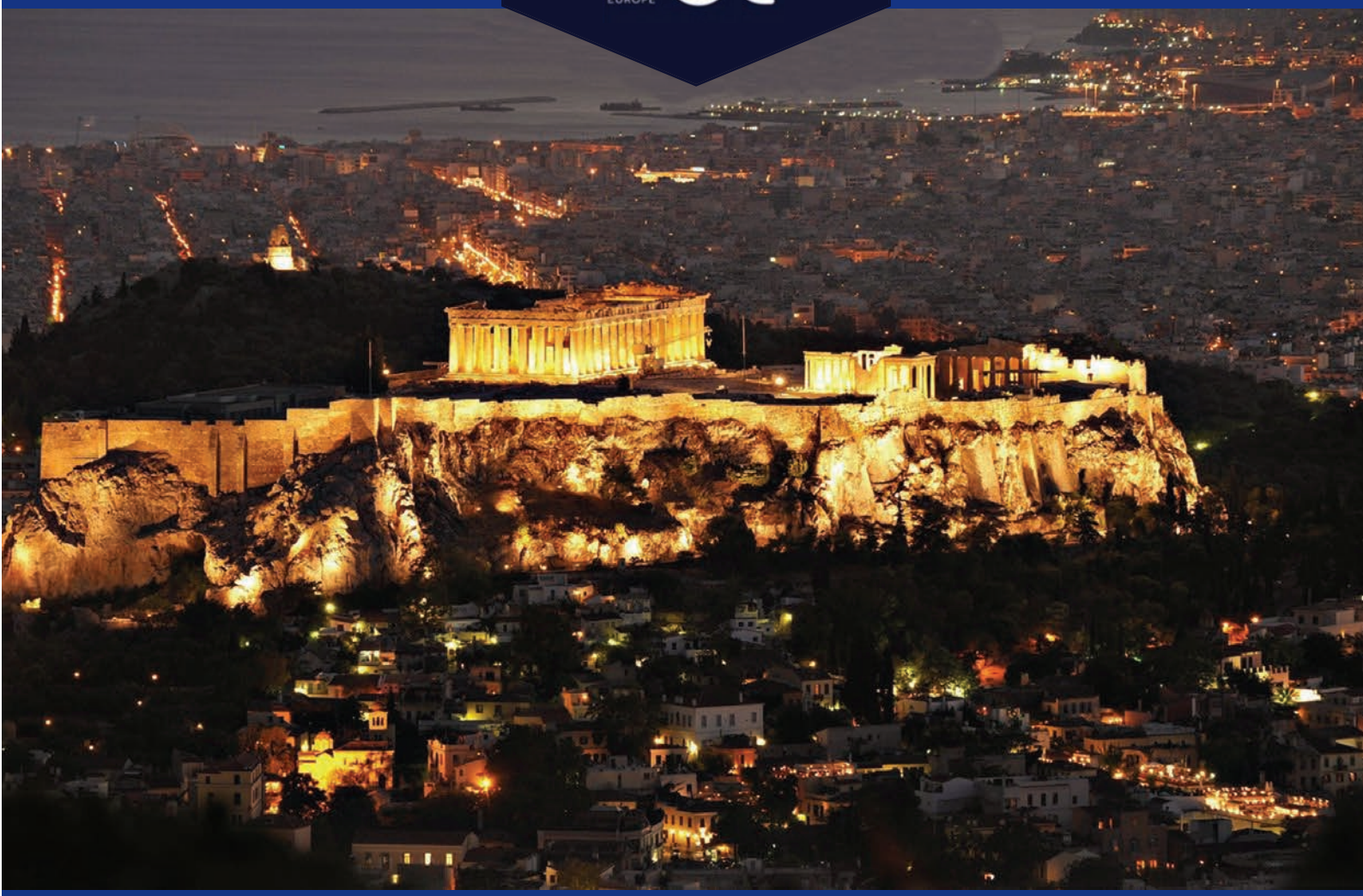
2nd MBE (Matrix Biology Europe) Conference

Athens-Greece, 11-14 June 2016 / Royal Olympic Hotel



Conference Proceedings

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Athens-Greece, 11-14 June 2016
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www.mbe2016.upatras.gr

Conference Proceedings

Welcome message

Dear Colleagues,

On behalf of the Organizing Committee, it is a pleasure to welcome you in the 2nd Matrix Biology Europe (MBE) Conference (being the XXVth FECTS Meeting), Athens (Greece), 11 – 14 June 2016.

MBE is the most important Matrix Biology conference in Europe. This international event is held every two years, and gives the opportunity to senior and young researchers to present their research and to interact with outstanding experts in the Matrix Biology field.

The scientific program covers all aspects of Matrix Biology, including the role of proteoglycans, collagen modifications, and matrix degrading enzymes in health and disease, the importance of cell-matrix interactions and signaling in matrix biology, pathology and the tumor environment as well as the advances in matrix disease mechanisms and pharmacological targeting. Tissue engineering, matrix-related epigenetics and systems biology, and the role of matrix in stem cell niche and inflammation are also addressed.

The Conference is guided by the Hellenic Matrix Biology Section of the Hellenic Society of Biochemistry & Molecular Biology and is organized by the Department of Chemistry of the University of Patras. The Organizing and Scientific Committees have put together internationally recognized experts as well as younger group leaders as invited speakers. The scientific program will involve several Plenary Lectures related to Workshops, parallel Workshops, Plenary Lectures devoted to specific matrix topics of high interest, the Rupert Timpl Award lecture, presentations by nominees of the Dick Heinegard European Young Investigator Award, a Plenary Workshop and several opportunities for presenting the posters in two Poster session. The Plenary Lectures and Workshops will provide you with an update of important new knowledge covering key areas of the field.

International Travel Fellowships and Young Investigators Awards sponsored by the International Society for Matrix Biology (ISMB), and the European Societies for Matrix Biology as well as Awards for Excellence in Matrix Biology sponsored by Matrix Biology journal/Elsevier were also available upon selection procedure based on scientific excellence.

In respect to the various social events, all participants are invited to attend the site visit in the Acropolis New Museum.

Many thanks to the ISMB, the HSBMB, the University of Patras and the private sectors that sponsored this conference.

Iam sure that your active participation will establish this MBE conference as an exciting and memorable scientific meeting.

Nikos Karamanos

Chairman of the MBE 2016 Conference

Committees

Chairman

- **Nikos K. Karamanos,** *University of Patras, GR*

Members of the Scientific Committee

- **Ruud Bank,** *University of Groningen, NL*
- **John Couchman,** *University of Copenhagen, DK*
- **Sylvie Ricard-Blum,** *UMR 5086 CNRS - Université Lyon, FR*
- **Antonio Rossi,** *University of Pavia, IT*
- **Liliana Schaefer,** *Goethe University, DE*

Members of the Organizing Committee

- **Alexios J. Aletras,** *University of Patras, GR*
- **Despina Deligianni,** *University of Patras, GR*
- **Chrysostomi Gialeli,** *Lund University, SE*
- **Dimitrios Kletsas,** *National Center for Scientific Research "Demokritos", GR*
- **George Koliakos,** *Aristotle University of Thessaloniki, GR*
- **Vasso Kostourou,** *Biomedical Sciences Research Center "Alexander Fleming", GR*
- **Dragana Nikitovic,** *University of Crete, GR*
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- **Dionyssios Sgouras,** *Hellenic Pasteur Institute, GR*
- **Paschalis Sideras,** *Biomedical Research Foundation, Academy of Athens, GR*
- **Spyros S. Skandalis,** *University of Patras, GR*
- **Achilleas D. Theocharis,** *University of Patras, GR*
- **Demitrios H. Vynios,** *University of Patras, GR*

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Program at a glance

<table>
<tr><th>Day1 (Saturday, 11/6)</th><th>Day2 (Sunday, 12/6)</th><th>Day3 (Monday, 13/6)</th><th>Day4 (Tuesday, 14/6)</th></tr>
<tr><td rowspan="9">16.00-20.00
Registration

OLYMPIA HALL
18.00-18.30
Opening Ceremony

18.30-19.30
Opening Lecture
R.V. Iozzo (USA)

19.30-20.30
Posters I

20.30-22.00
Welcome Reception
(Roof Garden-ROH)</td><td colspan="2">OLYMPIA HALL
09.00-09.30 PL: J.R. Couchman (DK)
09.30-10.00 PL: K.E. Kadler (UK)
10.00-10.30 Coffee Break</td><td colspan="2">OLYMPIA HALL
09.00-09.30 PL: M.J. Humphries (UK)
09.30-10.00 PL: J.P. Spatz (DE)
10.00-10.10 Memorial to Ruth Chiquet-Ehrismann (J. Adams-UK)
10.10-10.40 Coffee Break</td><td colspan="2">OLYMPIA HALL
09.00-09.30 PL: R. Faessler (DE)
09.30-10.00 PL: J.E. Turnbull (UK)
10.00-10.30 Coffee Break</td></tr>
<tr><td colspan="2">10.30-12.30 Parallel Workshops</td><td colspan="2">10.40-12.50 Parallel Workshops</td><td colspan="2">10.30-12.30 Parallel Workshops</td></tr>
<tr><td>OLYMPIA HALL
Workshop 1
(3×20' Invited
+ 5×12' STs)</td><td>ATTICA HALL
Workshop 2
(3×20' Invited
+ 5×12' STs)</td><td>OLYMPIA HALL
Workshop 5
(3×20' Invited
+ 5×12' STs)</td><td>ATTICA HALL
Workshop 6
(3×20' Invited
+ 6×12' STs)</td><td>OLYMPIA HALL
Workshop 7
(3×20' Invited
+ 5×12' STs)</td><td>ATTICA HALL
Workshop 8
(3×20' Invited
+ 5×12' STs)</td></tr>
<tr><td colspan="2">12.30-13.30 Posters I / Light Lunch</td><td colspan="2">12.50-14.00 Posters II / Light Lunch</td><td colspan="2">12.30-12.40 Break</td></tr>
<tr><td colspan="2">OLYMPIA HALL
13.30-14.00 PL: F-X. Maquart (FR)
14.00-14.30 PL: A. Passi (IT)
14.30-14.45 Break</td><td colspan="2">14.00-16.00 Free time / Visit-guided tour to the New Acropolis Museum (optional)</td><td colspan="2">OLYMPIA HALL
12.40-13.40 Plenary Workshop
D. Kletsas (GR)
V. Hascall (USA)
A. Day (UK)</td></tr>
<tr><td colspan="2">14.45-16.45 Parallel Workshops</td><td colspan="2" rowspan="2">OLYMPIA HALL
16.15-16.45 PL: L. Schaefer (DE)
16.45-18.15 Dick Heinegard European Young Investigator Award Presentations (6×15' STs)

18.15-19.00 MBE General Assembly</td><td colspan="2" rowspan="3">13.40-14.10
Awards/
Closing Ceremony</td></tr>
<tr><td>OLYMPIA HALL
Workshop 3
(3×20' Invited
+ 5×12' STs)</td><td>ATTICA HALL
Workshop 4
(3×20' Invited
+ 5×12' STs)</td></tr>
<tr><td colspan="2">16.45-18.00 Posters I / Coffee
MBE Contact Persons Meeting</td></tr>
<tr><td colspan="2">OLYMPIA HALL
18.00-18.30 Rupert Timpl Award Lecture : A. Nystroem (DE)
18.30-19.00 PL: I. Sagi (IL)</td><td colspan="2">19.00-20.30 Posters II / Coffee
21.00-23.00 Conference Dinner</td><td colspan="2">PL: Plenary Lecture; ST: Selected Talk</td></tr>
</table>

Conference Program

SATURDAY, JUNE 11th, 2016

16.00–20.00 **Registration**

OLYMPIA HALL

Chair Persons F. RAMIREZ (USA) & N. KARAMANOS (Greece)

18.00–18.30 **Opening Ceremony**

— *Rector of the University of Patras, Prof. V. Kyriazopoulou*

— *Chairman of the Organizing Committee, Prof. N. Karamanos*

— *President of the ISMB, Prof. F. Ramirez*

— *President of the HSBMB, Dr. D. Kletsas*

18.30–19.30 **Opening Lecture (L1): Renato V. IOZZO** *(USA)*
Novel proteoglycan functions in regulating autophagy and angiogenesis

19.30–20.30 **POSTER SESSION I (P1-P61, P133, P134)**

20.30–22.00 **Welcome Reception** (Roof Garden)

SUNDAY, JUNE 12th, 2016

OLYMPIA HALL

Chair Persons: R. SANDERSON (USA) & B. BRODSKY (USA)

09.00–09.30 **Plenary Lecture (L2): John R. COUCHMAN** *(Denmark)*
Syndecan control of ion channels: a conserved mechanism for regulation of cell adhesion and migration

09.30–10.00 **Plenary Lecture (L3): Karl E. KADLER** *(UK)*
Circadian clock regulation of the major control systems for collagen secretion and turnover

10.00–10.30 **Coffee Break**

10.30–12.30 **PARALLEL WORKSHOPS 1 & 2**

OLYMPIA HALL

10.30–12.30 **WORKSHOP 1:**
Proteoglycans in health and disease

Chair Persons: P. ROUSSELLE (France) & S. SKANDALIS (Greece)

Invited Lectures (L4–L6)

10.30–10.50 **Paraskevi HELDIN** *(Sweden)*
Functional role of Hyaluronan–CD44 interactions

10.50–11.10 **Ida Gjervold LUNDE** *(Norway)*
Syndecans: new sweethearts in cardiac remodeling and failure?

11.10–11.30 **Mauro PAVAO** *(Brazil)*
Heparin: new clinical uses for an old anticoagulant

Selected Talks (ST1–ST5)

11.30–11.42 **Patricia ROUSSELLE** *(France)*
Perlecan expression influences the keratin 15–positive cell population fate in the epidermis of aging skin

11.42–11.54 **Sissel B. RONNING** *(Norway)*
Syndecan–4 is important for normal skeletal muscle fibre development

11.54–12.06 **Sophie DOGNE** *(Belgium)*
Hyal–1 deficiency protects the endothelium in a mouse diabetic model

12.06–12.18 **Elvira DIAMANTOPOULOU** *(UK)*
The role of versican in semicircular canal development in the zebrafish inner ear and in cancer progression

12.18–12.30 **Stephane BREZILLON** *(France)*
Lumican inhibits SNAIL–induced melanoma cell migration specifically by blocking MMP–14 activity

ATTICA HALL

10.30–12.30 WORKSHOP 2:

Collagen modifications: role in matrix quality/quantity and disease

Chair Persons: T. PIHLAJANIEMI (Finland) & P. BONALDO (Italy)

Invited Lectures (L7–L9)

10.30–10.50 **Frank ZAUCKE** *(Germany)*
A novel function for cartilage oligomeric matrix protein in collagen secretion

10.50–11.10 **Paolo BONALDO** *(Italy)*
Collagen VI, a key matrix protein at the crossroad of skeletal muscle and peripheral nerves

11.10–11.30 **Fabio QUONDAMATTEO** *(UK)*
When Anatomy meets Collagen..... How the Anatomist adds to the phenotype of mutants

Selected Talks (ST6–ST10)

11.30–11.42 **Barbara BRODSKY** *(USA)*
The effect of Gly substitutions in the collagen triple–helix on binding to integrin and fibronectin

11.42–11.54 **Tiina PETAISTO** *(Finland)*
Collagen XVIII regulates energy metabolism and brown adipose tissue function

11.54–12.06 **Wayne A. CABRAL** *(USA)*
Absence of the ER cation channel TMEM38B/TRIC–B disrupts intracellular calcium homeostasis and dysregulates collagen synthesis in recessive osteogenesis imperfecta

12.06–12.18 **Melanie MENARA** *(France)*
Succinate mediates extracellular matrix remodeling to promote an invasive phenotype: a new function for oncometabolites

12.18–12.30 **Josephine A. ADAMS** *(UK)*
Novel role of Thrombospondin–1 as a binding partner of collagen cross–linking sites: Functional implication in modulation of myofibroblastic differentiation

12.30–13.30 **POSTER SESSION I (P1–P61, P133, P134) / Light Lunch**

OLYMPIA HALL

Chair Persons: I. SAGI (Israel) & A. ROSSI (Italy)

13.30–14.00 **Plenary Lecture (L10): François–Xavier MAQUART** *(France)*
Mechanisms of the anti–tumor effects of NC1(XIX), the C–terminal domain of type XIX collagen

14.00–14.30 **Plenary Lecture (L11): Alberto PASSI** *(Italy)*
Epigenetic control of hyaluronan synthases

14.30–14.45 **Break**

14.45–16.45 **PARALLEL WORKSHOPS 3 & 4**

OLYMPIA HALL

14.45–16.45 **WORKSHOP 3:**
Enzyme control of matrix function in health and disease

Chair Persons: Y. ITOH (UK) & D. SGOURAS (Greece)

Invited Lectures (L12–L14)

14.45–15.05 **Yoshifumi ITOH** *(UK)*
Sensing and cutting in pericellular microenvironment: essential processes for cellular invasion

15.05–15.25 **Ralph D. SANDERSON** *(USA)*
The heparanase/syndecan–1 axis in cancer: Mechanisms and therapy

15.25–15.45 **David J.S. HULMES** (*France)*
Why collagen I is normally a heterotrimer

Selected Talks (ST11–ST15)

15.45–15.57 **Christine BROSTJAN** *(Austria)*
Proteolytic processing of human thrombospondin–1

15.57–16.09 **Joan C. MARINI** *(USA)*
First X–linked form of osteogenesis imperfecta, caused by mutations in MBTPS2, demonstrates a fundamental role for regulated intramembrane proteolysis in normal bone formation

16.09–16.21 **Laurent MULLER** *(France)*
Lysyl oxydase like–2 (LOXL2) regulates angiogenesis through scaffolding of endothelial basement membrane

16.21–16.33 **Raymond P. BOOT–HANDFORD** *(UK)*
Stimulation of intracellular proteolysis reduced disease severity in an ER stress–related cartilage pathology

16.33–16.45 **Laura DUPONT** *(Belgium)*
Embryonic lymphangiogenesis and placental angiogenesis are altered in absence of Adamts3

ATTICA HALL

14.45–16.45 **WORKSHOP 4:**
Epigenetics, Systems Biology and Stem Cell Niche

Chair Persons: S. RICARD–BLUM (France) & J. KAPYLA (Finland)

Invited Lectures (L15–L17)

14.45–15.05 **Sylvie RICARD–BLUM** *(France)*

The interaction network connecting angiogenesis and Alzheimer's disease: focus on endostatin, lysyl oxidase and membrane collagens

15.05–15.25 **Sandra WILEY** *(USA)*
Phosphorylation of secreted proteins by a new family of kinases

15.25–15.45 **Martin GOTTE** *(Germany)*
MicroRNA miR–142–3p inhibits breast cancer cell invasiveness and stem cell properties by targeting integrin alpha V, KLF4 and multiple cytoskeletal elements

Selected Talks (ST16–ST20)

15.45–15.57 **Shireen R. LAMANDE** *(Australia)*
A dominant TRPV4 mutation underlies osteochondrodysplasia in Scottish fold cats

15.57–16.09 **Florence FLICK** *(France)*
Chromatin plasticity directs the fate of healthy cells and cancer cells on soft matrices

16.09–16.21 **Zoi PIPERIGKOU** *(Greece; Germany)*
MicroRNA targeting as a regulatory mechanism of breast cancer cells with different estrogen receptor status

16.21–16.33 **Mario RASPANTI** *(Italy)*
Different sugar epitopes drive the cells to different fates

16.33–16.45 **Magdalena KROCHMAL** *(Greece)*
Comprehensive meta–analysis of proteomics data in search for novel signatures associated with extracellular matrix remodeling in chronic kidney disease

16.45–18.00 **POSTER SESSION I (P1-P61, P133, P134) / Coffee / MBE Contact Persons Meeting**

OLYMPIA HALL

Chair Persons: L. SCHAEFER (Germany) & R. FAESSLER (Germany)

18.00–18.30 **Plenary Lecture (L18): Rupert Timpl Award Lecture**
Alexander NYSTROEM *(Germany)*
Delineation of disease modifiers allows for treatment of basement membrane–linked skin disorders

18.30–19.00 **Plenary Lecture (L19): Irit SAGI** *(Israel)*
Extracellular matrix proteolysis: a bystander or a partner in a crime?

MONDAY, JUNE 13th, 2016

OLYMPIA HALL

Chair Persons: R. BANK (The Netherlands) & E. PAPADIMITRIOU (Greece)

09.00–09.30 **Plenary Lecture (L20): Martin J. HUMPHRIES** *(UK)*
CDK1 inhibition triggers adhesion remodelling prior to mitosis

09.30–10.00 **Plenary Lecture (L21): Joachim P. SPATZ** *(Germany)*
Collective cell migration induced by mechano– and synthetic Biology

10.00–10.10 "*Ruth Chiquet–Ehrismann: A Tribute to her Research*" by **Josephin ADAMS** (UK)

10.10–10.40 **Coffee Break**

10.40–12.50 **PARALLEL WORKSHOPS 5 & 6**

OLYMPIA HALL

10.40–12.40 WORKSHOP 5:
Cell adhesion, signaling and the tumour environment

Chair Persons: B. GEIGER (Israel) & V. KOSTOUROU (Greece)

Invited Lectures (L22–L24)

10.40–11.00 Francesco RAMIREZ *(USA)*
ECM mechanosignaling

11.00–11.20 Peter FRIEDL *(The Netherlands; USA)*
Mechanics of cancer cell invasion in vivo

11.20–11.40 Benny GEIGER *(Israel)*
Cell adhesion, signaling and the tumor environment

Selected Talks (ST21–ST25)

11.40–11.52 Ritva HELJASVAARA *(Finland)*
Collagen XVIII regulates EGFR–HER2 signaling in breast cancer and its knockdown augments the effect of anti–ErbB drugs

11.52–12.04 Christos G. ZERVAS *(Greece)*
A novel mechanosensing role of Integrin–Linked Kinase, Parvin and PINCH in cell–matrix adhesion reinforcement in Drosophila

12.04–12.16 Christine JEAN *(France)*
FAK activity within cancer–associated fibroblasts is a key regulator of pancreatic ductal adenocarcinoma invasion

12.16–12.28 Pugazendhi M. ERUSAPPAN *(Norway)*
Signaling function of Integrin alpha 11 cytoplasmic tail

12.28–12.40 Lamprini SKONDRA *(Greece)*
Effect of moesin on cell migration induced by pleiotrophin

ATTICA HALL

10.40–12.50 WORKSHOP 6:
Tissue engineering from a matrix perspective

Chair Persons: F. QUONDAMATTEO (UK) & D. DELIGIANNI (Greece)

Invited Lectures (L25–L27)

10.40–11.00 Garry P. DUFFY *(Ireland)*
Living implants to reverse disease: Diabetes Reversing Implants for long term Viability and Efficiacy (DRIVE)

11.00–11.20 Julie FRADETTE *(Canada)*
Soft–tissue reconstruction solely based on cell derived matrix organization: the benefits of the self–assembly approach of tissue engineering

11.20–11.40 John WHITELOCK *(Australia)*
Perlecan is critical for tissue and organ development but does it have a role in tissue engineering?

Selected Talks (ST26–ST31)

11.40–11.52 Won Bae JEON *(South Korea)*
Signaling mechanisms for the enhanced survival of adipose stem cells in elastin–like extracellular matrix

11.52–12.04 **Attila ASZODI** *(Germany)*
FGF–2 treatment primes ADMSC chondrogenesis by increasing the expression of integrin alpha10

12.04–12.16 **Mona E. PEDERSEN** *(Norway)*
Eggshell membrane – An equivalent of extracellular matrix (ECM) in avian egg has modulating wound healing properties

12.16–12.28 **Maurice van DALEN** *(The Netherlands)*
Amyloid micronetworks in cartilage repair: a protein specific response

12.28–12.40 **Inna KORNIENKO** *(Russia)*
Low–immunogenic matrix suitable for transplantation

12.40–12.50 **Iratxe MADARIETA** *(Spain)*
Biologic scaffold materials composed of adipose tissue

12.50–14.00 **POSTER SESSION II (P62-P132, P135, P136) / Light Lunch**

14.00–16.00 **Free time / Visit – guided tour to the New Acropolis Museum *(optional)***

OLYMPIA HALL

Chair Persons: V. HASCALL (USA) & D. KLETSAS (Greece)

16.15–16.45 **Plenary Lecture (L28): Liliana SCHAEFER** *(Germany)*

16.45–18.15 **Dick Heinegard European Young Investigator Award Presentations *(ST32–ST37)***

16.45–17.00 **Nikolaos AFRATIS** *(Greece)*
Syndecan–4 is a key modulator of epithelial–to–mesenchymal transition in breast cancer cells

17.00–17.15 **Collin EWALD** *(Switzerland)*
Reduced insulin/IGF–1–signalling implicates extracellular matrix remodelling in longevity

17.15–17.30 **Edward R. HORTON** *(UK)*
Integrative analysis of multiple integrin adhesion complex proteomes defines a core consensus adhesome and reveals how it might work

17.30–17.45 **Albin JEANNE** *(France)*
Matricellular TSP–1 as a target of interest for facing tumor progression: towards a therapeutic use for TAX2 peptide

17.45–18.00 **Zsuzsa JENEI–LANZL** *(Germany)*
Mesenchymal progenitor cell chondrogenesis, hormones, and neuronal pathways – a role of G protein–coupled receptors

18.00–18.15 **Luca MONTI** *(Italy)*
Animal models of Desbuquois Dysplasia type 1 demonstrate CANT1 role in proteoglycan metabolism

18.15–19.00 **MBE General Assembly**

19.00–20.30 **POSTER SESSION II (P62-P132, P135, P136) / Coffee**

21.00–23.00 **Conference Dinner**

TUESDAY, JUNE 14th, 2016

OLYMPIA HALL

Chair Persons: D. HULMES (France) & N. SAVION (Israel)

09.00–09.30 **Plenary Lecture (L29): Reinhard FAESSLER** *(Germany)*
Genetic analysis of integrin signalling in mice

09.30–10.00 **Plenary Lecture (L30): Jeremy Ewan TURNBULL** *(UK)*
Next generation heparin therapeutics: targeting proteoglycan functions

10.00–10.30 **Coffee Break**

10.30–12.30 **PARALLEL WORKSHOPS 7 & 8**

OLYMPIA HALL

10.30–12.30 **WORKSHOP 7:**
Cell/matrix interactions in matrix biology and pathology

Chair Persons: M. DURBEEJ (Sweden) & D. VYNIOS (Greece)

Invited Lectures (L31–L33)

10.30–10.50 **Rashmin C. SAVANI** *(USA)*
CD44 is critical for TLR activation of the inflammasome and the evolution of lung injury: from mice to man

10.50–11.10 **Madeleine DURBEEJ** *(Sweden)*
Laminin α2 chain–deficient muscular dystrophy: pathogenesis and development of treatment

11.10–11.30 **Antonella FORLINO** *(Italy)*
Osteogenesis Imperfecta: not only an extracellular matrix disease

Selected Talks (ST38-ST42)

11.30–11.42 **Pearl LEE** *(Australia)*
A potent cell adhesive peptide from tropoelastin that mediates attachment to both integrins alphaVbeta5 and alphaVb3

11.42–11.54 **Jacek DROBNIK** *(Poland)*
Inhibition of α2β1 integrin by TC–I15 increases collagen accumulation by cultured fibroblasts isolated from the heart atrium of patients with aortic stenosis

11.54–12.06 **Vassiliki KOSTOUROU** *(Greece)*
Talin 1 dysfunction is associated with the Systemic Capillary Leak Syndrome

12.06–12.18 **Davide VIGETTI** *(Italy)*
The long non–coding RNA HAS2–AS1 is a new regulator of breast cancer cells invasiveness

12.18–12.30 **Maria Francesca SECCHI** *(Italy)*
The role of Heparanase in chronic liver disease fibrogenesis

ATTICA HALL

10.30–12.30 WORKSHOP 8:

Advances in matrix disease mechanisms and pharmacological targeting

Chair Persons: S. LAMANDE (Australia) & M. FRANCHI (Italy)

Invited Lectures (L34–L36)

10.30–10.50 **Ruud A. BANK** *(The Netherlands)*
Combatting fibrosis: new ways to inhibit myofibroblast formation or pyridinoline cross–linking

10.50–11.10 **Achilleas D. THEOCHARIS** *(Greece)*
Serglycin as a key proteoglycan in cellular effectors, signaling and functional properties of ERalpha silenced breast cancer cells

11.10–11.30 **Kirsi Johanna RILLA** *(Finland)*
Hyaluronan–coated extracellular vesicles as potential biomarkers and matrix messengers

Selected Talks (ST43–ST47)

11.30–11.42 **Taina PIHLAJANIEMI** *(Finland)*
Properties and functions of collagen XIII and other MACIT collagens

11.42–11.54 **Dragana NIKITOVIC** *(Greece)*
Receptor for hyaluronic acid– mediated motility (RHAMM) regulates HT1080 fibrosarcoma cell proliferation via a β–catenin/c–myc signaling axis

11.54–12.06 **Qing–Jun MENG** *(UK)*
The circadian clock gene Bmal1 is required for cartilage tissue homeostasis

12.06–12.18 **Marco MACCARANA** *(Sweden)*
Isolated iduronic acids in chondroitin/dermatan sulfate are important for neural crest cell migration

12.18–12.30 **Dionyssios N. SGOURAS** *(Greece)*
Expression of factors responsible for extracellular matrix remodeling leading to epithelial to mesenchymal transition in Helicobacter pylori infection

12.30–12.40 **Break**

OLYMPIA HALL

Chair Persons: J. TURNBULL (UK) & G. CHRISTENSEN (Norway)

12.40–13.40 PLENARY WORKSHOP

Invited Lectures (L37–L39)

12.40–13.00 **Dimitrios KLETSAS** *(Greece)*
Ionizing radiation–mediated premature senescence of stromal fibroblasts: Implications in tumor development

13.00–13.20 **Vincent C. HASCALL** *(USA)*
The non–reducing terminal trisaccharide of heparin blocks glucose uptake in hyperglycemic dividing cells

13.20–13.40 **Anthony J. DAY** *(UK)*
Age–related changes in the retinal matrix and induction of disease pathways relevant to AMD

OLYMPIA HALL

Chair Persons: R.V. IOZZO (USA) & N. KARAMANOS (Greece)

13.40–14.10 **Awards/Closing Ceremony**

ABSTRACTS

- Plenary Lectures (L)

- Workshop Invited Lectures (L)

- Workshop Selected Talks (ST)

- Posters (P)

2nd Matrix Biology Europe Conference, 11-14 June, 2016, Athens, Greece

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Opening Lecture

Plenary Lecture (L1) Novel proteoglycan functions in regulating autophagy and angiogenesis

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Proteoglycans contribute to the growth and homeostasis of multicellular organisms and control various biological processes that are utilized by cancer cells during invasion and metastasis. We have recently uncovered a new biological function for decorin and perlecan, in regulating autophagy. Decorin and the C-terminal portion of perlecan, called endorepellin, interact with the VEGF receptor 2 and trigger a canonical signaling cascade that leads to activation of AMPKa, suppression of mTOR activity, induction of Peg3 and Beclin 1 and ultimately induction of autophagy. This process occurs specifically in endothelial cells and the ultimate consequence is inhibition of angiogenesis. We have also made the novel discovery that decorin itself is an autophagy-inducible gene in the mouse heart, and that mice deficient in decorin demonstrate abnormal cardiac autophagy. Our results offer a new concept, that is, extracellular matrix constituents can regulate a key intracellular catabolic process, autophagy, via modulation of receptor tyrosine kinases, independent of nutrient deprivation. From a translational viewpoint, these data suggest that modulation of proteoglycan expression, and subsequently, autophagy, in the tumor microenvironment may be an appealing and effective chemotherapeutic target for quelling tumor angiogenesis and overall cancer progression.

Plenary Lecture (L2) Syndecan control of ion channels: a conserved mechanism for regulation of cell adhesion and migration

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The syndecans are transmembrane heparan sulphate proteoglycans with a long evolutionary history. While mammals possess four syndecan genes, invertebrates have one which is expressed in nervous and other tissues. The mammalian syndecans are widespread and almost all cells express at least one of the family. Work by many laboratories over the past 25 years has shown roles for syndecans in a range of processes, including the regulation of cell adhesion, actin cytoskeleton and migration. Mis-expression has been noted in many diseases, including vascular and musculoskeletal diseases as well as various cancers. All syndecans can link to the actin cytoskeleton, and in some cases promote junction formation. Since the phenotype of a single syndecan gene knockout is mild in the mouse, we hypothesized that there is redundancy between family members, but no molecular basis for this had been established. Through the use of microarrays, genetic experiments in the mouse and *C.elegans*, and imaging, we have established that a major function of invertebrate and vertebrate syndecans is the regulation of a class of calcium channel. The transient receptor potential channels of the canonical type (TRPC) are widespread regulators of resting calcium levels and are responsive to stretch activation. In this way, syndecans control the cytoskeleton and junction assembly. We propose that this syndecan function may also be important in disease progression, particularly where changes in cell adhesion and migration are implicated

Plenary Lecture (L3) Circadian clock regulation of the major control systems for collagen secretion and turnover

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Circadian rhythms are endogenous 24-hour oscillations in biological processes found throughout the animal kingdom. Regulation of extracellular matrix (ECM) is a fundamental property of cellular processes; matrix accounts for one-third of vertebrate mass and fibrosis occurs when this increases. Here we show that the circadian clock regulates major control systems in membrane and protein transport at the endoplasmic reticulum/Golgi and plasma membrane/matrix interface that determine the amount of matrix. In my talk I will present a novel mechanism of how the circadian clock regulates tissue homeostasis via transcriptional and translational networks and membrane trafficking.

This work is supported by The Wellcome Trust.

Workshop 1: Proteoglycans in health and disease

Invited Lecture (L4) Functional role of Hyaluronan-CD44 interactions

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Clinical and experimental studies have revealed that hyaluronan and its receptor CD44 modulate cellular behavior. Our aim is to elucidate the mechanism of regulation of hyaluronan and the importance of its interaction with CD44 in regulation of cell fate.
We and others have shown that the hyaluronan synthesizing enzyme HAS2 is transcriptionally upregulated in advanced cancers. Furthermore, HAS2 activity and stability is modulated by post-translational modifications including ubiquitination and phosphorylation.
We have demonstrated that TGFb stimulates the production of hyaluronan in normal mammary breast epithelial cells by upregulation of HAS2 and that efficient TGFb-induced epithelial to mesenchymal transition (EMT) requires the expression of HAS2. To elucidate further the molecular mechanisms by which TGFb induces HAS2 at the transcriptional level, we investigated the possible involvement of a natural antisense transcript of HAS2 (HAS2-AS). We found that HAS2-AS expression is regulated in a similar manner as HAS2 upon stimulation with TGFβ, and HAS2-AS is needed for the HAS2 expression in TGFβ-mediated EMT. Interestingly, by microarray analysis and RT-PCR, we have demonstrated that HAS2 mRNA is regulated by the high mobility group A2 (HMGA2) protein, which also is induced by TGFb. ChIP analysis revealed that HMGA2 and Smad2/3 bound to the HAS2 promoter and most likely potentiate the expression of HAS2 and HAS2-AS.
The posttranslational modifications of HAS2, both mono- and poly-ubiquitination are of main regulatory significance for the pool of hyaluronan in tissues. We have demonstrated that the deubiquitinating enzymes, USP17 and USP4, efficiently removed ubiquitination of HAS2. USP17 was found to remove Lys48- and Lys63-linked polyubiquitin chains and thus to stabilize HAS, whereas USP4 mainly removed the mono-ubiquitination of HAS2. Immunofluorescence stainings demonstrated a colocalization between HAS2 and USP17 in aggressive breast cancer cells. Interestingly, in a HAS2 stable knockdown clone of the aggressive breast cancer cells, the endogenous USP17 expression was decreased. Thus the activity and stability of HAS2 are regulated by mono- and poly-ubiquitination, respectively.
We also investigate the mechanisms through which hyaluronan signals via its receptor CD44 alone or in co-operation with the receptors for the growth factors TGFb and PDGF-BB. Using a proteomic approach we identified proteins interacting with the cytoplasmic part of CD44 such as IQGAP1 and iASPP. Our data demonstrate a role of IQGAP1/CD44 and iASPP/CD44 interactions in fibroblast migration and survival. We observed a reverse correlation between iASPP/CD44 and iASPP/p53 complexes. Interestingly, the CD44 expression levels affected the cellular localization and the function of the p53/iASPP complexes.

Invited Lecture (L5) Syndecans: new sweethearts in cardiac remodeling and failure?

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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 metabolizing tissues. We have no cures for heart failure other than transplantation, and current therapies only slow the progression. Five years from diagnosis, 50% of heart failure patients are alive. An increased understanding of underlying molecular mechanisms is needed for development of new future therapies. Remodeling of the heart, i.e. cardiomyocyte growth, dysfuction and death, and increased extracellular

matrix deposition and fibrosis, are main processes initiated by pathological stimuli, in addition to inflammation. We have studied proteoglycans in inflammation, cardiomyocyte growth and dysfunction, cardiac myofibroblast differentiation and fibrosis. In particular, we have focued on the role of syndecan-4 in these processes in the heart, with focus on pressure overload and lipopolysaccharide (LPS)-induced cardiac dysfunction and failure. Others have studied syndecan-1 and -4 in the setting of myocardial infarction, while syndecan-2 and -3 have not been studied in the heart. In this presentation, the roles of syndecans in cardiac dysfunction and failure will be summarized and discussed.

Invited Lecture (L6) Heparin: new clinical uses for an old anticoagulant

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Heparin is one of the oldest drugs still used in large scale in the clinic. It is a sulfated glycosaminoglycan obtained exclusively from pig and bovine intestine. Its main clinical use is as a blood clot inhibitor. Heparin chains contains several domains with specific sulfation patters that form the binding sites to different ligands, such as antithrombin, growth factors, adhesion molecules, lipases, etc. As a result, heparin displays other important pharmacologic effects such as anti-inflammatory, anti-viral and anti-neoplasic. In fact, several clinical trials have been designed to evaluate the effects of heparin treatment beyond anticoagulantion. We have investigated the anti P-selectin effect of heparin and heparin analogs and their effect on experimental colite in rats. These glycans drastically reduces colon inflammation by inhibiting cellular recruitment, production of pro-inflammatory cytokines after intra-peritoneal administration. Additionally, LPS-mediated macrophage activation is also drastically attenuated.

ST01/P01 Perlecan expression influences the keratin 15-positive cell population fate in the epidermis of aging skin

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The epidermis, primarily made of keratinocytes, is continuously renewed by the proliferation of stem cells and the differentiation of their progeny, which undergo terminal differentiation as they leave the basal layer and move upward toward the surface, where they die and slough off. Basal keratinocytes append the dermal-epidermal junction, a cell surface-associated extracellular matrix that forms as a concerted action of both epidermal and dermal cells. The dermal-epidermal junction provides both a structural support to keratinocytes and a specific niche that mediates signals influencing their behaviour. It consists of laminins, type IV collagen, nidogens, and perlecan, which are necessary for tissue organization and structural integrity. The appearance and mechanical functions of skin undergo profound changes over time with both increasing chronological age and cumulative exposure to external factors, such as ultraviolet radiation. In addition to an overall thinning of the epidermis and dermis, a major change is the flattening of the dermal-epidermal junction resulting from retraction of both epidermal papillae and microprojections of basal cells into the dermis. Epidermal stem cell niches weaken with age, due to the reduced expression of cell adhesion molecules and factors that control their self-renewal, therefore affecting the self-renewing properties of the epidermis basal layer.
We have obtained a series of compelling findings showing that the heparan sulphate (HS) proteoglycan perlecan, plays a role in the maintenance of epidermal homeostasis during skin aging. We found

decreased expression of perlecan in the epidermal and microvessel basement membranes during chronological skin aging. Our *in vitro* studies with keratinocytes from aged donors confirmed these findings and also indicated reduced levels of perlecan transcription. The use of *in vitro* skin models comprising epidermal keratinocytes from young and elderly donors showed that perlecan influences epidermal thickness. We provided evidence that keratinocyte-associated perlecan might contribute to basal keratinocyte phenotype maintenance by down-regulating the expression of early and intermediate differentiation markers and up-regulating the expression of keratin 15 (K15). This finding was particularly important, because K15-expressing keratinocytes are typically considered to be self-renewing stem cells. Our results further revealed that the mechanism involved the perlecan HS moities, known to participate in the presentation of growth factors to their high-affinity receptors. Finally, we found defects in keratin 15 expression in the epidermis of aging skin. This study highlighted a new role for perlecan in maintaining the self-renewal capacity of basal keratinocytes.

Reference:
Perlecan expression influences the keratin 15-positive cell population fate in the epidermis of aging skin. Aging, 2016, Mar 17.

ST02/P02 Syndecan-4 is important for normal skeletal muscle fibre development

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The cell surface proteoglycan syndecan-4 has been reported to be essential for muscle differentiation, but few molecular mechanisms are known. Syndecan-4$^{-/-}$ mice are unable to regenerate damaged muscle, and display deficient satellite cell activation, proliferation, and differentiation. We have previously shown that syndecan-4 is important for myoblast fusion and myotube formation in primary skeletal muscle cells. Physical activity is a factor that influence adaptations in muscle, and has long been known to cause an increase in adult skeletal muscle size. Interestingly, the syndecan-4 gene expression is reported to be up-regulated in muscle after exercise. In the present study we have investigated: 1) muscle fibre development and 2) effect of exercise in syndecan-4$^{-/-}$ mice.
Our recently data showed that syndecan-4$^{-/-}$ mice had significantly lower bodyweight and lower weight of the *tibialis anterior* (leg muscle) compared to wild type (wt) mice. A fraction of the syndecan-4$^{-/-}$ muscle fibres had also smaller cross-sectional area, but the number and type of fibres (slow/fast) did not differ. Also, the mRNA expression of transcriptional activator proteins, like *myod* (myoblast determination protein) and *myogenin,* were significantly lower in syndecan-4$^{-/-}$ compared to wt mice at 22 weeks. At protein level, syndecan-4$^{-/-}$ showed no differences in known signalling proteins like Erk 1/2, Erk5, Fak, NFAT, and PKCα. Although syndecan-4 is important for collagen cross-linking in heart muscle, no differences were observed in collagen I or IV protein level or cross-linking analysed by immunofluorescence. Neither was there any difference in the mRNA level of different extracellular matrix (ECM) components like *collagen I*, *collagen III*, *lox*, *decorin*, *biglycan* and *fibromodulin*. Exercise of syndecan-4 $^{-/-}$ mice (tread mill) increased mRNA levels of *myogenin, desmin, lox* (lysyl oxidase, important for collagen crosslinking) (ongoing experiments). Altogether, our results so far suggest that syndecan-4 is important for normal muscle fibre development with only minor impact on the ECM (sedentary mice). However, some ECM components might be changed in the syndecan-4$^{-/-}$ mice after exercise.

ST03/P03 Hyal-1 deficiency protects the endothelium in a mouse diabetic model

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Background. Hyaluronic acid (HA) is a major component of the glycocalyx. In diabetes, the size and permeability of the glycocalyx are altered. In addition, type 1 diabetic patients have increased plasma levels of both HA and its somatic hyaluronidase Hyal-1, which is endocytosed by endothelial cells.
Objective. To investigate the potential implication of Hyal-1 in the development of diabetes-induced endothelium dysfunction.
Methods. The following measurements were obtained in Hyal-1 deficient (KO) and wild-type (WT) mice 4 weeks after they became diabetic following daily injections of streptozotocin during 5 days: plasma HA and Hyal-1 activity, markers of endothelial dysfunction, glomerular barrier properties (urinary albumin/ creatinine and 70/40-kDa dextran ratio), myocardial arterioles glycocalyx (using transmission electron microscopy), and mesenteric artery endothelium-dependent vasodilation. The expressions of various components of the endothelium-derived hyperpolarizing factor (EDHF) pathway were evaluated by real time PCR.
Results. KO mice had higher plasma HA concentration than WT mice. Plasma HA increased with diabetes in WT mice but was unaffected in diabetic KO mice. A slight but significant increase of Hyal-1 activity was detected in WT mice during diabetes. ICAM-1 but not VCAM-1 was significantly up-regulated by diabetes in both KO and WT mice. On the other hand, P-selectin increased during diabetes only in WT mice. The glycocalyx had a greater thickness in KO vs WT mice and this difference persisted after 4 weeks of hyperglycemia. At that time, HA did not accumulate in the aortic wall but glycocalyx HA had almost vanished in WT mice whereas it was not affected in diabetic KO mice. Moreover, the 70/40-kDa dextran ratio, as well as the albumin/creatinine ratio, in urine were increased in diabetic WT mice but not in diabetic KO mice, suggesting better protection of the glomerular barrier. Endothelium-dependent vasodilation of mesenteric arteries did not differ between healthy WT and KO mice. However, in WT diabetic mice, EDHF-mediated vasorelaxation was severely impaired whereas KO diabetic mice had a partially preserved EDHF pathway. Among various components of the EDHF pathway, only SK3 channels were up-regulated in KO mice, both at basal level and after diabetes induction. Activation of SK3 channels using Cyppa confirmed a higher SK3-dependent relaxation in KO vs WT mice, with and without diabetes.
Conclusion. Decreased levels of Hyal-1 orient the diabetic endothelial response towards a preserved glycocalyx, less inflammation, a preserved glomerular barrier, and lesser damage to the EDHF-dependent vasodilation pathway. The mechanisms of this protection could be partly due to SK3 up-regulation. Links between Hyal-1 deficiency and SK3 regulation, as well as between the glycocalyx and the EDHF pathway, need to be further investigated.

ST04/P04 The role of versican in semicircular canal development in the zebrafish inner ear and in cancer progression

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Versican is a chondroitin sulphate proteoglycan of the extracellular matrix in all vertebrates, implicated in cellular processes such as cell adhesion, proliferation and migration. Versican core protein genes are expressed in the zebrafish inner ear, where morphogenesis of semicircular canals requires a fusion event between epithelial projections, forming pillars of epithelium that span the ear lumen. Transcripts for *vcana* and *vcanb* are strongly expressed before this fusion event and sharply down-regulated as soon as the fusion is complete, and thus Versican is likely to influence epithelial outgrowth and cell adhesion during semicircular canal morphogenesis. In the zebrafish *gpr126* mutant ear, *versican* genes fail to be

down-regulated, remaining expressed at high levels; epithelial fusion fails, and the ear shows concomitant morphological defects.
Versican has also been reported to be up-regulated in many types of epithelial cancer in humans, and its increased expression has been linked with enhanced metastasis and poor patient outcome. Here we speculate that Versican may represent one mechanism whereby tumour cells alter their microenvironment to facilitate the malignant growth, migration and invasion. Using the zebrafish *gpr126* mutant as an *in vivo* screening tool, we have identified several chemical compounds that can modify otic *versican* expression. We aim to test whether the positive hit compounds can also manipulate *versican* expression in a mammalian cancer cell context. To this end, we will examine the effects of the compounds on cell viability, proliferation and migration of stromal (fibroblast) and tumour cell lines that over-express Versican *in vitro*. A greater understanding of the function and regulation of Versican will not only shed light on the biological processes underlying development and morphogenesis, but may also contribute to the development of therapeutic methods to inhibit tumour invasion.

ST05/P05 Lumican Inhibits SNAIL-Induced Melanoma Cell Migration Specifically by Blocking MMP-14 Activity

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Lumican, a small leucine rich proteoglycan, inhibits MMP-14 activity and melanoma cell migration *in vitro* and *in vivo*. Snail triggers epithelial-mesenchymal transitions endowing epithelial cells with migratory and invasive properties during tumor progression. The aim of this work was to investigate lumican effects on MMP-14 activity and migration of Snail overexpressing B16F1 (Snail-B16F1) melanoma cells and HT-29 colon adenocarcinoma cells. Lumican inhibits the Snail induced MMP-14 activity in B16F1 but not in HT-29 cells. In Snail-B16F1 cells, lumican inhibits migration, growth, and melanoma primary tumor development. A lumican-based strategy targeting Snail-induced MMP-14 activity might be useful for melanoma treatment.

P06 Ext proteins in breast cancer cell lines

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Introduction: Breast cancer remains one of the major cause cancer deaths among women in the world. Research into the biology of the disease, aimed at identification of better biomarkers, drug targets and improved delivery of therapeutic molecules, has been intensified. Heparan sulfate (HS) proteoglycans are ubiquitously expressed in all tissues, where they function as adhesion molecules and co-receptors. Thus, they modulate cell/matrix interactions and growth factor signaling. We study the regulation of the EXT-family of proteins, EXT1, EXT2, EXTL1, EXTL2, and EXTL3, in cancer cell lines. Whereas the EXT1, EXT2 and EXTL3 proteins are clearly involved in HS chain elongation, the roles of EXTL1 and EXTL2 are unclear[1]. There are many types of breast cancer that differ in their capability of metastasizing to other body tissues. In this study, we aim to determine the gene expression profiles of

the EXT family and to correlate the gene expression profiles to HS structure and function. The cell lines we analyze include one normal mammary gland epithelial cell line, one luminal (less aggressive) and two triple negative (more aggressive) breast cancer cell lines.
Methods: We used real-time PCR to determine the expression levels of the *EXT/L*s in the breast cancer cell lines. Cell surface HS was assessed by flow cytometry and the ability of the different cell lines to respond to growth factor stimuli was determined by western blotting after FGF2 stimulation.
Results: Real time PCR revealed that *EXT1* and *EXT2* levels were significantly reduced in the luminal (MCF7) but not the two triple negative (MDA-MB231 and HCC38) cell lines, whereas, an opposite pattern was found for the expression of EXTL2. All cancer cell lines showed a reduced expression of *EXTL3* and none of the cell lines expressed *EXTL1*. Using an HS specific antibody revealed that the relative amounts of cell surface HS was similar in the cancer cell lines and the normal mammary gland epithelial cell line. Our findings further show that intracellular signaling, measured as phosphorylation of ERK upon stimulation with FGF2, was strongly cell type specific and correlated with published data of the expression levels of FGF-receptor isoforms on some of these cell lines[2, 3].
Conclusion: Our data indicate that *EXT/L* gene profiles may not correlate to the amount of HS or the function of HS in these cell lines.
References:
1.Busse-Wicher M., et al. Matrix Biol. 2014;35:25-33.
2.Nurcombe V., et al. J Biol Chem. 2000;275(39):30009-30018.
3.Delehedde M., et al. Exp Cell Res. 1996;229(2):398-406.

P07 Glypican-6 is upregulated in heart failure and mediates cardiomyocyte growth through ERK1/2 signaling

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Pressure overload is a leading cause of heart failure, a disease affecting millions of people worldwide. Cell surface proteoglycans are important regulators of signaling events and affect processes such as wound healing and cancer progression, but their roles in cardiac disease has just recently been explored. We have investigated the six-membered glypican (GPC1-6) family, evolutionary ancient heparan sulfate proteoglycans anchored to the extracellular part of cell membrane, *in vivo* in mice with increased left ventricular (LV) afterload, in cardiac biopsies from patients and in cultured cardiac cells.
Our results show that mice subjected to increased LV afterload by aortic banding (AB; n=88) had elevated LV GPC6 mRNA levels during hypertrophic remodeling (1 and 3 weeks of AB; 2.8- and 1.9-fold) and dilated, end-stage heart failure (16 and 18 weeks of AB; 2.0- and 2.4-fold). Immunoblotting confirmed GPC6 protein upregulation. There were minor alterations in GPC1-4 mRNA, while GPC5 was not expressed. GPC6 mRNA was 1.8-fold higher in LV tissue from explanted hearts of patients with end-stage, dilated heart failure (NYHAIII-IV, n=18) than in controls (n=7). A negative correlation with LV ejection fraction in patients and positive correlations with lung weight and LV weight after AB in mice suggested that increased GPC6 levels are associated with worsening heart failure. In primary cell cultures from neonatal rat hearts, GPC6 mRNA was 3.8-fold higher in fibroblasts than cardiomyocytes, indicating fibroblasts as the main source of cardiac GPC6. Adenoviral overexpression of GPC6 yielded GPC6 of different size, i.e. >250 kDa in fibroblasts and 150-250 kDa in cardiomyocytes, reflecting differences in glycosylation. Viral overexpression of GPC6 in cardiac fibroblasts did not affect collagen expression (COL1A2 and COL3A1 mRNA), myofibroblast transdifferentiation (ACTA2 mRNA),

proliferation (PCNA mRNA) or migration (scratch assay), suggesting GPC6 does not affect fibrosis in the heart. Importantly, overexpression of GPC6 induced mRNA expression of the pro-hypertrophic marker gene ACTA1 and the hypertrophy and heart failure marker genes encoding natriuretic peptides, NPPA and NPPB, as well as increased protein synthesis 1.5-fold (radioactive leucine incorporation assay). Overexpression of GPC6 induced ERK phosphorylation, and co-treatment with the ERK inhibitor U0126 inhibited the GPC6-induced increase in NPPA, NPPB, ACTA1 and protein synthesis.
In conclusion our data suggests that glypican-6 plays a role in clinical and experimental heart failure progression by regulating cardiomyocyte hypertrophy through ERK signaling.

P08 Fourier transform infrared microspectroscopy and imaging of non-inflammatory and inflammatory breast cancer cell lines and tissues: glycosaminoglycans as potential spectral markers?

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Introduction: Inflammatory breast cancer (IBC) is the most lethal form of breast cancer. Late diagnosis of IBC is the leading cause of poor prognosis due to lack of specific biomarkers. Fourier-transform infrared (FTIR) microspectroscopy and imaging can be used as label-free approach to detect the early modifications caused by cancer cells due to their capacity to detect any biochemical changes before any histological changes. Glycosaminoglycans (GAGs) play an important role in breast cancer progression. Their structure and content were found to be different in normal and cancer cells and even between different cancer cell types. Previously, we identified the infrared spectral signatures of standard GAGs and differentiated cell types based on their capacity of GAG synthesis.
Aims: The aim of the present work was to differentiate between non-IBC and IBC cell lines and tissues using FTIR microspectroscopy and imaging based on GAG spectral contribution.
Materials and Methods: Four human breast cancer cell lines, MDA-MB-231, SK-BR3 and MCF-7 (non-IBC) and SUM-149 (IBC) were used in this study as well as their respective conditioned media. Cells were analyzed as suspensions and as single isolated cells using FTIR microspectroscopy and imaging, respectively. Extracted GAGs from conditioned media were also analyzed. A total of 20 breast cancer patients (10 non-IBC and 10 IBC) and 3 healthy volunteers from Ain Shams University hospital in Egypt were enrolled in this study after signing consent form. Breast carcinoma and normal breast tissues from healthy volunteers were collected during modified radical mastectomy surgery and during breast reduction surgery, respectively. Breast tissues were analyzed using FTIR imaging. Cell data were analyzed by exploratory unsupervised chemometric methods based on hierarchical cluster analysis (HCA) and principal component analysis (PCA). Tissue images were pre-processed and analyzed using common K-Means and Fuzzy C-Means Cluster-Algorithms.
Results: HCA and PCA analysis of GAG spectra of different breast cancer cell line conditioned media showed low intra-group variability and high inter-group variability enabling us to differentiate between higher and lower invasive and metastatic cell lines. Moreover, non-IBC and IBC cell line conditioned media were differentiated. In the same manner, HCA and PCA analysis of GAG spectra obtained from different breast cancer cell lines showed low intra-group variability and high inter-group variability sufficient to distinguish between different breast cancer cell lines and also between non-IBC and IBC

cell types. Breast tissue analysis, by common K-Means and Fuzzy C-Means Cluster-Algorithms, allowed firstly to discriminate between healthy tissues and breast carcinoma, and secondly to distinguish non-IBC and IBC tissues. Adjacent normal tissues surrounding tumor mass were different in both breast cancer types in comparison with healthy tissues.
Conclusion: Our study demonstrates that FTIR microspectroscopy/Imaging has the potential to discriminate between healthy and cancer tissues but more importantly between non-IBC and IBC at both cell lines and tissues based on GAG spectral contribution.

P09 Inflammatory oxidants modify components of the extracellular matrix of human atherosclerotic lesions

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The extracellular matrix (ECM) of the vascular basement membrane is critical to the functional and mechanical properties of arteries, with matrix proteins interacting with growth factors and enzymes to regulate endothelial and smooth muscle cell activity. These interactions are perturbed in atherosclerosis, where activated neutrophils, monocytes and macrophages generate oxidants, including peroxynitrous acid, that can give rise to endothelial cell dysfunction, alter ECM composition, and contribute to vascular fibrosis such as arterial stiffening and ECM remodelling and plaque formation.
The studies reported here aimed to investigate the effects of peroxynitrous acid, on isolated ECM proteins and ECM synthesised by human coronary artery endothelial cells (HCAEC), as well as examining the presence of oxidised ECM in human atherosclerotic lesions. Perlecan, fibronectin and laminins were detected in the native HCAEC-ECM *in vitro,* and in the basement membrane of human atherosclerotic lesions. A loss of antibody reactivity against perlecan protein core and the cell binding epitopes of fibronectin and laminin was detected upon exposure to peroxynitrous acid, with increased antibody recognition of 3-nitrotyrosine, 6-nitrotryptophan and dityrosine, well-established products of peroxynitrous acid, also detected. These changes were confirmed and quantified by both parent amino acid loss, and product formation, by UPLC with fluoresence and electrochemical detection. The location of some of these modified amino acids on the ECM materials has been mapped by LC-MS/MS peptide mass mapping, with a number of modifications detected in critical functional domains.
Peroxynitrous acid treatment decreased HCAEC adhesion and spreading (diminished F-actin stress fibre formation and crystal violet staining) on ECM materials, when compared to untreated matrix. HCAEC exposed to peroxynitrous acid-modified ECM, up-regulate multiple inflammation-associated genes including matrix metalloproteinases 7 and 13, and down-regulate integrins associated with cell adhesion. The biological relevance of the observed ECM changes has been examined in advanced human atherosclerotic lesions, with co-localisation of laminins and perlecan with 3-nitrotyrosine epitopes detected. Peroxynitrous acid-modified laminins was also detected in proteins extracted from human atherosclerotic lesions.
In conclusion, peroxynitrous acid modifies HCAEC-generated ECM by inducing structural and functional changes to perlecan, laminins and fibronectin, which are important in determining endothelial cell function. These data suggest a mechanism through which peroxynitrous acid can modify the arterial ECM basement membrane in atherosclerotic lesions and subsequently lead to ECM remodelling.

P10 Syndecan-4 expression is upregulated in endometriosis and contributes to an invasive phenotype

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Introduction: Endometriosis is characterized by ectopic growth of endometrial tissue, and is associated with pain symptoms and reduced fertility[1]. The heparan sulfate proteoglycan Syndecan-4 modulates several processes potentially relevant to the disease including cell motility and proteolysis[2,3]. In the present work, we study the expression of Syndecan-4 in endometriotic tissue, and its role in the behavior of endometriotic cells in vitro.
Methods: Eutopic endometrial tissue (62 controls/44 endometriosis) was investigated by immunohistochemistry for the expression of Syndecan-4. The human endometriotic cell line 12Z was transiently transfected with Syndecan-4 small interfering RNA and investigated for changes in cell behaviour.
Results: Syndecan-4 expression was significantly higher in the glands and stroma of endometriosis patients compared to controls ($P<0.001$), whereas no menstrual-cycle dependent expression was observed. In 12Z cells, Syndecan-4 depletion did not affect cell viability, but resulted in a significantly reduced matrigel invasiveness ($p<0.05$), and reduced expression of the small GTPase Rac1, the transcription factor ATF-2, and MMP3.
Discussion: The upregulation of Syndecan-4 in the eutopic endometrium of endometriosis patients may facilitate the pathogenetic process by promoting invasive cell growth via Rac1, MMP3 and ATF-2.
References:
1. Schneider C, Kässens N, Greve B, Hassan H, Schüring AN, Starzinski-Powitz A, Kiesel L, Seidler DG, Götte M. Fertil Steril. 2013;99:871-881.e1.
2. Götte M. FASEB J. 2003;17:575-91.
3. Multhaupt HA, Yoneda A, Whiteford JR, Oh ES, Lee W, Couchman JR. J Physiol Pharmacol. 2009;60 Suppl 4:31-8.

P11 Evaluation of the activity of Lumican in the functional properties of matrix molecules of breast cancer cells of different estrogen receptor patterns

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Lumican is a small leucine-rich proteoglycan that has been shown to contribute to several physiological processes, but also to exert anticancer activity and to compromise the activity of membrane type matrix metalloproteinase (MMP-14) [1,2]. On the other hand, it has been recently shown that suppression of the estrogen receptor-α (ERα) significantly alters the functional properties of breast cancer cells in terms of growth, migration, proliferation as well as the gene expression profile of matrix effectors related to cancers progression and cell morphology [3]. The aim of the present study was to evaluate the effects of lumican in four breast cancer cell lines [shERαMCF-7, with knockdown of ERα and shERβMDA-MB-231, with suppression of ERβ and their control ones, respectively]. Lumican proved to be a powerful anticancer effector in terms of proliferation, migration, invasion, and activity of MMP-14 in all four cell lines. The effect of lumican was found to be related to the type of breast cancer cells and the estrogen receptor status. Further works are necessary to investigate the mechanisms of action and the signaling transduction pathways involved. As a perspective, the effect of lumican on the regulation of breast

cancer metastasis will be studied to elucidate whether lumican could be a good candidate as a potent anticancer agent in solid tumors including invasive breast cancer.
Keywords: Lumican; breast cancer; estrogen receptors, matrix metalloproteinases
References:
1. Pietraszek K., Chatron- Colliet A., Brezillon S., Perreau C., Jakubiak- Augustin A., Krotkiewski H., Maquart FX., Wegrowski Y., Lumican: A new inhibitor of matrix metalloproteinase- 14 activity, FEBS Letters, 2014, 588 (23): 4319- 4324.
2. Theocharis AD., Skandalis SS., Tzanakakis GN., Karamanos NK., Proteoglycans in health and disease: novel roles for proteoglycans in malignancy and their pharmacological targeting. FEBS journal, 2010, 277 (19): 3904- 3923.
3. Bouris P., Skandalis SS., Piperigkou Z., Afratis N., Karamanou K., Aletras AJ., Moustakas A., Theocharis AD., Karamanos N.K., Estrogen receptor alpha mediates epithelial to mesenchymal transition, expression of specific metric effectors and functional properties of breast cancer cells, Matrix Biological, 2015, 43: 42- 60.

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P12 Glycocalyx in endothelium inflammation: changes in both glycosaminoglycans and proteoglycans

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Inflammation is an extremely complex series of events that tissues undertake after harmful stimuli; besides the alteration of the cellular behavior, inflammation also causes the modification of tissue architecture and of the extracellular matrix (ECM). The tissue response to the injury can be therefore the starting point for the onset of several diseases, e.g. vascular pathologies, atherosclerosis etc. Noteworthy ECM modifications caused by cytokines (e.g. TNF-alfa or IL-1) and/or altered molecules such as oxidized LDL are also involved in the recruitment/activation of inflammatory cells.
In the arterial vessel wall, endothelial cells regulate the vascular tone, the thrombosis/fibrinolysis balance, and the adhesiveness of inflammatory cells; most of these abilities are due to molecules of the so-called glycocalyx: i) hyaluronan (HA) that promote monocyte/macrophage adhesion through interactions with the hyaluronan receptor CD44 present on inflammatory cell surfaces; ii) heparin/heparan sulfate proteoglycans (HS/HE PGs) such as syndecans.
HUVEC cells were used as a model to study the mechanism that regulates syndecans synthesis after treatment with the proinflammatory cytokine TNF-alfa. HS/HE polymerization enzymes (EXT1, EXT2, NDST1) and syndecans core protein expression were evaluated by qPCR, and HS/HE disaccharides by means of HPLC techniques. HA metabolism was investigated by the expression of the synthetic enzymes HAS2 and HAS3 as well as the quantification of HA by ELISA assay.
The HUVECs response to TNF-alfa was modulated within the 48 hours: EXT1, EXT2, NDST1 increased their expression at 24 hours and EXT1 and EXT2 were back to control levels at 48. In parallel, syndecans core proteins had an increase in syndecans -3 and -4 expression at 24 hour that were changed by syndecans -1 and 4 at 48 hours.
HS/HE disaccharide composition demonstrates an higher amount of N-sulfated modification, according to the synthetic enzymes expressions.
The main HA synthetic enzymes HAS2 was upregulated, while HAS3 role in HA accumulation is still under evaluation.
The inflammation of the endothelial cells induced dramatic changes in the ECM they produce, in particular, besides the HA increased production, they modified the composition and expression of the

HS/HE PGs, in a specific temporal order, starting with GAG polymeric enzymes and syndecans-1 core protein to the syndecans-4 PG. Those events are therefore involved in the monocyte recruitment within the inflammatory site. As reported in several studies syndecans-4 expression is related to NO production and release from endothelial cells, we observed that in TNF-alfa stimulated HUVEC, the expression of NOS enzymes is highly increased, accounting for the changes in permeability and adhesiveness in in vivo.

P13 IGF-IR / biglycan signaling axis regulates osteosarcoma cell growth

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Osteosarcoma (OS) is the most common bone primary tumor. It affects both children and adolescents; with a second peak incidence in the middle aged population. Biglycan is a member of the small leucine rich proteoglycans family (SLRPs) and an important structural as well as a functional component of the bone extracellular matrix. IGF-IR is a hetero-tetrameric transmembrane glycoprotein with tyrosine kinase activity. Upon ligand binding, IGF-IR downstream signaling is activated. IGF-IR/IGF-I signaling regulates both bone development and homeostasis. Previously, we had demonstrated that biglycan affects OS cell migration. In the present study by utilizing *in vitro* cell proliferation assay, siRNA transfection, Real-time PCR and western blotting we show that IGF-IR / biglycan downstream signaling regulates OS cell growth in a cyclin B1 and p53 dependent manner. Specifically, treatment with IGF-I (10 ng) strongly enhanced both OS cell growth ($p \leq 0.01$) and biglycan expression ($p \leq 0.01$). The reduction of biglycan expression was strongly correlated with inhibition of MG63 osteosarcoma cell growth ($p \leq 0.01$). Biglycan- deficient cells had an attenuated IGF-I –dependent growth response ($p \leq 0.05$); while exhibiting decreased cyclinB1 ($p \leq 0.01$) and increased p53 expression ($p \leq 0.01$). The latter results correlate well with a G2 cell cycle arrest. The present study therefore suggests that IGF-IR / biglycan signaling axis affects OS cell cycle progression.

P14 The effect of serglycin's suppression on various cellular aspects of glioblastoma cell line LN-18

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Serglycin, the only insofar characterized intracellular proteoglycan, possesses dominant roles in hematopoietic and in a variety of non-hematopoietic cells. It was only until recently that serglycin's functional importance has been emerged in malignancies. In particular, serglycin is overexpressed and be secreted by aggressive tumor cells and plays critical role in several cellular processes such as proliferation, migration and invasion. Previous study of our laboratory reveals that the overexpression of serglycin correlates with an enhanced aggressive and metastatic phenotype of breast cancer cells. Assessment of serglycin's expression by immunohistochemistry in different kind of tumors, among others breast cancer and glioblastoma (GBM), reveals a strong staining for serglycin in malignant tissues in contrast to normal ones. Taking into account the above, our goal was the investigation of the contribution of serglycin to the aggressiveness of GBM cells, which are characterized by rapid cell proliferation and high infiltration to the surrounding normal brain tissues. For this reason, we used the GBM cell line LN-18, which expresses high levels of serglycin, and we suppressed its expression levels establishing stably transfected cell line. Then, we examined crucial functional properties as well as the expression of molecules related with them including various extracellular matrix (ECM) molecules. Our data revealed alterations at the morphology and at some cellular properties of the LN-18 transfected cells such as cell migration, proliferation capacity and cellular invasiveness. Also, we observed variations at the expression levels of ECM molecules including proteolytic enzymes (MMPs and uPA)

and cell surface receptors, but also at the expression and secretion levels of interleukins. These results imply that serglycin may conduce to the aggressive potential of GBM cells, strengthening the view of the regulatory role of serglycin in several malignancies.

P15 IGF-I regulates HT1080 cell migration through Syndecan 2 and Erk1/2 dependent signaling

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Fibrosarcoma is a tumor of mesenchymal origin, predominantly derived from fibroblasts. Targeted therapy has not been developed for this rare, soft tissue tumor. Insulin like growth factor–I (IGF-I) and its respective IGF-I receptor (IGF-IR) are implicated in cancer development. Syndecan 2 (SDC2) is a transmembrane, heparan sulfate containing- proteoglycan acting as a co-receptor for various growth factor receptors. In the present study, utilizing an *in vitro* chemotactic migration assay, siRNA transfection, Real-time PCR, confocal microscopy as well as western blotting, we show that IGF-I in a SDC2 / Erk1/2 –dependent manner enhances HT1080 fibrosarcoma cell migration. In detail, IGF-I (10ng/ml) increased chemotactic migration of HT1080 cells towards; fibronectin (FN) and Fetal Bovine Serum (FBS) with statistical significance of $p<0.01$ and $p<0.001$, respectively. Utilization of an antibody specific for SDC2 demonstrated that IGF-I treatment significantly increased SDC2 protein expression in HT1080 cells as compared to control ($p<0.001$). Well in accordance with these data, immunofluorescence showed a stronger SDC2 signal in IGF-I treated cells. SDC2-deficient cells treated with IGFI exhibited significantly decreased cell migration as compared to siScr controls ($p<0.001$). Confocal microscopy and immunoprecipitation demonstrated a co-localization of IGF-IR and SDC2 in HT1080 cells. The inactivation of Erk1/2 through the addition of Erk1/2 inhibitor led to a strong suppression of IGF-I-dependent migration ($p<0.001$). Transfection with siSDC2 showed that SDC2 was crucial for both IGFI-induced ($p<0.001$) and basal ERK1/2 activation ($p<0.001$). These data suggest that SDC2 may be a co-receptor to IGF-IR facilitating its Erk1/2 –dependent downstream signaling to affect HT1080 cell migration.

P16 The role of Collagen XVIII in murine kidney development and function

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Collagen XVIII (ColXVIII) is a heparan sulfate proteoglycan with a complex multidomain structure and it is ubiquitously expressed in endothelial and epithelial basement membranes (BMs) throughout the body. In adult mouse kidney the short ColXVIII variant localizes to the endothelial side of the glomerular BM, whereas the two longer variants reside at the podocyte side. The absence of the short variant leads to loosening of the tubular BMs, and the lack of the longer variants causes podocyte foot process effacement. In an embryonic day 10.5 (E10.5) kidney ColXVIII is expressed throughout the epithelial ureter bud (UB) in the kidney. At E11.5 when the UB starts to branch the expression of ColXVIII becomes restricted to the stalk region of UB and is lost from the ureter tips, which suggests that re-specification of ColXVIII expression is associated with the initiation of branching.
We use in our studies ColXVIII total knock-outs which lack all three ColXVIII variants, and promoter specific knock-out mice which lack either the short variant or the two longer variants. The expression of ColXVIII and other molecules in embryonic kidneys are studied using whole mount and section *in situ* hybridizations, electron microscopy (EM), immunohistochemical stainings, optical projection tomography, organ and cell cultures as well as quantitative PCR.
Our results indicate that only the short form of ColXVIII is expressed in the UB at E11.5 when the UB has branched once. Similarly, in E14.5 and E16.5 kidneys only the short isoform is expressed during the

early phase of glomerular and tubular development, whereas the two longer variants are expressed only in the more matured tubules and glomeruli. The isoforms have different localizations in the glomeruli so that the longer variants are expressed by the podocytes and the short variant is expressed by the cells of the Bowman's capsule and especially in the layer of parietal epithelial cells.
Analysis of the ureteric tree development by Wnt11 *in situ* and cytokeratin-8 (Troma-1) stainings from E14.5-16.5 and new born pups indicate that especially the short isoform is important for the normal development of the ureteric tree but also the longer variants have a role in regulating the ureteric branching pattern. EM studies from E14.5, E16.5 and new born pups' kidneys show that the lack of the short isoform causes broadening of the BM of the proximal and distal tubules, and the lack of the two longer forms leads to podocyte foot process effacement already in the embryonic stage.

P17 Loss of ERα triggers the synthesis of hyaluronan and induces the expression and redistribution of CD44 and moesin in breast cancer cells

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ERα knock-down in MCF-7 breast cancer cells resulted in the transition of cells from the epithelial to mesenchymal phenotype. The strong morphological changes were followed by significant changes in the expression of multiple matrix macromolecules [1]. In the present study, the expression of hyaluronan synthesizing enzymes (HASes) and hyaluronan as well as its major cellular receptor CD44 were examined. The results revealed that ERα-suppressed cells produced significantly higher hyaluronan amounts, which was found to be the result of the specific up-regulation of HAS2, but not HAS1 and HAS3. Moreover, the expressions of CD44s and specific CD44v were significantly up-regulated in these cells.
Moesin is an ERM family protein that connects the actin cytoskeleton to transmembrane receptors such as CD44. The distribution and role of CD44 and moesin in breast cancer cells of different ER status were investigated. The results revealed a low to moderate expression of these proteins in the epithelial ER-positive (ERα+/ERβ-) MCF-7 cells, while their expression was higher in the mesenchymal ER-negative (ERα-/ERβ+) MDA-MB-231 cells. The distribution of these proteins was similar in MCF-7 cells (only 30-40% of the cells expressed CD44 and moesin mainly at the cell periphery). On the other hand, MDA-MB-231 cells showed ubiquitous distribution for both proteins with a preference to localize at their migrating front. Interestingly, a substantial increase of CD44 and moesin as well as their localization at the cell migrating front/ruffles of ERα-suppressed MCF-7 cells (MCF-7/SP10+) were observed similarly to MDA-MB-231 cells. However, ERβ knock-down in MDA-MB-231 (MDA-MB-231/ERβ-) cells did not result in significant changes in the expression and distribution of CD44 and moesin in these cells. Notably, the selective removal of cell surface heparan sulfate (HS) chains from MCF-7/SP10+, MDA-MB-231/ERβ- and their parental cell lines revealed significant changes mainly in the ERα-suppressed cells, which were characterized by an increased migratory potential and more localized distribution of both CD44 and moesin proteins within the cell migrating front/ruffles suggesting a role of cell surface HS proteoglycans in these events.
References:
1. Bouris P, Skandalis SS, Piperigkou Z, Afratis N, Karamanou K, Aletras AJ, Moustakas A, Theocharis AD, Karamanos NK. Matrix Biology, 43:42-60 (2015)

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P18 Hyaluronan in vitro modulation of macrophages in colorectal adenocarcinoma microenvironment

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Hyaluronan (HA), a glycosaminoglycan normally present in the extracellular matrix, acts as a modulator of immune and angiogenic responses(1). At homeostasis, high-molecular weight HA (HMW) is predominant, having hydrodynamic properties whereas the low-molecular weight (LMW) form is present mainly during inflammation(1). Macrophages are able to bind HA, previous studies showed that LMW HA polarize macrophages to M1 phenotype in vitro whereas HMW HA is associated with a M2 activation(2). HA present in tumor microenvironment serves as a signal for recruiting tumor associated macrophages(3), that are able to regulate angiogenesis releasing pro-angiogenic factors(1,4).
Aim: To evaluate the effect of HA, in its HMW and LMW form, on colorectal adenocarcinoma tumor-pulsed human macrophages using the THP-1 cell line.
Methods: The human monocytic cell line THP-1 was differentiated into active macrophages with PMA (25ng/ml). 72h later, were pulsed with tumor lysates(TL) (prepared with LoVo colorectal adenocarcinoma cell line) and treated with HA (20ug/ml) LMW (1,5-1,8x10^6Da) or HMW (1-3x10^5Da) for 24h. Macrophages were characterized with CD80 and MHC II by flow cytometry. Gene expression levels of CD44 and TLR4, were analyzed through RT-qPCR. IL10, IL12 and VEGF biosynthesis were evaluated by ELISA assay and MMP-2 by zymography.
Results: When THP-1 macrophages were treated with TL alone and together with HA, HMW or LMW, an up-regulation of MHC II and CD80 was observed, levels similar to M1 control. When pulsed with TL plus HA HMW, the biosynthesis of IL-12, IL-10 and VEGF diminished. However, in the presence of TL alone and TL plus HA LMW, their synthesis was higher. We found increased levels of MMP-2 activity when pulsed with TL plus HA HMW and a lower increase when treated with TL plus HA LMW, in comparison to basal conditions. We found significate differences in TLR4 expression when treated with TL alone and together with HA LMW. Conclusion: HA is able to modulate macrophages phenotype in the tumor microenvironment as well as their angiogenic capacity, key aspects for tumor development. Our results provide the first evidence that in tumor context HA is able to modulate not only macrophages phenotype, but also their cytokines profile and involvement in tumor angiogenesis.
Bibliography: (1)Spinelli FM et al. The immunological effect of hyaluronan in tumor angiogenesis. Clin & Trans Immunol 2015; 4:e52; (2)Rayahin JE et al. (2016) High and Low Molecular Weight Hyaluronic Acid Differentially Influence Macrophage Activation. ACS Biomaterials Science and Engineering 7:481–493; (3)Kobayashi N et al. Hyaluronan deficiency in tumour stroma impairs macrophage trafficking and tumour neovascularization. Cancer Res 2010; 70:7073–7083; (4)Murdoch C & Lewis CE. Macrophage migration and gene expression in response to tumour hypoxia. Int J Cancer 2005; 117:701–708.

Workshop 2: Collagen modifications: role in matrix quality/quantity and disease

Invited Lecture (L7) A novel function for cartilage oligomeric matrix protein in collagen secretion

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Cartilage oligomeric matrix protein (COMP) is an abundant component in the extracellular matrix (ECM) of load-bearing tissues such as tendons and cartilage. In the extracellular space COMP accelerates collagen fibrillogenesis and regulates the diameter of collagen fibrils. COMP binds to both collagens and non-collagenous matrix proteins and serves adaptor functions by bridging different ECM structures. It has been shown previously that COMP is also a constitutive component of healthy human skin and its expression is strongly induced in fibrotic conditions. In skin, COMP is synthesized by fibroblasts and binds directly and with high affinity to the fibril forming collagen I and to the FACIT collagen XII that decorates the surface of collagen I fibrils.
An in-depth analysis of COMP-deficient mouse skin revealed that lack of COMP-collagen interaction in the extracellular space leads to changes in collagen fibril morphology and density resulting in altered skin biomechanical properties. The ultrastructural analysis of fibroblasts showed strongly dilated cisternae in the ER indicating a secretion defect. We found that secretion of collagen I and collagen XII by COMP-deficient fibroblasts into culture medium was strongly reduced in comparison to wild-type fibroblasts. In contrast, fibronectin was properly secreted even though it has been described as another direct interaction partner of COMP. The hypothesis that COMP is directly and specifically involved in efficient collagen secretion is further confirmed by the observation that collagen secretion could be rescued in COMP-deficient fibroblasts by restoring COMP expression. To determine the in vivo relevance of COMP for collagen secretion, COMP-null and control mice were subjected to repeated intradermal injections of bleomycin. Interestingly, the fibrotic response elicited by bleomycin injection in COMP-null mice was clearly attenuated. Histological analysis of COMP-deficient skin revealed significantly reduced dermal thickness compared to bleomycin-treated control skin. In agreement with reduced dermal thickness, the levels of total collagen were also significantly diminished in COMP-deficient fibrotic lesions.
Thus, our studies reveal that COMP has two different functions – first, an intracellular activity that leads to efficient export of collagen from the ER, which depends on the intracellular formation of a COMP–collagen complex; second, COMP exerts an extracellular activity that ensures the correct spatial arrangement of collagen fibrils in the ECM. The novel intracellular function of COMP might be exploited for novel therapeutic approaches, which would target COMP in fibroblasts that are engaged in high levels of collagen production during the development of fibrosis.

Invited Lecture (L8) Collagen VI, a key matrix protein at the crossroad of skeletal muscle and peripheral nerves

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Collagen VI (ColVI) is a major extracellular matrix component made of three major genetically distinct α chains and abundantly deposited in the basement membrane of both skeletal muscles and peripheral nerves. Mutations in *COL6A1*, *COL6A2* and *COL6A3* genes are known to cause different forms of muscle diseases, including Bethlem myopathy, Ullrich congenital muscular dystrophy and myosclerosis

myopathy. ColVI null (*Col6a1*$^{-/-}$) mice display a myopathic phenotype characterized by latent mitochondrial dysfunction, spontaneous apoptosis, defective autophagy regulation and compromised muscle regeneration [1-3].
We recently demonstrated that the absence of ColVI in peripheral nerves leads to hypermyelination, altered Remak bundles, sensory-motor functional deficits and decreased nerve conduction velocities, thus pointing at ColVI as a crucial molecule for peripheral nerve structure and function [4, 5]. Given the muscle and nerve defects displayed by *Col6a1* null mice, we explored the role of ColVI in the neuromuscular junction (NMJ). Our unpublished studies revealed that ColVI is indeed deposited at the synapse. Immunofluorescence and immunoelectron microscopy indicated the α3(VI) chain, rather than the alternative α4(VI), α5(VI) and α6(VI) chains, as the main component of the α1α2αX ColVI assemblies deposited at the NMJ. Labeling of post-synaptic AChR clusters with α-bungarotoxin showed that *Col6a1*$^{-/-}$ muscles display abnormal NMJ morphology. Moreover, our results revealed altered expression of synaptic genes and abnormal electrophysiological parameters in the NMJs of *Col6a1*$^{-/-}$ mice. These findings indicate a specific role for ColVI at the NMJ, and further studies will allow shedding new light on the contribution of the NMJ defects to the etiopathology of ColVI-related myopathies.

References:
1. *Irwin WA, Bergamin N, Sabatelli P, Merlini L, Megighian A, Reggiani C, Braghetta P, Columbaro M, Volpin D, Bressan GM, Bernardi P, Bonaldo P (2003) Nature Genetics 35, 367-371.*
2. *Grumati P, Coletto L, Sabatelli P, Cescon M, Angelin A, Bertaggia E, Blaauw B, Urciuolo A, Tiepolo T, Merlini L, Maraldi NM, Bernardi P, Sandri M, Bonaldo P (2010) Nature Medicine 16, 1313-1320.*
3. *Urciuolo A, Quarta M, Morbidoni V, Gattazzo F, Molon S, Grumati P, Montemurro F, Tedesco FS, Blaauw B, Cossu G, Vozzi G, Rando TA, Bonaldo P (2013) Nature Comm. 4, 1964:1-13, 2013.*
4. *Chen P, Cescon M, Megighian A, Bonaldo P (2014) FASEB J. 28, 1145-1156.*
5. *Chen P, Cescon M, Zuccolotto G, Nobbio L, Colombelli C, Filaferro M, Vitale G, Feltri ML, Bonaldo P (2015) Acta Neuropathol. 129, 97-113.*

Invited Lecture (L9) When Anatomy meets Collagen..... How the Anatomist adds to the phenotype of mutants

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Essentially, when an Anatomist and Collagen meet, this happens in front of a microscope and very often this is an electron microscope. In this context, the focus of investigation is fundamentally on collagen fibrils and basement membranes. These are in fact the most typical anatomically recognisable structures where the contribution of collagens in determining their integrity and their functionality becomes essential. Therefore, for this reason, these are the structures that catch the eye of an Anatomist who is interested in collagen biology. This presentation will focus on recent in vivo work, mainly done on skin of mice lacking rac1 in keratinocytes. The talk will highlight novel aspects on how structural investigations of fibrils and basement membranes can add to the phenotype of mutants which initially may not necessarily seem to be related to the collagen world. This can, in turn, open further discussion in helping identifying novel regulatory roles and pathways for the mutated proteins, and/or, in highlighting novel aspects of changes in matrix arrangements in pathological conditions in vivo.

ST06/P19 The effect of Gly substitutions in the collagen triple-helix on binding to integrin and fibronectin

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The replacement of one Gly in the essential repeating tripeptide sequence of the type I collagen triple-helix results in the dominant hereditary bone disorder Osteogenesis Imperfecta (OI). Although

misfolding and autophagy are observed in many cases, it has been hypothesized that some OI collagens are secreted and incorporated into fibrils, where they may interfere with known collagen interactions. Here, a recombinant bacterial system is used to investigate the effect of Gly substitutions on the collagen interaction with integrin, which requires a native triple-helix, and the interaction with fibronectin, which can bind to denatured as well as native collagen. Insertion of the human collagen integrin binding sequence or fibronectin binding sequence within a bacterial collagen-like protein leads to the expected binding and activity. For integrin, Gly to Ser mutations within the two Gly residues in the essential GFPGER sequence prevented integrin binding and cell attachment, as did Gly replacements up to 4 tripeptides N-terminal to GFPGER. For fibronectin, Gly to Ser mutations within the essential (Gly-Xaa-Yaa)6 sequence perturbed binding to native and denatured collagen constructs, while mutations in adjacent sequences had no effect. Molecular dynamics simulations shed light on the interactions that were disrupted and led to loss of binding. The structural consequences of Gly substitutions depended strongly on the stability of the binding sequence. The collagen constructs with mutations in the integrin binding sequence showed no loss of stability and maintained a trypsin resistant triple-helix, while constructs with mutations within the less stable fibronectin binding sequence showed decreased stability, multiple domains, and susceptibility to trypsin digestion. The results of this study help explain the observed pattern of OI mutations near known collagen binding sites.

ST07/P20 Collagen XVIII regulates energy metabolism and brown adipose tissue function

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Collagen XVIII (ColXVIII) is a basement membrane (BM) associated proteoglycan which is ubiquitously expressed throughout the mammalian body as three different isoforms. These isoforms, called short, medium and long, are expressed via two different promoters and have tissue-specific expression patterns. For example, in adipose tissue the long and the medium isoforms are expressed mainly around the adipocytes while the short isoform is located in the BM of blood vessels. We recently showed that a specific lack of the medium/long isoforms in mice leads to reduced adiposity, increased fat deposition in the liver and elevated serum levels of very low density lipoprotein. White adipose tissue (WAT) serves as a storage where excess of energy is collected in the form of triglycerides and released as fatty acids when required, and it also contributes to metabolism by secreting endocrine, paracrine and autocrine signals. Brown adipose tissue (BAT) is histologically and functionally distinct from WAT, its main function being in heat production and the maintenance of body temperature in cold exposure. Instead of storing energy, BAT consumes fatty acids and produces heat through the uncoupling protein 1 (Ucp1), which is located in the inner membrane of mitochondria. We studied the role of ColXVIII in metabolism by using promoter-specific knockout mice that lack either all three (KO mice), or exclusively the short form (P1 mice), or the medium and long forms (P2 mice) of ColXVIII. The body weight of the KO mice is lighter than wild type (WT) control mice while the food consumption remains the same. Interestingly, we found that the KO mice have altered BAT functions but the amount of interscapular BAT is not affected. The BAT and thermogenesis specific genes (Ucp1, CIDEA, Prdm16) were upregulated in the absence of all or the long/medium isoforms of ColXVIII. Ultrastructural analysis supported these results as the BAT of KO mice was found to have an expanded mitochondrial area compared with the controls. Additionally, lack of ColXVIII alters the expression of genes related to glucose homeostasis and insulin sensitivity: glucose transporter 4 (GLUT4) and adiponectin mRNA levels were decreased in WAT while the GLUT2 mRNA level was increased in liver. We performed glucose tolerance and insulin glucose clamp studies, and observed impaired glucose tolerance and reduced insulin sensitivity in the KO and P2 mice. These mutant mice develop symptoms similar to prediabetic condition, and also their energy metabolism is altered. Our data highlights the importance of ColXVIII in the regulation of metabolic pathways and its involvement in metabolic disorders.

ST08/P21 Absence of the ER cation channel *TMEM38B*/TRIC-B disrupts intracellular calcium homeostasis and dysregulates collagen synthesis in recessive osteogenesis imperfect

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Recessive osteogenesis imperfecta (OI) is caused by defects in proteins involved in post-translational interactions with type I collagen. Recently, a novel form of moderately severe OI caused by null mutations in *TMEM38B* was identified. *TMEM38B* encodes the ER membrane monovalent cation channel, TRIC-B, proposed to counterbalance IP_3R-mediated Ca^{2+} release from intracellular stores. However, the molecular mechanisms by which *TMEM38B* mutations cause OI are unknown. We identified 3 probands with moderately severe OI caused by recessive defects in *TMEM38B*. Although *TMEM38B* transcripts in proband fibroblasts and osteoblasts are reduced to 19-86% of control levels, TRIC-B protein is undetectable in proband cells. TRIC-B deficiency causes impaired release of ER luminal Ca^{2+}, associated with deficient store-operated calcium entry, although SERCA and IP_3R, the channels for ER Ca^{2+} uptake and release, respectively, have normal stability. The disturbed Ca^{2+} flux is consistent with ER stress and increased BiP. In the absence of TRIC-B, synthesis of proband type I collagen is dysregulated at multiple steps. Collagen helical lysine hydroxylation is reduced, while telopeptide hydroxylation is increased, despite increased LH1 and decreased Ca^{2+}-dependent FKBP65, respectively. Although PDI levels are maintained, procollagen chain assembly is delayed in proband cells. The resulting misfolded collagen is substantially retained in TRIC-B null cells, consistent with a 50-70% reduction in secreted collagen. Lower-stability forms of collagen that elude proteosomal degradation are not incorporated into extracellular matrix, which contains only normal stability collagen, resulting in matrix insufficiency. These data support a role for TRIC-B in intracellular Ca^{2+} homeostasis, and demonstrate that absence of *TMEM38B* causes OI by dysregulation of multiple Ca^{2+}-regulated collagen-specific chaperones and modifying enzymes in the ER.

ST09/P22 Succinate mediates extracellular matrix remodeling to promote an invasive phenotype: a new function for oncometabolites

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Paragangliomas (PGL) and pheochromocytomas (PCC) are rare neuroendocrine tumors derived from the autonomous nervous system and from chromaffin cells of the adrenal medulla, respectively. Up to 40% of cases are genetically determined and there are 13 predisposition genes. Among them, *SDHx* genes (*SDHA*, *SDHB*, *SDHC* and *SDHD)* encode tricarboxylic acid cycle enzyme succinate dehydrogenase, catalyzing the oxidation of succinate into fumarate. *SDHx* mutations lead to succinate accumulation which acts as a competitive inhibitor of 2-OG dependent dioxygenases such as HIFs prolyl-hydroxylases and DNA/histone demethylases driving pseudo-hypoxic and hypermethylator phenotypes of these tumors. *SDHB* mutations are associated with malignancy and poor prognosis. To be invasive, a primary tumor needs to activate angiogenesis, acquire metastatic capacities through epithelial to mesenchymal transition (EMT) and modify its microenvironment in order to facilitate migration and invasion. To study the microenvironment, we used OMICS data generated on the COMETE collection of human PCC/PGL (200 samples) and our in vitro model of immortalized mouse chromaffin cells (imCCs) displaying a complete loss of SDHB protein and a massive accumulation of succinate. We previously showed that EMT is specifically activated in *SDHB*-related tumors and in *Sdhb*-/- imCCs. Here, we show that these cells promote angiogenesis in vivo and in vitro. Transcriptome analysis showed up-regulated extracellular matrix (ECM) related genes in *SDHB-mutated* tumors including fibronectin, collagens and matrix metalloproteinases (MMPs). It is now well known that modifications within ECM and its remodeling participate to cancer invasion. We first showed that *Sdhb*-/- ECM increases migration and adhesion of WT imCCs. Conversly, WT ECM reduces migration and adhesion of *Sdhb*-/- imCCs. Transcriptome analysis of imCCs shows an overexpression of fibronectin and collagen type IV in *Sdhb*-deficient cells. Loss of fibronectin slightly decreases *Sdhb*-/- imCCs migration. Interestingly, fibronectin coating dramatically increased migration and adhesion of WT imCCs. Collagen type IVa2 (ColIVa2) is overexpressed but fragmented in *Sdhb*-/- compared to WT imCCs which display a continuous ColIV network. This disrupted organization could be caused by MMPs activity which are induced by (pseudo)-hypoxia and/or by the succinate-mediated inhibition of collagen hydroxylases, which belong to the family of 2-OG dependant dioxyenases. 2-OG treatment decreased migration of *Sdhb*-/- imCCs as well as *Mmp9* and *Col4a2* expression, but restored the network of ColIVa2. Hence, it is still unclear wether ColIV fragmentation is mediated by MMP-mediated degradation and/or collagen hydroxylation. In conclusion, we show here for the first time that SDHB deficient cells modify their microenvironment to promote their invasive phenotype through modifications of the composition and the structure of their ECM.

ST10/P23 Novel role of Thrombospondin-1 as a binding partner of collagen cross-linking sites: functional implication in modulation of myofibroblastic differentiation

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Thrombospondins (TSPs) are multi-functional glycoproteins of the extracellular matrix (ECM) that modulate cell-ECM interactions and signaling. Gene knockout mice for individual TSPs have many distinct phenotypes, yet an emergent common phenotype is alterations to morphology of collagen fibrils, associated in some instances with mechanical tissue weakness. We have demonstrated increased fibril areas and increased frequency of enlarged fibrils in *Thbs1−/−* mice. However, the underlying molecular mechanisms of TSP-collagen interaction are unknown for any TSP. By screening libraries of collagen triple-helical peptides, the Collagen II and III Toolkits, for TSP1-binding peptides, we identified six active peptides, five of which contain the motif KGHR. Screening of substituted peptides established

that the K, H and R residues are each essential for TSP1 binding. The KGHR motif is highly conserved and the lysine residue is required in cross-linking of fibrillar collagen molecules into fibrils. TSP1 bound to pepsin-digested collagens I, II and III *in vitro* and more strongly to native collagen I fibrils. Binding to collagen I fibrils was effectively and specifically inhibited by KGHR-containing collagen triple-helical peptides, establishing a physiological relevance of the TSP1-binding motif in cross-linked, native collagen fibrils. Elevated fibrillar collagen and increased collagen cross-linking are key drivers of pathological tissue stiffening in organ fibrosis, and TSP1 is also elevated in several fibrotic conditions including scleroderma. Treatment of human dermal fibroblasts that were negative for alpha-smooth muscle actin (SMA) with KGHR-containing collagen triple-helical peptide correlated, over 24 h to 96 h, with altered collagen ECM patterning, as determined by quantified immunofluorescence microscopy. Over the same timecourse, the peptide specifically promoted myofibroblastic differentiation, demonstrated by elevation of smooth muscle actin-positive cells. These data implicate the TSP1-collagen interaction as novel regulatory mechanism for the KGHR site and an unsuspected mechanism in homeostatic restraint of myofibroblastic differentiation.
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P24 Carbamylation of matrix proteins as a marker of aging

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Aging is a progressive process determined by genetic and acquired factors, which have consequences on extracellular matrix (ECM) components. Among these factors, nonenzymatic post-translational modifications (NEPTMs), such as glycoxidation, are responsible for protein molecular aging. Carbamylation is a more recently described NEPTM that is caused by the binding of isocyanate, formed during urea dissociation or by the myeloperoxidase-mediated catabolism of thiocyanate, to amino groups of proteins. This reaction preferentially occurs on lysine residues generating homocitrulline (HCit), the most characteristic carbamylation-derived product (CDP). This modification is considered an adverse reaction because it induces alterations of protein and cell properties. The intensity of this reaction is particularly amplified during renal chronic diseases. However, to date no data are available on the involvement of carbamylation in aging and particularly regarding ECM proteins.
To address this issue, we studied the carbamylation of two tissues, skin and aorta, which contain a high proportion of ECM proteins, in three mammalian species. HCit was quantified by LC-MS/MS and our results showed that HCit concentrations progressively increased with age in total skin extracts of murine, bovine and human species (29.0-, 11.5- and 8.1-fold increase between young and old subjects, respectively). The same evolution was observed in total aorta extracts of mice and bovines (25.9- and 1.8-fold increase, respectively), but any significant increase was found in human aorta, which is the only species to develop atherosclerosis where the remodeling intensely occurs. Interestingly, the carbamylation reaction impacts preferentially matrix proteins probably because of their long half-life. The rate of carbamylation of type I collagen and elastin from skin or aorta was very high in bovines and

humans. We also showed that HCit increase in collagen was more pronounced in species with shorter lifespans suggesting that species with longer lifespans develop efficient turnover, repair or degradation mechanisms restricting tissue accumulation of CDPs. To study the potential turnover of CDPs *in vivo*, we used a mouse model of dietary cyanate-induced carbamylation. We observed that skin collagen exhibited the weakest HCit elimination. This could be due to a slower turnover or to the contribution of some mechanisms taking part in the renewal and remodeling of ECM proteins.
This study shows for the first time that carbamylation is a physiological process responsible for the accumulation of CDPs in the organism. As previous studies had shown the involvement of NEPTMs in structural alterations of matrix proteins, it is likely that carbamylation participates in the gain of stiffness and the loss of elasticity of skin and aorta. These results suggest that this accumulation is associated with long-term dysfunctions of aging or may represent a potential risk factor of cardiovascular complications.

P25 The role of collagen XVIII in squamous cell carcinoma of the lung

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Interactions between cells and extracellular matrix (ECM) regulate all cellular properties and processes, including cell polarity, survival, proliferation, and differentiation. In cancer, the ECM architecture is commonly disorganized and ECM remodeling is deregulated. Collagen XVIII is a basement membrane (BM) collagen and proteogylan, and it is found in association with most vascular and epithelial BMs. It has a complex multidomain structure and includes a C-terminal anti-angiogenic endostatin domain. Collagen XVIII is involved in tumour formation: it is upregulated in many human solid tumours and its loss from the epithelial BMs has been reported to precede the invasion of human cutaneous and oral squamous cell carcinoma (SCC). Lung cancer is the most common cancer among men worldwide in terms of both incidence and mortality, and among women has the third highest incidence. SCC of the lung is a subtype of non-small cell lung cancer (NSCLC). Approximately 85% of lung cancers are NSCLC, and of these, approximately 30% are SCCs. SCC is strongly associated with smoking. We have studied here the expression and localization of collagen XVIII in human SCC of the lung, and its correlation with the clinicopathological parameters of the patients. Our study consists of 120 SCC samples and a careful documentation of the clinicopathological data of the patients. We found that expression of collagen XVIII correlates with tumour differentiation. Expression is high in well-differentiated SCC samples and low in poorly differentiated cases. Immunohistochemistry showed tumour cell-specific, cytoplasmic staining for collagen XVIII. In cell culture studies, collagen XVIII knockdown reduced proliferation of a human SCC cell line. Expression of collagen XVIII correlates with desmoplasia, necrosis and keratinization, but it does not correlate with survival rate of patients.

P26 Ageing of human optic nerve head connective tissue

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The lamina cribrosa (LC) within the human optic nerve head (ONH), provides a supportive framework for axons as they leave the retina and exit the eye. Deformation and disruption of the LC is an important driver of axonal damage in the ocular disease, glaucoma, resulting in irreversible blindness. Age is a major risk factor in glaucoma. However, how ageing predisposes ONHs to an increased susceptibility to the development of glaucomatous optic neuropathy is not fully understood. The aim of this study was to

determine if LC collagen and elastin microstructure alters with age. Human optic nerve heads were serially cryosectioned either transversely, (100µm-thick sections from 3 young [<25years] and 3 elderly [>80years] ONHs) or longitudinally (160 µm thick, n= 7 ONHs, aged 11-88years). All sections were subjected to second harmonic generation (SHG, 800nm/400nm) and two photon excited fluorescence (TPEF, $\lambda_{ex/em}$ = 800nm/500-550nm) microscopy using a mode-locked, multiphoton, Ti:Sapphire laser (Chameleon®, Coherent UK Ltd, UK) with a 140 fs pulsewidth and a LSM510 META laser scanning microscope (Zeiss Ltd, UK)., 3D reconstructions of serial transverse ONH SHG image datasets were undertaken, in order to determine the distribution of fibrillar collagen throughout the LC and to quantify LC thicknesses and volumes.
SHG and TPEF signals, attributed to fibrillar collagen and elastin, respectively, co-distributed within the LC connective tissue beams of both longitudinal and transverse ONH tissue sections. In the young LC and peripapillary sclera, fibrillar collagen was wavy-like in appearance, an observation not evident in elderly LC collagen. The TPEF signal, associated with distinct elastic fibres in the elderly LC and peripapillary sclera, was absent from the young ONHs. Additionally, the TPEF signal was observed as 'granular-like' in the postlaminar optic nerve of elderly samples, not apparent in the young. Although SHG and TPEF signals appeared to be greater in the elderly LCs, LC thickness was significantly thinner in the elderly ($p<0.001$). In addition, anterior and posterior LC surface area was greater in the elderly ($p<0.05$), but LC volume remained unchanged ($p>0.05$).
In conclusion, SHG and TPEF signals, emitted from fibrillar collagen and elastin respectively, demonstrated age-related changes in their distribution, appearance and organisation. Together with LC microstructure differences, such changes will likely influence LC biomechanics as a function of age, and perhaps increase an elderly ONH's susceptibility to glaucomatous optic neuropathy.

P27 Thermodynamic calculations of Advanced Glycation End-product cross-linked collagen packing

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Connective tissues within the extracellular matrix can undergo structural modification as the result of a series of glycation reactions, in particular, those non-enzymatic reactions that go on to form cross-links between collagen peptides, known as advanced glycation end-products (AGE). Recent developments have indicated that the accumulation of AGEs, due to the long turnover of collagen, can contribute to a number of age related pathologies, such as osteoporosis and cardiovascular diseases. The structure, and dynamics of AGEs and how their accumulation affects the collagen microenvironment at an atomistic scale is still within its infancy.
The inclusion of post-translational modifications, novel therapeutic compounds, and metal binding centres, within a computational Molecular Dynamics model requires a staggering amount of bespoke atomistic structure and charge calculations, typically from quantum mechanical models or direct from experimental data, if available. An established mathematical practice combined with our quantum mechanical modelling has simplified the process required to fully parameterise a Molecular Dynamics forcefield for the simulation of AGE cross-linked collagen molecules. In particular, we have fully parameterised force constants and atomic charges for the arginine-lysine derived cross-links, GODIC, MODIC, DOGDIC, and glucosepane.
Using the periodicity defined from the crystal structure of a synthesised short heterotrimer collagen molecule we have mapped the thermodynamic free energy of association between packed collagen molecules before and after intra-molecular cross-linking of glucosepane. The bulky seven membered ring structure of glucosepane contains additional polar sites compared with its constituent amino acids. There is a significant difference in sidechain chemistry, which may contribute to a change in the

propensity for collagen molecule self-association and the ordering of water molecules and subsequent hydrogen-bond networks between collagen polypeptide-chains.

P28 A novel endoplasmic reticulum complex regulating collagen lysyl hydroxylation

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Sc65 (Synaptonemal Complex 65) is an endoplasmic reticulum protein that belongs to the Leprecan family which include the prolyl 3-hydroxylases (P3H1, P3H2, P3H3) and cartilage associated protein (CRTAP). We and others have shown that mutations in both *CRTAP* and *LEPRE1* (encoding P3H1) genes cause recessive forms of Osteogenesis Imperfecta. CRTAP forms a complex with P3H1 and cyclophilin B (CYPB) responsible for collagen prolyl 3-hydroxylation. Lack of Crtap causes decreased collagens prolyl 3-hydroxylation. Sc65 is a non-enzymatic protein that shares high homology with CRTAP, suggesting Sc65 may also have a role in bone homeostasis. We demonstrated that mice null for Sc65 exhibit a low bone mass phenotype with decreased BV/TV and loss of cortical and trabecular bone. To ascertain Sc65 function in bone, co-immunoprecipitation (co-IP) of Sc65 candidate interactors followed by mass spectrometry was performed in mouse fibroblasts. These experiments identified several fibrillar procollagen α-chains as likely substrates of Sc65 supporting the idea that Sc65 plays a role in collagen modification, similar to other Leprecans. Direct co-IP assays showed Sc65 interaction with lysyl-hydroxylase 1 (LH1, Plod1), prolyl 3-hydroxylase 3 and cyclophilin B. Sc65 and P3H3 form a stable complex in the ER, as demonstrated by size exclusion chromatography, that affects the activity of lysyl-hydroxylase 1 potentially through direct interactions or interaction with cyclophilin B. A dramatic reduction of LH1 and P3H3 was shown by western blot in primary osteoblasts and skin fibroblasts from *Sc65*-KO mice. At the biochemical level, mass spectrometry of type I collagen peptides demonstrated severe under-hydroxylation at helical cross-linking sites K87 and K930/933 in collagen α1(I) and α2(I) chains both from bone and skin, which are known LH1 preferred substrate residues, but with no effect on sites of prolyl 3-hydroxylation. *Sc65*-KO mice also show fragile skin with less tensile strength than control mice, consistent with a collagen cross-linking abnormality. Ultrastructural studies revealed a less orderly packed mutant dermal collagen in *Sc65*-KO mice. Collectively, these results indicate that Sc65 is a novel adapter molecule that stabilizes a unique ER-resident complex that is essential for proper collagen lysyl-hydroxylation. Loss of Sc65 leads to complex instability and defective fibrillar collagen modifications which negatively impacts bone and skin.

P29 Regulation of post-Golgi LH3 trafficking is essential for collagen homeostasis

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Arthrogryposis, Renal dysfunction, and Cholestasis (ARC) syndrome is a multisystem disorder caused by mutations in genes encoding two proteins VPS33B or VIPAR, which appear to be critical regulators of cell polarity. VPS33B and VIPAR may function as part of a multi-protein complex that interacts with an active form of RAB11A, associated with recycling endosomes (RE).
A novel interacting partner of VIPAR was identified, LH3, a posttranslational modification enzyme with lysyl hydroxylase (LH), collagen galactosyltransferase (GT), and glucosyltransferase (GGT) activities. These modifications are critical for the process of fibre crosslinking and basement membrane formation

in development and disease. Clinical features of a patient described with LH3 deficiency overlap with ARC and include severe growth retardation, hypotonia, arthrogryposis, osteopaenia and bone fractures.
Here we demonstrate how a novel trafficking pathway dependent on VIPAR and VPS33B is involved in the sorting of LH3 into post Golgi secreting carriers where it exerts its enzymatic function on collagen lysines. Engagement of firstly RAB10 at the level of TGN, and subsequently RAB25 at the RE, are necessary for this pathway to function.
Defects in LH3 mediated posttranslational modifications were observed in collagen type IV in VPS33B/VIPAR and LH3 knockdown mIMCD3 cells. This is consistent with the essential role of LH3 in the synthesis of type IV and the stability of basement membranes. A significant decrease in LH, GT and GGT modifications were observed also in Collagen type I derived from ARC patients fibroblasts compared with the controls.
These findings establish a role for VPS33B/VIPAR in the intracellular trafficking of LH3 and collagen homeostasis; the novel pathway is crucial for collagen post-translational modification in multiple cells and for different types of collagen and is at least partially responsible for the complex phenotype in ARC.

P30 Type III collagen is important for type I collagen fibrillogenesis and for dermal and cardiovascular development

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Type III collagen, a major fibrillar collagen consisting of three identical α1(III)-chains, is expressed in early embryos, throughout embryogenesis and in a wide variety of adult tissues. Mutations in *COL3A1* cause vascular Ehlers-Danlos Syndrome (vEDS), a severe, life-threatening disorder, characterized by thin, fragile skin and propensity to arterial and intestinal rupture. Most mutations result in glycine substitutions in the procollagen triple helical domain. However, the mechanisms by which mutant type III collagen causes dermal and vascular fragility are not well understood.
To study the role of type III collagen in development and disease, we generated two transgenic mouse models using a BAC transgenic approach. The *Col3a1*$^{MUT+}$ mice highly express a *Col3a1* transgene that harbours a typical glycine substitution (p.(Gly183Ser); c.547G>A) in the α1(III)-procollagen triple helical domain, whereas the *Col3a1*$^{WT+}$ mice overexpress WT *Col3a1*. *Col3a1*$^{MUT+}$ male mice display a thin, fragile skin and develop severe transdermal skin wounds by 3-4 months of age. Biomechanical testing reveals significant fragility of the skin and aorta of *Col3a1*$^{MUT+}$ animals, whereas tissues from *Col3a1*$^{WT+}$ mice show a normal tensile strength. Collagen fibrils in the skin and aorta of *Col3a1*$^{MUT+}$ mice are loosely packed and display a highly variable diameter. The adventitia is significantly thinner and smooth muscle cells make less connection with elastic fibers and show more intracellular space. These ultrastructural abnormalities are not observed in *Col3a1*$^{WT+}$ mice indicating that the observed phenotype in *Col3a1*$^{MUT+}$ mice is due to the Gly substitution, and not merely caused by type III collagen overexpression. Furthermore, biochemical and biophysical testing of mutant type III collagen, extracted from skin tissue, reveals a decrease in temperature stability. However, posttranslational modification is normal, and ER stress markers are not upregulated.
Together, our findings underscore a key role for type III collagen in collagen fibrillogenesis in skin and arterial tissue. These two novel transgenic animal models, overexpressing either mutant or wild type *Col3a1*, provide an important new tool for pathogenesis exploration and

advanced understanding of the role of (abnormal) type III collagen in collagen fibrillogenesis and vEDS in general.

P31 Distinct structure and composition of the vascular basement membrane in pulmonary hypertension

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Rationale: Vascular remodelling of the intrapulmonary arterial wall is a key characteristic in the pathogenesis of pulmonary hypertension (PH). Imbalance and enhanced expression of collagens and other extracellular matrix proteins have been implicated to contribute to the structural changes occuring in the vessel wall. Futhermore, neointima formation is a prominent process during vascular remodelling where the disturbance of the BM can lead to enhanced cell migration. In this context, collagens associated with the formation of the endothelial basement membrane (BM) could be of interest. Therefore, the aim of this study is to analyse and compare the composition of basement membrane in remodelled small and large pulmonary arteries from diverse forms of PH, namely idiopathic pulmonary arterial hypertension (IPAH), and PH associated with chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF).
Methods: To characterize the endothelial BM immunohistochemical staining for collagen IV was performed. BM gene expression profiles of donor, IPAH, COPD and IPF lungs were determined on both small pulmary arteries (50-500µm diameter) generated from laser capture microdissected material and larger (2-4mm diameter) isolated pulmonary arteries. Structural disintegrity of the BM was visualized by electron microscopy.
Results: Immunohistochemistry of BM-associated collagens revealed remodelling of the BM in IPAH, COPD and IPF patients compared to healthy donors. Concomitant, using electron microscopy, thickening and splitting of the BM was observed in vessels from PH samples. Gene expression analysis of BM components displayed a distinct profile associated with PH that was further dependent on PA size. Cleavage products of BM components, such as ColXVIII-derived endostatin, were elevated in PH and its levels correlated with worse patients‘ outcome.
Conclusion: PH is associated with changes in structure and composition of the BM. It is likely that those changes in the BM are implicated in disease pathogenesis of PH. Thus restoring the BM to normal conditions might stop or reverse pulmonary vasuclar remodelling.

P32 Severe extracellular matrix abnormalities in mice lacking collagen prolyl 4-hydroxylase isoenzyme II in combination with a reduced amount of isoenzyme I

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Collagen prolyl 4-hydroxylases (C-P4Hs I-III) catalyze the formation of 4-hydroxyproline (4Hyp) residues required for thermal stability of triple-helical collagen molecules in body temperatures. The vertebrate C-P4Hs are $\alpha_2\beta_2$ tetramers differing in their catalytic α subunits. β-subunit is protein disulphide isomerase. C-P4H-I is the major isoenzyme in most cells and its inactivation by a genetic deletion of α subunit gene (*P4ha1*) leads to embryonic lethality. To study the *in vivo* roles of C-P4H-II, we generated *P4ha2*$^{-/-}$ mice. *P4ha2* is strongly expressed in chondrocytes and in osteoblasts but, surprisingly, knockout mice were without any obvious phenotypic abnormalities. We then proceeded for double mutants and found out that *P4ha1*$^{+/-}$*;P4ha2*$^{-/-}$ mice were smaller than their littermates, had moderate chondrodysplasia and developed kyphosis. A transient cell death phenotype was detected in the inner, hypoxic region of the developing growth plate. Also the columnar arrangement of proliferative chondrocytes was impaired and extracellular matrix was disorganized. The amount of 4Hyp in growth plate was reduced and type II collagen had lower melting temperature as expected. Interestingly, no signs of uncompensated ER stress were detected in the mutant growth plate chondrocytes. In addition to distorted femur/tibia length ratio, *P4ha1*$^{+/-}$*;P4ha2*$^{-/-}$ mice exhibited shorter long bones, reduced cortical bone diameter and loss of trabecular bone likely due to reduced osteoblast number. In conclusion, our data show that C-P4H-I can to a large extent compensate for the lack of C-P4H-II in proper endochondral bone development, but their combined partial and complete inactivation, respectively, affects several tissues and causes, for example, growth retardation, moderate chondrodysplasia and trabecular bone loss.

P33 Collagen prolyl 4-hydroxylase mutant mice reveal importance of 4-hydroxyproline in collagen fibril formation and matrix organization in skin

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Collagen biosynthesis is a multi-stepped process with many intra- and extracellular steps and co- and posttranslational modifications needed to form mature collagen molecules and superstructures. One important event is the hydroxylation of certain proline residues in a collagenous repetition sequence -X-Pro-Gly of collagen. As a result 4-hydroxyproline (4Hyp), which is required for collagen thermostability, is formed. Enzymes responsible for this are collagen proline 4-hydroxylases (C-P4Hs). C-P4Hs are $\alpha_2\beta_2$ tetramers and three isoforms of C-P4Hs can be found in vertebrates. These isoforms differ in their α-subunits (α(I), α(II) and) α(III)), but the β-subunit is always protein disulphide isomerase PDI. To study the role of different isoforms in the skin, we have generated knock out mouse lines with either genetic deletion of *P4ha2* alone (*P4ha2-/-*) or combined with heterozygosity of *P4ha1* (*P4ha1+/-;P4ha2-/-*). It is known from the previous studies that a genetic deletion of C-P4H-I α-subunit gene (*P4ha1*) leads to embryonic lethality and genetic deletion of C-P4H-II α-subunit gene (*P4ha2*) causes no obvious phenotypic abnormalities. Histological studies showed that dermis of the skin is thinner in both *P4ha2-/-* and *P4ha1+/-;P4ha2-/-* mice. Masson's trichrome staining was less prominent, suggesting reduced collagen amount in the dermis and Hart's staining showed compensatory increase of elastic fibers in *P4ha1+/-;P4ha2-/-* mice. Analysis with transmission electron microscopy revealed a decrease in collagen fibril diameter and less collagen fibril bundles in *P4ha2-/-* and *P4ha1+/-;P4ha2-/-* mice. The thickening of capillary basement membrane was also observed. The scanning electron microscopy analysis also showed the decrease in fibril diameter and in the amount of collagen. Biochemical assays confirmed the observations in collagen quantity. Hydroxyproline and Sircol assays indicated that the amount of collagen is lower in the skins of both *P4ha2-/-* and *P4ha1+/-;P4ha2-/-* mice. The melting temperature of type I collagen was about 1 degree lower as expected for collagen prolyl hydroxylase mutants. We also studied the wound healing by making a 3mm skin punch on the back of the mouse and analyzed the healing from the start to the point of wound closure. It seems that

there is no difference in the speed of healing, but the *P4ha1+/-;P4ha2-/-* mutant mice had inflammation during the first 24-48 hours after wound making.

P34 Identification of novel HSP47 clients

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Introduction: Heat-shock protein 47 (HSP47), identified as collagen-specific chaperone, is essential for procollagen biosynthesis and secretion. The disruption of *hsp47* gene results in abnormal procollagen folding in the ER, thus inducing an embryonic lethal phenotype in mice[1]. To date, HSP47 has been shown to interact with the major collagen types, including types I-V[2]. However, it has not been elucidated whether other collagens require HSP47 assistance for proper folding. Moreover, it is unclear if other clients than collagens do also interact with HSP47. The recently solved crystal structure[3] gave insight into the collagen binding interface but the binding stoichiometry remains to be determined.
Methods: The interaction of HSP47 with collagens and other potential clients was characterized by ELISA style binding assays and surface plasmon resonance (SPR) measurements. The stoichiometry of the HSP47 collagen I interaction was visualized by electron microscopy.
Results: Using a set of recombinant and tissue-derived collagens, we could identify novel collagenous HSP47 clients. The fibrillar collagen XI bound HSP47 with high affinity in the nanomolar range. Furthermore, HSP47 was found to interact with FACIT collagens IX and XII with a higher affinity for collagen IX. Interestingly, also the ecto-domain of transmembrane collagen XXIII and XVII bound HSP47 with high affinity. Negative staining electron microscopy revealed binding of around 20 HSP47 molecules per procollagen I triple helix. In addition to collagens, we could show a direct interaction between HSP47 and fibrillin 1 (FBN1) by SPR measurements.
Discussion: Our data show that HSP47 interacts with members of other collagen subfamilies including FACIT (Fibril Associated Collagens with Interrupted Triple helices) and MACIT (Membrane Associated Collagens with Interrupted Triple helices) collagens. This suggests a global role of HSP47 in folding and stabilization of all collagen types. The binding stoichiometry has been determined for procollagen I, however, the exact binding motif has to be unraveled. We could further extend the HSP47 client repertoire with FBN1 as novel interactor. This leads to the assumption that HSP47 plays a role in the folding and secretion of other ECM proteins. However, the functional relevance of this interaction has to be demonstrated *in vivo.*
References:
1. Nagai N et al. (2000). J Cell Biol 150, 1499–1506
2. Natsume T et al. (1994). J. Biol. Chem. 269, 31224–31228
3. Widmer, C et al. (2012). Proc. Natl. Acad. Sci. U.S.A. 109, 13243–13247

P35 A comparison of preferential sites for glucosepane and DOGDIC formation in fibrillar type I collagen and their effect on the properties of fibrallar collagen

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During ageing advanced glycation end products (AGEs) accumulate in tissues with low turnover rates such as tendons. These AGEs form non-enzymatically and covalently crosslink collagen molecules, altering tissue properties, such as collagen stiffening properties which are believed to play a significant role in several age-related diseases such as osteoporosis and cardiovascular disease. Glucosepane is thought to be the most abundant AGE crosslink although other AGE crosslinks such as DOGDIC have also been recognised. Previously we identified sites where glucosepane is likely to form, although it is not known whether DOGDIC formation is more favourable at these sites or likely to occur at other sites.

This study aims to identify sites involved in DOGDIC formation within the collagen molecule and compare these to glucosepane formation sites. In addition, the effects of DOGDIC and glucosepane on the mechanical and biological properties of the collagen molecule are considered.
A distance-based criterion search identified lysine and arginine residues within 5Å of each other over a collagen molecule. Fully atomistic MD simulations, exploiting the D-band periodicity to replicate the dense fibrillar environment, were then conducted under pseudo physiological conditions in AMBER12. A site is a likely candidate for DOGDIC for glucosepane formation if the total energy of the collagen molecule is lower in the presence of a cross-link compared to an unbound glucose. Using a candidate cell and matrix interaction domains map for collagen, we determine the biological impact of the AGEs presence. Constant velocity steered MD simulations were conducted using NAMD to calculate changes in the stiffness of the collagen molecule.
Of the 24 positions identified based on the distance criteria, 6 sites were found to be energetically favourable compared to the unbound glucose collagen model, for glucosepane and 6 for DOGDIC with only 1 duplicate site. The local environment around the site has a significant effect on the energetics, with the sites within the gap region being more likely to have exothermic formation enthalpies. A number of favourable sites have potential for huge implications on the biological function of collagen, as they are within sites where key collagen-biomolecule and collagen-cell interactions occur. For example, the formation of glucosepane was found to be energetically favourable within close proximity of the Matrix Metalloproteinase-1 binding site, which could potentially disrupt collagen degradation. The presence of AGEs within the collagen is also observed to alter the mechanical properties of the molecule.
Our model representing a realistic 3-dimensional model of a collagen fibril has identified 11 likely sites for AGE formation within collagen, with the positioning of these sites likely to have a significant effect on tissue function and integrity.

P36 Long term effects of growth hormone on cartilage homeostasis

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Introduction: Acromegaly, a growth hormone (GH)-secreting pituitary adenoma causing a transient GH excess production, has been shown to result in a 4- to 12-fold increased risk to develop osteoarthritis (OA) when compared to the general population. Strikingly, the increased OA risk is also observed among patients with controlled and with long-term cured acromegaly. Furthermore, acromegaly-related OA has been characterized by particular changes of the cartilage. We hypothesized that a transient excess of GH results in irreversible cellular signalling, likely mediated via epigenetic mechanisms, with concurrent negative effects on joint tissue homeostasis.
Materials and Methods: To determine lasting effects of GH on matrix deposition and on mechanical properties of cartilage, an in vitro 3D chondrogenesis model was applied to human articular chondrocytes (hACs). In this model, a temporal exposure to recombinant GH during the proliferation phase at passage 1 was applied (exposed) and compared to control hACs (unexposed) of the same individual. As a measure for matrix deposition, surface area of the pellets was determined at subsequent time points during chondrogenesis. In addition, deposition of cartilage extracellular matrix (e.g. glycosaminoglycans) was determined with quantitative PCR and with immuno-histochemistry. Also the mechanical properties of the cartilage particles were measured (MACH-1, Biomomentum).
Data was generated from 3 different donors, 3-4 particles each.
Results: Significantly reduced matrix deposition was observed within 3 days of chondrogenesis following exposure to GH (p-value=0.006). After 14 days, however, GH-exposed constructs showed a significant catch-up in matrix deposition (45.5% increased surface area) as compared to controls (35% increased surface area; p-value=0.002). In addition, when exposing 3D-cartilage constructs with and without temporal GH exposure to a repeated 10% mechanical stress, control particles were found to be

significantly more flexible and better able to adapt as compared to particles from chondrocytes exposed to GH (p-value=0.03).
Conclusion: Exposure of hACs to a temporal excess of GH in vitro results in altered chondrogenic capacity as well as altered mechanical properties of deposited matrix. We hypothesize that GH exposure affects epigenetic regulatory mechanisms leading to permanent changes in cellular signalling which is currently under investigation.

P37 The role of extracellular matrix in zebrafish regeneration: from a global transcriptomic analysis to the multifaceted role of collagen XIV

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The epimorphic regeneration allows the complete regrowth of tissues or organs after amputation. A better understanding of the mechanisms underlying regeneration can helps regenerative and reconstructive medicine. Although long viewed as essential for a wide variety of developmental and physiological processes, only few studies have so far addressed the role of the extracellular matrix (ECM) during regeneration. We thus decided to fill this gap by investigating specifically the role of the matrix proteins during regeneration. With this aim, we took advantage of the regenerative capacity of the zebrafish caudal fin. A whole-transcriptome RNA-sequencing analysis was conducted on adult caudal fin before amputation and at 2, 3 and 10 days post-amputation (dpa). Using bioinformatics we have established the "matrisome" of regeneration and we have demonstrated that ECM proteins play a key role throughout caudal fin regeneration. Among all the identified matrix genes, *col14a1a*, encoding the collagen XIV-A (COLXIV-A), caught our attention. In zebrafish, this gene is only expressed during embryogenesis (Bader *et al.*, 2013). Interestingly, the protein COLXIV belongs to a pool of proteins implicated in the recruitment of progenitor cells during digit regeneration in mice (Agrawal *et al.*, 2010; Marçal *et al.*, 2012). Using qPCR and western-blotting respectively, we showed that both the gene *col14a1a* and the protein COLXIV-A are sharply upregulated at 2 and 3 dpa. At 2 dpa COLXIV-A was synthetized by the epidermal basal layer and progressively deposited in the newly formed basement membrane (BM). Using *vivo*-morpholino antisense strategy, we then demonstrated that COLXIV-A knockdown significantly impairs the regeneration process. We further demonstrated that the lack of COLXIV-A expression during regeneration does not affect cell proliferation but instead disturbs the balance between apoptosis and autophagy in the regenerate, a key biological process in regeneration (Varga *et al.*, 2013). The structure of the BM was also altered. Electron microscopy revealed that the epidermal BM was significantly thinner in absence of COLXIV-A. Using atomic force microscopy we also demonstrated that the topography and the stiffness of the epidermal BM are affected. Based on previous data, we hypothesized that ECM remodeling during regeneration can result in the release of a bioactive fragment from COLXIV-A that can be involved in stem and/or progenitor cells recruitment at the amputation site. We have recently obtained promising preliminary results that highlight the multifaceted role of COLXIV-A in regeneration.

P38 A modified technique to assess extracellular matrix turnover rate using aspartic acid racemisation

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Determining the rate of protein turnover is an important aspect of understanding normal metabolism and many disease processes. The synthesis and degradation of most proteins can be conveniently assessed using labelled precursors and pulse chase experiments. The long half-life of collagen in many

connective tissues however, makes this technique impractical and other approaches are necessary. Measuring age-related chemical modifications, such as amino acid racemization, can be used to assess protein turnover in tissues where turnover is slow. All amino acids are incorporated into tissues in the L-form, but over time they slowly convert to the D- form through a spontaneous optical conformation. This process is extremely slow for all amino acids; aspartic acid (Asp) racemisation occurs most rapidly making this amino acid a convenient choice. The previously established method for the quantification of Asp racemization in tissues uses High Pressure Liquid Chromatography (HPLC) separation of the two isomers and fluorescence detection after pre-column derivatisation with o-phthaladehyde (OPA)/N-acetylcysteine (NAC)[1]. This technique however, is relatively insensitive, the products are not chemically stable and the chromatographic separation is poor at higher concentrations of the analytes.
In the current study, we have developed a new fluorescence-based HPLC method for the quantification of D- and L- Asp, which overcomes the shortfalls of the previous technique. Amino acids are pre-column derivatised with naphthalene-2,3-dicarboxaldehyde (NDA) using cyanide ions as a nucleophile. Naphthalene-2,3-dicarboxaldehyde (NDA), a reagent analogous to OPA, is known to react rapidly with primary amines in the presence of CN−, as the nucleophile, to form 1-cyanobenz[f]isoindole (CBI) derivatives of amines, which possess fluorescent and electroactive properties. The corresponding CBI-derivatives are injected without any further clean-up, separated using a binary eluting system flowing under gradient conditions, and detected fluorimetrically at excitation and emission wavelengths of 420 and 490 nm.
The technique has been validated by demonstrating a complete separation of a mix of amino acids including D- and L aspartic acid in the presence of Boc-Arg-OH, as an internal standard. Compared to the products formed by derivatisation with OPA, the CBI derivatives exhibit improved chemical stability and were stable for up to eight hours. The products show enhanced fluorescence quantum efficiency relative to derivatisation with OPA, increasing sensitivity by an order of magnitude. The selected derivatization reagent proved to be chromatographically more selective than OPA, thus allowing baseline separation of all derivatized amino acids in 20 minutes instead of the previous run time of 35 minutes. We demonstrate the application of the technique to extracellular matrix proteins using human Achilles and anterior tibialis tendon.
References:
1. Yekkala et al., (2007) J. Sep. Sci. ***30****, 118-121*

P39 Tensile response of tendon collagen fibrils in relation to cross-links

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Introduction: Tendon is mainly made up of aligned type I collagen fibrils, and while the mechanical behavior of tendons has been studied at the macroscopic level, only few studies have been performed at the level of collagen fibrils. Our previous results found that collagen fibrils from human patellar tendon are not linearly elastic, but rather display three distinct phases, which was not the case for rat-tail tendon (1). Although the chemical stability of covalent cross-links (a prominent distinction between the two tissues) affected the macroscopic mechanical properties it did not alter the properties of fibrils. The present work further investigates the influence of enzymatic and non-enzymatic cross-links and maturation on collagen fibril mechanics.
Method: Achilles and tail tendons of 4- and 16-week old rats were used and also treated with methylglyoxal to induce non-enzymatic cross-links. Tail tendons were mechanically tested macroscopically and individual collagen fibrils were mechanically tested on a custom made device. Acid solubility was also measured.
Preliminary Results: Achilles tendons were less soluble in acid than tail tendons. For both tissues acid solubility was reduced with age, and methylgyoxal treatment made all tissues completely insoluble. At

the macroscopic level tail-tendons gained strength and stiffness with age and with methylgyoxal treatment. At the fibril level we were unable to detect any mechanical differences between tissue types or age but there was a marked increase in strength and stiffness following methylglyoxal treatment. The behavior of the treated fibrils also changed to display the three phases previously seen in human fibrils.
Conclusion: At the macroscopic level there appears to be a relation between chemical and mechanical stability of tendon tissue. This relation does not appear to hold at the level of collagen fibrils, which also suggests that there is a disparity between macroscopic and microscopic mechanical properties. Finally it seems that the previously observed difference between human and rat collagen fibrils may be caused by non-enzymatic cross-links rather than enzymatic cross-links as we had previously proposed.
References:
1. Svensson RB, Mulder H, Kovanen V, Magnusson SP. Fracture mechanics of collagen fibrils: Influence of natural cross-links. Biophys J 104: 2476-2484, 2013.

Plenary Lecture (L10) Mechanisms of the anti-tumor effects of NC1(XIX), the C-terminal domain of type XIX collagen

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Basement membranes are highly specialized forms of extracellular matrix that represent a barrier between the epithelium and the underlying connective tissue. They consist of type IV collagen and associated collagens (collagens XV, XVIII and XIX), structural glycoproteins (mainly laminin and entactin,) and proteoglycans, mainly perlecan. The interactions of the various basement membrane components with the cells, via surface receptors as integrins, may regulate many biological activities such as cell migration, proliferation or differentiation.
Activated stromal cells produce proteases and angiogenic factors. In addition to stromal ECM breakdown, proteases exert various pro- or anti-tumorigenic functions and participate in the release of various ECM fragments, named matrikines or matricryptins, capable to act as endogenous angiogenesis inhibitors and to limit tumor progression.
Several basement membrane collagen-derived matrikines have been shown to decrease tumor growth in various cancer models. These matrikines exert their anti-tumor effects through anti-angiogenic and/or anti-tumor activities at different levels. In our laboratory, we were interested in NC1(XIX), an anti-tumor matrikine derived from type XIX collagen.
Type XIX collagen is a minor collagen that localizes to basement membrane zone. Since several NC1, C-terminal, domains of other chains from basement membrane collagens were reported to exhibit anti-tumor activity, we decided to study the effects of the NC1 domain of collagen XIX [NC1(XIX)] on tumor progression, using an experimental in vivo model of mouse melanoma. We observed a 70% reduction in tumor volume in NC1(XIX)-treated mice, compared to the corresponding controls. Histological examination of the tumors showed a strong decrease in tumor vascularization in treated mice. In vitro, NC1(XIX) inhibited the capacity of tumor cells to invade Matrigel®. It also strongly inhibited the migratory capacities of melanoma cells in the scratch wound model and in Ibidi® devices. Similar results were obtained with UACC 903 human melanoma cells. NC1(XIX) also inhibited the capacity of Human Microvascular Endothelial Cells to form pseudo-tubes in Matrigel®. This effect was accompanied by a strong inhibition of MT1-MMP (MMP-14) and VEGF expression. We also demonstrated that plasmin, one of the most important enzyme involved in tumor invasion, was able to release a fragment of NC1(XIX), which retained all the anti-tumor activity. Molecular modeling studies showed that NC1(XIX) and the anti-tumor fragment released by plasmin adopted locally the same type I β-turn conformation, suggesting that the anti-tumor effect was conformation-dependent. Recently, we identified avb3 as a receptor of NC1(XIX) and demonstrated that NC1(XIX) inhibited the FAK/PI3K/Akt/mTOR pathway and increased GSK3b activity by decreasing their phosphorylation. Taken together, these results suggest that NC1(XIX) has the potential for the design of new anti-cancer drugs.

Plenary Lecture (L11) Epigenetic control of hyaluronan synthases

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Epigenetics has emerged as a key aspect in gene expression and in extracellular matrix (ECM) several genes has this control. The synthesis of hyaluronan (HA) is controlled by gene expression of hyaluronan synthases 1,2 and 3 and in particular 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) (1). Smooth muscle cells (SMC) in the presence of different stimuli, as inflammation, oxLDL, mechanical stress, produced an

altered ECM where hyaluronan (HA) is abundant as demonstrated in areas of atherosclerotic lesions. The control of the HA synthesis is therefore 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 (2). 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 (3). Eventually we found a long non-coding RNA (NAT) positively controls in cis the HAS2 expression involving p65 and NFkB pathway (4). Beside the antisense effect, another epigenetic control has been described 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 data produced indicate that epigenetics is a key point of the hyaluronan metabolism and ECM composition.

References:

1.Vigetti D et al. (2010) J Biol. Chem 285(32):24639-45
2.Vigetti D et al. (2011) J Biol. Chem. 286(10):7917-24.
3.Vigetti D et al. (2012) J Biol. Chem. 287(42):35544-55.
4.Vigetti D, et al. (2014) J Biol Chem. 289(42):28816-26.

Workshop 3: Enzyme control of matrix function in health and disease

Invited Lecture (L12) Sensing and cutting in pericellular microenvironment: essential processes for cellular invasion

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When cells migrate in tissue, surrounding extracellular matrix (ECM), especially fibrillar collagen, acts as a physical barrier that prevents cell migration. Cells thus require to degrade ECM in order to invade the tissue. It has been shown that one of the membrane-bound matrix metalloproteoinases, MT1-MMP, promotes cellular invasion in collagen-rich ECM. MT1-MMP has been shown to modify cellular microenviroment by cleaving pericellular ECM, and it also changes changes cellular response to microenvironment by cleaving ECM receptors. On the other hand, mechanism of activation of MT1-MMP genes and its functions *in vivo* are still not well understood. In rheumatoid arthritis (RA), MT1-MMP is highly upregulated in synovial pannus tissue that erode cartilage by invading it. Administration of highly-selective MT1-MMP biologic inhibitor inhibited cartilage erosion in mouse model of arthritis, suggesting that MT1-MMP is indeed invasion promotor of synovial cell in vivo. In vitro, passaged human synovia cells constitutively express detectable level of MT1-MMP, but MT1-MMP in those cells are not functionally active. To activate MT1-MMP, cells need to be stimulated with collagen, a major substrate for MT1-MMP. We found that recognition of collagen by the cells is essential for cells to activate MT1-MMP function and for the enzyme to cleave collagen. In this talk, I will discuss our recent finding on the collagen receptor that mediate MT1-MMP activation, modification of pericellular microenvironment to activate MT1-MMP functions and implication of this to the progression of RA.

Invited Lecture (L13) The heparanase/syndecan-1 axis in cancer: Mechanisms and therapy

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Heparanase is an endoglucuronidase that cleaves the heparan sulfate chains of proteoglycans, releasing biologically active fragments of heparan sulfate, and leaving shortened (trimmed) chains on the proteoglycan core protein. There is mounting evidence that this enzymatic action of heparanase impacts cell signaling and promotes tissue remodeling that drives tumor growth, invasion, metastasis, osteolysis and angiogenesis. Using in vitro and in vivo models of multiple myeloma, we have discovered that mechanistically, heparanase regulates tumor progression by upregulating expression of a number of growth factors (*e.g.,* VEGF, HGF) and by elevating the secretion and altering the composition of tumor-derived exosomes. In addition, many of the functions of heparanase can be traced to its impact on enhancing shedding of the syndecan-1 proteoglycan from the surface of tumor cells. These findings indicate that heparanase is a viable target for anti-cancer therapy. In addition, its potential as a therapeutic target is enhanced by the fact that there is a single enzymatically active heparanase in humans and the finding that heparanase knockout mice appear to be healthy. Thus, therapeutic neutralization of heparanase activity would likely have limited negative side effects.
Roneparstat (previously known as SST0001) is a rationally developed heparanase inhibitor produced by the modification of porcine mucosal heparin, the final product being 100% N-acetylated and 25% glycol split. These modifications endow Roneparstat with high anti-heparanase activity (IC_{50} ~ 3 nM) while retaining only negligible anti-coagulant activity. Preclinical studies of SST0001 in animal models of multiple myeloma indicated anti-tumor efficacy and pharmacodynamic effects consistent with its anti-heparanase activity in vivo (*e.g.,* reduced angiogenesis and diminished expression of HGF, VEGF and MMP-9). This led to an ongoing Phase I study of Roneparstat in advanced refractory multiple myeloma

patients. In recent studies we have discovered that treatment of tumor cells with anti-myeloma drugs such as bortezomib and melphalan significantly upregulated expression and secretion of heparanase by myeloma tumor cells and that tumor cells with high levels of heparanase expression have enhanced resistance to killing by these drugs. This indicates a key role for heparanase in promoting myeloma chemoresistance and provides strong rationale for testing the heparanase inhibitor Roneparstat in combination with anti-myeloma drugs. Using a SCID mouse model of disseminated myeloma we found that Roneparstat in combination with either bortezomib or melphalan dramatically decreased both the number of animals with detectable tumor and the tumor burden when compared with animals treated with either of these drugs alone. Together these findings support further clinical testing of Roneparstat in myeloma patients and indicate that anti-heparanase therapy increases the efficacy of cytotoxic drugs used to treat myeloma.

Invited Lecture (L14) Why collagen I is normally a heterotrimer

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Fibrillar collagens such as collagen I are synthesized in precursor form, procollagen, with large N- and C-terminal propeptide extensions. While a homotrimeric form of collagen I (three identical chains) is found in embryonic tissues as well as in many diseases (cancer, fibrosis, osteoarthritis, osteoporosis, osteogenesis imperfecta, Ehlers-Danlos syndrome), the overwhelmingly predominant form is a heterotrimer, consisting of two a1 chains and one a2 chain. The main role of the C-propeptides is to initiate trimerization with the correct chain stoichiometry during intracellular assembly which then proceeds in a zipper-like manner towards the N-terminal end of the molecule. Proteolytic release of the propeptides during or after secretion into the ECM then controls the assembly of collagen molecules into fibrils. Following the first determination of the 3D structure of a fibrillar collagen C-propeptide trimer (Bourhis et al, 2012, Nature Struct Mol Biol 19, 1031), that of procollagen III (an obligate homotrimer), we have now determined, by X-ray crystallography, the 3D structure of the C-propeptide trimer of homotrimeric procollagen I (submitted for publication). While overall sequence identity among the C-propeptides of the fibrillar procollagens is approximately 50 %, there is considerable variation in the interface regions connecting neighbouring chains, thus accounting for the specificity of chain assembly. Site-directed mutagenesis revealed critical aspartate residues that are essential for homotrimerization. Concerning the heterotrimer, though we were unable to obtain crystals suitable for high resolution structure determination, biophysical analysis showed the low resolution structure to be similar to that of the homotrimer. We were therefore able to model the structures of the a1:a2 and a2:a1 interfaces in the heterotrimer. This revealed a unique set of charge interactions, in addition to the known absence of a crucial disulphide bond, that together account for the precise $(a1)_2a2$ stoichiometry seen in heterotrimeric procollagen I. One of the amino acid residues involved, a glutamate in the a1 chain, is mutated to lysine in a patient with osteogenesis imperfecta type IV. Analysis of the effect of this mutation using an *in vitro* expression system led to a reduction in the amount of heterotrimer with relatively little effect on homotrimer production. As also found with the a1 chain, single mutations in the a2 chain revealed back-up mechanisms stabilizing interchain interactions, such that multiple mutations were required to prevent homo- or heterotrimerization completely. These results therefore reveal the molecular basis for the heterotrimerization of collagen I.

ST11/P40 Proteolytic Processing of Human Thrombospondin-1

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Background: Thrombospondin-1 (TSP-1) is engaged in various biological processes such as angiogenesis and hemostasis. Previous analyses of plasma samples of cancer patients after surgery revealed a full-length 185 kDa and a smaller 160 kDa TSP-1 isoform. The latter prevailed post surgery indicating that processing of TSP-1 to 160 kDa was triggered. Therefore, we aimed at characterizing the potential source and generation of the 160 kDa TSP-1 isoform.
Methods: TSP-1 secreted by primary human endothelial cells or by platelets isolated from whole blood, was detected by immunoblotting. Furthermore, processing of the full-length protein was assessed in co-cultures of either endothelial cells or platelets with isolated leukocytes. The contribution of the neutrophil proteases elastase and cathepsin G to proteolytic fragmentation of TSP-1 was tested through application of protease inhibitors.
Results: Platelets and endothelial cells consistently released full-length TSP-1 into the supernatant, but both cell types co-cultured with neutrophils led to the appearance of 160 kDa TSP-1 protein. Moreover, incubation of endothelial-derived 185 kDa TSP-1 with elastase and cathepsin G resulted in 160 kDa fragments generated by cathepsin G, and 160 and 140 kDa fragments produced by elastase. The hypothesis that neutrophil degranulation and hence release of elastase and cathepsin G trigger the generation of the 160 kDa TSP-1 isoform, was verified through application of inhibitors for the neutrophil proteases to co-cultures of endothelial cells or platelets with neutrophils. This led to an almost complete block of the conversion of the full-length protein to the 160 kDa isoform.
Conclusions: Processing of endothelial TSP-1 by neutrophil proteases was identified as a potential source of 160 kDa fragments comparable to the TSP-1 isoform detectable in post-operative human plasma. The impact of this proteolytic processing on TSP-1 function in hemostasis is currently under investigation.

ST12/P41 First X-linked form of osteogenesis imperfecta, caused by mutations in *MBTPS2*, demonstrates a fundamental role for regulated intramembrane proteolysis in normal bone formation

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Osteogenesis imperfecta (OI) is a heritable bone dysplasia with collagen-related defects. Dominantly inherited OI is caused by structural defects in type I collagen or *IFITM5*, while recessive forms are caused by deficiency of proteins that interact with collagen to catalyze or facilitate its post-translational modification, folding or cross-linking. We have identified the first X-linked form of OI, caused by a defect in regulated intramembrane proteolysis (RIP). The best described RIP-mediated signaling in humans involves sequential cleavage of diverse substrates by site-1 protease (S1P) and site-2 protease (S2P), encoded by *MBTPS1* and *MBTPS2,* respectively. S1P and S2P are located in the Golgi membrane, where they cleave regulatory proteins transported from the ER membrane in times of ER stress or decreased sterol metabolites, releasing mature N-terminal fragments that shuttle to the nucleus to activate gene transcription.
In two pedigrees with moderately severe OI, linkage analysis and next generation sequencing identified novel missense mutations in *MBTPS2* that co-segregate with the phenotype in each pedigree. The mutations predict S2P p.N459S and p.L505F substitutions, respectively, located in or near the NPDG motif required for metal ion coordination. Neither *MBTPS2* transcripts nor S2P protein were decreased in proband fibroblasts and osteoblasts. However, proband and *Mbtps2*-deficient CHO cells co-transfected with mutant *MBTPS2* and luciferase reporter constructs demonstrated deficient cleavage or activation of RIP substrates OASIS, ATF6 and SREBP. Consistent with diminished activation of OASIS signalling, proband fibroblasts have significantly reduced secretion of type I collagen (20-73% vs normal control cells). Furthermore, the proportion of collagen with mature crosslinks is decreased in matrix deposited by cultured proband fibroblasts. Also supporting defective collagen crosslinking, type I collagen extracted from proband bone tissue contained less than half the normal level of hydroxylation of the Lys87 (K87) residue crucial for intermolecular crosslinking. This alteration in collagen K87 hydroxylation is associated with decreased levels of Lysyl Hydroxylase 1 (LH1) in proband osteoblast lysates. In addition, proband urinary LP/HP crosslink ratios are increased. These data suggest that impaired collagen crosslinking undermines bone strength in X-linked OI. S2P-deficient osteoblasts also demonstrated broadly defective differentiation with decreased expression of transcripts related to osteoblast maturation, including *ALPL*, when induced to differentiate in culture. Transcripts encoding OASIS (*CREB3L1*) and SMAD4, which form a complex to upregulate expression of matrix-associated genes, were also significantly reduced in proband versus normal control osteoblasts. These are the first human studies to demonstrate the fundamental role of RIP in bone development, in addition to its function in cholesterol metabolism.

ST13/P42 Lysyl oxydase like-2 (LOXL2) regulates angiogenesis through scaffolding of endothelial basement membrane

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Angiogenesis plays a crucial role in development and cardiovascular ischemic pathologies and is associated with extensive extracellular matrix (ECM) remodeling. LOXL2 belongs to the lysyl oxydase family of secreted enzymes involved in crosslinking of elastin and collagens. We previously showed that LOXL2 is expressed in growing vessels of zebrafish embryo and of newborn rat retina. In addition, knocking-down LOXL2 prevented proper formation of intersomitic vessels (ISV) in zebrafish embryos and of capillaries in 3D hydrogel culture models. Considering that LOXL2 is colocalised with type IV

collagen in the vascular basement membrane, we hypothesized that LOXL2 could regulate angiogenesis through organization of the basement membrane.
LOXL2 depletion altered organisation of type IV collagen and fibronectin in the ECM. Using proximity ligation assay and SPR, we detected direct interaction of LOXL2 with these proteins both intracellularly and in the ECM. In agreement, time-lapse TIRF microscopy demonstrated the direct incorporation of LOXL2-GFP in the ECM at the site of exocytosis. These experiments also provided evidence for the rapid release of LOXL2-GFP from the ECM, as expected from such cross-linking enzyme. In addition, both SPR and TIRF microscopy demonstrated that a second pool of enzyme was more stably associated to the ECM, suggesting the participation of LOXL2 to scaffolding of the basement membrane. Atomic force microscopy (AFM) measurement demonsrated that LOXL2 also regulates endothelial ECM stiffness. In order to further understand the mechano-transduction involved in the regulation of angiogenesis by LOXL2, cell contractility and adhesion were investigated. Contractility in response to rigidity was not affected by LOXL2-depletion, as measured by stiffness clamp AFM. Similarly, formation of focal adhesion by LOXL2-depleted cells was not altered on fibronectin or collagen coatings. Their maturation into fibrillar adhesion was however altered on cell autonomous ECM, as a consequence of defective ECM deposition. Finally, we analysed the role of LOXL2 in vascular morphogenesis using a co-culture model of capillary formation by endothelial cells seeded on top of a fibroblast monolayer. Whereas LOXL2 depletion only slightly altered formation of the capillary network, capillaries were discontinuous and thinner, and displayed collapsed tubulogenesis.
Altogether, our data demonstrated that LOXL2 regulates the generation of endothelial basement membrane, which in turn affects the mechano-transduction properties involved in capillary formation, suggesting the scaffolding role of LOXL2 in the ECM in endothelial sprouting and vascular morphogenesis.

ST14/P43 Stimulation of intracellular proteolysis reduced disease severity in an ER stress-related cartilage pathology

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Previous studies (1-3) have demonstrated that ER stress is an integral part of the disease mechanism in a dwarfism caused by mutations in the type X collagen gene, namely metaphyseal chondrodysplasia type Schmid (MCDS).
We have used a cell culture model of MCDS to screen a number of drugs for their ability to reduce the levels of ER stress induced by the expression of an MCDS-causing mutant form of collagen X (N617K). One drug, carbamazepine (CBZ), was found to reduce the ER stress induced as measured by the levels of the BiP, Xbp1s and CHOP mRNA. CBZ reduced the levels of ER stress induced by 4 different MCDS causing collagen X mutations. In all cases, intracellular accumulation of the mutant collagen X was reduced by CBZ but for 2 mutations, increased autophagy was primarily responsible whereas for the other 2 mutations, increased proteasomal degradation occurred. CBZ treatment of our Col10a1.pN617K MCDS mouse line caused a reduction in disease severity based on histomorphometric analyses of the growth plate and caused a significant increase in long bone growth in MCDS animals.
CBZ, a drug already in clinical use for bipolar and control of seizures represents a potential therapy for treating MCDS and other ER stress-related connective tissue disorders.
References:
1. Wilson, R., Freddi, S., Chan, D., Cheah, K. S. and Bateman, J. F. (2005) J Biol Chem, 280(16), pp. 15544-52.
2. Tsang, K. Y., Chan, D., Cheslett, D., Chan, W. C., So, C. L., Melhado, I. G., Chan, T. W., Kwan, K. M., Hunziker, E. B., Yamada, Y., Bateman, J. F., Cheung, K. M. and Cheah, K. S. (2007) PLoS Biol, 5(3), pp. e44.

3. Rajpar, M. H., McDermott, B., Kung, L., Eardley, R., Knowles, L., Heeran, M., Thornton, D. J., Wilson, R., Bateman, J. F., Poulsom, R., Arvan, P., Kadler, K. E., Briggs, M. D. and Boot-Handford, R. P. (2009) PLoS Genet, 5(10), pp. e1000691.

ST15/P44 Embryonic lymphangiogenesis and placental angiogenesis are altered in absence of Adamts3

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Nineteen secreted metalloproteases form the family of ADAMTS proteases. The only documented enzymatic activity of a sub-class comprising ADAMTS2, 3 and 14 is the cleavage of the aminopropeptide of fibrillar procollagens, a process required for the proper formation of collagen fibrils. Based on genetic disorders, in vitro analyses and co-localization studies, it is generally accepted that ADAMTS2 is mainly responsible for processing of type I and type III procollagens in the adult, while ADAMTS3 was considered as the main enzyme for the processing of type I and type II procollagens in the embryo and of type II procollagen only during the adult life.
We created a knockout mouse model (Adamts3$^{-/-}$) to verify this hypothesis. Adamts3$^{+/-}$ mice are viable and fertile, but intercrosses demonstrated lethality of Adamts3$^{-/-}$ embryos around 15 days of gestation (15 dpc). Procollagen processing was unaffected in these embryos. Surprisingly, massive lymphedema was observed already at 13.5 dpc, at a development stage during which the lymphatic network is forming. This absence of lymphangiogenesis in Adamts3$^{-/-}$ embryos has been linked to impaired pro-VEGF-C cleavage into VEGF-C able to activate VEGF-R3.
Apoptotic foci in ventral zones of the liver were also identified. Further analyses performed on liver at increasing developmental stages (E13.5 to E14.5) showed increased expression of genes known to be stimulated by hypoxia and/or involved in the glycolysis pathway. A marked enlargement of the blood vessels close to the apoptotic areas was also evidenced which further illustrated the existence of local hypoxia and/or metabolic alterations. Since placenta is a critical organ allowing O_2 and nutrient exchange between the maternal and fetal blood, its structure was evaluated by immunohistochemistry. The gross morphology of Adamts3$^{-/-}$ placenta was normal but a significant reduction of blood vessel diameter was observed, which could hamper fetal blood circulation. Since insufficient activation of the VEGF-C/VEGF-R3 axis is the cause of the lymphatics phenotype, the potential expression of VEGF-R3 in placenta blood vessels was evaluated. Many placenta blood vessels were positive for VEGF-R3, irrespective of the genotype, which provides a direct link between the reduced diameter of placental blood vessels and the ADAMTS3-VEGF-C pathway.
This study is the first demonstration that an aminoprocollagen peptidase is crucial for developmental processes independently of its primary role in collagen biology and has physiological functions potentially involved in several human diseases related to angiogenesis and lymphangiogenesis.

P45 Processing of thrombospondin-1 by bone morphogenetic protein-1 strongly alters its ability to promote cell adhesion and to activate TGF-β

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Bone Morphogenetic Protein (BMP-1) is an extracellular metalloprotease known to be involved in the maturation of several extracellular proteins, including fibrillar procollagens and growth factors of the TGF-b and IGF superfamilies. As a consequence, it is essential for embryonic development and tissue remodeling and has been clearly involved in several lethal diseases ranging from osteogenesis imperfecta to fibrosis and cardiovascular diseases.
In this context, we studied the effect of BMP-1 overexpression in two cell lines (HT1080 and HEK 293-EBNA) and observed significant changes in cell morphology, proliferation, migration and adhesion. Since these observations were not obviously explained by the cleavage of known BMP-1 substrates, we decided to analyze BMP-1 activity in these cells in more detail. First, the effect on cell adhesion was investigated and revealed a drastic cell detachment due to modifications in the extracellular matrix. Then, the analysis of cell conditioned medium led to the identification of a new BMP-1 substrate which was a good candidate to explain the observed phenotype. This substrate is thrombospondin-1 (TSP-1), a multifunctional matricellular protein known to modulate cell-matrix interactions in several biological processes including platelet aggregation, angiogenesis and wound healing. TSP-1 also controls TGF-b activation and thereby, could explain the effect of BMP-1 on cell proliferation.
We confirmed the direct cleavage of TSP-1 by BMP-1 *in vitro* and determined that the cleavage site was located upstream from a domain known to play an important role in the interaction of TSP-1 with various cell receptors. Importantly, we also found that cleaved TSP-1 becomes unable to promote cell adhesion in contrast to full-length TSP-1. Moreover, siRNA-mediated extinction of TSP-1 in HT1080 cells reveals a similar morphology to that seen in BMP-1-overexpressing cells. These results strongly suggest that the loss of adhesion induced by BMP-1 is due to TSP-1 cleavage.
Given that the BMP-1 cleavage site in TSP-1 is also near the TGF-β activation domain, we analyzed the ability of cleaved TSP-1 to activate latent TGF-β. Interestingly, we observed that TGF-β-dependent signaling was increased two-fold when the cleaved and intact TSP-1 forms were compared, probably in relation with a better accessibility of the TGF-β binding site after TSP-1 cleavage. All these results demonstrate that the phenotypic changes observed upon BMP-1 overexpression are mostly the consequence of TSP-1 cleavage. Work is now in progress to evaluate the potential involvement of TSP-1 cleavage by BMP-1 in the context of wound healing where both proteins are known to be induced.

P46 Unveiling new functions of ADAMTS2, 3 and 14 in extracellular matrix organization and cell signalling using a N-terminomics approach

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ADAMTS2, 3 and 14 are highly homologous metalloproteases that were initially identified based on their aminoprocollagen peptidase activity. Various more recent studies indicate that they are also involved in male fertility, angiogenesis, pathologies related to blood coagulation, lymphatics development and osteoarthritis, which suggests the existence of substrates other than fibrillar collagens.
In order to evaluate this hypothesis, we analysed the substrates repertoire of ADAMTS2, 3 and 14 using a N-terminomics approach (iTRAQ-TAILS) that allows the relative quantification of N-termini of proteins in the secretomes of control cells or cells producing ADAMTS2, 3 or 14. These analyses identified 8, 17 and 22 potential "new" substrates, respectively for ADAMTS2, 3 and 14.
Validation assays by other methods are currently ongoing. As a few examples, we have already confirmed that the 3 enzymes efficiently cleave the assembly domain of fibronectin and that DKK3, a regulator of the Wnt/β-Catenin pathway is a substrate of ADAMTS2 and 14. LTBP1 (Latent transforming growth factor beta binding protein 1) and TGFb-R3, two key modulators of the TGFb pathway, are also substrates of the 3 enzymes. In order to evaluate if these cleavages might affect cell

response to TGFb, RT-PCR quantifications and immunofluorescence analyses were performed on control and ADAMTS2-depleted fibroblasts incubated with TGFb1 or TGFb2. In depleted fibroblasts, the induction of CTGF and α-SMA by TGFb is reduced, which confirms a biological impact of ADAMTS2 on the TGFb signaling pathway.
Since their initial identifications, ADAMTS2, 3 and 14 have always been considered as responsible for only the processing of the aminopropeptide of fibrillar collagens. This work expands this dogmatic view by demonstrating that they cleave also diverse molecules related to cell signaling or contributing to the architecture of the extracellular matrix and its turnover. It shows also for the first time that ADAMTS2 (and possibly ADAMTS3 and 14, by analogy) is a regulator of TGFβ signaling, a crucial pathway involved in multiple processes. Finally, these 3 ADAMTS could also regulate the Wnt/β-Catenin signaling pathways, although this would require further investigations to be firmly demonstrated.

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P47 The propeptide of lysyl oxidase, a new partner of cross-linking enzymes and proangiogenic factors

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Prolysyl oxidase (proLOX) is an extracellular proenzyme activated by Bone Morphogenetic Protein-1, which releases its propeptide and the mature enzyme. LOX initiates the covalent crosslinking of several collagen types and of elastin. The propeptide is internalized by different cell types, inhibits the transforming activity of the *H-Ras* oncogene in fibroblasts, and contributes to the secretion of LOX (*Grimsby et al. Biochemistry 2010, 111:1231-43*). Its interaction with tropoelastin is involved in the deposition of lysyl oxidase onto elastic fibers (*Thomassin et al. J Biol Chem 2005, 208:42848-55*). The goal of our work is to identify new partners of the propeptide of human LOX to determine if it is able to exert other biological roles that those reported above.
The propeptide of human prolysyl oxidase was expressed under a recombinant form in human embryonic kidney-293 EBNA cells. Its partners were identified with protein and glycosaminoglycan arrays probed by surface plasmon resonance imaging. More than twenty new partners of the propeptide have been identified including extracellular proteins (collagens I, II, III, dermatopontin, latent TGF-b binding protein 1), a proteoglycan (fibromodulin), glycosaminoglycans (heparin/heparan sulfate, chondroitin sulfate, dermatan sulfate, and hyaluronan), enzymes (transglutaminase-2, matrix metalloproteinase-2, superoxide dismutase, lysyl oxidase-like 2), the membrane protein Tumor Endothelial Marker-8 (TEM-8), and two growth factors. Intriguingly, the propeptide interacts with mature LOX and both share ECM partners (i.e. collagens, tropoelastin, fibronectin, fibromodulin, and dermatopontin).
The propeptide interacts with two proangiogenic growth factors (EGF and VEGF) and with the proangiogenic membrane protein TEM-8, which prompted us to study its ability to regulate angiogenesis. Although the propeptide did not influence tube formation by endothelial cells *in vitro*, we are currently investigating if it binds to activated endothelial cells *via* TEM-8, heparan sulfate, and if is able to interfere with EGF-induced signaling. We have built the interaction network of the propeptide using MatrixDB, the database developed by the team (matrixdb.univ-lyon1.fr*, Launay et al., 2015 Nucleic Acids Res 43:D321-7*) and are currently analyzing it with the Functional Enrichment analysis tool FunRich (www.funrich.org) to get new insights on molecular functions of the propeptide, and on the biological processes and pathways it may be involved in.

P48 Integration of proteomics and peptidomics data towards identifying kidney-specific proteolytic events associated with Chronic Kidney Disease progression

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Introduction: Detection of early stages as well as prediction of kidney disease progression remains a challenge with the currently available clinical methods. To address this issue, we investigated the proteome and peptidome of urine and plasma for the identification of biomarkers associated with Chronic Kidney Disease (CKD) progression using high resolution mass spectrometry. We focused on the integration of obtained -omics data for the identification of proteolytic events specific for the kidney. This includes prediction of proteases related to CKD progression, hence contributing to the elucidation of mechanisms underlying disease pathophysiology.
Methods: The following datasets originating from published but also ongoing work in our teams were used for data integration: i) urine tryptic peptides and ii) urine naturally occurring peptides from studies related to the prognosis of CKD progression; iii) plasma tryptic peptides from a study related to CKD staging; and iv) plasma naturally occurring peptides not specific for any disease. Protease prediction was performed using Proteasix software (proteasix.org).
Results: Peptide frequency (i.e. the percentage of samples in which the peptide was detected) and protein sequence coverage for each of the datasets was assessed. Peptides were considered as kidney-specific if they have been detected only in urine and not in plasma. Among the latter, for differentially expressed peptides in CKD progression, protease prediction was performed. As an example, a naturally occurring peptide of collagen alpha-1 (III) chain (position 910-948) was identified uniquely in the urine peptidomics datasets and thus, was considered as kidney-specific. Furthermore, this peptide was found as down-regulated in CKD progressors compared to non-progresssors, putatively linking it with disease progression. Protease prediction revealed five matrix metalloproteinases potentially generating this peptide: MMP1, MMP8, MMP12, MMP13 and MMP16.
Conclusions: In this study, we demonstrated that integration of proteomics and peptidomics data can predict peptides that originate from kidney and increase the confidence in protease predictions, opening new research avenues for future experiments. This ultimately, hopefully may lead to the discovery of novel molecular targets for therapeutic interventions. To confirm the validity of our findings, we currently aim at detecting shortlisted peptide candidates in kidney tissue using targeted proteomics approaches.

P49 Expression of ADAMTS4 and ADAMTS6 in Snail overexpessing melanoma and colon adenocarcinoma cells: effect of lumican

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SNAIL is one of the major transcription factors controlling EMT in various cancer cells affecting migration, invasion and proliferation. Previous studies in our laboratory showed that Lumican inhibits SNAIL-induced melanoma cell migration specifically by blocking MMP-14 activity. Beyond matrix MMPs, increasing interest has been focused on ADAMTS family of secreted MMPs. Many ADAMTS

possessing anti-tumor activity have been found to be modified epigenetically in cancer, suggesting their direct impact in cancer development. Preliminary observations in our laboratory suggested an increase at transcriptional level of ADAMTS6 in aggressive cancers. Moreover, it has been shown that ADAMTS4 and fragments thereof affect tumor growth in a different manner. The aim of the present study was to investigate the effect of SNAIL on the expression and cellular distribution of ADAMTS4 and ADAMTS6, as well as the effect of Lumican on the SNAIL-regulated ADAMTS4 and ADAMTS6 expression. B16F1 expressed the latent and active forms and variously sized fragments of ADAMTS4 and ADAMTS6 in both cell extract and conditioned media. SNAIL induced the expression of a 53kDa ADAMTS4 fragment in conditioned media. In the presence of Lumican the same band was found to be increased in cell extracts. In addition, SNAIL decreased the latent form of ADAMTS6 in conditioned media. In the presence of Lumican, most of the ADAMTS6 fragments were either decreased or lost. Similar expression as described above in B16F1 cells was observed for ADAMTS4 in HT29 cells. However, SNAIL decreased the 53kDa band in cell extracts. Lumican, on the other hand, seemed to induce the expression of a 30kDa fragment in cell extracts. In addition, HT29 cells showed an about similar expression of ADAMTS6 as described above in B16F1 cells. However, SNAIL induced the expression of a 53kDa fragment in conditioned media, which was additionally induced by Lumican. Moreover, Lumican induced a 30kDa fragment in cell extracts and reduced significantly the expression of a 97kDa band. In conclusion, SNAIL overexpression affected ADAMTS4 and ADAMTS6 expression in a different manner in the cells examined. Lumican, on the other hand, seemed to act by increasing selected ADAMTS4 fragments and decreasing selected ADAMTS6 fragments. Taken together, it seems that different mechanisms are responsible for the regulation of ADAMTS expression in aggressive cancers.

P50 Extracellular matrix involvement during zebrafish regeneration: highlight on the MMP/TIMP balance

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Regeneration enables the perfect restoration of lost or damaged tissues, organs and appendages. In adult humans, the regenerative potential is restricted to few tissues such as bone marrow, superficial layers of the skin, intestinal mucosa, nailbeds and to the liver. Thus, injury to other body parts usually leads to permanent damage from scarring to disability. To overcome this problem, one approach is to identify the key molecules guiding the stunning regeneration observed in organisms which maintain their regenerative potential throughout their life. The zebrafish model is able to regenerate numerous tissue and organs such as brain, spinal cord, heart and caudal fin. Notably due to their easy molecular and genetic manipulations, countless studies focused on larval zebrafish regeneration. However, those regeneration studies are controversial because of the intense developmental process occurring during this early life stage. We therefore performed for the first time, a transcriptomic analysis of genes differentially expressed during adult caudal fin regeneration using the RNA-sequencing technology. We used this data to investigate the genes encoding the extracellular matrix proteins and their associated receptors and we then specifically focused on the MMP/TIMP balance. We showed that *mmp-9* and *timp-2b* genes are highly upregulated throughout the all regeneration process. These results were then confirmed by qRT-PCR, western blot, zymography and reverse zymography. Importantly, we also showed that these genes are regulated during caudal fin regeneration in larvae. Localization and identification of cells expressing *mmp-9* gene and producing Mmp-9 protein were then analyzed by *in situ* hybridization, *in situ* zymography and immunofluorescence experiments and we showed that depending on the regeneration stage, different type of cells expressed Mmp-9. Finally, we specifically blocked Mmp-9 activity through the use of inhibitors and we showed that Mmp-9 corresponds to a key molecule driving the regeneration process following zebrafish caudal fin amputation. All these results highlight the central role of extracellular matrix proteins and specifically Mmp-9 during regeneration with obvious interest in regenerative medicine.

P51 Expression of ADAMTS in breast cancer cells

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ADAMTS is a family of extracellular metalloproteinases that contain at least one trombospondin-1 domain. Nineteen members of this family have been described so far. During the last decade increasing number of studies focused to their role in various types of cancer, and the results obtained suggested that ADAMTS functions were depending to the major ADAMTS form survived from extracellular proteolytic activities. The present study was undertaken to examine ADAMTS expression in breast cancer cells. Cells of different metastatic potential (MCF-7 and MDA-MB-231) were used to examine any relation between ADAMTS expression and metastatic potential. The same cells, stably transfected to suppress ERα and ERβ expression, respectively, were also included. In this case, ERα suppression gave to MCF-7 cells a mesenchymal-like phenotype. The results showed that: ADAMTS-1, -3, -6, -12, -15 and -16 were highly expressed in control MDA-MB-231 and shERαMCF-7 cells, consequently they were positively related with metastatic potential. ADAMTS-2, -7, -10, -13, -14, -18 and -19 were highly expressed in control MCF-7 cells and were reversibly related with metastatic potential. This is the first indication that ADAMTS members are expressed differentially in the various breast cancer cells, and of the existence of a relation between ERα/ERβ and ADAMTS expression. The results taken together suggest that specific ADAMTS members might be used for characterization of tumor aggressiveness, as

well as for specific treatment of selected cancers.

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P52 Laminin 111 Phosphorylation an in silico study

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Laminins are cross-shaped trimeric extracellular matrix proteins, consisted by an α-chain, an β-chain and an γ-chain. There are 5 genetic variants for α-chains (LAMA1, LAMA2, LAMA3, LAMA4, LAMA5), 4 for β-chains (LAMB1, LAMB2, LAMB3, LAMB4) and 3 for γ-chains (LAMC1, LAMC2, LAMC3), which are variously combined, creating the 16 isoforms of Laminin. The first laminin isoform was discovered 47 years ago and is today known as Laminin 111 (LAMA1, LAMBAMC1).

Laminins have been correlated with many important physiological processes, like cell differentiation, migration and adhesion. Furthermore, they modulate a host of cellular functions, like cell survival and growth, cell polarity, cell migration and tumor invasion and metastasis. Laminin play a key role on extracellular matrix-cell signaling. Laminin-111, is playing a critical role in the embryonic development and implantation, since it is the first extracellular matrix protein that is produced in the early life of an embryo. In adult tissues, Laminin-111 is expressed in kidney, liver, testis and ovary epithilium and in blood vessels. Moreover, specific sequences on Laminin-111 chains have been shown to proceed the function of cell-attachment, tumor metastasis, and kidney tubulogenesis. It has been reported that several extracellular matrix proteins can be phosphorylated by ecto- or exo-protein kinases, among them also laminin 1. This phosphorylation can alter functionally the extracellular matrix providing an additional way for intercellular communication.

Study of the bibliographic databases revealed that LAMA1 includes 41 sites responsible for various physiological and biological functions. LAMB1 includes 14 sites responsible for various functions and LAMC1 has 9 cell attachment sites, with no other functions identified for this chain. For LAMA1 a total

of 19 (11 on Ser and 8 on Thr), for LAMB1, 9 (5 on Ser and 4 on Thr), and for LAMC1 15 (7 on Ser and 8 on Thr) phosphorylation sites have been identified.
A total of 6 phosphorylation sites were identified within the reported active sites in LAMA1, 3 (2 Ser and 1 Thr) in LAMB1, 2 (all Thr) and in LAMC1 1 (Ser).
From the analysis of the motifs on these phosphorylation sites, we found 19 different kinases that can recognize those motifs. Four of these kinases have been reported to have ectoprotein kinase activity.
These results provide additional evidence for a possible physiological significance of laminin 111 phosphorylation by extracellular kinases.

P53 Expression of hyaluronidases in head & neck cancers

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Hyaluronidases (Hyals) are a class of six different enzymes with similar activity being implicated in cancer. In head & neck tumors, various isoforms of Hyals are found to be involved in cancer growth and progression. Among these, PH-20 and Hyal-1 seem to play a key role. To further elucidate the implication of Hyals in Head & Neck cancer, the present work focused on the examination of their expression in laryngeal cancer patients, and in relation to the anatomic site of cancer. Samples of 24 patients subjected to laryngectomy were obtained, two from each patient, one from the central part of cancer (C) and a second from a macroscopically normal part (N) and applied to mRNA isolation for the examination of Hyals expression. Fourteen samples were from glottic (G) and ten from superglottic (SG) cancer. Thirteen of the patients were of stage III and eleven of stage IV. RT-PCR analysis showed that the majority of enzymes largely differentiated between C and N samples. The expression of Hyal-1-wt, Hyal-1-v1, Hyal-1-v2, Hyal-2-wt, Hyal-3-wt was increased about 6-, 2-, 4-, 2- and 2-times, in C samples compared to N, respectively. On the other hand, Hyal-1-v5 expression was decreased to 10% in C samples compared to the one of N. PH-20 and Hyal-3-v3 expression was similar in both C and N samples. Hyal-1-v3, Hyal-1-v4, Hyal3-v1 and Hyal3-v2 were not detected. Depending on the anatomic site, PH-20 and Hyal-3-v3 were expressed in SG by 150% and 50% higher than in G samples, respectively, whereas all other enzymes detected showed lower expression in SG samples. The obtained data suggested that analysis of the various isoforms of Hyals might be a useful tool for head & neck cancer diagnosis. In addition, certain enzymes seemed to be expressed characteristically in either G or SG cancer. More data, however, is required, especially of early cancer patients, to verify the applicability of this analysis.

Workshop 4: Epigenetics, System Biology and Stem Cell Niche

Invited Lecture (L15) The interaction network connecting angiogenesis and Alzheimer's disease: focus on endostatin, lysyl oxidase and membrane collagens

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Interactions provide most biomolecules with functions, and influence each other *in vivo*. We have thus developed a roadmap to build extracellular and pericellular interaction networks in order to identify new functions of ECM proteins, and to determine how these interaction networks are rewired at the molecular level in diseases. 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 created a database (MatrixDB, http://matrixdb.univ-lyon1.fr/) to store extracellular interaction data and build interaction networks *(Launay et al., Nucleic Acids Res 2015 43:D321-7)*. Networks are contextualized and analysed with Cytoscape, an open source software platform (http://www.cytoscape.org/), and with the Functional Enrichment analysis tool FunRich (http://www.funrich.org/). This approach has been used to build the extracellular interaction network connecting angiogenesis and Alzheimer's disease. We initially focused on endostatin, a matricryptin of collagen XVIII, and lysyl oxidase, which are anti-angiogenic and pro-angiogenic respectively. Furthermore they are both found in high amount in vessel walls and amyloid plaques in Alzheimer's disease brain. Lysyl oxidase initiates the covalent cross-linking of collagens and elastin, plays a key role in cancer progression and influences cell signaling but no receptor has been identified so far for this enzyme. We have shown that lysyl oxidase binds to Tumor Endothelial Marker 8 (TEM-8), overexpressed at the surface of activated endothelial cells, to a5b1 and avb3 integrins and to the ectodomains of membrane collagens XXIII and XXV. Endostatin also interacts with TEM-8, a5b1 and avb3 integrins and with membrane collagens but it binds to the ectodomains of membrane collagens XIII and XVII. We have demonstrated that the ectodomains of collagens XIII and XXIII inhibit the formation of endothelial cell tubes in an *in vitro* Matrigel angiogenesis assay. Given that these membrane collagens are anti-angiogenic and also expressed by neurons, they may contribute to the interaction network connecting angiogenesis and Alzheimer's disease *via* endostatin and lysyl oxidase. The network will be contextualized by integrating the interaction data we collected by SPRi between endostatin, lysyl oxidase, membrane collagens and different molecular forms of the Ab(1-42) amyloid peptide. This will allow us to identify the molecular connections and pathways connecting angiogenesis and amyloidogenesis in Alzheimer's disease, and thus potential therapeutic targets, which could be either proteins or protein-protein interactions.

Invited Lecture (L16) Phosphorylation of secreted proteins by a new family of kinases

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Protein phosphorylation is a common post-translational modification that is critical for regulating nearly every aspect of cellular life. Until recently, all of the known kinases responsible for phosphorylating proteins were localized within the cell, primarily in the cytoplasm and nucleus. Although the first phosphoprotein ever reported was the secreted milk protein casein, the identification of kinases within the lumen of the secretory pathway capable of phosphorylating proteins destined for secretion or facing the extracellular face of the plasma membrane has remained elusive. Our lab has identified two families of kinases, a dozen in total, which localize within the lumen of the secretory pathway and are frequently secreted. Of particular interest is Fam20C, the *bona fide* Golgi casein kinase that has a preference for

phosphorylating substrates within an S-x-E/pS motif. Mutations in Fam20C cause Raine Syndrome, a devastating neonatal osteosclerotic bone dysplasia. Using quantitative phospho-mass spectrometry of conditioned media from Fam20C wildtype and CRISPR/Cas9 knock-out cells, we have identified over a hundred new substrates for the Fam20C kinase. Interestingly, GO Term analysis maps many of these substrates to processes involved in regulating the extracellular matrix. Fam20C depleted breast cancer cells have enhanced adhesive properties and are deficient in their ability to migrate both into a wound and through Matrigel, suggesting a potential role for phosphorylation of Fam20C substrates in mediating breast cancer metastasis. Current work exploring the contribution of Fam20C phosphorylation to the formation of ECM secreted by MEFs will also be discussed.

Invited Lecture (L17) microRNA miR-142-3p inhibits breast cancer cell invasiveness and stem cell properties by targeting integrin alpha V, KLF4 and multiple cytoskeletal elements

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MicroRNAs are post-transcriptional regulators of gene expression which influence tumorigenesis and tumor progression. miR-142-3p expression is dysregulated in several breast cancer subtypes, but its function at the molecular level is not known in detail. Here, we demonstrate that miR-142-3p is regulated in response to irradiation (2Gy) in human breast cancer cells, suggesting a potential role in the response to radiotherapy. Supported by transcriptomic Affymetrix array analysis and confirmatory investigations at the mRNA and protein level, we demonstrate that overexpression of miR-142-3p in MDA-MB-231, MDA-MB-468 and MCF-7 breast cancer cells leads to downregulation of Integrin-αV, WASL, RAC1, and CFL2, molecules implicated in cytoskeletal regulation and cell motility. ROCK2, IL6ST, KLF4, PGRMC2 and ADCY9 were identified as additional targets in a subset of cell lines. miR-142-3p overexpressing cells showed decreased Matrigel invasiveness and decreased activity of the stemness-associated enzyme ALDH. Confocal immunofluorescence microscopy, nanoscale atomic force microscopy and digital holographic microscopy revealed an altered actin distribution, a loss of membrane protrusions and a reduced cell volume and size. Interference with additional components of the extracellular matrix environment (Syndecan-1, Hyaluronan) resulted in similar alterations of membrane protrusion formation. Luciferase activation assays confirmed direct miR-142-3p-dependent regulation of the 3'-untranslated region of ITGAV and WASL. siRNA-knockdown of ITGAV and WASL resulted in a significant reduction of cellular invasiveness, highlighting their contribution to the invasion phenotype. While WASL-knockdown significantly reduced the number of membrane protrusions compared to controls, knockdown of ITGAV resulted in a decreased cell volume. Our data identify ITGAV, WASL, and several additional cytoskeleton- associated molecules as novel invasion-promoting targets of miR-142-3p in breast cancer. Whether regulation of the pluripotency-associated transcription factor KLF4 and alterations in ALDH1 activity are linked to alterations in matrix-dependent signaling, or due to direct regulation by miR-142-3p is currently under investigation.

ST16/P54 A dominant *TRPV4* mutation underlies osteochondrodysplasia in Scottish fold cats

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Objective: Scottish fold cats, named for their unique ear shape, have a dominantly inherited osteochondrodysplasia involving malformation in the distal forelimbs, distal hindlimbs and tail, and progressive joint destruction. This study aimed to identify the gene and the underlying variant responsible for the osteochondrodysplasia.
Design: DNA samples from 44 Scottish fold and 54 control cats were genotyped using a feline DNA array and a case-control genome-wide association analysis conducted. The gene encoding a calcium permeable ion channel, transient receptor potential cation channel, subfamily V, member 4 (TRPV4) was identified as a candidate within the associated region and sequenced. Stably transfected HEK293 cells were used to compare wild-type and mutant TRPV4 expression, cell surface localisation and responses to activation with a synthetic agonist GSK1016709A, hypo-osmolarity, and protease-activated receptor 2 stimulation.
Results: The dominantly inherited folded ear and osteochondrodysplasia in Scottish fold cats is associated with a p.V342F substitution (c.1024G>T) in TRPV4. The change was not found in 648 unaffected cats. Functional analysis in HEK293 cells showed V342F mutant TRPV4 was poorly expressed at the cell surface compared to wild-type TRPV4 and as a consequence the maximum response to a synthetic agonist was reduced. Mutant TRPV4 channels had a higher basal activity and an increased response to hypotonic conditions.

ST17/P55 Chromatin plasticity directs the fate of healthy cells and cancer cells on soft matrices

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Consequences of changes of the physical environment on cell adhesion have been widely investigated, but few is known about their impacts on chromatin plasticity. Chromatin integrates a multitude of biochemical signals interpreted by gene activation or gene silencing. We develop a cell-culture model based on polyelectrolyte multilayer films mimicking the elastic moduli of the extracellular matrix[1,2], on which epithelial Ptk2 cells are grown. On stiff matrices, chromatin is in its euchromatin form, whereas a soft matrix partially induces remodeling in its heterochromatin form. On a very soft matrix cells die by necrosis. Can we influence the cell survival if we modify the organization of the chromatin? We use a drug that inhibits histone deacetylases, trichostatin A, to maintain chromatin in euchromatin. Consequently, cells with chromatin organized in euchromatin fall into a quiescent state allowing them to

survive on a soft matrix previously lethal. Interestingly, these cells, transferred on a rigid substrate, enter in mitosis[3]. In parallel, colon cancer SW480 cells have been alternatively grown on soft and rigid matrices, simulating the process of metastasis dissemination, in which tumor cells face changing physical environments. After 24h of culture on a very soft matrix, only 4% of cells survive. The percentage of surviving cells increases to 10%, 50% and 80% after the second, third and fourth seeding on a soft matrix, respectively. On the stiff substrate, used as a control surface, SW480 cells display nuclei containing 15% of heterochromatin and are weakly motile. In contrast, cells successively seeded on a soft, a stiff and again a soft matrix show a significant increase in motility and the percentage of heterochromatin reaches 23%. Although a soft matrix remodels partially euchromatin into heterochromatin in both types of cells, their fate are totally opposite. A soft matrix guides Ptk2 cells into a quiescence state whereas selects aggressive SW480 cells.

References:
[1]Kocgozlu, L. et al. J. Cell Sci. 123, 29-39 (2009). [2]Rabineau, M. et al. PLoS ONE 8, e78468 (2013). [3]Rabineau, M. et al. Biomaterials 37, 144-155 (2015).

ST18/P56 MicroRNA targeting as a regulatory mechanism of breast cancer cells with different estrogen receptor status

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Human breast cancer is a heterogeneous, complex malignancy accounting for the second leading cause of cancer death among women. Estrogens and their receptors, ERα and ERβ, have pivotal roles in the growth, development and progression of breast cancer. It is well established that interactions among cancer cells and tumor microenvironment are in a dynamic interplay and regulated by extracellular matrix (ECM), while its components facilitating in tumor growth and the initiation of invasion and metastasis. We have recently demonstrated that the induced loss of ERα in the low invasive MCF-7 epithelial breast cancer cells results in epithelial to mesenchymal transition (EMT), striking changes in functional breast cancer cells' properties since they significantly increase the metastatic potential as well as the expression patterns of certain ECM mediators. Recent data indicate that the suppression of ERβ in the triple negative and highly metastatic MDA-MB-231 mesenchymal breast cancer cells reduces the aggressiveness of these cells and affects their functional properties as well as the expression and activity levels of certain ECM components. These data demonstrate the critical roles of ERα and ERβ in the development and progression of breast cancers. 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 ERα and ERβ, respectively). Following this evaluation of the affected onco-microRNAs, the observations of the functional properties and the ER status of breast cancer cells were correlated in order to understand the role of certain miRNAs in breast cancer. Interestingly, we demonstrated that the loss of ERα in breast cancer cells lead also to striking changes in the expression profile of specific microRNAs, including miR-200b and miR-10b, that have been correlated with the initiation of metastasis as well as the induction of the EMT process. Specifically, we observed that the loss of ERα resulted in a significant upregulation on miR-10b and in the strong downregulation of miR-200b. On the other hand, the loss of ERβ resulted in the modulation of microRNAs implicated in the inhibition of cancer progression and the retention of EMT. Interestingly, our data showed that breast cancer cells that lack ERβ exhibited an elevated level of miR-145 as well as downregulated levels of miR-200b and miR-10b. These novel results suggest that the alterations in cell behavior and in ECM composition that the suppression of estrogen receptors caused in MCF-7 as well as in MDA-MB-231 breast cancer cells can be correlated with differentiated profiles of certain onco-miRNAs, providing them as key players in the pathobiology of breast cancer.

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ST19/P57 Different sugar epitopes drive the cells to different fates

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Carbohydrates exceed any other class of macromolecules in complexity and in their ability to provide an endless variety of cell- and molecular recognition structures. In the present research different sugars were covalently linked to collagen substrates and tested against different cell lines. In a first approach chondroitin-6-sulfate chains were N-linked to collagen molecules by reductive amination. The reaction product was washed until no unreacted chondroitinsulfate was detectable by FTIR, resuspended in ultrapure water, deposited onto freshly cleaved mica and observed in Tapping Mode AFM. The molecules formed an irregular monolayer but were readily visible as caterpillar-shaped structures; each collagen molecule apparently bound 20-30 side chains out of 104 possible lysyl and hydroxylysyl residues. A collagen film obtained by solvent casting and neoglycosylated as above showed a network of thick, cross-banded collagen fibrils randomly oriented in a felt-like matrix of tangled collagen molecules and uniformly covered with thin, slender chondroitinsulfate chains.
Other similar collagen films were then functionalized with maltose, 3'-siallyllactose and 6'-siallyllactose. All were checked by AFM and, although these smaller sugars were not directly visible,all films showed the same network of randomly-oriented banded fibril. The correct exposure of the sugar epitope on the collagen film was checked by Enzyme-linked lectin assay (ELLA). Exposure of neuroblastoma F11 cells to maltose-treated collagen stimulated, through a signal pathway still to be elucidated, the cells to differentiate toward a functional neuronal phenotype, featured by neurite outgrowth, differentiation markers and the capability to generate action potentials. For the first time these cells were driven from proliferation to differentiation without any differentiating agents except the neoglycosylated extracellular matrix.
Exposure of mesenchymal stem cells to sialoside epitopes on collagen surface also provided different and specific signalling: in particular, 3′-sialyllactose upregulated the expression of the RUNX2 and ALP genes, which are obvious markers of osteoblast commitment, where 6′-sialyllactose upregulated the ACAN (aggrecan) gene, which codes for the most common proteoglycan of cartilage. Since the cells were grown without any osteogenic or chondrogenic supplements, the increased gene expression was uniquely a consequence of the neoglycosylation of the collagen surface. It is noteworthy how the mesenchymal stem cells were able to discriminate between the two functionalizations which differed only by the position of the glycosidic linkage. The research is still in progress.

ST20/P58 Comprehensive meta-analysis of proteomics data in search for novel signatures associated with extracellular matrix remodeling in chronic kidney disease

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Renal fibrosis is a pathological process underlying the progression of chronic kidney disease (CKD) and a common endpoint of end-stage renal disease (ESRD). At present, renal biopsy is the only method for the diagnosis of renal interstitial fibrosis. Non-invasive detection of fibrosis and more particularly its progression, remains a challenge. Moreover, molecular signatures vary among different CKD etiologies, hindering the search for accurate biomarkers. ECM accumulation and remodeling mechanisms in renal fibrosis are relevant sources of biomarkers for renal fibrosis. Collagens constitute the main structural element of ECM and have been linked with various CKD conditions in a series of publications. Moreover, the analysis of urine profiles for non-invasive detection of fibrosis has been proposed in recent publications. The aim of this study was to integrate existing literature data on urinary collagens and associate their presence with different disease etiologies. A database integrating peptidomics and proteomics published data on CKD was generated (www.peptiCKDdb.com). The database currently incorporates manually curated data from 107 studies of body fluids and kidney tissue on human CKD. A comprehensive meta-analysis of 26 urinary peptidomics datasets contained in the database was performed. In the course of analysis, the majority of urinary collagen fragments were found downregulated, which is in line with the fact that there is an exaggerated accumulation of collagens in kidney fibrosis. Detailed investigation of deposited data identified consistent downregulation of peptide sequences of collagen alpha-1(1) in most urine samples from patients with kidney diseases. Consequently, these peptides could be considered as putative general biomarkers of fibrosis in renal pathology. Moreover, due to the high variability of features among different diseases, unique peptides occurring only in one disease were investigated, as possibly being the most specific and indicative of early fibrotic events. For instance, a total of 13 peptides of collagen alpha-1 type I, II and III were found uniquely related to IgA nephropathy. Given that the change in abundance of specific collagen fragments might be directly correlated with altered proteolytic activity, we further used Proteasix tool (www.proteasix.org) to investigate proteases, which activity might have an impact on of ECM dynamics. Based on the analysis of down- and upregulated collagen peptides, possibly inhibited (e.g. MMP1, MMP8, MMP12) and activated metalloproteinases (e.g. MMP9, MMP20) were predicted. Collectively, we found common and potentially unique peptide signatures for the determination of fibrotic events in chronic kidney disease. Their role as diagnostic, prognostic or treatment-response, fibrosis-specific biomarkers remains to be further determined.

P59 The heparan sulfate proteoglycan Syndecan-1 regulates colon cancer stem cell function via a focal adhesion kinase – Wnt signaling axis

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The cell surface heparan sulfate proteoglycan Syndecan-1 acts as a coreceptor for growth factor-, morphogen-, and chemokine-mediated signaling, thereby regulating cancer cell proliferation, motility and invasiveness. In colon cancer, loss of Syndecan-1 is associated with increased invasiveness, metastasis and dedifferentiation. We demonstrate that siRNA-mediated Syndecan-1 downregulation in

human colon cancer cell lines enhances a cancer stem cell phenotype. Phenotypic marker analysis revealed an increase in the side population, enhanced ALDH1 activity, and higher expression of CD133, LGR5, EPCAM, NANOG, SOX2, KLF2, and TCF4/TCF7L2 in Syndecan-1-depleted cells. Syndecan-1 knockdown enhanced sphere-formation capacity, cell viability, matrigel invasiveness and gene expression changes suggestive of epithelial-to-mesenchymal transition. In vivo, growth of Syndecan-1-depleted HT-29 xenografts was significantly increased compared to siRNA controls. Mechanistic studies supported by transcriptomic Affymetrix analysis revealed that decreased Syndecan-1 expression is associated with an increased activation of beta1-integrins, and focal adhesion kinase (FAK). Wnt signaling was increased upon Syndecan-1 siRNA depletion as revealed by TOP Flash assays. Pharmacological inhibitors of FAK and WNT-signalling blocked the enhanced stem cell phenotype. Sequential side population cell enrichment by flow cytometric sorting substantially enhanced the stem cell phenotype of Syndecan-1-depleted cells, which showed increased resistance to doxorubicin chemotherapy and irradiation in vitro. We conclude that reduced Syndecan-1 expression cooperatively enhances activation of integrins and FAK, which then generates signals for increased invasiveness and cancer stem cell properties. Our findings may provide a novel concept to target a stemness-associated signaling axis as a therapeutic strategy to reduce metastatic spread and cancer recurrence.

P60 Modification of Mesenchymal Stem Cell-Derived Exosomes: Potential for Breast Cancer Therapy

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Introduction: Mesenchymal Stem Cells (MSCs) are multipotent stromal cells known to migrate and engraft into the tumour microenvironment. MSCs offer immense potential as vehicles for targeted delivery of therapies. Exosomes are small membrane-derived vesicles that are secreted by most cells including MSCs, and are readily taken up by recipient cells. Exosomes selectively package genetic material such as microRNAs, important regulators of genes involved in breast cancer progression. Engineering MSCs to secrete exosomes enriched with tumour suppressing microRNAs within the tumour microenvironment holds exciting potential. The aim of this study was to genetically modify MSCs to secrete exosomes enriched with a tumour suppressing microRNA.
Methods: MSCs were transduced with a lentivirus encoding miR-379 (MSC-379) or a non-targeting control(MSC-NTC) along with a red fluorescent protein(RFP). Transduced cells were analysed by fluorescence microscopy and RQ-PCR. Exosomes secreted by engineered and control MSCs were isolated by differential centrifugation, micro-filtration and ultracentrifugation, followed by characterisation by Transmission Electron Microscopy (TEM). The microRNA content of secreted exosomes was subsequently extracted and analysed by RQ-PCR. Isolated MSC-exosomes were transferred onto recipient breast cancer cell lines HCC-1954 and MDA-MB-231. Cells were subsequently imaged by confocal microscopy to confirm uptake of MSC-derived RFP exosomes. Furthermore, an MTS assay was carried out to determine the effect of engineered MSC-derived exosomes on recipient breast cancer cell viability.
Results: Successful transduction of MSCs was confirmed through visualisation of RFP expressing cells by fluorescence microscopy, and a >500-fold increase in miR-379 expression detected by RQ-PCR. Exosomes were successfully isolated from both MSC-379 and MSC-NTC cells, with vesicles of 30-120nm visualized using TEM. RQ-PCR analysis revealed that exosomes derived from MSC-379 cells had a 4-fold increase in expression of the microRNA when compared to MSC-NTC derived exosomes. RFP-labelled exosomes from the transduced MSCs were successfully taken up by breast cancer cell lines, as visualised by confocal microscopy. Initial data revealed a reduction in proliferation of MDA-MB-231 breast cancer cells when exposed to MSC-379 derived exosomes, compared to those receiving MSC-NTC derived exosomes.
Conclusion: This promising data highlights the ability to genetically engineer the content of exosomes secreted by MSCs, which were then readily taken up by breast cancer cells in vitro. Combined with the

tumour-homing capacity of MSCs, this approach has immense promise for targeted therapy of breast cancer.

P61 Cell Lineage Perspectives of Cutaneous Scarring

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Fibroblasts remain poorly characterized but are central to the fibrotic response to injury. We reveal the existences of multiple embryonic lineages in mammalian cutaneous tissues that underlie the diversity of scar formations across developmental stages and between anatomic sites. We further identify a highly fibrogenic lineage defined by embryonic expression of *Engrailed-1* that plays a central role in dermal development, wound healing, radiation-induced scarring, and cancer stroma formation. These results hold promise for the development of therapeutic tactics aimed at ameliorating wound healing and fibrotic diseases.

Rupert Timpl Award Lecture

Plenary Lecture (L18) Delineation of disease modifiers allows for treatment of basement membrane-linked skin disorders

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Dystrophic epidermolysis bullosa (DEB) is a rare skin fragility disorder caused by mutations in the *COL7A1* gene encoding collagen VII. Deficiency of collagen VII leads to chronic skin fragility, progressive soft tissue fibrosis, and it predisposes the individual to development of early-onset lethal skin cancer. An obvious therapy approach for DEB is to replace the gene or protein at fault, and indeed this has been a focus of the majority of DEB therapy development efforts. However, there are inherent difficulties with these approaches, and wider implementation of such therapies in the clinic remains elusive. DEB, together with most connective tissue disorders, is a progressive disease driven and modified by secondary disease mechanisms. Our studies identified increased TGFβ signaling in wounded DEB skin; targeting it or its downstream consequences both ameliorated disease signs and slowed down tumor progression. Treatment with the angiotensin II type 1 receptor antagonist losartan efficiently limited TGFβ activity in injured DEB mouse paws and in effect attenuated progression of fibrosis, as was seen by longer and fewer fused toes, softer skin and lower expression of fibrotic markers. Global unbiased mass spectrometry-based proteomics revealed molecular events linked to tissue inflammation as major drivers of disease progression in DEB. In addition, by targeting TGFβ activity, the dermal stiffening induced by the activity or bypassing tissue stiffness sensing by keratinocytes also reduced tumor progression ex vivo and in vivo. Collectively, our studies show that increased understanding of secondary disease mechanisms and disease modifiers for DEB reveals druggable targets and creates opportunities to effectively limit the symptoms and the natural progression of DEB and other connective tissue disorders.

Plenary Lecture (L19) Extracellular Matrix Proteolysis: a bystander or a partner in a crime?

Irit Sagi
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When it comes to basic and clinical research of disease-associated tissue destruction, most groups have directed their efforts to investigating cellular responses during disease progression, neglecting altogether the main component undergoing physical rupture, namely, the extracellular matrix (ECM). In a similar manner, while the regulatory role of the ECM in the progression of invasive diseases, such as cancer, is becoming acknowledged, its function in inflammation and infectious diseases is often overlooked despite the common notion that tissue destruction is imperative to disease progression. The Matrix metalloproteinases (MMPs) play a pivotal role in ECM remodeling during homeostasis as well as in dysregulated diseased tissues. These pleiotropic zinc endopeptidases cleave core matrisome proteins and non-matrix substrates including cytokines, chemokines, adhesion molecules, growth factors, as well as their receptors. MMPs thus affect cell-cell signaling milieu and cellular contacts along with their key roles in proteolytic alteration of matrix architecture and its bio- physical properties. Up regulation in cancer microenvironment brought MMPs to the lime light as candidate targets, but owing to their evolutionarily conserved central role in tissue proteolysis, they have also been implicated as candidate drug and theranostic (therapeutic as well as diagnostic) targets in diabetes, sepsis, neurodegenerative diseases, dentine erosion, ocular maladies, cardiovascular diseases, skin disorders, arthritis, respiratory tract disorders, glomerulonephritis and inflammatory bowel disease. We have found that MMPs can induce distinct morphological changes in collagen rich matrix and affect differential cellular response. This provides us with significant opportunities to influence the cellular microenvironment and interfere in infectious disease processes that affect intimate cellular-ECM communications at the very near microenvironment of immune cells. By implementing our discoveries into the design of novel inhibitory

antibodies targeting dysregulated ECM proteolysis we can now impact invasive infectious diseases by protecting ECM molecular integrity and morphology. Our studies highlight ECM remodelling as an integral part of directional pathological signalling in infectious diseases rather than, as is often considered, a passive bystander.

Plenary Lecture (L20) CDK1 inhibition triggers adhesion remodelling prior to mitosis

Humphries, M.J.
University of Manchester, UK

The cell cycle is a tightly regulated process that results in genome duplication and accurate distribution of DNA and proteins into daughter cells following mitosis. Progression through the cell cycle is anchorage-dependent, requiring cell-extracellular matrix interactions via integrin transmembrane receptors and the formation of actin-associated adhesion complexes. Before cell division, adhesion complexes are disassembled and cells round up, and following cytokinesis cells re-spread and adhesion complexes are re-established. The molecular mechanism that links the cell cycle machinery to the initial loss of adhesion prior to mitosis is unknown. Here we demonstrate that CDK1, the master regulator of the cell cycle, has an interphase role in promoting adhesion complex and actin cytoskeleton organisation. Disassembly of adhesion complexes and remodelling of actin in G2 is associated with elevated cyclin B1, CDK1-cyclin B1 binding and CDK1 inactivation via Wee1 phosphorylation. Thus, CDK1 inhibition is the trigger that initiates adhesion remodelling prior to mitosis.

Plenary Lecture (L21) Collective Cell Migration Induced by Mechano- and Synthetic-Biology

Joachim P. Spatz
Max Planck Institute for Medical Research, Germany

The collective movement of epithelial cells drives essential multicellular organization during various fundamental physiological processes like embryonic morphogenesis, cancer, and wound healing. Two hallmarks of collective behavior in migrating cohesive epithelial cell sheets is the emergence of so called leader cells and the communication between adjacent cells to move correlated to each other. Here we discuss these two phenomena:
(i) The geometry-based cue imposed by the matrix environment like local curvature of the collective's perimeter is capable of triggering leader cell formation and promoting enhanced motility at defined positions. Cytoskeletal tension was found to be important for geometry induced leader cell formation. Together our findings suggest that high curvature leads to locally increased stress accumulation, mediated via cell-substrate interaction as well as via cytoskeleton tension. The stress accumulation in turn enhances the probability of leader cell formation as well as cell motility.
(ii) Within this cohesive group each individual cell correlates its movement with that of its neighbours. We investigate the distinct molecular mechanism that links intercellular forces to collective cell movements in migrating epithelia. More specifically, we identified the molecular mechanism whereby *Merlin*, a tumor suppressor protein and Hippo pathway regulator that functions as a mechanochemical transducer, coordinates collective migration of tens of hundreds of cells. In the context of collective cell migration, the transmission and mediation of cellular tension is of major importance.
References:
1. T. Das, K. Safferling, S. Rausch, N. Grabe, H. Böhm, and J.P. Spatz: A molecular mechanotransduction pathway regulates collective migration of epithelial cells. Nature Cell Biology 2015, 17, 276-287.
2. S. Rausch, T. Das, J.R.D. Soiné, T.W. Hofmann, C.H.J. Böhm, U.S. Schwarz, H. Böhm, and J.P. Spatz: Polarizing cytoskeletal tension to induce leader cell formation during collective cell migration. Biointerphases 2013, 8, 32.

Workshop 5: Cell adhesion, signaling and the tumour microenviroment

Invited Lecture (L22) ECM mechanosignaling

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Marfan syndrome (MFS) is a prototypical connective tissue disease caused by mutations in a structural component of the ECM (fibrillin-1) that are associated with secondary cellular and molecular abnormalities. Studies of mouse models of MFS have demonstrated the multiple roles of fibrillin-1 assemblies (microfibrils and elastic fibers) in supporting organ function and homeostasis. One of the newly discovered functions of fibrillin assemblies is to transmit mechanical stimuli across tissues and present them to cell to be translated into biochemical signals. Although generally thought to be a secondary manifestation of MFS, ventricular dysfunction without evidence of valvular disease has raised the possibility of an inherent myocardial insufficiency in this disease. Genetic experiments in mice have confirmed this possibility by showing that fibrillin-1 is a component of the ECM/sarcomere multi-protein complex that regulates cardiac muscle adaptation to demanding loading conditions by modulating the AT1 receptor /β-arrestin 2/ /Erk1/2 mechanosignaling cascade. Similar studies have also indicated that mechanical stress is the principle trigger of thoracic aortic aneurysm (TAA) development through improper activation of AT1 receptor expression by endothelial cells in the intimal layer. Interestingly, these experiments showed that the AT1 receptor expressed on smooth muscle cells of the medial layer responds differently to ECM-induced aortic wall stress. Collectively, these findings have important implications for the development of therapeutic strategies against life-threatening cardiovascular manifestations in MFS.

Invited Lecture (L23) Mechanics of cancer cell invasion in vivo

Peter Friedl[1,2]
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Single-cell or collective invasion results from coordination of cell shape, deformability and actin dynamics relative to the tissue environment. When monitored in vivo, using intravital multiphoton second and third harmonic generation and fluorescence microscopy, tissue microniches provide invasion-promoting tracks that enable collective migration along tracks of least resistance. As main routes, non-destructive contact-guidance is mediated by preformed multi-interface perimuscular, vascular and –neural tracks of 1D, 2D and 3D topography. 3D ultrastructural analysis reveals predefined tissue conduits (“highways”) of defined geometry, nanotropography and molecular composition as predominant routes of invasion by contact guidance comvbined with a cell “jamming” mechanism. Consistently, spheroids of mesenchymal melanoma or sarcoma tumor cells switched from single-cell to collective invasion modes when confronted with 3D collagen matrices of increasing density, including gain of cell-to-cell junctions, supracellular polarization, suggesting cell jamming imposed by tissue confinement. Targeting of beta1/beta3 integrins induces unexpected plasticity of invasion, including collective and amoeboid single-cell dissemination, followed by enhanced systemic dissemination and micrometastasis, implicating a role of integrins in cell-cell cooperation and integrin-independent dissemination as effective route to metastasis. In conclusion, cancer invasion is maintained by physicochemical programs that balance cell-intrinsic adhesion and mechanocoupling with encountered physical space and molecular cues.

Invited Lecture (L24) Cell adhesion, signaling and the tumor environment

Benny Geiger, Yair Elisha, Yael Sagi, Or-Yam Revach, Ariel Livne
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Integrin- and cadherin-mediated adhesions, through which cells stably interact with the extracellular matrix (ECM) and neighboring cells, are structurally stable and molecularly dynamic cellular structures, located at the interface between the extracellular environment, and the cytoskeleton. These adhesions are extensively investigated for over 50 years, yet despite the vast effort invested in their characterization, their structure, diverse functions and molecular dynamics are still highly challenging. In this workshop I will focus on the roles of cell adhesions in regulating invasive migration of cancer cells. I will address four topics in this talk, illustrated in the figure below. First I will address the general adhesion-mediated signaling paradigm (Left panel) that highlights the sensory role of cell adhesions and their diverse effects on cell fate and behavior. Next I will discuss the capacity of stromal cell to induce in invasive phenotype in neighboring cancer cells (right, top panel). Finally, I will discuss two invasive mechanisms, presented by different cancer cells The first is a collective migration whereby invading cells and connected to each other via membrane tethers, and consequently, migrate collectively (right, bottom, left picture); and the second, is based on the formation of specific invasive organelles, namely, invadopodia, which locally degrade the pericellular matrix, and induce the formation of cytoskeleton-based protrusion into the surrounding matrix. In the talk, different aspects of the cellular plasticity involved in the interaction of normal and cancerous cells with their microenvironment will be elaborated.

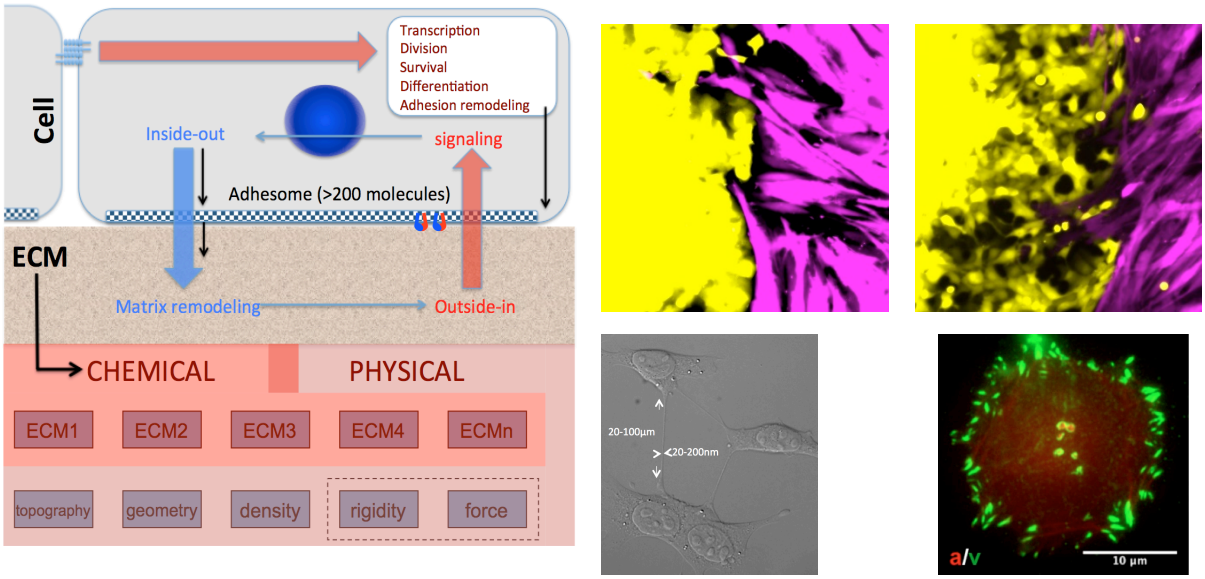

Figure: *The panel on the left illustrates the capacity of cells to sense, through their adhesion receptors, the chemical and physical properties of the ECM. The invasion of cancer cells into an adjacent stromal layer is shown in the upper right panels (1 day and 5 days). The bottom left image shows a unique form of collective cell migration, coordinated by E-cadherin mediated tethers. The bottom right image shows melanoma cells, forming invadopodia with actin core (red) and vinculin-containing adhesions (green)*

ST21/P62 Collagen XVIII regulates *EGFR-HER2* signaling in breast cancer and its knockdown augments the effect of anti-*ErbB* drugs

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Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer deaths among females worldwide. Tumour growth is significantly influenced by the local microenvironment consisting of different type of cells and the extracellular matrix (ECM). Collagen XVIII (Col18) is a ubiquitous, structurally complex basement membrane collagen/heparan sulphate proteoglycan. It is expressed as three variant forms that differ in their N-terminal regions and tissue specificity. The C-terminal part of Col18 called endostatin, present in all three isoforms, is widely studied due to its anti-angiogenic properties. In contrast, very little is known about the specific functions of the three isoforms and their diverse N-terminal domains. We have observed an up-regulation for Col18 in several human carcinomas. Here we explored the role of Col18 in breast cancer and found that, high Col18 expression is associated with poor prognosis and reduced survival in human patients. Col18 knockout mice crossed with MMTV-PyMT mouse model of mammary carcinogenesis showed a markedly reduced tumour burden and an improved survival. Deficiency of Col18 led to significantly reduced breast cancer stem cell populations in animal and cellular models. Further investigation identified a molecular mechanism of action for the N-terminal domain of Col18 in solid cancers. Hence, the thrombospondin-1 domain was found to interact with the ErbB-integrin signaling complex, and regulate the downstream signaling cascade which controls cell survival and proliferation. Importantly, we also showed that inhibition of Col18 action in human ErbB2-positive breast cancer cells substantially reduced cell proliferation and triggered a synergistic effect when combined with current ErbB-targeting drugs. In conclusion, our studies demonstrate a novel supporting role for Col18 in breast tumourigenesis by enhancing EGFR/ErbB2 signaling and proliferation of cancer cells, and in maintaining the cancer stem cell niche in a solid tumour. We propose that Col18 should be further investigated as a novel therapeutic target that provides new treatment opportunities in breast cancer patients.

ST22/P63 A novel mechanosensing role of Integrin-Linked Kinase, Parvin and PINCH in cell-matrix adhesion reinforcement in *Drosophila*

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Cell-matrix adhesion is established by modulating the binding properties of cell surface receptors with their ligands extracellularly and the cell cytoskeleton. One family of cell adhesion receptors that play a major role in cell-extracellular matrix (ECM) adhesion, is the integrins. The linkage of integrins to the cytoskeleton is mediated by the integrin adhesome, a network of interconnecting cytoplasmic proteins. The tripartite protein complex containing Integrin Linked Kinase (ILK), Parvin and PINCH, namely IPP-complex, is one of the most well conserved integrin adhesome modules. We have previously shown an essential function of the IPP-complex in the maintenance of the integrin-actin link at muscle attachment sites in *Drosophila* embryo. Here, we validated the mechanosensitive mode of IPP-complex

function by genetic analysis and advanced imaging methods, using FRAP and live imaging in whole *Drosophila* embryos. We demonstrated that IPP-complex relays force-elicited signals that tune integrin response to tension and restrain integrin turnover. Thus, integrins reside trapped in the plasma membrane and become amenable to engage in stable interactions with both ECM and cytoskeleton during *Drosophila* development. Our studies contribute important insight into the molecular mechanisms that regulate cell attachment strength both in development and in pathophysiological conditions like muscular dystrophies and cancer metastasis.

ST23/P64 FAK activity within Cancer-associated fibroblasts is a key regulator of pancreatic ductal adenocarcinoma invasion

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Pancreatic ductal adenocarcinoma (PDAC) remains a deadly cancer. One of the PDAC hallmarks is to be composed for 80% of a fibrotic stroma. Cancer-Associated Fibroblasts (CAFs) are the most abundant stromal cells, and promote tumorigenesis and metastasis. Notably, by producing large amounts of extracellular matrix (ECM) proteins and ECM remodeling enzymes, CAFs generate ECM tracks used by tumor cells to invade. On another hand, activation of the Focal Adhesion Kinase (FAK), a non-receptor protein tyrosine kinase, is involved in connective tissue remodeling by normal fibroblasts.
In human pancreatic tissue, we identified that FAK activity expressed by fibroblastic cells is very low or null in normal tissues and highly increased in cancer-associated fibroblasts (CAFs) from human PDAC. Notably, FAK increased activation in CAFs correlates with TNM (Tumor, lymph Node, Metastasis) stage T3 (tumor pathologic staging in which cancer has grown outside the pancreas into nearby surrounding structures but not into major blood vessels or nerves). Pharmacological and genetic FAK inhibition within activated fibroblasts results in a significant decrease of tumor cell invasion both *in vitro* and *in vivo*. Indeed, spheroid invasion assay composed of pancreatic tumor cells and fibroblasts expressing either FAK-Wild-Type (FAK-WT) or FAK-Kinase-Dead (FAK-KD) shows that loss of FAK activity specifically within fibroblasts inhibits fibroblast and tumor cell invasion into collagen I matrix. Moreover, orthotopic syngenic co-grafting of pancreatic tumor cells with either FAK-WT or FAK-KD fibroblasts show that FAK inactivation within fibroblasts dramatically decreases lung metastasis number and size. Finally, pharmacological FAK inhibition in primary cultures of CAFs isolated from PDAC patients drastically diminishes collagen I and LOXL2 (Lysyl oxidase like 2, enzyme implicated in the last step of collagen fiber maturation) expression and deposition.
In conclusion, our results show that FAK activity within CAFs is a crucial regulator of CAF-induced tumor metastasis, effect likely due to the formation of collagen tracks used by tumor cells to invade. Thus, we propose that targeting FAK activity in PDAC patient represents a promising strategy.

ST24/P65 Signaling function of Integrin alpha 11 cytoplasmic tail

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Integrin a11b1, a major receptor for fibrillar collagens, is expressed by mesenchymal cells and mediates cell adhesion, cell migration and collagen remodelling. Integrin a11b1 also stimulates myofibroblast differentiation and involved in wound healing. Integrin a11b1 is highly expressed in tumor stroma and enhances tumorigenicity.
Because the molecular mechanisms through which this collagen receptor mediates fibroblast cell functions are poorly understood, human integrin alpha 11 with truncated (deletion 1172-1188) cytoplasmic tail and a point mutation of the conserved lysine (K1185A) were generated and expressed in C2C12 cells, which are lacking endogenous collagen receptors.

Deletion of the alpha 11 cytoplasmic tail and the point mutation of the conserved lysine-1185 reduced ability of cells to form focal contacts and to remodel collagen lattices. In addition, focal adhesion kinase (FAK) and basal extracellular signal-related kinase (ERK) phosphorylation levels were reduced in these cells on collagen. We conclude that the cytoplasmic tail of integrin alpha 11 is required for focal adhesion formation, efficient reorganization of collagen fibrils, and that the conserved lysine-1185 residue is involved in these processes.

ST25/P66 Effect of moesin on cell migration induced by pleiotrophin

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Moesin is a member of the ERM protein family that appears to function as a cross-linker between cell membrane and actin-based cytoskeleton. It is important for cell-cell recognition, cell movement and at first, was characterized as a possible heparin receptor. Pleiotrophin (PTN) is a heparin-binding growth factor that through its receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ), integrin alpha v beta 3 ($\alpha_v\beta_3$) and cell surface nucleolin induces angiogenesis and regulates the angiogenic effects of vascular endothelial growth factor A. In the present study, we used two different types of cells: human umbilical vein endothelial cells that express $\alpha_v\beta_3$ integrin and migrate in response to PTN and rat glioma C6 cells that do not express $\alpha_v\beta_3$ and have decreased migration in response to PTN. By using MALDI-TOF, IP/Western blot, immunofluorescence and proximity ligation assays, we have found that PTN and $\alpha_v\beta_3$ integrin, but not RPTPβ/ζ or cell surface nucleolin, interact with moesin in endothelial and glioma cells. In both types of cells, moesin is mainly localized on the cell membrane and is also exposed to the extracellular site of the cell membrane. Cell stimulation with PTN increases moesin phosphorylation and decreases membrane moesin, as well as moesin-$\alpha_v\beta_3$ interaction in endothelial cells. Moesin-$\alpha_v\beta_3$ interaction was also decreased in CHO cells expressing a mutated β_3 subunit, where Tyr773 was substituted to Phe. Down-regulation of moesin by siRNA in endothelial cells leads to increased cell migration and in that case, PTN cannot cause further increase. In contrast, in glioma cells that do not express $\alpha_v\beta_3$, down-regulation of moesin by siRNA decreased migration. In these cells, PTN significantly increased migration when moesin was down-regulated. Collectively, our data suggest that moesin is a significant regulatory cell migration mechanism that also regulates the effect of PTN.

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P67 Effect of cell surface nucleolin on vascular endothelial growth factor A signaling related to endothelial cell migration

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The multifunctional protein nucleolin (NCL) is expressed on the surface of activated endothelial and tumour cells and plays a significant role in tumour growth and angiogenesis. Cell surface NCL localization is stimulated by several growth factors, among which vascular endothelial growth factor A (VEGF-A) and the expression of $\alpha_v\beta_3$ integrin by cells is a significant regulator of this translocation. In the present work, we studied whether VEGF-A directly interacts with NCL and if cell surface NCL affects the angiogenic effects of VEGF-A in human endothelial cells. By using IP/Western blot and proximity ligation assays, we have found that VEGF-A directly interacts with NCL outside the cell nucleus. A peptide that specifically binds to and inhibits cell surface but not nuclear NCL completely abolished VEGF-A-induced endothelial cell migration. The same peptide inhibited VEGF-A-mediated decrease in the expression and secretion of the growth factor pleiotrophin by endothelial cells, an effect that seems to be independent of the VEGF receptor 2 (VEGFR2). Finally, the cell surface NCL inhibiting peptide inhibited VEGF-A-stimulated ERK1/2 activation. Interestingly, in the rat glioma C6 cell line that does not express cell surface NCL but responds to VEGF-A through VEGFR2, VEGF-A does not stimulate ERK1/2 activation, despite VEGFR2 expression in these cells, suggesting that ERK1/2 activation by VEGF-A requires cell surface NCL. Collectively, our data suggest that cell surface NCL is a VEGF-A binding molecule that has a significant regulatory role in VEGF-A angiogenic actions.

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P68 Deciphering the cell and non cell-autonomous contribution of LOXL2 to tumor angiogenesis in 3D systems

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Hypoxia drives the remodeling of tumor microenvironment, composed of cancer cells and of stroma containing scaffolding extracellular matrix and growing capillaries (Germain *et al*., Curr Opin Hematol, 2010). The stoma plays a prominent role in the progression, growth and spread of cancers. Deciphering the regulation of hypoxia-induced angiogenesis by mutual crosstalks between tumor and endothelial cells is of major interest. Lysyl oxidase like-2 (LOXL2) belongs to the lysyl oxidase family of extracellular matrix crosslinking enzymes. We previously reported that LOXL2 is a hypoxia-target, which is secreted by endothelial cells, regulates developmental angiogenesis and is required for type IV collagen assembly (Bignon *et al.*, Blood, 2011). Moreover, LOXL2 is up-regulated in many cancers in both tumor and stromal cells and inhibition of extracellular LOXL2 impedes the development of pathologic microenvironment (Barry-Hamilton *et al*., Nat. Med. 2010). Microenvironmental LOXL2 has been reported as participating to tumor angiogenesis (Zaffrayar-Eilot *et al*, 2013).

We here investigated the cell autonomous (endothelial) and non-cell autonomous (tumor) contribution of LOXL2 in angiogenesis of clear cell renal cell carcinoma (ccRCC), a vascularized and metastatic

cancer. Analysis of human primary tumors or metastasis revealed that tumor and stromal cells expressed *loxl2* mRNA. Furthermore, LOXL2 was secreted by cultured human endothelial cells and tumor cells from ccRCC.
The functional role of LOXL2 was investigated using *in vitro* and *in vivo* 3D angiogenesis models of endothelial capillaries sprouting from spheroids in Matrigel/fibrin gel. We demonstrated that LOXL2 overexpressed by endothelial cells increased capillary formation and gel stiffness, indicating a cell autonomous role of LOXL2 associated with modification of the mechanical properties of the microenvironment. Adding recombinant LOXL2 into gels containing endothelial spheroids also increased *in vitro* capillary morphogenesis, revealing a non-cell autonomous effect of LOXL2. Moreover, conditioned medium from tumor cells overexpressing LOXL2 stimulated endothelial cell migration and proliferation and *in vivo* capillary formation without affecting the gel stiffness. These data demonstrate a non-cell autonomous role of LOXL2 associated with modification of the endothelial cell behavior.
The contribution of tumor cells on capillary morphogenesis was further deciphered using an *in vitro* 3D angiogenesis model of endothelial sprouting from Cytodex beads in fibrin gel. Control conditioned medium of tumor cells promoted the growth of tortuous and dilated capillaries whereas LOXL2-depleted conditioned medium reduced the disorganized angiogenesis.
The cell and non-cell autonomous contribution of LOXL2 to tumor angiogenesis involves therefore distinct mechanisms, regulating the mechanical properties of the microenvironment or the capillary morphogenesis.

P69 Transcriptional expression of CD44 is related with metastatic potential of breast cancer cells

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CD44 receptor is a transmembrane glycoprotein that appears in several isoforms with different extracellular domains. It belongs to the family of molecules that attach themselves to cells and mediate on contact and traction between neighboring cells and between cells and the extracellular matrix. In addition it may direct intracellular signaling for development and mobility, and thus it is implicated in many types of cancer including breast cancer. The high number of CD44 isoforms is due to the complexity of the gene in containing twenty exons, ten of which, exon 6 to exon 15 that are named v1 to v10, are alternatively expressed. CD44v isoforms are expressed in both normal and tumor cells at different levels, indicating that they are also an essential component for normal cellular functions. However, exon splicing mechanisms can lead to the overexpression of CD44v isoforms in cancer cells. It is of great importance that their role and the degree of expression vary in different malignancies. In certain cancers, CD44v isoforms are considered to be tumor progression promoters, while in other cancers, they may be involved as tumor suppressors. The present study was undertaken to examine CD44 expression in breast cancer cells. Cells of different metastatic potential (MCF-7 and MDA-MB-231) were used to examine any relation between CD44 expression and metastatic potential. The same cells, stably transfected to suppress ERα and ERβ expression, respectively, were also included. In this case, ERα suppression gave to MCF-7 cells a mesenchymal-like phenotype. The results in transcriptional level showed that expression of total CD44 isoforms was about similar in all cells. However, different CD44v isoforms were expressed in the various cells, the expression of which was clearly related with ER status and cell metastatic potential. These results are in good agreement with earlier findings and suggest that the differential transcriptional expression of CD44v isoforms might be used for identification of breast cancer subtypes and thus to provide a more targeted therapy to the patients.

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P70 Heparan sulfate 2-O sulfotransferase-dependent signalling pathways determine breast cancer cell-matrix interactions, cell motility, viability and invasive growth

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Heparan sulfate proteoglycans (HSPGs) interact with a variety of signaling molecules via their sulfated glycosaminoglycan side chains, thereby facilitating signal transduction as coreceptors. The function of heparan sulfate 2-O-sulfotransferase (HS2ST1), an enzyme which transfers the sulfate group to the 2-O-position of the heparan sulfate glycosaminoglycans in breast cancer is largely unknown. Hence, a comparative study on the functional consequences of HS2ST1 overexpression and siRNA knockdown was carried out in the breast cancer cell lines MCF7 and MDA-MB-231. Our results reveal that HS2ST1 overexpression inhibited Matrigel chamber invasiveness and cell motility as evidenced by video microscopy, while its knockdown reversed the phenotype. Altered 2-O-Sulfation of HS resulted in significant changes in cell binding to fibronectin and laminin. Phosphokinase array screening revealed a general decrease in signal transduction via multiple pathways. Altered MAPK signaling was a result of altered expression of an upstream element, epidermal growth factor receptor (EGFR). In addition, we observed an increase in E-cadherin expression in HS2ST1 overexpressing cells while its expression decreased upon HS2ST1 knockdown, correlating to the invasive phenotype. Moreover, the mRNA expression of Wnt 7a, a positive regulator of E-cadherin was upregulated upon HS2ST1 overexpression while HS2ST1 overexpression in these cell lines lead to low expression of the Wnt pathway-related transcription factor, Tcf4. Fluorescent ligand binding studies revealed altered binding of FGF-2 to HS2ST1 expressing cells compared to control cells. Following specific ligand stimulation, EGF- and FGF-2-induced MAPK activation was reduced in HS2ST1 overexpressing cells. Pharmacological inhibition of the MAPK signaling pathway reduced the upregulation of EGFR observed in HS2ST1-siRNA transfected cells, providing a link between the cellular phenotype and altered signal transduction. We conclude that modulation of breast cancer cell invasiveness by HS2ST1 is based on altered cytokine-heparan sulfate interactions which influence matrix binding and multiple signaling pathways. The cellular phenotype changes due to a combined effect of altered E-cadherin and EGFR expression, leading to altered signaling via the MAPK and additional pathways. This study shows for the first time the involvement of HS2ST1 in controlling the invasiveness of breast cancer cells.

P71 Sox9 influences apoptosis, adhesion and differentiation capacity in a human chondrosarcoma and osteosarcoma cell line

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Introduction: Sox9 is known as a master transcription factor for chondrogenesis. From the mesenchymal condensation of chondroprogenitors to the hypertrophic maturation of chondrocytes, Sox9 has an essential role in inducing expression of chondrogenic genes. Moreover, accumulating evidence from recent studies points to a critical role of Sox9 as a proto-onkogene in plenty of different tumor types. Especially in chondrosarcoma and osteosarcoma, Sox9 is presumed to influence the development and progression of the tumors.

Methods: We have introduced a transient Sox9 knockdown in a human chondrosarcoma cell line (HTB 94) and an osteosarcoma cell line (Saos-2) using specific Sox9 siRNA. Functional assays to investigate proliferation (BrdU), apoptosis (Caspase 3/7), senescence (ß-galactosidase), adhesion (crystal violet staining) and cytotoxicity (LDH) were performed. In addition, the capacity of both cell lines to differentiate into the chondrogenic or osteogenic lineage was analyzed via quantification of the expression of marker genes.
Results: A reproducible knockdown of 80% - 90% was achieved in both cell lines on mRNA and protein level. In cells with Sox9 knockdown a significant increase in apoptotic activity and an enhanced adhesion capacity was observed whereas senescence, cell growth and viability were unaltered. The ability to differentiate into the osteogenic lineage (Saos-2 cells) and into the chondrogenic lineage (HTB 94 cells) was clearly altered after Sox9 knockdown. Saos-2 cells with Sox9 knockdown, had an increased expression of Mmp13 and osteocalcin in early osteogenic differentiation stage, and a decreased expression of Vegfa after 21 days. HTB 94 cells with Sox9 knockdown, decreased expression of COL1A1, COL2A1 and Integrin alpha 11(ITG11) during the first two weeks of differentiation, while in a later phase, increased expression of COL10A1 points to an acceleration of the transition into a hypertrophic status.
Conclusion: Based on these studies, we conclude a critical effect of Sox9 on apoptosis and on adhesion in human chondrosarcoma and osteosarcoma cell lines, what may point to a role of Sox9 as a pro-survival factor for sarcoma cells.
Our findings furthermore indicate an influence of Sox9 on the balance between self-renewal and differentiation capacity, what could be the central point of action for Sox9 as a proto-onkogene.

P72 *In vitro* studies of the novel protein 7z3e2: role on hyaluronan regulation in breast tumor microenvironment

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Extracellular matrix (ECM) is a complex network of macromolecules and secreted factors that ensures the tissue integrity and elicits signals to and from the embedded cells. Dysregulation of the composition of the ECM is associated with several pathologies, including cancer. Among various ECM glycosaminoglycans, hyaluronan (HA) has a remarkable structural importance but also a role in regulating cellular processes through a binding with membrane receptors and activation of signaling pathways. The role of HA in tumor cells' functions depends on its molar mass which is regulated by the enzymes that synthesize HA, i.e. hyaluronan synthases (HAS), and hyaluronidases (HYALs). Alterations of these metabolic enzymes are correlated with breast cancer progression.
Induction of HAS2 in the cells of the stroma on the breast tumor surroundings and increase of HA in the tumor microenvironment showed a stimulation of metastasis and proliferation. Recently, in our laboratory we discovered a new protein in the conditioned medium of the low invasive breast tumor cell line BC8701, called "Uncharacterized protein of c10orf118" or "Q7z3e2". Further studies on the two well-known breast cancer cell lines MCF-7 (low invasive cells) and MDA-MB231 (high invasive cells) demonstrated a higher expression and secretion of Q7z3e2 in MCF-7 cells. As reported in the literature, co-culture of breast cancer cells with fibroblasts results to an induction of HAS2 in fibroblasts and an increase of the secreted HA. In a recent experiment performed in our laboratory, it was noticed that when fibroblasts were treated with a recombinant protein of Q7z3e2 (part of protein sequence 1-211 a.a.) or with the conditioned medium of MCF-7 which was previously treated with anti-Q7z3e2 that blocks this protein, HAS2 was induced and HA was increased.
Thus, we hypothesize that this novel protein is implicated in breast tumor cellular mechanisms and its secretion is concerned in a cross-talk of breast tumor and stromal cells.

P73 Role of integrin β1 and pleiotrophin in the chick cerebellum morphogenesis

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Neural developmental events include a series of processes such as, cell proliferation, migration, adhesion and cell differentiation contributing to the regional brain cytoarchitecture. Key players in these processes involved in cell proliferation and migration during brain ontogenesis are the morphoregulatory molecules, integrin, mediating cell–cell and cell–matrix interactions and pleiotrophin (PTN), a heparin growth factor. Pleiotrophin also known as HARP ((heparin affin regulatory peptide), is a growth factor expressed in astrocytes, glial and neuronal cells, whereas integrin β1 has a crucial role in both vasculogenesis and angiogenesis. The present study aimed to investigate the role of the morphogenetic molecules, integrin β1 and pleiotrophin, in two well-defined layered structures, cerebellum and optic tectum, that provide an excellent model for developmental studies in the central nervous system (CNS), using chick embryo, as a model organism. The distribution pattern of both PTN and integrin β1 was determined during the intermediate embryonic stages E10-E16, when cerebellum fissure formation and optic tectum organization take place, by means of immunohistochemistry. In addition, the possible involvement of these molecules in the granular cells proliferation and migration from their site of origin, the external granule cell layer (EGL), was investigated in the cerebellum, using double BrdU (S-phase marker) immunofluorescence. Co-expression of pleiotrophin and BrdU+ cells was observed in the proliferating zones of both cerebellum and periventricular layer of optic tectum at all the developmental ages studied. Moreover, correlation between integrin β1 and BrdU+ cells revealed the possible involvement of integrin β1 in the proliferation of granule cells. Our data, showed a specific spatio-temporal expression pattern during cerebellar ontogeny further supporting the role of integrin β1 and PTN on angiogenetic procedures that take place in development. In addition, the expression of pleiotrophin was higher in the secondary proliferation zone of cerebellum (EGL layer), with the folia floors including the lowest expression, suggesting the possible implication of PTN in the complex processes guiding the fissure formation. Furthermore, integrin β1 was expressed in the blood vessels radially distributed in the optic tectum and in the primary cerebellum folia floor, as well as, within the site that will develop to be the future secondary folia floor of the cerebellum. At the later developmental stages examined (E15-E16), in addition to the folia floor organization, strong angiogenesis was also extended in the white matter, possibly contributing to total fissure configuration. These findings further suggest the possible contribution of PTN and integrin β1 in the cerebellum and optic tectum maturation and cytoachitectonic organization.

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P74 Inhibition of intracellular kinases affects cell aggressiveness and expression of proteolytic network molecules in ERα suppressed breast cancer cells

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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. The aim of this study was to examine the effect of the inhibition of principal signaling pathways on cell functions of ERα suppressed

breast cancer cells as well as on the expression of molecules of the proteolytic network. Epithelial MCF-7 breast cancer cells with low metastatic potential were stable transfected with specific shRNA lentiviral particles for the suppression of ERα. We compared gene expression profiles between ERα suppressed (SP10+) and control lentiviral particles transfected (Csh) cell lines by Ion AmpliSeq Targeted Sequencing Technology. Our data showed that ERα suppression strongly increased gene expression of tyrosine kinase receptors, such as EGFR, PDGFR and AXL. ERα suppressed cells also expressed significantly higher mRNA levels for several proteolytic enzymes such as MMP2, MMP11 and MMP14, tissue metalloproteinase inhibitors TIMP4 and TIMP2 as well as molecules involved in plasminogen activation system, such as uPA, uPAR and PAI. Inhibition of intracellular pathways downstream of tyrosine kinase receptors, such as PI3K, MEK, JAK/STAT and Src, significantly reduces proliferation, migration and invasion of ERα suppressed cells. These alterations in cell functions were accompanied by changes in the expression levels of MMPs and plasminogen activation system molecules. The strongest effect has been found by the inhibition of Src. Our data indicate that the above signaling pathways are activated in ERα suppressed breast cancer cells and differently affect the expression of molecules of the proteolytic network.

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P75 Signaling through the collagen receptor, DDR1, is required for epithelial polarisation and morphological remodelling in 3D matrix

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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 with disorganised MT1-MMP distribution. Furthermore, polarisation of the epithelial cell membrane into an apical and a basal domain failed in absence of DDR1 signaling, suggesting that DDR1 affects apicobasal polarity in general. 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.
In conclusion, we find that DDR1 acts as a crucial sensor of the microenvironment and provides epithelial cells with spatial information necessary to polarise and undergo morphological remodelling in 3D.

P76 Hypoxia promotes microvascular endothelial cell interaction with extracellular matrix proteins

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The microvascular endothelium represents an important interface between the blood vessel lumen and tissues and plays a major role in a variety of basic processes, including wound healing, inflammation, tumor metastasis, and angiogenesis. In addition, the angiogenic functions of microvascular endothelial cells are regulated by interactions with the extracellular matrix. It is well known that the hypoxic environment of microvascular endothelium and the activation of Hypoxia-Inducible Factors (HIFs) are the basic inducers of angiogenesis; however, the mechanisms involved are still poorly understood. Here, we have investigated the effect of hypoxia on the adhesion and migration of dermal microvascular endothelial cells (HMEC-1) towards laminin-1 and collagen IV, as well as on the expression of genes that mediate the cell-matrix interaction, such as *integrin β1, plasminogen activator inhibitor-1 (PAI-1), carbonic anhydrase IX (CAIX) and vascular endothelial growth factor (VEGF)*.
We found that HMEC-1 exhibit increased adhesion to collagen IV compare to laminin-1. Additionally, the migration ability of HMEC1 towards laminin-1 and collagen IV is significantly enhanced under hypoxic conditions of 1% O_2 as well as in the presence of DMOG, a prolyl-4-hydroxylase inhibitor, compare to normoxic conditions. Our results also demonstrate that hypoxia up-regulates *integrin β1*, *PAI-1*, *CAIX* and *VEGF* mRNA level in HMEC-1. This allows us to examine the distinct or common effects of the HIFα isoforms, HIF-1α and HIF-2α, on HMEC-1 adhesion and migration towards laminin-1 and collagen IV and on the expression of the above mentioned genes.
Our study aims to elucidate the differential effects of HIF-1α and HIF-2α on the microvascular endothelial cell/matrix interaction within the hypoxic environment and to understand the HIF-mediated angiogenic endothelial functions, contributing to future molecular therapeutic interventions.

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P77 The signaling pathways of AXL and PDGFR receptor tyrosine kinases mediate the mesenchymal phenotype of ERα-suppressed breast cancer cells

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Estrogen receptor alpha (ERα) and its signaling pathway are of high significance in hormone-dependent breast cancer since about 70% of breast cancers are detected as ERα positive. In a previous study we have shown that the suppression of ERα induced EMT and strongly enhanced the aggressiveness of MCF-7 breast cancer cells. These changes on the differentiation and the behavior of the cells were followed by significant alterations on the expression of a plethora of genes. Such genes included many factors related to the process of epithelial-mesenchymal transition (EMT), such as TGFβ signaling molecules, as well as various receptor tyrosine kinases (RTKs). The role of TGFβ on the induction of EMT in cancer is well established. There are numerous studies showing that the activation of the TGFβ pathway triggers EMT, while its inhibition reverses this process in many cases. In addition, the signaling pathways of various RTKs are also associated with EMT. For example, it has been reported that the RTK AXL contributes to the mesenchymal phenotype of aggressive ERα negative breast cancer cells. Moreover, some studies indicate that the RTK PDGFR may also contribute to EMT in several types of cancer, including breast cancer. In our case, the suppression of ERα strongly enhanced the expression of AXL and PDGFR, and of TGFβR to a lesser extent. Considering the above, the aim of our study was to

investigate whether these receptors regulate the mesenchymal phenotype of the ERα suppressed MCF-7 breast cancer cells, referred as MCF-7/SP10. For this reason we evaluated the effect of specific inhibitors of TGFβR-I, AXL and PDGFR on the morphology and the protein expression of several EMT markers. Our data showed that, although MCF-7/SP10 cells are responsive to TGFβ, the constitutive inhibition of TGFβR-I is not able to reverse EMT. On the other hand, the inhibition of AXL, and PDGFR to a lesser extent, reduces the expression of some mesenchymal markers and slightly affects the phenotype of these cells. In conclusion, it seems that the signaling pathways of several RTKs, such as AXL or PDGFR may regulate the EMT caused by the suppression of ERα, however their inhibition does not fully reverse the mesenchymal phenotype of the MCF-7/SP10 cells, indicating that the regulation of EMT in these cells is a multivariable process.

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P78 Integrin alpha2 in nonactivated conformation can induce Focal Adhesion Kinase signalling

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Integrins are not only adhesion molecules that anchor the cell to its surroundings but they also contribute to the adhesion dependent cell signaling. In addition, several pathogens, such as viruses, use integrins to bind to cell surface. Our previous study showed that human echovirus 1 (EV1) binds to the nonactivated conformation of alpha2beta1 integrin (Jokinen et al., 2010). Here we show that this interaction also leads to Focal adhesion Kinase (FAK) mediated cellular signaling.
FAK regulates the formation and disassemble of integrin mediated adhesion sites, the focal adhesions. It has previously been described that the recruitment of FAK to integrin adhesions follows integrin activation and ligand induced integrin clustering. However, it was of interest to find out, whether FAK can be activated by nonactivated alpha2-integrins. EV1–integrin adhesions were studied using a novel approach, in which alpha2-integrin expressing SaOS-cells are plated on EV1-coated surface where they form adhesions only through nonactivated integrins. Indeed, FAK tyrosine 397 and 576/577 phsphorylation could be observed even without integrin activation, and also the phosphorylation of paxillin Y118. Loss-of-function mutation in alpha2-integrins (alpha2E336A), integrin inactivation with magnesium and calcium chelating agent EDTA, or silencing integrin activating protein talin 1 could not prevent FAK activation.
In conclusion, this study showed that integrin clustering, not conformational activation, must take place to activate FAK.

P79 Suppression of ERα induces the activation of TGF-β and IL-8 pathways in breast cancer cells

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Breast cancer is one of the major causes of death in the modern world. Changes in the levels of estrogen receptor (ERα) is the most important discriminator factor of breast cancers. We have shown that the loss of ERα leads to transition of epithelial cells to mesenchymal, which is associated with increased aggressiveness. It has been proposed that ERα signaling antagonizes signaling pathways that promote EMT including TGF-β and IL-8 signaling pathways. In breast cancer, TGF-β seems to have a dual role;

initially it suppresses tumorigenesis by inducing growth arrest and promoting apoptosis, however, in advanced breast cancer, where TGF-β often is overexpressed, it promotes tumorigenesis by induction of EMT. IL-8 is a proinflammatory chemokine that induces the activation of multiple signaling pathways promoting tumorigenesis. The aim of this study was therefore to evaluate the effect of the suppression of ERα in TGF-β and IL-8 signaling pathways. Specifically, MCF-7 epithelial breast cancer cells with low metastatic potential were stably transfected with lentiviral particles bearing shRNA against ERα and control shRNA and study on the expression levels of molecules involved in the reported signaling pathways was performed. Then, we examined the activation of the signaling pathways as well as the effect of their activation and inhibition in cell functional properties and in the induction of EMT. Our data showed that the loss of ERα induces the activation of TGF-β signaling pathway and increases the expression of molecules involved in TGF-β signaling, such as TGF-β1 and TGF-β receptors. Inhibition of TGF-β pathway seems to affect cell proliferation and migration. ERα suppression induces the expression of inflammatory mediators, especially that of IL-8 and can trigger the activation of IL-8 signaling pathway. The inhibition of IL-8/CXCR2 signaling axis rapidly rearranges cell cytoskeleton and affects cell spreading and morphology as well as significantly reduces cell proliferation, migration and invasion. The inhibition of both pathways is not sufficient to induce morphological alterations, even though it changes the expression of EMT markers. The above data suggest a correlation between TGF-β and IL-8 signaling with ERα status.

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P80 Collagen XIII regulates breast cancer in humans and mice

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Background: Collagen XIII is a transmembrane collagen previously implicated in cell adhesion and in adhesion-dependent cell functions. Bioinformatics data suggests that collagen XIII is upregulated in solid tumours, and particularly in breast cancer patients upregulation of collagen XIII is associated with poor prognosis.

Aims: To elucidate the roles of collagen XIII in breast cancer, we have crossed the transgenic MMTV-PyMT mouse mammary tumor model with collagen XIII deficient mice (*Col13a1*$^{-/-}$), and compared primary tumour development and progression. In addition, we investigated the expression level as well as the functional roles of collagen XIII using human breast cancer cell lines.

Results: High expression of collagen XIII associates with poor prognosis in human breast cancer patients, and with decreased disease-free survival. In the MMTV-PyMT model, tumour growth is significantly delayed in the *Col13a1*$^{-/-}$ mice. Immunofluorescence staining showed that collagen XIII associates to plasma membranes of cancer cells. High collagen XIII expression was detected in an invasive human breast cancer cell line MDA-MB-231, and its silencing using siRNAs resulted in a significant reduction in tumour cell proliferation.

Conclusion: Transmembrane collagen XIII plays a vital role in breast cancer. Collagen XIII deficient mice display a delay in tumour growth suggesting its active involvement in the disease process. Knocking down collagen XIII reduces proliferation of breast cancer cells *in vitro*, suggesting a role for this collagen in cell-cell or cell-matrix signaling.

Upcoming studies: We will continue our efforts to characterize the roles of collagen XIII in breast cancer initiation and progression, and aim to identify the signaling pathways regulated by collagen XIII,

as well as its detailed mechanisms of action. Treatment of human breast cancer cells harboring XIII knockdown with various cancer drugs will reveal whether lack of this transmembrane collagen sensitizes tumour cells for cancer therapy.

P81 Lysyl oxidase promotes survival and outgrowth of colon cancer cells in the bone marrow, enabling bone metastasis formation

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Lysyl oxidase (LOX) catalyzes the cross-linking of collagens and elastin in the extracellular matrix, thereby regulating the tensile strength of many tissues, such as in bone. In cancer, LOX plays a critical role in facilitating tumor growth and metastasis formation in soft tissues. Whether tumor-derived LOX also enables bone metastasis is unknown. In this study, we first showed by immunohistochemistry using patients' tumor specimens, that LOX was expressed in the desmoplastic tumor stroma of pairs of colorectal carcinomas and their matching bone metastases. Preclinical experiments showed that LOX overexpression in different colon carcinoma cell lines enhanced the formation of osteolytic lesions in animals by promoting both skeletal tumor burden and osteoclast-mediated bone resorption. Additionally, LOX treatment of animals enhanced bone metastasis caused by parental colorectal carcinoma cells. Conversely, the pretreatment of animals with the LOX inhibitor b-aminopropionitrile or the silencing of LOX in colorectal carcinoma cells drastically reduced the formation of osteolytic lesions. Furthermore, we demonstrated that LOX was involved in the early nidation of tumor cells into the bone marrow. In vitro, LOX directly enhanced the attachment of colon cancer cells to type-I collagen, but not to fibronectin. LOX-overexpressing colorectal carcinoma cells were more prone to adhere to components of the osteoblastic niche, such as osteoblasts. Thus, LOX may promote engraftment of colon cancer cells in the osteoblastic niche. Tumor-derived LOX also promoted osteoclast differentiation by enhancing the secretion of osteolytic factors from colon cancer cells such as IL-6. Additionally, the activation of an IL-6 autocrine loop led to cancer cell survival. In conclusion, our findings provide novel evidence that LOX endows colon cancer cells with the ability to thrive in the bone marrow microenvironment and stimulate osteoclast-mediated bone destruction.

P82 Adhesion strength and modeling of human bone cells on advanced biomedical substrates

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A deep understanding of the mechanism of osteoblasts adhesion on advanced nanostructured materials is fundamental for the successful design of novel implants. The present study evaluates the specific biomarkers and the adhesion strength of human osteoblasts when seeded on advanced biomaterials such as titanium dioxide nanotubes (TNTs) and carbon nanotubes (CNTs). Further on, a predictive model for the estimation of the interphase quality which exists between osteoblasts and various substrates is presented, while theoretical results are compared to the experimental values. The highest number of adherent cells maintained on a substrate after shear stresses were applied was observed in the case of CNTs, followed by TNTs, titanium and polystyrene (Fig.1a). Modeling results (Fig.1b) were in agreement with the experimental results and proved to be useful in predicting the quality of adhesion between human cells/ tissues and various substrates, thus giving solutions to existing problems related to the surface processing of biomaterials for advanced implant design. Nanostructured topographies favor cells proliferation but determine their strong adhesion to substrates, which may restrict cells appropriate development. Predictive modeling results give solutions for an improved functionality of biomaterial surfaces.

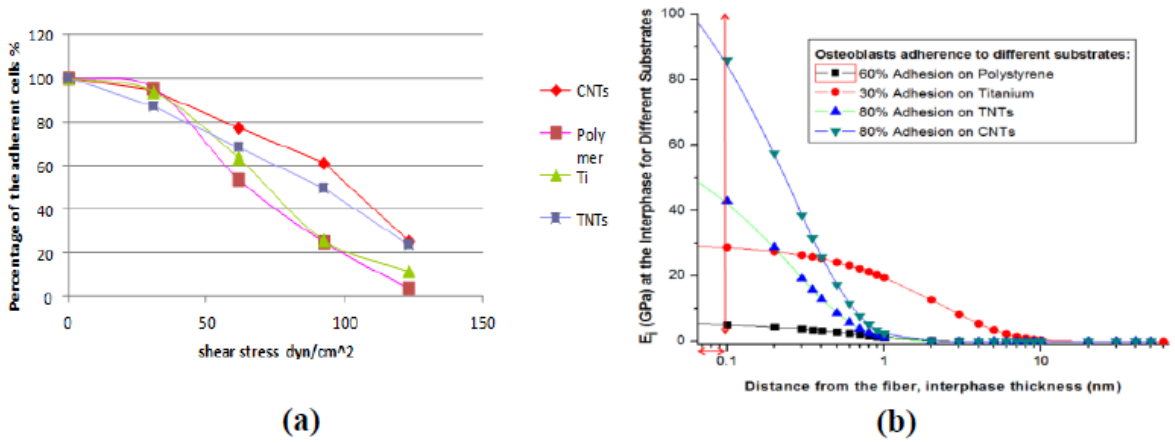

Figure 1 Comparative experimental and theoretical study: (a) The percentage of the adherent cells on four types of substrates as a function of the applied shear stress (b) Representation of the elasticity modulus variation within the interphase between different substrates and human osteoblasts

References

1.*Reutelingsperger CPM, et al. J Mater Sci: Mater Med 1994;5:361}7*

2.*G. C. Papanicolaoua, et al (2011) Composite Interfaces 18 (2011) 23–35*

Workshop 6: Tissue engineering from matrix perspective

Invited Lecture (L25) Living Implants to Reverse Disease: Diabetes Reversing Implants for long term Viability and Efficiacy (DRIVE)

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Diabetes mellitus is a chronic disease characterised by high blood glucose due to inadequate insulin production and/or insulin resistance which affects 382 million people worldwide. Pancreatic islet transplantation is an extremely promising cure for insulin-sensitive diabetes mellitus (ISDM), but side effects of lifelong systemic immunosuppressive therapy, short supply of donor islets and their poor survival and efficacy in the portal vein limit the application of the current clinical procedure to the most at-risk brittle Type I diabetes (T1D) sufferers. The DRIVE consortium (www.drive-project.eu) will develop a novel suite of bio-interactive hydrogels (β-Gel) and on-demand drug release systems to deliver islets in a protective macrocapsule (β-Shell) to the peritoneum with targeted deposition using a specialised injection catheter (β-Cath). Pancreatic islets will be microencapsulated in β-Gels; biofunctionalised injectable hydrogels containing immunosuppressive agents and polymeric microparticles with tuneable degradation profiles for localised delivery of efficacy cues. These β-Gels will be housed in a porous retrievable macrocapsule, β-Shell, for added retention, engraftment, oxygenation, vascularisation and immunoprotection of the islets. A minimally invasive laparoscopic procedure (O-Fold) will be used to create an omental fold and at the same time deliver β-Shell. An extended residence time in β-Gel will enhance long-term clinical efficacy of the islets and result in improved glycemic control. The novel β-Gels will also be developed as human three-dimensional in-vitro models of in-vivo behaviour. Islet harvesting and preservation technologies will be developed to facilitate their optimised yield, safe handling and transport, and ease of storage. DRIVE will also enable the future treatment of a broader range of T1 and insulin-sensitive T2 diabetics by working with induced pluripotent stem cell experts to ensure the compatibility of our system with future stem cell sources of β-cells.

Invited Lecture (L26) Soft-tissue reconstruction solely based on cell-derived matrix organization: the benefits of the self-assembly approach of tissue engineering

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Tissue engineering is needed to repair large tissue defects and is a promising alternative/complement to autologous grafting. It combines the use of cells and scaffolds providing the structural properties. Many combinations of cell types and biomaterials have been investigated in order to engineer tissues with optimal functional properties. An increased use of naturally-derived biological matrices has been recently noted. Along these lines, our core technology is based on the self-assembly approach of tissue engineering. The latter exploits the capacity of mesenchymal cells to secrete and assemble extracellular matrix (ECM) elements upon ascorbic acid and serum supplementation *in vitro*, leading to manipulatable cell sheets combined to form tridimensional constructs devoid of exogenous or synthetic biomaterials. Examples of tissue substitutes produced by the self-assembly approach include blood vessels, connective and adipose tissues as well as skin and bladder substitutes resulting from the combination of self-assembled stroma seeded with their respective epithelial cells (keratinocytes and urothelial cells).
Along the years, we also determined that dermal fibroblasts (DFs) and mesenchymal stem/stromal cells (MSCs) from different origins [adipose tissue (ASCs), bone marrow (BM-MSCs), umbilical cord Wharton's jelly (WJ-MSCs)] reveal distinct intrinsic capacities *in vitro* for matrix production and

organization. ASCs are particularly responsive to various culture conditions and very efficient for matrix assembly. ASC- and DF-constructs generally featured an increased thickness when compared to BM-MSC and WJ-MSC constructs. While shared similarities were noted in the nature of the ECM expressed by these various engineered constructs (collagens type I and III, fibronectin), the newly deposited ECM also reflected the cell origin and their state of differentiation (basement membrane collagen IV around adipocytes, laminin, etc). Stromal compartments reconstructed from different types of mesenchymal cells also displayed varying capacities to appropriately support epithelial cell proliferation and differentiation.
Finally, the in vivo behavior of the substitutes reconstructed using the self-assembly approach is characterized by rapid engraftment and physiological remodeling after grafting. This has been especially demonstrated for skin constructs used in a clinical setting for the treatment of venous ulcers and burn patients coverage. The highly natural ECM featured by the substitutes is undoubtedly part of the success of these various engineered tissues in preclinical and clinical studies, by avoiding the stimulation of foreign body responses upon grafting and thus favoring long-term survival. In conclusion, mesenchymal cells from various origins can be used to generate tissue-specific substitutes featuring excellent mechanical and therapeutic properties for a wide range of regenerative medicine applications.

Invited Lecture (L27) Perlecan is critical for tissue and organ development but does it have a role in tissue engineering?

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Perlecan is an ubiquitous, extracellular matrix heparan sulfate proteoglycan present in peri-cellular spaces and basement membranes. Mice lacking perlecan have major problems with the development of tissues and organs due to a lack of structural organisation leading to multiple organ failure. The animals that died early in utero displayed neural and vascular malformations, whereas those that survived to birth displayed severe chondrodysplasia. We were interested in determining whether native perlecan and/or bioengineered active domains of perlecan might be useful in the tissue engineering of cartilage and as a basement membrane substitute on synthetic vascular grafts. We were also interested in determining the fundamental biological role of perlecan in endochondral ossification and angiogenesis.
We engineered and expressed various regions of the perlecan protein core including the N-terminus encompassing the 3 glycosaminoglycan attachment sites in domain I, and the C-terminus encompassing the glycosaminoglycan attachment site, the a2b1 integrin binding site and endorepellin. Both recombinant molecules were decorated with heparan sulfate (HS) and chondroitin sulfate (CS) chains, similar to chondrocyte and smooth muscle cell-derived full length perlecan but in contrast to endothelial cell-derived perlecan, which is decorated only with HS.
We discovered that the HS decorating chondrocyte derived perlecan mediated the formation of FGF 18 – FGF receptor 3 complexes and that the signalling through was controlled by the microstructure of the HS and inhibited by heparanase cleavage of the HS. This suggests that this is a mechanism that cells may use to determine their proliferation and differentiation rates during different stages of cartilage development.
When vascular grafts coated with full length endothelial-cell derived perlecan were implanted into the carotids of sheep, we demonstrated significantly better endothelialisation and less thrombus formation. In vitro studies using the recombinants, as well as smooth muscle cell and endothelial cell-derived

perlecans concluded that perlecan was important for endothelial cell adhesion and proliferation and that the a2b1 binding site in domain V, in isolation, promoted the attachment of both endothelial cells and platelets but not smooth muscle cells. The full length perlecans, promoted the attachment of platelets, endothelial and vascular smooth muscle cells, suggesting that there might be another attachment sites in the protein core for smooth muscle cells. These data support the idea that we might be able to coat vascular grafts with various recombinant regions and domains of perlecan to encourage endothelialisation, prevent thrombus formation and prevent intimal hyperplasia to provide for a successful vascular graft replacement in the clinic.
The results will be discussed further with respect to the use of perlecan as a dynamic heterogeneous biological molecule in tissue engineering projects.

ST26/P83 Signaling mechanisms for the enhanced survival of adipose stem cells in elastin-like extracellular matrix

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Stem cell transplantation is promising for treating various type of disease. However, acute loss of stem cell population after in vivo transplantation is a critical obstacle to improving stem cell therapy. In most cases, extracellular matrix (ECM) environment of the injury sites to where stem cells are targeted to be transplanted is pathologically impaired or in physiologically abnormal condition. Therefore, the demand for provisional matrix that is permissive to stem cells and suitable to replace injured or missing matrix is very high. We biosynthesized an artificial ECM (aECM) with the amino acid sequence of TGPG[VGRGD$(VGVPG)_6]_{20}$WPC to emulate elastic function of elastin and cell-binding property of fibronectin. We performed Western blot analysis on Erk and Akt phosphorylation which was known to be related with the viability of adipose stem cells. The p-Erk/Erk and p-Akt/Akt ratio of the cells cultured with aECM were approximately 1.7- and 2.3-times greater than those of control cells, respectively. A phosphorylation inhibition study was performed using PD98059 (Mek inhibitor) and Wortmannin (PI3K inhibitor). In adhesion assay, treatment of PD98059 and Wortmannin caused a 69.2% and a 70.7% decline of cell adhesion to aECM, respectively. In proliferation assay, PD98059 or Wortmannin resulted in an about 50% decline in viable cell numbers. We implanted adipose stem cells and aECM together into the full-thickness excisional wounds generated on the dorsal skin of C57BL/6 mice. The half life of stem cells at wounds sites was 1.9 days (44 h) and 1.7 days (40 h) in the presence and absence of aECM, respectively. Both p-Erk/Erk and p-Akt/Akt ratios were statistically higher in the aECM-treated mice compared to control mice. The combined administration of stem cells and aECM upregulated the expression of provided dermal tissue components and improved overall wound healing than the use of stem cells alone. In vitro and in vivo results showed that aECM improved the adhesion and viability of adipose stem cells at least in part through the activation of Mek/Erk and PI3K/Akt pathways.

ST27/P84 FGF-2 treatment primes ADMSC chondrogenesis by increasing the expression of integrin alpha10

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Purpose: Adipose-derived mesenchymal stem cells (ADMSC) are considered to be an attractive source for cartilage repair because of their self-regeneration potential, accessibility and abundance. Previously it was shown that FGF-2 treatment of bone marrow-derived MSCs in monolayer upregulates the

expression of alpha10 integrin and enhances their chondrogenic differentiation. The aim of this study was to develop a similar pre-conditioning strategy which primes ADMSC towards chondrogenesis.
Methods: Human ADMSCs and BMSCs were cultured in monolayer for 5 days with or without FGF-2 under normoxia or hypoxia. Afterwards, MSCs were subjected for chondrogenic differentiation in pellet culture for 28 days in the presence or absence of TGFß1 and BMP2 (T/B). Differences in integrin and chondrogenic gene expression patterns were investigated by RT-PCR. Protein expression levels were determined for integrin α10 by immunoblotting. Safranin-O (SO) staining for proteoglycans and immunohistochemistry (IHC) for collagen II and aggrecan were performed to demonstrate chondrogenesis. Biomechanical properties and structure of cartilaginous matrix were examined by atomic force microscopy (AFM).
Results: FGF-2 supplementation of ADMSC and BMSC monolayers increased the expression of Itga10 and Sox9, while decreased the expression of Itga11 and Agc1, independently of the oxygen tension. Additionally, α10 protein levels were elevated in FGF-2 treated MSCs. SO staining and IHC demonstrated superior chondrogenic differentiation of BMSCs with T/B administration in pellet cultures compared to ADMSCs. ADMSCs showed patches proteoglycan deposition and increased Col2a1 mRNA expression in T/B-treated pellets upon FGF-2 and hypoxic preconditioning. AFM measurements confirmed collagen fibers development in peripherally differentiated ADMSC pellets and entirely differentiated BMSC controls.
Conclusion: We showed that FGF-2 pretreatment of ADMSCs increases the expression of the chondrogenic markers Itga10 and Sox9 in monolayer and primes subsequent chondrogenesis in pellet culture. We suggest that modulation of Itga10 expression in ADMSCs may help to develop better cell-based therapeutic strategies to treat osteoarthritis.

ST28/P85 Eggshell Membrane - An equivalent of extracellular matrix (ECM) in avian egg has modulating wound healing properties

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Components of the extracellular matrix (ECM) play an important role in normal wound healing and the unregulated degradation of ECM components impairs healing. This has led to the development of new therapies that aim to reduce the degradation of ECM or to re-establish undamaged ECM.
In Eastern societies, eggshell membrane (ESM) preparations have been used for centuries to assist healing of burn and cutaneous wounds. Biovotec AS, a biotech company, is developing a novel, low cost wound healing product based on the raw material of ESM. To review possible mechanism, we characterized the composition of the ESM and investigated the effect of ESM in cell models mimicking the wound healing processes. SEM analysis and histological collagen staining of ESM powder shows fibrous protein structure of primarily collagen. Glycosaminoglycan (GAG) analysis by HPLC revealed a high content of hyaluronic acid (81%) in addition to chondroitin-sulphate GAGs, while heparan sulphate and keratan sulphate were not detected. HA has been frequently reported to be involved in anti-inflammatory mechanisms. Consistent with this, we demonstrated that ESM was capable of reducing the LPS-induced NF-κB-luciferase activity in U937 cells. In addition, ESM was shown to have a role in regulating pro- and anti-inflammatory cytokine production in macrophage-like THP1 cells. In dermal fibroblasts, ESM increased α-SMA expression and influenced the differentiation from fibroblast to myofibroblast. Furthermore, our experiments show that ESM has an inhibitory effect on matrix degrading enzymes, as revealed by assays with recombinant MMP-2 and MMP-9. Finally, ESM applied to a db/db mouse model of delayed wound healing showed a comparable healing effectiveness to the positive control rhPDGF at day 20 post-wounding.

In conclusion, ESM presents properties of promoting wound healing and may potentially become a novel biological wound dressing that can be used for normal wounds and those at risk of delayed healing.

ST29/P86 Amyloid micronetworks in cartilage repair: a protein specific response

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Articular cartilage regenerates in 22% of cartilage defects using ACI [1]. Adding a material that mimics the extracellular matrix of articular cartilage could result in improved cartilage tissue formation. Such a material should be fibrous, form a hydrogel, be as strong as collagen, and preferably be chondroinductive. Amyloid fibrils are a self-assembling, polypeptide-based material characterized by inter-protein β-sheets. These fibrils are used in nature for cell adhesion [2], protection [3], and have collagen-like mechanical properties [4]. Also, they can assemble into hydrogel-like micronetworks [5]. Therefore, we hypothesize that amyloid micronetworks are a potential scaffold material for cartilage tissue engineering.
The self-assembly of amyloid fibrils and networks was validated for the proteins α-synuclein (αS), β-lactoglobulin (βLG) and lysozyme (LZ) with ThT, CD-spectroscopy, fluorescence microscopy, AFM and SEM. The effect of monomers (-M) or amyloid networks (-N) of the proteins on bovine chondrocyte (bCh) viability was studied after 3 days culture using MTT assays and flow-cytometry with Calcein AM. qPCR was performed to investigate the chondrocyte phenotype. Matrix formation was investigated by culturing bCh mixed with micronetworks for 5 weeks and analysing sectioned samples with histology and SEM.
All fibrils and networks stained positively for ThT. The fibrils were rich in β-sheets, micrometres long with diameters of several nanometres. The networks consist of fibrils organized in structures of several tens of micrometres in diameter: αS and LZ formed particle-like networks, while βLG assembled into sheet-like networks. The presence of amyloid networks decreased the metabolic activity, this effect was most pronounced in the presence of LZ-N. However, the percentage calcein positive cells did not vary between conditions. Aggrecan and collagen 2 gene expression increased in the presence of LZ, while collagenases MMP1 and MMP3 expression increased with αS or βLG present. αS-N and βLG-N lowered aggrecan production, while LZ-N increased the amount aggrecan present and SEM revealed the presence of collagen fibrils.
Our results indicate that amyloid micronetworks could enhance cartilage tissue formation. The amyloid micronetworks support bCh viability and matrix formation. Interestingly, the cell response is influenced by the type of protein used, even though all proteins have a β-sheet rich structure. LZ-N induced cartilage gene expression and matrix formation. This suggests that the chondroinductivity of the networks can be optimized by choosing or designing a polypeptide.
[1] DOI:10.1186/ar613; [2] DOI:10.1111/j.1365-2958.2010.07269.x; [3] PMID:11124142; [4] DOI:10.1038/nnano.2011.102; [5] DOI:10.1021/nn406309c

ST30/P87 Low-immunogenic matrix suitable for transplantation

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Transplantation is an effective treatment option for patients suffering from different end-stage diseases; however it is associated with a constant shortage of donor organs and lifelong immunosuppressive therapy. Using of pig organs is promising to replace human organs for transplantation. Matrix derived from porcine organs is a convenient substitute for the human matrix. As an initial step to create a new ex vivo tissue engineered model, we optimized protocols to obtain organ-specific acellular matrices and

evaluated their potential as tissue engineered scaffolds for culture of normal cells and tumor cell lines. Our protocols include decellularization by perfusion in a bioreactor system and immersion-agitation on an orbital shaker with use of various detergents (SDS, Triton X-100) and freezing.
Completeness of decellularization in terms of residual DNA amount is important predictor of probability of immune rejection of materials of natural origin. However, according to our data and the data of the literature, the signs of cellular material still remain even after harsh decellularization protocols. In this regard, the matrices obtained from tissues of low-immunogenic pigs with α3Galactosyl-tranferase gene knock out (GalT-KO) may be a promising alternative to native animal sources. We studied induced effect of frozen and fresh fragments of GalT-KO skin on healing of full-thickness plane wounds in 80 rats. Commercially available wound dressings (Ksenoderm, Hyamatrix and Alloderm) as well as allogenic skin were used as a positive control and untreated wounds were analyzed as a negative control. The results were evaluated on the 4th day after grafting, which corresponds to the time of start of normal wound epithelization. It has been shown that a non-specific immune response in models treated with GalT-Ko pig skin was milder than in all the control groups. These preliminary data may contribute to develop personalized transplantable organs from GalT-Ko pigs with significantly limited potential of immune rejection.
Ideally, a bioengineered organ must be biocompatible, non-immunogenic and support cell growth. Porcine organs are attractive for xenotransplantation, if severe immunologic concerns can be bypassed. Our results indicate that genetically modified pig tissues with knock-outed α3Galactosyl-tranferase gene may be used for production of low-immunogenic matrix suitable for transplantation.

ST31/P88 Biologic scaffold materials composed of adipose tissue extracellular matrix

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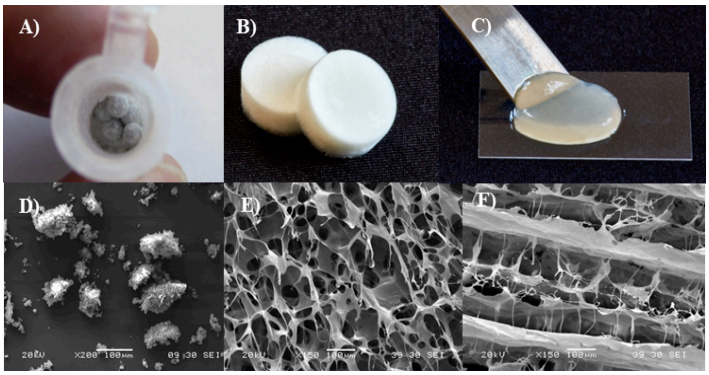
The extracellular matrix (ECM) represents the secreted product of the resident cells of each tissue or organ, including both functional and structural molecules arranged in a unique three-dimensional ultrastructure that supports the phenotype and the function of cells.[1] Biologic scaffold materials composed of ECM have received significant attention for their potential therapeutic applications. There are numerous commercially available biologic scaffold materials derived from a variety of animal tissues have been successfully applied in both preclinical and clinical studies.[2]
Adipose tissue represents a potentially abundant source of human origin ECM which can be easily obtained in large quantities by liposuction.[3] Interestingly, the adipocyte ECM composition includes a highly conserved specialized ECM how is the Basement Membrane (BM).[4] Methods for the obtaining of decellularized adipose tissue (DAT) have been previously described.[5-7] However, the method may affect the retained components, biologic and structural properties and host response after implantation.
Here we describe adipose tissue derived biologic scaffold materials obtained by an enzymatic decellularization protocol developed by our laboratory. The DAT showed absence of cell nuclei and preservation of tissue structure (adipocyte cytoplasmic space, dense fibrous and vascular structures) and a very similar pattern expression of the tissue ECM and BM proteins. Besides, very low remnant DNA (<0.02 ng/mg) and absence of *in vitro* cytotoxic effect was observed. confirmed the conservation of more than 140 proteins were confirm by chromatography (collagens, glycoproteins, proteoglycans and other ligand and anchoring), including specific BM ones (collagen type IV, laminin, heparan sulphate proteoglycan-2 and nidogens).



The DATs were milled to obtain a powder (fig. A, D). Dissolution in acetic acid and freeze drying permitted the obtaining of homogenous and interconnected porous scaffolds (fig. B) which maintained the structure and form in

aqueous media. Freezing temperature (–20ºC/-80ºC) allowed controlling the pore diameter (100-200 µm) and microstructure of the scaffolds (fig. E, F). Besides, acid-pepsin dissolved DAT self-assembled in physiological temperature (37ºC) and formed an *in situ* injectable hydrogel (Fig. C).
At present, these biologic scaffold materials are introducing in cell culture methods to conduct them as microcarriers and 3D cell and organoids, for drug screening and regenerative medicine applications in the near future.

References: [1]Reing J. E., et.al. Tissue Eng. Part A 15, 605–614. [2]Badylak SF. Biomaterials. 2007 Sep; 28(25):3587-93. [3]Fraser JK, et.al. Trends Biotechnol. 2006 Apr;24(4):150-4. [4]Edwin C. M. et.al. Cell Mol Life Sci. 2010 April; 67(8): 1277–1292. [5] Brown BN, et.al. Tissue Eng Part C Methods 2011; 17 (4): 411-21.[6]Choi JS, et.al. Tissue Eng Part C Methods 2009; 15:1-10.[7]Flynn LE et.al. Biomaterials. 2010 Jun; 31(17):4715-24.

P89 Tension is required for tenogenic gene expression in rat tail tenocytes

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Tendon is a connective tissue that transmits forces from muscle to bone. The tissue has a characteristic ultrastructure of highly aligned extracellular collagen fibrils, interspersed with a proteoglycan-rich interfibrillar matrix. The cells, namely tenocytes, are aligned in a similar fashion with the axis of tension. However, in the pathological state of tendinopathy, this cell- and matrix-orientation is partially lost. In this study we evaluated the gene expression profile of tenocytes as well as the extracellular matrix setup in an *in vitro* model of early tendon development.
Rat tail tenocytes were seeded in a fibrin gel, either constrained with silk sutures or left free floating, and the cells contracted the gel within 7 days. Ultrastructural analysis by confocal microscopy and transmission electron microscopy confirmed aligned tenocytes and extracellular collagen fibrils in constrained samples, while the free-floating samples contained disordered collagen fibrils and cells. Gene expression analysis proved that mechanical anchorage and the resulting cell derived tension are required for stable expression of the tendon markers scleraxis, mohawk and tenomodulin. Moreover, the matrix proteins collagen type I, fibromodulin and osteoglycin are upregulated in constrained samples. Unconstrained samples on the other hand, showed upregulation of decorin, integrin alpha 2, BMP6 and MMP13. BMP6 is described to induce cartilage formation, while MMP13 degrades collagenous matrix. Hence, the unconstrained condition promotes genes that are not beneficial for tenogenic gene expression.
In conclusion, this study shows that rat tail-derived tenocytes require anchor points to align with an axis of tension and to develop their tendon-specific gene expression. These factors are vital to establish the alignment of the extracellular collagen. Thus, it is likely that tenocytes in tendinopathic areas of tendon tissue lose their tendon-specific phenotype and cannot actively contribute to the tissue's regeneration.

P90 A biomaterial for cartilage engineering: biophyscochemical characteristics selected for promoting stem cell proliferation and chondrogenesis

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Tissue engineering requires combining three components: cells, biochemical factors from the microenvironment and scaffold. We designed an innovative biomaterial associating structural, mechanical and biological properties for tridimensional cell development. It consists of a tridimensional

scaffold, made of biosourced, biocompatible and biodegradable polymers such as poly(lactic acid), whose surface is functionalized with a nanolayer of biomolecules naturally occurring in the extracellular matrix [1]. Our biomaterial is highly tunable and scalable and it may thus be used to study of the effects of several of its characteristics on cell behavior. We mainly studied the behavior of mesenchymal stem cell (MSC) since they provide great potentialities for many tissue engineering applications. Here we focus on cartilage engineering, studying the ability of MSC to differentiate into chondrocytes. For that purpose, surface scaffold was functionalized with hyaluronan (HA), an abundant glycosaminoglycan in cartilaginous tissue. We used either high molecular weight (HMW) or low molecular weight (LMW) HA since it is known that biological functions of HA greatly depend on its chain size [2]. In addition, MSC chondrogenesis into our biomaterials was examined in the presence of either TGF-β3 or BMP-2.
We showed that MSC proliferation and chondrogenesis strongly depended on the polymeric composition, the shape (film or microsphere), the porosity and the pore diameter of the scaffold. It also varied according both the conditions used for functionalization of the scaffold surface with HA and the chain size of HA. The best results were obtained with porous asymmetric films which display an interconnected network of macro- and micropores and whose surface was functionalized with HMW HA. Moreover, as shown by flow cytometry analysis, MSC differentiate into chondrocytes in the presence of BMP-2 as well as in the presence of TGF-β3. However, it should be noted that mature chondrocytes with deposition of a hyaline cartilaginous matrix, characterized by collagen II and aggrecan synthesis and absence of collagen X, were finally obtained only in the presence of TGF-β3.
All together, these results show that the characteristics of our biomaterial can be finely adjusted according to the targeted application. In the case of cartilage engineering, our aim is now to include the differentiation factor TGF-β3 into our biomaterial.
References:
1- Al Tawil E., Monnier A., Nguyen Q.T., Demange E., Kassim Y., Deschrevel B, French Patent, n° 14 51478, Bulletin Officiel de la Propriété Industrielle, 2015, 35, FR 3 017 877 and curent PCT extension.
2- Deschrevel B., Tranchepain F., Vincent J.C., Matrix Biology, 2008, 27, 475-486.

P91 Preparation and characterization of nanofibrous polymer scaffolds for cartilage tissue engineering

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Polymer substrates obtained from poly(lactic acid) (PLA) nanofibres modified with carbon nanotubes (CNTs) and gelatin (GEL) for cartilage tissue engineering were studied. The work reports physical, mechanical, and biological results of the studies. The hybrid structure of PLA and gelatine nanofibres, carbon nanotubes- (CNTs-) modified PLA nanofibres, and pure PLA-based nanofibres were manufactured in the form of fibrous membranes. The fibrous samples with different microstructures were obtained by electrospinning method. Microstructure, physical and mechanical properties of samples made from pure PLA nanofibres, CNTs-, and gelatin-modified PLA-nanofibres were investigated. The scaffolds were then tested in vitro in culture of human chondrocytes collected from patients. The study was carried out in accordance with local ethics guidelines, and cartilage samples were collected after obtaining written informed consent from the donors. To assess the influence of the nanofibrous scaffolds on chondrocytes, tests for cytotoxicity and genotoxicity were performed. The study demonstrates viability of cells in the presence of pure polymer fibrous structure and its modified form, that is, after modification with CNTs and gelatine. Also, the materials studied were not genotoxic for tested chondrocytes using the Comet test assa and can probably be safely used in humans. Summarizing the work revealed that the nanofibrous structures studied here were neither genotoxic nor

cytotoxic, and their microstructure, physical and mechanical properties created promising scaffolds for potential use in cartilage repair.

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P92 To differentiate or not to differentiate? The impact of BMP-12 treatment on cells clinically relevant features

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Introduction: Tendon and ligament injuries represent one of the most common medical problems related to musculoskeletal system. With the regenerative medicine and cell transplantation development, there are prospects to use mesenchymal stem cells (MCS)-based therapy in patients with damaged ligaments and tendons. Researchers working on MSC-based therapies stand before a choice the use of undifferentiated cells, or suitably modified in the direction desired for a particular application path of differentiation. BMP-12 was previously demonstrated to induce tenogenic pathway in MSCs what resulted in increased production of collagen I, collagen III, tenascin. The aim of this study was to determine whether directing MSCs to the tenogenesis by BMP-12 do not affect other cells properties which are believed to be desirable.

Material and methods: Human adipose-derived mesenchymal stem cells (ADSCs) isolated from 6 donors were conventionally identified using flow cytometry and multilineage differentiation assay. Cells were induced into tenogenic pathway by the treatment with BMP-12 (50 or 100 ng/mL for 7 days). The effect of this treatment on ADSCs immunological properties (mixed lymphocyte reaction, MLR), proliferative potential (BrdU), viability and resistance to oxidative stress (MTT assay) or capacity to migrate (transwell assay) were performed in comparison to non-treated cells.

Results: Isolated cells displayed typical MSCs characteristics. The addition of ADSCs to the MLR significantly inhibited allo-activated lymphocytes proliferation ($p<0.01$). This inhibition was impaired when BMP-12 treated cells were used (54% and 35% depending on BMP12 dose in comparison to non-treated cells, $p\geq0.05$). Treatment of cells with BMP-12 did not affect significantly cells viability, resistance to oxidative stress, proliferative potential and migration capacity in comparison to the non-treated cells ($p>0.05$ in all tests).

Conclusions: Although our results suggest impairment of ADSCs immunomodulatory properties after BMP-12 treatement, partial differentiation of the cells may be beneficial for the tendinopathy treatment as most of cell clinically relevant features remains unchanged.

P93 Evaluation of consumption rate as a criteria for computational modeling of tissue organoid devepolment

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In vitro and in vivo organoid cultivation requires simultaneous provision of necessary vascularization and nutrients perfusion of cells during organoid development. However many aspects of this problem are still unsolved. Functionality of vascular network intergrowth is limited during early stages of organoid development since a function of the vascular network initiated on final stages of in vitro organoid cultivation. Therefore a microchannel network should be created in early stages of organoid

cultivation in hydrogel matrix aimed to conduct and maintain minimally required level of nutrients perfusion for all cells in the expanding organoid. The network configuration should be designed properly in order to exclude hypoxic and necrotic zones in expanding organoid at all stages of its cultivation.

We study the distribution of nutrient intake cells in a collagen gel in a scarce environment. It was set for 5 days without replacement of culture medium (unfed culture) for normoxia (21% oxygen) and for hypoxia (5% oxygen) conditions. We use enzymatic glucose-oxidase method for glucose measurements. Contraction was evaluated using free-floating fibroblast-populated collagen gel that contract by migratory forces. Type I collagen gel has been a major material extracellular matrix for 3-D culture systems and for investigating cell differentiation and maturation mimicking in vivo environment. Diffusion based mathematical model is used for perfusion evaluation under gel contraction and cells growth conditions.

In vitro vascularization is currently the main issue within the field of tissue engineering. As perfusion and oxygen transport have direct effects on cell viability and differentiation, researchers are currently limited only to a tissues of few millimeters in thickness. These limitations are imposed by mass transfer and are defined by the balance between the metabolic demand of the cellular components in the system and the size of the scaffold. Current approaches include growth factor delivery, channeled scaffolds, perfusion bioreactors, microfluidics, cell co-cultures, cell functionalization, modular assembly, and in vivo systems. These approaches may improve cell viability or generate capillary-like structures within a tissue construct. Thus, there is a fundamental disconnect between defining the metabolic needs of a tissue through quantitative measurements of oxygen and nutrient diffusion and the potential ease of integration into host vasculature for future in vivo implantation.

Preliminary mathematical background is analyzed capable for numerical estimates of the biotransplantat perfusion basing on the properties of matrix, cells, their initial placement and recipient tissue.

Plenary Lecture (L28) Bimodal signaling of biglycan in inflammation

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Biglycan, a ubiquitous proteoglycan, acts as a danger signal when released from the extracellular matrix. As such, biglycan triggers the synthesis and maturation of interleukin-1β (IL-1β) in a Toll-like receptor (TLR)-2-, TLR4-, and reactive oxygen species (ROS)-dependent manner. Here, we discovered that biglycan autonomously regulates the balance in IL-1β production *in vitro* and *in vivo* by modulating expression, activity and stability of NADPH oxidase (NOX) 1, 2 and 4 enzymes via different TLR pathways. In primary murine macrophages, biglycan triggered NOX1/4-mediated ROS generation, thereby enhancing IL-1β expression. Surprisingly, biglycan inhibited IL-1β due to enhancement of NOX2 synthesis and activation, by selectively interacting with TLR4. Synthesis of NOX2 was mediated by adaptor molecule Toll/IL-1R domain-containing adaptor inducing IFN-β (TRIF). Via myeloid differentiation primary response protein (MyD88) as well as Rac1 and Erk phosphorylation, biglycan triggered translocation of the cytosolic NOX2 subunit $p47^{phox}$ to the plasma membrane, an obligatory step for NOX2 activation. In contrast, by engaging TLR2, soluble biglycan stimulated the expression of heat shock protein (HSP) 70, which bound to NOX2, and consequently impaired the inhibitory function of NOX2 on IL-1β expression. Notably, a genetic background lacking biglycan reduced HSP70 expression, rescued the enhanced renal IL-1β production and improved kidney function of *Nox2*$^{-/y}$ mice in a model of renal ischemic-reperfusion injury. Here, we provide a novel mechanism where the danger molecule biglycan influences NOX2 synthesis and activation via different TLR pathways, thereby regulating inflammation severity. Thus, selective inhibition of biglycan-TLR2- or biglycan-TLR4 signaling could be a novel therapeutic approach in ROS-mediated inflammatory diseases.

Dick Heinegard European Young Investigator Award Presentations

ST32 Syndecan-4 is a key modulator of epithelial-to-mesenchymal transition in breast cancer cells

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

calcium levels. These changes of intracellular calcium seem to be regulated by the interaction of syndecan-4 with transient receptor potential channels (TRPCs). Moreover, the substitution on syndecan's-4 action after point mutation on specific TRPC causes epithelial-to-mesenchymal transition (EMT) on MCF-7 breast cancer cells. So the differences on calcium levels contribute significantly to cell cytoskeleton rearrangement. The ongoing study characterizes syndecans as key regulators of cell phenotype, highlighting their involvement in EMT and/or MET behavior of cancer cells, and potential regulatory role in breast cancer progression.

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

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ST33 Reduced insulin/IGF-1-signalling implicates extracellular matrix remodelling in longevity

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Interventions that delay ageing mobilize mechanisms that protect and repair cellular components, but it is unknown how these interventions might slow the functional decline of extracellular matrices, which are also damaged during ageing. Reduced insulin/IGF-1 signalling (rIIS) extends lifespan across the evolutionary spectrum. Here we show that rIIS can promote *C. elegans* longevity through a program that requires the Nrf (NF-E2-related factor) orthologue SKN-1 acting in parallel to DAF-16. SKN-1 is inhibited by IIS and has been broadly implicated in longevity. When IIS is decreased, SKN-1 most prominently increases expression of collagens and other extracellular matrix genes. Diverse genetic, nutritional, and pharmacological pro-longevity interventions delay an age-related decline in collagen expression. These collagens mediate adulthood extracellular matrix remodelling, and are needed for ageing to be delayed by interventions. The importance of collagen production in diverse anti-ageing interventions implies that extracellular matrix remodelling is a generally essential signature of longevity assurance, and that agents promoting extracellular matrix youthfulness may have systemic benefit.

ST34 Integrative analysis of multiple integrin adhesion complex proteomes defines a core consensus adhesome and reveals how it might work

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Cell adhesion to the extracellular matrix is mediated by the integrin family of adhesion receptors. Integrin receptor engagement initiates the formation of multimolecular protein complexes, termed integrin adhesion complexes (IACs), at the cell membrane. IACs are complex signalling hubs that are enriched in tyrosine-based phosphorylation events and form a mechanochemical connection between integrin receptors and the actin cytoskeleton. Proteomic analyses of isolated IACs have revealed an

unanticipated molecular complexity; however, a global view of the consensus composition of IACs, and a description of how the complex network of interactions in IACs influences global cell function, is currently lacking. Here, we have integrated several IAC proteomes and generated a 2,412-protein 'meta-adhesome' database. Analysis of this dataset reveals diverse functional classes of proteins in IACs and establishes a consensus adhesome of 60 frequently identified proteins. The consensus adhesome likely represents a core cell adhesion machinery, centered around four axes that link integrins to actin comprising ILK-PINCH-kindlin, FAK-paxillin, talin-vinculin and α-actinin-zyxin-VASP, and includes underappreciated IAC components. To investigate the robustness of this IAC network, the effects of pharmacological perturbation of the key IAC kinases focal adhesion kinase (FAK) and Src on IACs were examined. IAC composition was insensitive to inhibition of FAK and/or Src. In contrast, phosphorylation of IAC proteins was substantially reduced by kinase inhibition. Furthermore using fluorescence recovery after photobleaching, we found that FAK inhibition increased the exchange rate of a phosphotyrosine (pY) reporter (dSH2) at IACs. We conclude that the IAC is a stable structural connection, linking integrins to actin, which is nonetheless able to relay signals to functional end-points via phosphorylation. Current studies are focused on the role of cell-matrix interactions during cancer progression.

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ST35 Matricellular TSP-1 as a target of interest for facing tumor progression: towards a therapeutic use for TAX2 peptide

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Although thrombospondin-1 (TSP-1) contribution to tumor progression is intricate due to its pleiotropic nature, TSP-1 is widely recognized as a key actor within tumor microenvironment and therefore represents a target of interest for the development of innovative anti-cancer therapy. Recent multiscale work starting from molecular modeling to *in vitro* validation led us to identify an innovative cyclic peptide, named TAX2, acting as an orthosteric antagonist for TSP-1:CD47 interaction. While competing with CD47 for TSP-1 binding, TAX2 results in a CD36-dependent anti-angiogenic effect by increasing TSP-1 bioavailability for CD36 engagement. We previously demonstrated using syngeneic melanoma models that TAX2 treatment of immunocompetent mice disturbs subcutaneous allografts vascularization and inhibits lung metastases development and growth. Adoptive transfer experiments of xenogenic A375 human melanoma tumors into nude mice revealed that TAX2-treated tumors are less infiltrative, together with a reduction in the number of tumor-infiltrating blood vessels and a decrease in blood flow which are comparable to those obtained with the positive control bevacizumab. In order to confirm the proof-of-concept of TAX2 anticancer properties, a comprehensive systems biology approach was conducted combining multiple genomics databases mining and human tissue microarray analyses in the purpose of identifying human tumors that overexpress TAX2 molecular targets. Therefore, we sought to identify TAX2 effects considering human MIA-PaCa-2 pancreatic carcinoma, SK-N-BE(2) and SK-N-SH neuroblastoma as well as A2780 and SK-OV-3 ovarian carcinoma xenografts. In these models, TAX2 systemic administrations (10 mg/kg/day range) restricted tumor growth by at least 2-fold while concomitantly increasing mice survival. ADME evaluation of TAX2 and pharmacokinetic studies in SD rats revealed suitable stability, absence of TAX2-related enzyme inhibition and absence of cardiac toxicity. *In vivo* toxicity assessment highlighted that TAX2 administered at up to 400 mg/kg showed no measurable toxicity when looking at BW gain, food consumption, clinical signs, clinical pathology (clinical chemistry and hematology), gross pathology, organs weights and histopathology. Of note,

TAX2 administration at biologically active doses does not significantly alter coagulation parameters, platelet counts or bleeding time. Altogether, these preclinical results suggest that TAX2 may represent a valuable therapeutic alternative for facing tumor progression and spreading.

Nominated by the French Society (SFBMEc) for the Dick Heinegard European Young Investigation Award.

ST36 Mesenchymal Progenitor Cell Chondrogenesis, Hormones, and Neuronal Pathways – a Role of G Protein-Coupled Receptors

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During the last few decades first evidence emerged that besides aging, genetics, inflammation, or trauma also endocrine and sympathetic nervous factors influence cartilage homeostasis, regeneration capacity, and osteoarthritis (OA) development. Estrogen (E2), for instance, crucial for normal longitudinal growth and growth plate fusion (1) plays an important role in postmenopausal OA manifestation (2). Similarly, the sympathetic neurotransmitter norepinephrine (NE) has been shown to mediate a number of effects on chondrocyte growth and differentiation (3).
Recently, we analyzed the effects of those neuroendocrine factors, E2 and NE, on the chondrogenic differentiation potential of non-OA and OA-cartilage progenitor cells. Mesenchymal stem cells (MSCs) derived from non-OA trauma patients underwent chondrogenic differentiation in 3D pellet cultures. In MSCs treated with high E2 concentrations chondrogenic extracellular matrix deposition was significantly inhibited, whereas hypertrophic markers were increased (4). Further experiments using specific inhibitors of classical E2-receptors (ERα and ERβ) demonstrated that these receptors are not involved in the observed inhibition of chondrogenesis. Since GPR30, a G protein-coupled receptor (GPCR), has been recently shown to mediate E2 effects, we analyzed the effects of G1 (GPR30 agonist) and G15 (GPR30 antagonist) on chondrgenic differentiation as next. Chondrogenesis was disturbed by G1 similarly to the findings with NE treatment and this effect was significantly reversed by G15. Our results demonstrated for the first time that GPR30 signaling is responsible for the inhibitory effect of E2 on chondrogenesis (4).
After detecting relevant amounts of NE in the synovial fluid, also acting via GPCRs, we analyzed noradrenergic influences on chondroprogenitor differentiation in MSC and OA-chondroprogenitor (CPC) pellet cultures. In high concentrations, NE inhibited chondrogenesis and increased the expression of hypertrophic markers in both MSC and CPC cultures suggesting that β2-adrenergic receptor (β2AR) signaling is involved in these effects (5). In further experiments using specific β2AR agonists and antagonists, we confirmed that inhibition of chondrogenesis and concomitantly accelerated hypertrophy is mediated by β2AR signaling (5). Detailed signaling pathways need to be examined in future experiments.
Taken together, our studies confirm the importance of neuroendocrine factors in progenitor cell chondrogenic function and therefore in cartilage regeneration processes and provide new insights into OA pathophysiology by connecting the neuroendocrine domain to existing OA research areas. Future studies might unravel the potential of novel neuroendocrine-chondrogenic therapeutic options for OA treatment.
References:
1 Karimian E et al. Estrogen signaling in growth plate cartilage. Endocr Dev. 2011 21:42-51.
2 Martín-Millán M et al. Estrogens, osteoarthritis and inflammation. Joint Bone Spine, 2013 80(4):368-73.

3 Grässel SG. The role of peripheral nerve fibers and their neurotransmitters in cartilage and bone phisiology and pathophysiology. Arthritis Res Ther. 2014 16(6):485
4 Jenei-Lanzl Z. et al. Estradiol inhibits chondrogenic differentiation of mesenchymal stem cells via nonclassic signaling. Arthritis Rheum. 2010 62(4):1088-96
5 Jenei-Lanzl Z. et al. Norepinephrine inhibition of mesenchymal stem cell and chondrogenic progenitor cell chondrogenesis and acceleration of chondrogenic hypertrophy. Arthritis Rheumatol. 2014 66(9): 2472-81.

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ST37 Animal models of Desbuquois Dysplasia type 1 demonstrate CANT1 role in proteoglycan metabolism

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Desbuquois dysplasia (DBQD) is a rare recessive chondrodysplasia, characterized by growth retardation, multiple dislocations and advanced carpal ossification. Two forms of DBQD have been described on the basis of the presence (type 1) or absence (type 2) of characteristic hand anomalies. DBQD type 1 is caused by mutations in the *Calcium-Activated Nucleotidase 1* gene (*CANT1*), while DBQD type 2 is caused by mutations in the *xylosiltransferase 1* (*XYLT1*) gene.
CANT1 is a nucleotidase of the ER/Golgi that hydrolyzes UDP, suggesting its involvement in protein glycosylation; for this reason its role in proteoglycan metabolism has been hypothesized.
To better characterize CANT1 role in the etiology of DBQD, we generated a *Cant1* knock-in mouse carrying the R302H substitution reproducing the R300H mutation detected in patients. Morphometric analyses demonstrated that mutant mice are smaller with shorter and thinner tibiae, femurs and ilia compared to wild type animals. Limb extremities of KI mice reproduced the hand anomalies described in patients: additional carpal ossification centers and the delta phalanx. Thus the KI mouse develops a skeletal phenotype reminiscent of DBQD type 1. To better investigate CANT1 role in proteoglycan (PG) synthesis we generated a *Cant1* knock out mouse by excision of exon 3 and 4. The KO mouse showed the same growth defects and hand anomalies of patients already observed in the KI mouse. To study PG synthesis, rib chondrocytes were metabolically labeled with ^{35}S-sulfate and the amount of newly synthesized PGs was evaluated. KO cells showed reduced PG synthesis compared to wild types both in presence and in absence of β-D-xyloside, an enhancer of glycosaminoglycan (GAG) synthesis. Gel filtration chromatography of GAGs released from newly synthesized PGs after β-elimination demonstrated that the hydrodynamic size of GAG chains was reduced in KO chondrocytes compared to the controls. Ultrastructural analysis of KO and wild type cartilage and cultured chondrocytes by TEM demonstrated the presence of dilated vacuoli containing electrondense proteinaceus material suggesting a role of CANT1 in protein secretion. Pulse-chase labeling of cells with ^{35}S-sulfate demonstrated reduced PG secretion in mutant cells compared to the controls.
In conclusion we generated and validated two different mouse models for the study of DBQD type 1 and we demonstrated that CANT1 play a role in PG synthesis.

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Nominated by the Italian Society for the Study of Connective Tissue for the Dick Heinegard European Young Investigation Award.

Plenary Lecture (L29) Genetic analysis of integrin signalling in mice

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Integrins are α/β heterodimeric receptors that mediate cell-extracellular matrix (ECM) and cell-cell interactions. In order to interact with their ligand, integrins must shift from inactive to active conformations. This conformational switch is induced by intracellular signals (inside out signalling). The consequence of integrin ligation is the attachment of the contractile cytoskeleton to the plasma membrane, the clustering of integrins and recruitment of cytoskeletal and signalling molecules into focal adhesion sites (FAs), and finally the activation of various intracellular signalling pathways (outside-in signalling). Since integrin cytoplasmic domains lack both an actin-binding site as well as enzymatic activity, all integrin-mediated effects are accomplished by integrin-associated molecules. At the meeting I will discuss the *in vivo* consequences caused by mutating integrin tail-associated proteins, and how these studies helped understanding the way integrins work.

Plenary Lecture (L30) Next Generation Heparin Therapeutics: Targeting Proteoglycan Functions

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Heparan sulfate proteoglycans are complex sulfated glycoproteins present on almost all cell surfaces in multicellular organisms. They have variant HS chain structures which represent a molecular code that is a subset of the glycome called the ‘heparanome’. These sulfation patterns confer the ability to interact selectively with a wide interactome of proteins, especially growth factors and matrix proteins, that influence many cellular processes important in cell development and regulation. Importantly, they act as “master regulators” which co-ordinate many cellular processes relevant to disease processes. We have been using chemical biology tools and glycomics approaches to decode the molecular basis of the functional diversity of HS. This has yielded new insights into this code in a variety of biological contexts, including neurodegeneration (AD), cancer matastasis and regulation of cancer stem cells. In order to undertake translational development we have also developed synthetic chemistry and glycomimetic routes to novel heparin compounds, resulting in discovery of drug leads for Alzheimers disease and cancer.

Workshop 7: Cell/matrix interactions in matrix biology and pathology

Invited Lecture (L31) CD44 is critical for TLR Activation of the Inflammasome and the Evolution of Lung Injury: From Mice to Man

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Background: Exposure of neonatal mice to hyperoxia results in increased IL1ß, TGFß, and inflammation, in association with impaired alveolarization, features of Bronchopulmonary Dysplasia (BPD). The NLRP3 inflammasome, an intracellular complex that activates IL1ß, is a critical contributor to the development of BPD. Hyaluronan (HA) stimulates the expression of IL1ß and CD44 is necessary for both IL1ß and TGFß activation and signaling *in vitro*.
Objective: We hypothesized that neonatal CD44 knockout mice would be protected from hyperoxia-induced BPD phenotype and that CD44 would be elevated in a preterm baboon model of BPD and in the lungs of preterm infants with BPD.
Design/Methods: Neonatal WT and *Cd44*$^{-/-}$ mice were exposed to either 21% or 85% O_2 from PN3 to PN15. Lungs were harvested on PN15. Lung morphometry was determined by radial alveolar count (RAC). Expression of IL1ß, TGFß, the TGFß target gene PAI1, and CD44 were determined by qRT-PCR. Myeloperoxidase (MPO) and N-Acetyl Glucosaminidase (NAG) activities of lavage cells were determined to assess neutrophil and macrophage influx respectively. CD44 expression was determined in lung samples obtained from baboons delivered preterm and ventilated in a neonatal intensive care unit and compared to gestational age controls, as well as RNA from paraffin-embedded lung sections of infants that had died of BPD, compared to infants that had died early from extreme prematurity, or term stillborn infants. The role of CD44 in TLR4 signaling was examined in bone marrow-derived macrophages (BMDM) obtained from WT and *Cd44*$^{-/-}$ mice.
Results: In WT mice kept in 21% O_2 (controls), expression of TGFß and CD44 decreased over the first two weeks of life. By PN15, exposure of WT mice to 85% O_2 from PN3 to PN15 resulted in decreased alveolarization (RAC 50% of 21% O_2 mice), and increased expression of CD44 (5-fold), TGFß (10-fold), and PAI1 (30-fold). Compared to WT mice, CD44 KO mice exposed to 85% O_2 had normal distal lung architecture, with normal RAC (P<0.001), and also had decreased IL1ß, TGFß1, and PAI1 (P<0.01). Compared to gestational age controls, lungs obtained from ventilated preterm baboons had increased CD44 expression. Further, compared to controls, human infants that died with BPD had 6-fold higher CD44 mRNA (P < 0.05, n=4/group). Interestingly, cultured BMDM from *Cd44*$^{-/-}$ mice failed to signal LPS-stimulated IL1ß production.
Conclusions: We conclude that CD44 is necessary for TLR4 activation of the NLRP3 inflammasome and loss of CD44 protects neonatal mice from hyperoxia-induced lung injury. Both ventilated preterm baboon and human infants with BPD have increased CD44 expression. CD44 is a novel therapeutic target for TLR4-mediated diseases.

Invited Lecture (L32) Laminin a2 chain-deficient muscular dystrophy: pathogenesis and development of treatment

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Laminin-211 is major constituent of the skeletal muscle basement membrane. It stabilizes skeletal muscle and influences signal transduction events from the myomatrix to the muscle cell. Mutations in the gene encoding the a2 chain of laminin-211 leads to congenital muscular dystrophy type 1A (MDC1A), a life-threatening disease characterized by severe hypotonia, progressive muscle weakness and joint contractures. Common complications include severely impaired motor abilities, respiratory

failure and feeding difficulties. Using adequate animal models and patient muscle cells, we have identified important disease driving mechanisms and developed novel therapeutic approaches for laminin a2 chain-deficient muscular dystrophy.
We have previously demonstrated that transgenically expressed laminin a1 chain significantly ameliorates muscular dystrophy and reduces peripheral neuropathy and cardiomyopathy in laminin a2 chain-deficient dy^{3K}/dy^{3K} mice. We have also shown autophagy dysfunction and increased proteasome activity in MDC1A muscle cells. Accordingly, separate treatment with proteasome and autophagy inhibitors significantly improved the dystrophic phenotype of dy^{3K}/dy^{3K} mice. More recently, we have performed comparative proteomic analyses of affected muscles from dy^{3K}/dy^{3K} mice in order to obtain new insights into the molecular mechanisms underlying MDC1A. A large number of differentially expressed proteins in diseased compared to normal muscles were identified. A majority of the downregulated proteins were involved in different metabolic processes and mitochondrial metabolism while upregulated proteins were related to inflammation and fibrosis. These data imply that metabolic alterations could be novel mechanisms that underlie MDC1A and might be targets that should be explored for therapy. Indeed, new data indicate reduced mitochondrial respiration and enhanced glycolysis in laminin a2 chain-deficient muscle cells. Finally, preliminary data show that treatment with metformin has beneficial effects in laminin a2 chain-deficient mice.

Invited Lecture (L33) Osteogenesis Imperfecta: not only an extracellular matrix disease

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Osteogenesis imperfecta (OI), also known as brittle bone disease, is a heritable skeletal dysplasia characterized by bone fragility, frequent fractures and short stature. In its classical form it is caused by dominant mutations in the collagen type I coding genes, COL1A1 and COL1A2. Defects in other proteins involved in collagen type I synthesis, post translational modification, maturation and secretion as well as in osteoblasts differentiation had been more recently described as causative for the disease. The bone phenotype of OI patients was traditionally attributed to the presence of altered collagen type I in the bone extracellular matrix, but it is now becoming clear that for OI, as well as for other skeletal dysplasia, a cellular function impairment, due to mutant protein retention, may have an effect on patients outcome. By using the OI murine model Brtl, carrying a typical glycine substitution in one α1 chain of collagen type I and a moderately severe or lethal OI phenotype, we demonstrated that the severity of the disease could be modulated by a different ability of bone as well as of skin cells to cope with the stress caused by mutant collagen retained in the endoplasmic reticulum. Furthermore an abnormal cytoskeleton organization in mice with lethal outcome was evident in different tissues including calvarial and long bone, affecting osteoblast proliferation, collagen deposition and altering the signaling of TGFβ, one of the growth factor involved in bone development. Interestingly a more severely compromised cytoskeleton assembly was detected in fibroblasts obtained from lethal, but not from non lethal, OI patients carrying an identical glycine substitution either with the same or a different amino acid. All these data supported that a compromised cellular functionality can affect both cell signaling and cellular trafficking in mutant lethal mice, altering bone properties.
Furthermore mutant Brtl bone marrow progenitor cells ability to differentiate toward osteoblasts as well as mineralization of differentiated mutant osteoblasts were reduced with respect control cells. In mutant mesenchymal stem cells (MSCs), the expression of early and late osteoblastic markers was significantly reduced with respect to wild type. Conversely, mutant MSCs generated more colony-

forming unit-adipocytes compared to WT, with more adipocytes per colony, and increased number and size of triglyceride drops per cell.
We demonstrated that the reduction of mutant collagen synthesis by allele specific shRNA relieved cellular stress *in vitro* and the stimulation of MSCs by bortezomib ameliorated osteoblast differentiation *in vitro* and bone properties *in vivo*. Thus many evidences suggest an involvement of intracellular events in modulating OI phenotype and cellular stress seems to be an appealing new pharmacological target for OI.

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ST38/P94 A potent cell adhesive peptide from tropoelastin that mediates attachment to both integrins alphaVbeta5 and alphaVb3

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It is important to know how integrins interact with the extracellular matrix, as these interactions mediate processes such as alterations in cell morphology, migration, proliferation and differentiation (1). For many years it was not known that tropoelastin binds integrin ligands because it does not contain the classic integrin-binding RGD sequence. As a result, we were surprised to find that integrin $\alpha_V\beta_3$ recognizes the extreme C-terminal RKRK motif of human tropoelastin (2). However, this interaction does not account for the full cell-binding activity of tropoelastin. More recently, to help understand this additional binding, we examined the cell interaction performance of tropoelastin constructs, with the goal of identifying a new cell binding site in tropoelastin and the major receptor involved in this novel region. We probed cell interactions with tropoelastin by deleting the C-terminal cell binding sequence in order to unmask these other cell binding interactions. Through this path, we found a novel site on tropoelastin responsible for dermal fibroblast attachment and spreading. This site is located centrally in the molecule (3, 4). Inhibition studies demonstrate that this cell adhesion is not mediated by either the traditional elastin binding protein or by glycosaminoglycans. Cell interactions are divalent cation-dependent, which is expected for integrin dependence.
We have now narrowed down the precise sequence responsible for these cell interactions. A peptide made to a region of tropoelastin is powerful: it inhibits cells interacting with tropoelastin constructs in solution, and it can independently bind to cells to promote cell spreading. Through the mutation of amino acid residues in the peptide sequence, we have identified key amino acids that are crucial in driving this tropoelastin-cell interaction. Functional blocking with monoclonal antibodies reveals that α_V- integrin(s), and specifically integrin $\alpha_V\beta_5$ and integrin $\alpha_V\beta_3$, are critical for cell adhesion to this peptide. We have discovered a common α_V integrin binding theme for tropoelastin: $\alpha_V\beta_3$ at the C-terminus and both $\alpha_V\beta_5$ and $\alpha_V\beta_3$ in the central region of tropoelastin. Each α_V binding region contributes to cell attachment and spreading but they differ in their effects on cytoskeletal assembly.
References:
1. Bridgen D.T., Gilchrist C.L., Richardson W.J., Isaacs R.E., Brown C.R., Yang K.L., Chen J., and Setton L.A. (2013) Integrin-mediated interactions with extracellular matrix proteins for nucleus pulposus cells of the human intervertebral disc. J. Orthop. Res. 31, 1661-67.
2 Bax, D.V., Rodgers, U.R., Bilek, M.M. and Weiss, A.S. (2009) Cell adhesion to tropoelastin is mediated via the C-terminal GRKRK motif and integrin alphaVbeta3. J. Biol. Chem. 284, 28616-23.
3. Lee, P., Bax, D.V., Bilek, M.M. and Weiss, A.S. (2014) A novel cell adhesion region in tropoelastin that mediates attachment to integrin alphaVbeta5. J. Biol. Chem. 17, 1467-77.
4. Yeo, G.C., Aghaei Ghareh Bolagh, B., Brackenreg, E.P., Hiob, M.A., Lee, P. and Weiss, A.S. (2015). Fabricated elastin. Advanced Healthcare Materials, 4, 2530-56.

ST39/P95 Inhibition of a2b1 integrin by TC-I15 increases collagen accumulation by cultured fibroblasts isolated from the heart atrium of patients with aortic stenosis

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Disturbances of extracellular matrix metabolism may result in changes of heart chambers shape and heart failure development. Enlargement of atria and fibrosis of atrial muscle are the risk factors of atrial fibrillation. The aim of the study was to test hypothesis that a2b1 integrin is involved in regulation of the extracellular matrix accumulation in the heart atrium. Thus, the effect of TC-I15 the a2b1 integrin inhibitor on accumulation of collagen and glycosaminoglycans in human fibroblasts cultures was examined.
The fibroblast were isolated from the heart atrium of patients with aortic stenosis. By using flow cytometry an expression of integrin subunits (a1, a2, a10, a11 a and b1) was investigated. The cells were treated with the a2b1 integrin inhibitor TC-I15 at concentrations of 10^{-5}M, 10^{-6} M and 10^{-7} M. The intracellular collagen content and glycosaminoglycans level in the culture were measured.
Obtained results confirmed the expression of all subunits of integrin binding to collagen on fibroblasts derived from the human heart atrium. Application of the a2b1 integrin inhibitor increases intracellular collagen content in the fibroblasts culture. This effect was found when TC-I15 was applied at concentrations of 10^{-5}M, 10^{-6} M, whereas the lower applied concentration was not effective (10^{-7} M).
Conclusion: The inhibition of a2b1 integrin increases intracellular collagen content in the human fibroblasts cell culture. The present data supports hypothesis of the involvement of a2b1 integrin in regulation of collagen accumulation in the heart fibroblasts.

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ST40/P96 Talin 1 dysfunction is associated with the Systemic Capillary Leak Syndrome

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The importance of integrin-mediated cell-matrix adhesion adhesion in controlling endothelial cell function and blood vessel morphogenesis is well established. Talin is a key regulator of integrin function. We have previously shown that Talin is indispensable for embryonic angiogenesis. Our current data demonstrates an important role for Talin1 in regulating capillary function. Along with collaborators, we have identified and characterized the first familiar case for Systemic Capillary Leak Syndrome (SCLS), which is a rare medical condition manifested by acute life-threatening episodes of increased capillary permeability, leading to plasma extravasation, edema, vascular collapse and hypotensive shock. Exome sequencing revealed a heterozygous point mutation in TLN1 gene that generates a truncated mutant form of Talin1 protein. To investigate the involvement of Talin1 in this vascular pathology, we generated an in vitro model of SCLS disease using primary endothelial cells, isolated from endothelial-specific Talin1 heterozygous mice, transfected with Talin constructs carrying relevant mutations. Our data supports a critical contribution of Talin1 C-terminal region in the pathology of SCLS.

ST41/P97 The long non-coding RNA HAS2-AS1 is a new regulator of breast cancer cells invasiveness

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Hyaluronan is a ubiquitous glycosaminoglycan of extracellular matrix important for tissue homeostasis and development that can regulate different cellular behaviours like adhesion, motility, growth and inflammation. Its presence is critical in tumor microenvironment, where the up-regulation of HAS2 and the overproduction of HA are often associated with tumor 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 (HA) in different pathologies. HAS2-AS1 is a long-non coding RNA (lncRNA) transcribed in the opposite strand of HAS2 gene on chromosome 8. It has an alternative splicing site which generates two RNA isoforms of different lengths (HAS2-AS1 long and HAS2-AS1 short), that have 257 or 174 nucleotides of perfect complementary sequence to the first exon of HAS2, respectively. LncRNAs play important roles in cancer, like chromatin remodeling, as well as transcriptional and post-transcriptional regulation, through a variety of chromatin-based mechanisms and the interaction with other RNA species. Here we show that the knockdown of HAS2-AS1 in the aggressive triple negative breast cancer cells (MDA-MB-231) increases proliferation respect to the estrogen receptor (ER) positive breast cancer cell line MCF-7. Furthermore, we report that the silencing of HAS2-AS1 in MDA-MB-231 is linked to a higher migration and invasion rate. Moreover, quantitative PCR analysis reveals that the abrogation of HAS2-AS1 brings to higher levels of HAS2, HAS3, CD44 and hyaluronidase 2 (HYAL2) mRNA, suggesting a possible role of HAS2 antisense in tumor progression. Since little is known about HAS2-AS1, in our future experiments we want to elucidate the cellular pathway linked to its abrogation and to study how it is involved in tumor development.

ST42/P98 The role of Heparanase in chronic liver disease fibrogenesis

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Background/Aims: Chronic liver injury of any etiology is characterized by progressive fibrosis which culminates in nodules formation and cirrhosis. Heparanase (HPSE) is an endoglycosidase that participates in the remodelling of extracellular matrix and basement membranes by the cleavage of heparan sulfate chains of proteoglycans. Although the role of HPSE in the establishment of kidney fibrosis is well documented, its involvement in liver fibrosis is unknown. Therefore, we aimed to measure circulating plasma levels of HPSE in patients with different stages of chronic liver diseases. Moreover a mouse model of chronic liver injury (CCl4)-induced was developed to better understand the role of HPSE in liver fibrogenesis.
Materials and methods: HPSE plasma activity was measured in the plasma from 13 healthy individuals, 33 patients diagnosed with different autoimmune chronic liver diseases and 35 patients with chronic viral hepatitis. HPSE plasma activity was correlated to fibrosis stage assessed by transient elastography (Fibroscan). Male Balb/cJ mice were intraperitoneally injected with CCl_4 in olive oil twice a week for 1, 2, 8 and 12 weeks. Control group received no treatment. H-E and Azan-Mallory stainings were performed to assess liver histopathological changes and fibrosis. Real time RT-PCR, Western blot and immunofluorescence were performed to evaluate HPSE expression. Results: Both in human chronic autoimmune liver diseases and in viral hepatitis, HPSE plasma activity was significantly higher in patients with mild, significant and severe fibrosis compared to healthy control group. However, there was no significant difference in patients with cirrhosis. Moreover, HPSE plasma activity negatively

correlated with liver stiffness. Following 1 and 2 weeks of CCl_4 treatment, murine liver tissue showed centrolobular necrosis with extensive inflammatory cells infiltration and mild central fibrosis. Moderate cell infiltration and micronodular cirrhosis were observed after 8 and 12 weeks of CCl4 treatment. HPSE protein and mRNA levels were significantly up-regulated in murine liver tissue after 1 and 2 weeks of CCl_4 exposure. However, HPSE expression progressively decreased to equal control mice levels after 8 and 12 weeks of treatment. While no detectable HPSE staining was evident in control murine livers, immunofluorescence assay revealed a strong immunopositivity for HPSE after 1 and 2 weeks but not after 8 and 12 weeks of CCl_4 administration. Moreover, HPSE protein was restricted to centrolobular necrotic area and it co-localized with F4/80, a macrophage marker.
Conclusions: *ex vivo* and *in vivo* data indicated an up-regulation of HPSE in early chronic liver disease suggesting its possible involvement in the initial phase of fibrotic process. Co-localization studies suggested that inflammatory activated macrophages could be a relevant source of HPSE in the injured liver.

P99 Novel biochemical insight into T190M and C667F primary dystroglycanopathies

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Dystroglycan (DG) is a transmembrane complex expressed both in skeletal and cardiac muscle as well as in epithelial tissue and in central and peripheral nervous system. DG links extracellular matrix proteins, such as laminin, to the cytoskeleton and it is composed by two subunits, α-DG and β-DG, derived from a protein precursor of 895 amino acids in humans [1]. The maturation of DG takes place in the endoplasmic reticulum, where the two subunits are liberated, and subsequently within the Golgi apparatus where the mucin-like domain of α-DG is extensively decorated with sugars. In particular, a-DG harbors O-mannosylated-glycans and several mutations of the enzymes that catalyse their association to α-DG cause a number of muscular dystrophy phenotypes, collectively known as secondary dystroglycanopathies [1]. Conversely, mutations associated to primary defects of DG gene are still very few. Among these, we focused our attention on the murine counterparts (T190M and C667F) of two human mutants, namely T192M and C669F, associated respectively to a limb-girdle muscular dystrophy (LGMD2P) and to a Muscle-Eye-Brain disease-like phenotype [2, 3]. To better characterize the biochemical behavior of these mutants, Western blot assay, confocal microscopy and fluorescence recovery after photobleaching (FRAP) analysis were used. The T190M mutant is characterized by a lower degree of glycosylation that severely reduces the a-DG ability to bind laminin [2]. We have analyzed the influence that hypoglycosylation of a-DG might play on the membrane dynamics of b-DG and on filopodia formation. We found that an altered a-DG glycosylation reduces laminin binding affinity, enhancing the b-DG membrane dynamics, and affects the cytoskeletal architecture. The C667F mutation hits the extracellular domain of b-DG, inhibiting the formation of a disulfide bridge [3, 4]. Western blot analysis of total protein extracts from transfected EBNA cells showed that the mutation partially inhibits the post-translational cleavage of the DG precursor. Moreover, confocal microscopy experiments show that the mutant is mostly engulfed in the endoplasmic reticulum. Although more extensive analyses are necessary to better understand the pathological effects induced by the C667F mutation, our data suggest a novel intracellular mechanism behind this dystroglycanopathy.

Bibliography:
1. Bozzi et al. (2009) Functional diversity of dystroglycan. Matrix Biol.; 28 :179-187
2. Hara et al. (2011) A dystroglycan mutation associated with limb-girdle muscular dystrophy. N. Engl. J. Med.; 364: 939–946
3. Geis et al. (2013) Homozygous dystroglycan mutation associated with a novel muscle–eye–brain disease-like phenotype with multicystic leucodystrophy. Neurogenetics; 14: 205-213
4. Sciandra et al. (2012) Dystroglycan is associated to the disulfide isomerase ERp57. Experimental cell research; 318: 2460-2469

P100 Interactions of Discoidin Domain Receptors with fibrillar and non-fibrillar collagen forms: ligand binding versus receptor activation

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The Discoidin Domain Receptors (DDRs) are proteins of the Receptor Tyrosine Kinase (RTK) family. There are two DDR members, DDR1 and DDR2, which are unique RTKs in the sense that they are activated by a component of the extracellular matrix, collagen, whereas typical RTKs are activated by small diffusible proteins such as growth factors. The DDRs control fundamental functions in cells such as proliferation, differentiation, and migration. They fulfill very important roles in development, DDR1 is essential for mammary gland development whereas DRR2 controls bone growth. Both DDRs are drug targets for a number of diseases ranging from fibrotic diseases, arthritis and atherosclerosis to cancer. Previous literature has shown that the DDRs can bind to monomeric collagen (i.e. collagen as single triple helices), which induces receptor phosphorylation. It is not clear however, whether DDRs can get activated by the main tissue forms of collagens, fibrils or fibres.
In this project, we are testing DDR activation using native collagen I fibrils produced by fibroblast cultures and *in vitro* reconstituted collagen I fibrils. Our data show that both native and reconstituted fibrils can induce DDR activation, inducing phosphorylation signals with the same strength as single triple helices. Even though DDR1 activation by monomers is independent of β1 integrin function, interestingly we have seen that DDR1 activation induced by fibrils depends on β1 integrin function. Current and future experiments are aimed to test whether DDR activation by collagen I fibrils recruits different signalling molecules compared to DDR activation by monomeric collagen I and which cellular functions are regulated by DDRs when activated by collagen I fibrils.
A widely expressed collagen that has not been tested for its interactions with the DDRs is collagen VI. Collagen VI is initially secreted to the extracellular matrix as tetramers, where it forms its final structure, microfibrils. We tested DDR binding and activation using collagen VI from two different sources, skin and cornea. Our data show that full-length or triple-helical region of collagen VI tetramers and microfibrils bind to both DDRs, but can promote only DDR1 activation, not DDR2. Collagen I binding to DDR2 is not inhibited by collagen VI, hence DDR2 seems to have two different binding sites for collagens I and VI. Current experiments are designed to further explore the collagen VI binding site on DDR2.

P101 Molecular mechanisms of ANGPTL4-induced regulation of vascular integrity

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Ischemic injuries occur in various pathologies such as myocardial infarction, stroke and retinopathies. Deciphering the mechanisms involved in the regulation of vascular integrity is of major interest in order to develop relevant therapeutic vasoprotective approaches to achieve tissue protection. Our team identified angiopoietin-like 4 (ANGPTL4) as a hypoxia-induced target, accumulated in the extracellular matrix (ECM) by binding to heparan sulfate proteoglycans (Chomel et al. 2009). Furthermore, we demonstrated that ANGPTL4 is a key regulator of vascular integrity
by protecting inter-endothelial cell junctions (Galaup et al. 2012, Bouleti et al. 2013). These junctions are regulated by many proteins and complexes such as VE-cadherin and integrins. Since ANGPTL4 receptors and downstream signaling pathways, which mediate vasoprotective effect, remain poorly investigated, the present project aims to decipher the molecular pathway leading to ANGPTL4-mediated vascular integrity. Using *in vitro* binding (Surface Plasmon Resonance and Proximity Ligation Assay) we showed that ANGPTL4 bound αvβ3 integrin. Exposure to hypoxia of the endothelial cells increased this binding. Using functional *in vivo* assays, we showed that this interaction was necessary to mediate vasoprotective effects triggered by hypoxia. Mechanistically, binding of ANGPTL4 to αvβ3 led to Src recruitment and its sequestration away from VEGFR2 combined to a diminished Src signaling downstream VEGFR2, thereby inducing stabilization of both VEGFR2/VE-cadherin and VEGFR2/αvβ3 complexes. Thus, ANGPTL4 strengthened maturation of adherens junctions, involving VE-cadherin and tight junctions, involving ZO-1. Altogether, our results identify a novel mechanism by which ANGPTL4 counteracts hypoxia-driven vascular permeability through αvβ3 binding, modulation of VEGFR2/Src kinase signaling and endothelial junction stabilization.

P102 Insights into the structure of the N-terminal region of α-dystroglycan: a concerted crystallographic and SAXS study

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Dystroglycan (DG) is a glycoprotein complex that links the cytoskeleton with the extracellular matrix. DG is composed of two subunits: α-DG and β-DG. a-DG is a highly glycosylated extracellular protein, whereas β-DG is an integral membrane protein that also interacts with dystrophin in the cytoplasm. a-DG's glycosylation is essential for high-affinity binding of extracellular matrix proteins such as laminins. The hypoglycosylation of α-DG weakens this interaction affinity resulting in severe pathological states. The N-terminal region (a.a 50-313 in mouse) of α-DG plays a crucial role in the glycosylation of the α-DG mucin-like domain, being required by the glycosyltransferase LARGE during the extension of the O-glycans implicated in laminin binding. Furthermore, pathological missense mutations, observed at the N-terminal region of α-DG, are responsible of a-DG hypoglycosylation state. We have been investigating the structures of N-terminal region of the WT human α-DG (WT-hα-DG) and of the two point mutants V72I (V72I-mα-DG) and D109N (D109N-mα-DG) of mouse α-DG by X-ray crystallography, and in solution by Small Angle X-ray Scattering (SAXS). The purpose of this study is to gain evidences about the structural determinants of N-terminal α-DG that are functionally relevant for its glycosylation pathway. The crystal structure of the hα-DG does not significantly deviate from the already determined crystal structure of WT mouse α-DG (WT-mα-DG). The overall fold is conserved and differences are restricted to the most flexible part of the protein, i.e. the loop encompassing residues 159-179, which is only partially visible after crystal structure refinement. In addition, the crystal structures of the two mutants V72I-mα-DG and D109N-mα-DG display the same overall structure of WT-mα-DG, suggesting for negligible effects of the point mutations on the overall fold of α-DG. The two mutants show limited and local structural dissimilarities with respect to WT-mα-DG, probably influencing the interaction with potential binding partners. In contrast, the solution structural models

obtained by SAXS analysis depict a different scenario, where the WT-α-DG is quite flexible in solution, assuming more than one conformation, with the most populated close to the crystal conformation and a second less populated one, which is more extended with respect to the principal conformation. The comparison of SAXS data from WT-mα-DG and its mutants demonstrates the presence of a perturbation, in both the conformations as well as in the partition among different populations. SAXS data thus suggest a more complex and dynamical situation with respect to the crystallographic evidences that may have functional implications for DG post-translational processing and for the interactions with its binding partners.

P103 Identification of a common binding site for BMPs on TSG-6; a protein with endogenous protective effects in cartilage and bone

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Background: TSG-6 (Tumor Necrosis Factor-Stimulated Gene-6) is expressed during inflammation and is known to protect joint tissues from damage in mouse models of rheumatoid arthritis. The latter was attributed primarily to TSG-6's inhibition of neutrophil extravasation, which we have shown is mediated through its interaction with the chemokine CXCL8. Our recent work has revealed that the Link module of TSG-6 (Link_TSG6) acts directly on chondrocytes to inhibit cytokine-induced expression of matrix-degrading enzymes *in vitro*, and that it reduces cartilage breakdown both in human OA explant tissue and in a rat model of OA. Furthermore, Link_TSG6 inhibits osteoclast-mediated bone resorption *in vitro* and suppresses bone loss in a mouse model of osteoporosis. Thus, Link_TSG6 represents an attractive therapeutic target for musculoskeletal disorders. However, the mechanisms underlying its protective activities are not fully understood. TSG-6 is known to interact with at least seven BMPs, which are important mediators of bone and cartilage maintenance. The aim of this study was to fully characterise these protein-protein interactions and identify reagents to investigate their functional consequences.
Methods: Surface Plasmon Resonance (SPR) was used to study the interactions of full-length recombinant human (rh)TSG-6, Link_TSG6 (wild-type and mutants) and CUB_C_TSG6 (the isolated CUB_C domain of TSG-6) with BMP-2, BMP-7 and BMP-14 (GDF5), where the latter proteins were immobilized on C1 chips. Single-site mutants of Link_TSG6 were expressed in *E. coli* and 1D NMR showed that the mutant proteins have wild-type 3D structures.
Results: SPR, with single-cycle kinetic analyses, showed that rhTSG-6 binds tightly to BMP-2 (K_D ~7 nM) and BMP-7 (K_D ~3 nM); Link_TSG6 bound to the BMPs with similar affinities to the full-length protein, whilst CUB_C_TSG6 showed little/no binding. Analysis of a panel of Link_TSG6 mutants identified residues where mutation resulted in similar reductions or enhancements in the affinities for BMP-2, BMP-7 and BMP-14 and/or similarly altered the kinetics of these interactions.
Conclusions: This study has revealed that the high affinity interactions of TSG-6 with BMP-2 and BMP-7 are mediated via its Link module and has identified residues that likely contribute to a common BMP-binding surface on Link_TSG6. Mutants of Link_TSG6 with impaired/enhanced affinities for BMPs have been identified and work is on-going, using *in vitro* assays, to determine if/how modulation of these interactions influences the effects of Link_TSG6 on chondrocyte phenotype.

P104 Syndecans as key partners in the interaction between breast cancer cells and endothelium

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

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P105 Impact of substrate modulus of elasticity on human osteoblasts' differentiation and proliferation

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Introduction: Stiffness is a measure of the ability of a material to resist deformation. The influence of the substrate stiffness on human bone cells morphology, motility and differentiation has been known for decades and recently re-emphasized as an essential *in vivo* control parameter in cells biochemical signaling. In the body, tissue stiffness ranges several orders of magnitude, from adipose tissue (Young's Modulus E ~ several kPa) to bone (E ~ GPa). In order to evaluate the importance of substrate elasticity in biomaterial design, it is critical to test a wide variety of substrata that span physiologically relevant ranges of elasticity [1]. However, for valid results, the substrate chemistry and structure should be constant. In this context, traditional polymeric materials and composites, PMMA and glass ionomers, have been manufactured and the stiffness, of the order of GPa, influence on cells behavior has been investigated.

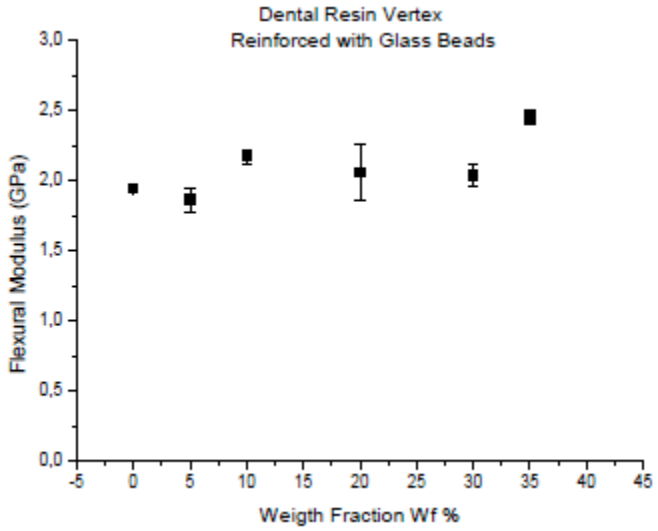

Fig. 1- Flexural modulus of elasticity as a function of filler weight fraction

Materials & Methods: Dental resin vertex repair material and dental resin vertex reinforced with glass beads of 120 μm diameter have been prepared. The elasticity modulus of both pure and reinforced materials has been investigated through 3-point bending tests. Human bone marrow stromal cells (hMSCs) obtained from patients undergoing hip surgery were used for the experiments. Sigma Aldrich detection kits were used to determine the Alkaline Phosphatase and the Total Protein Levels, as a measure of cell proliferation.

Results & Discussion: The dental resin was gradually reinforced with glass beads until the maximum fraction (35% weight volume fraction). The maximum modulus of elasticity was noticed at the maximum fraction of reinforcement (Fig. 1).
Thus, the pure dental resin with theminimum modulus and the one with 35% reinforcement and maximum modulus were compared. The hBMCs on each material expressed differentially both alkaline phosphatase activity and total protein content.
Conclusions: The modulus of elasticity of dental resin has been 30% increased due to the glass beads reinforcement. The reaction of cultured cells to different modulus of elasticity shows that differentiation and proliferation vary according to the mechanical properties of the substrate.
References:
1. M Ventre, F Causa, P Netti, J.R. Soc. Interface, 9, 2012, 2017-2032.
2. XQ Brown, K Ookawa, JY. Wong, Brain Research, 2005, 41–49.
3. WT Hsieh, YS Liu, YH Lee,et al., Acta Biomater, 2016, 32, 210-22.

P106 Ionizing radiation-mediated premature senescence and paracrine interactions with cancer cells enhance the expression of syndecan1 in human breast stromal fibroblasts: the role of TGF-β

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The cell surface proteoglycan syndecan1 (SDC1) is overexpressed in the malignant breast stromal fibroblasts, creating a favorable milieu for tumor cell growth. In the present study, we found that ionizing radiation, a well-established treatment in human breast cancer, provokes premature senescence of human breast stromal fibroblasts *in vitro*, as well as in the breast tissue *in vivo*. These senescent cells were found to overexpress SDC1 both *in vitro* and *in vivo*. By using a series of specific inhibitors and siRNA approaches, we showed that this SDC1 overexpression in senescent cells is the result of an autocrine action of TGF-β through the Smad pathway and the transcription factor Sp1, while the classical senescence pathways of p53 or p38 MAPK - NF-kB are not involved. In addition, the highly invasive human breast cancer cells MDA-MB-231 (in contrast to the low-invasive MCF-7) can also enhance SDC1 expression, both in early-passage and senescence fibroblasts via a paracrine action of TGF-β. The above suggest that radiation-mediated premature senescence and invasive tumor cells, alone or in combination, enhance SDC1 expression in breast stromal fibroblasts, a poor prognostic factor for cancer growth, and that TGF-β plays a crucial role in this process.

P107 ERβ as a key regulator of extracellular matrix effectors and breast cancer cells aggressive phenotype

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

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P108 Extracellular matrix stiffness controls the breast circadian clock

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The cell-autonomous circadian clocks drive ~24 hr rhythms in fundamental biological processes that control behaviour and tissue physiology. At the molecular level, circadian oscillations rely on a feedback loop driven by a core clock mechanism containing transcription activators (Clock and Bmal1) and repressors (Periods and Cryptochromes). The amplitude of circadian rhythms in multiple tissues dampens with ageing, leading to a compromised temporal control of physiology. Age-associated clock suppression has been proposed as a predisposing factor for various human diseases. However, our understanding of how cellular clocks within a given tissue maintain robust circadian outputs, and how this robustness is lost during ageing, remain largely unknown. Here we have combined tissue-mechanics and real-time clock imaging studies to reveal that the breast epithelial clock is regulated by the mechano-chemical stiffness of the cellular microenvironment. Moreover it is controlled by the periductal extracellular matrix (ECM) *in vivo*, which contributes to the dampened circadian rhythm during ageing. Mechanistically the Rho/ROCK pathway, which transduces extracellular stiffness into cells, regulates the activity of the core circadian clock complex. Importantly, genetic perturbation, or age-associated disruption of self-sustained clocks, compromises the self-renewal capacity of mammary epithelia. Thus our results reveal that the mammalian circadian clocks are mechano-sensitive, providing a potential mechanism for how ageing influences their robustness and function. The stiffening of the ECM may contribute to age-related dampening of clock rhythm, which could impact on a spectrum of downstream rhythmic target genes that are involved in tissue homeostasis and function.

P109 Advanced Glycation End products (AGEs) combined with in vitro UVA exposure lead to an aggravation of skin aging by creating an elastosis- like phenomenon

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Skin aging is the result of superimposed intrinsic (individual) and extrinsic aging (e.g. like UV exposure). Previous work has considered the combined effects of UVs and AGEs. Modified radical species production, kinetic appearance of AGEs, co localization of AGEs and elastosis (characterized by accumulation of abnormal elastin content and degraded fibers) were observed. AGEs such as pentosidine are formed by an oxidative process, and often referred to as a glycoxidation product. Recently we demonstrated that the *in vivo* damaging effects of UV radiations might be more detrimental in aged skin than in young skin due, in part, to an increased accumulation of pentosidine and, in turn, to the exacerbation of alterations related to chronological aging (1). Consequently, we first investigated the biological effect of pentosidine in fibroblasts grown in monolayers. Soluble pentosidine induced up-regulation of IL8 and MMP12 mRNA (which are respectively inflammatory and elastasic markers). The tropo-elastin protein expression (elastin precursor) was also increased. In order to be closer to the *in vivo* situation we investigated the combination of the glycation reaction and UVA effects on the reconstructed skin model. We found that UVA exposure combined with glycation could intensify the response for specific markers: for example, MMP1 or MMP3 mRNA, proteases involved in extracellular matrix degradation, or IL1a protein expression, which is a proinflammatory cytokine. Moreover we have also observed that glycation and UVA irradiation could promote an environment favoring the onset of an elastosis-like phenomenon (mRNA coding for elastin, elastase and tropoelastin expression were increased). In conclusion, fibroblasts in monolayer cultured with soluble pentosidine and tridimensional

in vitro skin constructs exposed to the combination of AGEs and UVA are powerful skin aging approaches, as shown by increased alterations of the dermal compartment including an elastosis-like environment.
Keywords: AGEs, pentosidine, glycation, UVA, skin aging, reconstructed skin, elastosis.

P110 Development of an in vitro assay system to investigate peritoneal cell mediated fibrin remodelling associated with post-operative adhesion formation

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Introduction: Peritoneal adhesions, fibrous bands of extracellular matrix (ECM)-rich tissue joining normally separated organs, are a frequent side effect of abdominal surgery. Despite being a major cause of infertility and bowel obstruction, there are currently no adequate therapeutic interventions. Adhesion formation is strongly associated with fibrin persistence and with excessive collagen production by peritoneal cells. We hypothesise that peritoneal cells actively participate in the adhesion process by inducing coagulation, inhibiting fibrinolysis and undergoing epithelial-mesenchymal transition (EMT) with upregulation of ECM deposition. This study aimed to test the hypothesis *in vitro* using an assay system where peritoneal cells were cultured in a fibrin-rich environment in the presence of the pro-fibrotic mediator, TGF-β1.
Methods: Peritoneal cells were isolated by trypsin digestion of human omentum; human omental mesothelial cells (HOMCs). Lung fibroblasts (HPFs) were used as a control and sourced commercially. Cells were cultured on 2D fibrin film (FF) or 3D fibrin gel (FG), with or without 1ng/ml TGF-β1. Expression of key ECM and coagulation/fibrinolysis genes was assessed by real-time PCR and production quantified by western blotting. Collagen abundance was also characterised colorimetrically (Sircol assay, Biocolor).
Results: HPFs consistently showed increased α-SMA and procollagens expression in response to TGF-β1 (N=3) whereas the phenotype of TGF-β1 exposed HOMCs was patient dependent (N=4), suggesting that the fibrogenic response of mesothelial cells is varied. However, TGF-β1 induced upregulation of tissue factor (TF, coagulation cascade inducer) and plasminogen activator inhibitor 1 (PAI-1, fibrinolytic inhibitor) and downregulated tissue factor pathway inhibitor (TFPI, coagulation inhibitor) in both HPFs and HOMCs. HOMCs treated with TGF-β1 expressed decreased E-cadherin and increased N-cadherin, β-catenin, vimentin and snail1; consistent with trends observed during EMT. Culturing cells on fibrin in 2D or 3D did not have a significant effect in comparison to tissue culture plastic.
Discussion: The *in vitro* assay developed shows that HOMCs may play a dual role during peritoneal remodelling by regulating fibrin persistence and undergoing EMT to become fibroblast-like cells, which consistently show increased fibrogenic response to TGF-β1. HPFs and HOMCs expressed significantly higher levels of PAI-1 and lower levels of TFPI in response to TGF-β1, which may serve as potential therapeutic targets/molecular biomarkers for post-operative adhesion formation.

P111 Mechanisms of damage to the arterial wall extracellular matrix protein fibronectin by myeloperoxidase-derived oxidants

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The extracellular matrix (ECM) is comprised of a complex mixture of sugar polymers (glycosaminoglycans), proteoglycans and proteins, such as fibronectin, that are important for the structure and function of the artery wall. The ECM regulates cell activity via cell-ECM interactions and binding of cytokines and enzymes. Fibronectin possesses multiple important functional domains, including a cell-binding fragment (CBF) and heparin-binding fragments (HBF). During chronic inflammatory diseases such as atherosclerosis, activated leukocytes and particularly neutrophils and monocytes, generate reactive oxidants including hypochlorous acid (HOCl) and hypothiocyanous acid (HOSCN) that can modify proteins and glycosaminoglycans.
Aims: The hypothesis for this study was that HOCl and HOSCN would modify fibronectin and other ECM materials resulting in endothelial cell dysfunction (an early and defining marker of atherosclerosis) and contribute to plaque rupture, the major cause of most heart attacks and strokes.
Results: Our data indicate that there is a loss of antibody reactivity against the CBF and HBF domains of fibronectin, as detected by Western blotting and enzyme-linked immunosorbent assay (ELISA), when the protein is exposed to increasing molar ratios of the reagent oxidants HOCl or HOSCN. HOCl t reatment also resulted in a decrease in tryptophan and methionine concentrations and a corresponding increase in methionine sulfoxide, changes that are characteristic of protein modification by HOCl, as well as protein cross-linking / aggregation. Some of the cross-links were repaired on treatment of the oxidized protein with the reductant DTT, consistent with disulfide bond formation. Oxidative modification of the protein was accompanied by a loss of adhesion of human coronary artery endothelial cells to HOCl- and HOSCN-modified fibronectin, consistent with modification to the CBF of the protein. Evidence of this modification on fibronectin CBF by HOCl was also found in advanced human atherosclerotic lesions by immunofluorescence.
Extension of these studies to more complex and biologically-relevant myeloperoxidase (MPO), hydrogen peroxide (H_2O_2), and chloride ion (Cl^-) system provided data consistent with that obtained with reagent HOCl and fibronectin, with significant oxidation and cross-linking detected on the target protein. With this enzyme system, increasing concentrations of myeloperoxidase or H_2O_2 resulted in an increased extent of modification of the CBF and HBF domains on fibronectin. The presence of SCN^- in this system decreased damage relative to that observed with $MPO/H_2O_2/Cl^-$.
Conclusions: Overall, our data support the hypothesis that fibronectin, and particularly its functional CBF and HBF domains are susceptible to modification by HOCl or HOSCN, with this resulting in modification to its structure, function and biological properties. These changes may contribute to endothelial dysfunction and rupture of atherosclerotic lesions.

Workshop 8: Advances in matrix disease mechanisms and pharmacological targeting

Invited Lecture (L34) Combatting fibrosis: new ways to inhibit myofibroblast formation or pyridinoline cross-linking

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An estimated 35% of deaths in the Western world can be attributed to fibrosing disorders. The pathogenesis of fibrosis remains poorly understood. Understanding the pathways leading to an excessive accumulation of collagen produced by myofibroblasts, the hallmark of fibrosis, will be of help to define intervention points for novel therapeutics to prevent fibrosis. Myofibroblasts are mostly derived from fibroblasts that are activated by cytokines such as TGFβ. Although there is a vast amount of knowledge about the activation of fibroblasts into myofibroblasts, hardly anything is known whether myofibroblasts can be reversed into normal fibroblasts. If the latter is indeed possible, treatments may even become available in advanced stages of fibrosis (that is, under conditions where many myofibroblasts are already present). We have recently found that two low molecular weight molecules (CAPE and ACHP) are able to inhibit the formation of myofibroblasts out of fibroblasts, and that in some cases already existing myofibroblasts can be reversed into fibroblasts. The same was observed with conditioned medium derived from fetal stem cells. These findings should be further explored in preclinical fibrosis models.
A common denominator of fibrosis is the presence of elevated levels of pyridinoline cross-links that function to stabilize collagen fibrils. Formation of these cross-links is initiated by lysyl hydroxylase 2 (LH2) (encoded by *PLOD2*) which specifically hydroxylates lysine residues of the collagen telopeptide area. Collagen containing pyridinoline cross-links are difficult to degrade by collagenases. Remarkably, mutations in the PPIase enzyme FKBP65 (encoded by *FKBP10*) in BRuck syndrome results in diminished pyridinoline levels. We have dissected the molecular principle by which FKBP65 affects cross-link formation. We found that FKBP65 complexes with the LH2 splice-variants LH2A and LH2B, but not with LH1 and LH3. Partially ablating the catalytic activity of FKBP65 did not affect its complex formation with LH2, but it depleted the dimeric (active) active form of LH2 (i.e., LH2 was not able to form homodimers anymore). Furthermore, we found that LH2A and LH2B cannot form heterodimers with each other, but did so with the other lysyl hydroxylases LH1 and LH3. Collectively, our results indicate that FKBP65 is linked to pyridinoline cross-linking by specifically mediating dimerization of LH2, and that FKBP65 does not interact with LH1 and LH3. Our results provide a mechanistic link between FKBP65 and the loss of pyridinolines, and may hold the key to future treatments of fibrotic diseases related to collagen cross-linking anomalies. Reducing the levels of pyridinoline cross-links in scarring pathologies may enable the formation of a reversible, non-fibrotic matrix.

Invited Lecture (L35) Serglycin as a key proteoglycan in cellular effectors, signaling and functional properties of ERalpha silenced breast cancer cells

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Estrogen receptor alpha (ERα) signaling pathway plays pivotal roles in cancer cell growth, survival, as well as in gene expression regulatory mechanisms in hormone-dependent breast cancer. ERα silencing in low aggressive MCF-7 cells (MCF-7/SP10+ cells) induced epithelial to mesenchymal transition associated with increased aggressiveness (1). Gene expression experiments by using Ion AmpliSeq Targeted Sequencing Technology revealed that ERα silencing markedly affected the expression of various signaling molecules, cell surface receptors, proteolytic enzymes and extracellular matrix (ECM)

components such as collagens and proteoglycans. Recent studies have shown the functional importance of serglycin in malignancies (2). Serglycin is among the proteoglycans that significantly up-regulated following ERα silencing. Suppression of serglycin expression in MCF-7/SP10+ cells reduced their aggressive properties, whereas ectopic over-expression of serglycin in MCF-7 cells (MCF-7VSG) markedly promoted their aggressiveness. Notably, MCF-7VSG cells synthesize and secrete elevated amounts of matrix degrade enzymes such as MMP-1, -2, -7, -9, MT1-MMP and uPA. Serglycin over-expression is accompanied by activation of various signaling pathways such as PI3K, NF-κB, Src-family kinases and Rac1. Inhibition of these signaling pathways resulted in reduced proliferation, migration and invasion of serglycin over-expressing cells. Of great importance to aggressive breast cancer cell phenotype is the activation of TGFβ and IL-8 signaling pathways in MCF-7/SP10+ cells. In this regard it is noted that serglycin regulated the biosynthesis and secretion of IL-8 in MCF-7/SP10+ and MCF-7VSG cells. The expression of both serglycin and IL-8 is correlated with the absence of ERα and increased tumor grade in breast cancer tissues. IL-8 signaling pathway was activated in MCF-7/SP10+ and MCF-7VSG cells and its inhibition by using CXCR2 inhibitor SB225002 markedly reduced breast cancer cell properties. Furthermore, serglycin over-expression enhanced resistance of breast cancer cells against a series of chemotherapeutic drugs. In conclusion, ERα silencing promotes breast cancer through activation of various signaling pathways and regulation of functional ECM molecules such as serglycin. Up-regulation of serglycin plays a central role in breast cancer cell biology.

References

1. Bouris P, Skandalis SS, Piperigkou Z, Afratis N, Karamanou K, Aletras AJ, Moustakas A, Theocharis AD, Karamanos NK. Estrogen receptor alpha mediates epithelial to mesenchymal transition, expression of specific matrix effectors and functional properties of breast cancer cells. Matrix Biol. 43:42-60, 2015.
2. Korpetinou A, Skandalis SS, Labropoulou VT, Smirlaki G, Noulas A, Karamanos NK, Theocharis AD. Serglycin: at the crossroad of inflammation and malignancy. Front Oncol. 13;3:327, 2014.

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Invited Lecture (L36) Hyaluronan-coated extracellular vesicles as potential biomarkers and matrix messengers

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Extracellular vesicles (EVs) are plasma membrane-derived particles, secreted by many cell types into the extracellular matrix and body fluids. EVs represent their cellular origin and shuttle biological signals between cells. They transfer compounds, such as proteins, RNA and even DNA between cells during tissue healing, inflammation and cancer progression.

Hyaluronan is a unique glycosaminoglycan synthesized by specific plasma membrane-bound enzymes, hyaluronan synthases (HASs). Hyaluronan is essential for maintenance of normal tissues, but it promotes cancer progression by creating a favorable niche for the growth of tumor cells. In many pathological states, like cancer and inflammation, the amount of hyaluronan increases in the extracellular matrix and in the body fluids.

Activity of hyaluronan synthesis induces the growth of extremely long protrusions that act as specific sites for hyaluronan secretion. Furthermore, activity of HAS induces shedding of EVs that carry hyaluronan on their surface. This is a novel mechanism for cells to secrete hyaluronan and to carry it even long distances from the original cell. Interestingly, EVs are suggested to interact with their target cells by utilizing receptors on the vesicular surface, such as CD44, which is the most common receptor for hyaluronan. These CD44-hyaluronan interactions may act as a general mechanism regulating EV interactions with target cells.

In this work the hyaluronan-coated EVs secreted by different cell types, like tumor cells and mesenchymal stem cells were characterized by nanoparticle tracking analysis, immunoblotting and qPCR. Their binding mechanisms and effects on target cell properties like hyaluronan secretion were studied in monolayer and organotypic cell cultures by using live cell confocal microscopy, immunoelectron microscopy and correlative light and electron microscopy. EVs were found to carry both proteins and mRNAs for CD44 and HASs and they enhanced hyaluronan secretion of the target cells. Furthermore, the interaction of hyaluronan with CD44 regulated the binding of the EVs to the target cells.
The results of this work suggest that the EVs carrying hyaluronan on their surface are potential mediators in inflammation, healing of tissues and cancer progression and they may act as specific vehicles in extracellular matrix remodeling. Moreover, hyaluronan-coated EVs act as potential biomarkers, carriers and targets of therapy in many diseases associated with enhanced hyaluronan secretion.

ST43/P112 Properties and functions of collagen XIII and other MACIT collagens

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Among the 28 collagen types known in mammals, collagens XIII, XXIII and XXV form a subgroup of structurally related collagens. These collagens, named as MACITs (Membrane-Associated Collagens with Interrupted Triple-helices), are type II transmembrane proteins, composed of a short N-terminal cytosolic domain, a transmembrane domain and three collagenous domains (COL1-COL3), flanked and interrupted by non-collagenous sequences (NC1-NC4). The ectodomains of the three collagens can be shed by furin convertases. While the physiological functions and molecular mechanisms of the MACITs are not fully known, studies with genetically-engineered mouse models have suggested requirements for collagen XIII in the maturation of neuromuscular junctions (NMJ) (Latvanlehto et al., 2010) and for collagen XXV in the development of the NMJ and the survival of motor neurons (Tanaka et al., 2014). Our recent work shows that both transmembrane and shed forms of collagen XIII are needed for the maintenance and plasticity of the NMJ. Presence of the transmembrane form of collagen XIII alone rescues imperfect adhesion, Schwann cell invaginations and compromised acetylcholine vesicle accumulation phenotype seen in the lack of both collagen XIII forms. However, lack of shedding leads to an oversized and fragmented postsynaptic apparatus, and loss of plasticity. In addition to its autocrine and paracrine effects, collagen XIII keeps *acetylcholinesterase* in its precise localization at the NMJ. Furthermore, lack of both collagen XIII forms leads to denervation and distal axonopathy. Analyses of the muscles of collagen XIII modified mice revealed progressive atrophy in the lack of total collagen XIII and differential roles for collagen XIII in the plasticity of fast and slow twitch motor units. Interestingly, Logan et al. (2015) have recently reported on the first disease-causing mutations in the human COL13A1 gene, which were found to cause congenital myasthenic syndrome type 19. Thus also in man collagen XIII is critical for formation and maintenance of the NMJ. Collagen XIII occurs widely in tissues and its role is not restricted to the musculoskeletal system. For example, in experimental skin carcinogenesis, lack of collagen XIII or collagen XXIII markedly affect tumour formation, albeit in markedly distinct manners.

ST44/P113 Receptor for hyaluronic acid- mediated motility (RHAMM) regulates HT1080 fibrosarcoma cell proliferation via a β-catenin/c-myc signaling axis

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Fibrosarcoma belongs to the sarcoma cancer group, which are spindle cell malignancies of mesenchymal origin, and owe their name to the predominant cell line that is present within the tumor. High levels of hyaluronan (HA) synthesis in various cancer tissues, including sarcomas, are correlated with tumorigenesis and malignant transformation. RHAMM (receptor for hyaluronic acid-mediated motility) is overexpressed during tumor development in different malignancies. Previously, we have determined that HA metabolism regulates, in a cell-specific manner the migration capability of fibrosarcoma cells; whereas RHAMM/HA interaction regulates fibrosarcoma cell adhesion via the activation of FAK and ERK1/2 signaling pathways. In the present study by utilizing in vitro cell proliferation assay, siRNA transfection, flow cytometry, immunoprecipitation, western blotting and immunofluorescence we demonstrate that LMWHA/RHAMM downstream signaling regulates fibrosarcoma cell growth in a β-catenin/c-myc dependent manner. Specifically, the reduction of RHAMM expression was strongly correlated with an inhibition of HT1080 fibrosarcoma cell growth ($p \leq 0.01$). Both basal and LMWHA dependent growth of HT1080 cells was attenuated by β-catenin deficiency ($p \leq 0.01$). β-Catenin cytoplasmatic deposition is positively regulated by RHAMM ($p \leq 0.01$). Immunoflourescence and immunoprecipitation suggest that RHAMM/β-catenin form an intracellular complex. Transfection experiments identified c-myc as candidate downstream mediator of RHAMM/β-catenin effects on HT1080 fibrosarcoma cell proliferation. Thus, the present study suggests that RHAMM is a novel β-catenin intracellular binding partner, protecting β-catenin from degradation and supporting the nuclear translocation of this key cellular mediator, which results in c-myc activation and enhanced fibrosarcoma cell growth.

ST45/P114 The circadian clock gene *Bmal1* is required for cartilage tissue homeostasis

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Introduction: The circadian clock generates 24h rhythms in gene expression that coordinate daily physiology and metabolism. Disruptions to circadian clocks have been associated with a wide spectrum of human diseases. But how circadian rhythm regulates tissue-specific physiology and pathology in cartilage is still largely unknown. To address this we generated a chondrocyte-specific *Bmal1* ko mouse model.
Materials and Methods: Specific disruption of cartilage circadian rhythm was confirmed by real-time monitoring of clock gene activity. Standard histology was used to assess the integrity of the articular cartilage. RNA sequencing was used to determine genes differentially expressed between wild type and knockout mouse cartilage. Immunohistochemistry and western blotting was used to determine changes in protein levels in mouse and human cartilage samples.
Results: The expression of the core clock factor BMAL1 was disrupted in human osteoarthritic cartilage and in aged mouse cartilage. Targeted *Bmal1* deletion in mouse chondrocytes abolished their circadian rhythm and caused progressive degeneration of the articular cartilage. RNA sequencing revealed rhythmic expression of genes in several key anabolic and catabolic pathways in wild-type articular cartilage, and constant up- or down-regulation in the *Bmal1* knockout cartilage. The Tgfβ/SMAD and Nfat signalling pathways were among the most affected.
Discussion: Our study provides genetic evidence for the key role of the circadian clock transcription factor *Bmal1* in the homeostasis of cartilage. These results suggest that the circadian clock orchestrates rhythmic expression of key pathways relevant to cartilage metabolism. Dampening of the circadian rhythms with old age may be a contributing factor for osteoarthritis development.

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ST46/P115 Isolated iduronic acids in chondroitin/dermatan sulfate are important for neural crest cell migration

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Chondroitin/dermatan sulfate (CS/DS) is present in most of the ECMs and cell surfaces. Not surprisingly, it displays a variety of functions also affecting the outside-in signaling. We previously reported that CS/DS in cellular models, carried by cell surface proteoglycans (PGs), are important for an effective migration in the direction of the haptotactic stimuli, one of which was identified as the hepatocyte growth factor. Recently, the *Xenopus* model was utilized to address the question, which migratory cells would be mostly affected *in vivo* during development. Dermatan sulfate epimerase 1 (DS-epi1), and not DS-epi2, was the major iduronic acid-converting enzyme in early *Xenopus* development. DS-epi1 knockdown by morpholino injection resulted in tadpoles with reduced cartilaginous head structures, lack of dorsal fin tissue, and greatly decreased number of melanocyte. These effects are due to alteration of the neural crest cell (NCC) development and migration. DS-epi1 knockdown does not affect the formation of early neural progenitors but impairs the correct activation of transcription factors involved in the epithelial-mesenchymal transition (EMT) of NCC and reduces the extent of NCC migration. A role of NCC-bound CS/DS-PG was clear after transplantation experiments. Cranial NC explant and single cell cultures indicate a requirement of DS-epi1 in cell adhesion, spreading and extension of polarized cell processes on fibronectin. Altogether our work reveals the importance of CS/DS in NCC migration *in vivo*. The structure of CS/DS responsible for this pro-migratory activity contained few iduronic acid (IdoA) residues per chain.
We previously observed that CS/DS chains with high content of long consecutive IdoA blocks are required for a correct collagen fibril maturation in the mouse skin. We therefore studied the mode of action by which DS-epi1 and the 4-O-sulfotransferase D4ST1 are producing such long blocks. Incubations of chondroitin with recombinant DS-epi1 alone were monitored by hydrogen-deuterium mass spectrometry and revealed that the substrate was processively modified from the reducing to the non-reducing end. However, the processivity was only partial, resulting in at most five consecutive IdoA residues per chain. For the formation of long IdoA blocks, addition of D4ST1 was required. Functional and crosslinking experiments *in vitro* and FRET analysis *in vivo* indicated that DS-epi1 and D4ST1 are physically interacting.
Basic studies on the biosynthesis and biological functions of CS/DS could impact on our understanding of pathological mechanisms. For instance, the Ehlers-Danlos syndrome (EDS) is a heritable connective tissue disorder caused by several genes affecting collagen maturation. Recently approximately 30 patients lacking a functional D4ST1 and two patients with a defective DS-epi1 were reported as belonging to the Musculocontractural subtype EDS group. NC defects in the EMT and cell migration may account for the craniofacial anomalies and other congenital malformations in these patients, which may facilitate the diagnosis and development of therapies for this distressing condition.

ST47/P116 Expression of factors responsible for extracellular matrix remodeling leading to epithelial to mesenchymal transition in *Helicobacter pylori* infection

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H. pylori infection is the main etiologic factor for gastroduodenal ulceration and a primary risk factor for the development of gastric adenocarcinoma. The infection has been reported to induce up-regulated expression of a number of matrix metalloproteinases (MMPs), most notably MMP-1, -2, -7, -9 and -13 all of which contribute to the degradation of extracellular matrix (ECM). Cytotoxin-associated gene A (CagA) has emerged as the major *H. pylori* virulence factor increasing the risk of gastric cancer by 10-fold. Following its intracellular translocation, CagA is hierarchically tyrosine-phosphorylated in repeating EPIYA motifs and deregulates cellular polarity, inducing the appearance of an elongation and scattering phenotype, reminiscent of the epithelial to mesenchymal transition (EMT). In epithelial cells, stromelysin-1 (MMP-3) has been proposed to induce a sequence of molecular alterations leading to stable EMT conversion and carcinogenesis. In this study, we investigated the putative role of CagA protein, in the activation of MMP-3, as well as, other factors involved in mesenchymal transition and stemness. We utilized isogenic mutants of the P12 *H. pylori* reference strain, expressing CagA protein with variable numbers of terminal functional EPIYA phosphorylation and corresponding deficient EPIFA motifs. Also included were *cagA*- and *cagE*-gene knock-out strains, the latter having impaired functionality of type IV secretion system. Following *in vitro* infection of AGS and MKN-45 gastric epithelial cell lines, increased transcriptional activation of MMP-3 gene was determined by quantitative Real Time RT-PCR, in total cell lysates. This was associated to expression and functional translocation of CagA and the number of repeating terminal EPIYA phosphorylation motifs. Corresponding increase in enzymatic activity was detected in culture supernatants, utilizing zymography. Interestingly, in total cell lysates of *H. pylori*-infected AGS cells, expression of stromelysin-2 (MMP-10), which shares an 82% homology in amino acid sequence with MMP-3, was observed to be infection-independent. Significant increase in the transcriptional activation of mesenchymal markers *Snail, Vimentin and ZEB1* as well as, stem cell marker CD44, was observed in a CagA EPIYA phosphorylation-dependent manner. Collectively, our results suggest that following *H. pylori* infection, CagA can induce a variety of factors involved in epithelial to mesenchymal transition in an EPIYA phosphorylation-dependent manner, thereby contributing to carcinogenesis and metastasis in the gastric mucosa.

P117 Effects of flavonoids on proteasome expression and activity in fibroblasts of various origin

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Background: Flavonoids represent a wide class of phenolic phytochemicals which constitute an important component of the human diet, possessing antioxidant properties. The main subclasses of flavonoids are anthocyanins, flavanols, flavanones, flavonols, flavones and isoflavones. Flavonoids have been shown to act as scavengers of various oxidizing species and exhibit 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 (CT-L) (subunit β5), trypsin-like (subunit β2) and caspase-like (subunit β1) activities.
Nasal polyposis and eye pterygium are two inflammatory diseases of the nasal mucosa and cornea, respectively. Chronic UVB exposure is considered as a pathogenetic factor of pterygium.
The purpose of this work was to study the effect of flavonoids as modulators of the 20S proteasome in fibroblasts of various origin.
Biologic Materials: Fibroblasts were isolated from nasal polyps tissues, pterygium specimens and skin biopsies, by middle treatment with collagenase.
Results: Fibroblasts were cultured in the presence of flavones Baicalein and Quercetin, and the isoflavones Genistein and Biochanin at final concentration of 100μM. Real-time PCR showed that both the flavones enhanced the expression of proteasome subunit β5 in all fibroblasts tested with baicalein and quercetin to be more effective in polyps fibroblasts, and in skin and pterygium fibroblasts, respectively. In contrast, the isoflavones genistein and biochanin suppressed the expression of β5 proteasome subunit in polyps fibroblasts, while they enhanced its expression in skin and pterygium

fibroblasts. All the flavonoids had not any deleterious effect on the fibroblasts survival except of biochanin which caused a reduction (50%) of cells number. Using a fluorometric method, it was found that only the quercetin caused significant enhancement of CT-L activity in all fibroblasts tested. All other flavonoids caused suppression of this activity in polyps fibroblasts or had not any effect in pterygium and skin fibroblasts, except of biochanin which showed stimulatory effect in polyps fibroblasts.
Conclusions: The flavonoids exhibit different effects on proteasome expression and activity, dependent from their subclass. It appears that the number of hydroxyl groups may be the more significant factor in the modulation of proteasome activity since the quercetin, containing five hydroxyl groups, is the more effective. Therefore, it is reasonable to propose that the utilization of quercetin may be beneficial in the treatment of disorders related to proteasome reduced activity, because of oxidized proteins accumulation under exposure in UV radiation, such as pterygium and skin aging.

P118 N-acetylcysteine ameliorates the skeletal phenotype in a mouse model of Diastrophic Dysplasia

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Diastrophic Dysplasia (DTD) is an autosomal recessive chondrodysplasia caused by mutations in the *SLC26A2* gene encoding for a sulfate/chloride antiporter of the cell membrane.
Functional impairment of the sulfate transporter causes low levels of intracellular sulfate leading to cartilage proteoglycan (PG) undersulfation. Intracellular sulfate is mainly dependent on extracellular uptake, but in normal conditions a small fraction comes from the catabolism of sulfur-containing amino acids and their derivatives. In this work we studied cartilage PG sulfation and the clinical phenotype of a mouse model of Diastrophic Dysplasia (dtd mouse) after treatment with N-acetyl-L-cysteine (NAC) as alternative intracellular sulfate source.
Since the diastrophic phenotype already develops in the fetal period we administered NAC in the drinking water of pregnant mice for the whole pregnancy and we demonstrated transplacental delivery of the drug to fetuses by HPLC analysis of fetus homogenates.
To evaluate NAC treatment cartilage PG sulfation was studied by chondroitin sulfate HPLC disaccharide analysis in wild-type and mutant newborns from females treated with NAC or the placebo. A marked increase of PG sulfation was found in mutant mice from females treated with NAC (78% sulfated disaccharides) respect to mutant mice from untreated females (63% sulfated disaccharides). These data were confirmed by urinary glycosaminoglycan HPLC disaccharide analysis underlining an increase of the sulfated disaccharides in dtd treated newborns (65% sulfated disaccharides) compared to untreated ones (53% sulfated disaccharides). A skeletal phenotype amelioration towards the normal bone morphology in dtd mice born from treated females compared to mutant mice from untreated females was revealed by differential staining with alcian blue and alizarin red. Morphometric studies confirmed that femurs and ilia of dtd treated mice were longer and thinner and tibias were straighter than untreated mice. Moreover, a marked increase of proliferating chondrocytes in tibia epiphyses of dtd treated mice compared to dtd untreated ones approaching wild-type values was found by PCNA immunohistochemistry.
In conclusion we demonstrated that NAC ameliorates cartilage PG sulfation and the skeletal phenotype of dtd mice, suggesting a potential pharmacological treatment of diastrophic dysplasia.
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P119 The complement inhibitor CSMD1 acts a tumour suppressor for breast cancer

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Human CUB and Sushi multiple domains 1 (CSMD1) is a large (~390 kDa) membrane-bound complement inhibitor composed by 14 N-terminal CUB domains separated by single complement control protein (CCP) domains and followed by a tandem repeat of 15 CCP domains. It has a single membrane-spanning domain at the C-terminus and a small cytoplasmic tail with a putative tyrosine phosphorylation site. Therefore, CSMD1 may also serve as a receptor for unknown ligands resulting in signal transduction. CSMD1 has been suggested to act as a putative tumour suppressor gene since allelic loss on the short arm of the chromosome 8 (8p23), region that it occupies, has been reported in a number of malignancies. However, the claim that CSMD1 might act as tumour suppressor gene remains unconfirmed with a direct experimental approach.
This study aims to fully characterise the role of CSMD1 as a tumour suppressor gene in breast cancer progression *in vitro* and *in vivo*. For these purposes, we overexpressed the full-length human CSMD1 on the surface of cells BT-20 and MDA-MB-231 and down-regulated CSMD1 using ribozyme in T47D cells. *In vitro* characterization of cellular functional properties revealed that stable overexpression of CSMD1 significantly reduces cell motility and migration, adhesion and invasion of human breast cancer cells, whereas stable knockdown of CSMD1 enhances cell motility, cell adhesion and clonogenic forming abilities. Additionally, CSMD1 expressing cancer cells lose their tumorigenic potential and stem cell-like properties as the number of colonies that emerge in soft agar colony assay and aldehyde dehydrogenase (ALDH) activity are diminished. Actin cytoskeleton staining as well as scanning electron microscopy revealed distinct morphological architecture of CSMD1 expressing cells depicting their altered cellular functions. Using a xenograft model, we showed that expression of CSMD1 hindered the ability of the cancer cells to metastasize to a secondary site *in vivo*. Further, microarray analyses of gene expression in tumors formed *in vivo* showed that CSMD1 expression revealed altered extracellular matrix expression. Together, our results confirm that the levels of CSMD1 in breast cancer cells were negatively linked with the aggressive cancer cell behaviour, hence demonstrating the role of CSMD1 as tumour suppressor.

P120 Targeting ER stress in collagen IV pathologies

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Collagen IV is a major structural component of the basement membrane. Mutations in the genes *COL4A1* or *COL4A2*, encoding the alpha 1 and alpha 2 chains of collagen IV, cause vascular, eye and kidney disease including intracerebral haemorrhage (ICH). Moreover, common *COL4A2* variants are associated with sporadic ICH, for which there are no specific treatments. *COL4A1/ COL4A2* mutations lead to basement membrane defects and intracellular retention of collagen IV but their relative contribution to disease development remains unclear. Interestingly, intracellular retention and

subsequent endoplasmic reticulum (ER) stress are associated with disease development and in cell culture can be rescued by the chemical chaperone phenyl butyric acid (PBA). To investigate the role of ER-stress in disease development and *in vivo* efficacy of PBA, PBA was administered to *Col4a1* mutant mice from conception. This revealed that PBA was able to rescue the lethality due to ICH in newborn pups. In adult mice preventative PBA treatment reduced the severity of ICH and blood in the urine, indicating efficacy for vascular defects. The reduced ER stress levels and increased incorporation of collagen IV in the basement membrane due to PBA, modulated defects in the renal tubules but not the glomerular and eye defects. Combined with the absence of BM defects in renal tubules and a lack of chronic ER-stress in early onset glomerular defects, these in depth analyses revealed that the renal tubular defects are associated with ER stress while the glomerular disease is due to BM defects. Interestingly, a 1 month PBA treatment of 3 month old mice indicates PBA is effective as a preventative therapy. These data highlight that against expectation *Col4a1* mutations exhibit tissue- and cell-specific disease mechanisms. Moreover, preventative chaperone treatment may represent a putative therapeutic approach for adult ICH and renal tubular disease due to *Col4a1* mutations.

P121 Biomarkers associated with *Drosophila* type IV collagen *col4a1* mutations as potential therapeutic targets in collagenopathy

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In an allelic series of *Drosophila* dominant temperature-sensitive mutations within the type IV collagen *col4a1* gene that we have generated, all lesions affected glycine residues and caused G->A transitions consistent with the mutagen EMS. The mutations resulted in a systemic phenotype affecting the striated muscle of the oviduct (1), the visceral muscles (2), the innate immune system (3), and the integrity of the epithelia of the Malpighian tubules (4), providing morphological, biochemical, molecular and immunological markers for these mutations. We have also identified severe myopathy in +/- heterozygotes resembling features of actin aggregate-associated myopathy. Using interallelic/intragenic complementation we have generated trans-heterozygous double mutants in which actin aggregate myopathy occurred in numerous allelic combinations. This phenotype preferentially associated with allelic combinations consisting of N-terminal integrin-binding mutations in both +/- and compound heterozygotes. As actin aggregate myopathy is known to associate with mutations within the sarcomeric actin genes, we have analyzed myofibrillar proteins in *col4a1*mutants and found proteolytic cleavage of sarcomeric actin at the SKR/GILKY site, yielding 30 and 17 kDa fragments in the integrin-binding mutants, but not in members of the allelic series carrying C-terminal mutation sites. This observation strongly supports the occurrence of actin aggregate-like myopathy in integrin-binding mutants as proteolytical cleavage of actin is a feature associated with COL4A1 mutant protein.
We hypothesized that *col4a1* mutations disrupt the three-dimensional structure of the COL4A1 containing triple helical COL4 protein and that this disruption can be mitigated by chemical chaperon treatment. We therefore utilized osmolytes that successfully alleviated some of the phenotypic consequences of the *col4a1*integrin binding-site mutation including increased lifespan of the mutants and improvement of striated muscle of the oviduct among the myopathic features.
References:
1. Kelemen-Valkony, I., et al., Matrix Biology 31:(1) pp. 29-37, 2012.
2. Kelemen-Valkony I, et al., Myopathies, Book Chapter, ISBN:978-1-62257-372-1, 2012.
3. Kiss, M., et al., Matrix Biology 49, 120–131, 2016.
4. Kiss, AA, et al., Data in Brief 7 868–872, 2016.

P122 The effects of UVB radiation on cytokines, metalloproteinases and their endogenous inhibitor, and proteasome expression in human pterygium fibroblasts is mediated by macrophage migration inhibitory factor (MIF)

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Background: Pterygium is a condition characterized by epithelial overgrowth of the cornea, inflammatory cell infiltration and an abnormal extracellular matrix accumulation. Various cytokines, growth factors, metalloproteinases and their tissue inhibitors are implicated in pathogenesis of this disease. Chronic UVB exposure is considered as a pathogenetic factor of disease. Macrophage migration inhibitory factor (MIF) is a predominantly pro-inflammatory cytokine that plays a key role in several inflammatory diseases. In this study the presence of MIF in pterygium was investigated, and evaluated its involvement in the expression of various pterygium pathogenetic factors, under UVB exposure, in cultured human pterygium fibroblasts.
Biologic Materials: Normal conjunctival specimens were obtained from the superotemporal bulbar conjunctiva of healthy individuals undergoing cataract surgery. Pterygium specimens were obtained from patients after the surgical removal of primary tissue.
Results: RT-PCR analysis showed that the expression of MIF was higher in pterygium than in normal conjunctiva. Upon UVB exposure of pterygium fibroblasts at irradiation doses 0-50 mJ/cm^2, an irradiation dose-dependent up-regulation of MIF, IL-6 and MMP-2, and down-regulation of TIMP-1 and proteasome subunit β5 (PSMB5) expression was observed. When UVB exposure took place in the presence of MIF inhibitor ISO-1, the concentration of IL-6 and MMP-2 were decreased at the control levels, while the TIMP-1 and PSMB5 expression was up-regulated and restored almost at the control levels. The PSMB5 expression was also up-regulated and restored almost at the control levels, when UVB exposure was performed after pre-incubation of cells with the src kinases inhibitor PP2, while the UVB-induced production of MIF was not affected, indicating that a src kinase-induced by MIF is responsible for the down-regulation of PSMB5 expression. UVB had very low deleterious effect on the fibroblasts survival.
Conclusions: It appears that, in pterygium fibroblasts the MIF-induced by UVB irradiation is involved in the expression of factors which play a significant role in pterygium development, in particular of proteasome which is responsible for the degradation of many intracellular proteins, including abnormal, denatured, or otherwise damaged proteins that may be produced from the UVB effect. Thus, it would be concluded that the implication of UVB irradiation in pterygium pathogenesis is partially mediated by the up-regulation of MIF expression. Therefore, it is reasonable to propose that the utilization of MIF inhibitors together with other drugs in clinical practice may be beneficial in the treatment of pterygium.

P123 Identification of new targets for the treatment of classical Osteogenesis Imperfecta using zebrafish models

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Osteogenesis imperfecta is a bone disease mainly caused by collagen type I mutations and characterized by bone fragility and skeletal deformities. No definitive cure is available and the search for novel

treatments is necessary. The small teleost *D. rerio* is particularly appealing for drug screening approaches because of its small size and high fecundity.
Due to the presence of three different genes coding for collagen 1 alpha chains, the composition of zebrafish collagen type I is still puzzling and needs to be clarified before using zebrafish as a model for collagen related diseases. Electrophoresis, mass spectrometry and aminoacid analysis on collagen type I extracted from bone, skin and scales revealed the presence of all the three chains in equal amount and let us to suppose the existence of an α1α2α3 heterotrimer or the coexistence of the $(\alpha1)_2\alpha2$ and the $(\alpha3)_2\alpha1$ trimers.
A zebrafish OI model (*Chihuahua*) carrying in heterozygosis the G574D substitution in the α1 chain of collagen type I is available. At a morphological level, *Chihuahua* fishes show a typical fin fold bending in the embryonal stage and a smaller size starting from 3 months compared to WT. X-ray and microCT revealed reduced mineralization in adult *Chihuahua* fishes and skeletal staining highlighted multiple bone fractures and severe skeletal deformities, such as alterations in neural spine inclination and fused vertebral bodies. Finally, a delayed mineralization was observed in mutant embryos. Collagen type I extracted from mutant tissues presents a delay in migration and a broadening of the bands in SDS-PAGE, suggesting an overmodification, while DSC revealed a lower Tm of mutant collagen compared to WT. The presence of an ER enlargement in osteoblasts and fibroblasts of mutant fishes was evident by electron and confocal microscopy. The collagen overmodification and ER retention, the skeletal deformities and the abnormal mineralization found in *Chihuahua* reproduce the main features reported in OI patients, validating this model for the study of the disease and for the evaluation of novel possible targets.
A chemical chaperone was tested using Chihuahua fishes. The efficacy of the drug was evaluated in term of ability to straighten the fin fold and to increment the low mineralization level typical of mutant embryos. After 10 days of treatment an increase in the fin fold angle of mutant fishes was observed, such as an improvement of the mineralization of the skull. These results suggest that ER stress is an appealing new target for the development of treatments for Osteogenesis imperfecta.
All experiments were performed in agreement with EU Directive 2010/63/EU for animals.
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P124 Switch of mesenchymal into epithelial phenotype of breast cancer cells: Impact of anti-inflammatory drug dexamethasone

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Epithelial mesenchymal transition (EMT) is an essential mechanism in breast cancer invasion and metastasis. This process is remarkably regulated by a wide range of transcription factors and different signaling pathways. The aim of the present study was to investigate the impact of anti-inflammatory drug dexamethasone (DEX) on EMT mechanism in the human breast cancer cell lines with different metastatic potential; MCF-7 (Luminal) and MDA-MB-231 (triple negative) cells. Both cell lines were treated with DEX (0.1 mg/ml) for 24 hours and qPCR was performed to decipher its effect on expression of EMT markers; TGF-β, Snail, Slug, Twist, E-cadherin and vimentin. Our results indicate DEX downregulated expression of Slug trasnscript by 11.6% in MDA-MB-231 and 20% in MCF-7 cells compared to untreated cells. E-cadherin mRNA level was upregulated by 1.5 and 1.9 folds in MDA-MB-231 and MCF-7 cells, respectively, compared to untreated cells. Vimentin mRNA level was decreased by 12.6% in MDA-MB-23l cells compared to untreated cells. Snail and Twist expressions on mRNA level were decreased by 12.5% and 29% respectively in MCF-7 cells compared to untreated cells. We concluded that the EMT mechanism could be abrogated and reversed into mesenchymal-epithelial transition (MET) in breast cancer cell lines by treatment with DEX. More studies are needed to fully identify signaling pathways involved in DEX-mediated switching EMT into MET in breast cancer cells.

P125 Structural characterization of Collagen VI von Willebrand factor type A domains and the functional consequences of mutations

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Introduction: Collagen VI is an integral extracellular matrix protein involved in key functions such as cellular adhesion and anchorage of large interstitial structures. Disruption to the assembly or export of Collagen VI microfibrils results in the conditions of Bethlem Myopathy and Ullrich Congenital Muscular Dystrophy which represent the mild and severe ends of a spectrum respectively. Collagen VI is a heterotrimeric protein composed of three classical and three novel polypeptide chains, α1, α2 and α3 and α4, α5 and α6 respectively. These chains are composed of protein protein interaction subunits, termed von Willebrand factor Type A (VWA) domains. This project focusses herein on the classical chains, chiefly Collagen VI α2 and α3. Interestingly, both chains have been shown to contain mutations in their VWA domains which may lead to a disease phenotype. Here, we attempt to structurally and functionally characterize both wild-type and mutated VWA domains of the Collagen VI α2 and α3 chains.
Methods: The individual VWA domains of the α2 and α3 chain were selected according to the intron/exon boundaries of the human and murine Collagen VI. In total, 46 constructs were produced utilizing a parallel cloning and screening protocol, which were then overexpressed in eukaryotic and bacterial protein expression systems to reveal suitable candidates and systems for further characterization. Purification proceeded through affinity chromatography and size exclusion chromatography to obtain a homogenous protein species. The pure protein was then subject to X-ray crystallography trials, in which screens for ideal buffer conditions for crystal formation were investigated. Furthermore, SAXS analysis will be applied to longer constructs (of 2 – 7 VWA domains in tandem). For a disease scenario, patient primary fibroblasts containing mutations in the α2 and α3 chain VWAs will be subject to western blot analysis of cell culture supernatant and lysate to observe effects on mature collagen VI production, with immunocytochemical staining to reveal the effect of these mutations on the macro-structural level of the ECM.
Results: The parallel cloning and expression screen yielded information on those domains most likely to be stable for downstream characterisation. Pure homogenous protein was obtained for several α3 chain VWA domains, the N2, N3, N6, N4-N5 and N5-N6 which proceeded to crystallography trials, with screens being checked periodically for the formation of crystals.
Discussion: Further inspection of the method shows optimization of the purification procedure in future is necessary. This is desirable in order to maximize protein concentration and in turn increase the probability of obtaining a crystal for further structural determination.
References: Beecher, N., Roseman, A., Jowitt, T., Berry, R., Troilo, H., Kammerer, R., et al. (2011). Collagen VI, Conformation of A domain Arrays and Microfibril Architecture. Journal of Biological Chemistry, 286, 40266-40275

P126 Dissecting the molecular basis of Fibrillin-LTBP interactions and their importance for TGF-β regulation

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Genetic diseases such as Marfan syndrome and Loeys-Dietz syndrome have suggested a link between the fibrillin microfibril network and transforming growth factor beta (TGF-β) signalling. This has been

further elucidated by various studies with mouse models in which Marfan-like phenotypes have been normalised (or in some cases exacerbated) by inhibition of TGF-β. It is also well established that the latent TGF-β binding proteins (LTBPs) co-localise with fibrillin fibres, while three of the four LTBP isoforms have been shown to interact directly with fibrillin fragments in biochemical assays. However, there remain a great number of unanswered questions regarding how these LTBP-fibrillin interactions contribute to regulation of TGF-β in the extracellular matrix, and also how reductions in fibrillin-1 lead to the dysregulated TGF-β activity seen in Marfan syndrome.
In order to investigate this we have conducted various biochemical and biophysical studies of fibrillin-1 and LTBP-1 fragments, and studied their interactions by SPR, NMR, and ELISA. By collecting NMR titration data and testing interaction models with site directed mutagenesis we have identified specific binding sites on both fibrillin and LTBP proteins, and generated a structural model for their interaction. We are also using these molecular insights to study the role of fibrillin in the integration of LTBP1 into the matrix in cell culture. Furthermore we are developing cell culture assays to test the mechanisms by which fibrillin and LTBPs regulate TGF-β bioavailability and activation by co-culture with integrin β6 expressing cells and TGF-β reporter lines.

P127 ERα knock-down severely affects the subcellular distribution of β-catenin in breast cancer cells. A possible implication of HSPGs

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Recent data from our lab point to the conversion of ERα–positive breast cancer cells (MCF-7/c cells) into an endocrine resistant state (MCF-7/SP10+ cells) triggered by the functional loss of ERα, which directly and potently initiates a complex series of processes that lead to epithelial-to-mesenchymal transition (EMT). These cell morphological changes are accompanied by dynamic changes in the expression of critical matrix effectors, resulting in an aggressive re-programmed and highly metastatic cell [1]. In order to study the underlying molecular mechanisms, the distribution of the transcriptional co-activator β-catenin in these cells was investigated. The results revealed a substantial translocation of β-catenin in the nucleus of ERα-suppressed cells (MCF-7/SP10+) compared to its membrane localization in the control cells (MCF-7/c). Activation of β-catenin is triggered by several signaling pathways. Given the co-receptor functions of cell surface heparan sulfate proteoglycans (HSPGs), such as syndecans, glypicans, and CD44, their possible implication in the subcellular distribution of β-catenin as well as in the phenotype and properties of MCF-7/SP10+ cells was investigated. The selective degradation of cell surface HS abrogated the nuclear localization of β-catenin in MCF-7/SP10+ cells associated with an increased migratory potential of these cells.
These results indicate an involvement and functional role of HSPGs in the distribution and activity of β-catenin in ERα-suppressed breast cancer cells.
References:
[1] Bouris P, Skandalis SS, Piperigkou Z, Afratis N, Karamanou K, Aletras AJ, Moustakas A, Theocharis AD, Karamanos NK. Matrix Biology, 43:42-60 (2015)

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P128 Macrophages migration inhibitory factor (MIF) attenuates the suppressive effect of dexamethasone on IL-6 production by nasal polyps fibroblasts via modulation of proteasome expression and activity

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Background: Nasal polyposis (NP) is a chronic inflammatory disease of the nasal mucosa, that characterized by inflammatory cell infiltration, tissue remodelling, extracellular matrix accumulation, and oedema. MIF is a predominantly pro-inflammatory cytokine that plays a key role in several inflammatory diseases. It is also able to antagonize the inhibitory effects of glucocorticoids on the expression of various cytokines and growth factors, including IL-6. IL-6 is a multifunctional cytokine, implicating in a variety of inflammatory conditions, which occurs in high levels in nasal polyps. Proteasome is an intracellular multi-subunit protease complex that degrades intracellular proteins. It possesses multiple endopeptidase activities including chymotrypsin-like (CT-L) (subunit β5) activity. In most of cases its β5 subunit expression is mediated by Nrf2-ARE pathway. In this study we investigated the effects of MIF on the production of IL-6 by polyps fibroblasts and its antagonism on the inhibitory effects of glucocorticoids on the expression of this cytokine.
Results: RT-PCR analysis showed that the expression of MIF, as well as of IL-6, was higher in polyp tissues than in normal nasal mucosa. MIF was able to enhance the expression of IL-6 in polyps fibroblasts in a dose- and time-depended mode. Using protein kinases and MAPKs inhibitors was found that the tyrosine kinases, and the MAPK Erks and JNK inhibitors significantly suppressed the MIF-induced IL-6 production from polyps fibroblasts. MIF was also able to up-regulate the expression of proteasome β5 subunit possibly through the Nrf2/ARE pathway, since was also up-regulated the expression of Nrf2. In contrary, the CT-L activity of this proteasome subunit was down-regulated. When fibroblasts were cultured with dexamethasone (DEXA), a dose-dependent suppression of IL-6 production was observed, which was restored almost at the control levels in the presence of MIF. DEXA stimulated also the MKP-1 expression which, however, it was not affected in the presence of MIF. In contrast, DEXA suppressed the expression and enhanced the CT-L activity of β5 proteasome subunit, which were restored in the presence of MIF.
Conclusions: It appears that DEXA enhances the proteasome activity, possibly through the induction of an endogenous proteasome activator, leading in suppression of proteasome expression and IL-6 production by degradation of Nrf2 and phosphorylated c-Jun, respectively. On the other hand, MIF is antagonized this effect by suppression of proteasome activity, possibly via the induction of an endogenous proteasome inhibitor, leading in enhancement of proteasome expression. This proteasomal inhibition subsequently led to an accumulation of phosphorylated c-Jun and activation of AP-1, known to control IL-6 expression. Therefore, it is reasonable to propose that the utilization of MIF inhibitors together with glucocorticoids in clinical practice may be beneficial in the treatment of NP.

P129 The Xbp1 arm of the unfolded protein response (UPR) has a pro-survival role in a chondrodysplasia triggered by mutant protein aggregation

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Multiple epiphyseal dysplasia type 5 (MED; EDM5) is an autosomal dominant skeletal dysplasia characterised by short-limbed dwarfism. EDM5 results from mutations in *MATN3*, leading to misfolding and intracellular aggregation of mutant matrilin-3 protein, thus triggering an unfolded protein response (UPR) in growth plate chondrocytes. The expression of mutant matrilin-3 elicits a specific disease

signature comprising up-regulation of numerous chaperones, PDIs and novel foldases (such as Armet and Creld2), and the alternative splicing of Xbp1 (Xbp1s).
In order to study the role of Xbp1s in the pathobiology of EDM5, MED (*Matn3* V194D) knock-in mice were crossed with the cartilage specific *Xbp1* null line and the resulting phenotype was analysed using bone measurements, histology, immunohistochemistry and quantitative methods to assess proliferation and apoptosis. Microarray analysis was used to generate a full transcriptomic profile of the double mutant and the parent lines, all of which were generated on the C57BL/6J background.
Both parent lines presented with a mild to moderate decrease in bone lengths and chondrocyte proliferation. Interestingly, double mutant mice presented with abnormal bell-shaped thoraxes and dramatically reduced bone lengths, severe growth plate disorganisation and dramatically decreased chondrocyte proliferation (66%), suggesting a synergistic effect. Moreover, the retention of mutant matrilin-3 appeared increased upon Xbp1 deletion, whilst the levels of several chaperone proteins, including Creld2 and PDIA6, were noticeably decreased. Microarray analysis revealed that the genes regulated by the Xbp1 arm of the UPR were specifically involved in modulating cell stress and cell integrity. Interestingly, Xbp1s is also induced in other aggregation conditions such as Alzheimer's and Huntington disease and several differentially expressed genes were shared with the aggregation disease signatures. Finally, we have overexpressed several forms of Xbp1 in *Matn3* V194D primary chondrocytes and shown that Xbp1 can facilitate a decrease in the intracellular retention of mutant matrilin-3, suggesting a potential novel therapeutic avenue for EDM5.
To summarise, Xbp1s arm of the UPR appears to play a protective role in the pathobiology of EDM5, and the Xbp1s signalling pathway may represent a novel target for the therapy of aggregation conditions.

P130 Short stature and altered BMP signalling in mice with a Gremlin-2 3'-UTR mutation

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Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-β (TGF-β) superfamily of signalling molecules that are secreted into the extracellular matrix to regulate BMP ligand-receptor engagement. BMP gene knockout studies in mouse show embryonic lethality and severe defects in cardiac, bone, kidney, lung, eyes and brain which shows that BMP signalling is important in the embryogenesis and homeostasis of numerous organ systems; however, little is known about the importance of BMP signalling in tendon development.
Gremlin 1 and 2 members of the CAN (cerberus and DAN) family of secreted BMP antagonists that bind with high affinity to BMP2, 4 and 7 and block receptor engagement. Little is known about Gremlin-2 (encoded by *grem2*) especially its role in tendon development and homeostasis.
We previously showed that the expression of *grem2* in tendon is under the control of the circadian clock; the transcript is maximally expressed at 09:00 (3 hours into daylight) and down-regulated at 21:00 (Yeung et al., 2014). We also showed that disruption of the rhythmic expression of *Grem2* occurs in arrhythmic mice that have calcific tendinopathy (Yeung et al., 2014). The aim of the present study was to investigate the function of *grem2* in tendon development. Our approach was to use CRISPR/Cas9 mediated genome editing to generate mutations in *grem2*. In this abstract and poster, we show results from the *grem2* (-/-) knockout (KO) mouse and a *grem2*-3'UTR (a 50 bp deletion in the untranslated region) knock-in mouse.
X-ray analysis showed that the UTR mouse was smaller (by 25%) than littermates at 6 weeks postnatal. Interestingly the KO mice had normal size at 6 weeks postnatal. Western blot analysis of the KO and 3'UTR mice showed increased phosphorylation of Smad1 (P-Smad Ser463 and Ser465), Smad5 and Smad9 (at their corresponding sites) in Achilles tendon of 6-week old mice. Data from tendons of KO and 3'UTR collected at 09:00 and 21:00 indicated increased level of P-Smad1 at 09:00 and no P-Smad1

signal at 21:00. Preliminary data from electron microscopy suggest that the collagen fibrils in the tendons of *grem2* 3'UTR are larger in diameter compared to wild-type littermates.
Our conclusions to date are: 1) Gremlin-2 can regulate Smad-dependent BMP signalling in tendon, 2) the 3'-UTR mutation most likely causes decreased stability of the *grem2* mRNA, 3) the 3'-UTR mutation is not functionally equivalent to a KO because of the UTR mice are shorter than KO mice, 4) mutations in the 3'-UTR do not affect clock regulation of the gene, and 5) elevated BMP signalling in the presence of reduced levels of Gremlin-2 possibly explain the observed increases in fibril diameter in the tendons of 3'UTR mice.
Reference:
Yeung CY, Gossan N, Lu Y, Hughes A, Hensman JJ, Bayer ML, Kjær M, Kadler KE, Meng QJ. (2014) Gremlin-2 is a BMP antagonist that is regulated by the circadian clock. Sci Rep. 4:5183.

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P131 Effect of β-tryptase on the production of extracellular matrix components from human nasal polyps fibroblasts

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Background: Nasal polyposis is a chronic inflammatory disease of the nasal mucosa, that characterized by inflammatory cell infiltration, modifications of epithelial differentiation, tissue remodelling, extracellular matrix (ECM) accumulation and oedema.
Mast cells usually are present in diseases characterized from deposition of extracellular matrix proteins and seem to be key players in fibrosis. One mast-cell product, the serine protease tryptase, is of special interest and has been shown to exert fibro-proliferative action, most likely via activation of the protease-activated receptor 2 (PAR-2).
In the this study the presence of tryptase in polyps was investigated, and evaluated its involvement in various polyposis pathogenetic factors expression in cultured human polyps fibroblasts.
Biologic Materials: Nasal polyps were resected from patients suffered from chronic sinusitis with polyposis. Normal nasal mucosa was taken during nasal septoplasty-inferior turbinoplasty of healthy subjects.
Results: By histochemistry it was ascertained the presence of mast cells in the coverage epithelium as well as in the stroma of polyps tissues. Tryptase activity was also detected in all polyps tissue extracts tested. RT-PCR analysis showed that the expression of COX-1, COX-2, ICAM-1 and PAR-2 was higher in polyps than in normal nasal mucosa. Tryptase caused up-regulation of collagen type I, COX-1, COX-2, ICAM-1 and fibronectin expression in cultured human polyps fibroblasts, through activation of PAR-2, while it had not any effect on IL-6 and IL-8 production. Using MAPK inhibitors it was found that MEK1/2, JNKs and p38 are implicated in the expression of collagen type I, ICAM-1 and fibronectin, while only MEK1/2 and JNKs in the expression of COX-1.
Conclusions: It appears that mast cells are attracted in inflamed nasal mucosa where, among other, they secrete tryptase which in turn stimulates the stromal fibroblasts to produce factors that contribute in the ECM accumulation and fibrosis, and consequently in polyps formation and development.

P132 Effect of S-Allylmercapro-N-Acetylcysteine on Stromal Bone Marrow Cells and Bone Structure in Adult Mice

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Background: Reduced activity of the cellular antioxidant defense mechanisms and/or increase in environmental or cellular metabolism-derived free radicals is defined as "oxidative stress" which is associated with the development and progression of degenerative diseases. It is suggested that oxidative stress plays a central role in the pathogenesis of osteoporosis. We have previously shown that S-allylmercapto-N-acetylcysteine (ASSNAC) activates Nrf2-mediated processes, increasing glutathione cellular level and resistance to oxidation in cultured endothelial cells (Izigov et al., FRBM 50:1131, 2011).
Aims: This study was designed to test the ability of ASSNAC to: (i) increase glutathione level in bone marrow (BM) cells; (ii) increase the number of stromal BM cells (SBMC); and (iii) to improve bone structure and strength in adult female mice.
Methods: Female mice were daily injected (i.p.) with or without ASSNAC (50 mg/kg/day) at the age of 12 to 20 weeks and the following parameters were studied: (1) the number of SBMC by counting adherent cells from BM extracts; (2) the populations of BM cells by fluorescently labeled antibodies to CD45 and CD73, measured by fluorescence activated cell sorting (FACS); (3) the level of glutathione in BM cells by the Enzymatic Recycling Method; (4) morphometric parameters of the femur bone by the Micro-Computed Tomography (μCT).
Results: ASSNAC treatment of adult female mice resulted in a significant increase in: the number of SBMC (160%), the percentage of bone-forming stromal cells ($CD73^+/CD45^-$; 234%) and glutathione level (210%) in BM cells. Furthermore, it affected the following parameters of the femur cortical bone structure: an increase in bone length (3%), its diameter (3%) and possibly their mechanical strength based on structural midshaft moment of inertia [MOI, 13% (polar), 11% (areal)]. There were no significant changes in the femur trabecular bone.
Conclusions: Treatment of healthy adult mice with ASSNAC protects the femur from oxidative stress as well as enhances the number of SBMC and the percentage of bone-forming SBMC in the BM. In addition, it improves the microarchitecture of the femur. These findings suggest the potential of ASSNAC in protecting adult healthy bones from bone loss associated with oxidative stress and may improve bone quality and mechanical strength [based on the improved MOI].

Plenary Workshop

Invited Lecture (L37) Ionizing radiation-mediated premature senescence of stromal fibroblasts: Implications in tumor development

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Cancer research is traditionally focused on the neoplastic cell. However, accumulating evidence indicate the importance of stroma in tumor development. Fibroblasts represent the most abundant stromal cell type, involved in the formation and remodeling of the extracellular matrix and the paracrine interactions with cancer cells. Normal fibroblast can become senescent after exposure to several genotoxic stresses due to the activation of a DNA damage response (DDR), i.e. the stimulation of the ATM-Chk2-p53-p21WAF1-pRB axis, leading to a permanent growth arrest. On the other hand, senescent cells express a catabolic phenotype, most probably affecting locally tissue homeostasis. In this work we studied the effect of ionizing radiation (IR), a classical anticancer treatment regime, on stromal fibroblasts. We found that repeated doses of IR provoke double strand DNA breaks, activate DDR ultimately lead to premature senescence of human lung stromal fibroblasts. These cells express of catabolic phenotype and enhance the considerably the growth of human lung cancer cells in vitro and in immunocompromised (SCID) mice in vivo. This effect seems to be due to the increased production of matrix metalloproteases (MMPs) by senescent cells, as an inhibitor of MMPs significantly reduced the growth of tumors in xenografts composed on cancer and senescent cells. We also found that IR provokes premature senescence in human breast stromal fibroblasts, both in vitro and in in vivo. These senescent cells are characterized by an intense catabolic phenotype; they further overexpress the cell surface proteoglycan syndecan 1 (SDC1), a poor prognostic factor when expressed in the malignant breast stroma. This overexpression is independent of the major senescence-related pathways of p53 and p38 MAPK – NF-kB, and it seems to be the outcome of an autocrine TGF-β loop acting via the Smad pathway and the transcription factor Sp1. Furthermore, the highly invasive human breast cancer cells MDA-MB-231 (and not the low-invasive MCF-7 cells) increase further SDC1 expression in young and senescent stromal fibroblasts by a paracrine action of TGF-β. The above suggest that the synergism of a side effect of radiotherapy (i.e. premature senescence) with aggressive tumor cells leads to a reactive stroma which is in favor of an enhanced tumor growth.

Invited Lecture (L38) The non-reducing terminal trisaccharide of heparin blocks glucose uptake in hyperglycemic dividing cells

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Our previous studies showed that: 1) kidney glomerular mesangial cells [1] and bone marrow stromal (stem) cells [2] dividing from G0/G1 in hyperglycemic glucose (3-5 x normal, 15-25 mM) initiate hyaluronan (HA) synthesis into intracellular compartments (ER, Golgi, transport vesicles) after entering S phase; 2) after completing cell division, the cells extrude a monocyte-adhesive HA matrix; 3) in vivo, the mesangial cells in streptozotocin diabetic rats that divided no longer sustain glomerular function; and 4) both daughter cells of the dividing bone marrow stromal (stem) cells become pathological adipocytes. Our recent paper [3] showed that the non-reducing terminal trisaccharide (Hep-Tri) purified from

heparan lyase digests of heparin is sufficient to prevent the intracellular hyaluronan synthesis response with a Kd of <20 nM.
Our ongoing studies tested glucose uptake in the mesangial cells stimulated to divide from G0/G1. Interestingly, cells dividing in normal glucose (5 mM) with or without heparin completely blocked glucose uptake after entering G1 suggesting that normal cells do not want to address cytosolic glucose metabolism during cell division. In high glucose (25 mM, equivalent to DMEM high glucose), glucose uptake was initiated within the first hour from G0 and increased steadily for at least 3 hours. In high glucose plus heparin, glucose uptake started, but was totally blocked by 1 hour from G0. Our proposed model is that: 1) glucose influx in hyperglycemic dividing cells activates glucose metabolism and increases cytosolic UDP-glucose and UDP-glucuronate (a substrate for HA synthesis) within 4-5 hours from G0, 2) activation of the hexosamine biosynthetic pathway subsequently increases cytosolic UDP-N-Acetylglucosamine (the other substrate for HA synthesis) to an unacceptable level by the beginning of S phase, 3) this initiates a pathway that activates hyaluronan synthases in intracellular membrane compartments that leads to the loss of mesangial cell function and the depletion of bone marrow stromal (stem) cells; and 4) the interaction of Hep-Tri with a receptor on dividing cells, but not on confluent cells, activates signaling pathways that stop glucose uptake, which blocks the increase in UDP-sugars and allows the cells to complete division and sustain normal functions.
References: [1] Wang A et al. J. Biol. Chem. 289:9418-29, 2014. [2] Wang A et al. J. Biol. Chem. 289:11410-20, 2014. [3] Wang C et al. J. Biol. Chem. 290:29045-50, 2015.

Invited Lecture (L39) Age-related changes in the retinal matrix and induction of disease pathways relevant to AMD

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A major hallmark of age-related macular degeneration (AMD), the leading cause of blindness in the developed world, is the presence of particulate material (drusen) in the central retina. This accumulates at the interface between Bruch's membrane (BM), a multi-laminar matrix, and the retinal pigment epithelium (RPE). Multiple genetic and environmental risk factors have been identified for AMD, however, the role of normal ageing is less well characterized. We have found previously that the Y402H polymorphism in complement factor H (FH), a complement regulator and major AMD risk factor, alters the specificity of the FH protein for heparan sulphate (HS) and reduces its binding to the human BM; furthermore, there is a reduction of HS within this matrix during normal ageing, which when combined with the Y402H polymorphism might lead to impaired complement regulation and promotion of drusen formation due to local chronic inflammation.
Recently we have investigated the age-related accumulation of metal ions in the BM and how this affects the function of the adjacent RPE. We analysed eyes from human donors without known AMD (aged 11-88 years) and quantified the level of 14 metal ions in BM, by inductively coupled plasma mass spectrometry (ICP-MS) (n=131). We determined gene expression changes in the adjacent RPE cells by quantitative PCR (n=81) and transcriptomics (n=24), and performed histological analysis on the macula regions from the contralateral eye (n=45).
ICP-MS revealed a significant linear increase in cadmium and cobalt ions, and a decrease in zinc ions, with age. Furthermore, we identified a population of older donors with high levels of aluminium; Walton staining localised aluminium to the BM and RPE (it was also present in drusen in 5 AMD donors). The amounts of metal ions in the BM (e.g. Al^{3+}, Cd^{2+}, Co^{2+}, Zn^{2+}) correlated with RPE gene expression changes in oxidative stress and complement pathways and in the major AMD risk factor, HTRA1. Age, and Cd^{2+} and Co^{2+} levels, also correlated with histological changes in carboxymethyl lysine (an oxidative stress marker) and the terminal complement complex in the BM. Analysis of the transcriptomics data further demonstrated that pathways associated with oxidative stress and complement correlate with certain metal ions in the adjacent BM, and additionally identified changes in

eumelanin biosynthesis, mitochondrial dysfunction, autophagy and proteasomal activity with age; in the case of Al^{3+}, this was also highly associated with vasculogenesis.
Our work has identified that several metal ions accumulate within the human retinal matrix during normal ageing and are associated with changes in multiple pathways implicated in AMD initiation and progression.

Late Breaking Abstracts

P133 Determination of the collagen consensus sequence for COMP binding

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Cartilage oligomeric matrix protein (COMP) is as member of the Thrombospondin family of proteins, which forms homopentamers induced by an N-terminal coiled-coil domain.Mutations in COMP can lead to skeletal disorders in humans, ranging from mild to severe forms of multiple epiphyseal dysplasia (MED) or pseudoachondroplasia (PSACH) (Briggs et al. 2014). In healthy situations COMP has been described to fulfil many functions by cross-linking various components in the extracellular matrix and plays an important role in the organisation of the different matrix protein networks. Beside non-collagenous proteins (for review see (Acharya et al. 2014)), COMP is described to bind directly to collagen type I and II (Rosenberg et al. 1998), type IX (Thur et al. 2001), as well as collagen type XII and type XIV (Agarwal et al. 2012). Binding of pentameric COMP to the fibrillar collagens leads to an increased rate of fibril formation *in vitro* (Halász et al. 2007), while the interaction of COMP with collagen XII and XIV may be important for the dermal-epidermal junction (Agarwal et al. 2012). Despite the importance of the collagen binding of COMP, the exact sequence of the binding interface on the collagen triple helix is unknown up to now.
We employed a collagen fragment library approach to determine the binding site of COMP to the N-terminal section of collagen II. We identified very recently peptides of with a low nanomolar avidity to pentameric COMP. Similar sequences exist at the C-terminal end of the collagenous domain of collagen II as well as in collagen XIV and XII. Studies in the near future will use these peptides to define a minimal consensus sequence for COMP binding. This will enable us a more focused biochemical and structural analysis of collagen-COMP interface and subsequently help to understand COMP's roles in collagen fibril formation and homeostasis.

P134 Inhibition of lysyl oxidase like 2 reduces collagen accumulation and collagen cross-links in CCl_4-induced liver fibrosis

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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 or cancer. Liver fibrosis occurs in various diseases like non-alcoholic steatohepatitis or virus-induced hepatitis. CCl_4-induced liver fibrosis is a useful model to study the role of extracellular matrix formation in the maintenance or progression of the disease. Therefore, inhibition of lysyl oxidases seems a possible mechanism to resolve fibrosis. The lysyl oxidase family contains 5 members and lysyl oxidase like 2 is validated drug target as it is upregulated in various fibrotic diseases. Pharmaxis has developed small molecule inhibitors that are selective for LOXL2 over LOX and have drug-like properties. A 6-week CCl_4-induced liver fibrosis in rat was used to test the efficacy of LOXL2 inhibitors prophylactically (for 6 weeks) as well as therapeutically (for the last three weeks). Treatment with LOXL2 inhibitors reduced the plasma concentration of a liver enzyme, hydroxyproline content in the liver as well as the fibrotic area as measured by picrosirius red. Mature collagen cross-links were measured by a pyridinoline (Pyd) ELISA assay. Pyd concentration was reduced in the presence of LOXL2 inhibitors and this effect was significant when LOXL2 inhibitor was given prophylactically. mRNA of collagen α1, TGF-β, TIMP-1, MMP-2 and LOXL2 were all upregulated in the liver of CCl4-treated animals but reduced in LOXL2 inhibitor treated animals

suggesting that the reduction in extracellular matrix accumulation and cross-linking is sufficient to improve liver fibrosis.

P135 Authentication of collagen VI antibodies - Immunoblot analysis for specificity and sensitivity

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Collagen VI is an extracellular matrix macromolecule involved in the pathology of muscular dystrophy due to mutations in the *COL6A1*, *COL6A2* and *COL6A3* genes. While many antibodies have been raised against individual collagen VI chains and tissue-purified collagen VI, few have been properly validated. This is critical to resolve because collagen VI antibodies are used widely in human genetic studies on patient material with collagen VI gene mutations. To address this deficiency, we authenticated more than 20 commonly used collagen VI antibodies by immunoblotting using a panel of cell lines transfected with cDNA for *COL6A1*, *COL6A2* and *COL6A3* individually and all three chains together. Cell lysates were resolved under reducing and non-reducing conditions and each antibody tested for specificity and sensitivity against individual chains and multimeric collagen VI by immunoblotting. Most antisera recognized their correct chain but with a range of sensitivities and specificities.

P136 The role of ERβ and EGFR in functional properties and the expression profiles of major ECM modulators in aggressive breast cancer cells

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Breast cancer is the most common tumor in women worldwide. Estrogens and their receptors (ERs) play crucial roles in breast cancer progression through the modulation of important extracellular matrix (ECM) macromolecules. To note, ERs are reported to interact with several cell receptors, such as the epidermal growth factor receptor (EGFR), promoting critical changes in signaling cascades that regulate breast cancer cells behavior. Moreover, estradiol (E2) regulates gene and protein expression of basic molecules of ECM, induces the crosstalk between ERs/IGFR/EGFR and play an important role in the progression of breast cancer through the modulation of functional cell properties. Even though the role of ERα in breast cancer is well established, the role of its isoform, ERβ, is not yet clarified. In the present study, transcriptional reprogramming but also induction of alterations in essential cell functions of ERβ-knocked down breast cancer cells [MDA-MB-231(ERβ+), high metastatic potential] as well as the effects of E2 and EGFR in breast cancer cells behavior were investigated. Importantly, we highlighted that the suppression of ERβ gene and the treatment of MDA-MB-231 breast cancer cells, with specific concentration of E2 and EGFR specific inhibitor (AG1478) significantly affect cell proliferation, motility, invasion and adhesion of MDA-MB-231 and the gene expression of important molecules of ECM, including proteolytic enzymes (MMPs, TIMPs), proteoglycans (syndecans, serglycin), cell receptors (EGFR, HER2) and EMT markers (vimentin). Recent data from our research group indicated that ERβ suppression in MDA-MB-231 breast cancer cells significantly reduced cell proliferation, migration, adhesion and invasion as well as it contributes in critical changes in expression profiles of important ECM modulators. In addition, this study demonstrated that the reduced aggressiveness, in terms of cell proliferation and migration, followed by the suppression of ERβ in MDA-MB-231 breast cancer cells was partly reversed upon treatment with E2. On the other hand, treatment with AG1478 did not caused any additional turnover in breast cancer cell aggressiveness. Both E2 and AG1478 regulated the gene expression profiles of matrix macromolecules with key roles in breast cancer progression. These novel results indicate the important role of the interaction among ERβ, E2 and EGFR in cell behavior and gene expression of ECM mediators in aggressive breast cancer cells.

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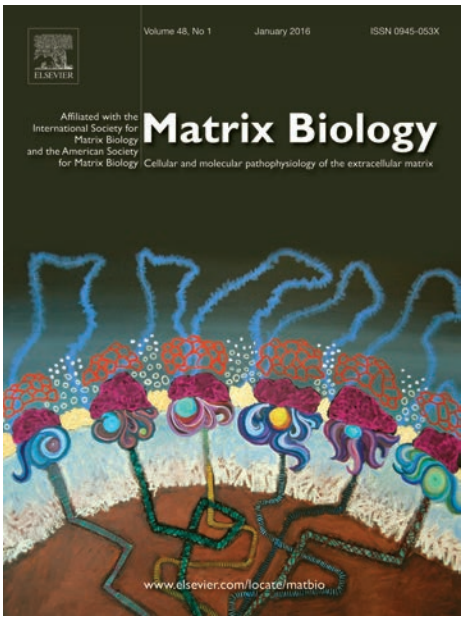

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