidify the media. In previous work, we demonstrated that in a continuous flow bioreactor HEPES plus $NaHCO_3$ supplemented media (pH 7.4) drastically improved cell proliferation and ECM deposition (5–6 fold), compared to media supplemented with HEPES alone (pH 6.9). Here we investigated whether increased matrix deposition was due to differences in chondrocyte extracellular pH or to the buffer system used.

Cartilaginous tissue constructs were generated from isolated bovine chondrocytes seeded in high–density 3D cultures and cultivated in a continuous flow bioreactor for 5 weeks at 37 °C under 5% CO_2. The media was pre–incubated under 5% CO_2 to obtain a starting pH of pH 7.4 either with HEPES alone or with HEPES supplemented with 14mM $NaHCO_3$. Media osmolarity was adjusted to c.330 mOsm. After 5 weeks of culture, tissue weight, DNA and GAG content were determined. The pH of the spent media from the bioreactor was measured every two days.

Both buffer systems maintained extracellular pH relatively well. The pH of the spent media for all cultures was above pH 7.2 in the first two weeks but had fallen to pH 7.1 by the fifth week of culture. The cultures grown with HEPES and $NaHCO_3$ (pH 7.4), in the reactor showed increased matrix accumulation and cell proliferation compared to cultures where extracellular pH was maintained at pH 7.4 with HEPES alone; dry weight, GAG content and DNA were 20–30% greater in the presence of $NaHCO_3$.

These results show that the large increase in GAG and DNA content seen previously in long–term cultures incubated at pH 7.4 relative to cultures at pH 6.9 was due mainly to extracellular pH rather than to the presence of $NaHCO_3$. The results thus confirm earlier short term studies which demonstrated that both cartilage matrix accumulation and turnover were very sensitive to extracellular pH. Thus maintaining a flow of medium at pH 7.4 is important for optimizing cell proliferation and ECM accumulation in cartilage tissue engineering.

Having said this; there exists differences between the HEPES plus $NaHCO_3$ (pH 7.4) supplemented media and HEPES (pH 7.4) alone media, suggesting that there might be other factors that could contribute to increased cell proliferation and ECM accumulation. Further work is underway to determine these additional factors. It is still unknown whether the high levels of GAG seen with under conditions of this study are a result of increased GAG synthesis or reduced GAG degradation.

Proteoglycan loss is only associated with loading–induced lesions that progress spontaneously

P 28

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Introduction: Osteoarthritis (OA) is a disease of the joint resulting in progressive articular cartilage (AC) degeneration, pain and movement restriction. It is known that many individuals will acquire AC lesions, but not all will progress to develop OA. The factors that control this progression remain, however, unknown. We recently characterised an adjustable murine joint loading model and demonstrated that modifying the number of applied loading episodes could control whether the induced lesions worsened with time or not1. This offers an opportunity to define factors that drive progressive AC degeneration and herein we describe histological features, including proteoglycan (PG) loss, glycosaminoglycan (GAG) synthesis and chondrocyte cell death that may distinguish progressive from non–progressive lesions.

Methods: Right knees of 8 week–old male CBA mice were loaded either once and analysed 2h or 2 weeks thereafter (non–progressing lesions), or loaded repetitively 3 times per week for 2 weeks (progressing) and analysed immediately or 3 weeks after the last loading episode (limb use was habitual in the interim[1]. Coronal 6µm wax sections, adjacent to AC lesions in the lateral femur, were stained with Safranin O to reveal PG loss, H&E and TUNEL for cell death and labelled using immunohistochemistry for UGDH protein expression (rate–limiting in GAG monosaccharide synthesis).

Results: Repetitively loaded mouse knee joints, containing progressing AC lesions, exhibit loss of Safranin O staining intensity. This was not evident in joints loaded once only containing non–progressing AC lesions. In contrast, UGDH protein was lost from AC chondrocytes surrounding lesions induced by either single (non–progressing) or multiple loading episodes (progressing). In addition, empty lacunae and decreased cellularity was seen in H&E stained sections around the lesions in joints loaded once and left for 2 weeks as well as in joints loaded repetitively (non–progressive and progressive respectively). TUNEL staining showed that cells were undergoing active cell death as soon as 2 hours after the initial loading episode.

Discussion: This study shows that progressive load–induced lesions are associated with a decrease in PG content in the AC that is not only due to decreased PG synthesis. In addition, the localised but marked chondrocyte death evident in all loaded joints suggests that this PG degradation is not necessarily achieved by an active contribution from neighbouring cells. Further studies are currently being undertaken to determine the molecular processes involved, as these may ultimately be targeted to slow OA progression.

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P
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Basement membrane and integrin gene expression during chondrocyte differentiation

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Earlier work has shown that there is a distinct localization of typical basement membrane components around murine and bovine chondrocytes in articular cartilage, forming a functional equivalent of a basement membrane (Kvist et al., *Matrix Biol.* 27:22–33, 2008). In the present study, we investigate the role of basement membrane components and receptors during chondrogenesis.

The expression of a distinct set of genes by chondrocytes undergoing differentiation leads to the formation of a characteristic extracellular matrix for each differentiation step. On the one hand, this extracellular matrix undergoes extensive remodeling during chondrogenic ossification in the growth plate, on the other hand it provides the crucial physical properties of adult cartilage, e.g. articular cartilage.

We investigated expression levels of basement membrane and integrin genes during differentiation of several chondrogenic cell lines using quantitative real–time PCR.

We found that basement membrane and integrin genes are indeed expressed by chondrogenic cell lines. Furthermore, while some of these genes show a stable expression during the whole differentiation cascade, others show increased expression levels at specific differentiation steps. Using immunohistology, we confirmed differences in basement membrane protein localization in mouse growth plate cartilage. This indicates that the respective gene product is of importance to maintain or regulate a differentiation step or to drive further differentiation.

To investigate this, we are now studying effects of isolated basement proteins, or fragments thereof, on chondrocytic differentiation. In addition, we are investigating the effect of pre–laid extracellular matrices, produced by cells at various differentiation stages, on undifferentiated cells.

Cell Sub–Populations in the Nucleus Pulposus of the Bovine Intervertebral Disc

P 30

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Introduction: The nucleus pulposus is derived from the notochord of mesodermal origin, and is populated initially by clusters of large, vacuolated notochordal cells. In humans, these cells disappear by 8–10 yrs to be replaced by the chondrocyte–like cells (NP cells) of the mature nucleus pulposus. It is not known whether the notochordal cells differentiate into NP cells or alternatively, notochordal cells die to be replaced by cells invading from the surrounding annulus or cartilaginous endplate, both of mesenchymal origin. Recent studies which have found that cells isolated from adult human and bovine discs express notochordal markers (Minogue et al. 2010) suggest NP cells are of notochordal origin. However, immunohistochemical studies of bovine discs, showed that only around 10% of cells appeared positive for the notochordal marker, cytokeratin 8 (KRT–8) and that the positive cells, though of the same diameter as the chondrocyte–like cells, appeared in clusters. Here, we aimed to separate NP cells in KRT–8 positive and negative cells and examine the populations for expression of notochordal cell markers (KRT–8&19 and N–Cadherin). Methods: Bovine NP cells were isolated by enzymatic digestion from bovine tails and semi–separated by consecutive filtration through nylon filters of 40 and 25 um into two fractions: cells which form clusters and are retained on the filters (›25um) and the cells which pass the filters (‹25um). Outer annulus (OA) cells isolated from bovine tail discs were used for comparison. Total RNA was isolated from all cell types. SYBR Geen qRT–PCR was performed for genes encoding KRT–8 and 19 and N–Cadherin. Cells were also stained by KRT–8 antibodies (Insight Biothechnology Ltd, UK).

Results: Cells in ‹25um cell fraction were single, and negative to KRT–8 immunostaining. The cell fraction retained by filters consisted of KTR–8–positive cell clusters together with trapped KRT–8–negative single cells. Results of qRT–PCR demonstrated genes encoding KRT–8,19 and N–Cadherin were highly expressed by ›25um cell fraction, whereas the ‹25um cell fraction and OA cells showed virtually no expression of these genes.

Discussion: These results demonstrate only a small fraction of cells from the mature disc express notochordal markers confirming human (Weiler et al. 2010) and bovine (Gilson et al. 2010) immunostaining studies. The origin of the cells of the mature human and bovine disc is thus still unclear.

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P 31

X–linked Alport syndrome investigation in Hellenic families. G624D mutation in *COL4A5* may explain many familial hematuria cases in Greek mainland that hardly can be diagnosed as Alport syndrome

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The X–linked Alport syndrome (ATS) is caused by mutations in *COL4A5*. ATS is heralded with continuous micro–hematuria which rapidly progresses to proteinuria, hypertension and chronic or end–stage renal disease (ESRD) by adolescence, frequently accompanied by sensorineural deafness and ocular complications. Milder forms of ATS also exist. We initially studied nine Hellenic families suspected clinically of X–linked ATS who presented with marked phenotypic heterogeneity. We identified four mutations in *COL4A5* in six families. Males currying E228X or c.2946delT mutation had the classical ATS symptoms with early onset of renal failure and deafness. However four males with the milder mutation G624D, belonging in two families originating from Greek mainland, reached ESRD after 39–yo and one patient showed Thin Basement Membrane Nephropathy (TBMN: a milder phenotype than ATS, classically attributed to *COL4A3/COL4A4* genes). Another 5/8 affected males with mutation P628L also developed ESRD between 30–57–yo, while three exhibit only mild chronic renal failure (CRF). Surprisingly, screening three more hematuric families from Greek mainland for G624D, were found positive for this mutation. The data support previous findings that certain mutations are associated with milder phenotypes, such as TBMN and familial microscopic hematuria. G624D may explain a lot of familial hematuria cases in Greece. We are designing a Greek genetic epidemiological study in order to find the exact frequency of the mutation in hematuric patients. Similar conclusions apply for missense mutation P628L. Interestingly, mutations G624D and P628L are near the 12th natural interruption of *COL4A5* triple–helical domain, which may explain the milder phenotype.

Anti–inflammatory and anti–remodeling effects of cardiac resynchronization therapy in patients with chronic heart failure

P 32

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Background: Remodeling reflects the structural and functional deterioration that occurs in chronic heart failure (CHF). Reverse remodeling is an accepted goal in the treatment of CHF. Cardiac resynchronization therapy (CRT) is an adjunctive treatment, indicated for CHF patients, who remain symptomatic, despite optimal drug therapy. In patients with CHF, CRT leads to reverse remodeling of the left ventricle (LV). Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are matrix–degrading enzymes that influence LV properties and serve as targets of potential anti–remodeling agents.

Objective: We aimed to investigate the effects of CRT on the serum levels of N–terminal pro–brain natriuretic peptide (NT–proBNP), pro–inflammatory cytokines (interleukins IL–1β, IL–6, IL–8, tumor necrosis factor TNF–α), MMP–2 and TIMP–2 in patients with CHF.

Methods and patients: 29 patients (21M/8F, aged 64 ± 11 years) with CHF (II–IV NYHA functional class) were investigated before and after 1 week, 3, 6, 12 months following the CRT treatment. In all patients, blood specimens were drawn from a peripheral vein. The serum levels of MMP–2, TIMP–2, IL–1β, IL–6, IL–8, TNF–α and NT–proBNP were measured at the same time by ELISA. Cardiac function was assessed echocardiographically.

Results: 12 months after CRT, the NYHA–class had improved with 2 classes and there was a significant reduction in serum levels of the NT–proBNP, IL–6, IL–8, MMP–2 and MMP–2/TIMP–2. There was a positive correlation between the changes in NT–proBNP and IL–6 ($r=0.76\pm0.23$, $p<0.05$), NT–proBNP and IL–8 ($r=0.72\pm0.23$, $p<0.05$). CRT positively influences the extracellular matrix remodeling by decreasing serum levels of MMP–2 and increasing TIMP–2. The MMP–2/TIMP–2 ratio had decreased from 6.84±7.55 before CRT, to 1.95±0.64 at 12 months after CRT. It was observed an immediate and persistent reduction in LV asynchrony, which was associated with an important and progressive improvement of the LV systolic function and an extensive LV reverse remodeling. There was a good correlation between the changes in the ejection fraction (EF) and MMP–2/TIMP–2 ($r=0.45$, $p<0.05$) and the septal–to–posterior wall motion delay (SPWMDSax) and MMP–2/TIMP–2 ($r=0.61$, $p<0.05$) after CRT.

Conclusions: After 12 months follow–up, CRT was associated with a discordant change in the serum levels of MMP–2 and TIMP–2. Changes in pro–inflammatory cytokines activity were related to the changes in the MMP–2 serum levels. This suggests that CRT reduces the peripheral markers of immune activation in patients responding to CRT. These changes in MMP–2/TIMP–2 ratio lead to reverse LV remodeling in patients with CHF.

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Matrix remodeling in relation to hemodynamic shear stress

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The vascular wall is constantly subjected to mechanical forces, which regulate vessel structure as well as influence the development of arterial wall pathologies. Shear stress is the frictional force acting on the endothelium as a result of blood flow, and has previously been identified as an important determinant of endothelial function and phenotype.

Atherosclerosis is a chronic inflammatory disease in the vascular wall, characterized by retention of lipids, accumulation of smooth muscle cells and macrophages as well as extracellular matrix remodeling. Despite the systemic nature of its associated risk factors, atherosclerotic lesions predominately form at specific regions, such as arterial branch points or the inner curvature of the aortic arch. In these regions blood flow is slow or re–circulating, resulting in low or oscillatory shear stress. In comparison, linear vessel segments, which are exposed to steady laminar shear stress, tend to be lesion free. Shear stress is thus an important factor for maintaining normal vascular homeostasis, and recent reports have also shown that the direction of flow and/or relative difference in shear stress are relevant determinants of the inflammatory response in the vessel wall.

The aim of the present study is to investigate matrix remodeling in relation to flow. More specifically, matrix morphology and shear dependent gene– and protein expression will be studied. Aortic arch geometry and blood flow velocities were determined in healthy male Wistar rats, using a 1.5T MRI scanner (Philips Achieva, Philips Medical Systems). Shear stress magnitude and direction of flow was estimated using a computational fluid dynamics approach. We have identified a region in the inner curvature of the aortic arch, showing re–circulating blood flow and low shear stress. A region of high shear stress, with unidirectional flow was found just after the A. subclavia sin. mRNA have been isolated from each region respectively, and are presently subjected to global gene expression analysis (microarray). Vessel wall morphology and the expression of candidate genes will further be investigated in tissue sections using immunohistochemistry, *in situ* hybridization.

Decreased fibroblast contractile activity and reduced fibronectin expression are involved in skin photoaging

P 34

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Skin aging is the result of both intrinsic and extrinsic factors. Extrinsic aging is mainly due to photo–damage caused by ultraviolet (UV) irradiation. On the histological level fragmentation of interstitial collagens is observed which contributes substantially to the reduction of skin's tensile strength during aging. While dermal fibroblasts from young donors are capable of maintaining the homeostasis of the extracellular matrix (ECM), (photo–)aged fibroblasts display decreased synthetic activity and are reduced in number. Their interaction with the surrounding ECM is impaired. This becomes apparent in a loss of mechanical tension which is exerted on the ECM by fibroblasts. The contractile activity of fibroblasts is thought to contribute to the skin's elasticity.

By utilizing the floating fibroblast–populated collagen lattice (FPCL) model we showed that the contractile forces exerted by human dermal fibroblasts (HDF) are affected by a single dose of UV irradiation. Non–irradiated HDF contracted the collagen matrix to approx. 52% of the original area whereas irradiated HDF reduced the gel size to approx. 78% of the original area which is a loss of contractile activity of 26%. This effect was not due to reduced cell viability.

Microarray analysis identified fibronectin (FN) as one of the interesting candidate gens which was downregulated after UV exposure. FN is a large multi–domain glycoprotein which is important for the organization and maintenance of the ECM. Its function for cell–ECM and cell–matrix adhesion site interactions links FN to cell contraction and matrix assembly. The effect of UV exposure of healthy human skin on FN expression has been discussed controversially. To shed light on this topic we confirmed a reduced expression of FN in skin biopsies 6 h and 24 h after treatment of volunteers with 2 MED solar simulated radiation (SSR) *in vivo* whereas FN was slightly induced after 72 h. Furthermore we detected lower FN expression levels in skin biopsies from chronical sun–exposed skin. The functional relationship between FN expression and gel contraction was proved by treatment of fibroblast with FN–siRNA. We were able to demonstrate that the contractility of FN–siRNA treated HDF was significantly reduced after 24 h in comparison to control–siRNA transfected cells. These results suggest an involvement of FN in the contractile activity of fibroblasts.

In summary, our results showed that acute UV irradiation reduces fibroblasts contractility and is associated with a reduced FN expression. FN functions as a general cell adhesion molecule by anchoring cells to the ECM. Our data indicate that FN plays an important role in maintaining the biomechanical properties of human skin.

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Impaired splicing of fibronectin is associated with Thoracic Aortic Aneurysm formation in patients with Bicuspid Aortic Valve disease

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Object: Bicuspid aortic valve (BAV) is a congenital disorder present in 1–2 % of the population which makes Thoracic Aortic Aneurysm (TAA) associated with BAV a very common implication. Histological observations of TAA show extra cellular matrix (ECM) breakdown, smooth muscle cell disappearance, and areas of mucoid degeneration. Fibronectin is a fundamental ECM component which may be involved in the formation and progression of aneurysm and manifests itself under different isoforms due to the alternative splicing. Interestingly, exons EDA and EDB appear to have essential roles in vascular morphogenesis during embryogenesis, as well as in processes like migration and proliferation, which are necessary mechanisms in tissue repair and maintenance of tissue integrity. Moreover, impaired regulation of transforming growth factor–β (TGFβ) signaling pathway has been linked to TAA and previous work indicated that TGFβ promotes the inclusion of EDA in fibronectin. In the present study, we analyze the expression of fibronectin spliceforms in dilated and non–dilated ascending aorta of tricuspid (TAV) and BAV patients.

Methods and results: The mRNA expression was analyzed in the ascending aorta by Affymetrix Exon arrays in patients with TAV (n=40) and BAV (n=69). EDA and extra domain B (EDB) expression was increased in dilated aorta from TAV patients compared with non–dilated aorta ($p<0.001$ and $p<0.05$, respectively). In contrast, EDA expression was not increased in dilated aorta from BAV patients ($p=0.25$) whereas EDB expression was upregulated ($p<0.01$).

The expression of EDA correlated with maximum aortic diameter in TAV (Rho=0.58) but not in BAV patients (Rho=0.15). Protein analyses of EDA–FN showed concordant results.

TGFβ treatment influenced the splicing of FN and enhanced the formation of EDA–containing FN in cultured medial cells from TAV patients but not in cells derived from BAV patients. Gene set enrichment analysis together with multivariate and univariate data analyses of mRNA expression suggested that differences in the TGFβ signaling pathway may explain the impaired EDA inclusion in BAV patients.

Conclusions: Decreased EDA expression may contribute to increased aneurysm susceptibility of BAV patients.

PRELP interacts with cells

P 36

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PRELP, a member of the extracellular matrix leucine–rich–repeat –proteins, has a positively charged amino–terminal domain rich in proline and arginine residues. It has previously been shown that the molecule binds to heparin an the heparan sulphate proteoglycan Perlecan via this domain. Current work has shown that the basic amino–terminal domain of PRELP mediates cell adhesion, that cells spread when bound and that heparin can act as an inhibitor of cell attachment to PRELP.

To identify cell surface proteoglycans/receptors binding to PRELP, a lysate from rat skin fibroblasts was applied on an affinity column with the amino–terminal part of PRELP immobilized. The cell surface proteoglycans syndecans 1 and 4, glypican as well as the extracellular matrix perlecan bound to the affinity matrix, and were identified using Western blot and mass spectrometry. In addition, the amino–terminal domain of PRELP was shown to bind both to monocytes and macrophages using flow cytometry with a biotin–tagged PRELP peptide.

PRELP's cell–binding properties and its localization in the pericellular–territorial matrix together with its ability to tightly bind to collagen suggest a role of PRELP as a cell anchoring protein.

In ongoing work we are investigating other types of PRELP binding cell surface receptors on cells.

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The protease nexin–1 is associated to collagen fibers in the medial layer in aneurysms of the ascending aorta

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Human aneurysms of the ascending aorta (AscAA) are characterized by fibrillar extracellular matrix breakdown, mucoid degeneration within the aortic media associated with progressive smooth muscle cell (SMC) rarefaction, presence of metalloproteinases (MMPs 1, 3 and 7) and serine proteinases (t–PA, u–PA and plasmin). Protease nexin–1 (PN–1), a potent inhibitor of serine proteases, is present in vascular cells and forms complexes with thrombin, plasminogen activators, and plasmin. PN–1 modulates the activity of endogenous and exogenous serine proteases and is found in the central and peripheral nervous system and in the vasculature. However, little is known about its function in the vessel wall. We thus examined the presence of PN–1 in the aortic wall in AscAA. Ascending aortas from 9 controls and 22 patients with AscAA, were analyzed by immunohistochemical or immunogold techniques. In AscAA samples, PN–1 immuno–reactivity was markedly increased. The degenerative mucoid areas were consistently negative for PN–1. When analyzed by electron microscopy the SMCs of the control group showed weak positivity for PN–1 associated with the extracellular matrix and absence of labeling in the cytoplasm. However, the SMCs of the AscAA group showed intense positivity within both the lumen of the Golgi apparatus, near the nucleus, and the rough endoplasmic reticulum. Immunolabeling was also observed within cytoplasmic vesicles, along the cell surface (plasma membrane), in the pericellular space and associated with molecules of collagen and proteoglycans. PN–1 secreted by smooth muscle cells remained essentially associated with glycosaminoglycans. The increase of expression of the PN–1 suggests that stimulation of an antiproteolytic response by SMCs may occur in AscAA in order to block the active MMPs and serine proteases and consequent advances of disease. The interaction between PN–1 and molecules of collagen may suggest a potential role in modulation of extracellular matrix in ascending aortic aneurysmas. LFB were sponsored by Paris 7 University, France.

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Triptolide inhibits integrin–beta1/FAK/c–Src signaling pathway and STAT3–mediated production of MMP2 in human cancer cells: a way to prevent invasion and metastases?

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Invasiveness and formation of metastases are the most fatal hallmarks of human aggressive malignancies; therefore, development of pharmacological approaches to prevention of tumor invasion and metastases is of paramount importance. Here we explored how triptolide, an anticancer activity–exhibiting diterpenoid triepoxide from the Chinese herb *Tripterygium wilfordii*, affects cancer cell–matrix interactions in *in vitro* cell adhesion/migration tests which model tumor invasion and metastasis. PC–3 and MCF–7 cell lines derived from human prostate and breast tumors with the high invasive and metastatic potential were the objects of our study. It was found that extremely low (3–5 nM) concentrations of triptolide potently repressed adhesion and spreading of cells of both the cancer cell cultures onto collagen–, fibronectin– or laminin–coated substrates. Moreover, 3–5 nM triptolide considerably (2.5–3–fold) reduced migration of these cancer cells through 3D collagen gel that suggests an impairment of their invasiveness. Studying molecular mechanisms of the discovered phenomena we have found that the same concentrations of triptolide inhibited the integrin–beta1–mediated phosphorylation of focal adhesion kinase (FAK) and c–Src in the cancer cell pools contacting with matrix proteins (collagen or laminin, or fibronectin) or RGD–peptide; correspondingly, this affected the focal adhesion formation and actin polymerization/bundling in the triptolide–treated cells thereby inhibiting their adhesion and motility. In addition, it was revealed that the triptolide–induced suppression of the cancer cell migration through collagen correlated with down–regulation of the expression of matrix metalloproteinase–2 (MMP2), a key (matrix–destructing) enzyme in the tumor invasion process. Importantly, the transcription factor initiating the MMP2 expression, signal transducer and activator of transcription–3 (STAT3), was found to be less phosphorylated in the triptolide–treated cancer cells. Thus, we demonstrate that very low concentrations of triptolide can significantly suppress adhesion/migration of human malignant cells onto/through the extracellular matrix; such effects appear to be due to the inhibition of integrin–beta1/FAK/c–Src and STAT3/MMP2 signaling pathways. Our findings allow to expect that, in the case of *in vivo* administration, clinically applicable (tolerable) doses of triptolide will target patients' malignant tumors, beneficially reducing their invasive and metastatic capability.

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Aggrecanases in colorectal cancer

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ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) are zinc–contained secreted proteinases which are known to influence homeostatic and pathologic processes such as morphogenesis, reproduction, osteoarthritis and several types of cancer, due to their involvement in cell proliferation, adhesion, migration and angiogenesis. Dysregulated expression of ADAMTS has been reported in breast, brain, lung and prostate cancer. In this study, the expression of ADAMTS–1, –4, –5, –8 and –20, the main aggrecanases, was estimated in colorectal cancer in transcriptional and translational level, in different anatomic sites and cancer stages. ADAMTS–4 and –5 seemed to follow the same expression pattern, as they were over–expressed, facilitating tumour invasion by degradation of proteoglycan–rich ECM. On the contrary, ADAMTS–1, –8 and –20 were all down–regulated, unable to exhibit their anti–angiogenic activity. Further study of ADAMTS, as well as the molecules with which they interact, will reveal their emerging role in tumour progress.

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Syndecan–1 and –2 cooperation in the migration and proliferation of a fibrosarcoma cell line

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Syndecans are transmembrane heparan sulphate proteoglycans. Their role in the development of malignant phenotype is ambiguous and depends on the type of cancer. Some earlier studies reported that certain syndecan paralogues can functionally cooperate with each other in actin stress fiber formation (Kusano et al., 2004), or in cell migration (Averbeck et al., 2007). Syndecan–1 increases the invasive behaviour of HT1080 fibrosarcoma cells (Pıterfia et al., 2006) similar way as it was described for syndecan–2 (Park et al., 2005). In the current work we investigated the possibility of a functional cooperation between syndecan–1 and –2 in HT1080 cell behaviour.

Syndecan–1 and –2 silencing by micro RNA constructs and overexpression experiments were performed. Proliferation and chemotaxis of stable transfectants were studied.

Stable overexpression of syndecan–1 promoted both the growth and the chemotaxis of HT1080 cells, moreover, it upregulated syndecan–2 expression. Results of syndecan–2 cDNA transfection and silencing of either syndecan–1 or –2 raised the possibility of a functional cooperation between syndecan–1 and –2. This cooperation was confirmed by additional cotransfection experiments.

Our results indicate that syndecan–1 can affect the behaviour of HT1080 cells via syndecan–2. Further experiments are planned in order to study the role of syndecans cooperation in their contradictious biological effects.

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Dual effects of unique dermatan sulfates from ascidians on tumor invasion: inhibition of epithelial–mesenchymal transition and hematogeneous metastasis

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Metastatic disease is responsible for most cancer–associated deaths and epithelial–mesenchymal transition (EMT) along with hematogeneous metastasis are critical steps in cancer progression. Therefore, inhibition of these events could be an effective approach to reduce the metastatic disease. Heparin has been show to modulate EMT–associated growth factors and also to inhibit P–selectin, leading to attenuation of metastasis. Previously, we showed that unique dermatan sulfates (DS) from Styela plicata and Pallusia nigra, composed by 2,4–O–sulfated and 2,6–O–sulfated disaccharide units, respectively, bind with high affinity to an EMT–related growth factor, hepatocyte growth factor (HGF), modulating its Met–dependent intracellular signaling. In the present work, the effect of the ascidian DSs on EMT–mediated cell migration and P–selectin–mediated metastasis in experiments in vitro and in vivo were evaluated. Cell migration was evaluated in the wound–healing assay, which was inhibited by glycans. The anti–selectin activity was evaluated by the binding of LS180 cells to immobilized P–selectin in the presence of glycans. Ascidian DSs inhibited the binding of tumor cells to P–selectin and were better inhibitors than unfractionated heparin. DS from porcine skin had no effect. The effect of DSs on formation of tumor cell–platelets aggregates in vivo was evaluated by injecting mice with LS180 cells 10 minutes after treatment with

PBS, UFH (1 mg/mouse) or ascidian DSs (100 µg/mouse). Ascidian DSs inhibited the adhesion of platelets to tumor cells in vivo in a concentration 10–fold lower than heparin. Metastasis was investigated by injecting 100 µg of each ascidian DSs or PBS 10 minutes prior injection of MC–38 cells and lungs analyzed for content of metastatic foci. Both ascidian DSs drastically reduced metastasis of MC–38 cells. Neither 2,4– nor 2,6–DS had any additional effect in P–sel–/– mice. In conclusion, ascidian DSs inhibit cell migration and reduce hematogeneous metastasis by a EMT– and P–selectin mediated events and could be used therapeutically to prevent tumor invasion and metastasis.

Studies on novel mammalian endo–type chondroitin sulfate hydrolases

P 42

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Purpose: Chondroitin sulfate (CS) chains are linear polymers composed of the repeating disaccharide units, —4GlcAβ1–3GalNAcβ1—, where GlcA and GalNAc represent D–glucuronic acid and *N*–acetyl–D–galactosamine, respectively, which are sulfated at different positions in various combinations. Structural diversity of CS is responsible for its multiple biological functions. Although the biosynthetic mechanism of CS has been well characterized, its catabolism has not been sufficiently understood. The cellular degradation of CS occurs predominantly in lysosomes. Following the fragmentation of polysaccharides by endo–type hydrolases, the oligosaccharide products are degraded sequentially from the non–reducing end by exo–type glycosidases and sulfatases. No endoglycosidases specific to CS had been reported. Hyaluronan (HA)–degrading enzymes, hyaluronidases, are considered to be responsible for the initial degradation of CS, because HA is similar in structure to nonsulfated, chondroitin (Chn). Recently we have identified human hyaluronidase–4 (hHYAL4) as a CS–specific hydrolase [1]. In this study, *in vivo* functions of hHYAL4 and mouse hyaluronidase–4 (mHyal4) were characterized.

Methods: An expression vector containing a cDNA fragment encoding a soluble form of the FLAG–tagged hHYAL4 or mHyal4 was constructed, and the recombinant proteins were transiently expressed in COS–7 cells. The purified enzymes were incubated with various substrates (HA, Chn, CS–A, CS–C, and CS–D), and the digests were analyzed by gel–filtration and anion–exchange HPLC. Characteristics of the enzymatic activity were compared between hHYAL4 and mHyal4. The cellular localization of mHyal4 in the COS–7 cells was analyzed by immunostaining using an anti–mHyal4 antibody. The expression of *hHYAL4* and *mHyal4* mRNAs in various tissues and organs was also examined by PCR.

Results and Discussion: Although both hHYAL4 and mHyal4 exhibited the activity to degrade CS variants into oligosaccharides, they depolymerized neither HA nor Chn. However, sulfate groups at different positions in CS were selectively recognized by hHYAL4 and mHyal4. A 6–O–Sulfate group on the GalNAc residue located on the non–reducing side of the cleavage site is essential for hydrolysis by hHYAL4, whereas mHyal4 has broader specificity than hHYAL4. Preliminary results showed the distribution of mHyal4 at the cell surface, suggesting that mHyal4 may degrade CS chains at the pericellular region. The mRNAs of *hHYAL4* and *mHyal4* were found to be expressed in placenta, skeletal muscle, and testis as well as in testis and 17–day embryos, respectively, being consistent with the previous observation of their restricted tissue distribution. Since CS exists ubiquitously in mammals, these enzymes do not seem to be involved in the generic catabolism of CS in lysosomes, but may rather regulate specific functions of CS in particular tissues or organs.

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P 43

Novel therapies to target inflammatory driven processes in cancer and chronic lung disorders

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Proteoglycans (PG) consist of a core protein with one or more covalently bound glycosaminoglycans (GAG) and are the major component of the extracellular matrix (ECM). GAGs, in turn, are complex molecules built up by repeating blocks of either hexose or hexuronic acid, linked to a hexose amine.[1, 2] GAGs are important factors in inflammatory diseases, such as chronic obstructive pulmonary disease, asthma and cancer.

A common GAG is dermatan sulfate (DS), which has been shown to play an important role in, among other things, inflammation, blood coagulation, cardiovascular diseases, fibrosis, wound healing and cancer development.[3]

The aim with the project is to investigate the structure and function of dermatan sulfate epimerase 1 (DS–epi1), one of two epimerases involved in the biosynthesis of DS, and to ensue generation of active site inhibitors for DS–epi1. Active inhibitors will eventually be tested *in vitro* and *in vivo*.

Preliminary results: Detailed analyses of oligosaccharides of different lengths have showed that the smallest size active as a substrate is a tetrasaccharide. However, oligosaccharides of longer length are much better substrates. These findings are of major importance for the design of candidate inhibitors, where a tetrasaccharide would act as a feasible starting point.

Experimental plan: 6XHIS–flagged recombinant DS–epi1 will produced. Expression systems will include *Saccharomyces cerevisiae* and HEK293 EBNA cells.

Next, modeling and synthesis of candidate small inhibitors will be performed. We intend to synthesize substituted glucuronic acid analogs similar to the natural substrate for DS–epi1. The carboxylic acid functionality and the hydrogen at position 5, as well as the hydroxyl group on position 4, will be exchanged for groups with different electronic properties. Finally, potential inhibitors will be evaluated in enzymatic assays and in lung and skin fibroblast cultures.

In a preliminary study the C6–difluorinated analog has been synthesized and tested in an epimerase assay. A small inhibition of activity was detected at high concentration.

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The role of decorin in hepatocarcinogenesis

P 44

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Liver cancer is one of the most frequent tumors in the world and may develop in cirrhotic or non–cirrhotic liver. Decorin a small ECM proteoglycan was proved to bind different receptor tyrosine kinases (e.g. EGFR, Met) resulting in inhibition of cell proliferation. Our aims were to reveal the role of decorin in both types of liver cancer and to identify the underlying molecular mechanisms.

Liver cancer with and without cirrhosis was induced by thioacetamide (TA) and diethylnitrosamine (DEN) respectively. We utilized decorin knock out (*Dcn–/–*) and wild type mice to determine whether the lack of decorin affects the tumorigenesis of the liver.

Interestingly, besides cirrhotic surrounding, tumors induced by TA and DEN showed different phenotypes. Tumors developed after TA exposure were rich in cytoplasm which showed strong eosinophil staining, whereas DEN–induced tumor cells had narrow basophil cytoplasm and often invaded the veins. *Dcn–/–* animals developed more tumors with cirrhotic surrounding induced by TA, while no significant difference between wild type and knock out mice could be revealed after DEN exposure, despite the higher tumor prevalence seen in decorin–null animals. In our experimental set–up based on the results of microarray analysis MAPK pathway and the cell cycle regulation were chosen for detailed examination. In *Dcn–/–* tumors members of the MAPK pathway (e.g. Erk1/2, Mek1, Raf1) were found to be more active. Furthermore, the lack of decorin had a crucial impact on cell cycle regulation by decreasing $p21^{Waf1/Cip1}$ via AP4 transcription factor leading to the activation of CDK4/6–cyclinD1 complex that finally phosphorylates retinoblastoma and triggers the cell cycle.

Our findings suggest that decorin has a protective role in tumorigenesis of cirrhotic liver, but has less effect in tumors induced by DEN. The lack of decorin results in enhanced cell survival and accelerated cell cycle allowing the cell to pass restriction point in G1 phase. Thus, decorin could be a useful tool to improve the management of liver cancer in the future.

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Alterations in overused supraspinatus tendon: a possible role of glycosaminoglycans and HARP/Pleiotrophin in early tendon pathology

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Injuries to the shoulder tendon due to overuse can lead to significant pain and disability in athletes and workers. Despite the prevalence and high social cost of these diseases, the early pathological events are not well known. A rat model of rotator cuff supraspinatus tendon overuse was presently used to explore the possible early relationship between glycosaminoglycan (GAG) composition in the extracellular matrix and biochemical alteration leading to phenotypic cellular transformation of tendon cells (tenocytes). Total sulfated GAGs increased after 4 weeks of overuse and remained elevated up to 16 weeks. GAG accumulation was preceded by up–regulation of decorin, versican and aggrecan proteoglycans (PGs) mRNAs and proteins and biglycan PG mRNA after 2 weeks. Also at 2 weeks, collagen I transcript decreased whereas mRNAs for collagen II, collagen III, collagen VI and the transcription factor Sox9 increased. All these modifications are characteristic of a shift towards the chondrocyte phenotype. HARP/Pleiotrophin, a cytokine known to regulate developmental chondrocyte formation, was increased in the injured tendon already at 2 weeks of overuse but greatly more at 4 weeks. However, this increase was observed only at the protein level without an associated up–regulation of its mRNA levels. GAGs are known to bind HARP/Pleiotrophin. Our present results also show increased affinity between HARP/Pleiotrophin and the altered GAGs in overused tendon which may suggest that its increased protein level is due to its enhanced sequestration in the cell environment. HARP/Pleiotrophin may thus contribute to the shift of tenocytes towards a chondrocyte phenotype. Identification of these early changes in the extra–cellular matrix may help to prevent the progression to more disabling and degenerative pathology.

Small leucine rich proteoglycans in the vertebral column of Atlantic salmon *(Salmo salar L.)* individuals displaying spinal fusions

P 46

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Vertebral deformities are reoccurring problems in Atlantic salmon and other farmed teleost species. We have previously characterized different stages during the development of vertebral fusions induced by elevated water temperature in Atlantic salmon. Spinal fusions are characterized morphologically as shape alterations of vertebral body endplates, reduced intevertebral space, transformation of intevertebral notochord tissue into cartilage and replacement of intevertebral cartilage by bone. In this study we have focused on the small leucine rich proteoglycans (SLRPs) in order to further understand mechanism in the development of spinal malformation. In mammals, SLRPs have been demonstrated a central role in regulating bone cells, mineralization and bone quality. Their expression and localization in non–deformed and fused vertebrae of Atlantic salmon was therefore compared. Samples characterized as fused ranged from incomplete fusions to complete fusions. Real–time PCR analysis demonstrated a highly different expression pattern of the SLRPs between non–deformed and fused samples. Decorin and fibromodulin were up–regulated, whereas biglycan and lumican was down–regulated. Immunohistochemistry data revealed their presence in osteoblast growth zone of the endbone and in the notochord. A more pronounced expression of biglycan and decorin was evident in the osteoblast area compared to notochord. This in contrast to lumican with a more pronounced expression in the notochord. In addition, bone quality parameters as mineral/matriks, collagen crosslinks and crystallinity of endplates in non–deformed and fused vertebra was determined by FTIR analysis. In fused samples, a more heterogenious mineral/matriks ratio and more unmature collagen due to collagen crosslinks was obtained. Our data identified for the first time the presence and localization of SLRP in salmon vertebrae, and their precence in tissue areas undergoing morphological changes during the spinal fusion process.

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Expression of the small leucine–rich proteoglycans (SLRPs) in Atlantic cod (*Gadus morhua* L.) skeletal muscle was altered in fish on a high–starch diet

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Aquaculture requires feed that ensures rapid growth and healthy fish. Higher inclusion of plant ingredients is desirable, as marine resources are limited. Atlantic cod (*Gadus morhua* L.) is considered mildly diabetic due to its low capacity to handle large glucose loads. In this study we investigated the effects of higher starch inclusion in feed on muscular extracellular matrix in farmed cod with an emphasis on the small leucine–rich proteoglycans and their GAGs.

Cod was fed two different diets containing high and low levels of starch. Starch was replaced by more complex fibers in the low–starch diet to keep the total carbohydrate inclusion 15% in both groups. Skeletal muscle was investigated for biglycan, decorin and lumican expression and distribution by immunohistochemical staining of muscle cryo sections and Western blot. Comparative semi quantification of protein content was performed using ELISA. The Abcam antibodies ab58562 (anti biglycan), ab35378 (anti decorin), ab70191 (anti lumican), and NorthStar Bioproducts/Seikagaku antibodies 2B6 and 5D4, against chondroitin–4–sulfated (C4S/DS) epitopes and keratan sulfate (KS) respectively, were used in the immunochemical analysis. The expression of SLRPs was also measured on mRNA level using real–time PCR, along with cytokines IL–1β, IL–8 and IL–10. Biglycan and decorin immunohistochemical staining showed a defined expression in the cod myocommata, corresponding to the perimysium of mammals. Lumican stained strongly and was ubiquitously distributed, as were C4S/DS and KS epitopes. The group fed a high–starch diet showed up regulation on mRNA level of biglycan, decorin and lumican compared to the group fed a diet containing more complex carbohydrates. ELISA confirmed the real–time PCR results on protein level for biglycan and lumican, indicating a stronger expression in fish on a high–starch diet. For decorin the protein levels were decreased in the high–starch group, in contrary to real–time PCR results. Disaccharide composition analysis using HPLC showed that there were alterations also on GAG level. The interleukins were furthermore up regulated on mRNA level, indicating an inflammatory response in the tissue. This study shows that the extracellular matrix of fish muscle is affected by diet, and effects of a higher inclusion of dietary fibers should be further investigated in different carnivorous fish species subjected to aquaculture.

Identification of genes associated to syndecan–1 overexpression and silencing by microarray analysis in mesothelioma cells

P 48

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Malignant pleural mesothelioma (MPM) is a highly malignant tumor, originating from the mesothelial cells lining the pleural cavities. MPMs exhibit biphasic morphology where epithelioid and fibroblast–like cells coexist. This is correlated to the expression of Proteoglycans. The expression of Syndecans is correlated with the epithelioid morphology, anchorage–dependent growth and inhibition of invasiveness. Its absence results in an epithelial to mesenchymal transformation and increased invasion. Syndecan–1 (sdc1) also demonstrates tumor promoter function in a panel of different cancers.

The aim of the study is to disclose genes or pathways regulated by Syndecan–1 in the MPM to better understand its importance for tumor cells. We coupled gene expression modulation with microarray analysis by over–expressing and silencing Sdc1 in malignant mesothelioma cells.

RNA samples were submitted to Affymetryx for a whole genome analysis. Genes with differential expression in cells with sdc1 overexpressed or silenced vs. their specific controls, were identified and ranked. Significant pair–wise statistics were made. According to our preset criteria, 2450 transcripts were differentially expressed due to sdc1 overexpression and 33 of these were induced by more than 10–fold. The list of most upregulated genes included SRGN, IL2, IL33, IL8, PDGFR or MTSS1, while among the most downregulated genes we found TGFb2, LUM, ADAMTS5, TUBa1a, etc. By clustering these genes according to GO terms, we identified differentially expressed genes which play a role in previously shown functions of sdc1 (e.g. 151 genes related to adhesion, as well as genes involved in the cell migration, or extracellular matrix organization). The analysis also revealed that sdc1 plays a complex role in regulating cell proliferation: 73/54 proliferation–related genes were up/down–regulated and 90 genes involved in cell cycle regulation were differentially expressed. Furthermore, expression of 26 growth factors and 25 growth factor receptors was altered. We also found evidences that sdc1 is involved in histone modification: expression of 17 histone modifying genes were altered, 11 of them downregulated.

Only 110 genes were influenced due to sdc–1 silencing, and expression of 14 genes was altered in both cases, genes involved in cell adhesion (FBL5, ALCAM, LRRC7, TLN1) or transcription (ETS1, SMARCD3). These can be considered primary binding partners of sdc–1 in this cell line. GO and KEGG molecular pathway analysis was performed to discover possible enrichment of genes with specific biological functions.

Overall, this study can help to elucidate the complex way how regulation by syndecan–1 participates to oncogenesis.

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Expression of syndecan–4 and correlation with metastatic potential in testicular germ cell tumours

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Syndecans are important regulators of cell proliferation, adhesion, migration and cell differentiation and are directly implicated in cancer progression. Thus we aimed to study the expression of syndecan–4 in patients with testicular germ–cell–tumours (GCTs) and to correlate with the clinicopathological findings. Over–expression of syndecan–4 was detected in GCTs by reverse–transcriptase–polymerase–chain–reaction. Immunohistochemistry was performed in 71 cases of paraffin–embedded tissues. In seminomas, high percentage of tumour cells exhibited membranous and/or cytoplasmic staining for syndecan–4 in all cases. Stromal staining for syndecan–4 was found in seminomas and it was associated with nodal metastasis (p=0.04), vascular/lymphatic invasion (p=0.01) and disease stage (p=0.04). Reduced tumour cell associated staining for syndecan–4 was observed in non–seminomatous germ–cell–tumours (NSGCTs) compared to seminomas and this loss of syndecan–4 was associated with nodal metastasis (p=0.01), vascular/lymphatic invasion (p=0.01) and disease stage (p=0.01). Stromal staining for syndecan–4 was also found in NSGCTs but did not correlate with any of the clinicopathological variables. The stromal expression of syndecan–4 in GCTs was correlated with the number of microvessels in the tumour stroma (p=0.03). Our results indicate that syndecan–4 is differentially expressed in seminomas and NSGCTs. Stromal staining for syndecan–4 in seminomas and loss of syndecan–4 by tumour cells in NSGCTs are related to metastatic potential of GCTs. Syndecan–4 expression in the tumour stroma is associated with increased neovascularization and might be a useful marker in GCTs.

Hyaluronan Synthase 2 Promotes Breast Cancer Cell Invasion by Suppression of Tissue Metalloproteinase Inhibitor 1

P 50

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Invasion and metastasis are the primary causes of breast cancer mortality, and increased knowledge about the molecular mechanisms involved in these processes is highly desirable. High levels of hyaluronan in breast tumours have been correlated with poor patient survival. The involvement of hyaluronan in the invasion of a clone of the breast cancer cell line MDA–MB–231 that forms metastasis in bone was studied using an *in vivo–l*ike basement membrane model. The metastatic to bone tumor cells exhibited a 7–fold higher hyaluronan synthesizing capacity compared to MDA–MB–231 cells, predominately due to an increased expression of hyaluronan synthase 2 (HAS2). We found that knockdown of HAS2 completely suppressed the invasive capability of these cells by the induction of tissue metalloproteinase inhibitor (TIMP)–1 and dephosphorylation of focal adhesion kinase. HAS2 knockdown–mediated inhibition of basement membrane remodelling was rescued by HAS2 overexpression, or transfection with specific siRNA of TIMP–1 or addition of TIMP–1 blocking antibodies. Thus, this study provides new insights into a possible mechanism whereby HAS2 and hyaluronan production enhance breast cancer invasion.

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Role of hyaluronan and tumour–associated antigen in human breast and ovarian cancer

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Qualitative and quantitative changes are frequently observed in the extracellular matrix stroma surrounding cancer. High level of hyaluronan (HA) is often associated with malignant progression in many cancers, such as breast cancer, colorectal cancer, and glioma (1). Hyaluronan–rich matrices around tumors favor the cancer cells migration and infiltration of newly formed blood vessels (2). Changes in hyaluronan content within primary tumours result from complex interactions between the cancer cells and associated stroma: growth factors and cytokines produced by the carcinoma may stimulate fibroblasts embedded in the stroma to increase the production of hyaluronan and associated hyaluronan–binding matrix components. In a recent paper carcinoma–associated fibroblasts are reported to be responsible for recruiting endothelial cells into tumour thereby boosting tumour angiogenesis (3). Human breast cancer cell line 8701BC, expresses the enzymes involved in HA metabolism, the synthases HAS2 and 3 and the hyaluronidases; nevertheless cells do not produce HA in ECM but only few molecules of HA are evident within the cells.

In co–culture in Transwell system with fibroblasts and tumour cells it was evident that 8701BC cells and fibroblasts increased the expression of the HA synthases, demonstrating a cross–talk between these cells. Further analysis using the proteomics of the conditioned media demonstrated the presence of a tumour–associated antigen. In order to elucidate the origin of this protein, we performed studies of the gene expression using the reverse transcription and Real–time PCR techniques. Results showed a high expression of this tumour–associated antigen in 8701BC cells and low expression in human skin fibroblasts. Moreover, we performed the Real–time PCR technique to study the gene expression in other tumour cell lines, including high and low metastatic cancer cells, obtained from breast and ovarian cancer. Results showed increased expression of this protein in most of the cell lines, especially in ovarian carcinoma cell line 3 (OVCAR3).

Further studies on the correlation of HA production from stroma cells and this tumour–associated antigen from cancer cells are in progress.

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Towards the Synthesis of a Tetrasaccharide Partial Sequence of Hyaluronan

P 52

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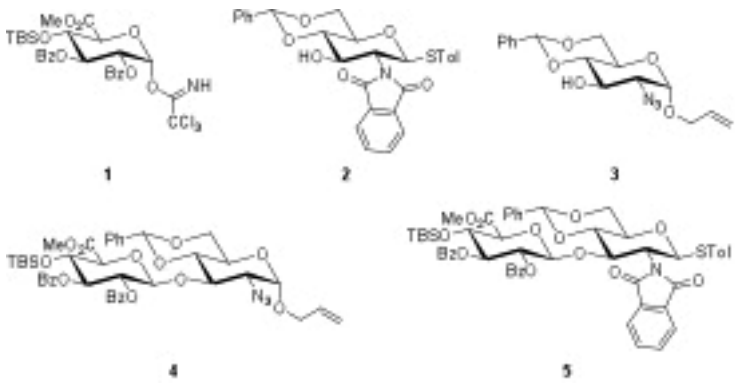
The extracellular matrix consists of different molecular components. Besides proteins such as collagen and elastin the ECMs major constituents are glycosaminoglycans (GAGs). These are long unbranched polysaccharides which are built out of repeating disaccharide units. Depending on the occurring disaccharide units the GAGs are differently classified. The most abounded GAG is Chondroitin sulphate. Other examples are Dermatan sulphate, Keratan sulphate and Heparin. Usually those GAGs are attached to extracellular matrix proteins thus forming proteoglycans. Compared with other GAGs Hyaluronan (HA) is an exception, because it is not forming proteoglycans and it is neither biosynthesized in the endoplasmic reticulum nor in the Golgi apparatus but by integral membrane proteins (HA–synthases). HA consists of alternating residues of D–glucuronic acid and *N*–acetyl–D–glucosamine and is the only non–sulphated GAG.

In the frame of a research project aiming the investigation of protein–GAG–binding we envisaged an efficient synthesis of a tetrasaccharide partial sequence of HA that can be easily ^{13}C–labelled and/or attached to polymer matrices or functionalized surfaces.

In our presentation we will describe the synthesis of the fully protected monosaccharides (**1, 2, 3**) and the synthesis of the two needed disaccharide units (**4, 5**) as well. Furthermore progress towards the synthesis of the repeating tetrasaccaride unit will be discussed.



1 2 3 4 5

P 53

UDP– Sugars regulate HAS2 expression

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The three hyaluronan synthase isomers (HAS1–3) produce hyaluronan using the cytosolic pools of UDP–N–Acetylglucosamine (UDP–GlcNAc) and UDP–Glucuronic acid (UDP–GlcUA) as substrates. Earlier we found that the concentrations of these UDP–sugars control hyaluronan synthesis (1), and now show that the content of UDP–GlcNAc also regulates the expression of *Has2* gene. Interestingly, while elevated cellular UDP–GlcNAc concentration, created by incubation in 6 mM glucosamine, increases hyaluronan synthesis, it simultaneously downregulates the expression of *Has2*. Conversely, the expression of *Has2* was upregulated by lowering the content of UDP–GlcNAc by incubation in 20 mM mannose, or by suppressing hexosamine biosynthesis with glutamine fructose–6–phosphate amidotransferase 1 (GFAT1) siRNA. The results indicate that while the content of UDP–GlcNAc as a HAS substrate is one of the limiting factors in hyaluronan synthesis, this metabolite also controls the content of the HAS2 enzyme through transcriptional regulation. The transkription factors YY1 and SP1 with their coregulators have a role in this feedback system, since glucosamine and mannose treatments change the extent and site of YY1 and SP1 binding on Has2 promoter. Phosphokinase arrays also showed that Stat 1 and 4 phosphorylations correlate positively, while Stat 2, 5 and 6 correlate negatively with *Has2* expression in the cells treated with mannose and glucosamine. Accordingly, reducing Stat 5 with siRNA increased Has2 expression. We also discovered a possible autocrine/paracrine signaling system in which extracellular UDP–sugars can increase *Has2* expression. UDP–sugars secreted into extracellular space perhaps stimulate *Has2* expression through the G–protein coupled P2y–receptors. A proper rate of hyaluronan synthesis is thus adjusted through multiple delicate feedback systems between the availability of hyaluronan building blocks and the expression level of the synthase enzyme *Has2*.

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Serum Hyaluronidases in colorectal cancer

P 54

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Hyaluronidases are a class of enzymes degrading predominantly the glycosaminoglycan hyaluronan. The best characterized of its isoforms, Hyal–1, is an enzyme active in acidic pH values with a pH optimum of 3.7, and it is present in human serum. In the present study, a semi–quantitive HA–substrate gel procedure was applied specifically for the detection and quantification of HA–degrading activity 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. HA–degrading activity was detected as a single lysis band of 54 kDa, in both cancer and healthy serum samples. Western blotting analysis revealed the presence of only Hyal–1. The levels of the enzyme in healthy donors and cancer patients before surgery did not show significant differences. However, a gradual slight increase in hyaluronidase activity was noticed in patients with the stage of the disease. The levels of the enzyme decreased significantly in patients seven days after surgery and thereafter gradually increased to reach the pro–surgery levels, but the general figure was related to the stage of cancer. In some cases the post–surgery levels overcame the pro–surgery ones without an indication of an abnormal condition for the patients. The obtained results suggested a possible over–concentration of Hyal–1 at early stages of colorectal cancer in the tumor area in accordance with previous findings and a putative role for it in wound healing after surgery.

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Hyaluronidases and hyaluronan synthases in nasal polyps

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Nasal polyps are benign lesions on the lining of nasal passages or sinuses. The etiology of the development of the polyps still remains unknown but the most important factor seems to be the increased hydration of epithelium and hyperplasia of the extracellular matrix, which may involves hyaluronan. In addition, hyaluronan of small molecular size is considered to be responsible for the inflammation. Biosynthesis of hyaluronan is regulated by three synthases: HAS1, HAS2, HAS3 and also by its receptor CD44, which is responsible for the control of the extracellular amounts of HA. Degradation of hyaluronan is regulated by hyaluronidases and it may involve HYAL1, HYAL2, HYAL3, HYAL4 and PH–20.

The aim of the present study was the investigation of the presence of hyaluronidases and hyaluronan synthases in nasal polyps. 20 patients with NP who underwent sinus surgery, were recruited from the Department of Otolaryngology–Head and Neck Surgery for this study.

Tissue was subjected to sequential extraction with PBS, 4M GdnHCl, 4M GdnHCl–1% Triton X–100. Zymographic analysis was applied for the detection of HA degrading activity in sequential extracts and Western Blotting for the identification of hyaluronidases. Also, RT–PCR was applied at fresh frozen tissue for studying the expression of different forms of hyaluronidase and hyaluronan synthases. Furthermore, hyaluronidases presence was investigated immunohistochemically.

Three different hyaluronidase isoforms were identified: Hyal–1, of lysosomal origin and with an acidic optimum pH, Hyal–2, of lysosomal origin and with an acidic optimum pH and PH–20, which is active in a wide range of pH values, from 4.0 to 7.0. The vast majority was observed extracellularly, suggesting that it directly acted to the ECM macromolecules and its presence seemed not to be originated from blood. Moreover, differential expression between NPs and normal nasal epithelia of the hyaluronidase isoforms variants was observed, suggesting a significant role of hyaluronidases in NPs progression. HAS–2 and –3 were the hyaluronan synthases expressed in NPs, suggesting the presence of variously–sized hyaluronan, able to express both its space–filling and inflammatory properties.

IQGAP1 in CD44 signaling: molecular mechanisms and cellular effects

P 56

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Hyaluronan, a major glycosaminoglycan, through its interactions with the cell adhesion receptor CD44 is involved in many normal bioactivities, including interactions between cell–to–cell, and between cells and extracellular matrix, triggering signals leading to cell survival and proliferation. Previous research in our laboratory has highlighted the critical importance of hyaluronan–activated CD44 in modulating the response of fibroblasts to PDGF–BB–mediated migration.

More recently we have demonstrated that the C–terminal domain of CD44 interacts with the IQ motif containing GTPase activating protein 1 (IQGAP1). IQGAP1 contains several protein–interacting domains, and has been shown to influence actin dynamics and intercellular adhesion. The functional role of the interaction between CD44 and IQGAP1 has been studied in telomerase immortalized BJ human dermal fibroblasts (hTERT–BJ). The analysis revealed that external signals, including hyaluronan and PDGF–BB stimulation, significantly augmented the formation of CD44–IQGAP1 complexes. Additionally, FACS analysis demonstrated the expression of "active forms of CD44" on the cell surface, i.e. CD44 able to bind hyaluronan. Phosphorylation at Ser and Tyr residues modulate the biological functions of CD44 and IQGAP1, respectively. We have observed that protein tyrosine phosphatases modulate the PDGF–BB– or hyaluronan–induced CD44–IQGAP1 complexes since treatment with pervanadate suppressed their interaction. Importantly, PDGFRβ is also a component of the complex.

The functional role of IQGAP1 in CD44–mediated migration was studied in scratch wounds in hTERT–BJ cultures deprived of IQGAP1, by using siRNA–mediated knock–down of IQGAP1. Interestingly, abrogation of IQGAP1 suppressed the hyaluronan–activated CD44–mediated migration by about 80%, but had no effect on PDGF–BB–induced cell migration. Furthermore, studies on the intracellular signaling involved under these conditions revealed that the activity of focal adhesion kinase was significantly decreased. The possible involvement of p38 and Src signaling pathways are currently under investigation.

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Isoforms of HA–receptor in laryngeal cancer

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Laryngeal cancer is a disease with increasing incidence in the population, and it is one of the most frequent head and neck malignancies. Several undergoing studies, correlate the expression of adhesion molecules with pathological, anatomical and clinical characteristics of laryngeal cancer. One of these molecules is CD44, the main transmembrane receptor for HA, which exists in variable alternatively spliced isoforms. The variety of CD44 isoforms is highly increased due to post–translational glycosylation. The aim of the study was to evaluate the transcriptional and translational expression of CD44, and CD44s, CD44V2, CD44V5–6, CD44V6–7 isoforms. The results of the study indicated that CD44 played a key role in cancer, having increased expressional heterogeneity in cancers of different or similar, in histological terms, cancers. Its expression appeared to depend to the stage and the anatomic site of cancer, even for the same type of cancer. It was very interesting that in laryngeal cancer the expression of all studied isoforms, CD44V2, CD44V5–6, CD44V6–7, CD44 as well as CD44s, was observed, in different extent for each one in different anatomic sites, and most of them appeared to possess similar ongogenicity action.

*Key words: CD44, laryngeal cancer, metastasis, hyalouronic acid, isoform*s

Studies on the functional role of CD44–iASPP complex as a key regulator of cell growth and apoptosis

P 58

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The lymphocyte homing receptor CD44 and its principal ligand hyaluronan are both expressed at high levels in regions of cell proliferation and invasion and play key roles in the pathogenesis and development of the inflamed synovium and malignant disease.

We identified several interaction partners to CD44, including NFkappaB interacting protein iASPP which is upregulated in rapid remodeling tissues and plays a key role in the regulation of the p53 tumor suppressor; iASPP binds and inhibits p53–mediated apoptosis. We have been able to demonstrate an endogenous interaction between CD44 and iASPP in primary cultures of human dermal fibroblasts, telomerase–immortalized human foreskin fibroblasts (hTERT–BJ), and hepatoma HepG2 cells, by performing co–immunoprecipitation experiments. Furthermore, we have characterized the epitopes involved in the interaction between the intracellular domain of CD44 and iASPP by overexpressing in 293 cells iASPP wild type and its various constructs . The analysis revealed that iASPP interacts with the intracellular domain of CD44 ($CD44_{icd}$) through the ankyrin repeats region which is close proximity to SH3 domain of iASPP known to interact with the proline residue 72 of p53 regulating the activity of p53. Using co–immunoprecipitation assay we have demonstrated that CD44, iASPP and p53 are in the same complex . Our preliminary data suggest that CD44 has an effect on iASPP –p53 complex formation, and that knocking down of CD44 by siRNA technology increase iASPP–p53 complex formation.

Taking into consideration this last observation we are planning to investigate whether CD44 has the influence on p53 induced apoptosis which could be inhibited by iASPP.

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The interplay between breast cancer & endothelium in cancer progression

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During carcinogenesis, tumor cells participate in several interactions with the tumor microenvironment as well as the surrounding cells. High level of hyaluronan is often associated with malignant progression in many cancers, such as breast cancer. The aim of the present study was to determine the cellular responses between the breast cancer cell lines, MDA–MB–231 & MCF–7, and the human umbilical vein endothelial cells (HUVEC) using conditioned media (CM) and co–culture Transwell systems. We assayed that conditioned media from cancer and HUVEC cells decrease and induce the cell migration of HUVEC and cancer cells, respectively. Real–Time PCR analysis showed that the gene expression of CD44, HAS2, VCAM–1 and ICAM–1 in HUVEC is up–regulated, especially by CM from MDA–MB–231. In MDA–MB–231 and MCF–7, CD44 and HAS2 are up–regulated and down–regulated, respectively, by CM from HUVEC. A similar effect is demonstrated during co–culture of HUVEC and cancer cells. HA expression in culture medium of HUVEC cells is up–regulated by CM from cancer cells, in reference with CM added, whereas in cancer cells is slightly downregulated by CM from HUVEC. In co–culture system, HA expression is similar, highlighting cell–cell communications. Moreover, the adhesion of cancer cells is favored by the presence of HUVEC cell monolayer and this effect is abolished by hyaluronidase. In conclusion, relatively little is known about the paracrine effects of tumor–endothelial cell interactions and it is of high interest to highlight key molecules participating in this cross–talk.

Growth Factors and Signaling Pathways Involved in the Amniotic Fluid–Induced Proliferation of Human Skin Fibroblasts

P 60

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Wound healing in adults is characterized by scar formation, whereas the early–gestation fetus has the remarkable ability of tissue regeneration without a scar. Experimental evidence indicates that this most probably reflects the intrinsic characteristics of fetal tissue; however, environmental factors may also contribute to this phenomenon.

Accordingly, aim of the present study was to investigate the effect of the in utero environment, i.e. the amniotic fluid, on cell proliferation – one of the major parameters of tissue repair. More specifically, the effect of second trimester human amniotic fluid on the proliferation of human both fetal and adult skin fibroblast–cultures was examined. The data we present here show, that amniotic fluid is a potent stimulant of DNA synthesis and proliferation of cells from both developmental stages. This effect is due to the presence of growth factors, especially basic fibroblast growth factor (bFGF) and platelet–derived growth factor (PDGF), since inhibitors of their respective receptor–kinases and specific neutralizing antibodies can significantly inhibit cell proliferation. Furthermore, amniotic fluid activates both the MEK/ERK and the PI3K/Akt signaling pathways in human skin fibroblast cultures. This activation seems to mediate its mitogenic effect, since pharmacological inhibitors of the two signalling pathways are able to attenuate amniotic fluid–induced fibroblast proliferation (Chrissouli *et al.*, 2010, *Wound Repair Regen.* **18**: 643–654).

Interestingly, we have not observed any significant differences between fetal and adult fibroblasts in their response to amniotic fluid, indicating that cells from both developmental stages respond equally to this complex mixture of regulatory molecules.

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Lumican modulates TGF–β2 signaling to regulate osteosarcoma cell adhesion

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Human osteosarcoma cell lines are known express and secrete the small leucine rich proteoglycan (SLRP) lumican. IN this study we investigated possible role of lumican on osteosarcoma cell adhesion and focused on mechanisms involved. Real–time PCR, western blot, adhesion assay, and siRNA methodologies were applied. Lumican–deficient Saos 2 cells were demonstrated to have increased adhesive capability onto fibronectin (FN) ($p \leq 0{,}01$). Upon neutralization of endogenous transforming growth factor β2 (TGF–β2) activity, no difference in the ability of lumican siRNA–transfected and scramble siRNA–transfected Saos 2 cells to adhere onto FN was detected (p=NS). Exogenous TGF–β2 was shown to stimulate Saos 2 cell adhesion to FN ($p \leq 0{,}01$). These results therefore, suggest that the inverse correlation existing between lumican expression and Saos 2 cell adhesion is dependent on active TGF–β2 signaling. Furthermore, the significant increase in Smad 2 activation present in lumican–deficient cells ($p \leq 0{,}01$) was annulled in the presence of the anti–TGF–β2 peptide, demonstrating that lumican is an upstream regulator of the TGF–β2 / Smad 2 signaling cascade. Crucial to FN–dependent adhesion, β1 integrin expression and pFAK activation were likewise identified as downstream TGF– β2 effectors regulated by lumican expression. In conclusion, this study demonstrates a novel out–in signalling circuit in human osteosarcoma cells: secreted to extracellular matrix lumican is an endogenous inhibitor of TGF–β2 activity, resulting in downstream effector modulation including pSmad 2, integrin β1 and pFAK to regulate osteosarcoma adhesion.

PTH induces osteosarcoma cell migration through FGF–2/Biglycan signaling axis

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Parathyroid peptide PTH (1–34), a powerful osteotropic agent, was established to have net anabolic effects on bone and also to regulate osteosarcoma cell migration. Fibroblast growth factor–2 (FGF–2) plays a significant role in PTH–induced bone anabolic action. Small leucine–rich proteoglycans (SLRPs) are an established component of the extracellular matrix (ECM), of great importance to osteosarcoma cell functions and their expression was found to be regulated by FGF–2. In the present study, we investigated the possible participation of FGF–2–signaling on the PTH–dependent osteosarcoma cell migration. We utilized MG63 osteosarcoma cell line as a model system with real–time PCR, western blotting, "wound healing" assay and RNA interference as techniques of choice. FGF–2 treatment of osteosarcoma cells resulted in a significant, similar to PTH, increase ($p \leq 0.01$) in MG63 cell migration. The mRNA expression analysis of cells treated with PTH, showed a strong increase of FGF–2 transcript levels ($p=0.0015$). Interestingly, the addition of FGF–2 to MG63 cells demonstrated a significant downregulation of the SLRP biglycan expression both at the mRNA ($p \leq 0.0001$) and protein (60%) level. In order to examine the significance of biglycan on MG63 cell migration, transfection with short interfering RNA (siRNA) specific for biglycan was performed, resulting in a significant increase ($p \leq 0.01$) in the migration capacity of biglycan–deficient MG63 cells. Finally, a direct correlation between PTH(1–34) action and biglycan expression was established by determining a significant decrease ($p \leq 0.01$) in biglycan transcript levels in PTH treated cells. In conclusion, this study demonstrates a novel synergistic mechanism of PTH(1–34) and FGF–2 action which results in specific alteration of the biglycan ECM content to regulate osteosarcoma cell migration.

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HS–chains modulate TGFB2 signaling in both SMAD– dependent and –independent manner to regulate fibrosarcoma cell adhesion

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Proteoglycans (PGs) and glycosaminoglycans (GAGs) are implicated in cancer progression. Fibrosarcoma is an uncommon soft tissue tumour originated from fibroblasts and its cell microenvironment is rich in PGs and GAGs. Heparan sulphate (HS) glycosaminoglycans are present on the surface of virtually all mammalian cells and are significant for a wide range of cellular functions, including cell adhesion, proliferation and migration. In the present study we investigated the role of HS on TGFB2–dependent fibrosarcoma cell adhesion and focused on possible mechanisms of their action. The experiments were conducted on HT1080 fibrosarcoma cell line utilizing real–time quantitative PCR, specific enzymic digestions, western blot and adhesion assays. Treatment of HT1080 cells with TGFB2 significantly ($p \leq 0{,}001$) enhanced their adhesion. Heparitinase digestion efficiently cleaved membrane associated HS–chains as demonstrated by the utilization of an antibody specific for the HS–stubs, but did not affect HT1080 cell adhesion. Interestingly, absence of cell–surface HS inhibited TGFB2–dependent adhesion ($p \leq 0{,}001$). Examination of the TGFB2 pathway–restricted downstream effector, SMAD 2, revealed a decrease in TGFB2–dependent pSMAD 2 phosphorylation in HS–chain deficient HT1080 cells ($p \leq 0{,}001$). Furthermore, TGFB2–dependent activation of the key adhesion molecule, focal adhesion kinase (FAK), was found to be downregulated ($p \leq 0{,}05$) in HS–deficient cells

In summary, this study suggests that membrane associated HS–chains modulate TGFB2 signaling in both SMAD– dependent and –independent manner to regulate fibrosarcoma cell adhesion.

Hyaluronan as regulator of the differential responses of fetal and adult dermal fibroblasts to TGF–β

P 64

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Transforming Growth Factor– β (TGF–β) is a pleiotropic factor which plays a major role in a large subset of cellular homeostatic processes, such as cell proliferation, differentiation, extracellular matrix formation and tissue repair. Especially concerning cell proliferation, its action is clearly cell type–dependent, as it inhibits epithelial and endothelial cells while it stimulates many mesenchymal cells. In addition, its action depends on the extracellular environment. As an example, it has been recently reported that the proliferative effect of TGF–β on dermal fibroblasts is annulled when hyaluronan (HA) levels are reduced.

We have already shown that the effect of TGF–β on human dermal fibroblasts' proliferation depends on their developmental stage, as it inhibits fetal cells while it stimulates adult fibroblasts [Pratsinis H. et al. Wound Rep. Regen. 12 (2004) 374–83; Giannouli C.C. and Kletsas D, Cell Signal. 18 (2006) 1417–1429]. Having in mind also the differential expression of HA in these two developmental stages, we aimed at understanding the role of HA in its differential effect of TGF–β. First, we have found that even after degradation of HA by hyaluronidase TGF–β is able to activate the intracellular signaling pathways, such as the Smad pathway. However, the presence or not of HA seems to regulate the proliferative effect of TGF–β in fetal dermal fibroblasts, as after HA degradation the inhibitory effect of TGF–β is annulled. In contrast, the enzymatic degradation of HA affects only to a partial extent the stimulation of adult fibroblasts following TGF–β treatment. The mechanisms underlying this phenomenon are currently under investigation.

P 65

Low molecular weight heparin through PKCa–mediated changes in actin cytoskeleton organization modulates melanoma motility

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Heparin is a complex mixture of linear glycosaminoglycan (GAG) chains, used as anticoagulative agent. Biochemical modifications of heparin produce a wide range of heparin derivatives including unfractionated heparin (UFH), low molecular weight heparin (LMWH) and modified heparins with different anticoagulant activities. Low molecular weight heparin (LMWH) has significant antimetastatic capabilities and affects cancer progression in humans. In this study, we evaluated its activity at the intracellular level and its effect on melanoma cell adhesion and migration. The obtained results indicated that LMWH presence, inhibited M5 melanoma cell adhesion and migration ($p \leq 0,01$). Treatment with LMWH also caused a marked down regulation of constitutive as well as the FN–induced phosphorylation ($p \leq 0,01$) of protein kinase alpha (PKCa). This was associated with a profound decrease in the cytoplasmic pPKCa ($p \leq 0,05$) and a simultaneous enhancement of nuclear pPKCa localization ($p \leq 0,01$). Furthermore, LMWH–treated cells had disorganized actin stress fibers correlated to a strong decrease in cell–substratum interface area ($p \leq 0,05$) and altered morphology. The decrease in the activation of PKCa, which is an important regulator of cell motility, was directly correlated to the reduced ability of the LMWH–treated melanoma cells to adhere onto and migrate towards the fibronectin (FN) substrate ($p \leq 0,01$). In conclusion, LMWH through the downregulation of pPKCa and redistribution to nuclear region, induces cytoskeleton changes correlated to M5 cell decreased adhesion / migration. This may provide clues for the pharmacological targeting of melanoma.

Invasion of breast cancer cells is related to the PDGF–R mediated expression of heparan sulfate proteoglycans

P 66

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Expression of proteoglycans (PGs), essential macromolecules of the tumor microenvironment, is markedly altered during malignant transformation and tumor progression. Expression of cell surface associated PGs, such as syndecans and glypicans, is also modulated in both tumor and stromal cells. Cell–surface associated PGs bind various factors that are involved in cell signaling thereby affecting cell proliferation, adhesion and motility. Abnormal PG expression in cancer and stromal cells may serve as biomarkers for tumor progression. The aim of this study was to examine the gene expression profiles of HSPGs and PDGF–R in breast cancer and normal cells. Furthermore, to examine STI571 in the PDGF–BB mediated gene expression of HSPGs and TIMP–1 and to evaluate the efficacy of molecular RTK inhibitor STI571 on cell invasion, migration and signaling pathways in breast cancer cells. The basic conclusions of this study are that the PDGF–R–mediated expression of certain HSPGs (syndecans–2/–4 and glypican–1) is associated with the invasion and migration potential of cancer cells. Therefore, STI571 may be of great value for the pharmacological targeting of breast cancer invasion and metastasis.

Acknowledgments
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Migration of ERb(+) breast cancer cells is depended on EGFR and IGF–IR signaling and expression of syndecans–2 and –4

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Breast cancer is characterized by significant quantitative changes of the extracellular network components. In a previous study (Kousidou *et al*, Mol Oncology, 2008) it has been shown that estradiol (E2) is a critical factor affecting gene expression of extracellular matrix macromolecules, such as the cell membrane syndecans and metalloproteinases, which may implicated in cancer growth and progression. It is established that E2 and its receptors (ERs) play a very important role in the growth and development of hormone–depended breast cancer. It has been also proposed that E2 may exert its effects via cross talk signaling pathways involving insulin–like growth factor receptor (IGF–R) and epidermal growth factor receptor (EGFR). However, it has not yet demonstrated whether in breast cancer cells, E2 may affect syndecans' expression and functional cell properties via a cross talk signaling with EGFR and IGF–IR. The aim of this project was therefore to evaluate whether such a cross talk between E2–ERs and EGFR, IGF–IR effects the expression of syndecans–2 and –4 as well as the migration of breast cancer cells. For this purpose the epithelial breast cancer cells with known ER expression status [MDA–MB–231 ERβ(+)] were used. The effects of E2 were evaluated in the presence of EGFR and IGF–IR specific tyrosine kinase inhibitors. In MDA–MB 231 cells, gene expression of syndecan–4, a critical regulator of tumor invasion and metastasis, is suppressed following treatment with E2. Inhibition of both EGFR and IGF–IR in presence or absence of E2, down–regulates the expression of Syndecan–4 at a level similar to that obtained using separate receptors inhibitions in E2–treated cells. A down–regulated expression pattern is also characterized Syndecan–2. This proteoglycan is statistically down–regulated after combined EGFR and IGF–IR inhibitions in presence and absence of E2. On the other hand, the migration ability of these cells significantly induced by EGFR signaling pathway. In addition, the migration of the E2–treated cells treated with EGFR and IGFR is suppressed. These results indicate that the down–regulation of Syndecan–4 and Syndecan–2 seem to be correlated with the migration ability of MDA–MB–231 cells.

Purification and characterization of recombinant blood coagulation factor XIII from different species

P 68

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Transglutaminases play an important role in stabilization of the extracellular matrix by crosslinking glutamine– and lysine–residues of proteins to form intra– and intermolecular isopeptide bonds. Blood coagulation factor XIII is a heterotetrameric protransglutaminase consisting of two catalytic A–subunits and two non–catalytic B–subunits. The A–subunit (FXIII–A) plays a crucial role in the final stage of the blood coagulation cascade. It crosslinks α– and γ–fibrin–chains to strengthen the fibrin clot and also integrates α_2–plasmin inhibitor in the clot to prevent it from early fibrinolysis by plasmin. Besides the role in blood coagulation, it is involved in matrix stabilisation, wound healing, angiogenesis, and bone remodelling. FXIII–A deficient mice show delayed wound healing and a decreased number of new blood vessels after injury compared to normal mice.

Since FXIII–A is the predominant transglutaminase involved in clot formation, selective inhibitors can be developed to block its fibrin–crosslinking. This may be desirable to prevent the development of thromboses. Non–crosslinked fibrin–chains assemble together by hydrophobic interactions to give a soft clot. This can easily be solubilised by plasmin, because of the lack of covalent connections between the fibrin chains and α_2–plasmin inhibitor.

The aim of the study was to investigate the differences in the inhibition of recombinant factor XIII–A of dog, pig, mouse and rat compared to human factor XIII–A with several different irreversible factor XIII inhibitors. For this, the factor XIII–A variants were recombinantly produced in insect cells.

The ability of the FXIII–A variants to assemble with recombinant human FXIII–B to a heterotetrameric complex was demonstrated by native PAGE. For further analysis the specific activities and K_m–values of all FXIII–A variants for a specific fluorescent FXIII–substrate were determined.

To compare the inhibition of FXIII–A with all inhibitors the second order rate constants were determined by the continuous assay of *Tian* and *Tsou*. The results showed that there are differences in the inhibition of the FXIII–A variants. Pig FXII–A was resistant to a previously described small–peptide inhibitor and two peptide–based inhibitors developed by Zedira. Only one α2–plasmin inhibitor–based molecule was able to inhibit it. The other FXIII–A variants from dog, mouse and rat were inhibited in a manner similar the human FXIII–A.

These results will be discussed in the light of differences in amino acids forming the active site pocket of FXIII–A.

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Simultaneous analysis of heparan sulfates, chondoroitin/dermatan sulfates and hyaluronan disaccharides by glycoblotting assisted sample preparation followedby a single ZIC HILIC chromatography

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Although various analytical techniques have been developed for GAGs, there continues to be a widespread need for sensitive, rapid, and highly quantitative analysis of GAGs. Disaccharide analysis is the first step in characterizing and assessing changes in the GAG compositional profile, which is typically comprised of isolation of proteoglycans, GAG release, enzymatic depolymerization, labeling of disaccharides by chromophore/fluorophore. Disaccharide composition can be determined using a combination of on–line separation methods such as capillary electrophoresis, ion–pairing RPC, HILIC in conjunction with UV absorbance, fluorescence or MS detection. However, to date no analytical techniques are available to simultaneously analyze disaccharides of heparan sulfates (HS), chondoroitin (CS)/dermatan sulfates (DS) and hyaluronan (HA). In this study, we aimed to establish a novel analytical technique applicable to all major classes of GAGs to realize high throughput cellular/tissue glycosaminoglycomics. We previously reported that a zwitterionic type of hydrophilic–interaction chromatography (ZIC–HILIC) allowed excellent separation of aminopyridine–labeled N–glycans [1]. Due to the high structural recognition capability of ZIC–HILIC for glycan structures, we sought to develop a novel analytical procedure widely applicable to GAG disaccharide analysis. The standard of most common 8 disaccharides comprising CS/DS, 8 disaccharides comprising HS, and 1 disaccharide that forming HA were mixed in equal amount. In addition to 17 kinds of disaccahride standards, maltotriose was also mixed as an internal standard (IS) to enable absolute quantification. The mixture was derivatized with 2–aminobenzamide and were subjected to ZIC–HILIC chromatography using a high concentration of volatile organic solvent (40–90% acetonitrile) and a low concentration of volatile electrolytes (~8mM NH_4Ac) with fluorescence or MS detection. Upon optimizing the elution conditions, ZIC–HILIC chromatography allowed separating all 17 disaccharide standards and IS. To authors' knowledge, this is the first successful demonstration of simultaneous analysis of HS, CS/DS and HA disaccharides. Furthermore, instead of using guanidinium chloride extraction followed by anion–exchange chromatography, a classical protocol for the isolation of proteoglycan required prior to analysis, we utilized chemoselective glycoblotting technique [2] to enrich GAG disaccharides from biological samples. We examined the benefits of a strategy exploiting glycoblotting technique using various cultured cell lines (e.g. NIH–3T3, HL60, RC4, CHO etc) as well as various rabbit ocular tissues as model studies. We demonstrate here that a combination of glycoblotting followed by a single ZIC HILIC chromatography provides rapid, sensitive and simultaneous analysis of all major classes of glycosaminoglycan disaccharides.

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Influence of the molecular composition of blood plasma on ELISA–based immunoassays

P 70

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ELISA tests are assays utilized to diagnose the presence and/or the concentration of specific antigens in a biological liquids by taking advantage of the specific interactions between an antibodies and antigens [1].They are widely utilized to detect and diagnose diseases. These tests are optimised to give reliable and reproducible results in spite of the variable molecular characteristics of the samples analysed. For instance plasma, the cell–depleted fraction of blood, contains different quantities of glycosaminoglycans, proteins, lipids, sugars and ions that reflect the different metabolism of each patient [2].

It is well known that many molecular components of patient blood samples are affected by several aspects such as dietary intake, physical activity, age etc. [3] it is still not clear how these differences impact on the reliability of immunoassays [4]. However, it is important to assess these elements because blood components assays are designed to test samples in conditions of normal tissue homeostasis while samples from patients may present a very different molecular composition as a consequence of their disease status.

We tested how varying the concentration of classes of lipids, proteins and glycosaminoglycans that are physiologically present in blood can influence the signal generated by the immunoassay consequently affecting the final result of the test (false positive/negative, wrong concentration measurement). The association of blood plasma components concentration and signal variations will contribute to unravel the mechanism that drive assays failure and contribute to develop more robust test.

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Fluorophore–Assisted Carbohydrate Electrophoresis as a useful tool to assay HA oligosaccharides

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Hyaluronan (HA) is a linear non–sulfated glycosaminoglycan composed of repeating disaccharide units (→4–D –glucuronic acid *β*–1,3–N–*acetyl*–D–glucosamine1→) with an overall molecular weight between 100 and 5000kDa. HA is a major constituent of the extracellular matrix (ECM) contributing to physiological processes, such as tissue remodeling as well as in pathological conditions, such as tumor progression. High molecular weight HA has been shown to inhibit angiogenesis, whereas degradation products of low molecular weight stimulate endothelial cell proliferation and migration. Moreover, oligosaccharide HA fragments have been shown to induce angiogenesis in several animal models. The aim of this project was to produce in a scale–up mode (at least 1g of HA) hyaluronan–derived Δ–oligosaccharides as well as to evaluate the available biochemical tools to monitor the HA–derived molecular sized following limited enzymatic digestion with chondroitinase ABC and ACII in combination. The identification of the obtained oligosaccharides of various sizes was performed by Fluorophore–Assisted Carbohydrate Electrophoresis (FACE) and Capillary Electrophoresis (CE). Furthermore, we purified each oligosaccharide fractions on Bio–gel P–10 gel filtration chromatographic column. The eluted fractions absorbing at 232 nm were pooled and freeze–dried to give various sizes (Δ–oligo– to Δ–disaccharides species). The residues obtained were then purified by rechromatography on the same column. This protocol provides the possibility of a scale–up production of various sizes of hyaluronan oligosaccharides in large quantities. The results obtained by FACE using labeling of oligosaccharides with 2–aminoacridone FACE was in agreement with those obtained using reversed polarity CE. Therefore, FACE is as a very useful tool for fast and reliable assay for screening the size of the various sizes of oligosaccharides with detection limits of 0.05 μg/ml.

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Analysis of chondroitin sulfate disaccharides in cell culture media using Fluorophore–Assisted Carbohydrate Electrophoresis and effects of pharmacological inhibitors in synthesis and sulfation pattern

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Chondroitin sulphate proteoglycans (CSPGs) play key roles in tumour progression. The high expression of CSPGs in fast growing tissues and cells is correlated with chondroitin sulfate (CS) chains and their sulfation pattern. The negatively charged CS chains interact with various ligands and receptors and activate signalling pathways which in turn stimulate tumour growth and progression. A methodology to analyze CS in secreted PGs and characterize its sulfation pattern in cell culture media is challenging in evaluating the effects of various pharmacological inhibitors. For this purpose we used the FACE set up (polyacrylamide gel electrophoresis for the analysis of fluorophore–labelled chondroitin sulfate) and evaluated various sample enrichment methodologies to characterize the variously sulfated moieties of CS. Several cells lines of colon and breast cancer as well as from foetal and adult fibroblasts were cultured and the obtained media were analysed by FACE. The method utilizes derivatization of reducing end of the variously sulfated chondroitin sulfate–derived disaccharides with 2–aminoacridone (AMAC), followed by electrophoresis in Tris–HCl buffer (pH 8.8), and polyacrylamide gel (*T* 50%/*C* 15%). The procedure followed for the treatment of samples ensured the complete liberation of the variously sulfated CS/DS–derived disaccharides. The electrophoretic method completely resolved 4–, 6–, di– and trisulfated Δ–disaccharides suggesting that FACE is a useful and sensitive tool for the rapid monitoring of CS in cell culture media. Moreover, it has been found that various RTK inhibitors affects both the synthesis of secreted CS by cancer cells as well as its sulfation pattern.

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Identification and characterization of hyaluronan oligosaccharides by capillary electrophoresis

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Hyaluronan (HA) is a high molecular weight glycosaminoglycan present in extracellular matrix (ECM). HA plays a vital role in maintaining tissue integrity, as well as in facilitating adhesion and differentiation of cells during inflammation, wound repair and embryonic development. Moreover, lots of studies have connected oligosaccharides of HA (oligo–HA) with a variety of biological processes, including tumorigenesis, morphogenesis, inflammation and host response to injury. Because of the wide significance of biological activities of hyaluronan and its fragments, the separation, identification and characterization of them from various biological samples and pharmaceutical formulations is of great interest. The aim of this study was to develop a highly sensitive and reliable assay that can be used for identification of hyaluronan oligosaccharides by Capillary Zone Electrophoresis (CZE). For this purpose a reversed polarity capillary electrophoresis (CE) approach has been used with 50 mM sodium dihydrogen orthophosphate, pH 3.00. Direct analysis of aqueous solutions and detection at 200 nm can be easily used to identify intact HA. Mixtures of different molecular sized oligosaccharides of HA (6, 8, 10, 12, and disaccharide) and HMW–HA were assayed. The 6, 8, 12 mers of HA, the Δdisaccharide as well as the high molecular weight HA were completely separated within 20 min at 30 kV and detection at 200 nm. Although the HA 10mer can be detected with the present set up, it not completely resolved and quantified. Intact HA was assayed to limited digestion digested with ACII and the produced oligosaccharides of HA were detected both at 200 nm and 232 nm. Furthermore, we developed a sample clean up methodology for identification and characterization of exogenous intact HA in serum. Considering the results of the developed methodology, capillary zone electrophoresis is useful tool for the separation of different molecular weight HA oligosaccharides, as well as for identification of high molecular weight intact HA in biological samples.

Chondroitin Sulfate – a Major Glycosaminoglycan in Brittle Stars

P 74

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Unbranched sulfated polysaccharides like chondroitin sulfate (CS) and heparan sulfate (HS) are evolutionary conserved from invertebrates to higher vertebrates. We use an invertebra model – *Ophiuroidea* (phylum *Echinodermata*) – to study various aspects of such carbohydrate chains in development and homeostasis of larval and adult stages mainly due the structural simplicity and high regenerative capacity of these animals. Ophiuroids, commonly called '*Brittle stars*', are penta–radially symmetric marine animals usually found at the bottom of the seabed. Autotomy and regeneration are regular phenomena in their natural habitat. Our present investigation focuses on characterisation of glycosaminoglycans (GAGs) from different species to gain insights on the functional role of GAGs in tissue regeneration processes.

Brittle stars (four species *Amphiura filiformis, A. chiajei, Ophiothrix nigra* and *O. fragilis*) were collected from 'Gullmarn' a fjord on the Swedish west coast. GAGs were isolated and analysed after enzymatic digestion with either CS or heparin lyases for composition. CS/DS chains with a high overall sulfation degree turned out to be the major form of GAGs in *brittle stars* whereas no detectable levels of HS could be found. These CS chains were further shown to bind to several growth factors of biological significance for cell signaling and developmental processes.

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Molecular Characterization of Saccharomyces cerevisiae Extracellular Matrix and Yeast Response to Different Sizes of Hyaluronan

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Yeast, *Saccharomyces cerevisiae*, presents itself as the best studied eukaryotic model, with the possibility to modify its genetic information with highly advanced molecular techniques. This microorganism displays a high degree of similarity and conservation of processes with higher eukaryotes. Several fundamental mechanisms such as cell cycle regulation, DNA replication, recombination and repair were first uncovered in this microorganism. From areas as cancer to neurological diseases, as Parkinson, yeast has provided vital information.

Different kinds of diseases in humans are connected to the Extracellular Matrix (ECM), like fibrosis and scleroderma. Therefore, the study of ECM components function as well as its regulation has been receiving lots of attention. Several of these, such as the structural molecule Hyaluronic Acid (HA) and diffusible growth factors, have the capacity to signal and redirect the behavior of the surrounding cells. HA has the ability to give different signal inputs depending solely on its size and concentration.

Yeast is a unicellular eukaryotic with the remarkable capacity to live as individual, small aggregates and colonies. Within a colony, yeast cells live or die according to their relative position, being able to differentiate into hyphae, as well as pseudohyphae, and stalks. All these accomplished through the communication between the cells within the colony, which are embedded on a yet uncharacterized ECM.

Besides ECM tridimensional structure aspect, none is known regarding its composition and organization. So an efficient method for the extraction, analysis and identification of S. *cerevisiae* ECM components, proteins and sugars, in colonies is currently in the last phase of implementation. The knowledge of the main constituents of the ECM will be a milestone in the establishment of this microbe as a model organism for ECM–related processes.

Yeast doesn't present the necessary enzymes and receptors to produce a response from HA. As yeast lacks the capacity to produce, degrade or "understand" the HA molecules, it is an excellent model for the manipulation of all aspects regarding the eukaryotic transduction of information from HA. The heterologous expression of HA receptors in yeast (CD44 and HMMR),the effect of this glycosaminoglycan on the main pathways, HOG, PKC or TOR, as well as changes in duplication time, chronological aging or life span, are also currently underway.

The main asset of our work resides on the use of a simpler and better understood model, the yeast, for the study of HA effects on eukaryotic cells, and how the information is transduced.

The characterization of yeast own ECM, and the understanding of the HA effect on this microorganism could lead yeast to a privileged position as a model organism for the study of ECM–related pathologies.

Proteolytic processing of TMEFF2 – a novel regulatory mechanism in prostate cancer?

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TMEFF2 (transmembrane protein with EGF–like and two follistatin motifs 2) is a type I protein expressed in brain and prostate and over–expressed in prostate cancer. The role of TMEFF2 in the development of prostate cancer is not clear. Several *in vitro* studies showed that over–expression of TMEFF2 suppresses prostate cancer growth. However, high levels of TMEFF2 expression were found in high grade tumours, suggesting that TMEFF2 supports prostate cancer development. The TMEFF2 ectodomain (TMEFF2–ECD) is released from the cell surface by ADAM10 and ADAM17 and the membrane–retained fragment undergoes regulated intramembrane processing by γ–secretase. The conflicting data about the role of TMEFF2 in prostate cancer may result from differential processing by proteases, especially those that are over–expressed in prostate cancer such as the type II transmembrane serine proteases (TTSPs), the GPI–anchored serine protease prostasin and other ADAMs.

HEK293 cells stably transfected with TMEFF2 tagged at the amino terminus with alkaline phosphatase (AP) and at the carboxy terminus with V5, were transiently transfected with potential TMEFF2 sheddases. Analysis of conditioned media for AP activity showed that TMEFF2 is also a substrate for ADAM9, ADAM12 and TTSPs, matriptase, matriptase–2 and hepsin. Moreover, western blot analysis of cell lysates using anti–V5 antibody showed that matriptase and hepsin but not matriptase–2 generate different in size TMEFF2 C–terminal fragments than ADAM–mediated processing. The increased release of TMEFF2–ECD from cells co–expressing matriptase–2 and TMEFF2 was inhibited by ADAMs inhibitors (GI25023X and GW280264X) which suggest that matriptase–2 regulates TMEFF2 shedding due to the activation of ADAMs rather than through direct TMEFF2 cleavage.

To establish the biological role of differential TMEFF2 processing, N–terminal TMEFF2 fragments predicted to be generated by TTSPs and ADAMs will be expressed in mammalian cells and tested for activation of different signalling pathways. The fate of TMEFF2 carboxy terminus following γ–secretase processing will be examined by confocal microscopy.

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The small leucine–rich proteoglycan populations in human intervertebral discs

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The small leucine–rich proteoglycans (SLRPs) contain a leucine–rich core protein (approx. 40kDa) and one or more glycosaminoglycan chains. They have many functions including tissue organisation and repair, protection of collagens from proteolysis, cellular proliferation and matrix adhesion. SLRPs are present in the intervertebral disc but their role in disc pathology is unclear. In degradative diseases such as osteoarthritis it has been shown that the core protein within the SLRPs becomes fragmented in cartilage, possibly altering their homeostatic functions resulting in loss of matrix integrity.

Human samples of disc were obtained from patients with scoliosis (n=7, 19–53y), degenerative disc disease (DDD) (n=6, 35–51y) and herniated discs (n=5, 33–68y). Proteoglycans were extracted in 4M GuHCl and characterised via enzymatic digestion following caesium chloride gradient centrifugation. The SLRPs (biglycan, decorin, fibromodulin, keratocan and lumican) were identified and their degree of fragmentation visualised by Western blotting using a combination of monoclonal and polyclonal antibodies to the core proteins.

All samples of disc demonstrated the presence of the SLRPs prior to caesium chloride centrifugation, but decorin was no longer detected following this step. In addition to the intact core proteins of biglycan, fibromodulin, keratocan and lumican there was evidence of fragmentation of all except for lumican. Biglycan fragmentation was present in the DDD and scoliosis samples, but not in the herniated discs. Fibromodulin fragments were also present in all the DDD samples, but only in some of the scoliosis samples, and again, not in the herniated discs. Keratocan fragments were greatest in the less severe DDD samples (Grades I and II) but their presence was variable in the scoliosis and herniated samples.

Although the number of samples investigated so far is low, fragmentation of the core protein of SLRPs appears to be a common occurrence in pathological intervertebral disc. In particular, the greatest amount of fragmentation of SLRP core proteins occurred within DDD samples. In scoliosis and herniated disc samples fragmentation was more varied. Overall these findings indicate that SLRP fragmentation differs within the various pathologies and may eventually provide information on the degenerative pathways involved. If changes within the extracellular matrix prior to collagen destruction can be identified then these changes may prove useful as early biomarkers of the different pathologies.

A Ion Mobility Assisted Data Independent Approach Towards the Qualitative and Quantitative Profiling of Biomarkers in Complex Protein Mixtures

Extra Talk

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A central goal in a proteomics study is to fully characterize a sample both qualitatively and quantitatively. A data-independent analysis yields reproducible fragmentation and peak area information for peptides in a complex mixture (1). The addition of ion mobility into this analysis inserts an orthogonal, gas-phase separation, occurring in the millisecond timescale, which is poised nicely between chromatographic and TOF mass spectrometry timescales. Peptides and their corresponding fragment ions are aligned by chromatographic retention time in such data-independent analyses. When ion mobility is used, peptides and their fragment ions also share the same mobility drift time which dramatically improves specificity. In this study, eukaryotic and prokaryotic samples were analyzed by one-dimensional (1D) and 2D chromatography using high-low pH RP-RP separations, both with and without ion mobility separation. The number of proteins identified in a sample doubled by using a five fraction 2D separation compared to 1D chromatography. Utilizing the mobility separation yielded an additional increase in the number of proteins and peptides by at least 20%, without any additional instrument time. The amount of information obtained from a sample depended on the amount loaded as well as the time dedicated to analysis of the sample. For one prokaryotic sample, 552 proteins were reproducibly identified with 1D chromatography without ion mobility from 0.75 ug of material. Loading 5 ug and performing 5 fractions, along with ion mobility, yielded 1260 reproducible protein identifications. Stoichiometry between proteins was determined by comparing the average intensity of the top three peptides to every protein to that of an internal standard (2). Agreement was found between the experimental stoichiometric ratios and those found in literature for many protein groups.

Reference

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Authors Index

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