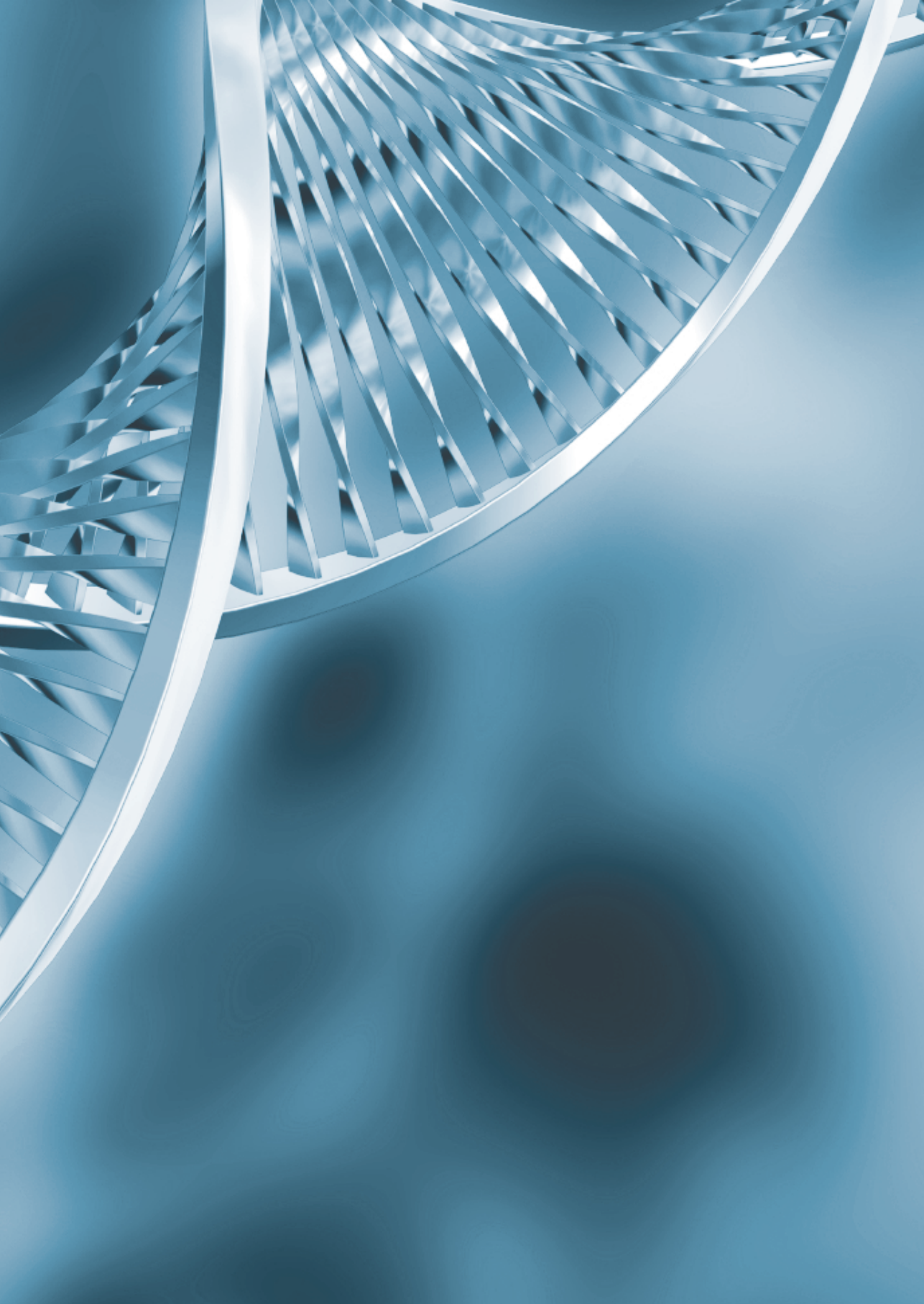
6th FEBS Advanced Lecture Course
FEBS - MPST 2017

Matrix Pathobiology, Signaling and Molecular Targets

Program & Abstracts

May 25th – May 30th, 2017
Spetses, Greece



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Spetses, May 25th – May 30th, 2017

Organizing Committee:

Nikos K. Karamanos
Chairman, University of Patras, GR

Renato V. Iozzo
Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, USA

John Couchman
Department of Biomedical Sciences, University of Copenhagen, DM

Liliana Schaefer
Institute of Pharmacology and Toxicology, Goethe University, GE

Dimitris Kletsas
Institute of Biology, NCSR "Demokritos", Athens, GR

Achilleas Theocharis
University of Patras, GR

Spyros S. Skandalis
University of Patras, GR

Chrysostomi Gialeli
Department of Translational Medicine, Lund University, SE

Young Scientists' Committee:

Nikolaos Afratis (Weizmann Institute of Science, IL)
Ilaria Caon (University of Insubria, IT)
Tabea Dierker (Uppsala University, SE)
Chrysostomi Gialeli (Lund University, SE)
Thomas Neill (Thomas Jefferson University, Philadelphia, USA)
Zoi Piperigkou (University of Patras, GR)
Mari Elen Strand (University of Oslo, NO)

Conference Venue:

Anargyrios and Korgialenios School of Spetses (A.K.S.S.)
at the Spetses Island – Greece

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

http://www.febs-mpst2017.upatras.gr
febs-mpst2017@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
e–mail: cpr@pat.forthnet.gr

Preface

Dear Colleagues and Friends,

On behalf of the Organizing Committee, it is a pleasure to invite you to the 6th FEBS Advanced Lecture Course (ALC) on Matrix Pathobiology, Signaling and Molecular Targets (6th FEBS-MPST 2017). This ALC follows the previous successful FEBS-MPST meetings started 10 years ago in 2007 at the University of Patras.

Matrix Biology is a fast growing field with significant impact in all areas of Biosciences. The 6-days FEBS-MPST 2017 offers oral sessions with invited plenary lectures, talks by confirmed/ senior speakers, general lectures and tutorials, selected talks and flash presentations related to the topics of the presented abstracts, poster presentations, panel discussions and speakers' corner/meet the expert. These sessions address both basic and applied science topics that appeal to the range of participants working in the fields of Matrix Biology, Biochemistry, Cell & Molecular Biology, Glycobiology, Structural Biology, Pharmacology, Biotechnology and Medicine.

The Organizing Committee has put together an outstanding group of internationally recognized experts as speakers. The lectures and tutorials provide the participants with an update of important new knowledge covering key areas of the field. We thank FEBS, the main sponsor of this ALC, ISMB, HSBMB and the private sectors for supporting the organization, Young Travel Fellowships and the Young Investigator Awards.

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

We are looking forward to welcome you in Greece for an exciting and memorable scientific meeting.

Nikos Karamanos
Chairman of the Organizing Committee

6th FEBS Advanced Lecture Course

Matrix Pathobiology, Signaling and Molecular Targets

Spetses, May 25th – May 30th, 2017

Program: Lecture (L), Selected Talk (ST), Flash Presentation (FP), Poster (P)

Thursday, May 25th

12:00 – 18:00 Registration

Chairpersons/discussion leaders: Tabea Dierker & Chrysostomi Gialeli
GENERAL LECTURES / TUTORIALS (L1-L6)

14:00 – 14:15 **S. Skandalis – L1** (University of Patras, Greece)
An introduction to ECM dynamic network – Proteoglycans

14:15 – 14:30 **N. Karamanos – L2** (University of Patras, Greece)
Syndecans – central players in cell functional properties and signaling

14:30 – 14:45 **D. Seidler – L3** (Hannover Medical School, Germany)
Glycosaminoglycans: structure, biosynthesis and key functions

14:45 – 15:00 **Y. Itoh – L4** (University of Oxford, UK)
Matrix Metalloproteinases: target or not to target

15:00 – 15:15 **R. Boot-Handford – L5** (University of Manchester, UK)
Collagens: types and functions

15:15 – 15:30 **P. Rousselle – L6** (CNRS, University Lyon 1, France)
ECM receptors: their importance in cell functions

Chairpersons/discussion leaders: Thomas Neill & Zoi Piperigkou
PANEL DISCUSSION

15:30 – 16:45 Groups of young scientists will discuss emerging aspects with tutors

16:45 – 17:15 Coffee break

Chairpersons/discussion leaders: Nikolaos Afratis & Ilaria Caon
CAREER DEVELOPMENT SESSION I

17:15 – 17:30 **G. Guner** (Chair, FEBS Education Committee; School of Medicine, Izmir, Turkey)
Ph.D. Training: Tips for success for a Ph.D. student

17:30 – 17:35 Discussion

17:35 – 17:55 **B. Vertessy** (Chair, FEBS Advanced Courses Committee; Budapest, Hungary)
Career planning: how to jump the transitions on your career path?

17:55 – 18:00 Discussion

18:00 – 18:20 **M. Papatriantafyllou** (Editor of FEBS Letters, Heidelberg, Germany)
How to write a scientific paper

18:20 – 18:25 Discussion

18:25 – 18:30 Break

Chairpersons: Renato Iozzo & Nikos Karamanos

OPENING SESSION

18:30 – 18:50 **N. Karamanos**, Chairman of FEBS ALCs - MPST 2007 - 2017
Ten years of FEBS ALCs on Matrix Pathobiology: achievements and prospectives

18:50 – 19:15 Welcome Addresses by
V. Kyriazopoulou, Rector of the University of Patras
B. Vertessy, Chair of the FEBS Advanced Courses Committee
D. Kletsas, President of the Hellenic Society of Biochemistry & Molecular Biology
L. Schaefer, President of ISMB

IUBMB LECTURE (L7)

19:15 – 20:15 **F.-X. Maquart – L7** (University of Reims, France)
Matrikines as important players in matrix physiopathology
Honorary Awards

20:30 Welcome Reception

Friday, May 26th

Chairperson/discussion leader: Liliana Schaefer

LECTURES / TUTORIALS – GLYCOBIOLOGY - GLYCOSAMINOGLYCANS & PROTEOGLYCANS (L8 - L9)

09:00 – 09:20 **P. Heldin – L8** (Ludwig Institute for Cancer Research, Uppsala University, Sweden)
The role of hyaluronan - CD44 interactions in inflammation and cancer

09:20 – 09:25 Discussion

09:25 – 09:45 **L. Schaefer – L9** (Goethe University, Germany)
Proteoglycan neofunctions: Regulation of inflammation & beyond

09:45 – 09:50 Discussion

SELECTED TALKS (ST1 – ST4)

09:50 – 10:00 **A. Abbadi – ST1 / P1** (Cleveland Clinic Foundation, USA)
Heparin inhibits the differentiation of M1 and promotes M2 macrophages under hyperglycemic stress

10:00 – 10:10 **T. Dierker – ST2 / P2** (Uppsala University, Sweden)
Identification of novel chondroitin sulfate sulfotransferases and proteoglycan core proteins in the nematode C. elegans

10:10 – 10:20 **K. Karamanou – ST3 / P3** (University of Reims, France)
Lumican effectively regulates the estrogen receptors-associated functional properties of breast cancer cells, expression of matrix effectors and epithelial-to-mesenchymal transition

10:20 – 10:30 **S. Skandalis – ST4 / P4** (University of Patras, Greece)
Effects of the inhibition of hyaluronan synthesis by 4-methylumbelliferone on breast cancer cells of different ER status

10:30 – 11:00 Coffee break

Chairperson/discussion leader: Irit Sagi

LECTURES / TUTORIALS – METALLOPROTEINASES & MATRIX AS A DYNAMIC STRUCTURAL NETWORK (L10 – L15)

11:00 – 11:20	**Y. Itoh – L10** (University of Oxford, UK) *Role of MT1-MMP in inflammatory arthritis: a potential therapeutic target?*
11:20 – 11:25	Discussion
11:25 – 11:40	**I. Sagi – L11** (Weizmann Institute of Science, Israel) *Matrix remodeling and proteolysis as key regulator of embryo implantation*
11.40 – 11.45	Discussion
11:45 – 12:05	**M. Franchi – L12** (University of Bologna, Italy) *Cancer cell morphology and local microenvironment*
12:05 – 12:10	Discussion
12:15	All together photo
12:20 – 14:00	Lunch
14:00 – 16:30	**Poster Session (I): P1 – P21 / Discussion groups (I)**

Chairperson/discussion leader: Dimitris Kletsas

SELECTED TALKS (ST5 – ST7)

16:30 – 16:40	**I. Gonçalves – ST5 / P22** (Lund University, Sweden) *ADAMTS-7 is associated with a high-risk plaque phenotype in human atherosclerosis*
16:40 – 16:50	**I. Solomonov – ST6 / P23** (Weizmann Institute of Science, Israel) *Distinct biological events generated by ECM proteolysis by two homologous collagenases*
16:50 – 17:00	**C. Gauthier-Rouviere – ST7 / P24** (University of Montpellier, France) *The new metastatic markers flotillins promote tumor cell invasion through MT1-MMP-dependent ECM degradation*
17:00 – 17:20	**D. Kletsas – L13** (NCSR "Demokritos", Greece) *Altered metalloprotease and proteoglycan expression in senescent human breast stromal fibroblasts: implications in tumour progression*
17:20 – 17:25	Discussion
17:25 – 17:40	**S. Fournel-Gigleux – L14** (University of Lorraine, France) *Glycosaminoglycan biosynthetic enzymes: actors and targets in pathobiology*
17:40 – 17:45	Discussion
17:45 – 18:00	**M. Dauchez – L15** (University of Reims Champagne-Ardenne, France) *Molecular modelling of the ECM: what could we do? What could we learn?*
18:00 – 18:05	Discussion

Chairperson/discussion leader: Spyros Skandalis
CAREER DEVELOPMENT SESSION II

18:05 – 18:25 **B. Vertessy** (Chair, FEBS Advanced Course Committee; Budapest, Hungary)
How to write a project proposal

18:25 – 18:30 Discussion

20:00 Dinner

Saturday, May 27th

Chairperson/discussion leader: Renato Iozzo
LECTURES / TUTORIALS – MATRIX PATHOBIOLOGY (L16 – L19)

09:00 – 09:20 **R. Iozzo – L16** (Thomas Jefferson University, USA)
Novel proteoglycan roles in regulating autophagy and angiogenesis

09:20 – 09:25 Discussion

09:25 – 09:45 **S. Brézillon – L17** (University of Reims, France)
Lumican delays melanoma growth in mice and drives tumor molecular assembly as well as response to extracellular matrix-targeted therapy

09:45 – 09:50 Discussion

09:50 – 10:10 **G. Christensen – L18** (University of Oslo, Norway)
Extracellular matrix and heart failure

10:10 – 10:15 Discussion

10:15 – 10:35 **A. Theocharis – L19** (University of Patras, Greece)
Serglycin implication in malignancies: unraveling novel molecular mechanisms

10:35 – 10:40 Discussion

10:40 – 12:30 **Speakers corner (I)**

12:30 Lunch Box

13:30 – 21:30 Excursion / Dinner

Sunday, May 28th

Chairperson/discussion leader: Ralph Sanderson
LECTURES / TUTORIALS – INTERACTIONS & FUNCTIONS OF MATRIX MACROMOLECULES (L20 - L21)

09:00 – 09:20 **P. Rousselle – L20** (University Lyon 1, France)
Dual function of syndecan-1 in epidermal regeneration and homeostasis

09:20 – 09:25 Discussion

09:25 – 09:45 **R. Sanderson – L21** (University of Alabama, USA)
Roles of heparanase and exosomes in regulating the tumor microenvironment

09:45 – 09:50 Discussion

SELECTED TALKS (ST8 – ST11)

09:50 – 10:00 **V. Juskaite – ST8 / P25** (Imperial College London, UK)
Collagen induces activation of DDR1 through lateral dimer association and phosphorylation between dimers

10:00 – 10:10 **M. Gross-Cohen – ST9 / P26** (Technion, Israel)
Heparanase 2 attenuates tumor vascularity and growth, associating with enhanced cell differentiation and inversely correlating with tumor grade and stage

10:10 – 10:20 **P. Sogaard – ST10 / P27** (University of Oxford, UK)
Signalling through the collagen receptor, DDR1, is required for epithelial polarisation and morphological remodelling in 3D matrices

10:20 – 10:30 **V. Abbonante - ST11 / P28** (University of Pavia, Italy)
Altered megakaryopoiesis in Type VI collagen KO mice

10:30 – 11:00 Coffee break

Chairperson/discussion leader: Alberto Passi
LECTURES / TUTORIALS – GLYCOBIOLOGY & METABOLIC REGULATION OF ECM MOLECULES (L22 - L23)

11:00 – 11:20 **A. Passi – L22** (University of Insubria, Italy)
Translational control and epigenetic events in hyaluronan synthase 2

11:20 – 11:25 Discussion

11:25 – 11:45 **D. Seidler – L23** (Hannover Medical School, Germany)
The function of 2 O-sulfation of chondroitin/dermatan sulfate under physiological and pathological conditions

11:45 – 11:50 Discussion

SELECTED TALKS (ST12 – ST15)

11:50 – 12:00 **A.J. Deen – ST12 / P29** (University of Eastern Finland, Finland)
UDP-N-Acetylglucosamine regulation of epithelial-to-mesenchymal transition in breast cancer

12:00 – 12:10 **S. Delbaere – ST13 / P30** (Ghent University, Belgium)
Zebrafish modeling of the β4GalT7-deficient type of Ehlers-Danlos syndrome

12:10 – 12:20 **I. Caon – ST14 / P31** (University of Insubria, Italy)
HAS2-AS1 regulates breast cancer cells aggressiveness through a molecular sponge effect

12:20 – 12:30 **U. Arasu – ST15 / P32** (University of Eastern Finland, Finland)
HAS3-induced Extracellular Vesicles (HAS3-EVs) promote EMT in human keratinocyte to acquire malignant properties

12:30 – 14:00 Lunch

14:00 – 16:00 **Poster Session (II): P22 – P47 / Discussion groups (II)**

Chairperson/discussion leader: Elvira Grigorieva

LECTURES / TUTORIALS – GLYCOBIOLOGY & METABOLIC REGULATION OF ECM MOLECULES (L24 - L25)

16:00 – 16:20 **M.I. Tammi – L24** (University of Eastern Finland, Finland)
Hyaluronan synthesis and O-GlcNAc-signaling in malignant tumors role of hexosamine biosynthesis and UDP-N-acetylglucosamine content

16:20 – 16:25 Discussion

16:25 – 16:45 **E. Grigorieva – L25** (Karolinska Institute, Sweden)
Heparan sulfate biosynthesis in health and disease

16:45 – 16:50 Discussion

SELECTED TALKS (ST16 – ST17)

16:50 – 17:00 **Z. Piperigkou – ST16 / P33** (University of Patras, Greece)
MicroRNA targeting as a regulatory mechanism of breast cancer cells with different estrogen receptor status

17:00 – 17:10 **J. Wragg – ST17 / P34** (University of Manchester, UK)
The impacts of ER stress associated chondrodysplasias on metabolomics

FLASH PRESENTATIONS (FP1 – FP5)

17:10 – 17:15 **K. Jandl – FP1 / P35** (Ludwig Boltzmann Institute for Lung Vascular Research, Austria)
Alterations in basement membrane proteoglycans defines vascular remodelling in pulmonary hypertension associated with lung diseases

17:15 – 17:20 **N. Mohammadzadeh – FP2 / P36** (University of Oslo, Norway)
Lack of the extracellular matrix proteoglycan lumican in mice exacerbates left ventricular dilatation and contractile dysfunction upon pressure overload

17:20 – 17:25 **D. Vitale – FP3 / P37** (Cit Noba, Unnoba-Conicet, Argentina)
Hyaluronan modulates tumor progression, angiogenesis and proliferation during Doxorubicin treatment

17:25 – 17:30 **M.E. Christopoulou – FP4 / P38** (University of Patras, Greece)
TGF-β1 suppresses the IL-1β-induced production of matrix metalloproteinase-1 from human pterygium fibroblasts through PKA activation

17:30 – 17:35 **C. Yeung – FP5 / P39** (University of Copenhagen, Denmark)
Understanding the tendon circadian clock as therapeutic target for treatment of age-related chronic tendinopathies

17:35 – 18:45 **Speakers corner (II)**

19:00 – 20:30 Free time

21:00 Dinner

Chairperson/discussion leader: Sylvie Ricard-Blum

LECTURES / TUTORIALS – ECM NETWORKS & ECM-BASED NANOTECHNOLOGY (L26 - L27)

09:00 – 09:20 **S. Ricard-Blum – L26** (University Lyon 1, France)
Extracellular interaction networks: from extracellular matrix assembly to the cell surface

09:20 – 09:25 Discussion

09:25 – 09:45 **M. Pavão – L27** (University of Rio de Janeiro, Brasil)
Non-anticoagulant Heparan sulfate from a marine bivalve mollusk inhibits P-selectin and heparanase: effect on cellular recruitment and tumor metastasis

09:45 – 09:50 Discussion

SELECTED TALKS (ST18 – ST21)

09:50 – 10:00 **J. Caldeira – ST18 / P40** (University of Porto, Portugal)
Pathobiology of the ageing matrix: on the path to intervertebral disc regeneration

10:00 – 10:10 **A. Buson – ST19 / P41** (Pharmaxis Ltd., Australia)
Inhibition of lysyl oxidase like 2 reduces collagen cardiac interstitial fibrosis in mice

10:10 – 10:20 **M. Weissmann – ST20 / P42** (Technion, Israel)
9E8 Anti-Heparanase antibody neutralizes heparanase activity and inhibits invasion of cancer cells after class switching from IgM to IgG

10:20 – 10:30 **S. van Helvert – ST21 / P43** (Radboud Institute Molecular Life Science, The Netherlands)
Strain stiffening and structural ECM remodelling by invading cancer cells

FLASH PRESENTATIONS (FP6 – FP11)

10:30 – 10:35 **A. Kocak – FP6 / P44** (Dokuz Eylul University, Turkey)
Protective effects of epigallocatechin-3-gallate on fibrosis in scleroderma model

10:35 – 10:40 **I. Kalograiaki – FP7 / P45** (Biological Research Center (CIB), Spain)
Structural studies of a tumour-associated carbohydrate epitope defined by the monoclonal antibody A10

10:40 – 10:45 **M. Szczygiel – FP8 / P46** (German Cancer Research Center (DKFZ), Germany)
Investigating the dynamics of tumor - stroma interactions in lung cancer

10:45 – 10:50 **M. Le Borgne-Rochet – FP9 / P47** (University of Montpellier, France)
P-cadherin promotes reorientation of collagen fibers during collective cell migration

10:50 – 10:55 **E. Bengtsson – FP10 / P48** (Lund University, Sweden)
Cartilage oligomeric matrix protein associates with a vulnerable plaque phenotype in human atherosclerosis

10:55 – 11:00 **R. Cocchiola – FP11 / P49** (University of Rome, Italy)
Glucosamine and its peptidyl-derivative NAPA: novel therapeutic strategy for chondrocytes matrix remodeling

11:00 – 11:30 Coffee break

Chairperson/discussion leader: Martin Götte

LECTURES / TUTORIALS – SIGNALING & DISEASE MOLECULAR TARGETING (L28 – L34)

11:30 – 11:50 **M. Götte – L28** (Muenster University, Germany)
Heparan sulfate proteoglycans as modulators of therapeutic resistance in breast cancer

11:50 – 11:55 Discussion

11:55 – 12:15 **I. Kovalszky – L29** (Semmelweis University Budapest, Hungary)
Cervical cancer cells downregulate tissue pathway inhibitor2 (TFPI2) in tumor-associated fibroblasts

12:15 – 12:20 Discussion

12:20 – 12:35 **T. Neill – L30** (Thomas Jefferson University, USA)
Decorin evokes tumor cell mitophagy via mitostatin and Parkin

12:35 – 12:40 Discussion

SELECTED TALKS (ST22 – ST24)

12:40 – 12:50 **K. Baghy – ST22 / P50** (Semmelweis University, Budapest, Hungary)
Decorin delivery hinder primary and metastatic tumor formation in the liver

12:50 – 13:00 **D. Manou – ST23 / P51** (University of Patras, Greece)
Suppression of serglycin decreases glioblastoma cell aggressiveness

13:00 – 13:10 **C. Gialeli – ST24 / P52** (Lund University, Sweden)
Complement inhibitor CSMD1 acts as a tumor suppressor in breast cancer by interacting with extracellular matrix receptor EGFR

13:10 – 14:30 Lunch

14:30 – 16:30 **Poster Session (III): P48 – P72 / Discussion groups (III)**

Chairperson/discussion leader: John Couchman

16:30 – 16:50 **J. Couchman – L31** (University of Copenhagen, Denmark)
Syndecans: signalling and regulation of cell adhesion

16:50 – 16:55 Discussion

16:55 – 17:10 **I. Gjervold Lunde – L32** (University of Oslo, Oslo, Norway)
Syndecan-4 promotes hypertrophic remodeling of the heart through NFAT signaling

17:10 – 17:15 Discussion

17:15 – 17:30 **D. Zeugolis – L33** (Remodel Laboratory, NUI Galway, Galway, Ireland)
Rebuilding the matrix: The influence of microenvironmental signalling in matrix synthesis and deposition

17:30 – 17:35 Discussion

17:35 – 17:50 **P. Bruckner – L34** (Muenster University, Germany)
The binding capacity of α1β1-, α2β1- and α10β1-integrins depends on non-collagenous surface macromolecules rather than the collagens in cartilage fibrils

17:50 – 17:55 Discussion

SELECTED TALKS (ST25 – ST27)

17:55 – 18:05 **N. Afratis – ST25 / P53** (University of Patras, Greece
University of Copenhagen, Denmark)
IGF-IR inhibits breast cancer cells aggressiveness via regulation of syndecan-4 and MMPs expression

18:05 – 18:15 **A. Kiss – ST26 / P54** (University of Szeged, Hungary)
Chronic inflammation, oxidative-nitrosative stress, mitochondrial dysfunction, improper integrin expression, loss of sarcomeres and proteolysis of myofibrillar proteins allow diagnosis of muscular dystrophy in Drosophila type IV collagen col4a1 mutants

18:15 – 18:25 **K. Rada – ST27 / P55** (Semmelweis University Budapest, Hungary)
Tumour-stroma interaction in the case of different hepatomas

20:30 Dinner

Tuesday, May 30th

Chairperson/discussion leader: Raymond Boot-Handford
LECTURES / TUTORIALS – MATRIX REGULATION IN HEALTH & DISEASE (L35 – L38)

09:00 – 09:20 **R. Boot-Handford – L35** (University of Manchester, UK)
ER stress as a pathogenic factor in ECM diseases

09:20 – 09:25 Discussion

09:25 – 09:45 **M. Onisto – L36** (University of Padova, Italy)
Role of heparanase in the onset of organ fibrosis

09:45 – 09:50 Discussion

SELECTED TALKS (ST28 – ST31)

09:50 – 10:00 **G. Bellin – ST28 / P56** (University/Hospital of Verona, Italy)
Heparanase regulates macrophage M1 polarization and tubular cells crosstalk after kidney ischemia/reperfusion injury

10:00 – 10:10 **E. Shimshoni – ST29 / P57** (Weizmann Institute of Science, Rehovot, Israel)
Unraveling silent pathological states by decoding their unique extracellular matrix signature in intestinal inflammation

10:10 – 10:20 **M. Pinto – ST30 / P58** (University of Porto, Portugal)
Decellularized human colorectal cancer matrices polarize macrophages towards a pro-invasive phenotype

10:20 – 10:30 **J. Toraskar – ST31 / P59** (Norwegian University of Science and Technology-NTNU, Norway)
Extracellular matrix Nephronectin in breast cancer metastasis

FLASH PRESENTATIONS (FP12 – FP17)

10:30 – 10:35 **E. Caravà – FP12 / P60** (University of Insubria, Italy)
Vascular inflammation and extracellular matrix modifications

10:35 – 10:40 **R. Constantini – FP13 / P61** (University of Pavia, Italy)
Towards the generation of an animal model of chondrodysplasia with joint dislocations gPAPP type

10:40 – 10:45 **S. Gencer – FP14 / P62** (áskódar University, Turkey)
TGF-í receptor I/II signaling at primary gilia membrane is regulated by ceramide to modulate cell migration

10:45 – 10:50 **M. Catela – FP15 / P63** (University of Reims, France)
Lumican effect on MMP-14 expression and migration of Snail overexpressing MC38 colon carcinoma cells

10:50 – 10:55 **D. Harmanci – FP16 / P64** (Dokuz Eylul University, Turkey)
The activity of serum superoxide dismutase enzyme and the serum level of lipid peroxidation product malondialdehyde in different forms of scleroderma

10:55 – 11:00 **V.R. Krishnaswamy – FP17 / P65** (Weizmann Institute of Science, Israel)
Intriguing role of dermatopontin in skin re-epithelialization: Implications on chronic cutaneous wounds

Chairperson/discussion leader: Rashmin Savani

11:00 – 11:15 **R. Savani – L37** (University of Texas, USA)
Regulation of inflammation by the hyaluronan receptor RHAMM: Studies in knockout and transgenic mice

11:15 – 11:20 Discussion

11:20 – 11:35 **A. Rossi – L38** (Department of Molecular Medicine, Unit of Biochemistry, Pavia, Italy)
In vivo models of chondrodysplasias caused by defects in proteoglycan biosynthesis: phenotyping and pharmacological approaches

11:35 – 11:40 Discussion

11:40 – 12:00 **Closing remarks & Awards**

12:00 – 13:00 **Farewell Party**

Afternoon **Departure**

Invited Lectures/Tutorials

L
01

An introduction to ECM dynamic network - Proteoglycans

Spyros S. Skandalis

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

Extracellular matrix (ECM) was initially characterized as the substance outside the cells possessing structural but not functional roles. During the last 30 years, the evolution of molecular and cell biological techniques has led to the demonstration that the ECM in not the passive residence of cells but is highly interactive and plays a major role in normal and pathological conditions. ECM components contain structural motifs further modified by specific post-translational events that determine their molecular interactions to form complex and dynamic networks associated with resident cells. Among the most important matrix molecules/effectors are cell surface and secreted proteoglycans (including glycosaminoglycans), matrix degrading enzymes (such as matrix metalloproteinases, and heparanase), collagens and non-collagenous proteins, and matrix receptors (such as integrins and CD44). They all play crucial roles in cell growth, differentiation, stem cell niche, autophagy, cytoskeleton reorganization, and tissue organization and integrity. Although ECMs undergo continues fine-tuned remodeling during normal development, their composition and structure are dysregulated during the onset and progression of multiple pathologies, including inflammation and cancer, creating an environment permissive to the survival and expansion of the transformed cells. Special emphasis is given on proteoglycans, which constitute complex hybrid molecules with emerging roles and functions in health and disease.

Syndecans-central players in cell functional properties and signaling

L
02

Nikos Karamanos

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

Syndecans consist a family of four transmembrane proteoglycans in mammals. They have a highly conserved structural organization, consisting of an N-terminal ectodomain, single transmembrane domain and C-terminal cytoplasmic domain. The association between syndecans and the actin cytoskeleton has been well investigated, which has consequences for the regulation of cell adhesion and migration. Specifically, ecto- and cytoplasmic domains are responsible for the interaction with extracellular matrix molecules and intracellular kinases, respectively. These interactions indicate syndecans as key molecules during cancer initiation and progression. Particularly syndecans interact with other cell surface receptors, such as growth factor receptors, integrins and ion-channels, which lead to activation of downstream signaling pathways, which are critical for the cellular behavior as well as the intracellular calcium regulation and homeostasis. The syndecan-mediated regulation of calcium metabolism is highly correlated with cells' adhesion phenotype through the actin cytoskeleton and formation of junctions, with implications during differentiation and disease progression.

L
03

Glycosaminoglycans: structure, biosynthesis and key functions

D.G. Seidler

Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Glycosaminoglycans (GAG) are linear polysaccharides composed of repeating disaccharides units and found on the cell surface and the extracellular matrix. The disaccharide units consist of an amino sugar and a hexuronic acid. GAGs are classified into four groups: heparin/heparan sulfate (HS), chondroitin/dermatan sulfate (CS/DS), keratin sulfate and hyaluronan. Except for hyaluronan the GAGs are synthesized via a tetra-saccharide linkage to a protein core forming proteoglycans. Specific glycosyltransferases transfer the first GlcNAc for heparin/HS or GalNAc for CS/DS synthesis. As the HS chains are polymerized, they undergo a series of modification reactions involving N-deacetylation and N-sulfation of the GlcNAc, epimerization of GlcA to IdoA, and O-sulfation at various positions. CS polymerization is catalyzed by bifunctional enzymes chondroitin synthases. Epimerization of GlcA to IdoA at the polymer level constitutes the formation of DS followed by O-sulfation patterns in CS and DS at various positions. The sulfation patterns of the GAGs introduce a micro-heterogeneity along the chain. The structural diversity enables GAGs to bind and interact with a wide variety of proteins, such as growth factors, chemokines, morphogens and extracellular matrix components. GAGs are produced by virtually all vertebrate cells and modulate biological processes like cell proliferation, adhesion, migration and survival. For example, mutations in genes encoding for enzymes involved in the biosynthesis of dermatan sulfate leads to the connective tissue disorder Ehlers-Danlos syndrome.

Matrix Metalloproteinases: target or not to target

L 04

Yoshifumi Itoh

Kennedy Institute of Rheumatology, University of Oxford, Oxford,UK

Matrix Metalloproteinases (MMPs) are structurally related zinc metalloproteinases. They are secreted from the cell (soluble MMPs) or bound to the cell surface (membrane-type MMPs; MT-MMPs) and degrade ECM and other proteins. At present, 23 mammalian MMPs have been identified and they are classified according to their substrate specificity and structural similarity. Activities of MMPs are tightly regulated in physiological conditions while dysregulated MMP activities contribute to progression of diseases. Because of their involvement in disease progression, including cancer and arthritis, many small molecule broad spectrum MMP inhibitors were developed in 1990s. However, all clinical trials of those inhibitors were not successful, and pharmaceutical companies have been traumatised. However, inhibition of MMPs may still be a valid therapeutic strategy to prevent progression of tissue destructive diseases if one can identify target MMPs and able to inhibit them in highly selective manner.

L
05

Collagens: types and functions

Ray Boot-Handford

Wellcome Centre for Cell-Matrix Research, Faculty of Biology, Medicine and Health, & Manchester Academic Health Science Centre, University of Manchester, UK

In this tutorial, I will cover, the basic features of collagen molecules, how collagens are synthesised, how fibrillar collagens are processed and assembled into fibrillar structures and how mutations in collagen and associated genes can impact upon these processes. In addition, I will briefly touch upon the evolution of collagen and collagen-like families.

ECM receptors: their importance in cell functions

L 06

Patricia Rousselle

CNRS, University Lyon 1, France
patricia.rousselle@ibcp.fr

All cellular processes are determined by adhesive interactions between cells and their local microenvironment. Integrins, which constitute one class of cell-adhesion receptor, are multifunctional proteins that link cells to the extracellular matrix (ECM) and organise integrin adhesion complexes at the cell periphery. Following adhesion and clustering, integrins recruit different cytoskeletal and cytoplasmic proteins, which anchor the newly formed complexes to the actin cytoskeleton. This ultimately leads to the local remodeling of the actin cytoskeleton and the formation of specialized adhesive structures. Integrin-based adhesions provide anchor points for assembling and organising the cytoskeleton and cell shape, and for orchestrating migration. Integrins also control the fate and function of cells by influencing their proliferation, apoptosis, differentiation an death. Integrins affect the expression and activity of other integrins and other types of adhesion molecules. Although integrins are the major cell surface receptors for the ECM, other adhesive systems, including transmembrane proteoglycans (such as syndecans), have recently drawn attention as an important class of adhesion receptors working in concert with integrins. Like integrins, syndecans lack intrinsic enzymatic activities and thus transmit intracellular signals by interacting with various effector proteins, including both structural and signalling molecules. Following ligand binding, syndecans participate in the formation of adhesion complexes comprised of several actin-associated proteins. They can co-operate synergistically with integrins to regulate adhesion-complex formation, cell spreading and directional migration or function as cell adhesion receptors that independently mediate cell signalling. Other transmembrane proteins such as ADAMS and Tetraspanins can also be mentioned as important modulators of cell-ECM interactions.

L 07

Matrikines as important players in matrix physiopathology

François-Xavier Maquart [1,2], Jean-Claude Monboisse [1,2], Bertrand Brassart[1], Laurent Ramont [1,2], Sylvie Brassart-Pasco [1]

[1] *Université de Reims-Champagne-Ardenne, CNRS UMR 7369, UFR Médecine, 51095 Reims Cedex, France ;* [2] *CHU de Reims, 51092 Reims cedex, France*

Matrikines are specific domains of extracellular matrix proteins which are able to regulate many cellular functions (Maquart et al, 1999). Our laboratory has been interested in the study of these peptides for more than 30 years now. Our first interest was devoted to the role of matrikines in wound repair, demonstrating the stimulating effect of GHK-Cu, a copper-tripeptide complex derived from collagen (Maquart et al, 1993). Elastin-derived peptides, also called elastokines, are also able to stimulate wound healing. More recently, we were able to demonstrate the anti-tumor activity of the NC1 domains of several basement membrane-associated collagens. Particularly, the first anti-tumor basement membrane collagen-derived matrikine, now called tumstatin, was characterized in our laboratory, in collaboration with N. A. Kefalides' group (Pasco et al, 2000). These matrikines exert their anti-tumor effects through anti-angiogenic and/or anti-invasive activities.

Type IV collagen is a major constituent of the basement membrane zone. It is composed of three α(IV) chains choosen among 6 different types called α1(IV) to α6(IV), which all include a C-terminal noncollagenic NC1domain. In this presentation, I'll focus on an anti-tumor matrikine derived from the NC1 domain of the α4(IV) chain of type IV collagen, that we called tetrastatin (Brassart-Pasco et al, 2012). We demonstrated for the first time that tetrastatin exerts a potent anti-tumor activity both in vitro on human UACC 903 melanoma cells and in vivo in a human melanoma model in nude mice (Brassart-Pasco et al, 2012. The anti-tumor effects of tetrasatatin were mediated through an inhibition of cell proliferation and invasive capacities. MT1-MMP activation was largely decreased and its cellular distribution was altered, with a decrease of its expression at the migration front and a loss of the cell migratory phenotype in tetrastin-overexpressing melanoma cells. In collaboration with Pr Sylvie Ricard-Blum (University Claude Bernard-Lyon I), we were able to show that tetrastatin binds to the αvβ3 integrin. Recent data permitted us to identify a 13 amino-acid sequence from tetrastatin (QS13) which reproduces the anti-tumor effects of tetrastatin and binds to specific sites on αvβ3 integrin.

Taken together, our results suggest that matrikines or derived peptides have a high potential for the design of new drugs.

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The role of hyaluronan - CD44 interactions in inflammation and cancer

L 08

Paraskevi Heldin[1,2], Constantinos Kolliopoulos[1], Merima Mehic[1], Chun-Yu Lin[1], Yanshuang Li[1], Aino Ruusala[1]

[1]*Ludwig Institute for Cancer Research,* [2]*Department of Medical Biochemistry and microbiology, Uppsala University, Biomedical Center, Box 595, SE-75124 Uppsala, Sweden*

Our aim is to elucidate the mechanism of regulation of hyaluronan levels and the importance of its interaction with CD44 in regulation of cell fate. The hyaluronan synthesizing enzyme HAS2 and its receptor CD44 are upregulated in advanced cancers and the activity and stability of HAS2 are modulated by post-translational modifications.

We have demonstrated that TGFβ stimulates the production of hyaluronan in normal mammary breast epithelial cells by upregulation of HAS2 and that efficient TGFβ-induced epithelial to mesenchymal transition (EMT) requires the expression of HAS2. To elucidate further the molecular mechanisms by which TGFβ induces HAS2 at the transcriptional level, we investigated the possible involvement of a natural antisense transcript of HAS2 (HAS2-AS). Our data revealed that HAS2-AS cooperates with the high mobility group A2 (HMGA2) protein, which also is induced by TGFβ during EMT, and promote the recruitment of transcriptional factors to drive HAS2 expression and breast cancer progression.

HAS2 undergoes both mono-ubiquitination at Lys 190 and poly-ubiquitination. We have demonstrated that the deubiquitinating enzymes USP17 and USP4 differentially removed the ubiquitination of HAS2. USP17 efficiently removed poly-ubiquitination while USP4 preferentially removed mono-ubiquitination of 6myc-HAS2. USP17 significantly stabilized 6myc-HAS2 protein levels, whereas USP4 did not. Thus, the activity and stability of HAS2 are regulated by mono- and poly-ubiquitination, respectively.

The expression of the hyaluronan cell surface receptor CD44 in tumor tissues correlates with high levels of p53. We have found that CD44 interacts with iASPP, a specific inhibitor of wt p53, both in normal and cancer cells expressing wt p53. Our data suggests a mutual regulation of iASPP-CD44 and iASPP-p53 complexes, and that p53 and CD44 levels regulate iASPP-CD44 and iASPP-p53 complex formation, respectively.

Dengue fever is a re-emerging infectious disease in tropical countries. Recently, we found that increased plasma hyaluronan levels correlate with the severity of Dengue virus infection. Currently, we characterize the molecular mechanisms involved, whereby hyaluronan signals via its receptor CD44 and modulate the outcome of an acute and a chronic inflammatory response after Dengue virus infection.

L 09

Proteoglycan neofunctions: Regulation of inflammation & beyond

Liliana Schaefer

Pharmazentrum Frankfurt/ZAFES, Institut fór Allgemeine Pharmakologie und Toxikologie, Klinikum der Goethe-Universität Frankfurt am Main, Frankfurt am Main, Germany

Inflammatory processes in the body can be triggered not only in response to external signals, but also in response to the release of endogenous molecules called damage-associated molecular patterns. One such molecule, the small leucine-rich proteoglycan biglycan, is ubiquitously distributed in the body and can be proteolytically released from the extracellular matrix following tissue injury. In its soluble form, biglycan acts as a ligand of Toll-like receptors (TLR)-2 and -4, leading to the activation of NF-κB and subsequently the transcription and secretion of several pro-inflammatory cytokines and chemokines in macrophages.

In continuation of this work, we generated a novel transgenic mouse model, in which biglycan was constitutively overexpressed and secreted by hepatocytes (BGN^{Tg}), thereby providing a constant source of biglycan released into the blood stream. Secondary polycythemia, a disease characterized by a selective increase in circulating mature erythrocytes, is caused by enhanced erythropoietin (Epo) concentrations triggered by hypoxia-inducible factor-2α (HIF-2α). While mechanisms of hypoxia-dependent stabilization of HIF-2α protein are well established, data regarding oxygen-independent regulation of HIF-2α are sparse. We discovered that although the BGNTg mice were apparently normal, they harbored an increase in mature circulating erythrocytes. In addition to erythrocytosis, the BGNTg mice showed elevated hemoglobin concentrations, hematocrit values and enhanced total iron binding capacity, revealing a clinical picture of polycythemia. In BGNTg mice markedly enhanced Epo mRNA expression was observed in the liver and kidney, while elevated Epo protein levels were found in liver, kidney and blood.

Mechanistically, we showed that the transgenic animals had an abundance of HIF-2α protein in the liver and kidney. Finally, by transiently overexpressing circulating biglycan in mice deficient in various Toll-like receptors (TLRs), we determined that this novel function of biglycan to promote Epo synthesis was specifically mediated by a selective interaction with TLR2. Thus, we discovered a novel biological pathway of soluble biglycan inducing HIF-2α protein stabilization and Epo production presumably in an oxygen-independent manner, ultimately giving rise to secondary polycythemia.

Role of MT1-MMP in inflammatory arthritis: a potential therapeutic target?

L 10

Yoshifumi Itoh

Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK

Rheumatoid arthritis (RA) is a systemic autoimmune disease, and a key pathological feature of which is destruction of joint tissues leading to disability. In RA joints, many proteolytic enzymes including matrix metalloproteinases (MMPs) are highly upregulated and thought to promote joint tissue destruction. Membrane-type 1 MMP, (MT1-MMP) is a type I transmembrane proteinase that promotes invasion of various cell types including cancer cells, endothelial cells, fibroblasts, macrophages and so on. MT1-MMP is highly expressed in inflamed pannus tissue, and it destroys cartilage by promoting invasion of pannus tissue. To examine if inhibition of MT1-MMP can be a potential therapeutic strategy, MT1-MMP inhibitory antibody, DX-2400, was administered to mice developing collagen-induced arthritis. DX-2400 inhibited cartilage erosion and spreading of the disease to non-effected joints, but it also exhibited synergistic efficacy when it is co-administered with biologic inhibitor for TNFα. Role of MT1-MMP in development of arthritis, mechanism of MT1-MMP regulation in pannus, and potential of MT1-MMP to be therapeutic target for RA will be discussed.

L
11

Matrix remodeling and proteolysis as key regulator of embryo implantation

Irit Sagi

Department of Biological Regulation, The Weizmann Institute of Science, Israel

Embryo implantation is strongly dependent on the receptivity of the endometrium and characterized, among others, by extracellular matrix (ECM) proteolysis, which has fundamental importance in trophoblast invasion. However, the molecular mechanisms underlying ECM proteolysis in embryo implantation are not yet clear. We show that topical administration of recombinant protease significantly improves the rate of embryo implantation regardless of genetic background. We find that this treatment promote transient changes in uterine collagen fibril spatial organization, with profound alterations in fibril directionality. In addition it increases vascular permeability, inflammation-like processes and angiogenesis at implantation sites. These mechanisms illustrate the favorable receptive endometrial environment accounting for ECM remodeling-induced embryo implantation. These findings emphasize the role of ECM proteolysis in embryo implantation from the molecular to the physical levels, and may encourage the use of ECM remodeling enzymes to overcome implantation failure in the future.

Cancer cell morphology and local microenvironment

L 12

M. Franchi

Department for Life Quality Studies, University of Bologna, Italy

In general, cell shape and cell surface morphology are stricktly related to cell function. Similarly cancer invasion is related to changes of morphology and structural asimmetry of cancer cells involving mechanical and molecular mechanisms, organization of the cytoskeleton, capability to remodel the tissue structures, cell-substrate adhesions, cell-cell adhesions and intercellular comunications (Friedl and Alexander, 2011). Analysis of cell morphology and cytoplasmic ultrastructural changes in cancer cell cultures may help in understanding and confirming different biochemical data concerning cellular signaling pathways. Cancer local invasion in extracellular matrix is a prerequisite for migration of cancer cells in blood and limph vessels and then colonization of distant organs. Extracellular matrix is a dynamic network of macromolecules contributing to cell behaviour, gene expression and diverse functional properties (Theocharis et al., 2015).The main component of extracellular matrix is collagen which is organized in collagen fibrils forming collagen fibres. Cancer cell invasion in extracellular matrix is a three dimensional event where cancer cells have to adapt their shape and size to penetrate and migrate into the guiding scaffold of collagen network. It is reported that mammary tumors are related to an increase of collagen deposition, straightening of collagen fibres firstly parallel and then perpendicular aligned to the tumor boundary (Conklin et al., 2011). Epithelial low invasive ERα-positive MCF-7 breast cancer cells as well as highly migrating and invasive ERα-knockdown cells (MCF-7/SP10+) were cultured in 2D and 3D cultures (millipore filter, millipore filter with Matrigel, type I and III collagen membrane). Invasivity test and scanning electron microscope analysis demonstrated that breast cancer cells have different behaviour and show different shape and different cytoplasmic surface depending on the diverse substrate. Data from this study confirm that collagen fibres of extracelluar matrix may influence the phenotype of breast cancer cells and suggest to study cancer cell migration in 3D cultures with natural extracellular matrix which can mimic the in vivo microenvironment.

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L 13

Altered metalloprotease and proteoglycan expression in senescent human breast stromal fibroblasts: implications in tumour progression

A. Papadopoulou[1], E. Liakou[1], E. Mavrogonatou[1], H. Pratsinis[1], P. Panayotou[2], V. Gorgoulis[3], N. Karamanos[4], D. Kletsas[1]

[1] *Laboratory of Cell Proliferation & Ageing, Institute of Biosciences & Applications,NCSR "Demokritos", Athens, Greece*

[2] *Department of Plastic Surgery and Burns Unit, KAT General Hospital of Athens, Greece*

[3] *Molecular Carcinogenesis Group, Department of Histology and Embryology, Medical School, National and Kapodistrian University of Athens, Athens, Greece*

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

Cancer research is traditionally focused on the neoplastic cell per se, however numerous studies have revealed that stroma plays an important role in tumor development and progression. Stromal fibroblasts, the most abundant cell type of tumor microenvironment, synthesize extracellular matrix (ECM) molecules and soluble mediators, highlighting their importance on paracrine interactions between them and tumor cells. We have already reported that repeated non-cytotoxic, curative, doses of ionizing radiation provoke senescence of stromal lung fibroblasts in a p53-dependent mode. These cells express an inflammatory phenotype and they further enhance significantly the growth of cancer cells in co-cultures in vitro and in immunocompromised mice in vivo. This effect seems to be largely due to the overproduction of MMPs by senescent fibroblasts, as an MMP inhibitor significantly reduced tumor growth. As radiotherapy is often used also in breast cancer treatment, we further studied the effect of ionizing radiation on breast stroma fibroblasts. We have found that ionizing radiation provokes the accumulation of prematurely senescent breast stromal fibroblasts both in vitro and in vivo. These cells express a catabolic and inflammatory phenotype. Interestingly, senescent cells overexpress also syndecan-1, a poor prognostic factor in breast cancer development. This overexpression seems to be independent of the activation of two major pathways in senescence, i.e. those regulated by p53 and p38 MAPK, and it is rather the effect of TGF-β, which in an autocrine fashion activates the Smad pathway and in collaboration with the transcription factor Sp1 activates syndecan-1. Interestingly, invasive breast cancer cells enhance further syndecan-1 expression in stromal cells, again via the paracrine action of TGF-β, showing a positive feedback loop in tumor progression. In addition, senescent fibroblasts are also characterized by a decrease of decorin expression, the latter having an antitumorigenic effect. These data indicate that the ionizing radiation-mediated accumulation of senescent stromal fibroblasts may represent a long-term side-effect of this anticancer therapeutic approach.

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Glycosaminoglycan biosynthetic enzymes: actors and targets in pathobiology

L 14

Sylvie Fournel-Gigleux[1], Xianqing Mao[1], Caroline Gauche[1], Michael Coughtrie[2], Jean-Christophe Bourdon[3], Sandrine Gulberti[1]

[1]UMR 7365 CNRS-Université de Lorraine (IMoPA), MolCelTEG Team, Biopôle, Campus Biologie-Santé, Faculté de Médecine, Université de Lorraine, CS 50184, 54505 Vandoeuvre-les-Nancy, France; [2]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC V6T 1Z3, Canada. [3]Division of Cancer Research, Medical Research Institute, University of Dundee, Dundee, DD1 9SY, UK

Key words: heparan sulfates, 3-O-sulfotransferases, cell microenvironment, cancer, epigenetic regulation

Heparan sulfate (HS) proteoglycan chains are key components of the microenvironment of cells that critically influence their behavior, in particular during the cancer process. It is abundantly documented that abnormal synthesis and processing of HS play a prominent role in tumor formation and metastasis albeit mechanisms remain mostly obscure. HS biological function critically depends upon their capacity to interact with HS binding proteins and is mainly controlled by the action of sulfotransferases. In this talk, we will discuss about 3-O-sulfotransferases, an intriguing class of enzymes catalyzing the final step of HS maturation, a relatively rare modification whose function remains enigmatic.

It is well documented by us and others that 3-OST genes are subject to epigenetic silencing in different types of cancer cells (Bui et al. FASEB J. 2010). We recently investigated the regulation and pathophysiological role of 3-OST3A, a gD-type 3-O-isoform in breast cancer. We will discuss the following questions: (1) How is 3-OST3A regulated? (2) Does it act as tumor suppressor or as oncogene? (3) What is its functional and clinical significance? We showed in a recent study (Mao/Gauche et al. Oncogene, 2016) that 3-OST3A is epigenetically repressed in most breast cancer cell lines tested. However, DNA methylation and histone modifications produced different repressive chromatin environments depending on the cell molecular subgroup. The functional significance of this regulation was investigating by a gain and loss of function study. cDNA and siRNA transfection revealed profound effects of 3-OST3A expression on cell behavior including apoptosis, proliferation, and tumor growth in xenografted mice. Importantly, 3-OST3A exerted dual activities acting as tumor-suppressor in lumA-MCF-7 and triple negative-MDA-MB-231 cells, or as an oncogenic factor in HER2+-SKBR3 cells. Interestingly in this latter cells, 3-OST3A influenced response to trastuzumab, an anti-Her2+ antibody used in breast cancer treatment. Curiously, among the array of HS binding proteins, few have shown a preference for 3-O-sulfated HS. Thus, we employed fluorescence-based assays including FRET-FLIM technologies to investigate the mechanisms underlying 3-OST3A tumor suppressive properties. We demonstrate that 3-OST3A expression strongly influences interactions between HS and fibroblast growth factor-7 (FGF-7). Remarkably, impaired binding of FGF-7 to HS chains modulated downstream signaling cascades, suggesting that altering 3-O-sulfation affects FGFR2IIIb-mediated signaling. This finding is reminiscent of the role of 3-O-sulfation in the control of FGF10-FGFR2IIIb in morphogenesis (Patel et al. Matrix Biol. 2017).

To establish the significance of our cell data, we conducted a clinical study in a cohort of breast cancer patients. We uncovered that, in HER2+ patients, high expression level of 3-OST3A in tumors is associated with a strong reduction of relapse-free survival. Our findings define 3-OST3A as a novel regulator of breast cancer pathogenicity, displaying tumor-suppressive or oncogenic activities not per se but in a cell- and tumor-dependent context. We demonstrate the clinical value of the HS-O-sulfotransferase 3-OST3A as a prognostic marker in HER2+ patients enabling to stratify patients for treatment in this highly aggressive type of breast cancer.

L
15

Molecular modelling of Extra-Cellular Matrix: what could we do? What could we learn?

S. Baud[1,2], N. Belloy[1,2], J. Jonquet[1,2], M. Gueroult[1], H. Wong[2], M. Dauchez[1,2,*]

[1] *CNRS UMR 7369 MEDyC, Faculty of Sciences, University of Reims Champagne-Ardenne, REIMS France*
[2] *Multiscale Molecular Modelling Platform(P3M), House of Simulation of Champagne-Ardenne (MaSCA), University of Reims Champagne-Ardenne, REIMS, France*
** Email : manuel.dauchez@univ-reims.fr*

The extracellular matrix (ECM) is composed of very large extracellular molecules and macromolecules secreted by cells; it is a biochemical reservoir and it provides structural supports to the surrounding cells. The composition of ECM varies according to multicellular structures and cellular types. Macromolecules of the ECM are often very huge macromolecules constituted of numerous multi-globular domains and patterns, with fibrous structures and/or glycosylated molecules. These domains and patterns could adopt numerous structures and have a fabulous adaptability and most of the time a large flexibility to perform their functions. Most of the three-dimensional structures at the atomic and molecular levels are provided by experimental data from crystallographic and NMR experiments. Both of these methodologies lead to some structure/function relationships and to an understanding of their roles.

Nevertheless, the dynamical aspects are up to now missing from the structure/function/dynamic relationships. In this presentation, we will describe some of our results obtained using two types of numerical simulations: molecular dynamics simulations and molecular docking experiments. We will discuss the interactions and motions observed in proteins in the case of some metalloproteinases (MMPs) and their natural inhibitors TIMPs. MMP2 and MMP9 cleave the elastin molecule into small fragments: the elastin-derived peptides (EDP). We will focus on the specific structural behaviour of these EDP. Some of these peptides interact with the Elastin Receptor Complex (ERC) and particularly with the Elastin Binding Protein (EBP). We will present how molecular docking could explore these interactions and propose new sequences designed in silico that could theoretically bind to EBP. Last, the use of "serious games" as a future tool used for the modelling of large systems and interactions will be briefly presented. All these examples result from the works done in our group in conjunction with experimental and biological data obtained by colleagues who worked on different pathologies as for instance cancers, cardiovascular diseases or diabetes and obesity (ref 1-7 below). The simulated data try to take into account as much as possible these biological data in order to validate different models.

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Dual control of autophagy and angiogenesis by soluble proteoglycans

L 16

Renato V. Iozzo

Department of Pathology, Anatomy, and Cell Biology, and the Cancer Cell Biology and Signaling Program, Sidney Kimmel Medical College and Cancer Center, Thomas Jefferson University, Philadelphia, PA USA

In adult life, soluble proteoglycans, such as decorin, play a direct role in modulating receptor tyrosine kinase activity. We have uncovered a new biological function for decorin in regulating autophagy. This process is mediated by a specific interaction with VEGF receptor 2 and subsequent downstream signaling through the mTOR pathway. We have recently discovered that systemic delivery of decorin for treatment of breast carcinoma xenografts induces Peg3, an imprinted gene encoding a zinc finger transcription factor postulated to function as a tumor suppressor. We found that de novo expression of Peg3 increased Beclin 1 promoter activity and protein expression. This process required the full-length Peg3, as truncated mutants lacking either the N-terminal SCAN domain or the zinc fingers failed to translocate to the nucleus and promote Beclin 1 transcription. Importantly, overexpression of Peg3 in endothelial cells stimulated autophagy and concurrently inhibited endothelial cell migration and evasion from a 3D matrix. Mechanistically, we found that Peg3 induced the secretion of the powerful angiostatic glycoprotein, Thrombospondin-1, thus providing a new mechanism whereby Peg3 can simultaneously evoke autophagy in endothelial cells and attenuate angiogenesis. Our findings provide a biological axis where soluble proteoglycans can regulate an intracellular catabolic pathway independent of nutrient deprivation.

L 17

Lumican delays melanoma growth in mice and drives tumor molecular assembly as well as response to extracellular matrix-targeted therapy

Albin Jeanne[1,2,3], Valérie Untereiner[2,4], Corinne Perreau[2,5], Isabelle Proult[2,5], Cyril Gobinet[2,5], Camille Boulagnon-Rombi[2,6], Christine Terryn[4], Laurent Martiny[1,2], Stéphane Brézillon[2,5] and Stéphane Dedieu[1,2]

[1] *Université de Reims Champagne-Ardenne (URCA), UFR Sciences Exactes et Naturelles, Campus Moulin de la Housse, 51100 Reims, France*
[2] *CNRS UMR 7369, Matrice Extracellulaire et Dynamique Cellulaire, MEDyC, 51 rue Cognacq Jay, 51100 Reims, France*
[3] *SATT Nord, 25 avenue Charles Saint-Venant, 59800 Lille, France*
[4] *Plateforme d'Imagerie Cellulaire et Tissulaire, URCA, 51 rue Cognacq Jay, 51100 Reims, France*
[5] *URCA, UFR Pharmacie, 51 rue Cognacq Jay, 51100 Reims, France*
[6] *CHU de Reims, Laboratoire Central d'Anatomie et de Cytologie Pathologique, 51100 Reims, France*

Extracellular matrix (ECM) remodeling may be viewed as a hallmark of cancer, as it strongly contributes to disease progression by creating a tumor-specific microenvironment. Among the molecules that control ECM assembly, lumican is a small leucine-rich proteoglycan (SLRP) being known as a regulator of collagen fibrillogenesis. However, little attention has been given so far in studying its influence on tumor-associated matrix architecture. Here, a comprehensive analysis of genomics and proteomics databases for melanoma was first performed so as to establish a correlation between lumican expression and patient outcome. The role of host lumican on tumor ECM organization as well as on disease progression was then investigated considering an immunocompetent model of B16F1 melanoma implanted in Lum-/- vs. wild type (WT) syngeneic mice. Innovative approaches were used such as K-means clustering and Gabor filtering combined with Fast Fourier Transform (FFT) respectively for Fourier transform infrared (FT-IR) microimaging and cross-polar microscopic images analysis, therefore allowing visualization and quantification of subtle biochemical and structural changes that occurs within a tumor tissue under lumican deficiency. The results obtained present lumican as a strong endogenous inhibitor of tumor growth, while identifying this proteoglycan as a major driver of tumor matrix coherent assembly. Besides, a previously validated thrombospondin targeting peptide named TAX2 was considered so as to figure out that extracellular lumican also impacts tumor response to ECM-targeted therapeutic strategies. Overall, our data afford brand-new knowledge about lumican influence on both tumor assembly and stromal reaction, while providing valuable insights on TAX2 therapeutic agent mechanism of action.

Extracellular matrix and heart failure

L 18

Geir Christensen[1]

[1] *Institute for Experimental Medical Research and Center for Heart Failure Research, Oslo University Hospital and University of Oslo, Oslo, Norway*

Patients with heart failure suffer from dyspnoea and other symptoms that dramatically reduce their quality of life. Despite optimal treatment, mortality after being diagnosed with heart failure is higher than for most cancers. Thus, development of new and better treatments for the increasing number of heart failure patients is one of the most important challenges facing the medical community. New treatments must be based on detailed insight into the mechanisms involved in the cardiac remodelling process leading to heart failure.

One hallmark of the failing heart is reduced filling of the left ventricle, termed diastolic dysfunction. Alterations in the extracellular matrix of the myocardium contribute significantly to impaired diastolic function by increasing cardiac stiffness and reducing distensibility. We and others have shown that proteoglycans are central to the alterations occurring in the extracellular matrix during cardiac remodelling. In particular, we have shown that amounts of the membrane-bound syndecans and glypicans are increased during cardiac remodelling. Together with integrins, syndecan-4 is central for transdifferentiation of fibroblasts through activation of the calcineurin-NFAT signalling pathway. Moreover, shedding of the extracellular part of syndecan-4 occurs in failing human hearts and experimental models of heart failure.

The extracellular part of syndecan-4 facilitates cross-linking of collagens by lysyl oxidase and causes inflammation known to occur in the failing heart. Also extracellular matrix-localized proteoglycans such as lumican, fibromodulin and decorin are increased in the myocardium during remodelling. Their roles are most likely related to reorganisation of collagen fibres and directing pro-fibrotic effects. We believe that degradation fragments of proteoglycans are detrimental during development of heart failure and we have shown increased amounts of the proteoglycan-degrading enzyme ADAMTS4 during remodelling. Therapeutically, inhibition of ADAMTS4 reduced mortality and improved cardiac function in animal models of heart failure. Accordingly, a clinical study based on this. Accordingly, a clinical study based on this novel therapeutic principle for treatment of heart failure is currently being planned.

L
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Serglycin implication in malignancies: unraveling novel molecular mechanisms

Achilleas D. Theocharis

Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group,

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

Serglycin is the only characterized intracellular proteoglycan so far. It has been shown to be expressed mainly in hematopoietic cells affecting the storage of several molecules within granules and, consequently their secretion and availability in the extracellular matrix. Recent studies have shown that serglycin is synthesized by a variety of malignant cells acting as a tumor promoter. It has been demonstrated that serglycin is not only constitutively secreted by numerous tumor cells such as breast, lung, prostate, hepatocellular cancer cells, glioblastoma and myeloma cells but is also located at the cell surface via binding to cell surface receptors. High serglycin levels predict poor prognosis in hepatocellular carcinoma and are independent prognostic indicator of metastasis-free survival and disease-free survival in nasopharyngeal carcinoma. Serglycin has been shown to act as tumor promoter via multiple molecular mechanisms. Serglycin facilitates the adhesion of tumor cells to extracellular matrix and stromal cells. In myeloma cells serglycin promotes their adhesion to collagen I and enhances the release of MMPs. Furthermore, myeloma-derived serglycin inhibits bone mineralization and complement system activity participating in disease progression. Serglycin is capable of interacting with cell surface receptors such as CD44 triggering signaling events that promote epithelial to mesenhymal transition and tumor cell's aggressiveness. In another model, knock-down of serglycin in glioblastoma is associated with the loss of mesenchymal phenotype and reduced production of proteolytic enzymes. Similarly, serglycin expression affects the levels of proteolytic enzymes and the aggressive phenotype in breast cancer cells. Serglycin also affects the release of inflammatory mediators in several tumor and stromal cells thus interfering in cellular signaling. For instance, serglycin is implicated in the establishment of an autocrine IL-8 positive signaling that drives breast cancer cell aggressiveness. In other in vivo models lack of serglycin either retards tumor cell growth or affects tumor metastases and angiogenesis by suppressing the release of chemokines such as CCL2 and angiogenic factors such as VEGF, HGF. Recent data unravels a prevailing role for this proteoglycan in tumor cell biology and provoke us to investigate it further.

Dual function of syndecan-1 in epidermal regeneration and homeostasis

L 20

Patricia Rousselle

CNRS, University Lyon 1, France

Cell adhesion to the extracellular matrix (ECM) stimulates signal transduction cascades known to impinge on cell growth, differentiation, and cell death. Although integrins are the major cell surface receptors for the ECM, other adhesive systems, including transmembrane proteoglycans (such as syndecans), have recently drawn attention as an important class of adhesion receptors working in concert with integrins. Like integrins, syndecans lack intrinsic enzymatic activities and thus transmit intracellular signals by interacting with various effector proteins, including both structural and signalling molecules. Following ligand binding, syndecans participate in the formation of adhesion complexes comprised of several actin-associated proteins that coordinate the cross-talk of ECM components with the cellular cytoskeletal machinery. Cell-matrix adhesion receptors also activate kinases that phosphorylate signaling and cytoskeletal proteins, and thus regulate cell shape and motility.

Among the four syndecans in mammals, which are expressed in a development-, cell-type, and tissue-specific manner, syndecan-1 is predominantly expressed by epithelial cells thus mediating the interaction of cells with their matrix, influencing attachment, migration and response to growth factors. A number of data has suggested that syndecan-1 is an important modulator of epidermal cell proliferation, migration and adhesion and may control their behaviour. We have been interested in understanding the function of syndecan-1 in skin epidermal cells both in quiescent and regenerating epidermis. In the epidermis, syndecan-1 is located in the pericellular region of keratinocytes and displays a modest expression in the basal cell layer, which becomes increasingly intense in the suprabasal layers. Remarkably, syndecan-1 is strongly induced in wound edge keratinocytes during wound healing and we have recently shown that its interaction with a specific region in the laminin 332 variant drives keratinocyte migration through formation of actin-containing protrusive adhesion contacts. Protrusive contacts are considered to function as membrane extensions by which cells make transient adhesions, sample the surround environment beyond the cell body and maintain a polarised edge during cell locomotion. Most interestingly, loss of syndecan-1 in keratinocytes delays wound healing and reduces migration. Through involvement of both its glycosaminoglycans moieties and cytoplasmic tail, our most recent data indicates that syndecan-1 not only participate to epidermal regeneration but plays an important role in epidermal homeostasis.

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L
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Roles of heparanase and exosomes in regulating the tumor microenvironment

Ralph D. Sanderson

University of Alabama, USA

Heparanase is an endoglucuronidase that cleaves the heparan sulfate chains of proteoglycans, releasing biologically active fragments of heparan sulfate, and leaving shortened (trimmed) chains on the proteoglycan core protein. This enzymatic action of heparanase impacts cell signaling and promotes tissue remodeling that drives tumor growth, invasion, metastasis, osteolysis and angiogenesis. Using in vitro and in vivo models of multiple myeloma, we have discovered that mechanistically, heparanase regulates tumor progression by: i) upregulating expression of a number of growth factors (e.g., VEGF, HGF), ii) enhancing the shedding of syndecan-1 from the surface of tumor cells and iii) elevating the secretion and altering the composition of tumor-derived exosomes. Together, these findings indicate that heparanase is a viable target for anti-cancer therapy. We previously reported that heparanase stimulates exosome biogenesis and alters exosome protein composition and exosome function to promote tumor progression. Recently, we found that when myeloma cells are exposed to the anti-myeloma drugs bortezomib, carfilzomib or melphalan, exosome secretion is dramatically enhanced. In addition, the exosomes induced by chemotherapy (i.e. chemoexosomes) have enhanced levels of heparanase compared to exosomes secreted by cells not exposed to chemotherapy. Heparanase is located on the surface of chemoexosomes where it enables these intact exosomes to degrade heparan sulfate localized within an extracellular matrix. Addition of purified chemoexosomes to myeloma cells enhanced ERK and P38 signaling, promoted tumor cell proliferation and chemoresistance and upregulated shedding of syndecan-1 from the myeloma cell surface. Chemoexosomes also enhanced proliferation of macrophages and stimulated macrophage production of TNF-α, a survival and proliferation factor for myeloma. These results reveal an important link between heparanase expression and exosomes and demonstrate a negative side effect of anti-myeloma therapeutic drugs. Therapeutic disruption of exosome or chemoexosome secretion or function represent a novel approach for improving myeloma patient outcome.

Epigenetic control of hyaluronan synthase 2

L 22

A. Passi, D. Vigetti, M. Viola, E. Karousou, I. Caon, E. Caravà, G. De Luca

Department of Medicine and Surgery,
University of Insubria, via J.H. Dunant 5, 21100 Varese, Italy

Epigenetic has emerged as a critical point in the control of gene expression and the hyaluronan (HA) synthase 2 shows some epigenetic aspects. The synthesis of hyaluronan (HA) is controlled by gene expression of hyaluronan synthases 1,2 and 3 and the HAS2 shows an intriguing and complex regulation with epigenetic relevance. Hyaluronan represents a perfect environment in which cells can migrate and proliferate as we described for human aortic smooth muscle cells (SMC). Smooth muscle cells (SMC) in the presence of different stimuli, as inflammation, oxLDL, mechanical stress, produced an altered ECM where HA is abundant as demonstrated in areas of atherosclerotic lesions. The control of the HA synthesis is critical not only in ECM assembly but also in various pathologies. In contrast with other glycosaminoglycans, which are synthesized in the Golgi apparatus, HA is produced on the plasma membrane by HA synthases (HAS1-3), using UDPGlcUA acid and UDPGlcNAc as substrates. UDP-sugar availability as well as the cellular energy are critical for the synthesis of HA and for HAS2 activity. The AMP activated protein kinase, a sensor of the energy status of the cell, leads to HAS2 T110 phosphorylation, which specifically inhibits HA secretion. However, the most general sensor of cellular nutritional status is the UDPGlcNAc produced by hexosamine biosynthetic pathway. This metabolic pathway is influenced by protein, fatty acid, nucleotide and glucose metabolisms and when activated leads to intracellular protein glycosylation (O-GlcNAcylation). We described that O-GlcNAcylation of serine 221 residue of HAS2 induces a dramatic stabilization of the enzyme on the membranes and an increase of HA production. Eventually we found a long non-coding RNA (NAT) that positively controls in cis the HAS2 expression involving p65 and NFkB pathway. This HAS antisense is also involved in the miRNA regulation acting as a sponge modulating the availability of some miRNA. Beside the antisense effect, another epigenetic control has been tested for P300 and histone acetylation. In fact transfection of P300 increased the HAS2 expression and HA synthesis whereas transfection of HDAC1 has opposite effects, indicating that this epigenetic control plays a role in this context. The sirtuins are also another part of the complex mechanisms involved in HAS 2 expression. The data produced in our laboratory indicate that epigenetic area is a key point of the hyaluronan metabolism and therefore of ECM composition.

L 23

The function of 2 O-sulfation of chondroitin/dermatan sulfate under physiological and pathological conditions

D.G. Seidler

Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Glycosaminoglycans, like chondroitin/dermatan sulfate (CS/DS) are discussed for several therapeutic aproaches due to their ability to bind growth factors and function as low affinity receptors. CS/DS are invovled in multiple biological functions. The micro-heterogeneity along the chain is due to O-sulfation which is introduced by specific sulfotransferases. The minor modification of 2-O sulfation is introduced by the enzyme uronyl 2-O sulfotransferase (Ust). We previously showed that modulation of Ust expression in CHO-K1cells influenced migration and proliferation in response to Fgf2. In addition, Ust knock-down also affected migration of fibroblasts and melanoma cells. The proliferation rate of the different cell types correlated also with the Ust expression. Therefore, we further evaluate the therapeutic potential of different CS/DS preparations. We used CHO-K1/Ust cells to obtain 2-O sulfated CS/DS preparations and tested these GAGs on melanoma cell proliferation. Depending on the 2-O sulfation CS/DS inhibited Fgf2- and Fgf7-induced proliferation of melanoma cells. In addition, the cell surface CS/DS sulfation are an important factor for the function of the exogenouse CS/DS. Overall, CS/DS prepartions from CHO-K1 cell lines can used to inhibit cell proliferation.

Hyaluronan synthesis and *O*-GlcNAc-signaling in malignant tumors – role of hexosamine biosynthesis and UDP-N-acetylglucosamine content

L 24

Oikari S.[1], Deen AJ.[1], Melero R.[1], Kärnä R.[1], Kettunen T.[2], Tiainen S.[2], Masarwah A.[3], Auvinen P.[2], Tammi M.I.[1]

[1]Institute of Biomedicine/Anatomy, University of Eastern Finland, [2]Cancer Center and [3]Department of Diagnostic Radiology, Kuopio University Hospital, Kuopio, Finland

Hyaluronan enhances cancer cell survival, epithelial to mesenchymal transition, migration, and metastasis, and is an established unfavorable prognostic factor in several cancers. However, factors involved in the high synthesis rate in cancers are not known. The UDP-sugar substrates of hyaluronan synthases (HAS1-3) can limit the synthesis. In addition, abundant UDP-GlcNAc increases the O-GlcNAc modifications on HASs, enhancing the stability (1) and plasma membrane residence (2) of HASs, and hence hyaluronan synthesis.

Supporting the importance of UDP-GlcNAc, the mRNA levels of all HASs were unchanged or reduced in human breast cancer biopsies compared to normal breast tissue, while the content of UDP-GlcNAc was ›10-fold higher, and the content of hyaluronan correlated with the level of UDP-sugars. The high level of tumor UDP-GlcNAc was probably not only due to the high uptake of glucose (Warburg effect), since the expression of glutamine-fructose-6P-aminotransferase 2 (GFAT2) and glucosamine-6P-deaminase 1 (GNPDA1) at the rate limiting step of UDP-GlcNAc biosynthesis (3) were upregulated. Sequencing of mRNA in breast cancer cell lines (MCF7, MDA-MB361 and MDA-MB-231) showed consistently higher levels in the more malignant cells also for other enzymes in the hexosamine synthesis pathway to UDP-GlcNAc (such as UDP-N-Acetylglucosamine pyrophosphorylase 1, UAP1). The synthesis of hyaluronan also correlated with UAP1 expression. These findings support the idea that a high supply of UDP-GlcNAc brings a specific advantage to tumor growth.

Similar positive correlations between UDP-GlcNAc and hyaluronan synthesis were also found in melanocyte-melanoma cell lines (2) and oral squamous cell carcinoma cell lines. These data indicate that the production of the precursors for hyaluronan synthesis, especially that of UDP-GlcNAc, has an important contribution to the hyaluronan accumulation of tumors.

The elevated UDP-GlcNAc supply also increases O-GlcNAc modifications of a number of cytoplasmic, nuclear and mitochondrial proteins. The O-GlcNAc signaling was indeed stimulated in human breast cancers (4). The deranged hexosamine biosynthesis thus drives tumor progression both through extracellular hyaluronan, and intracellular signaling through O-GlcNAc-modifications of transcription factors and other proteins controling the cell phenotype.

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L 25

Heparan sulfate biosynthesis in health and disease

E. Grigorieva[1,2]

[1]*Institute of Molecular Biology and Biophysics, Novosibirsk, Russia*

[2]*Karolinska Institute, MTC, Stockholm, Sweden*

Heparan sulfate proteoglycans (HSPGs) play an important role in cell-cell and cell-matrix communication and signalling, being an essential part of cell microenvironment. A functional specificity of the HSPG interactions with multiple cell surface and extracellular ligands in many cases depends on a fine structure of their heparan sulfate (HS) polysaccharide chains, generated by the system of HS synthesising and modifying enzymes. Many different enzymes (multiple glycosyltransferases, sulfotransferases and epimerase) participate in HS biosynthesis and catalyse the assembly and appropriate modification of HS chains. All the enzymes work together and each one provides the preferred substrate for the next reaction in the pathway, forming the highly informative HS chains. A physical complex of the enzymes, committed to the assembly of HS, was designated as "GAGosome". Because of non-template nature of HS biosynthesis, highly coordinated and successful work of all the components of the HS biosynthetic machinery is vital for HS biosynthesis and proper HS structure in normal cells.

During carcinogenesis, significant changes in HS structure, composition and functional activity occur and distortion of the HS biosynthetic machinery could be a primary candidate responsible for the changes. An involvement of individual HS metabolic enzymes in carcinogenesis are known: tumour-suppressor function is suggested for EXT family genes, *NDST4* and *GLCE*; significant association of *HS6ST2, 3-OST,* SULF1/2 and heparanase-1 with cancer progression and metastasis are demonstrated. These individual changes disbalance HS biosynthesis and create cell type-specific changes of HS-biosynthetic machinery in cancer cells *in vitro* and tissue-specific changes in different cancers *in vivo* suggesting a close involvement of HS biosynthetic system in carcinogenesis.

Extracellular interaction networks: from extracellular matrix assembly to the cell surface

L 26

Sylvie Ricard-Blum

Institute of Molecular and Supramolecular Chemistry and Biochemistry, UMR 5246, University Lyon 1, France (sylvie.ricard-blum@univ-lyon1.fr)

The extracellular matrix (ECM) determines the shape and organization of tissues, provides them with mechanical properties, and regulates cellular processes. ECM is remodeled in many diseases such as fibrosis, cancer and diabetes and its remodeling releases bioactive fragments, called matricryptins, which have biological activities of their own mediated by several receptors (Ricard-Blum and Vallet Biochimie 2016 122: 300-313, Ricard-Blum and Vallet Front Pharmacol. 2016 7:11). ECM assembly and functions are mostly mediated by protein-protein and protein-glycosaminoglycan (GAG) interactions. Since interactions provide most biomolecules with functions, and interactions influence each other in vivo, we have developed a roadmap to build extracellular and pericellular interaction networks in order to i) identify new functions of ECM proteins, ii) decipher the molecular mechanisms underlying molecular functions and biological processes in the ECM and at the cell surface, and iii) determine how these interaction networks are rewired in pathological situations with a focus on angiogenesis, Alzheimer's disease and host-pathogen interactions.

We have developed protein and glycosaminoglycan arrays to screen potential partners of ECM proteins by surface plasmon resonance imaging (SPRi), calculated kinetics and affinity of the identified interactions using SPR and Bio-Layer Interferometry (BLI) and created a database (MatrixDB, http://matrixdb.univ-lyon1.fr/) to store interaction data and build interaction networks specific of a biomolecule, a tissue, a biological process or a disease (Launay et al., Nucleic Acids Res 2015 43:D321-7). We have then contextualized the interaction networks by integrating data on proteins (3D structure, intrinsically disordered sequences, localization, and quantitative proteomics), GAGs (size, and sulfation, Ricard-Blum and Lisacek Glycoconjugate J 2017, in press), interactions (kinetics, affinity, binding sites, and influence of mutations). The topology of the contextualized networks, their molecular functions and the biological processes they regulate have been investigated with Cytoscape, an open source software platform (http://www.cytoscape.org/), and the Functional Enrichment analysis tool FunRich (http://www.funrich.org/).

We have identified more than 300 interactions during the screening process carried out by SPRi and we have built several ECM subnetworks including those of endostatin, an anti-angiogenic matricryptin of collagen XVIII (Faye et al. J Biol Chem 2009 284:22041-7, Peysselon et al., Matrix Biol 2014 35:73-81), the ECM protein procollagen C-proteinase enhancer-1 (Salza et al. Biochem J 2014 457:137-49), the four membrane collagens (XIII, XVII, XXIII, and XXV) and matricellular proteins. We have also built the interaction networks formed between the ECM and Leishmania parasites to promote host-pathogen interactions (Fatoux-Ardore et al. Infect Immun 2014 82:594-606), and between the ECM and the supramolecular assemblies of the β-amyloid peptide in Alzheimer's disease (Salza et al. J Alzheimers Dis 2017 56:991-1005). These subnetworks are interconnected and the ultimate goal is to build the 3D human extracellular and pericellular interactome and also tissue-specific and disease-specific extracellular and pericellular networks to better understand how acquired and genetic diseases affect the ECM, and how the pericellular matrix regulates molecular traffic at the periphery of the cell and the ECM-cell interplay.

6th FEBS Advanced Lecture Course

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Non-anticoagulant Heparan sulfate from a marine bivalve mollusk inhibits P-selectin and heparanase: effect on cellular recruitment and tumor metastasis

Mauro S. G. Pavão

Institute of Medical Biochemistry, Program of Glycobiology - Federal University of Rio de Janeiro - Rio de Janeiro, Brazil

Institute of Biomedical Research, Marcvlio Dias Naval Hospital - Rio de Janeiro, Brazil

The source of pharmaceutical heparin is limited primarily to porcine intestine and bovine lung. Heparin-like glycans with diverse disaccharide composition and variable anticoagulant activities have been described in several families of marine mollusks. We isolated a heparan sulfate from the tissues of the marine bivalve *Nodipecten nodosus*, and determined its anticoagulant properties, its anti-selectin activity and its *in vivo* effect on inflammation and tumor metastasis. Initially, we identified that the major glycan component of the mollusk visceras is a Heparan Sulfate-like glycosaminoglycan, representing about 4.6mg g-1 of dry tissue. The mollusk HS has an anticoagulant activity of 36 IU mg-1,5-fold lower than bovine heparin (180 IU mg-1), as measured by the aPTT assay. It also inhibits factor Xa (IC_{50}= 0.835 µg mL-1) and thrombin (IC_{50}= 9.3 µg mL-1) in the presence of antithrombin. *In vivo* assays demonstrated that at the dose of 1 mg Kg-1, the mollusk HS inhibited thrombus growth in photochemicaly injured arteries. No bleeding effect, factor XIIa-mediated kallikrein activity or toxic effect on fibroblast cells were induced by the invertebrate HS at the antithrombotic dose. Furthermore, the mollusk HS inhibited LS180 colon carcinoma cell adhesion to immobilized P-selectin in a dose-dependent manner. In addition, we demonstrated that this glycan attenuates leukocyte rolling on activated endothelium and inflammatory cell recruitment in thioglycollate-induced peritonitis in mice. Biochemical analysis indicated that the invertebrate glycan also inhibits heparanase, a key player in cell invasion and metastasis. Experimental metastasis of Lewis lung carcinoma cells was drastically attenuated by the mollusk HS through a mechanism involving inhibition of platelet–tumor-cell complex formation in blood vessels. These data suggest that the mollusk HS is a potential alternative to heparin for inhibiting P-selectin-mediated events such as metastasis and inflammatory cell recruitment.

Heparan sulfate proteoglycans as modulators of therapeutic resistance in breast cancer

L 28

M. Götte[1], A. Vijaya Kumar[1], C. Oberschelp[1,2], Th. Rottke[1,2], S. Masutti[1], H. Hassan[3], E.A. El-Ghonaimy[1,3], S.A. Ibrahim[3], L. Kiesel[1], B. Greve[2]

[1] *Department of Gynecology and Obstetrics, Münster University Hospital, 48149 Münster, Germany*
[2] *Department of Radiotherapy-Radiooncology, Münster University Hospital, 48149 Münster; Germany*
[3] *Department of Zoology, Faculty of Science, Cairo University, 12613, Giza, Egypt*

Heparan sulfate (HS) proteoglycans (PGs) are proteins containing highly sulfated glycosaminoglycan chains. HS is present in all cell types and tissues and functionally interacts with growth factors, tyrosine kinase receptors, matrix metalloproteinases and extracellular matrix (ECM) proteins to modulate cell adhesion, proliferation and motility. HSPGs do not only regulate physiological processes, such as organogenesis, angiogenesis, blood coagulation and lipid metabolism, but are also implicated in tumorigenesis and tumor progression.

Using in vitro overexpression and siRNA-mediated knockdown approaches in human breast cancer cell lines, we demonstrate that altered expression of HS sulfotransferases and the HSPG Syndecan-1 affect resistance to chemo- and radiotherapy in vitro. In the case of the HS sulfotransferase HS3ST2, upregulation of 3-O-sulfated HS residues resulted in altered activation of the MAPK and TCF7L2/Wnt signaling pathways, which in turn affected invasive behaviour and resistance to chemotherapy palcitaxel and doxorubicin. Both HS3ST2 overexpressing and Syndecan-1-depleted breast cancer cells showed an enhanced cancer stem cell phenotype. As this phenotype is characterised by an increased side population, and high expression of multidrug resistance proteins, this property may be linked to increased chemoresistance. In the case of Syndecan-1, we observed an increased resistance to radiotherapy, which was associated with increased activation of integrin-associated focal adhesion kinase signaling. In addition, expression of the small GTPase Rac1, of the ECM protein tenascin C, and the cell cycle regulators CCNA1, CCNB1 and CDK6 were differentially affected in Syndecan-1 depleted cells under radiotherapy.

We conclude that alterations in HS structure and core protein expression are linked to a dysregulation of signaling pathways which determine the response to chemo- and radiotherapy, rendering HSPGs important targets to overcome therapeutic resistance in breast cancer.

L
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Cervical cancer cells downregulate tissue pathway inhibitor2 (TFPI2) in tumor-associated fibroblasts

Alexandra Fullar[1], Gábor Lendvai[2], Kristóf Rada[1], Katalin Karászi[1], Eszter Regős[1] Kornélia Baghy[1], Ilona Kovalszky[1]

Semmelweis University Budapest, Hungary

[1]:1st Department of Pathology and Experimental Cancer Research

[2]: 2nd Department of Pathology

Background: TFPI2 a Kunitz type serine protease inhibitor was described in 1994. The protein is abundantly expressed in full term placenta and several other tissues such as kidney, liver, and pancreas.

The protein resides in the extracellular matrix and binds heparin/HS with high affinity. It cleaves plasmin, trypsin, chymotrypsin, and components of extrinsic clotting cascade. In the last ten years more and more evidence were gathered, indicating the role of TFPI2 in the inhibition of tumor invasion. These data resulted in the conclusion that TFPI2 acts as a tumor suppressor molecule. In our studies comparison of mRNA microarray of normal fibroblasts from uterus cervix to cervical cancer derived cancer associated fibroblasts (CAF) revealed, that the expression of TFPI2 is twelve times lower in the latter, being the most downregulated mRNA of CAFs.

Aims: Our aim was to clarify if cancer cells are capable to downregulate TFPI2 expression in normal and cancer associated fibroblast and if they do so, what is the mechanism they utilize for that purpose?

Methods: Immunohistochemistry, Western blots, promoter methylation, of TFPI2 gene and miR profile of tumor cells, as well as the normal and tumor associated fibroblast were studied.

Results: Immunohistochemistry revealed that surgically removed cervical cancer specimens are negative for TFPI2 protein. Western blot of primary CAF cultures showed considerable, significant decrease of protein expression, as well, although the extent of decrease was different in individual samples of origin. Direct co-culture of cervical cancer cells with normal, TFPI2 expressing, and tumor associated fibroblast proved the potential of cancer cells to downregulate the protease inhibitor in fibroblasts.

Promoters of TFPI2 in cancer cells were fully methylated, however, only modest or no promoter methylation was found in CAF samples

MicroRNA regulation has been expected, as an alternative option for mRNA inactivation. Studying a series of miRNA, upregulation of miR-23a, miR-17-5-p, miR-18-a and mir613-3p went parallel with the decrease of TFPI2, indicating, their possible role in the regulation of TFPI2 expression. Furthermore mir23a has a binding site at the 3' untranslated region of TFPI2. It is a further question how tumor cells are able influence TFPI2 activity of CAFs? Exosome mediated transfer, or paracrine mechanisms can be considered for the further experiments.

Decorin evokes tumor cell mitophagy via mitostatin and Parkin

L 30

T. Neill[1], R.T. Owens[2], and R.V. Iozzo[1]

[1]*Department of Pathology, Anatomy, and Cell Biology, and the Cancer Cell Biology and Signaling Program, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA USA*

[2]*LifeCell Corporation, Branchburg, NJ USA*

Tumor cell mitochondria are key organelles that reprogram cancer metabolism and energetics for aggressive cancer progression and metastasis. Soluble decorin possesses onco-suppressive properties by simultaneously repressing oncogenes and inducing tumor suppressor genes downstream of receptor tyrosine kinases. Recently, we found that decorin potently attenuated oxidative phosphorylation and mitochondrial DNA with a concomitant induction of mitochondrial autophagy (mitophagy) in breast carcinoma cells. We found that mitostatin, a decorin-inducible tumor suppressor, was essential for this novel process. Importantly, depletion of mitostatin abrogated decorin- or rapamycin-evoked mitophagy. Mechanistically, mitostatin bound Parkin and was required for Parkin recruitment to the outer mitochondrial membrane. This process establishes a functional interplay for early mitophagic induction. We further discovered that casein kinase II, an enzyme involved in the regulation of nuclear/cytoplasmic trafficking and cell cycle, was required for mitostatin/Parkin interaction. Collectively, our findings underscore the complexity of extracellular cues in regulating mitochondrial dynamics and establish mitostatin as a general effector of tumor cell mitophagy.

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Syndecans: signalling and regulation of cell adhesion

John R. Couchman

Biotech Research & Innovation Center, University of Copenhagen, Denmark

john.couchman@bric.ku.dk

In mammals there are four syndecan genes, each of which is expressed in a cell specific manner and in response to tissue injury, disease and repair. They have a long evolutionary history, there being one syndecan in invertebrate members of the Bilateria, which has important roles in neuronal guidance. Most mammalian cell types express at least one, and often more than one syndecan. They are transmembrane receptors, with the extracellular portion of the core protein bearing glycosaminoglycan chains, commonly heparan sulphate.

Over the past 20 years, much has been learned regarding their function, and roles in cell adhesion have emerged as a common theme. However, there remain large gaps in our knowledge, particularly with regard to signalling. The cytoplasmic domains of syndecans are small, and none has intrinsic kinase activity, so that signalling relies on the docking of proteins and lipids. In the case of syndecan-4, about which most is known, protein ligand binding to the heparan sulphate chains seems to trigger clustering that facilitates docking and activation of protein kinase Cα in the cytoplasmic compartment. There are many potential substrates for the kinase, but among them are regulators of the Rho family of GTPases that are essential for actin cytoskeletal organisation. In this way, syndecans can regulate, alongside integrins, the morphology of the cytoskeleton, with impact on cell migration, adhesion and survival.

In addition, it is now clear that syndecans can regulate cytosolic calcium levels, through the transient receptor potential canonical ion channels (TRPC). This regulation also has a protein kinase C component. Calcium is a potent regulator of the cytoskeleton also, not only with regard to cell-extracellular matrix interactions, but also cell-cell adhesion. In this talk, the mechanisms by which syndecans exert their control over cell behaviour will be explained, including genetic evidence that regulation of calcium can also be demonstrated in *Caenorhabditis* elegans where it controls neuronal guidance and worm behaviour. This suggests that regulation of calcium is an ancient property of syndecans that deserves further examination.

Syndecan-4 promotes hypertrophic remodeling of the heart through NFAT signaling

L 32

Ida Gjervold Lunde

Institute for Experimental Medical Research (IEMR),
Oslo University Hospital and University of Oslo, Oslo, Norway

Heart failure is a common, costly and deadly syndrome affecting millions of people worldwide. In heart failure, the heart fails to pump blood at a rate that meets the requirements of the tissues. Current medical therapy slows disease progression, but sadly, there is no other cure than transplantation. Five years from diagnosis, only 50% of patients are alive. Research into the underlying molecular basis of heart failure is necessary in order to understand the disease mechanisms, and thereby, design effective medical therapy. Remodeling of the heart, i.e. cardiomyocyte growth (hypertrophy) and death, and fibrosis, is triggered by pathological stimuli and over time, promotes dysfunction. We have studied the role of proteoglycans in the failing heart, and in particular, we have been interested in the role of syndecan-4 in remodeling. This presentation will discuss the role of syndecan-4 in cardiomyocyte hypertrophic growth and dysfunction. Focus will be on regulation of syndecan-4 levels in biopsies from patients with heart failure, in mouse hearts with hypertrophic growth, and in cultured cardiomyocytes. The role of syndecan-4 in hypertrophic remodeling is mainly understood from studies of mice lacking syndecan-4 (SDC4-KO). Pro-hypertrophic signaling pathways regulated by syndecan-4 will be highlighted, especially the calcineurin-NFAT pathway. Excitingly, recent and unpublished data from our study of a mouse with cardiomyocyte-specific overexpression of syndecan-4 (SDC4-TG) will be presented for the first time.

L 33

Rebuilding the matrix: The influence of microenvironmental signalling in matrix synthesis and deposition

D.I. Zeugolis[1, 2]

[1] Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), Biomedical Sciences Building, National University of Ireland Galway (NUI Galway), Galway, Ireland
[2] Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), Biomedical Sciences Building, National University of Ireland Galway (NUI Galway), Galway, Ireland

Physiologically and clinically relevant cell-based therapies and *in vitro* models depend on our ability to efficiently grow cells ex vivo. However, during *in vitro* propagation, bereft of their optimal in vivo tissue context, cells loose their phenotype, function and therapeutic potential. To this end, numerous *in vitro* microenvironmental signalling cascades (biochemical, biophysical or biological in nature) are under intense scientific research and technological innovation to recapitulate the complexity of the *in vivo* tissue setting. This talk will discuss the influence of surface topography, substrate rigidity, macromolecular crowding / excluding volume effect, oxygen tension and mechanical loading, alone or in combination, in permanently differentiated and stem cell cultures and how these in vitro microenvironment modulators affect cell phenotype and function and matrix synthesis and deposition. Development of physiologically relevant cell culture systems will lead to clinically relevant tissue facsimiles and *in vitro* models for drug discovery.

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L 34

The binding capacity of α1β1-, α2 β1- and α10β1-integrins depends on non-collagenous surface macromolecules rather than the collagens in cartilage fibrils

Peter Bruckner[1], Christian Woltersdorf[1], Melanie Bonk[1], Birgit Leitinger[2], Mikko Huhtala[3], Jarmo Käpylä[3], Jyrki Heino[3], Christian Gil Girol[1], Stephan Niland[1], Johannes A. Eble[1], Rita Dreier[1], Uwe Hansen[1,4]

[1]Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, D-48149 Münster/Germany, [2]National Heart and Lung Institute, Imperial College London, London SW7 2AZ, UK, [3]University of Turku, Department of Biochemistry, FI-20014 Turun Yliopisto / Finland, and [4]Institute of Experimental Musculoskeletal Medicine (IEMM), University of Münster, DE-48149 Münster/Germany

Interactions of cells with supramolecular aggregates of the extracellular Matrix (ECM) are mediated, in part, by cell surface receptors of the integrin family. These are important molecular components of cell surface-suprastructures regulating cellular activities in general. A subfamily of β1-integrins with von Willebrand-factor A-like domains (I-domains) in their α-chains can bind to collagen molecules and, therefore, are considered as important cellular mechano-receptors. Here we show that chondrocytes strongly bind to cartilage collagens in the form of individual triple helical molecules but very weakly to fibrils formed by the same molecules. We also find that chondrocyte integrins α1β1-, α2 β1- and α10β1-integrins and their I-domains have the same characteristics. Nevertheless we find integrin binding to mechanically generated cartilage fibril fragments, which also comprise peripheral non-collagenous material. We conclude that cell adhesion results from binding of integrin-containing adhesion suprastructures to the non-collagenous fibril periphery but not to the collagenous fibril cores. The biological importance of the well-investigated recognition of collagen molecules by integrins is unknown. Possible scenarios may include fibrillogenesis, fibril degradation and/or –phagocytosis, recruitment of cells to remodeling sites, or molecular signaling across cytoplasmic membranes. In these circumstances, collagen molecules may lack a fibrillar organization. However, other processes requiring robust biomechanical functions, such as fibril organization in tissues, cell division, adhesion, or migration, do not involve direct integrin-collagen interactions.

L
35

ER stress as a pathogenic factor in ECM diseases

Ray Boot-Handford

Wellcome Centre for Cell-Matrix Research, Faculty of Biology, Medicine and Health, & Manchester Academic Health Science Centre, University of Manchester, UK

Protein misfolding in the endoplasmic reticulum (ER) causes ER stress which triggers the unfolded protein response (UPR) – a homeostatic mechanism to restore proteostasis. Cells synthesising high levels of ECM proteins, such as chondrocytes, fibroblasts, osteoblasts etc, are particularly susceptible to increased ER stress. Using a mouse model of a dwarfism caused by mutations in type X collagen, I will show how ER stress is induced in the cartilage growth plate, the response of the growth plate to the increased ER stress, and how pharmacological reduction of growth plate ER stress can be developed as a possible treatment for this dwarfism. This paradigm illustrates how targeting ER stress may be of more widespread use in treating diseases caused by mutations in ECM genes that cause elevated ER stress.

Role of Heparanase in the onset of organ fibrosis

L 36

M.F. Secchi[1], G. Bellin[1,2], V. Masola[1,2], M. Onisto[1]

[1] *Dept. Biomedical Sciences, University of Padova, Viale G. Colombo 3, 35121 Padova, Italy*

[2] *Dept. of Medicine, University of Verona, Piazzale A. Stefani 1, 37126, Verona, Italy*

The organ fibrosis is a response to chronic injury and inflammation characterized by a gradual accumulation and decreased remodelling of ECM that can lead to organ failure. Heparanase (HPSE) is the only mammalian endo-glucuronidase capable of cleaving heparan sulfate (HS) chains of HS proteoglycans. Through its enzymatic and other non-enzymatic functions, HPSE contributes to extracellular matrix (ECM) turn-over and regulates several physiological and pathological processes. Since a role for HPSE in chronic liver disease has not been demonstrated so far, we aimed at investigating the involvement of HPSE in liver fibrosis/cirrhosis and its possible effects on the development of the disease. In a mouse model (CCl_4) of liver fibrosis real time RT-PCR and WB analyses showed a significant increase of HPSE expression in liver tissue after 1 and 2 weeks of treatment with a trend to decline after 8 and 12 weeks. Immunostaining analyses revealed that HPSE expression was restricted in the central-lobular areas with necro-inflammatory damage and fibrosis and it co-localized with F4/80 macrophage marker. *In vitro* experiments on U937 macrophages showed that TNF-α treatment significantly increased HPSE mRNA and protein expression as well as HPSE secretion. HPSE activity in the plasma of patients with different stages of chronic liver disease showed an increases in the patients with mild and severe fibrosis but not in patients with cirrhosis. On the whole the study indicated a direct involvement of HPSE in the onset of chronic liver disease and inflammatory macrophages as an important source of HPSE. Switching to talk about the kidney we have shown that, by regulating the availability and activity of growth factors (i.e. FGF-2 and TGF-β), HPSE promotes tubular epithelial mesenchymal transition (EMT) and renal fibrosis. More recently we demonstrate that ischemia/reperfusion (I/R) is an important cause of acute renal failure and fibrosis and that HPSE may have an important role in this process. Therefore, we aimed to confirm the role of HPSE in the I/R-induced renal pro-fibrotic machinery by employing an in vivo mouse model. Mouse (C57BL/6J) were subjected to mono-lateral I/R injury by vascular clamping for 30 min. and then treated or not with SST0001 (HPSE inhibitor) for 10 weeks. Renal fibrosis, histological alterations and HPSE expression were evaluated by microscopy. Preliminary results indicate a reduced fibrosis in those animals treated with SST0001 thus confirming that HPSE may be part of the biological network involved in renal tissue damage/repair following I/R injury.

L 37

Regulation of inflammation by the hyaluronan receptor RHAMM: studies in knockout and transgenic mice

Rashmin C. Savani[1,2,3], Zheng Cui[2], Jie Liao[1], Naeun Cheong[1], Christopher Longoria[1], Horace M. DeLisser[3]

[1] *University of Texas Southwestern Medical Center, Dallas, Texas, USA*
[2] *Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA*
[3] *University of Pennsylvania, Philadelphia, Pennsylvania, USA*

BACKGROUND: Acute lung injury results in inflammation and respiratory distress. Hyaluronan (HA) and its receptor RHAMM have been implicated in the inflammatory response to lung injury.

OBJECTIVE: We hypothesized that, compared to wild type (WT) mice, RHAMM knockout (KO) mice would be protected from, whereas mice with macrophage-specific transgenic overexpression of RHAMM (TG) would have worse inflammation and respiratory distress after intratracheal (IT) bleomycin.

DESIGN/METHODS: Transgenic mice with selective overexpression of RHAMM in macrophages were generated using the Scavenger Receptor A promoter driving a Myc-tagged full length RHAMM cDNA. For injury studies, WT, TG and KO mice were treated IT with either 0.5 U/kg (TG mice) or 1U/kg bleomycin (WT and KO mice) in saline or with saline alone. Untreated mice served as controls. Change in body weight was used as a measure of disease severity. Respiratory rate was measured by non-sedated, dual chamber plethysmography. Lavage HA content was determined by ELISA. Neutrophil and macrophage contents of lavage cells were determined by myeloperoxidase (MPO) and N-acetylglucosaminidase (NAG) activities respectively.

RESULTS: TG bone marrow-derived macrophages (BMDM) had increased cell surface RHAMM and Myc, but equal CD44 expression by FACS. TG BMDM also had a 2-fold increase in both chemotaxis to HA and proliferation. Baseline expression of RHAMM and CD44 was the same in TG and WT mice. However, 21 days after IT bleomycin, TG mice had increased Myc-tagged macrophage accumulation as compared to non-transgenic mice. Lavage HA concentrations were 6-fold higher in injured WT mice, but 30-fold higher in injured TG mice. Non-transgenic mice had respiratory distress with increased respiratory rates from day 7 to 21. However, TG mice had higher respiratory rates from 4 days after bleomycin and continued to increase respiratory rates up to day 21. At day 10, compared to WT mice, IT bleomycin in RHAMM KO mice resulted in less weight loss ($p<0.05$), and less increase in respiratory rate ($p=0.05$). Compared to injured WT animals, RHAMM KO mice had lower lavage concentrations of HA ($p<0.005$), as well as lower day 7 MPO ($p<0.01$) and day 28 NAG activities ($p<0.01$). No changes were seen in unmanipulated and saline-treated animals, and these groups were not different from each other.

CONCLUSIONS: We conclude that RHAMM is a critical component of the inflammatory response and respiratory distress after acute lung injury. We speculate that RHAMM is a potential therapeutic target to limit the consequences of acute lung injury.

In vivo models of chondrodysplasias caused by defects in proteoglycan biosynthesis: phenotyping and pharmacological approaches

L 38

A. Rossi

Department of Molecular Medicine, Unit of Biochemistry, University of Pavia, Italy

Heritable skeletal disorders are connective tissue diseases that primarily affect the development and homeostasis of the skeleton; unfortunately, in general no resolutive treatments are available for these painful conditions. Thus, the goal in this research area is the deep phenotyping of relevant in vivo and in vitro models in order to identify and test novel targets for innovative therapies.

In this respect among the skeletal disorders that are being studied in our group, Diastrophic Dysplasia (DTD) and Desbuquois Dysplasia (DBQD) are paradigmatic. Both dysplasias, with similar clinical features, are caused by defects in the biosynthesis of glycosaminoglycan (GAG) chains of proteoglycans (PGs).

DBQD type 1 is caused by mutations in the Calcium-Activated Nucleotidase 1 (CANT1) a Golgi/ER resident enzyme. To define the role of CANT1 in the etiology of DBQD, we generated Cant1 knock-in and knock-out mice. Morphological and clinical observations in the murine strains confirmed the skeletal defects described in the patients. PG synthesis was studied in rib knock-out chondrocytes; mutant cells showed GAG chains with reduced hydrodynamic size, GAG oversulfation, reduced PG synthesis and impaired secretion. This latter observation was confirmed by transmission electron microscopy of mutant vs. wild type cartilage showing the presence of dilated vacuoli suggesting a role of CANT1 in protein secretion.

Nowadays animal models are useful tools to elucidate the molecular mechanisms underlying genetic diseases as described above, but also to develop therapeutic strategies. The dtd mouse is a murine model of Diastrophic Dysplasia (DTD) a skeletal dysplasia caused by mutations in the sulfate-chloride antiporter (SLC26A2), crucial for sulfate uptake and GAG sulfation. Deep phenotyping of the model suggested that N-acetyl-L-cysteine (NAC) might play a role as an intracellular sulfate source for macromolecular sulfation. Because of the important prenatal phase of skeletal development and growth, we administered NAC in the drinking water to pregnant mice to explore a possible transplacental effect on the foetuses. A marked increase of proteoglycan sulfation was observed in dtd newborns from NAC treated pregnancies compared to the placebo group paralleled by a partial rescue of the abnormal bone morphology.

In conclusion, these different mouse models have recapitulated key aspects of disease pathology and identified new fundamental mechanisms paving the way for developing potential therapeutic approaches.

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Selected Talks/ Abstracts

ST1/
P1

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

Amina Abbadi[1], Vincent Hascall[1*], Minjia Yu[2],
Nansy Elbtoush, Xiaoxia Li[2], Aimin Wang[1]

[1]Department of Biomedical Engineering and [2] Immunology, Cleveland Clinic, Cleveland Clinic, Cleveland, Ohio

** hascalv@ccf.org*

Elevated glucose levels induce kidney glomerular mesangial cells (MCs) to divide initiating the synthesis of an abnormal intracellular hyaluronan (HA) and unique HA structures outside the cell that recruit inflammatory cells (a monocyte-adhesive HA matrix). This results in chronic inflammation that leads to fibrosis, proteinurea, and eventually kidney failure in rats. We have shown that heparin inhibits M1 markers such as the production of the transcription factor (IRF8), the expression of inducible nitric oxide synthase (iNOS) and Tumor Necrosis Factor (TNFa), while it promotes M2 markers such as the production of the transcription factor (IRF4), the expression of arginase and IL-10 in murine monocytes, derived from femur bones as well as the broncheoalveolar lavage, that are cultured under a hyperglycemic stress. Similarly, heparin inhibits expression of M1 markers such as CD80, iNOS and TNFa, while it induces M2 markers such as CD163, arginase and IL-10 in U937 cells (a human monocytic cell line) under a hyperglycemic stress. This suggests that the M1 macrophages in the uncontrolled diabetic glomeruli initiate inflammatory and fibrotic responses and are ineffective in removing the HA matrix synthesized by the MCs while the M2 macrophages in the glomeruli of diabetic rats treated with heparin effectively remove the HA matrix without initiating the inflammatory and fibrotic responses, which allows the MCs to maintain glomerular function while still synthesizing a monocyteadhesive HA matrix in response to the hyperglycemia. Interestingly, not only does heparin regulate the phenotypic activation of the monocyte/macrophages but it also regulate their metabolic responses and HA synthesis in response to a hyperglycemic stress that is independent of any possible cross talk between macrophages and any adjacent cellular compartment in the kidney. In summary, these results indicate that heparin inhibits M1 macrophage differentiation (pro-inflammatory phenotype) while it promotes M2 macrophage differentiation (anti-inflammatory phenotype) that is critical for the resolution of inflammation caused a by hyperglycemic stress providing a possible explanation for the sustained glomerular function in diabetic rats treated with heparin.

Identification of Novel Chondroitin Sulfate Sulfotransferases and Proteoglycan Core Proteins in the Nematode C. elegans

ST2/ P2

T. Dierker[1], F. Noborn[2], A. Hinas[3], L. Kjellén[1]

[1]*Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden*

[2]*Department of Clinical Chemistry and Transfusion Medicine, Institute of Biomedicine, Gothenburg University, Gothenburg, Sweden*

[3] *Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden*

The nematode *Caenorhabditis elegans* is a well-known model organism that during recent years also has been used for the study of glycosaminoglycans (GAGs). It has long been known that worms express heparan sulfate (HS) that is similar to mammalian HS with regard to both pattern and level of sulfation. However, we could recently show that the nematodes also produce chondroitin sulfate (CS) and not only non-sulfated chondroitin. We also identified the responsible CS-4-*O*-sulfotransferase, *chst-1*, and our data indicated the presence of at least one CS-6-*O*-sulfotransferase.

In our current study we aim to identify and characterize the remaining CS-sulfotransferases. To this end, we knocked down different candidate genes in animals lacking *chst-1* using RNAi. We then tested if egg-laying or development were altered and analyzed staining of whole worms with the CS-specific antibody CS-56. In addition, we used a glycoproteomics approach in order to identify novel CS core proteins.

We were able to narrow down the list of potential CS-sulfotransferase candidates to three genes and are currently confirming our results. Moreover, we could identify a number of novel CS core proteins in *C. elegans*. Our data will be important for the future development and establishment of *C. elegans* as a model system for GAG biology. It is already evident that these animals are more complex than previously thought and we believe that they can serve as important models for GAG-related processes even in humans.

ST3/
P3

Lumican effectively regulates the estrogen receptors-associated functional properties of breast cancer cells, expression of matrix effectors and epithelial-to-mesenchymal transition

Konstantina Karamanou[1,2,3], Marco Franchi[4], Zoi Piperigkou[2], Corinne Perreau[1,3], Francois- Xavier Maquart[1,3,5], Demitrios H. Vynios[2], Stéphane Brézillon[1,3]

[1]*Université de Reims Champagne Ardenne, Laboratoire de Biochimie Mıdicale et de Biologie Molıculaire, Reims, France*

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

[3]*CNRS UMR 7369, Matrice Extracellulaire et Dynamique Cellulaire, Reims, France*

[4]*Department for Life Quality Studies, University of Bologna, Rimini, Italy*

[5]*CHU de Reims, Laboratoire Central de Biochimie, Reims, France*

Lumican is a small leucine-rich proteoglycan that has been shown to contribute in several physiological processes, but also to exert anticancer activity. On the other hand, it has been recently shown that knockdown of the estrogen receptor α (ERα) in low invasive MCF-7 (ERα+) breast cancer cells and the suppression of ERβ in highly aggressive MDA-MB-231 (ERβ+) cells significantly alter the functional properties of breast cancer cells and the gene expression profile of matrix macromolecules related to cancer progression and cell morphology. In this report, we evaluated the effects of lumican in respect to the ERs-associated breast cancer cell behaviour, before and after suppression of ERs, using scanning electron and confocal microscopies, qPCR and functional assays. Our data pinpointed that lumican significantly attenuated cell functional properties, including proliferation, migration and invasion. Furthermore, it modified cell morphology, inducing cell-cell junctions, evoked EMT/MET reprogramming and suppressed the expression of major matrix effectors (matrix metalloproteinases and EGFR) implicated in breast cancer progression. The effects of lumican were found to be related to the type of breast cancer cells and the ERα/β type. These data support the anticancer activity of lumican and open a new area for the pharmacological targeting of the invasive breast cancer.

Effects of the inhibition of hyaluronan synthesis by 4-methylumbelliferone on breast cancer cells of different ER status

ST4/P4

Theodoros Karalis[1], Athanasios Chatzopoulos[1], Paraskevi Heldin[2], Demitrios H. Vynios[1], Nikos K. Karamanos[1], Spyros S. Skandalis[1]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Ludwig Institute for Cancer Research, Uppsala University, Sweden
skandalis@upatras.gr

Previous studies have demonstrated that inhibition of hyaluronan (HA), a major extracellular matrix (ECM) heteropolysaccharide, suppressed the tumorigenicity of various malignant tumors including breast cancer. 4-methylumbelliferone (4-MU) has been reported to specifically inhibit HA synthesis in several cell types. However, few studies have focused on the effects of HA inhibition by 4-MU in breast cancer cells. Here, we investigated the effects of 4-MU on HA synthesis, certain ECM components synthesis/activity as well as the functional properties of breast cancer cells of different estrogen receptor (ER) status (ERα+/ERβ- low-invasiveness MCF-7 and ERα-/ERβ+ highly aggressive MDA-MB-231 cells).

Immunofluorescence analysis showed strong staining for cell associated HA in MDA-MB-231 (ERα-/ERβ+) cells in contrast to the negligible HA amounts found in MCF-7 (ERα+/ERβ) cells. Treatment of MDA-MB-231 cells with 4-MU significantly reduced intracellular HA, while it caused a loss of lamellipodia and their spindle-like morphology. 4-MU treatment also significantly inhibited cell growth, motility and adhesive capacity of ERα-/ERβ+ MDA-MB-231 cells in a time- and dose-dependent manner. However, no significant effects were observed for ERα+/ERβ- MCF-7 cells. Interestingly, 4-MU markedly changed the transcripts coding for the three HA synthases (HAS1, 2, and 3) as well as the expression and activity of certain matrix metalloproteinases (MMPs) and components of the plasminogen activation system in ERα-/ERβ+ MDA-MB-231 cells.

These data suggest that 4-MU might represent a promising therapeutic candidate for specific breast cancer subtypes with regard to the ER status, a major classification and predictive marker in breast cancers, via suppression of HA synthesis and accumulation, and regulation of matrix-degrading enzymes and inflammatory mediators.

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ST5/
P22

ADAMTS-7 is associated with a high-risk plaque phenotype in human atherosclerosis

E. Bengtsson[1], K. Hultman[1,8], P. Dunér[1], G. Asciutto[2], P. Almgren[3], M. Orho-Melander[4], O. Melander[3,5], J. Nilsson[1], A. Hultgårdh-Nilsson[6], and I. Gonçalves[1,7]

[1]Experimental Cardiovascular Research Unit, Department of Clinical Sciences, Malmö, Lund University, Sweden
[2]Vascular Center Malmö, Skåne University Hospital, Malmö, Sweden
[3]Department of Clinical Sciences, Malmö, Lund University, Sweden
[4]Diabetes and Cardiovascular Disease Genetic Epidemiology Unit, Department of Clinical Sciences, Malmö, Lund University, Sweden
[5]Department of Internal Medicine, Skåne University Hospital, Malmö, Sweden
[6]Department of Experimental Medical Science, Lund University, Lund, Sweden
[7]Department of Cardiology, Skåne University Hospital, Sweden
[8]Present address: Novo Nordisk A/S, Bagsvaerd, Denmark

Several large-scale genome-wide association studies have identified single-nucleotide polymorphisms in the genomic region of A Disintegrin And Metalloproteinase with ThromboSpondin type 1 repeats (ADAMTS)-7 and associations to coronary artery disease. Experimental studies have provided evidence for a functional role of ADAMTS-7 in both injury-induced vascular neointima formation and development of atherosclerotic lesions. However, whether ADAMTS-7 is associated with a specific plaque phenotype in humans has not been investigated. Carotid plaques (n=206) from patients with and without cerebrovascular symptoms were analyzed for expression of ADAMTS-7 by immunohistochemistry and correlated to components associated with plaque vulnerability. Plaques from symptomatic patients showed increased levels of ADAMTS-7 compared with lesions from asymptomatic patients. High levels of ADAMTS-7 correlated with high levels of CD68-staining and lipid content, but with low smooth muscle cell and collagen content, which together are characteristics of a vulnerable plaque phenotype. ADAMTS-7 levels above median were associated with increased risk for postoperative cardiovascular events. Our data show that ADAMTS-7 is associated with a vulnerable plaque phenotype in human carotid lesions. These data support previous observations of a potential proatherogenic role of ADAMTS-7.

Distinct biological events generated by ECM proteolysis by two homologous collagenases

ST6/P23

Inna Solomonov[1], Eldar Zehorai[1], Dalit Talmi-Frank[1,2], Sharon G. Wolf[3], Alla Shainskaya[4], Alina Zhuravlev[1], Elena Kartvelishvily[3], Robert Visse[5], Yishai Levin[4], Nir Kampf[6], Diego Adhemar Jaitin[2], Eyal David[2], Ido Amit[2], Hideaki Nagase[5], and Irit Sagi[1]

[1]*Department of Biological Regulation, Weizmann Institute of Science, Rehovot 76100, Israel*

[2]*Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel*

[3]*Department of Chemical Research Support, Weizmann Institute of Science, Rehovot 76100, Israel*

[4]*Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Rehovot 76100, Israel*

[5]*Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences, University of Oxford, Oxford, OX3 7FY, United Kingdom*

[6]*Department of Materials and Interfaces, Weizmann Institute of Science, Rehovot 76100, Israel*

It is well established that the expression profiles of multiple and possibly redundant matrix-remodeling proteases (e.g., collagenases) differ strongly in health, disease, and development. Although enzymatic redundancy might be inferred from their close similarity in structure, their in vivo activity can lead to extremely diverse tissue-remodeling outcomes. We observed that proteolysis of collagen-rich natural extracellular matrix (ECM), performed uniquely by individual homologous proteases, leads to distinct events that eventually affect overall ECM morphology, viscoelastic properties, and molecular composition. We revealed striking differences in the motility and signaling patterns, morphology, and gene-expression profiles of cells interacting with natural collagen-rich ECM degraded by different collagenases. Thus, in contrast to previous notions, matrix-remodeling systems are not redundant and give rise to precise ECM–cell crosstalk. Because ECM proteolysis is an abundant biochemical process that is critical for tissue homoeostasis, these results improve our fundamental understanding its complexity and its impact on cell behavior.

ST7/ P24

The new metastatic markers flotillins promote tumor cell invasion through MT1-MMP-dependent ECM degradation

Cécile Gauthier-Rouvière[1], Damien Planchon[1], Mallory Genest[1], Eduardo Rios-Morris[1], Franck Comunale[1], Philippe Chavrier[2], Ivan Bièche[3], Stefan Linder[4] and Stéphane Bodin[1]

[1]*Universités Montpellier, CRBM, CNRS, UMR 5237, 1919 Route de Mende, 34293 Montpellier, France*

[2]*Department of Genetics*

[3]*Cell Dynamics and Compartmentalization Unit, nstitut Curie, Paris, France.*

[4]*Universitätsklinikum Eppendorf - Institut fór medecine, Hamburg, Germany*

Tumor cell invasion and consecutive metastasis formation are the main cause of death in cancer patients. Invading tumor cells are surrounded by stroma and extracellular matrix (ECM) that is remodeled or degraded, by specialized organelles called invadopodia, during the metastatic process. Invadopodia function depends on matrix metalloproteinases (MMPs) that degrade ECM. Among which, MT1-MMP plays a major role. In many metastatic cells, the invasive behavior strongly depends on MT1-MMP trafficking deregulation leading to the increase of its extracellular delivery. The mechanisms responsible of these deregulations are not fully understood.

Flotillin 1 and 2 are ubiquitous and highly conserved proteins associated with specific caveolin-independent membrane microdomains enriched in cholesterol and glycosphingolipids. Flotillins 1 and 2 are believed to exist as heterotetramers able to oligomerize. In physiological conditions, they are expressed at moderate levels and mainly localize at the plasma membrane where they form flotillin-microdomains that play a function in a large number of cellular processes, mainly through their role in membrane receptor clustering and in the regulation of clathrin-independent endocytosis. We demonstrated that flotillins directly regulate intercellular adherence by stabilizing the formation of cadherin complexes (Guillaume et al., J Cell Science, 2013).

Flotillins are overexpressed in many invasive cancers and considered as markers of poor prognosis. How overexpressed flotillins participate in the acquisition of metastatic properties remains to be determined. We discovered that flotillins are critical regulators of MT1-MMP trafficking.

We used a dual reciprocal approach consisting in the overexpression of flotillins in non tumoral cells and in the down-regulation of flotillins in metastatic cells. Using quantitative cell biology approaches and optogenetics coupled with in vivo studies, we show that flotillins downregulation in invasive cancer cells dramatically inhibit their invasive properties as monitored *in vitro* using a 3D-collagen invasion assay and in vivo using zebrafish xenografts. Reciprocally, ectopic overexpression of flotillins in non-tumoral cells is sufficient to induce their invasive behavior in vitro and *in vivo*. We show that flotillins are critical regulators of the trafficking of MT1-MMP, hence increasing the ECM degradative capacity of cancer cells.

Collagen induces activation of DDR1 through lateral dimer association and phosphorylation between dimers

ST8/ P25

Victoria Juskaite[1], Birgit Leitinger[1]

[1]*National Heart and Lung Institute, Imperial College London, London SW7 2AZ, United Kingdom*

The discoidin domain receptors, DDR1 and DDR2, are intriguing receptor tyrosine kinases (RTKs) that signal in response to collagen with unusually delayed autophosphorylation kinetics. Both DDRs play important roles in development and regulate cell adhesion, migration, invasion, proliferation, and survival. The DDRs are attractive drug targets for many diseases including organ fibrosis, atherosclerosis, arthritis and various forms of cancer, but very little is understood about how collagen binding translates to DDR activation.

Classical RTKs are activated through ligand-induced dimerisation. However, the DDRs are constitutive, non-covalent dimers and are unlikely to signal at the level of dimers. We hypothesised that collagen binding induces lateral association (clustering) of DDR receptors on the cell membrane. Cell imaging experiments demonstrate that collagen induces DDR1 aggregation on the cell surface, whilst FRET imaging reveal collagen-induced structural changes in extracellular and intracellular regions of DDR1. We also co-expressed different types of signalling-incompetent DDR1 mutants ('receiver') with functional DDR1 ('donor') and demonstrate phosphorylation of receiver DDR1 by donor DDR1 in response to collagen. Making use of enforced covalent DDR1 dimerisation, which does not affect receptor activation, we show that receiver dimers are phosphorylated *in trans* by the donor. This process requires kinase activity of the donor but not that of the receiver. The phosphorylation *in trans* does not require ectodomain contacts, but is abolished by mutations in the transmembrane domain.

Interestingly, different isoforms of DDR1 and DDR2 can act as the donor kinase to phosphorylate receiver DDR1 in response to collagen. Receiver DDR1 dimers are also phosphorylated *in trans* by donor DDR1 in response to a triple-helical collagen-like DDR-selective peptide and a multivalent DDR1 antibody, indicating that ligand multivalency and specific mode of activation is not required. Furthermore, collagen-coated beads induce recruitment and phosphorylation of DDR1, which does not extend beyond direct receptor-ligand contact area. Mutant DDR1 that cannot bind collagen is recruited to collagen-coated beads when co-expressed with functional receptor. Current work is examining collagen-induced structural changes between DDR1 dimers at receptor-ligand contact area by FRET imaging.

In conclusion, collagen induces recruitment of mutant DDR1 into DDR1 signalling clusters and induces phosphorylation between DDR dimers, which is consistent with a mechanism of activation by receptor clustering.

ST9/
P26

Heparanase 2 attenuates tumor vascularity and growth, associating with enhanced cell differentiation and inversely correlating with tumor grade and stage

Miriam Gross-Cohen[1], Sari Feld[1], Ilana Doweck[2], Gera Neufeld[1], Peleg Hasson[3], Gil Arvatz[1], Uri Barash[1], Inna Naroditsky[4], Ofer Nativ[5], Neta Ilan[1] and Israel Vlodavsky[1]

[1]Cancer and Vascular Biology Research Center and [3]Department of Anatomy and Cell Biology, the Bruce Rappaport Faculty of Medicine, Technion, Haifa 31096, Israel; [2]Department of Otolaryngology, Head and Neck Surgery, Carmel Medical Center, Haifa, Israel; [4]Department of Pathology, Rambam Health Care Campus, Haifa, Israel; [5]Department of Urology, Bnai-Zion Medical Center, Haifa, Israel

Heparanase is an endoglycosidase that specifically cleaves heparan sulfate side chains of proteoglycans, activity that is highly implicated in tumor metastasis, inflammation and angiogenesis, a consequence of HS cleavage and remodeling of the sub-endothelial and sub-epithelial extracellular matrix. Heparanase 2 (Hpa2) is a close homolog of heparanase that lacks intrinsic HS-degrading activity but retains the capacity to bind HS with high affinity. In head and neck cancer patients, Hpa2 expression was markedly elevated, correlating with prolonged time to disease recurrence and inversely correlating with tumor cell dissemination to regional lymph nodes, suggesting that Hpa2 functions as a tumor suppressor. The molecular mechanism associated with favorable prognosis following Hpa2 induction is unclear.

Here, we demonstrate that overexpression of Hpa2 in head and neck cancer cells markedly decreases tumor vascularity and growth, likely due to reduced expression of Id1, a transcription factor implicated in VEGF-A and VEGF-C gene regulation. Moreover, Hpa2 over expression induces cellular differentiation (i.e., induction of cytokeratin 13 & 15), thus maintaining a more normal epithelial phenotype. Notably, reduced tumor growth in response to Hpa2 occurred in heparanase-, and HS-independent manner.

Subsequent studies revealed that normal bladder, breast, and oral epithelium exhibit relatively high levels of Hpa2 and its expression is decreased substantially in the corresponding carcinomas, expression pattern typical of a tumor suppressor. Notably, bladder and breast tumors that retain high levels of Hpa2 were diagnosed as low grade. Moreover, overexpression of Hpa2 in 5637 bladder carcinoma cells resulted in smaller tumors that were diagnosed as low grade.

Taken together, our results support the notion that Hpa2 functions as a tumor suppressor in bladder and head & neck carcinomas. We further show that Hpa2 function is not restricted to modulation of heparanase activity or interaction with HSPGs but is apparently involved in regulation of selected genes that affect tumor vascularity (Id1, VEGF-A, VEGF-C), tumor fibrosis (LOX), and cell differentiation (cytokeratins 13 & 15), thus expanding significantly the repertoire of Hpa2 functions and its mode of action.

Signalling through the collagen receptor, DDR1, is required for epithelial polarisation and morphological remodelling in 3D matrices

ST10 /P27

Pernille Sogaard[1], Karl Matter[2] and Yoshifumi Itoh[1]

[1]*Kennedy Institute of Rheumatology, University of Oxford, Oxford OX3 7FY, UK*
[2]*UCL Institute of Ophthalmology, University College London, EC1V 9EL, UK*

During development of epithelial organs, epithelial cells collectively migrate and invade into their surroundings in a highly coordinated manner, where degradation of the extracellular matrix (ECM) must be restricted to distinct regions of protrusion. A prerequisite for such spatial coordination across growing structures is epithelial cell polarity. Cell polarity divides the plasmamembrane into an apical domain towards the lumen of structures and a basal domain facing the ECM. This allows the cells to restrict contact between ECM-degrading enzymes and the ECM according to time and space by targeting enzymes either to the apical or the basal side in distinct locations. An enzyme regulated in this manner is the transmembrane matrix metalloproteinase, MT1-MMP, which plays a key role in epithelial invasion. We previously found its default localisation to be apical, with a switch to basal localisation seen upon treatment with the morphogenic factor, hepatocyte growth factor (HGF). This switch in localisation requires signals from a collagen matrix, however, reception and interpretation of these signals are not understood.

We found that inhibition of the collagen-binding receptor tyrosine kinase, discoidin domain receptor 1 (DDR1), disturbed the apicobasal distribution of MT1-MMP in confluent monolayers of MDCK cells, causing it to be present both apically and basally independently of HGF-treatment. In 3D, DDR1 inhibition blocked MT1-MMP-dependent tubulogenesis of MDCK and MCF10A cells, which instead of tubular structures formed compact, multi-layered cell aggregates. Furthermore, polarisation of the epithelial cell membrane into an apical and a basal domain failed in absence of DDR1 signalling, suggesting that DDR1 affects establishment of apicobasal polarity. In support of this, effects of DDR1-signaling on cell polarity were not limited to MT1-MMP-dependent morphogenesis, but also proved essential for lumen formation of MDCK or CaCO-2 cells in 3D conditions not requiring ECM degradation. This role of DDR1 in establishing apicobasal polarity during cyst formation involves suppression of the RhoA-ROCK activity and inhibition of ROCK during cyst formation rescues polarisation and lumen formation in absence of DDR1 signalling.

In conclusion, we find that DDR1 acts as a sensor of the microenvironment, which provides epithelial cells with spatial information necessary to polarise and undergo morphological remodelling in 3D.

ST11 /P28

Altered megakaryopoiesis in Type VI collagen KO mice

Vittorio Abbonante[1, 2], Cristian Gruppi[1, 2], Christian Andrea Di Buduo[1, 2], Monica Battiston[3], Alessandra Zullian[4], Moreno Mazzucato[3], Luigi De Marco[3], Paolo Bonaldo[4], Alessandra Balduini[1, 2,3]

[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy. [2]Laboratory of Biotechnology, IRCCS San Matteo Foundation, Pavia, Italy. [3]Department of Biomedical Engineering, Tufts University, Medford, MA, USA. Department of Laboratory Diagnostics and Cell Therapy, National Cancer Center (IRCCS CRO), Aviano, Italy. [4]Department of Molecular Medicine, University of Padova, Padova, Italy

In the last decades the study of the role of Bone Marrow (BM) Extracellular Matrix (ECM) components in the regulation of megakaryopoiesis has gained relevant attention. Type VI collagen is an ECM protein broadly distributed in different tissues, where it tunes fundamental aspects of cell behavior. Previous works from our group and others have localized type VI collagen within BM and demonstrated its adhesive properties for hematopoietic cells. Recently, we demonstrated that human and mouse megakaryocytes possess the whole collagen synthesis machinery and that produce and release various types of collagen. Here we extend the list of collagens produced by human and mouse megakaryocytes to type VI collagen and we studied the role of this protein in regulating megakaryopoiesis in vitro and in vivo by using a mouse model null for type VI collagen (Col6a1-/-). We found a significant increase in megakaryocyte number within BM of Col6a1-/- mice with respect to WT mice. However, despite a higher number of megakaryocytes, the peripheral platelet count was comparable between the two genotypes. Defects in platelet formation were excluded as demonstrated by performing platelet formation assays both in vitro and in vivo. While, platelets from Col6a1-/- mice displayed a reduced half-life and increased tendency to activate and aggregate, with respect to WT platelets, in response to principal agonists. Searching for the mechanism, we found that Col6a1-/- platelets presented an increased expression and function of Store Operated Calcium Entry channels, Stim1 and Orai1, which was derived from alterations in megakaryocytes mTOR-signaling pathway. Consistently, in vitro and in vivo treatment with the mTOR inhibitor rapamycin rescued Stim1 and Orai1 expression and function in megakaryocytes and platelets resulting in an increase of peripheral platelet count only in Col6a1-/- mice. These findings confirm the important role of self-produced ECM components in the regulation of megakaryopoiesis.

UDP-N-Acetylglucosamine regulation of epithelial-to-mesenchymal transition in breast cancer

ST12 /P29

Ashik Jawahar Deen[*1,2], Sami Heikkinen[1], Riikka Kärnä[1], Suresh Raju[1], Päivi Auvinen[3], Timo Nykopp[3], Markku Tammi[1], Sanna Oikari[1]

[1]*Institute of Biomedicine, University of Eastern Finland, Kuopio, Finland*
[2]*A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland*
[3]*Kuopio University Hospital, Kuopio, Finland*
**ashik.jawahardeen@uef.fi*

Breast cancer is the second most common cancer worldwide and the most common cancer in women. Most cancer cells show metabolic reprogramming of their energy metabolism, especially enhanced glucose uptake and glycolysis. This project aims to study the role of the glucose metabolite, UDP-N-acetyl glucosamine (UDP-GlcNAc) in breast cancer cells and tissue samples in the initiation of epithelial-to-mesenchymal transition (EMT), a process which transforms normal cells to a malignant state and metastasis. The specific aim is to analyze EMT-associated transcription factors (TFs), that are regulated by the O-GlcNAc modification derived from UDP-GlcNAc, and the role of these TFs in promoting gene expression of proteins involved in EMT.

Cell lines from breast cancer representing different degrees of malignancy (MCF-7 (adenocarcinoma), MDA-MB-361 (metastatic adenocarcinoma) and MDA-MB-231 (metastatic adenocarcinoma)) are used in this work. Differences correlating with the degree of malignancy were also noted in mRNA levels of hexosamine biosynthesis (HBS) enzymes (GFPT, GNPDAs, UAP1) and hyaluronan synthesis among the breast cancer cell lines. Our preliminary data from breast cancer shows that cancer patients indeed have drastically increased levels of UDP-sugars, especially UDP-GlcNAc. Elevated GFPT2 mRNA accompanies this. Furthermore, our results show that HA, a known prognostic factor for breast cancer correlates with its UDP-sugar substrates. An initial RNA sequencing experiment on breast cancer cell lines with progressively increasing invasiveness (MCF-7 ‹ MDA-MB-361 ‹ MDA-MB-231), revealed significant differences in the mRNA levels of HBS enzymes, along with many other key molecules involved in EMT or its transcriptional regulation. Also, we have found that mRNAs of STAT, FOX and PAX families of TFs involved in EMT and malignancy, among others, are differentially expressed in MDA-MB-361 and MDA-MB-231, when compared to MCF-7 cells. Most importantly, cellular functions related to EMT, regulation of extracellular matrix and gene transcription are upregulated in the more invasive MDA-MB-361 and MDA-MB-231, when compared to less invasive MCF-7 cell line. Future experiments will address the question of how O-GlcNAcylation regulates TFs in breast cancer EMT.

By defining the transcriptional consequences of the deranged glucose metabolism in malignant cells, this basic information may unveil new diagnostic markers and therapeutic targets of breast cancer.

ST13 /P30

Zebrafish modeling of the β4GalT7-deficient type of Ehlers-Danlos syndrome

S. Delbaere[1], T. Van Damme[1], P. Coucke[1], S. Symoens[1], D. Syx[1], A. Willaert[1] & F. Malfait[1]

[1]*Center for Medical Genetics Ghent, Ghent University, De Pintelaan 185, 9000 Ghent, Belgium*

Proteoglycans consist of glycosaminoglycan (GAG) side chains attached to a core protein through a tetrasaccharide linker region. Biallelic mutations in B4GALT7, the gene encoding galactosyltransferase I (β4GalT7) which is an essential enzyme for the biosynthesis of the linker region, are the cause of the rare autosomal recessive variant of the Ehlers-Danlos syndrome (EDS). This disorder is mainly characterized by short stature, hypotonia and skeletal abnormalities, in addition to the typical features of EDS such as joint hypermobility and skin hyperextensibility. Our current knowledge about this severe and disabling disease is very limited, in part due to the lack of a relevant in vivo model. The aim of this study was to create a zebrafish model for the β4GalT7-deficient type of EDS as there is a need for thorough, functional research of this disorder.

We developed and characterized a knockdown (KD) zebrafish model for the β4GalT7-deficient type of EDS by using morpholino injections targeting the b4galt7 gene, as this model mimics the hypomorphic effects patients are suffering from. Embryos injected with a standard control morpholino were used as negative control.

Morphant embryos showed morphological abnormalities such as a small, round head, withdrawn jaw, more front-facing eyes, short stature and mild developmental delay compared to wild-type and control morpholino injected embryos. The total amount of sulfated GAGs, using the Blyscan assay, was severely reduced in morphant embryos and whole-mount immunohistochemistry showed that heparan and chondroitin sulfate proteoglycans were severely diminished in the heads of b4galt7 morphants. In addition, alcian blue staining demonstrated that cartilage structure in the heads of morphant embryos are absent or strongly misshapen and alizarin red staining indicated a lack of head bone structures. The Tg(Col2a1aBAC:mcherry) reporter line, which is cartilage specific, confirmed the impaired cartilage pattern and showed an impaired chondrocyte organization in b4galt7 KD embryos. Furthermore, morphant embryos suffered from a lack of muscle tone and immunohistochemical staining revealed a disturbed filamentous actin pattern in head and tail. The specificity of these results was confirmed by injection of a different morpholino targeting b4galt7 and by F0 b4galt7 CRISPR/Cas9 injected embryos.

To conclude, a b4galt7 morphant zebrafish model has been developed, which partly phenocopies the human phenotype of patients suffering from β4GalT7-deficient EDS. This model enables the in vivo investigation of the pathogenesis of this condition.

HAS2-AS1 regulates breast cancer cells aggressiveness through a molecular sponge effect

ST14 /P31

I. Caon[1], M. Götte[2], P. Moretto[1], M. Viola[1], E. Karousou[1], E. Caravà[1], G. De Luca[1], A. Passi[1], D. Vigetti[1]

[1]*Department of Medicine and Surgery, University of Insubria, Varese, Italy*

[2]*Department of Gynecology and Obstetrics, Münster University Hospital, Münster, Germany*

Hyaluronan is a ubiquitous glycosaminoglycan of extracellular matrix able to modulate cell adhesion, motility, growth and inflammation. In tumour microenvironment, the up-regulation of hyaluronan synthases 2 (HAS2) and the overproduction of hyaluronan are often associated with tumour progression and metastasis. Recently, it has been discovered that the natural antisense transcript for hyaluronan synthase 2 (HAS2-AS1) can modulate the expression of HAS2 and the production of hyaluronan [1, 2, 3]. HAS2-AS1 is a long non coding RNA (lncRNA) transcribed in the opposite strand of HAS2 gene. It has an alternative splicing site which generates two RNA isoforms of different lengths (HAS2-AS1 long and HAS2-AS1 short) that share a region of complementarity to the first exon of HAS2.

Here, we show that the knockdown of HAS2-AS1 in the triple negative breast cancer cells MDA-MB-231 increased proliferation, migration and invasion. On the contrary, the over-expression of the isoforms HAS2-AS1 short and HAS2-AS1 long decreased cell viability and invasion. Quantitative PCR analysis revealed that the abrogation of HAS2-AS1 enhanced the levels of HAS2, HAS3 and hyaluronidase 2 (HYAL2) mRNA, suggesting a possible role of HAS2-AS1 in tumour progression through alteration of HA metabolism.

LncRNAs can orchestrate gene expression through a variety of mechanisms, regulating transcription and translation, chromatin-remodelling and the interaction with other RNA species, i.e. microRNAs. HAS2-AS1 transcript contains a putative binding site for miR-186, a negative regulator of the pro-apoptotic receptor P2X7 [4]. In our preliminary results indeed, we demonstrate that the overexpression of HAS2-AS1 decreased the abundance of miR-186, while the transcript of P2X7 was raised. All together, these data suggest a "sponge effect" of HAS2-AS1, able to sequester miR-186 away and antagonize its function on the target site.

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2. Chao et al; J Biol Chem. 2005 Jul 29;280(30):27513-22.

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4. Zhou et al; J Biol Chem. 2008 Oct 17;283(42):28274-86

ST15 /P32

HAS3-induced Extracellular Vesicles (HAS3-EVs) promote EMT in human keratinocyte to acquire malignant properties

Uma T. Arasu*[1], Ashik J. Deen[1], Kai Härkönen[1], Arto Koistinen[2], Riikka Kärnä[1], Sanna Oikari[1], Elisa Lazaro-Ibanez[3], Pia Siljander[3,4], Kirsi Rilla[1]

[1]*Institute of Biomedicine*

[2]*SIB Labs, University of Eastern Finland, Kuopio, Finland*

[3]*Division of Pharmaceutical Biosciences, Faculty of Pharmacy*

[4]*Division of Biochemistry and Biotechnology, Department of Biosciences, University of Helsinki, Helsinki, Finland *E-mail: uma.thanigai@uef.fi*

Hyaluronan (HA) is the most abundant and essential polysaccharide of the extracellular matrix for maintenance of normal tissues, but it also creates a favorable microenvironment for growth of tumor cells. HA is synthesized by specific plasma membrane-bound enzymes, hyaluronan synthases (HASs). The HASs activate growth of extremely long filopodia coated with HA which induces shedding of extracellular vesicles (EVs) originating from the tips of filopodia. This plasma membrane derived EVs of different size secreted by many cell types are thought to carry and transfer cytosolic components, proteins, RNA, ribosomes and selected plasma membrane proteins. Interestingly EVs are suggested to interact with their target cells by utilizing receptors on the vesicular surface, such as CD44. These CD44-hyaluronan interactions may act as universal mechanism facilitating cellular binding and uptake of HAS3-EVs. The aim of this work was to study these interactions and their effects on target cells.

The HA-coated EVs secreted by GFP-HAS3 overexpressing cells were shown to carry GFP-HAS3 (both as protein and mRNA) using techniques like immunoblotting and qPCR. Moreover, confocal microscopy and correlative light and electron microscopy showed that GFP-HAS3 containing EVs (i.e. HAS3-EVs) induced morphological changes and increased the size of pericellular HA coat of the target cells. HA secretion of the target cells to the culture medium was also increased as analyzed by ELISA-like assay. Furthermore, adding an excess of HA oligosaccharides in the incubation medium to displace HA from the receptors, or treatment with CD44 blocking antibody down regulated the vesicle binding to the target cell, thereby showing that HA-CD44 interactions are important for EV binding to the target cells. Moreover the binding of HAS3-EVs induced changes to the cell cycle related proteins in the G1 and S Phase, pointing the cell towards obtaining EMT like changes.

HAS-induced EVs act as carriers for HA on their surface and are potential vehicles in preparing the premetastatic niches. Furthermore, CD44-HA interactions may act as universal mechanism facilitating cellular binding of EVs. This may result in signals to modify the target cells functions like paving way for benign cells to become metastatic. Moreover, HA-induced EVs could be utilized as diagnostic tools and targets of therapy.

MicroRNA targeting as a regulatory mechanism of breast cancer cells with different estrogen receptor status

ST16 /P33

Z. Piperigkou [1,2], M. Götte [2], N. K. Karamanos [1]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory ofBiochemistry, Department of Chemistry, University of Patras, Patras, Greece; [2] Department of Gynecology and Obstetrics, University Hospital, Münster, Germany

Estrogens and their receptors have pivotal roles in the development and progression of breast cancer. It is well established that interactions among cancer cells and tumor microenvironment are in dynamic interplay and regulated by extracellular matrix. We have recently demonstrated that the loss of ERα in MCF-7 breast cancer cells increased their aggressiveness through the induction of epithelial to mesenchymal transition, striking changes in their functional properties and expression patterns of certain ECM mediators. Recent data indicated that the suppression of ERβ in MDA-MB-231 breast cancer cells reduces their aggressiveness and affects their functional properties as well as certain ECM components. In the present study, we evaluated the effects of the estrogen receptors suppression on the microRNAs expression levels in four breast cancer cell lines (MCF-7 and MDA-MB-231 before and after suppression of ERβ). Our data indicated that the three different breast cancer cells exhibited alterations in the expression levels of certain microRNAs that are implicated in the inhibition of cancer progression and the retention of EMT, depending on the presence of ERα or ERβ. We demonstrated that the loss of ERβ in shERβ MDA-MB-231 cells resulted in differentiated expression profiles of miR-10b, miR-200b and miR-145, compared to MDA-MB-231 and MCF-7 breast cancer cells. Interestingly, our data revealed that breast cancer cells that lack ERβ exhibited elevated expression levels of miR-145 and decreased levels of miR-200b and miR-10b and that these miRNAs significantly regulate breast cancer cell behavior, EMT process and ECM composition. These novel results suggest that the alterations in cell behaviour and in ECM composition caused by the suppression of ERβ in aggressive MDA-MB-231 cells are closely related to certain epigenetic miRNA-induced alterations. Targeting the ERβ-regulated miR-10b and miR-145 is a promising tool for diagnosis and pharmaceutical targeting in breast cancer.

ST17 /P34

The impacts of ER stress associated chondrodysplasias on metabolomics

J.M.A. Wragg[1], K.A. Hollywood[2], D.K.Trivedi[3], R. Goodacre[3] & R.P. Boot-Handford[1]

[1] Wellcome Trust Centre for Cell-Matrix Research, Faculty of Biology, Medicine and Health, Michael Smith Building, University of Manchester, Manchester, M13 9PL

[2]SYNBIOCHEM, Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street, Manchester, M1 7DN

[3]School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street, Manchester, M1 7DN

Longitudinal bone growth via endochondral ossification (EO) can be disrupted by increased endoplasmic reticulum (ER) stress and the associated unfolded protein response (UPR), observed in cells expressing mutant protein coding extracellular matrix (ECM) components of cartilage. These cellular stresses cause reduced proliferation and differentiation of these cells, ultimately leading to the clinical phenotype of various forms of dwarfism including the chondrodysplasias. Gaining a biological understanding of these disease mechanisms at the biochemical level is important, as it is possible to reduce cellular ER stress resulting from these genetic mutations pharmacologically and therefore potentially reduce clinical consequences of the chondrodysplasias. Establishing quantitative metabolite profiles for these disease states allows assessment of which areas of metabolism are affected by each given mutation and comparison can then be made between disease states. Initial focus has centred on four collagen X mutations that have the downstream consequence of causing the metaphyseal chondrodysplasia type Schmid (MCDS) form of dwarfism: NC1del10, Y598D, G618V and N617K as well as a wild-type collagen X construct expressed as a control comparison. Work then focussed on a similar comparison between the matrilin-3 mutant V194D (causing multiple epiphyseal dysplasia) and its wild type control.

To facilitate this research stable 293-EBNA cell lines expressing each construct were generated via transfection and selection. To determine whether the disease phenotype exhibited by these cell models involved alterations in cell metabolism, metabolic profiles were generated from the seven cell culture models by established gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) techniques. Principal components analysis (PCA) was used to summarise the variance in the metabolic profiling data. We report the top 10 discriminating metabolite features (control to mutant) by their variable importance in projection (VIP) scores for the various cell models. Similar results were seen for all data acquired (LC-MS –ve and +ve ion mode data and GC-MS). Each group of biological replicates clustered closely together demonstrating reproducibility and the quality control samples cluster tightly in the centre of the plot highlighting that the analytical process was robust.

Pathobiology of the ageing matrix: on the path to intervertebral disc regeneration

ST18 /P40

J. Caldeira[1,2,3], C. Santa[4,5], H. Osório[1,3,6], M. Molinos[1,2,7], B. Manadas[5], R. Gonçalves[1,2], M. Barbosa[1,2,7]

[1] i3S - Instituto de Investigaçyo e Inovaçyo em Saïde, Universidade do Porto
[2] INEB - Instituto de Engenharia Biomıdica, Universidade do Porto, Rua Alfredo Allen, 208, 4200-180 Porto, Portugal
[3] IPATIMUP - Institute of Molecular Pathology and Immunology, University of Porto, Rua Jïlio Amaral de Carvalho, 45, 4200-135 Porto, Portugal
[4] III – Institute for Interdisciplinary Research, University of Coimbra, Casa Costa Alemyo – Polo II, Rua Dom Francisco de Lemos, 3030-789 Coimbra, Portugal
[5] CNC – Center for Neuroscience and Cell Biology, University of Coimbra, 3004-504 Coimbra, Portugal
[6] Department of Pathology and Oncology, Faculty of Medicine, University of Porto,4200-319 Porto, Portugal
[7] ICBAS - Instituto de Cıkncias Biomıdicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira n. 228, 4050-313 Porto, Portugal

Although not a cause of premature mortality, Low Back Pain (LBP), which affects 70-85% of the population, is often caused by Intervertebral Disc (IVD) degeneration and progresses with age, being accompanied by extracellular matrix depletion. It ranks highest in terms of disability, and sixth in terms of overall burden. An estimated 149 million days of work per year are lost because of LBP, with annual total costs estimated to be around 150 billion euros, two-thirds of which are due to decreased wages and productivity.

Conventional therapies, which involve pain modulators and invasive surgeries, are often unsuccessful. To date, promising strategies for disc regeneration, based on the maintenance and/or increase of matrix synthesis, are being explored in vivo: protein injection, gene transfer and cell implantation. Although rapid advances are being made in regulating the degenerative process, many challenges remain. Understanding IVD pathophysiology (particularly in terms of IVD matrix constituents and their alterations in development and disease) is key to unveil molecular cues that might be used to slow, halt or reverse the age-associated degenerative cascade.

In this work, we have performed iTRAQ LC-MS/MS analysis to investigate matrisome changes observed with development and ageing in healthy bovine IVDs. In total, 161 bovine proteins were identified. Of these, 77 molecules were common to the three different age groups, of which 36 defined the IVD matrisome. Differential expression levels obtained for Collagen Type XI, XII, XIV, Fibronectin and Prolargin were independently validated.

This study provides the first matrisome database of healthy discs during development and ageing. The shift in IVD matrisome signatures herein observed provides insight into factors that may explain age-associated IVD degeneration, and which are putative targets for therapy. It also defines early developmental stage microenvironments that we are currently trying to recapitulate in order to develop novel therapeutic strategies.

ST19 /P41

Inhibition of lysyl oxidase like 2 reduces collagen cardiac interstitial fibrosis in mice

A. Buson, L. Cao, M. Deodhar, A. D. Findlay, J. S. Foot, A. Greco, J. Moses, L. Perryman, B. Rayner*, H. C. Schilter, X. Tan, C. I. Turner, T. T. Yow, A. Zahoor, W. Zhou, W. Jarolimek

*Drug Discovery, Pharmaxis Ltd., 20 Rodborough Road, Frenchs Forest, NSW, Australia; *Heart Research Institute, Eliza Street, Newton, NSW, Australia*

Lysyl oxidases oxidise the primary amine group of lysine residues in extracellular matrix proteins which then can cross-link and stabilise the fibrosis. Fibrosis is the accumulation of cross-linked extracellular matrix and can progress to organ failure. Yan et al (DOI: 10.1038/ncomms13710) have recently shown that an enzyme that crosslinks collagen—Lysyl oxidase-like 2 (Loxl2)—is essential for interstitial fibrosis and mechanical dysfunction of pathologically stressed hearts. Other lysyl oxidase family members are less upregulated during cardiac fibrosis. Importantly, in diseased human hearts, LOXL2 is upregulated in cardiac interstitium; its levels correlate with collagen crosslinking and cardiac dysfunction. LOXL2 is also elevated in the serum of heart failure (HF) patients, correlating with other HF biomarkers, suggesting a conserved LOXL2-mediated mechanism of human HF.

Pharmaxis has developed small molecule inhibitors that are selective for LOXL2 over LOX. These compounds have nanomolar potency in blocking the enzymatic activity of LOXL2 in a mechanism-based manner. They have good oral bioavailability and an excellent developability profile.

In a model of myocardial infarction, echocardiography was performed on 61 mice at 24 hrs post-MI and 50 mice at 28 days post-MI. Echo specialist was blinded to each treatment when performing the sand analysis. Echo data showed that LOXL2 inhibitor or the positive pharmacological control losartan improved cardiac function in infarcted mice. Losartan and the LOXL2 inhibitor increased the ejection fraction improved the fractional shortening as well as left ventricular contraction.

This study shows that small molecule LOXL2 inhibitors have beneficial effects in the treatment of cardiac infarction. As mouse models reflect many aspects of the human disease in regards to the role of LOXL2 it is highly likely that these small molecule inhibitors will be beneficial in the treatment of patients.

9E8 anti-Heparanase antibody neutralizes Heparanase activity and inhibits invasion of cancer cells after class switching from IgM to IgG

ST20 /P42

Marina Weissmann[1], Steve R Roffler[2], Talal S Al-Qaisi[2], Neta Ilan[1], and Israel Vlodavsky[1]

[1]Cancer and Vascular Biology Research Center, the Bruce Rappaport Faculty of Medicine, Technion, Haifa 31096, Israel; and [2]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

Heparanase is the only enzyme in mammals that can cleave heparan sulfate (HS) side chains of proteoglycans in the extracellular matrix (ECM) and cell surface. This cleavage results in the release of ECM-bound growth factors and degradation of the ECM, allowing cancer cells to disseminate. Increased heparanase levels were found in a wide variety of solid and hematological malignancies, and correlated with poor patient's prognosis. In our previous study we described the first anti-heparanase neutralizing monoclonal antibody, 9E8, which was targeted to the HS-binding domain of heparanase. This antibody attenuated cellular invasion, experimental and spontaneous metastasis, and lymphoma growth within bones. Since this antibody is an IgM subtype, the aim of the current study was to convert the 9E8 antibody to IgG, while maintaining its neutralization and biological capacities. We adopted a methodology that mimics the germinal center reaction in-vitro, in an attempt to attain class switching and affinity maturation. In this process, Activation-Induced Cytidine Deaminase (AID) gene was introduced into the 9E8 hybridoma cells to generate somatic hyper-mutation. Cells that were mutated and undergone class switching to IgG, which was expressed on their cell surface, were sorted by flow cytometry. Subsequently, a population of hybridoma cells that bound to heparanase with high affinity was sorted sequentially. As a result, we were able to isolate two clones of hybridoma cells which express IgG antibodies with high affinity to heparanase. Both of these clones neutralized heparanase enzymatic activity and attenuated cellular invasion. Currently these antibodies are being tested in-vivo for their anti-lymphoma capacity.

ST21 /P43

Strain stiffening and structural ECM remodelling by invading cancer cells

Sjoerd van Helvert[1], Cornelia Veelken[1], Nader Atlasy[2], Anna Haeger[1], Henk Stunnenberg[2], Peter Friedl[1,3,4]

[1]*Dpt. of Cell Biology, Radboud Institute for Molecular Life Science, Nijmegen, The Netherlands*
[2]*Dpt. of Molecular Biology, Radboud Institute for Molecular Life Science, Nijmegen, The Netherlands*
[3]*David H. Koch Center for Applied Research of Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA*
[4]*Cancer Genomics Center (CGC.nl), 3584 CG Utrecht, The Netherlands*

In the development of metastatic cancer, tumor cells detach from their primary location and navigate through extracellular matrix (ECM), the physical and chemical organization of which varies greatly during cancer progression. ECM modulation is both transient and permanent, which likely has reciprocal effects on cell signalling and function. During migration, cells dynamically couple to ECM interfaces via adhesion receptors and generate traction. When deformed, biopolymer networks, including fibrillar collagen, undergo a nonlinear elasticity change termed strain stiffening. It remains poorly characterized, however, whether forces generated by moving cells suffice to induce strain stiffening. Strain stiffening at the leading edge of cells moving across fibrillar type I collagen was detected by combining large-field AFM nanoindentation with confocal reflection microscopy. In different cell models, gradient-like fiber realignment, densification, and elevation of Young's modulus ahead of the leading edge were observed. Moving fibroblasts generated larger anterograde strain fields with a higher amplitude and up to 6-fold increased cumulative strain stiffening compared with mesenchymal HT1080 fibrosarcoma cells and epithelial SCC38 cancer cells. Collectively moving SCC38 cells produced 4-fold increased strain stiffening compared with individually moving SCC38 cells in a β1 integrin- and actomyosin-dependent manner. This indicates that the extent of strain stiffening by moving cells scales with cell type, multicellular cooperativity, integrin availability, and contractility. By straining, migrating cells realign and densify fibrillar ECM and thus adopt an autonomous strategy to move on a "traveling wave" of stiffened substrate. To dissect irreversible ECM remodeling downstream of strain stiffening, deposition of ECM proteins by tumor cells along the 3D invasion path are being tested. Proteomics and RNA-seq data was used to generate a list of upregulated matrisome proteins in invading cancer cells. Using immunostaining of in vitro generated collagen-based 3D models, the sub-regions and candidate proteins are spatially mapped along the cancer-stroma interface. Understanding the dynamics of transient and permanent self-tuning of the microenvironment will lead to a better fundamental understanding of the co-evolution of tumor and ECM and has potential for personalized medicine.

Decorin delivery hinder primary and metastatic tumor formation in the liver

ST22 /P50

Zs. Horvth, H. Fehér, A. Reszegi, K. Karszi, V. Postnikov, E. Regs, I. Kovalszky, K. Baghy

1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary

Hepatocellular carcinoma and colorectal cancer represents the third and the second most common cause of cancer death worldwide. A key constituent of the hepatic microenvironment is the small leucine-rich proteoglycan decorin known to interfere with cellular events of tumorigenesis mainly by blocking various receptor tyrosine kinases and inducing p21WAF1/CIP1. Our previous studies showed that the lack of decorin favors primary hepatocarcinogenesis resulting in higher tumor incidence. In addition, decorin expression is decreased in primary human liver and colorectal cancer and in its liver metastases. Thus, we hypothesized that overexpression in the liver may inhibit tumor formation in both malignancies. To this end pLIVE vector coding human decorin cDNA and its control vector were targeted to the liver. For monitoring vector operation, a control vector coding for serum alkaline phosphatase (SEAP) was also applied. Vectors were ingested by hydrodynamic gene delivery. Primary hepatocarcinogenesis was induced by administering thioacetamide (TAA) to wild type and decorin-null animals. Liver metastases of colon cancer were generated by inoculating murine colon 38 tumor cells into the spleen followed by their colonization into the liver. Artificial decorin expression was tested by immunostaining, and SEAP was detected from sera by a chemiluminescent assay. After transfection, the excessive proteoglycan amount was mainly detected in hepatocytes around the central veins and located in the endoplasmic reticulum and Golgi complex. Depending on the transfection efficiency measured by SEAP assay, wild type experimental groups were subdivided into decorin negative, low and high decorin expressing categories. Upon TAA induced hepatocarcinogenesis the highest tumor count was observed in mice with no decorin production. In contrast, decorin delivery decreased the number of tumors by 72 and 78% in low and high decorin expressing groups respectively. In vivo gene delivery into Dcn-/- animals was less effective than that of wild type mice. In spite of that, artificial decorin expressed in knockout mice was able to decrease tumor formation by 83%. In colon carcinoma liver metastasis model, decorin overexpression caused a 63% reduction in the number of liver metastases in parallel with lower liver mass/body mass ratio reflecting on the tumor content of the organ. Furthermore, decorin, produced by the hepatocytes reduced the levels of active EGFR, MSPR and PDGFRα. In conclusion, decorin gene delivery is able to effectively inhibit the primary and metastatic tumor formation in the liver. Our results support the idea of decorin utilization as an anticancer agent in the battle of liver malignancies.

ST23 /P51

Suppression of serglycin decreases glioblastoma cell aggressiveness

D. Manou[1], P. Bouris[1], D. Kletsas[2], T.M. Reine[3], S.O. Kolset[3], A.D. Theocharis[1]

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

[2]Laboratory of Cell Proliferation & Ageing, Institute of Biosciences & Applications, NCSR "Demokritos", Athens, Greece

[3]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

Serglycin is the only representative of intracellular proteoglycans and can be found in granules and vesicles, but also as a secreted macromolecule of the extracellular matrix (ECM), in hematopoietic and in a variety of non-hematopoietic cells. Serglycin can interact with plenty of molecules such as growth factors, cytokines and enzymes. Serglycin regulates the storage, secretion and activation of its binding partners and therefore can control many physiological and pathological processes including cancer. Indeed, the last few years new data have been emerged for the role of serglycin concerning cancer cell behavior and tumor microenvironment. It seems that overexpression of serglycin is in line with the aggressive phenotype of many cancer cells, including breast, nasopharyngeal, lung and multiple myeloma cells. Serglycin was found to influence crucial cell functions in vivo and in vitro, such as cell migration, invasion and tumor development. Because of its binding molecules, serglycin can participate in the re-organization of the ECM. The goal of our study was the investigation of the contribution of serglycin to the aggressiveness of glioblastoma cells, which are characterized by enhanced cell proliferation and intense infiltration to the surrounding non-neoplastic brain tissue. For this reason, using shRNA lentiviral particles, we established stably transfected LN-18 glioblastoma cell line, with suppressed levels of serglycins' expression (LN-18/SRGNsh). We evaluated basic functional properties in these cells as well as the expression of molecules related with them including various ECM molecules. Our results revealed significant alterations at cell morphology and at some cellular properties of the LN-18 transfected cells, such as cell migration, proliferation and invasion. Notably, the suppression of serglycin decreased the ability of LN-18 cells to form tumors in vivo and affected the functional properties of HUVEC, treated with conditioned media from LN-18/SRGNsh cells. Moreover, we observed important variations at the expression levels of ECM molecules, including proteolytic enzymes (MMPs, uPA & PAI-1), but also at the expression and secretion levels of inflammatory and angiogenic molecules, such as ILs, VEGF, CCL-2. These results indicate that serglycin plays a pivotal role in the aggressive phenotype of glioblastoma cells, strengthening the view of the regulatory role of serglycin in several malignancies.

Complement inhibitor CSMD1 acts as a tumor suppressor in breast cancer by interacting with extracellular matrix receptor EGFR

ST24 /P52

Chrysostomi Gialeli[1], Safia Guleed[1], Michael Bartoschek[2], Matthias Mörgelin[3], Petter Storm[4], Kristian Pietras[2] and Anna M. Blom[1]

[1]*Department of Translational Medicine, Lund University, Malmö, Sweden*
[2]*Translational Cancer Research, Medicon Village, Lund, Sweden*
[3]*Colzyx AB, Medicon Village, Lund, Sweden*
[4]*Department of Clinical Sciences Malmö, Division of Diabetes and Endocrinology, Lund University, Malmö, Sweden*

In cancer, the complement system can either promote or inhibit disease progression. Thus, its regulation is of outmost importance. Human CUB and Sushi multiple domains 1 (CSMD1) is a large membrane-bound complement inhibitor of the classical and lectin pathways with a typical mode of action mediated by its complement control protein (CCP) domains. Based on our in vitro characterization of cell functional properties CSMD1 impedes breast cancer progression. Additionally, in vivo CSMD1 attenuates in high extent the formation of metastatic foci. Extracellular matrix (ECM), a dynamic and complex scaffold of macromolecules, initiates crucial biochemical and biomechanical signals that are regulating cancer progression. Hence, considering CSMD1 localization to the cell surface a crucial mediator of its actions may lay in its interplay with ECM macromolecules, as documented for several other complement system members.

Scanning electron microscopy revealed distinct architecture of CSMD1 expressing cells and less ECM deposition around them. In agreement, mRNA array analysis of breast tumors formed in mice injected with MDA-MB-231 CSMD1 and CTRL cells revealed large changes in ECM component expression. Furthermore, using Proteome Oncology/kinase array, we found altered expression of many macromolecules cancer-related, as well as diminished expression of signaling receptors and their phosphorylation in the presence of CSMD1. Epidermal Growth Factor Receptor (EGFR) is a transmembrane receptor with tyrosine kinase activity that plays crucial roles in cancer progression. A direct interaction of CSMD1 and EGFR was identified by co-immunoprecipitation. Furthermore, decreased secretion of cathepsin S, a cysteine protease, was found in MDA-MB-231 CSMD1 cells. Cathepsin S has been implicated in EGFR signaling by attenuating its endosomal signaling. In our experimental setup, upon stimulation with EGF and subsequent receptor dimerization, activation and recycling/degradation, the internalization of EGFR was significantly inhibited in the presence of CSMD1 monitored by biotin labeling of the cell surface proteins.

Our study unravels one possible underlying molecular mechanism of CSMD1 tumor suppressor action and may provide novel avenues for design of better treatment.

ST25 /P53

IGF-IR inhibits breast cancer cells aggressiveness via regulation of syndecan-4 and MMPs expression

N.A. Afratis[1,2*], P. Bouris[1], S.S. Skandalis[1], H.A. Multhaupt[2] , J.R. Couchman[2] , A.D. Theocharis[1] & N.K. Karamanos[1]

[1]*Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras 26110, Greece*
[2]*Department of Biomedical Sciences and Biotech Research and Innovation Center, University of Copenhagen, Denmark*

IGF-IR is highly associated with the behaviour of breast cancer cells and is highly expressed in ERα-positive breast cancer. In clinical practice, prolonged treatment with anti-estrogen agents results in resistance to the therapy with activation of alternative signaling pathways. IGF-IR, membere of Receptor Tyrosine Kinases, have crucial roles in these processes. Here, we report a nodal role of IGF-IR in the regulation of ERα-positive breast cancer cell aggressiveness and the regulation of expression levels of several extracellular matrix molecules. In particular, activation of IGF-IR, but not EGFR, in MCF-7 breast cancer cells results in the reduction of specific matrix metalloproteinases and their inhibitors. In contrast, IGF-IR inhibition leads to the depletion by endocytosis of syndecan-4. Global important changes in cell adhesion receptors, which include integrins and syndecan-4 triggered by IGF-IR inhibition, regulate adhesion and invasion. Cell function assays that were performed in MCF-7 cells as well as their ERα-suppressed counterparts indicate that ER status is a major determinant of IGF-IR regulatory role on cell adhesion and invasion. The strong inhibitory role of IGF-IR on breast cancer cells aggressiveness for which E2-ERα signaling pathway seems to be essential, highlights IGF-IR as a major molecular target for novel therapeutic strategies.

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* Current affiliation: Department of Biological Regulation, Weizmann Institute of Science

ST26 /P54

Chronic inflammation, oxidative-nitrosative stress, mitochondrial dysfunction, improper integrin expression, loss of sarcomeres and proteolysis of myofibrillar proteins allow diagnosis of muscular dystrophy in Drosophila type IV collagen col4a1 mutants

András A. Kiss [1], Szabolcs Zahorán[2], Nikoletta Popovics[1], Márton Kiss[1], Monika Radics[1], Edit Hermesz[2], Zsolt Boldogkői[1], Katalin Csiszár[3] and Mátyás Mink[1]

[1]Institute of Medical Biology, University of Szeged, Somogyi B. u. 4, H-6720 Szeged, Hungary; [2]Department of Biochemistry and Molecular Biology, University of Szeged, Kφzıpfasor 52, H-6726 Szeged, Hungary; [3]John A. Burns School of Medicine, University of Hawaii, 1960 East West Road, Honolulu, HI 96822, USA

Background. We have recently demonstrated chronic inflammation in col4a1 mutants with elevated concentrations of antimicrobial peptides and two oxidants, peroxynitrite and hydrogen peroxide.

Results. We measured the extent of nitrated proteins in muscle fibers and recorded an inverse relationship at elevated peroxynitrite concentration and temperature, characteristic for radical reactions. We observed less protein adduct formation by 4-hydroxy-2-nonenal at higher temperature in wild-type, but significantly more as a sign of robust lipid peroxidation in col4a1 mutants. Posttranslationally modified proteins were detected at both sides of the Z-discs at the level of costamers and co-localized with mitochondria. Morphologic features and uneven distribution of mitochondria strongly suggest their dysfunction. Integrins as anchors play a pivotal role in maintenance of sarcomere functions and mechanotransduction. We noted inappropriate and dislocated integrin expression in col4a1 mutants, apparently in those lines where the mutation affected integrin-binding sites of the COL4A1 protein. These lines are characterized by loss of the contracting units, fiber size disproportion and by actin stress fibers traversing over many sarcomeres. Further property of the integrin-binding site mutants is the proteolytic cleavage myosine heavy chain into an N-terminal p70 and a C-terminal, p150 kDa fragment, respectively. p70 yielded the sequence XKPVANQEXE, matching the N-terminus of muscle MHC in Drosophila devoid of methionine. Myofibrillar actin was cleaved at the SKR/GILTLKYPIEXGIIT site. Tropomyosine escaped electrophoretic detection in the integrin-binding site mutants.

Conclusion. The data presented here are consistent with the onset of muscular dystrophy in col4a1 mutants. Importantly, human patients with COL4A1 mutations develop muscle-eye-brain disease, characteristic for Walker-Warburg syndrome. Thus our system of col4a1 mutants allows screening of a large number of drugs to prevent or mitigate the dystrophic symptoms of type IV collagenopathy.

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ST27 /P55

Tumour-stroma interaction in the case of different hepatomas

K. Rada[1], I. Kovalszky[1]

[1] *Semmelweis University, 1st Department of Pathology and Experimental Cancer Research, Hungary*

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

In the presented research we addressed the question if fibrogenic response of Ito cells for the presence of hepatoma cells is homogenous, or it is influenced by the phenotype of hepatocellular carcinomas? On the other hand, does conditioned medium of Ito cells induce identical response in hepatomas with different phenotype?

Hepatoma cell lines were received from the Pathology Department of Heidelberg University. A fast growing dedifferentiated, (HLE) and a slowly growing, more differentiated (HUH7), cell line was selected for our experiments. Immortalized LX2 Ito cell line was a kind gift of Scott Friedman (USA).

The increased invasiveness of HLE cells were proved by their high proliferation rate, and their faster migration both in would healing assay and in Boyden chamber for Matrigel compared to HuH7 cells.

Both cell lines express vimentin, an intermedier filament described in hepatomas with poor prognosis. They also express syndecan-1, and produce CXCL12 chemokine. In the meantime LX2 cells express CXCR4 chemokine receptors.

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

The coculture of LX2 cells with the more aggressive HLE cells produced more laminin b1, TIMP1, type IV collagen, and more pronounced shedding of syndecan-1, whereas fibronectin, thrombospondin1 and type IV collagen were the characteristic matrix components in the LX2-HuH7 coculture.

As an effect of cocultivation the expression of a6b4 laminin receptor integrin increase in both cell lines whereas fibronectin receptor a5b1, and avb1 fibronectin/vitronectin receptors decrease in the HLE hepatoma.

Our results indicate that the response given by the stromal cells and tumor cells in coculture is not uniform, and is determined by the biological characteristics of tumor cells.

Heparanase regulates macrophage M1 polarization and tubular cells crosstalk after kidney ischemia/reperfusion injury

ST28 /P56

[1]Bellin G., [1]Zaza G., [1]Masola V., [1]Granata S., [2]Secchi M.F., [3]Dall'Olmo L., [4]Vischini G., [1]Lupo A., [4]Gambaro G., [2]Onisto M.

[1]Renal Unit, Department of Medicine, University/Hospital of Verona, Verona, Italy
[2]Department of Biomedical Sciences, University of Padova, Italy
[3]Azienda ULSS3 Serenissima-San Giovanni and Paolo Hospital, Venice, Italy
[4]Division of Nephrology and Dialysis, Columbus-Gemelli Hospital Catholic University, School of Medicine Rome, Italy

Recent studies demonstrate the involvement of Heparanase (HPSE), an endoglycosidase that cleaves heparan sulphate chains, in the biological network triggered by the ischemia/reperfusion (I/R) injury. This critical condition promotes immune-cells recruitment/activation in the transplanted kidney, leading to a delayed graft function. HPSE probably activates macrophages that contribute to major biological effects.

To assess this hypothesis, we tested the different capacity of U937 wild-type (WT) cells to undertake an M1/M2 polarization when stimulated with LPS or IL-4 with or without a specific HPSE inhibitor SST0001. Additionally, we evaluated the ability of renal proximal tubules cells (HK2) exposed to H/R to activate/polarize macrophages in presence of normal or reduced HPSE activity, measuring DAMPs, TLRs and partial-EMT markers gene and protein levels. Besides, to better investigate the role of HPSE in macrophage activation/polarization, in-vivo studies were performed. In this part, mouse (C57BL/6J) were subjected to monolateral I/R injury by vascular clamping and treated with SST0001 for 48h and 7 days. Renal inflammation, macrophages polarization and histological alterations were evaluated.

In-vitro results showed that HPSE sustained M1 macrophagic polarization promoting a hyper-expression of several pro-inflammatory cytokines (IL1-β, IL-6, TNF-α, Caspase-1). We proved that HPSE controlled tubular cell death, DAMPs generation, cytokines expression, TLRs up-regulation after I/R. Results showed a bi-directional crosstalk between tubular cells and macrophages mediated by HPSE: conditioned medium from post-ischemic HK2 cells polarized macrophages to M1 and M1 macrophages activated partial-EMT in HK2 cells. The lack of HPSE prevented both effects. In-vivo results showed that HPSE inhibition by SST0001 reduced inflammation and M1 polarization without affected M2 phenotype in ischemic mice. SST0001 treatment also ameliorated renal function (BUN and Creatinine) and reduced tubular cells apoptosis.

All together our results demonstrated that HPSE orchestrates part of the biological machinery involved in renal tissue damage/repair following I/R injury, through the macrophage activation/polarization and function. In future, the inhibition of HPSE could represent a new pharmacological tool to minimize acute graft injury and/or chronic pro-fibrotic damages in renal transplantation.

ST29 /P57

Unraveling silent pathological states by decoding their unique extracellular matrix signature in intestinal inflammation

Elee Shimshoni[1], Ran Afik[1], Anjana Shenoy[2], Miri Adler[3], Luca Puricelli[4], Alessandro Podestà[4], Tamar Geiger[2], Paolo Milani[4], Uri Alon[3] and Irit Sagi[1]

[1]*Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel*
[2] *Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv-Yafo, Israel*
[3]*Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel*
[4]*CIMAINA and Department of Physics, Universitŭ degli Studi di Milano, Milano, Italy*

The identification of molecular signatures that aid early diagnosis of complex tissue pathologies, such as inflammatory diseases, poses a major scientific and clinical challenge due to their genetic and phenotypic heterogeneity. We have been able to unprecedentedly discover a distinct tissue state occurring before the onset of inflammatory clinical symptoms by integrating quantitative proteomics with advanced microscopy analyses. Through monitoring colonic extracellular matrix (ECM) remodeling in colitis animal models we have unexpectedly revealed that pre-symptomatic tissues display a unique ECM signature in terms of molecular composition, morphology and stiffness. By applying advanced computational analyses we were able to project quantitative proteomics data onto an axis correlating with spatially resolved tissue damage originating from the ECM. These results bridge the gap between tissue structure and composition while outlining the predictive power of the cellular microenvironment in early diagnostics of inflammatory diseases.

Decellularized human colorectal cancer matrices polarize macrophages towards a pro-invasive phenotype

ST30 /P58

M.L. Pinto[1,2,3], E. Rios[1,4,5,6], A.C. Silva[1,2,3,7], S.C. Neves[1,2,8], H.R. Caires[1,2,3], A.T. Pinto[1,2,8], C. Durães[1,4], F.A. Carvalho[9], A.P. Cardoso[1,2,8], N.C. Santos[9], C.C. Barrias[1,2,3], D.S. Nascimento[1,2], P. Pinto-do-Ó[1,2,10], M.A. Barbosa[1,2,3], F. Carneiro[1,4,5,6] and M.J. Oliveira[1,2,5,]

[1]*i3S-Instituto de Investiganyo e Inovanyo em Saïde, Universidade do Porto (UP)*
[2]*INEB-Institute of Biomedical Engineering, UP*
[3]*ICBAS-Institute of Biomedical Sciences Abel Salazar, UP*
[4]*IPATIMUP-Institute of Molecular Pathology and Immunology of the UP*
[5]*Department of Pathology and Oncology, Faculty of Medicine, UP*
[6]*Department of Pathology, Centro Hospitalar Syo Joyo, Porto*
[7]*Gladstone Institutes, University of California San Francisco, United States of America*
[8]*FEUP-Faculty ofEngineering, UP*
[9]*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.*
[10]*Faculty of Medicine, UP, Porto, Portugal*

Macrophages are highly plastic cells and very sensitive to environmental factors, including the extracellular matrix (ECM). In a rather simplistic vision, macrophages were initially considered as being either pro-inflammatory (M1-like) or anti-inflammatory (M2-like), but nowadays a continuum of polarization status between these two extreme populations is the model most accepted. These cells are extremely abundant in solid tumors and tumor-associated macrophages frequently share features common to M2 macrophages, being key players in cancer progression, invasion and metastasis.

In the present work, we evaluated the impact of human colorectal tumor matrices on macrophage polarization and on macrophage-mediated cancer cell invasion. Accordingly, we developed an innovative 3D-organotypic model, based on the decellularization of normal and tumor tissues derived from colorectal cancer patients' surgical resections. Extensive characterization of these scaffolds revealed that DNA and other cell constituents were efficiently removed while native tissue characteristics, namely major ECM components, architecture and mechanical properties, were preserved. Notably, normal and tumor decellularized matrices distinctly promoted macrophage polarization, with macrophages in tumor matrices differentiating towards an anti-inflammatory M2-like phenotype (higher IL-10, TGF-β and CCL18 and lower CCR7 and TNF expression). Matrigel invasion assays revealed that tumor ECM-educated macrophages efficiently stimulated cancer cell invasion through a mechanism involving CCL18. Finally, the high expression of this chemokine at the invasive front of human colorectal tumors correlated with advanced tumor staging. Our approach evidences that normal and tumor decellularized matrices constitute excellent scaffolds when trying to recreate complex microenvironments to understand basic mechanisms of disease or therapeutic resistance.

ST31 /P59

Extracellular matrix Nephronectin in breast cancer metastasis

Jimita Toraskar[1], Gunbjørg Svineng[2], Tonje Steigedal[1]

[1]*Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, Norway*
[2]*Department of Medical Biology, Faculty of Health Sciences, UiT - The Arctic University of Norway, Tromsø, Norway*

The extracellular matrix (ECM) plays a crucial role in tumor progression and metastasis and both tumor cells along with stromal cells modify and dictate the components of the ECM in a manner favoring further growth and spread of the tumor cells. In a genomic analysis of a spontaneous breast cancer model, several ECM proteins such as Nephronectin (Npnt) have been identified to be linked to metastasis. Npnt, was initially recognized to be involved in embryonic development of kidneys and other endocrine organs via interaction with the integrin α8β1 receptor via a classical integrin-binding RGD-domain. In the present study we have shown that Npnt expression can be correlated with poor prognosis in a subset of human breast cancer patients. Using a low metastatic breast cancer cell line known as 66cl4 displaying low endogenous levels of Npnt we have made 66cl4 variants-stably overexpressing Npnt protein and mutated variants that disturbs the ability of Npnt protein to interact with integrins.

In an experimental metastasis assay we injected 66cl4 cells with forced Npnt expression via the tail-vein in Balb/c mice to monitor the cells's ability to seed in the lungs. We found that Npnt overexpression resulted in an increased ability to form metastatic lesions in the lungs and that this was dependent on the integrin binding and enhancer elements. Immunohistochemical analyses of the harvested lung tissues revealed phenotypically distinct patterns of Npnt staining. We observed a prominent granular Npnt-staining pattern and we affirm vesicular secretion of Npnt by characterizing microvesicles and exosomes from 66cl4 variants. We also speculate that vesicular secretion of Npnt and composition of the exosomes plays a vital role in survival of tumor cells in lungs. ECM proteins are known to interact with various transmembrane receptors like integrins and activate multiple signalling pathways. We have profiled the signalling pathways induced by extracellular recombinant Nephronectin on 66cl4 cells using Reverse Phase Protein Array. Several upregulated phosphoproteins indicate role of Npnt in cell survival and angiogenesis in breast cancer.

Flash Presentations/ Abstracts

FP1/ P35

Alterations in basement membrane proteoglycans defines vascular remodelling in pulmonary hypertension associated with lung diseases

Katharina Jandl[1,*], Julia Hoffmann[1*], Leigh M. Marsh[1], Helene Thekkekara Puthenparampil[1], Wilhelm Bloch[2], Bahil Ghanim[1,3], Walter Klepetko[3], Gabor Kovacs[1,4], Horst Olschewski[4], Andrea Olschewski[1,5], Grazyna Kwapiszewska[1,5]

[1]Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria, [2]Department of Molecular and Cellular Sports Medicine, German Sports University Cologne, [3]Division of Thoracic Surgery, Department of Surgery, Medical University of Vienna, Austria, [4]Division of Pulmonology, Department of Internal Medicine, Medical University of Graz, Austria, [5]Institute of Physiology, Medical University of Graz, Austria, [*]contributed equally

Objectives: Pulmonary hypertension (PH) is a frequent condition in pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and interstitial pulmonary fibrosis (IPF). Common pathological features include vascular remodelling of pulmonary arteries (PAs) resulting in increased pulmonary arterial pressure. Vascular remodelling is accompanied by extracellular matrix deposition within the PAs. Recently, our group identified that gene clusters belonging to the extracellular space, specifically the basement membrane, discriminate the two diseases. This study aims to identify the differentially regulated components of the basement membrane in COPD-PH and IPF-PH and to clarify their role in the process of vascular remodelling.

Material and Methods: Immunohistochemical staining was performed to detail the structural localization and expression of basement-membrane proteins. Gene expression was evaluated on laser-capture microdissected small pulmonary arteries and isolated large PAs. Capillary and PAs basement-membrane ultrastructure was visualized via electron microscopy. The levels of proteoglycans were assessed by real-time PCR, a dimethylmethylene blue assay as well as by alcian blue staining.

Results: Immunohistochemical staining of basement membrane components revealed distinct morphological alterations in PAs from COPD-PH and IPF-PH. Those alterations include increased abundance of collagen type IV in both diseases, with more difusse appearance than in control PAs. Gene expression analysis of small and large PAs revealed a distinct regulation of genes belonging to basement membrane collagens including increased expression of COL4A5 and COL14A1 and decreased expression of COL18A1 as well as proteoglycans such as versican and biglycan. Interestingly, structural changes in the basement membrane were compartment and disease specific. On ultrastructural level, the basement membrane of COPD-PH resembled that of healthy donors, whereas IPF-PH displayed thickened, thinned and split areas. In agreement with the striking differences in expression levels of genes belonging to basement membrane proteoglycans, total proteoglycan content in PAs from both COPD and IPF patients with PH was increased. Although the total proteoglycans levels were elevated at a similar extend in both diseases, their tissue distribution differed.

Conclusions: Alterations in basement membrane composition, structure and function observed in COPD and IPF associated with PH can discriminate between the two diseases. Restoring the ECM and the basement membrane can present an attractive novel therapeutic approach in the treatment of PH. However, disease specific alterations in the vascular basement membrane have to be taken into account when designing cohort-specific therapies.

Lack of the extracellular matrix proteoglycan lumican in mice exacerbates left ventricular dilatation and contractile dysfunction upon pressure overload

FP2/P36

Mohammadzadeh N.[1,2], Lunde IG.[1,2,3], Andenæs K.[1,2], Strand M.E.[1,2], Aronsen J.M.[1,4], Skrbic B.[1,2,5], Marstein H.S.[1,2], Bandlien C.[1,2,5], Nygård S. [1,2], Sjaastad I.[1,2], Chakravarti S.[6], Christensen G.[1,2], Engebretsen K.V.[1,2,5], Tønnessen T.[1,2,5]

[1]Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo, Norway
[2] Center for Heart Failure Research, University of Oslo, Norway
[3]Department of Genetics, Harvard Medical School, Boston, MA, USA
[4]Bjørknes College, Oslo, Norway
[5]Department of Cardiothoracic Surgery, Oslo University Hospital Ullevɛl, Norway
[6]Department of Medicine, Johns Hopkins University, Baltimore, USA

Heart failure (HF) in response to pressure overload is characterized by cardiac remodeling. Lumican (LUM) is an extracellular matrix (ECM)-localized small leucine-rich proteoglycan (SLRP) with increased expression in hearts of HF patients and mice subjected to pressure overload by aortic banding (AB). To test the hypothesis that lumican is important for cardiac remodeling, lumican knock-out (LUM-/-) mice were subjected to AB and neonatal rat heart primary cultures exposed to LUM.

LUM exists as a non-glycosylated 38 kDa core protein and a 50-75 kDa proteoglycan in the mouse left ventricle (LV, n=3). When overexpressed in HEK293 cells, WT LUM was secreted into the culture medium, while the non-glycosylated N88A/N127A/N160A/N252A mutant was retained intracellularly (n=8). LUM+/- intercrosses (n=47) showed that out of 236 pups, 8 were LUM-/- (3% vs. expected 25%, $p<0.0001$), suggesting that LUM-/- predominantly is embryonic lethal. Echocardiography of adult LUM-/- mice showed no cardiac phenotype (n=9-26). LUM-/- exhibited exacerbated LV dilatation 1-10 weeks post-AB (n=3-14), with exacerbated contractile dysfunction 4-10 weeks post-AB. LV wall thickness was reduced 2 and 6-10 weeks post-AB. Importantly, Sirius Red staining of mid-ventricular sections (n=3-4) showed reduced fibrosis in LUM-/- 12 weeks post-AB, with reduced LV expression of collagen (COL1A2) and a marker of myofibroblast differentiation (ACTA2). To identify LUM-dependent molecular mechanisms, transcriptional profiling was performed on LUM-/- hearts. RNA sequencing (n=3) showed differential expression (DE) of 714 transcripts 2 weeks post-AB (>1.33-fold up/<0.75-fold down, $p<0.001$). We identified 4 KEGG pathways and 69 enriched gene ontology categories (FDR<0.05), with extracellular space, inflammatory response and defense response as top three. Ingenuity Pathway Analysis (IPA) suggested miR-21 among top inactivated upstream regulators, while p38-MAPK, IL-1β, NFκB and IL6 were among the top activated. Interestingly, SPON2, a molecule with pro-fibrotic, pro-hypertrophic and pro-inflammatory effects was among the most downregulated genes in LUM-/- post-AB. Cultured cardiac fibroblasts (n=3 isolations, n=19-21) responded to LUM treatment (24h) with upregulation of molecules that are important in ECM remodeling, i.e. proliferation (PCNA), myofibroblast differentiation (ACTA2, SM22), fibrosis (COL1A2, COL3A1, POSTN, miR-21a-5p, SPON2), collagen cross-linking (LOX) and ECM degradation (MMP2).

In conclusion, our results from experiments in LUM-/- mice suggest that lumican plays an important role during development and cardiac remodeling in response to pressure overload, with exacerbated dilatation and contractile dysfunction as the most important phenotype. Our results indicate that lumican is a pro-fibrotic mediator, regulating cardiac fibroblast phenotype and ECM production, thereby affecting heart failure progression.

FP3/P37

Hyaluronan modulates tumor progression, angiogenesis and proliferation during Doxorubicin treatment

D. Vitale[1], F. Spinelli[1], I. Caon[3], G. Demarchi[2], A. Passi[3], C. Cristina[2], L. Alaniz[1,*]

[1]*Laboratorio de Microambiente Tumoral - Centro de Investigaciones y Transferencia del Noroeste de la Pcia. de Bs. As. (CIT NOBA, UNNOBA-CONICET), Junvn, Bs. As., Argentina*

[2]*Laboratorio de Fisiopatologva de la Hipofisis. - Centro de Investigaciones y Transferencia del Noroeste de la Pcia. de Bs. As. (CIT NOBA, UNNOBA-CONICET), Junvn, Bs. As., Argentina*

[3]*Dipartimento di scienze chirurgiche e morfologiche, Universita dell'Insubria, Varese, Italia*

[*]*laualaniz@yahoo.com.ar*

The microenvironment generated around the tumor can modulate chemotherapy response. Extracellular matrix (ECM) deregulation could be crucial for tumor progression and metastasis. Hyaluronan (HA), a ECM glycosaminoglycan, interact with its principal receptor (CD44) and induces signals that can promote cell proliferation, angiogenesis, and could be related with apoptosis and multidrug resistance. The objective of our study is to analyze the mechanisms triggered by HA through the interaction with CD44: modulation of signaling pathways associated with angiogenesis and drug resistance.

EL4 lymphoma T murine cell line was treated with low molecular weight HA (LMW HA 20-100 µg/ml) and Doxorubicin (DOX 0,5-1-2,5 µM). CD44 expression and HA binding capability were measured by flow cytometry. HA effect during DOX treatment was studied analyzing cell survival, angiogenesis and intracellular signaling. Apoptosis (by AnnexinV) and DOX accumulation assays were performed by flow citometry. Angiogenesis modulation was studied evaluating VEGF expression by ELISA assay and endothelial cells (EC) migration through wound healing assay. To analyze Wnt/β-Catenin pathway, Wnt ligand (β-Catenin) total expression was determined by Western Blot. Three independent experiments were performed for each test, analyzed by one-way or two-way ANOVA considering a significant $p\ value<0.05$.

EL4 cells showed high expression of CD44 and binds HA. When cells were treated with 0,5 and 1µM DOX, HA decreased drug activity (lower DOX accumulation and lower rates of apoptosis). β-Catenin expression showed an increment pattern with DOX plus HA treatment respect to basal conditions (without treatment); suggesting that this pathway could affect the antitumor action of this drug. Besides, these results can be related with DOX intrinsic resistance in EL4 cell line.

When angiogenesis was evaluated, we observed that DOX had a pro-angiogenic action in tumor cells, since EC migration was higher when it were cultured with supernatants of EL4 cells. In combination with HA, we found an increment in EC migration capability when they were stimulated with the supernatants of tumor cells treated with DOX plus HA. Finally, VEGF concentration was determined to evaluate potential factors involved in this mechanism, although no differences were found between treated and not treated cells.

In resume, we found that HA affect DOX treatment in lymphoma T cells, and the mechanisms implicated could be related with modulation of drug efflux, activation of proliferation pathways and angiogenesis promotion. The angiogenic effect is not directly related to an increase in VEGF biosynthesis levels, but could be intervening other mechanism that will be our target for future studies.

TGF-β1 suppresses the IL-1β-induced production of matrix metalloproteinase-1 from human pterygium fibroblasts through PKA activation

FP4/P38

M.E. Christopoulou[1], I. Trilivas[2], E. Giannopoulou[3], N. Pharmakakis[2], A. J. Aletras[1]

[1]*Laboratory of Biochemistry, Department of Chemistry, University of Patras*
[2]*Department of Opthalmology, Medical School, University of Patras*
[3]*Department of Pharmacology, Medical School, University of Patras*

Background: Pterygium is a condition characterized by epithelial overgrowth of the cornea, inflammatory cell infiltration and an abnormal extracellular matrix accumulation. Chronic UV exposure is considered as a pathogenetic factor of this disease. Many cytokines and growth factors, such as IL-1β, IL-6, IL-8, TGF-β and PDGF as well as matrix metalloproteinases (MMPs) and their inhibitors, whose the production from pterygial cells is induced by UV radiation, may be implicated in pterygium formation and growth. IL-1β is considered as one of the main pro-inflammatory cytokines, while TGF-β1 as an anti-inflammatory cytokine, which in many cases is implicated in fibrosis. IL-1β is among the main stimulators of many MMPs production by a variety of cells, while TGF-β1 induces the expression of TIMP-1, and in some cases it antagonizes the stimulatory effect of IL-1β on the expression of MMP-1 and MMP-3.

The aim of this study was to investigate the contribution of TGF-β1 in the abnormal extracellular matrix accumulation, occurred in pterygium, via its ability to suppress the IL-1β-induced production of MMPs from pterygium fibroblasts.

Materials and Methods: Pterygium specimens were obtained from patients after the surgical removal of primary tissue and the fibroblasts were isolated from these tissues by mild treatment with clostridium collagenase. The expression of MMPs in cells was ascertained by qPCR and their detection in cell cultures conditioned media by ELISA and western blot analysis.

Results: When pterygium fibroblasts were cultured with IL-1β, a significant stimulation of MMP-1 expression was observed, which significantly inhibited by MAPK inhibitors U0126, SP600125 and SB203580, manly by JNK inhibitor SP600125, but not by PI3-K inhibitor LY294002. The IL-1β-induced expression of MMP-1 was enhanced in the presence of PKA inhibitor H-89, while it strongly suppressed in the presence of TGF-β1 or forskolin, an adenylate cyclase activator. The PKA inhibitor H-89 almost totally reversed the suppressive effect of TGF-β1 and forskolin. The suppressive effect of TGF-β1 was also reversed in the presence of Na3VO4, a non specific phosphatases inhibitor, while it was not affected in the presence of the adenylate cyclase inhibitor di-deoxy-adenosine. TGF-β1 as well as forskolin had no any effect on MKP-1 expression, a MAPK phosphatase. TGF-β1 and forskolin exhibited also the same effect on the IL-1β-induced expression of MMP-3.

Conclusions: TGF-β1, via a cAMP-independed mechanism, activates the PKA, which in turn activates a phosphatase that then mediates in the suppression of IL-1β-induced production of MMP-1 by pterygium fibroblasts through an unknown mechanism. This effect may partially contribute in the abnormal extracellular matrix accumulation observed in this disease.

FP5/ P39

Understanding the tendon circadian clock as therapeutic target for treatment of age-related chronic tendinopathies

C.-Y. C. Yeung[1], A. Adamson[2], P. Schjerling[1], J. Fahrenkrug[3], M. Kjær[1]

[1]*Institute of Sports Medicine Copenhagen, Bispebjerg Hospital, Faculty of Health Sciences, University of Copenhagen, Copenhagen NV 2400, Denmark*
[2]*Faculty of Biology, Medicine and Health, University of Manchester, Manchester M13 9PT, United Kingdom*
[3]*Department of Clinical Biochemistry, Bispebjerg Hospital, Faculty of Health Sciences, University of Copenhagen, Copenhagen NV 2400, Denmark*

Chronic tendinopathies and associated chronic musculoskeletal disorders including osteoarthritis affect 1-in-4 people over the age of 40 and can be attributed to insufficient homeostasis of the collagen-rich matrix in tendon. Musculoskeletal tissues (tendon, cartilage, muscle) are matrix-rich peripheral clocks with endogenous 24-hour rhythms that are responsible for driving time-dependent expression of tissue-specific genes that regulate matrix homeostasis. However, peripheral clocks dampen and their outputs are diminished with age, which could explain why chronic musculoskeletal disorders are prevalent in the older population. Therefore our aim is to understand how the circadian rhythm is generated in tendon and why it dampens such that we could 'reset' the clock for the prevention and management of chronic tendinopathies.

We hypothesise that tenocyte communication via gap junctions is critical for maintaining a robust circadian rhythm. We established the presence of gap junction proteins connexin 43 in primary mouse tenocyte cultures by immunofluorescence and FRAP (fluorescence recovery after photobleaching). Gap junctions were disrupted using chemical inhibitor 18alpha-glycyrrhetininc acid and CRISPR/Cas9-mediated deletion of the *Gja1* gene. The effects on the circadian rhythm was assessed by real-time bioluminescence recordings on tenocytes isolated from circadian reporter (*Per2::Luciferase*) mice, and the impact on the time-dependent expression of tendon clock-regulated matrix homeostasis genes, including *Grem2, Mia3, Mmp14*, was assessed by quantitative PCR. Our ongoing work is to elucidate whether gap junction conductivity or gap junction-cytoskeleton signalling is the mechanism via which tenocyte circadian rhythms are synchronised. Further, we will examine if and how age-related tissue stiffening and/or inactivity contribute to the dampening of the tendon circadian clock.

Protective effects of epigallocatechin- 3 -gallate on fibrosis in scleroderma model

FP6/ P44

A. Kocak[1], D. Harmancı[1], M. Birlik[2], S. Sarioglu[3], O. Yilmaz[4], G. Guner Akdogan[5]

[1]*Molecular Medicine Department, Institute of Health Sciences, Dokuz Eylul University*

[2]*Department of Internal Medicine, Division of Rheumatology & Immunology, School of Medicine, Dokuz Eylul University*

[3]*Department of Medical Pathology, School of Medicine, Dokuz Eylul University*

[4]*Department of Laboratory Animal Sciences, Institute of Health Sciences, Dokuz Eylul University*

[5]*Department of Basic Medical Sciences, School of Medicine, Izmir University of Economics, Turkey*

Background: Systemic sclerosis (SSc) or scleroderma is an autoimmune disease that shows involvement in internal organs or in the skin characterized by fibrosis. Dermis thickening and uncontrolled extracellular matrix (ECM) accumulation are seen in this disease, whose pathogenesis is not fully understood (1, 2). TGF-β/ Smad 2/ Smad 3 pathway plays a pivotal role in SSc pathogenesis via induction of profibrotic molecules including collagen (3-5) and decreasing of matrix metalloproteinases (MMPs) synthesis (6, 7). The occurrence of the myofibroblast phenotype at wound healing and fibrosis is thought to be responsible for the contracted regions of the affected tissues (8).

Objective: The aim of this study to investigate the potential effects of epigallocatechin-3-gallate (EGCG) against fibrosis in an experimental scleroderma model induced with bleomycin (BLM) .

Methods: 32 Balb / c female mice were randomly selected into four groups. For 21 days:(1) Control group (n :8) was given 100 uL *subcutaneous (sc)* saline (SF) once a day and 100 uL *intraperitoneal (ip)* SF twice a week, (2) BLM group (n:8) was given 100 uL (100 ug) sc BLM once a day and 100 uL *ip* SF twice a week,(3) BLM + EGCG group (n:8) was given 100 uL (100 ug) *sc* BLM once a day and 100 uL (1.6mg/kg) ip EGCG twice a week, (4) EGCG group (n:8) was given 100 uL sc SF once a day and 100 uL (1.6mg/kg) *ip* ECCG twice a week.

Effects of EGCG on fibrosis were investigated through a physical examination prior to sacrificing of animals. Histochemical (hematoxylin & eosin) and Masson trichrome staining of dermal areas were performed. Myofibroblast activity was measured using alpha smooth muscle actin antibody (αSMA). Expression levels of MMP-1, MMP-8 , MMP-13 and p-SMAD protein were examined by Western blot. Expression levels of TGF-β1 (TGF-β) mRNA were examined by reverse qPCR. All of the statistical analyses were performed using SPSS 13.0 software. The quantitative data were expressed as the means ± SEM. The quantitative variables were compared using the two-sample Student's t-test or a one-way ANOVA. Statistical significance was defined as $p<0.05$.

Results: When compared to sham, control and experimental groups (EGCG-treated group) were observed to have reduced connective tissue fibrosis in dermis area (p :0.000), according to Masson Trichrome results. EGCG group showed a significant reduction in fibrosis at the dermal surface area (p :0.022) with respect to hematoxylin measurements. MMP-1, MMP-8, p-SMAD 2/3 protein levels and TGF- β mRNA expression were slightly decreased in EGCG Group compared with the other tested groups ($p<0.05$). Otherwise, MMP-13 was not changed between groups.

FP7/
P45

Structural studies of a tumour-associated carbohydrate epitope defined by the monoclonal antibody A10

Ioanna Kalograiaki[1], Ana I. Manzano[1,2], José I. Tudela[2], Marcos Viñuela[3], F. Javier Cañada[1], José L. Subiza[2]

[1]*Group of Nuclear Magnetic Resonance & Molecular Recognition, Department of Chemical & Physical Biology, Biological Research Center (CIB), Spanish National Research Council (CSIC), Madrid, Spain*

[2]*Department of Research & Development, Inmunotek SL, Alcala de Henares, Spain*

[3]*Department of Immunology, Hospital Clvnico San Carlos, Madrid, Spain*

Tumour cells display an aberrant glycosylation profile at both structural and expression levels, compared to their non-transformed counterparts [1]. The continuous cross-talk between these carbohydrate neoantigens and the immune system may result in cancer suppression or promotion. This concept of glycosylation-dependent prognosis evolved with the support of manifold clinical and histopathological studies. Of relevance, many monoclonal antibodies (mAbs) selective for adenocarcinomas were reported to be directed against mucus glycoproteins, particularly targeting their oligosaccharide chains. Among them, A10, an IgM mAb raised against murine Ehrlich tumour (ET) cells, stands out for efficiently inhibiting their growth both in vitro and in vivo [2]. When tested for reactivity against human tissues, A10 reacted strongly with a high proportion of adenocarcinomas as well as their mucin-enriched fractions, but not with non-mucosal malignancies or healthy tissues [3]. The epitope defined by A10, namely Ca10, is isolated by tangential flow filtration from ET culture supernatants. It was found to be sensitive to oxidation by sodium periodate, underscoring its carbohydrate nature, then resistant to protease and O-glycosidase treatments. Prominent presence of oligo-GlcNAc was evident in nuclear magnetic resonance (NMR) heteronuclear (1H-13C HSQC) and total correlation (TOCSY) spectra. Diffusion-ordered spectroscopy and dynamic light scattering measurements revealed the presence of carbohydrate fragments in the filtered extract along with its major component of nominal size › 300 kDa. Characterisation of the carbohydrate-fraction of Ca10 by NMR upon enzymatic lysis, size-exclusion chromatography and matrix-assisted laser desorption/ionization is currently under study.

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Investigating the dynamics of tumor – stroma interactions in lung cancer

FP8/P46

Magdalena Szczygiel[1], Martin Böhm[1], Bastian Kister[1], Michael Meister[2], Ursula Klingmüller[1]

[1]*Division Systems Biology of Signal Transduction, German Cancer Research Center (DKFZ), Heidelberg, Germany*

[2]*Translational Research Unit, Thoraxklinik at University Hospital Heidelberg, Germany*

Lung cancer is the leading cause of cancer-related deaths worldwide. Its fatal outcome is frequently related to late detection, early metastatic spread and frequent resistance to applied therapies. Recent reports indicate that metastatic spread and therapy resistance can be critically influenced by the tumor microenvironment. Cancer-associated fibroblasts modulate the extracellular matrix (ECM) and secrete a multitude of proteins including chemokines and growth factors. One of them is transforming growth factor beta (TGFβ), which regulates the epithelial-to-mesenchymal transition (EMT), a process that promotes tumor survival and spread.

An important open question is to identify differences in the communication between cancer cells and cancer-associated fibroblasts in different matrix niches.

We combine fibroblasts-derived matrix generation and tumor cell-fibroblast co-culture to generate time-resolved proteomic data of tumor–stroma–ECM interaction. We show that human fibroblast cell lines can be induced to become myofibroblasts and efficiently deposit extracellular matrix *in vitro*. ECM produced by TGFβ-treated fibroblasts shows higher expression of components that are also differentially present in tumor-derived ECM of lung cancer patients. The analysis of the co-culture secretomes of the lung adenocarcinoma cell line H1975 with CCL-171 fibroblasts on different ECM surfaces by multiplexed bead-based assays show differential up-regulation of several proteins related to tumor progression.

These results suggest the existence of multiple feedback loops between cancer cells and fibroblasts. Such bidirectional interactions may further increase fibroblast differentiation and induce EMT in tumor cells that would facilitate tumor spread and therapy resistance.

FP9/
P47

P-cadherin promotes reorientation of collagen fibers during collective cell migration

Maïlys Le Borgne-Rochet[1], Franck Comunale[1] and Cécile Gauthier-Rouvière[1]

[1]*Centre de Recherche de Biologie cellulaire de Montpellier, Centre National de la Recherche Scientifique, Unitı Mixte de Recherche 5237, Université Montpellier, 34293 Montpellier, France*

Collective cell migration (CCM) is essential for organism development, wound healing, and metastatic invasion, the main cause of cancer-related death, and it involves cell–cell adhesion molecules of the cadherin family. We have previously shown that increased P-cadherin expression levels are correlated with tumor aggressiveness in carcinoma and aggressive sarcoma (Thuault et al., 2012) and determined that P-cadherin specifically induces polarization and CCM through an increase in the strength and anisotropy of mechanical forces (Plutoni et al., 2016). We show that this mechanical regulation is mediated by the P-cadherin/ β-PIX/Cdc42 axis; P-cadherin specifically activates Cdc42 through β-PIX, which is specifically recruited at cell–cell contacts upon CCM. This mechanism of cell polarization and migration is absent in cells expressing E- or R-cadherin. Thus, we identified a specific role of P-cadherin through β-PIX–mediated Cdc42 activation in the regulation of cell polarity and force anisotropy that drives CCM. However, how P-cadherin induces this mechanical regulation is still unknown. Here, we showed by transcriptomic analysis that P-cadherin has a specific collagen signature and upregulates numerous genes involved in collagen reorientation such as proteoglycans. Using 2D and 3D migration systems, we demonstrated that P-cadherin induces type I collagen fibers reorientation in the direction of migration. These results are specific to P-cadherin and are not observed with cells expressing E- and R-cadherin. We are currently investigating how P-cadherin expression allows collagen fibers reorientation and the role of proteoglycans identified by transcriptomic analysis.

Cartilage oligomeric matrix protein associates with a vulnerable plaque phenotype in human atherosclerosis

FP10 /P48

E. Bengtsson[1], K. Hultman[1,4], P. Dunér[1], J. Nilsson[1], A. Hultgårdh-Nilsson[2], I. Gonçalves[1,3]

[1]*Experimental Cardiovascular Research Unit, Department of Clinical Sciences, Malmö, Lund University, Sweden*

[2]*Department of Experimental Medical Science, Lund University, Lund, Sweden*

[3]*Department of Cardiology, Skɛne University Hospital, Sweden*

[4]*Present address: Novo Nordisk A/S, Bagsvaerd, Denmark*

Objective

Cartilage oligomeric matrix protein (COMP) is present in non-atherosclerotic and restenotic human arteries. COMP maintains the contractile smooth muscle cell phenotype *in vitro* and overexpression of COMP in rats retards smooth muscle cell dedifferentiation in response to vascular injury. Recently, it was shown that COMP deficiency in bone-marrow derived cells results in increased atherosclerotic calcification in apoE-/- mice. In this study we investigated the association of COMP with plaque phenotype in human atherosclerosis.

Methods and Results

COMP levels were analyzed by immunohistochemistry in 212 carotid plaques, removed by endarterectomy. 111 plaques were from patients with cerebrovascular symptoms and 106 were from asymptomatic patients. COMP was significantly increased in atherosclerotic lesions from symptomatic patients compared to lesions from asymptomatic patients (9.7% of plaque area (IQR 4.7-14.3) vs 5.6% of plaque area (IQR 2.8-9.8); p=0.0001). COMP was positively associated with lipids (r=0.32, p<0.001), and displayed a trend to an association to macrophages determined by CD68 staining (r=0.13, p=0.059), but was negatively associated to collagen (r=-0.16, p<0.05), elastin (r=-0.14, p<0.05) and smooth muscle cells (r=-0.25, p<0.001), which together indicate an association to a vulnerable plaque phenotype. Furthermore, COMP was negatively associated with MMP-2 (r=-0.26, p<0.001), and MMP-3 (-0.28, p<0.0001), which previously have been shown to facilitate migration of smooth muscle cells. COMP did not associate with calcification (r=-0.08, p=0.28) of the lesions measured by van Kossa staining, but was positively associated with markers of apoptosis: caspase-3 (r=0.24, p<0.001), caspase-8 (r=0.19, p<0.01), and TRAIL (r=0.14, p<0.05).

Conclusion

These data show that COMP are associated with a vulnerable plaque phenotype in human atherosclerotic lesions.

FP11 /P49

Glucosamine and its peptidyl-derivative NAPA: novel therapeutic strategy for chondrocytes matrix remodeling

R. Cocchiola[1], M. Lopreiato[1], L. Politi[1], R. Scandurra[1], A. Scotto d'Abusco[1]

[1]*Dept. Biochemical Sciences, Sapienza University of Rome, Italy*

Cartilage degradation, due to an imbalance between anabolic and catabolic rate of chondrocyte metabolism, is the main feature of Osteoarthritis (OA). To date, OA is mainly treated with Non Steroidal Anti-Inflammatory Drugs (NSAIDs), in order to reduce arthritis-related symptoms. In the last decades an increasing number of patients have started to use supplements, such as Glucosamine (GlcN) and chondroitin sulfate, as potential chondroprotective agents. Several *in vivo* clinical trials as well as *in vitro* experiments have been performed reporting inconsistent outcomes. Previously, in our lab we analyzed the anabolic effects of GlcN and its N-acetyl-phenylalanine derivative (NAPA) in a rabbit OA model, finding that intra-articular administration of GlcN and NAPA was very effective in reducing cartilage changes in injured rabbit knee. GlcN and NAPA intra-articular administration allows higher concentrations to be reached in the joints compared to oral administration, thus providing an explanation for the ability of both molecules to interfere with OA progression. We also studied the effects of GlcN and NAPA on inflammatory pathways, finding that both molecules can interfere with MAP kinase and NF-kB pathways, by interfering with IKKa activity. Finally, we studied the effectiveness of GlcN and NAPA on the biosynthetic activity and hence the matrix production of human primary chondrocytes cultured in micromasses, which represent a good tridimensional culture model. We explored the ability of GlcN and NAPA to stimulate the synthesis of collagen type II (Col2), Aggrecan (ACAN) and Small Leucine-Rich Proteoglycans (SLRPs). After 6 weeks, micromasses stimulated with GlcN + NAPA still showed a large amount of ECM compared to untreated cells. Moreover, Collagen type II was more abundant and better organized compared to that produced by untreated cells. Finally, cells resulted viable in both treated and untreated micromasses, even if in the middle of untreated micromasses, few dead cells were observed, whereas in the treated micromasses only viable cells and cells completely surrounded by ECM were detected.

Vascular inflammation and extracellular matrix modifications

FP12 /P60

E. Caravà, M. Viola, D. Vigetti, E. Karousou, P. Moretto, G. De Luca, A. Passi

Department of Medicine and Surgery, University of Insubria, Varese, Italy

Atherosclerosis is an inflammatory disease of the wall of large- and medium-sized arteries. It's an extremely complex series of events that causes the modification of tissue architecture and of extracellular matrix (ECM). The accumulation of oxidized-LDL (oxLDL) in the tunica intima and the endothelium damage are early stages of vessel inflammation[1]. Our research is focused on the study of the effect of different type of inflammation stimuli, such as oxLDL, TNFα and or 22-oxysterol, on ECM metabolism of endothelial cell (HUVEC). In particular, we aimed in understand the changes of the ECM components like hyaluronan (HA), glycosaminoglycans (GAGs) and HE/HS proteoglycans family.

We treated HUVEC with TNFα for 24 or 48 hours and we evaluated the expression of the syndecans protein core, the expression of HS/HE polymerization enzymes (EXT1, EXT2, NDST1) and the characteristics of HS/HE disaccharides. HA metabolism was investigated by the expression of the synthetic enzymes HAS2 and HAS3 and the epigenetic mediator HAS2-AS1. Syndecan4 seems the isoform implicated in the early response because its expression increases after 24h, followed by upregulation of Syndecan1 at 48h. EXT1, EXT2, NDST1 increase their expression at 24 hours and were back to control levels at 48h. Accordingly, HS/HE disaccharide composition shows a higher amount of N-sulfated modification. HAS2, HAS3 and HAS2-AS1 increase their expression at 24h. Summarizing, endothelial glycocalyx is highly modified during inflammatory condition, in both GAGs and proteoglycans.

A reported in several studios, Syndecans4 expression is related to NO production and release from endothelial cells. NO synthases (NOSs) have a central role in modulating vascular tone and can be altered by various forms of endothelial cells injury[2]. In our model, we observed a decrease of endothelial NOS (eNOS) expression in TNFα stimulated HUVEC, in according with changes in permeability and adhesiveness in vivo due to endothelial lesion.

HUVEC treated with oxLDL respond in a different way since HAS2 increases like in TNFα treatment, while HAS2-AS1 decreases and HAS3 remains at control level. This difference ought to be compared with SMC as in the tunica intima, the SMC are subjected to the action of oxLDL. We treated AoSMC with oxLDL or 22-oxysterol to compare their effect on HAS2 and HAS3. HAS2 expression with oxLDL increases and also 22-oxysterol can modulate HASs, even though with different ratio between HAS2 and HAS3. In facts, HAS3 expression in both treatments remains at control level.

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FP13 /P61

Towards the generation of an animal model of chondrodysplasia with joint dislocations gPAPP type

R. Costantini[1], L. Monti[1], C. Paganini[1], Stephan Sonntag[2], A. Forlino[1], A. Rossi[1]

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

[2]*PolyGene AG, Rümlang, Switzerland*

Chondrodysplasia with joint dislocations gPAPP type is a recessive skeletal disorder characterized by short stature, brachydactyly, congenital joint dislocations, micrognathia, cleft palate and facial dysmorphism. The disorder is caused by mutations in the *IMPAD1* gene encoding for gPAPP, a Golgi-resident phosphoadenosine phosphate 3'-phosphatase that hydrolyzes phosphoadenosine phosphate (PAP), the by-product of sulfotransferase reactions, to AMP; thus, this enzyme is involved in the GAGs sulfation pathway. The function of IMPAD1 has been confirmed for the first time by the study of knock-out mice, generated by the gene trap approach, which are lethal at birth preventing to study the role of IMPAD1 in post-natal skeletal development (Frederick JP et al. PNAS, 105, 11605-12; Sohaskey ML et al. Development, 135, 2215-20, 2008). Since patients with chondrodysplasia gPAPP do not have a lethal phenotype, it is crucial to study the role of IMPAD1 in skeletal growth after birth using an in vivo model with a less severe phenotype. In collaboration with PolyGene AG, Rumlang, Switzerland, we have generated the first Impad1 conditional knock-in for a missense mutation (Asp175Asn) reported at the homozygous state in a patient with chondrodysplasia gPAPP. The conditional knock-in was first mated to a global Cre deleter mouse (*EIIaCre*) to get the full knock-in. Unexpectedly, the phenotype of mutant homozygous mice was lethal at birth with severe hypoplasia of the skeleton: the length of the axial skeleton and of the limbs were reduced. By X-rays and differential staining with alcian blue and alizarin red the femur, tibia and fibula were markedly shorter compared to wild-type animals; furthermore, rib cages of mutant mice displayed malformations characterized by reduced sternal length and diminished rib spacing. On the molecular basis, the mouse lethality at birth was investigated by looking at the level of mutant Impad1 mRNA and at possible alternative splicing of the targeted allele due to the strategy used in the set-up of the conditional knock-in targeting vector. Results demonstrated that the lethality is due to the presence of an additional mutated exon that led to an aberrant alternative splicing mechanism. Based on these results a new knock-in mouse line will be generated using a different gene targeting vector.

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TGF-β Receptor I/II Signaling at Primary Cilia Membrane is Regulated by Ceramide to Modulate Cell Migration

FP14 /P62

Salih Gencer[1,2], Besim Ogretmen[2]

[1]*Üsküdar University- Medical University of South Carolina, Molecular Biology and Genetics, Istanbul, Turkey*

[2]*Medical University of South Carolina, Biochemistry and Molecular Biology, Charleston, USA*

Mechanisms that regulate TGF-beta receptor I/II (TβRI/II) trafficking to primary cilia membrane for mediating signal transduction remain unknown. Here, we show that ceramide synthase 4 (CerS4) generated ceramide, bioactive sphingolipid, stabilized Smad7-TβRI association, which then inhibited the trafficking of TβRI/II to primary cilia membrane. Expression of a mutant TβRI, which is resistant to Smad7 binding/inhibition, restored receptor signaling to increase migration in response to CerS4/ceramide induction. Genetic or molecular alterations of CerS4 abundance prevented Smad7-TβRI inhibitory complex, and increased association between Arl6 transporter and TβRI via novel cilia targeting signal (31-ATALQ-35). Mutation of the cilia targeting signal abolished the trafficking of the receptor to the cilia membrane in response to CerS4 knockdown in various cell types. Localization of TβRI/II to primary cilia activated sonic hedgehog (Shh) receptor smoothened (Smo), inducing migration/invasion and liver metastasis both in wild type and CerS4-/- knockout mice in response to endogenous CerS4/ceramide knockdown in 4T1 mammary cancer cells, injected in the mammary pads. Smad7 overexpression or primary cilia inhibition by shRNA-mediated knockdown of intraflagella transport protein 88 (IFT88) prevented TβRI-Smo crosstalk and attenuated liver metastasis of mammary cancer cells stably transfected with shRNA against CerS4/ceramide. Overall, these data define a key mechanism for the regulation of TβRI/II targeting selectively at the primary cilia membrane by CerS4/ceramide-Smad7 inhibitory complex to control Shh-mediated cell migration and invasion without affecting canonical TGF-β signaling.

FP15 /P63

Lumican effect on MMP-14 expression and migration of Snail overexpressing MC38 colon carcinoma cells

Maria Catela[1,3], Izabella Papiewska-Pajak[2], Corinne Perreau[1], M. Anna Kowalska[2,4], Demitrios Vynios[3] and Stéphane Brézillon[1]

[1]CNRS UMR 7369, Matrice Extracellulaire et Dynamique Cellulaire (MEDyC), Universitı de Reims Champagne Ardenne, Laboratoire de Biochimie Mıdicale et Biologie Molıculaire, Reims, France
[2]Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland
[3]Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece
[4]Division of Hematology, The Children's Hospital of Philadelphia, Philadelphia, PA, United States of America

Lumican is a small leucine-rich proteoglycan (SLRP) which inhibits MMP-14 activity and melanoma cell migration *in vitro* and *in vivo*. Additional data from our laboratory have shown that lumican inhibits growth and melanoma primary tumor development of Snail-B16F1 cells. Snail is known to trigger epithelial-to-mesenchymal transitions endowing epithelial cells with migratory and invasive properties during tumor progression. Colorectal cancer is characterized by a high tendency to spread metastases to the lung and liver.
The aim of this study was to investigate lumican effect on MMP-14 expression and migration of Snail overexpressing MC38 colon carcinoma stable clone.
Snail induced epithelial-to-mesenchymal transition as demonstrated by modified cell morphology and down-regulation of E-cadherin and CDX2 expression. Moreover, gene and protein expression of MMP-14 was upregulated in Snail overexpressing MC38 cells. Induction of MMP-9 expression was also observed. Functional assays showed that Snail significantly increased MC38 cell migration. The presence of lumican did not significantly alter the migration of control and Snail overexpressing MC38 cells.
Taken together, our preliminary data suggest that Snail does induce mesenchymal phenotype of MC38 carcinoma cells as indicated by the downregulation of E-cadherin and CDX2 expression. In addition, Snail increased MC38 cell migration associated with an induction of MMP-14 and MMP-9 expression. In contrast to Snail overexpressing B16F1 clone, lumican did not inhibit the migration of Snail overexpressing MC38 cells, but further work is necessary to better understand the mechanism.

The activity of serum superoxide dismutase enzyme and the serum level of lipid peroxidation product malondialdehyde in different forms of scleroderma

FP16 /P64

A. Kocak[1], D. Harmancı[1], M. Birlik[2], G. Guner Akdogan[3]

[1]*Molecular Medicine Department, Institute of Health Sciences, Dokuz Eylul University*
[2]*Department of Internal Medicine, Division of Rheumatology & Immunology, School of Medicine Dokuz Eylul University*
[3]*Department of Basic Medical Sciences, School of Medicine, Izmir University of Economics, Turkey*

Systemic sclerosis or scleroderma (SSc) is an autoimmune multisystemic connective tissue disease characterized by skin and internal organ fibrosis. Underlying mechanism is still unclear in scleroderma. Reactive oxygen species (ROS) may play a role in scleroderma pathogenesis. Superoxide dismutase (SOD) scavenges oxygen radicals or inhibits lipid peroxidation. Malondialdehyde is a lipid peroxidation products.

In order to examine antioxidant status and lipid peroxidation in scleroderma patients, activity of SOD, and the level of malondialdehyde as an index of lipid peroxidation have been studied in scleroderma patients serum. Blood samples were collected from 22 scleroderma patients individually. Scleroderma patients were divided into two groups diffuse type (n=8) and limited type (n=14) to determine differences between these subgroups. Serum SOD activity levels were determined with SOD activity kit and serum MDA levels were assessed with the high-performance liquid chromatography.

We found a significant increase in SOD activity in the limited type compared to the diffuse type ($p<0.05$). Statistically significant increases in serum MDA levels were found in diffuse group in comparison to limited group. It is suggested that antioxidant status may be changed in scleroderma patients. This alteration may lead to increase lipid peroxidation. In summary, oxidative stress may contribute the progression of scleroderma, but further investigations are necessary.

FP17 /P65

Intriguing role of dermatopontin in skin re-epithelialization: implications on chronic cutaneous wounds

Venkat Raghavan Krishnaswamy[1, 2] & Purna Sai Korrapati[1]

[1]*Biomaterials Department, CSIR-Central Leather Research Institute, Chennai, India*
[2]*Present address: Prof. Irit Sagi lab, Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel*

Chronic cutaneous wound (CCW) is a major health care burden wherein the healing process is slow or rather static resulting in anatomical and functional restriction of the damaged tissue.

Re-epithelialization is a key event in wound healing and impairment in the process is explicitly associated with CCW and various other pathological conditions. Epidermal keratinocyte migration and proliferation during re-epithelialization is largely regulated by the cytokines and growth factors from the provisional matrix and dermis. This study essentially dealt with assessing the role of a matricellular protein, Dermatopontin (DPT), in re-epithelialization and its expression pattern in normal skin and CCW. The results indicated that, DPT promotes keratinocyte migration in a dose dependent fashion by inducing lamellipodia formation. However, the protein failed to induce cell proliferation even at higher concentrations and prolonged incubations. The expression analysis on normal skin revealed for the first time that DPT is negligible in the epidermis both at mRNA and protein levels in spite of its prominent role in epidermal migration. Interestingly, DPT mRNA was found to be augmented in contrast to its protein levels that are reduced both in tissue and exudates of CCW. Further investigations imparted that DPT was degraded at higher rates by certain proteases in chronic wound thus elucidating the cause for the contradiction in its mRNA and protein levels. In conclusion, DPT has a profound role in wound healing specifically during re-epithelialization by promoting keratinocyte migration via paracrine action from the underlying dermis. The susceptibility of DPT protein to specific proteases present at high levels in the chronic wound milieu resulted in the degradation of DPT, thus affecting the healing trajectory and eventually hindering the barrier function of the skin. Simultaneous delivery of pro-healing molecules like DPT with specific protease inhibitors holds a promising strategy in combating CCW. Further studies on the signaling pathways of DPT and exploring the other functions of this multi-faceted protein may facilitate in unraveling novel molecules for cutaneous tissue regeneration.

Posters/ Abstracts

P5

In Silico screening identified ADAMTS12 as a new actor in liver injuries

B. Dekky, D. Bonnier, K. Bévant, V. Legagneux and N. Théret

INSERMU1085-IRSET Institut de Recherches en Santı-Environnement-Travail, Université de Rennes 1, Rennes, France

All chronic liver diseases are associated with the development of fibrosis characterized by excessive deposit of Extra-Cellular Matrix (ECM), leading to distortion of the hepatic architecture and significant impairment of liver functions. During liver injury, Hepatic Stellate Cells (HSC) undergo an ''activation'' process that consists of the transition from quiescent vitamin A–rich cells in the healthy liver to proliferating, fibrogenic, and contractile myofibroblasts. Activated HSC are a major cellular source of ECM component and are also responsible for synthesizing matrix metalloproteases (MMP) and Adamalysins (A disintegrin and metalloprotease) thereby contributing to ECM remodeling. At the molecular level, the Transforming Growth Factor β (TGF-β) is widely regarded as the major pro-fibrogenic agent since it induces activation of HSC and regulates expression of ECM proteins and metalloproteases.

During last years, our group has demonstrated an association between ADAMTS1 and chronic liver disease. Up-regulation of ADAMTS1 in fibrosis was found to be associated with HSC activation and ADAMTS1 regulates the activity of the TGF-β by activating the latent form of TGF-β in the microenvironment. Today, adamalysins and more specially the secreted ADAMTS are considered as major regulators of chronic diseases and cancer, however few have been investigated and molecular mechanisms remain to be explored. In the present study, we develop a large *in silico* screening for all adamalysins expression in tissue samples from ICGC database to identify new adamalysins co-regulated with ADAMTS1.

Based on the screening of 345 biopsies of patients with hepatocellular carcinoma, we identified a cluster of co-regulated genes that include ADAMTS1, and ADAMTS12. We next investigated the relevance of ADAMTS12 in chronic liver diseases. For that purpose we explored the expression of ADAMTS12 in liver tissue samples from 50 patients with hepatocellular carcinoma. Our results showed that ADAMTS12 expression is associated with the inflammatory score of the underlying fibrotic liver and immunostaining analyses revealed localization of protein in myofibroblasts and some Kupffer cells. At the cellular level, we showed that ADAMTS12 is expressed by activated hepatic stellate cells but not by parenchymal cells. In accordance with these observations, ADAMTS12 expression is absent in five hepatocellular carcinoma cell lines. Using mouse fibrosis model, we further found that ADAMTS12 expression is associated with the early response to liver injuries.

Altogether, our data identified ADAMTS12 as a new potential actor in liver response to injury.

ADAMTS expression in laryngeal cancer

P6

K. Kakatsou[1], T. Papadas[2], D. H. Vynios[1]

[1]*Biochemistry, Biochemical Analysis and Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece*

[2]*Department of Otolaryngology, School of Health Sciences and University Hospital of Patras, Greece, Department of Medicine, University of Patras, Greece*

Background: Laryngeal cancer is one of the less frequent human cancers, but symptoms have not been always perceived by the patient quickly, only when cancer has reached an advanced state. Thus, the investigation of therapeutic targets is something of concern to the scientific community worldwide and much attention has been given to the ADAMTS family. ADAMTS are members of the secreted, extracellular metalloprotease family. ADAMTS are members of the secreted, extracellular metalloprotease family possessing disintegrin structure and thrombospondin motif(s). ADAMTSs possess proteolytic activities to different substrates, some are proteoglycanases, others N-propeptide procollagenases, some can degrade COMP and ADAMTS-13 can degrade vWF.

Aim: The present work is designed to study the expression of ADAMTS-2,-3,-7,-12,-13 and -14 in this type cancer for understanding their biological role.

Methods: Two samples were taken from each one of the patients, one from the tumor site and the second from a macroscopically normal distal site. Expression of ADAMTS was examined at transcriptional level by RT-PCR.

Results: The results have shown differences in expression of ADAMTS studied in the cancerous samples compared with the respective macroscopically normal. For N-propeptide procollagenases, ADAMTS-2 (detected in all the samples) increased by 27% and ADAMTS-3 (detected in 71% and 57% of the cancerous and macroscopically normal samples, respectively) by 52%, whereas ADAMTS-14 (detected in all samples) decreased by 57%. For COMP proteases, ADAMTS-7 (detected in 63% and 38% of the cancerous and macroscopically normal samples, respectively) increased by 62% and ADAMTS-12 (detected in 13% of both cancerous and macroscopically normal samples) by 176%. The vWF protease ADAMTS-13 increased by 48% in cancerous samples (detected in 50% of samples) compared to normal ones (detected in 25% of samples).

Discussion: The results suggested that cancer regulates the expression of ADAMTS in a specific manner, depending to the requirements for its growth. It is of high importance that ADAMTS-2 and -14 were present in all samples of laryngeal carcinoma examined, with significant alterations, suggesting a central role for these enzymes in cancer growth. It will also be quite interesting to examine the differences of the remaining ADAMTS proteins in a larger cohort of patients to give the opportunity to use additional members of this enzyme family as biological markers for laryngeal carcinoma.

P7

The role of MT1-MMP in *Salmonella* infection

Dvir Mintz, Roi Avraham, Irit Sagi

Weizmann Institute of Science, Israel

Pathogens face many barriers in their road to the site of infection, *Salmonella* enterica is a bacteria that can invade into host macrophages and use them to disseminate throughout the body and colonize the spleen and liver.

Importantly, the molecular mechanisms and the functions of MMPs in pathogenesis remain unknown. It is suggested that bacterial proteases mediate degradation of structural matrix components of the tissue by their own, or by activation of MMPs zymogens. Such mechanisms will help infected macrophages to pave the way to the lymphatic vessels as well as aid the systemic dissemination of the intracellular *Salmonella*.

We looked at membrane type 1-matrix metalloproteinase 1 (MT1-MMP) RNA and protein levels as well as its enzymatic activity in macrophages (RAW and J774 cells) infected with *Salmonella typhimurium* strain SL1344. The macrophages showed elevated RNA and protein levels and increased activity of MT1-MMP in comparison to control, dead bacteria, and LPS treated macrophages.

We are currently aiming to shed light on the pathway which unable *Salmonella* to hijack MT1-MMP regulation and use it for the bacteria own benefit.

Effects of flavones and isoflavones on proteasome expression in chondrocytes from articular cartilage of patients with osteoarthritis

P8

Z. Kordou, M-E. Christopoulou, and A. J. Aletras

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

Background: Flavones and isoflavones represent two subclasses of flavonoids, a wide class of phenolic phytochemicals which constitute an important component of the human diet, possessing antioxidant properties and exhibiting various biological effects, including cancer prevention, inhibition of bone resorption, hormonal and cardioprotective action.

The proteasome is the major cellular non-lysosomal threonine protease, implicated in the removal of normal as well as abnormal, denatured or otherwise damaged proteins. It possesses multiple endopeptidase activities, including chymotrypsin-like (subunit β5, PSMB5), trypsin-like and caspase-like activities.

Osteoarthritis (OA) is a joint degenerative disease highly associated with ageing. Chondrocytes, in response to exogenous stimuli, produce free radicals, which are considered as main pathogenetic factors of OA.

The purpose of this work was to study the effect of flavones and isoflavones as modulators of proteasome expression in chondrocytes from articular cartilage of patients with OA.

Materials and Methods: Chondrocytes were isolated upon treatment of articular cartilage consecutively with testicular hyaluronidase, pronase and clostridium collagenase. The PSMB5 expression was ascertained by qPCR and the detection of cytokines and MMPs in cell cultures conditioned media by ELISA.

Results: Chondrocytes were cultured in the presence of flavones baicalein and quercetin, and of isoflavones genistein and biochanin at final concentration of 10 and 100 μM. All the flavones and isoflavones tested, at concentration of 100 μM caused suppression or did not affect the expression of PSMB5. In contrast, at concentration of 10 μM the two flavones significantly enhanced the expression of PSMB5, with quercetin to be more effective, while the two isoflavones suppressed the expression of this subunit. All the flavonoids had not any deleterious effect on the chondrocytes survival in both concentration tested. The enhanced expression of PSMB5 by quercetin was suppressed in the presence of MAPK and PI3-K inhibitors as well as in the presence of an inhibitor of NF-κB activation or a ROS scavenger. Quercetin was also able to decrease the production of IL-6 and of metalloproteinases MMP-1, MMP-3 and MT1-MMP in a dose-depended manner.

Conclusions: The flavonoids exhibit different effect on PSMB5 expression, dependent from their subclass. It appears that in this effect the number of hydroxyl groups may be significant factor, since the quercetin, containing five hydroxyl groups, is the more effective. In addition to up-regulate PSMB5 expression, quercetin suppressed also the MMPs and IL-6 production, factors that are pathogenetics in OA. Taken into account that proteasome play a pivotal role in the protection of cells against senescence, it is reasonable to propose that quercetin may exhibit a chondroprotective role and to be beneficial in the treatment of OA.

P9

The role of syndecan-1 in angiotensin II mediated liver fibrosis

E. Regős[1], H. H. Abdelfatah[2], K. Baghy[1], I. Kovalszky[1]

[1]*Ist Institute of Pathology and Cancer Research, Semmelweis University, Budapest*
[2]*University of Cairo, Giza, Egypt*

Aim: Syndecan-1 is a transmembrane proteoglycan and according to our previous studies the amount of SDC1 enhances on the hepatocytes membrane during liver fibrogenesis. In our in vivo studies we proved the protecting function of SDC1 overexpression against liver fibrogenesis. The renin-angiotensin-aldosteron system plays a major role in kidney, cardiovascular diseases and also in liver fibrogenesis. In some studies angiotensin-type-1 receptor (AT1R) blockers attenuate liver fibrogenesis. According to previous studies hepatocytes downregulates AT1R expression in advanced human liver fibrosis. Schelling and co-workers identified SDC1 as an essential mediator in angiotensin II (AngII) induced, TGFβ mediated, cardiac fibrosis. However the connection between syndcan-1 and angiotensin II in the liver is still not known.

Material and methods: We used LX2 myofibroblast and Hep3b or syndecan-1 transfected Hep3b hepatoma cell lines. The cells were co-cultures in 6-well plate, and after serum starvation the cells were treated with 1 μg/ml AngII or with 2ng/ml TGFβ, which served as positive controll for myofibroblast activation. After 48 hours of cytokine administration the cells and culture media were harvested. We measured the amount of αSMA and TIEG mRNA by qRT-PCR. From signaling pathway molecules pERK1/2 was detected from the cells by Western-blot, and from the cell culture media we analysed the produced collagen-1 and thrombospondin-1. In order to investigate the clinical aspect of our investigation 30 FFPE samples were stained with syndecan-1 and angiotensin receptor-type I and compared with clinical data.

Results: The amount of TIEG and αSMA mRNA was significantly lower in the SDC1 overexpressing co-culture model compared to wild type ones. The same difference could be observed in the case of collagen-1 and thrombospondin-1 in the culture media and also in the activation of the ERK1/2 in the cell lysate. On the FFPE samples angiotensin type-1 receptor is expressed in hepatocytes as well as syndecan-1. The syndecan-1 expressing hepatocytes have less angiotensin type-1 receptor. In the clinical samples we didn't find correlation between syndecan-1 expression and etiology, however the amount of SDC-1 significantly rose with the severity liver dysfunction.

Conclusion: Our data suggests that excess amount of syndecan-1 on the hepatocyte cell surface attenuates the myofibroblast activator effect of TGFβ and AngII. In our clinical samples the syndecan-1 expression raised with the severity of liver dysfunction, which changes oppositely to angiotensin type-1 receptor, assuming another protecting function of SDC-1 in liver fibrosis.

Engineering novel protein agents to study and manipulate the extracellular matrix

P 10

M. Levin[1], I. Sagi[1]

[1]*Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel*

The extracellular matrix (ECM) consists of a complex molecular network responsible for dynamic interactions between molecules secreted from and by the cells. This biological network provides structural support for cells and serves as a reservoir of biological factors that mediates extra- as well as intra- cellular signaling, thus governing almost every physiological process in the tissue, including migration, differentiation and proliferation. ECM architecture and function is constantly remodeled by a wide range of proteases, in which the MMPs and ADAMs family members are the most prominent. These proteases dictate ECM activity through direct degradation of ECM components as well as "shedding" of cell surface receptors, cytokines, growth factors and other biologically active proteins, which connects these enzymes to a wide set of pathological conditions, marking them as highly important therapeutic targets. Yet, *in-vitro* substrate promiscuity and apparent structural homology shared by enzyme catalytic sites, makes it very difficult to produce potent inhibitors or molecular modulators with high specificity. Nevertheless, modulating dysregulated MMPs and ADAMs activity selectively *in-vivo* is highly desired in both pathogenesis and developmental biology. Accordingly, based on ADAMs and MMPs characteristics, we have developed a new inhibitory strategy targeting protease activity towards individual physiological substrates. Using protein engineering and directed evolution methodology, we have established a novel system for the production of highly specific molecular agents that will target key MMPs and ADAMs substrates. The latter are designed to compete on molecular pathways governed by selective enzyme-substrate interactions. Producing an innovative and highly specific system for pathway inhibition.

P
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Syndecan-1 expression on breast cancer cells promotes M2 macrophages polarization

Noura El-Husseiny[1], Hossam Taha Mohamed[1], Ramy Gadalla[1], Hebatallah Hassan[1], Eslam A. El-Ghonaimy[1], Noha Ahmed Mahana[1], Mohamed El-Shinawi[2], Martin Götte[3], Mona Mostafa Mohamed[1] and Sherif Abdelaziz Ibrahim[1]

[1]Department of Zoology, Faculty of Science, Cairo University, 12613 Giza, Egypt
[2]Department of General Surgery, Faculty of Medicine, Ain Shams University, 11566 Cairo, Egypt
[3]Department of Gynecology and Obstetrics, Münster University Hospital, Albert-Schweitzer-Campus 1, D11, 48149 Münster, Germany

Introduction:
The transmembrane heparan sulfate proteoglycan Syndecan-1expression is induced in a variety of cell types during development and tumor progression. It plays an essential role in regulation, recruitment and activation of monocytes in tumor microenvironment. A growing body of evidence indicates tumor associated macrophages (TAMs) are classified into major phenotypes, tumor inhibiting M1 and tumor promoting M2. The role of Syndecan-1 in macrophage polarization is poorly studied.

Materials and Methods
In the present study, Sydecan-1 expression was silenced in MDA-MB-231 and SUM-149 cells using siRNA approach. The transfected cells were used in direct and indirect coculture with breast cancer patients –derived monocytes and U937 cells to test their effect on macrophage polarization. The expression levels of markers of the M1 state such as IL-1 beta, as well as those of markers of M2 activation, such as IL-10 were measured by RT-qPCR. Furthermore, the M1 marker HLA-DR and the M2 markers CD163 and CD206 were assessed by flow cytometry.

Results:
Our findings indicate that the Syndecan-1-silenced cells promoted macrophage polarization M1 by upregulating expression of IL-1 beta by 32% and suppressed M2 by suppression of IL-10 expression by 28% in both coculture conditions. Relative to controls, CD206 and CD163 markers were downregulated and HLA-DR marker was upregulated upon coculture with Syndecan-1-silenced cells as assessed by flow cytometry.

Conclusion:
Overall, these findings highlight the potential role played by Syndecan-1 in promoting M2 polarization, and understanding molecular mechanism(s) exerted by Syndecan-1 in such regulation is highly warranted.

Increased plasma hyaluronan levels correlate with the severity of Dengue virus infection - characterization of the molecular mechanisms involved

P 12

Chun-Yu Lin[1,2,3], Konstantinos Kolliopoulos[1], Chung-Hao Huang[2], Jyrki Tenhunen[4], Jaap van der Heijden[4], Yan-Shuang Li[1], Carl-Henrik Heldin[1], Yen–Hsu Chen[2*] and Paraskevi Heldin[1,3*]

[1] Ludwig Institute for Cancer Research, Science for Life Laboratory, Uppsala University, Box 595, SE-751 24 Uppsala, Sweden

[2] School of Medicine, Graduate Institute of Medicine, Sepsis Research Center, Center for Dengue Fever Control and Research, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan

[3] Dept. of Medical Biochemistry and Microbiology, Uppsala University, Box 582, SE-751 23 Uppsala, Sweden

[4] Anesthesiology and Intensive Care, Dept. of Surgical Sciences, Uppsala University Hospital, Uppsala University, Akademiska sjukhuset ing 70 1 tr, 751 85 Uppsala, Sweden

Dengue fever is a re-emerging infectious disease in tropical countries. It is not hard to make a timely diagnosis of dengue fever by the diagnostic tests nowadays, however, it is hard to distinguish which patients will get into severe dengue in the early phase of illnesses. In the present study, we demonstrate that the aberrant levels of circulating hyaluronan during the critical phase, more than 100-fold increase compared to normal levels, is a predictor of the severity of the disease in dengue patients, as early as within 72 hours of disease onset.

Dengue virus non-structural protein 1 (NS1) is a pathogen-associated glycoprotein and can be used as indicator for early acute infection and secondary dengue infection. Because during the blood feeding of dengue virus-infected mosquitoes, cells from skin along with cells in microvascular endothelium might be exposed to or infected with dengue virus, we undertook to characterize the molecular mechanisms of aberrant hyaluronan production and its role in inflammation and vascular leak by investigating the effects of NS1, TGFβ and/or TNFα, in primary dermal fibroblasts and microvascular endothelial cells.

Time-course and concentration-dependent studies of NS1-infected dermal fibroblast cultures revealed increased hyaluronan synthases (HAS1, 2, 3) and HYAL1 expressions, as well as increased hyaluronan production. This effect was further potentiated in the presence of TGFβ or TNFα. Interestingly, such a treatment induced a later response in microvascular endothelial cells and whereas HYAL1 expression dramatically increased the HYAL2 expression was significantly reduced. Importantly, NS1 treatment induced the expressions of mRNAs of both CD44 and TLR4, and CD44 expression was even higher in the presence of a TLR4 antagonist suggesting a compensation of TLR4 inactivation by CD44 upon NS1 treatment. Studies on the signaling pathways revealed distinct differences in NS1-activated fibroblasts versus microvascular endothelial cells, indicating a context-dependent transduction pathway in response to NS1 stimuli. Most interestingly, NS1 treatment disrupted the vessel-like network. These studies might provide novel insights into susceptibility to dengue infection and involvement of CD44 in the induction of vascular leakage.

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Hyaluronidases and Hyaluronan Synthases in colon cancer cell cultures

S. Vasileiou, E. Bouga, A. D. Theocharis, D. H. Vynios

Laboratory of Biochemistry, Department of Chemistry, University of Patras, 26500 Patras, Greece

Hyaluronan (HA) is a major glycosaminoglycan that is abundant in the extracellular matrix (ECM) of connective tissue and plays crucial role in many physiological processes, necessary for maintenance of the integrity of ECM. It is synthesized by transmembrane hyaluronan synthases (HAS1, HAS2 and HAS3 isoenzymes), each one of which catalyses hyaluronan synthesis by a distinct function and produces a polymer of different molecular mass. Hyaluronan is degraded by hyaluronidases, six genes of which, Hyal1-2-3-4, PH20 and pHyal1, with high degree of homology, have been identified. Hyaluronidase-mediated degradation of hyaluronan increases the permeability of connective tissue and therefore facilitates cancer progression.

Our study focused on the expression of hyaluronan synthases and hyaluronidases in colon cancer cells of different aggressiveness using RT-PCR and western blotting.

The results confirmed the expression of HAS3 in all cells, whereas HAS2 was expressed only in DLD1 cells and none of the cells expressed HAS1. The presence of serum in the culture medium highly increased the expression of HAS2 by 250%. On the other hand, serum seemed to down-regulate HAS3 expression in Caco2 and DLD-1 cells by 25%, whereas up-regulate HAS3 in HT-29 cells by 200%. The cells also expressed HYAL1, HYAL2, HYAL3 and PH20, as well as the isoforms HYAL1-v2 and HYAL1-v5. The hyaluronidase isoenzymes were variably affected by the addition of serum in the culture media, more characteristic being the up-regulation of HYAL1 and HYAL2 in DLD-1 cells by about 200% and of PH20 in HT-29 cells by 100%. The expression of HYAL1 and PH20 was also assessed by western blotting, where, HYAL1 appeared as a single band migrating at 48kDa and PH20 as a double band at 65 and 52kDa. In the absence of serum both enzymes were observed in the culture media, whereas in the presence of serum some minor amounts of enzymes were detected in cell lysates.

These observations indicate that hyaluronan synthases and hyaluronidases are associated with tumour progression and may be used as pharmacological tools to study their physiological and pathophysiological involvement in colon cancer progression.

Effects of posttranslational modification site mutations on intracellular trafficking of hyaluronan synthase 2 (HAS2)

P 14

[1]Melero-Fernandez de Mera R.M., [1]Kärnä R., [1]Arasu U.T., [2]Vigetti D., [2]Passi A., [3]Heldin P., [1]Tammi M., [1]Deen A.J.

[1]Institute of Biomedicine, University of Eastern Finland, Kuopio, Finland
[2]Department of Biochemistry, University of Insubria, Varese, Italy
[3]Ludwig Institute for Cancer Research, Uppsala, Sweden

Hyaluronan is synthesized and secreted by hyaluronan synthases (HAS1-3) inserted in the plasma membrane (PM). Therefore, vesicular trafficking of HAS to PM is crucial for its enzymatic activity. HAS2 can be ubiquitinated (1), phosphorylated (2) and O-GlcNAcylated (3), and these posttranslational modifications also influence the rate of hyaluronan (HA) production. We studied whether the effects of these HAS2 posttranslational modifications on HA synthesis are mediated by changes in the trafficking of the enzyme. Site-directed mutagenesis was used to block ubiquitination (K190R), phosphorylation (T110A) and O-GlcNAcylation (S221A) in Dendra2- and EGFP-HAS2 plasmids transfected into COS1 cells. As compared with wild type HAS2, the K190R and S221A mutants showed minor differences in their distribution between ER, Golgi and PM, while the T110A-HAS2 remained mostly in the ER. HA synthesis was not affected by the S221A, while the K190R and T110A mutants were completely inactive. The level of HAS2 in PM, accessible to biotinylation from cell exterior, was considerably lower than that of HAS3 (4). Among the HAS2 mutants, cell-surface biotinylation experiments indicated that T110A was completely absent from PM, while S221A was close to the level of wild type HAS2, and K109R was markedly increased in PM. Rab10 silencing increased HA secretion in wild type and S221A HAS2, as reported before for HAS3 (5). Green-to-red photoconversion of Dendra2-HAS2 constructs indicated that S221A half-life for was longer than that of wild type, and T110A mutant was barely degraded at all. Addition of glucosamine increased the half-life of wild type HAS2, while it had no effect on T110A or K290R, and decreased the half-life of S221A. Examination of Dendra2-HAS2 constructs in Golgi showed that S221A disappearance from Golgi was slower than that of wild type, and K190R was slower than S221A. Co-transfection of wild type HAS2 with K190R suppressed HA synthesis in a concentration dependent manner, whereas co-transfection with S211A mutant was the same as HAS2 wild type alone, and T110A had no effect on wild type, either. Co-transfection of HAS3 with the HAS2 mutants generated a tendency to reduced HA production, in a concentration dependent manner. The results suggest that posttranslational modifications have major influences on the intracellular trafficking of HAS2, and HA synthesis as a consequence.

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P 15

Src kinases affect aggressiveness and the expression of proteolytic network molecules in aggressive breast cancer cells

A. Papargyriou[1], Z. Piperigkou[1], P. Bouris[1,2], D. Manou[1], N. K. Karamanos[1], A. D. Theocharis[1]

[1]*Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece*
[2]*Ludwig Institute for Cancer Research, Biomedical Center, Uppsala University, Uppsala, Sweden*

Breast cancer is by far the most prevalent malignant disease and cause of death among women worldwide. Estrogen receptor alpha (ERα) plays a pivotal role in the growth and progression of hormone-dependent breast cancer. ERα signaling either crosstalk or antagonizes with other signaling pathways in breast cancer thus regulating the expression of important extracellular matrix molecules and cancer cell properties. Matrix metalloproteinases (MMPs) degrade components of extracellular matrix and facilitate angiogenesis, cell invasion and metastasis in breast cancer. Components of the plasminogen activation system play a crucial role in the activation of MMPs in breast cancer. Tyrosine kinases are important cellular allosteric enzymes that regulate cell growth, proliferation, metabolism, differentiation and migration. The Src family kinases (SFKs) has nine known members, all of which are non-receptor tyrosine kinases involved in signal transduction in both normal and cancer cells. c-Src is the best-studied member of the SFKs, its activation is significantly associated with tumor progression. The aim of this study was to examine the effect of the inhibition of the SFKs on cell functions of aggressive, triple negative breast cancer cell lines as well as on the expression of molecules of the proteolytic network and how they affect the cytoskeleton. We used different cell lines: MDA-MB-231 and shERβ MDA-MB-231 (ERβ-suppressed MDA-MB-231 breast cancer cells). Our data indicate that the inhibition of the SFKs significantly reduces cell proliferation, migration and invasion of ERα-negative breast cancer cells. These alterations in basic functional properties were accompanied by changes in the expression levels of MMPs and plasminogen activation system molecules. SFKs play a crucial role in cell morphology and cytoskeleton organization, as the inhibition of SFKs resulted in significant alterations in cell morphology. These data demonstrate the critical role of SFKs in regulating the behavior of aggressive breast cancer cells suggesting that targeting c-Src could be a potential target for improving the focused therapy of breast cancer.

Synthesis and biological applications of Heparan Sulfate-like polymers

P 16

Anastasia Tsichli[1], Theodoros Karalis[1], Valadoula Deimede[2], Georgios Bokias[2], Nikos K. Karamanos[1], Spyros S. Skandalis[1]

[1]*Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece*

[2]*Advanced Polymers Hybrid Nanomaterials Research Laboratory, Department of Chemistry, University of Patras, Greece*

Heparan Sulfate (HS) and Heparin (Hep) are linear negatively charged heteropolysaccharides that exhibit a large number of important biological and pharmacological activities. These polysaccharides are characterized by high heterogeneity, as they can undergo a large number of post-translational modifications per disaccharide unit in the Golgi apparatus of the cells. Through their ability to bind and modulate the binding of various molecules to their cellular receptors they can modulate the activation of multiple signaling pathways that define numerous cell functions. The density and distribution of negative charges within these chains, and particularly the sulfate groups, determine their specific interactions.

The aim of this study was the synthesis of polymeric materials with structures similar to HS/Hep (HS-like polymers) with main regard to their sulfation rate and the subsequent evaluation of their biological effects in breast cancer cell models.

The results showed that oversulfated HS/Hep-like polymeric structures, but not undersulfated or neutral ones, altered the functional properties of breast cancer cells with different estrogen receptor (ER) expression profile. Oversulfated HS-like polymers induced significant morphological changes in ERα-positive tumor cells with the characteristic creation of compact cell aggregates with tight intercellular junctions and reduced cell protrusion formation without affecting cell proliferation. In contrast, the incubation of ERα-negative tumor cells with oversulfated polymers resulted in significant induction of cellular apoptosis and reduction of cell population.

Therefore, synthesis of HS/Hep mimetics with specific structural features, such as the above polymers, and their introduction to biomaterials and nanoparticles may generate novel therapeutic strategies for the targeted treatment of diseases (such as breast cancer) in which HS/Hep-bearing macromolecules have emerged as crucial markers for the disease development.

P 17

The balance of iASPP-CD44 and iASPP-p53 complexes modulate cell migration and proliferation

Aino Ruusala[1], Kaustuv Basu[1], Inna Kozlova [1], Spyridon Skandalis [1, 2] and Paraskevi Heldin [1]

[1] *Ludwig Institute for Cancer Research, Science for Life Laboratory, Uppsala University, Biomedical Center, Uppsala, Sweden*

[2] *Current address: Laboratory of Biochemistry, Dept. of Chemistry, University of Patras, Greece*

The expression of the hyaluronan cell surface receptor CD44 in tumor tissues correlates with high levels of p53. However, under physiologic conditions wild-type p53 suppresses CD44 in conjuction with induction of apoptosis; p53 binds to the CD44 promoter by a non-canonical p53 consensus sequence.

We have found that CD44 interacts with iASPP, a specific inhibitor of wt p53, both in normal and cancer cells expressing wt p53. Formation of iASPP/CD44 complexes was promoted by hyaluronan stimulation. Characterization of the epitopes involved in the interaction between CD44 and iASPP indicated that the ankyrin binding domain in CD44 accounts for its interaction with iASPP. Furthermore, sequestration of CD44 at the plasma membrane increases its interaction with iASPP. We found that iASPP is required for hyaluronan-induced CD44-dependent migration, suggesting a role of iASPP in tumor progression.

Addressing the question how the expression of wt p53 influences the interaction between iASPP and CD44 in normal dermal fibroblasts, we found that high cellular levels of p53 abrogated the complex between iASPP and CD44. In contrast, in p53-depleted cells a stronger interaction between iASPP and CD44 was observed. Next, we investigated how the presence of CD44 affects the formation of iASPP-p53 complexes. Interestingly, the ablation of CD44 promoted the translocation of p53 and iASPP from the nucleus to the cytoplasm and the formation of iASPP-p53 complexes. Moreover, the depletion of CD44 promoted the proliferation of normal dermal fibroblasts and the activation of the serine/threonine kinase Akt, as determined by the phosphorylation of Akt at Ser 473. We hypothesize that such a signaling leads to the stabilization of p53 and consequently increased interaction with iASPP, promoting cell cycle progression.

Our observations suggest a mutual regulation of iASPP-CD44 and iASPP-p53 complexes, and that p53 and CD44 levels regulate iASPP-CD44 and iASPP-p53 complex formation, respectively.

Synthesis and biological evaluation of hyaluronic acid of defined molecular weight

P 18

A.G. Tavianatou[1], C. Barbera[2], R. Beninatto[2], I. Caon[3], E. Karousou[3], D. Galesso[2], N.K. Karamanos[1]

[1] *Biochemistry, Biochem. Anal. & Matrix Pathobiology Res. Group, Dept. of Chemistry, University of Patras, Greece*

[2] *Fidia Farmaceutici S.p.A., Abano Terme (PD), Italy*

[3] *Dept. of Medicine and Surgery, University of Insubria, Varese, Italy*

Hyaluronic acid (HA) plays pivotal roles in various biological processes. HA fragments of different molecular weight (MW) exhibit different biological properties. HA chains of high MW influence the cellular behavior (proliferation, migration, invasion) in a different mode of action than HA fragments of low MW (LMW), a phenomenon that depends on the interaction with the HA receptors. HA fragments of different MW influence wound healing process, angiogenesis and cancer cells behavior. LMW HA and HA oligomers (o-HA) enhance the proliferation and migration of microvessel endothelial cells promoting the angiogenesis process in vitro and in vivo. They can also induce the β-defensin 2 production, necessary in skin epithelium during inflammation stages of the wound healing process. On the other hand, sulfated HA fragments exert antitumor activity in prostate cancer cell lines and preclinical models of bladder cancer [1]. The o-HA also inhibit cancer cells growth and invasiveness, but the detailed mechanism is still unclear. The aim of this study was to produce HA fractions of defined LMW in order to evaluate their effects on endothelial (HMEC-1) and breast cancer cells (MCF-7 and MDA-MB 231) behavior. These HA fractions were produced by chemical and enzymatic hydrolysis followed by chromatographic purification. Of these, nine HA fractions in the oligomers-200 kDa range and one sulfated HA were used. Cell proliferation, wound healing and angiogenesis invasion assays were used in order to evaluate the effect of the fractions on cell motility and invasiveness. Real-time PCR was used to evaluate the effect of the HA fragments on gene expression. According to the results, three of the fractions tested promote the proliferation of endothelial cells. In particular, the HA trimer was able to inhibit the proliferation of endothelial cells in a dose dependent manner. Furthermore, o HA up to pentamers do not affect the invasiveness of the endothelial cells, whereas fractions higher than 5kDa promoted this effect. Interestingly, the MDA-MB 231 cancer cells' morphology changed into a less invasive, MCF-7-like morphology after treatment. A deeper understanding of the mechanisms by which HA fractions influence these processes will be helpful to the development of therapeutic strategies.

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P 19

Autoantibodies against aldehyde-modified collagen type IV are associated with risk for development of myocardial infarctions

Jenifer Vallejo[1], Pontus Dunér[1], Jan Nilsson[1] and Eva Bengtsson[1]

[1]Department of Clinical Sciences Malmö, Lund University, Sweden. Jan Waldenströms gata 35, 20502 Malmö, Sweden

Background:
Oxidation of LDL particles entrapped in the extracellular matrix of the arterial wall is a key factor in the development of atherosclerosis. Lipid oxidation products, such as malondialdehyde (MDA), react with surrounding extracellular matrix proteins and cause modifications recognized by the immune system. MDA modification of collagen type IV is increased in carotid lesions from symptomatic patients and correlates with autoantibodies against MDA-collagen type IV in plasma.

Objectives:
We asked whether autoantibodies against MDA-collagen type IV predict risk for development of myocardial infarction (MI).

Methods:
Plasma levels of MDA-collagen type IV IgM and IgG were analyzed by ELISA in 385 subjects with incident MI during 13 years follow-up and 410 age- and sex-matched controls in the Malmφ Diet and Cancer study.

Results:
MDA-collagen type IV IgG levels were higher in individuals with incident MI than in controls. Subjects in the highest tertile of MDA-collagen type IV IgG had an increased risk to develop MI [hazard ratio (HR) 1.56 (95% confidence interval (CI), 1.22 – 2.00); p for trend 0.0004]. This association remained significant after adjusting for factors in the Framingham risk score and diabetes. High levels of MDA-collagen IV IgG were associated with increased carotid intima-media thickness and elevated plasma levels of MMP-10 and -12.

Conclusions:
Immune responses against MDA-collagen type IV are associated with more severe carotid disease and increased risk for development of MI. These immune responses may reflect LDL oxidation in the artery wall, but could also in themselves affect the atherosclerotic disease process.

EGFR as a critical factor of functional properties and ECM composition in ERα-negative breast cancer cells

P 20

Z. Piperigkou, K. Kyriakopoulou, E. Kefali, E. Riti, C. Pentavra, N. K. Karamanos

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

Breast cancer is the most common type of cancer among women worldwide. Estrogens and their receptors play pivotal role in breast cancer progression affecting the development and growth of breast cancer cells. There are two main forms of estrogen receptors (ER), ERα and ERβ, which belong to the superfamily of nuclear receptors for steroid/thyroid hormones. Studies have shown that the ratio of ERβ and ERα is reduced in tumor tissue compared to normal tissue. Estrogens trigger cell proliferation in breast cancer cells in the presence of ERα while the expression of ERβ appears to reduce cell proliferation and tumor formation induced by ERα. It is known from previous studies of our research team that there is a significant crosstalk between EGFR signaling pathway and ERs in breast cancer cells. The presence of EGFR is critical for breast cancer progression, but its expression is inversely correlated with the expression of ERα. Moreover, the cross-talk between EGFR and ERs is crucial for regulating cell phenotype, functional properties, as well as the expression of ECM macromolecules in breast cancer cells. The goal of this study was to evaluate the role of EGFR in the basic functional properties, signaling and expression of ECM mediators in the highly aggressive ERα-negative MDA-MB-231 breast cancer cells. Our data demonstrated that EGFR is critical for the regulation of breast cancer cell proliferation, migration, invasion and adhesion in vitro. Moreover, the inhibition of EGFR signaling cascade triggered striking changes in the gene expression and activity profiles of critical ECM components suggesting its crucial role in the pathobiology of aggressive breast cancer cells.

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Cyclic tensile stress stimulates mitogen-activated protein kinases in human annulus fibrosus cells inducing the expression of proinflammatory genes

H. Pratsinis[1], A. Papadopoulou[1], C. Neidlinger-Wilke[2], M. Brayda-Bruno[3], H.-J. Wilke[3], D. Kletsas[1]

[1] *Laboratory of Cell Proliferation & Ageing, Institute of Biosciences & Applications, NCSR "Demokritos", 15310 Athens, Greece*

[2] *Institute of Orthopaedic Research and Biomechanics, Centre of Musculoskeletal Research, University of Ulm, Helmholtzstr. 14, D-89081 Ulm, Baden-Wórttemberg, Germany*

[3] *Department of Orthopedics and Traumatology e Vertebral Surgery III e Scoliosis, I.R.C.C.S. Istituto Ortopedico Galeazzi, Via R. Galeazzi 4, 20161, Milano, Italy*

Intervertebral disc (IVD) degeneration is considered as the main reason underlying low back pain, the leading cause of disability in all developed countries. Mechanical forces due to occupational and lifestyle factors are considered to contribute to IVD degeneration. Especially in the area of annulus fibrosus (AF), cells are experiencing predominantly intermittent tensile forces. Hence, the effects of cyclic tensile stress (CTS) on AF cell cultures have been studied using a variety of models. On the other hand, studies examining CTS-induced mechanotransduction in AF cells are rather scarce. Accordingly, objective of the present work was to study the role of mitogen-activated protein kinases (MAPKs) in human AF cells subjected to cyclic tensile stress (CTS) within physiological range.

To this end, primary cultures from outer AF were subjected to CTS using fibronectin-coated silicone dishes and a custom-built cell-stretching device allowing for the regulation of both magnitude and frequency of the strain. MAPK phosphorylation was studied by Western analysis, using antibodies recognizing the three family members (i.e. ERK, SAPK/JNK, and p38), as well as, corresponding specific pharmacologic inhibitors. Gene expression was assessed using qRT-PCR. Cell proliferation was determined using both tritiated thymidine incorporation into DNA and flow cytometric analysis of cell cycle-phase distribution, and collagen synthesis using tritiated proline incorporation and the protease-free collagenase method.

All three members of the MAPK family were found to be phosphorylated immediately after CTS application, with a second phosphorylation-peak appearing at later time points. In general, MAPK activation was proportional to the CTS magnitude, with the exception of ERK phosphorylation which peaked at a magnitude of 4%. Furthermore, all three MAPKs exhibited more intense phosphorylation at the highest frequency tested (1.5 Hz). CTS did not stimulate significantly DNA synthesis in AF cells. Moreover, CTS did not affect matrix metalloprotease-1, -2, and -3 gene expression and activity in the supernatant, neither collagen gene expression and overall accumulation. On the other hand, CTS stimulated the expression of the proinflammatory genes, cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), and interleukin-8 (IL-8). This stimulation was more intense at the highest magnitude (8%) tested and at the median frequency (1 Hz) and time interval (12 h). Blocking of all three MAPKs attenuated the CTS-induced stimulation of COX-2 and IL-8. On the other hand, IL-6 expression was mediated only by SAPK/JNK and p38 MAPK.

In conclusion, activation of MAPKs in human AF cells due to CTS was described for the first time, along with evidence that CTS initiates an inflammatory response. Delineating the mechanisms of IVD cell responses to mechanical stress will contribute to the understanding of disc pathophysiology and, possibly, to the design of novel therapeutic interventions.

Receptor tyrosine kinases drive epithelial-mesenchymal transition in ERα-suppressed MCF-7 breast cancer cells

P 66

P. Bouris[1,2,3], A. Sopaki-Valalaki[1,2,3], C-H. Heldin[2], N.K. Karamanos[3], A.D. Theocharis[3], and A. Moustakas[1,2]

[1] *Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, SE 75123 Uppsala, Sweden*

[2] *Ludwig Institute for Cancer Research, Science for Life Laboratory, Uppsala University, SE 75124 Uppsala, Sweden*

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

Breast cancers are classified based on their dependency on the expression status of the estrogen receptor α (ERα), whose molecular function in tumor cells requires deeper understanding. Breast cancer progression to the metastatic stage can also be associated with the differentiation switch known as epithelial-mesenchymal transition (EMT). We have previously established human breast carcinoma MCF-7 cells with stable suppression of ERα, which resulted in a permanent and stable mesenchymal morphology, followed by enhanced cell proliferation, migration, invasion and corresponding changes in the expression of several extracellular matrix molecules. Using an unbiased genome-wide screen of mRNA expression we identified that loss of ERα led to prominent increase in the expression profile of several known mediators of EMT, including transforming growth factor β (TGF-β) and the receptor tyrosine kinases Axl and platelet-derived growth factor receptor β (PDGFRβ). Functional analysis of the mesenchymal and metastatic ERα-suppressed breast cancer cells, convincingly established that TGF-β signaling, although primed, is not a major factor that maintains the mesenchymal state and invasiveness in this model. In contrast, Axl and PDGF receptor β are major contributors of the mesenchymal and invasive phenotype of these breast cancer cells. The new findings suggest that the constitutive activation of signaling pathways such as Axl and PDGFRβ act in a dominant manner over and possibly downstream of TGF-β signaling in the context of human breast cancer with loss of ERα activity. For this reason, pharmacological intervention against Axl and PDGFRβ more effectively reversed the phenotype towards epithelial differentiation, compared to inhibition of TGF-β signaling. This work suggests that the therapy of ERα-independent breast cancer may benefit from combinatorial treatment, targeting one of these pathways together with conventional anti-cancer drugs.

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Engineering optimal culture conditions to maintain tenogenic phenotype of tenocytes

Andrea Rampin[1, 2, 3], Athina Tzora[1,3], Ioannis Skoufos[1], Nikolaos Diakakis[2], Nikitas Prassinos[2], Dimitrios I. Zeugolis[1, 3, 4]

[1]*Bioprocessing and Biotechnology Laboratory, , Department of Agricultural Technology, Technological Education Institute of Epirus, Arta, Greece*
[2]*School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[3]*Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway, Ireland*
[4]*Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Ireland*

With the aging of human population, the impact of tendon related injuries on global healthcare is constantly growing. Current surgical intervention based therapies (tissue grafts, synthetic/natural biomaterials, direct cells injection, combinations of cells and carrier system) fail to restore the complete functionality of the complex extracellular matrix (ECM) of tendons, so in the last decade a growing number of promising studies focused on tissue engineering by self-assembly (TESA), in tendons and in various other tissue models. Nevertheless, only a skin and a blood vessel device have been commercialised so far, because of the intrinsic limitations of the ex vivo culture conditions, which fail to mimic physiological conditions surrounding cells in vivo. This is true especially for cultured tenocytes, which are commonly subject to sudden transplantation from their highly crowded and organized ECM, to the ECM-free and highly diluted in vitro culture conditions. The culture of tenocytes in the diluted in vitro conditions has been associated with chondrogenic drift, growth arrest and senescence in a number of mammal species, hindering their application in tendon tissue engineering. As tenocytes are considered the most favoured cells for tendon-TESA, maintenance of their phenotype during in vitro expansion is crucial. This study aims to tackle this technical issue by assessing the influence of macromolecular crowders (MMCs) on tenocytes phenotype maintenance, growth and ECM deposition.

Activated SMAD1 is increased in symptomatic atherosclerotic plaques

P 68

C. Tengryd[1], E. Bengtsson[1], P. Dunér[1], A. Edsfeldt[1,3] C. -H. Heldin[2], J. Nilsson[1], I. Gonçalves[1,3]

[1]*Department of clinical sciences Malmö, Lund University*
[2]*Ludwig Institute for Cancer Research, Uppsala University, Uppsala and*
[3]*Department of cardiology, Skεne University Hospital, Sweden*

Introduction The rupture of atherosclerotic plaques with subsequent thrombosis is the leading cause of death in the world today. Rupture-prone plaques, also called vulnerable plaques, are lipid rich, have plenty of inflammatory cells, intra-plaque hemorrhage and are poor in smooth-muscle cells and collagen. The cytokine TGF-β has implicated in atherosclerosis although its exact role has not been unraveled. The best-studied signaling pathway of TGF-β is the SMAD-signaling pathway. The protein SMAD1 is phosphorylated by serine/threonine receptor ALK1 at the cell surface and thereafter it activates target genes in the nucleus. SMAD1 has been found to induce expression of pro-atherogenic genes in macrophages after TGF-β1 stimulation and the SMAD1/ALK1 pathway is important in several aspects of cardiovascular disease including angiogenesis. The aim of this study was to examine activated SMAD1 in human atherosclerotic plaques and its relation to plaque components and plaque vulnerability.

Method 204 plaques from carotid endartectomy patients were analyzed with immunohistochemistry for lipids (ORO), smooth muscle cells (α-actin), macrophages (CD68), collagen (Masson) elastic fibers (elastin), intraplaque hemorrhage (Glycophorin A), calcification (van Kossa) and activated SMAD1 (p-SMAD1). Matrix metalloproteinases were analyzed in plaque homogenate with ELISA.

Results p-SMAD1 was increased in symptomatic compared to asymptomatic plaques (6.83 (2.09-12.14) vs 3.56 (1.62-7.75), median (interquartile range), respectively; p=0.004). p-SMAD1 was present in macrophage-rich areas, around and close to the necrotic core of the plaques. p-SMAD1 correlated to age (r=0.168; p=0.017) and number of events before operation (r=0.185; p=0.008). p-SMAD1 correlated with lipids (r=0.450; p=2x10-9), macrophages (r=0.259; p=2x10-4), intra-plaque hemorrhage (r=0.333; p=1x10-6) and elastin (r=0.171; p=0.018) but did not correlate with vascular smooth muscle cells, collagen or calcification. MMP1 (r=0.326; p=4x10-6) and MMP9 (r=0.412; p=3x10-9) also correlated with p-SMAD1.

Conclusion Activated SMAD1 was increased in symptomatic plaques and correlated to age and the number of events and several components of plaque vulnerability, namely lipids, macrophages and intra-plaque hemorrhage. These results suggest that SMAD1 and its coactivators or downstream signaling proteins might be potential drug targets to affect key processes in atherosclerosis development or vulnerability.

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Vascular calcification during chronic kidney disease: role of the RAGE/Cathepsin S/elastin peptides axis

Wahart A.[1], Neveux M.[1], Ortillon J.[1], Belmokhtar K.[1], Schmelzer C.[2], Heinz A.[2], Velard F.[3], Boulagnon-Rombi C.[1], Romier-Crouzet B.[1], El Btaouri H.[1], Blaise S.[1], Maurice P.[1], Bennasroune A.[1], Debelle L.[1], Gillery P.[1], Rieu P.[1], Touré F.[1*], Duca L.[1*]
(*both authors contributed equally to this work)

[1]UMR CNRS/URCA 7369 MEDyC, Université de Reims Champagne-Ardenne, France
[2]Faculty of Natural Sciences I, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
[3]EA 4691 BIOS, SFR-CAP Santı (FED 4231), Université de Reims Champagne-Ardenne

Vascular calcification is a common feature of patients suffering from chronic kidney disease (CKD). We recently reported a role for the Receptor for Advanced Glycation End products (RAGE) in the uremic vascular calcification process. Following engagement by uremic toxins, RAGE mediates active osteodifferentiation of vascular smooth muscle cells (VSMCs) and vascular calcifications. This process is mainly found in aorta where an intense elastolysis is observed leading to the release of elastin derived peptides (EDPs). Using animal models, several reports from the literature have suggested the possible involvement of Cathepsin S in elastolysis and vascular calcification during CKD. However, the mechanisms implicated and the putative role of RAGE in Cathepsin S expression and the subsequent elastolysis have not been described.

To answer these questions, we used a mouse model of uremic vasculopathy, induced by 5 of 6 nephrectomy in the ApoE-/ - or APoE-/-/RAGE-/- backgrounds. Moreover, we used primary cultures of VSMCs isolated from control or RAGE deleted animals for in vitro studies.

We found that induction of CKD increases the calcification process in the cardiac valves of ApoE-/- mice whereas ApoE-/-/RAGE-/- mice are protected. Moreover, sera and aortas analysis showed that Cathepsin S expression and elastolysis seem to be greater in ApoE-/- compared to the ApoE-/-/RAGE-/- animals. Using recombinant Cathepsin S, we found that this protease was able to directly drive insoluble elastin degradation and bioactive EDPs production as demonstrated by electron microscopy scanning and mass spectrometry analysis. Finally, we showed that an in vitro calcification process is triggered when VSMCs are incubated with inorganic phosphate. This phenomenon is increased in the presence of EDPs enhancing expression of osteoblast differentiation specific genes.

In conclusion, we report that in uremic conditions, increased Cathepsin S expression may promote elastolysis. This process generates bioactive EDPs which could accelerate osteogenic differentiation of VSMCs and apparition of vascular calcification. RAGE engagement may be involved in the initial steps leading to increased Cathepsin S production.

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Characterization of adipose-derived stem cells and optimal in vitro culture conditions to promote their differentiation towards the tenogenic lineage

Sergio Garnica-Galvez[1, 2, 3], Ioannis Skoufos[1], Athina Tzora[1, 3], Nikitas Prassinos[2], Nikolaos Diakakis[2], Dimitrios I. Zeugolis[1, 3, 4]

[1]*Bioprocessing and Biotechnology Laboratory, Department of Agricultural Technology, Technological Education Institute of Epirus, Arta, Greece*
[2]*School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[3]*Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway, Ireland*
[4]*Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Ireland*

Tendon injuries constitute an unmet clinical need for both human and equine patients which lead to an excess of the expenditures in the human healthcare system and make a negative impact on the horse industry. Current treatments involve invasive surgical procedures with a high risk of relapse: tissue grafts from autologous healthy tendons, natural or synthetic biomaterial-based substitution that yields to a thinner and weaker neo-tissue formation and direct cell injections, which offer a low control on repair location and distribution involve. However, the ex vivo expansion of tenocytes for tendon regeneration leads to phenotypic drift, growth arrest and senescence, besides being needed painful medical interventions for their extraction. Adipose-derived stem cells (ADSCs) are a promising cell source to solve these issues due to their high proliferation rate and their demonstrated tenogenic potential. Also, a high extraction yield of ADSCs can be achieved with minimally invasive surgical procedures. ADSCs constitute a heterogeneous cell population whose features depend on several factors such as cell surface marker expression or the extraction body area, so it is necessary their characterization to achieve successful outcomes. On the other hand, equine species are not only a patient but they are also an appropriate animal model due to the similarities between equine flexor tendons and human Achilles tendons. Furthermore, as well as the human patients, horses must be undergone to rehabilitation periods. However, the characterization of horse ADSCs is difficult due to the lack of specific antibodies against equine antigens and also, there is no a fixed criteria for equine mesenchymal stem cells (MSCs), unlike the human MSCs. Additionally, the exact biological and biophysical cues to trigger stem cell tenogenic differentiation, and the specific phenotypic markers of tenocytes, remain still unclear. For these reasons, this project aims to develop an optimal engineered in vitro microenvironment to promote ADSC differentiation towards the tenogenic lineage.

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Development of in vitro microenvironments for directing dermal fibroblasts towards tenogenic lineage

Adrian Djalali-Cuevas[1,2,3], Ioannis Skoufos[1], Athina Tzora[1,3], Nikitas Prassinos[2], Nikolaos Diakakis[2], Dimitrios I. Zeugolis[1,3,4]

[1]*Bioprocessing and Biotechnology Laboratory, Department of Agricultural Technology, Technological Education Institute of Epirus, Arta, Greece*
[2]*School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[3]*Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway, Ireland*
[4]*Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Ireland*

Introduction: Healthcare expenditure for human tendon injuries exceeds €145 billion per year. Tissue engineering is emerging as a potential solution for developing alternatives to tissue grafts for tendon repair and regeneration. Tenocytes play a central role in tendon tissue engineering, but are phenotypically unstable under the standard mammal cell culture conditions typically used for their expansion. Autologous dermal fibroblasts can be obtained in abundance with minimally invasive procedures and can be addressed to a variety of cell lineages. Thus, if a clinically relevant differentiation procedure is developed, dermal fibroblasts can provide an abundant alternative source of tenocytes for tendon regeneration procedures. In this line, the present study assesses the effects of different modulators of the tenocytes cell microenvironment for directing human adult dermal fibroblasts towards the tenogenic lineage.

Experimental methods: Primary human normal adult dermal fibroblasts (hADFs) were cultured in DMEM high glucose with 10% FBS. The cell culture medium was supplemented with different concentrations of growth factors along with high molecular weight polymers to generate a macromolecular-crowded cell microenvironment. Cells were cultured for 3, 7, and 14 days under the different conditions and cell viability, metabolic activity and proliferation were assessed for every treatment. Also, for the assessment of the induction of a tenogenic phenotype on dermal fibroblasts, a variety of tenocyte-related cell markers were assessed by means of immunofluorescence, in vitro collagen deposition was determined my means of SDS-PAGE, and matrix metalloproteinase secretion into the cell culture medium was determined by means of gelatine zymography.

Results and discussion: None of the treatments negatively affected to cell viability, which indicates a non-cytotoxic effect of these on hADFs. Cell proliferation and metabolic activity showed different results depending on the experimental conditions. SDS-PAGE analysis demonstrated that macromolecular crowding increased extracellular matrix deposition. The combination of macromolecular crowding with the different growth factors exerted different effects on the different tenogenic-related phenotype readouts.

Conclusions: Cell culture medium supplementation with growth factors and macromolecular crowders appears to be a safe approach to generate cell microenvironments. Future work and detailed analysis will shed more light on the effectiveness of this strategy for directing cell phenotypes towards a desired lineage.

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Intervertebral discs possess an osmo- and mechano-sensitive circadian clock that temporally coordinates matrix homeostasis

M. Dudek[1], N. Yang[1], J.P. Ruckshanthi[1], J. Williams[1], P. Wang[1], R.P. Boot-Handford[1], J.A. Hoyland[1] and Q.J. Meng[1]

[1]*Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medical and Health Sciences, University of Manchester, UK*

Circadian clocks are the internal timing mechanisms which drive ~24 hr rhythms in physiology and behaviour. In mammals and humans, the central pacemaker Suprachiasmatic Nuclei in the hypothalamus synchronizes peripheral molecular clocks in almost all major body organs. Circadian rhythms coordinate tissue-specific physiology with rest/activity cycles and also show frequent age-related changes, contributing to increased disease risk. Existing evidence suggests that the intervertebral disc (IVD) is a highly rhythmic tissue, experiencing a diurnal cycle of loading followed by low-load recovery. Under load the pressurized interstitial fluid will flow to regions of lower pressure through the annulus fibrosus and the endplate, resulting in decreased disc height and an increase in osmolarity of the nucleus pulposus. When unloaded the process is reversed by high osmotic pressure inside the disc causing flow of fluid to the nucleus pulposus. We have recently identified a functional circadian clock in the intervertebral disc which controls rhythmic expression of over 600 genes, including key molecules in extracellular matrix homeostasis. We also found that genetic disruption of the clock leads to accelerated aging and degeneration of the discs. Using reporter mouse tissues and primary human cells, our new data reveal that the IVD clock is highly sensitive to changes in the osmolarity and to mechanical forces. Imposing a rhythmic 24 hour change in extracellular osmolarity or rhythmic mechanical loading is able to reset the circadian rhythm and rescue the dampened rhythm in desynchronised and ageing IVDs. These novel findings elucidate the importance of daily mechanical loading and associated changes in osmolarity for a well-functioning circadian clock and homeostasis of IVDs. Current work aims to identify key underlying pathways linking the intracellular clock of IVDs with the mechanical/osmotic environment, which holds potential to uncover a new way of counteracting the aging process and delaying the occurrence of age-related IVD degeneration.

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