British Society for Matrix Biology
Autumn meeting September 9th-10 2019
University of East Anglia
"Cell Adhesion Networks in Health and Disease"

Organisers:
Stephen Robinson – Quadram Institute/ University of East Anglia

Monday September 9th

11:30-12:45	**REGISTRATION**	**Conference Office (TPSC main foyer)**
12:45-1:00	OPENING remarks	
	Welcome and Introduction	Stephen Robinson (QIB/UEA)

1:00-2:40	**SESSION 1: Adhesion signalling networks (TSPC lecture theatre)**	
Chair	Kasia Pirog	
1:00-1:40	Vascular adhesion signalling uses a novel NRP1 domain	Christiana Rhurberg (UCL)
1:40-2:20	Therapeutic potential of syndecan derived peptides	James Whiteford (QMUL)
2:20-2:40	Identifying disease mechanisms of vascular Ehlers Danlos Syndrome	Ramla Omar (U Glasgow)

2:40-4:00	**Networking, posters, trade stands (TPSC main foyer)**

4:00-5:40	**SESSION 2: Novel approaches to studying adhesion networks (TSPC lecture theatre)**	
Chair	Tom Van Agtmael	
4:00-4:40	Integrin-HER2 Crosstalk in Breast Cancer Invasion	Mark Morgan (U. Liverpool)
4:40-5:20	Integrin-dependent control of epithelial cell junction dynamics	Maddy Parsons (KCL)
5:20-5:40	An innovative proteomic workflow identifies ECM-binding ADAM17 substrates	Simone Scilabra (Fondazione Ri.MED)

BSMB Medal Lecture (TSPC lecture theatre) **Chair: John Couchman**		
5:45-6:30	Young Investigator Award: The John Scott Lecture A troubled teenager turning 20: new trends and concepts in ADAMTS research	Salvatore Santamaria (Imperial College London)
7:00-7:30	**Drinks Reception (Sainsbury Centre for the Visual Arts)**	
7:30-11:30	**Conference Dinner (Sainsbury Centre for the Visual Arts)**	

Tuesday September 10th

9:00-10:30	**SESSION 3: The Mentor/Mentee relationship – what do we need to drive each other's success? (TPSC 0.1 Breakout Room)**
Chair	**Stephen Robinson**

10:30-11:00	**Coffee break (TSPC main foyer)**

11:00-12:40	**SESSION 4: Adhesion network trafficking (TSPC lecture theatre)**	
Chair	**TBC**	
11:00-11:40	Adhesion-regulated adhesion. Auto-regulation of Matrix Receptors through Trafficking	Mark Bass (U Sheffield)
11:40-12:20	Metabolic control of invasive migration. A novel role for mitochondrial dysfunction in exosome release and the acquisition of invasive behaviour	Nick Rabas (Beatson)
12:20-12:40	Unravelling Extracellular Matrix Endocytosis in Breast Cancer	Keqian Nan (U Sheffield)

12:40-2:00	**Lunch Buffet + Networking, posters, trade stands (TSPC main foyer)**

2:00-3:40	**SESSION 5: studying adhesion networks *in vivo* (TSPC lecture theatre)**	
Chair	Kim Midwood	
2:00-2:20	Forward genetics approach to identify modifiers for collagen type IV-related disorders in the nematode *C. elegans*	Anna Gatseva (U Glasgow)
2:20-3:00	Talin mechanosensing is required for vascular development and pathological angiogenesis	Vasso Kostourou (Fleming Institute, Greece)
3:00-3:40	TBC	Kairbaan Hodivala-Dilke (BCI)

3:40-4:00	**Presentation of Prizes** **Close of Meeting**
4:00-7:00	**BSMB Committee Meeting (SCI 0.69)**

Meeting Sponsors:

Identification of a novel therapeutic peptide from Syndecan-3

S. Arokiasamy[1], G. De Rossi[2], J.R. Whiteford[1]

[1]William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, United Kingdom

[2]UCL Institute of Ophthalmology,11-43 Bath Street, London EC1V 9EL, UK.

Introduction

Syndecans are single transmembrane domain proteins with multifunctional roles in many cellular processes, such as adhesion, proliferation and migration. The mammalian syndecan family consists of four members - syndecan-1,-2,-3 and -4. All four have a conserved molecular architecture consisting of a single transmembrane domain, a conserved cytoplasmic domain and a GAG chain bearing ectodomain. The type and characteristics of the GAG chains can vary depending on the particular syndecan family member or the cell types expressing them, this coupled with unique sequences in the cytoplasmic 'variable' region are thought to govern syndecan functionality. We have characterised a peptide sequence within the syndecan-3 extracellular core protein which has biological activity. Specifically, it is potently anti-angiogenic and we have been assessing its potential as a possible novel therapeutic for neovascular eye diseases.

Materials and Methods

A variety of *in vivo* and *in vitro* disease models which recapitulate neovascular eye diseases and cancer were used. This included scratchwound assays, rat aortic ring assays and mouse choroid sprouting assays.

Results

The extracellular core protein of syndecan-3 exhibits anti-angiogenic properties. We have shown that sequences within this molecule are potently anti-angiogenic and that this likely occurs due to interactions with β1 integrins. We have also shown that this peptide has anti-angiogenic effects in an oxygen-induced model of retinopathy. Taken together, our results show that syndecan-3 derived peptides have potential therapeutic uses in neovascular eye diseases and other diseases where angiogenesis is a feature.

Discussion

The identification of sequences within syndecan-3 ectodomain that have anti-angiogenic properties is a novel and emerging avenue of research. Whilst we have shown that it has therapeutic potential, further research is required to identify key signalling pathways to deepen our understanding of its biological activity.

Targeting a disease "off switch"

M. Balderstone[1], S. Arokiasamy[1], G. De Rossi[2], J.R. Whiteford[1]

[1]William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, United Kingdom

[2]UCL Institute of Ophthalmology,11-43 Bath Street, London EC1V 9EL, UK.

Introduction

Angiogenesis is the formation of new blood vessels from pre-existing vasculature. It is a feature, along with vascular permeability, in numerous diseases. This includes diabetic retinopathy, the most common microvascular complication of type 1 and type 2 diabetes mellitus. Current treatments for diabetic retinopathy include intra-vitreal injection of anti-VEGF (vascular endothelial growth factor) drugs; however, they are associated with a high level of patient non-response and have high production and drug administration costs.

We have identified a novel pathway which, upon stimulation by shed syndecan-2, blocks VEGF-induced vascular permeability. This pathway is initiated through protein tyrosine phosphatase receptor, CD148, present on endothelial cell surfaces. We have already identified a peptide derived from syndecan-2, QM107, which interacts with CD148 to block vascular permeability. Building on this, we developed a methodology to ascertain where on the CD148 extracellular domain QM107 interacts and have been working to refine where precisely the peptide binds.

CD148 extracellular domain consists of 9 fibronectin type-III repeats; the binding site for its known ligand, shed syndecan-2, was previously identified as the first 5 repeats, called the CD148 short form (CD148SF). To further refine where the binding site is, we also split this into the first 2 repeats (CD148NT) and remaining 3 repeats (CD148CT) and expressed all 3 proteins.

Materials and Methods

In vitro solid phase binding assays were used, with biotin-labeled peptide and streptavidin-HRP detection. Recombinant CD148-derived proteins were produced through transformation and induction of BL21 Competent *E. Coli* cells.

Results

Solid-phase binding assays using the 3 CD148-derived proteins identified CD148CT as containing the binding site for QM107. This confirms that our peptide binds and interacts with CD148 to exhibit its effects on vascular permeability and demonstrates for the first time where it binds on the CD148 extracellular domain.

Discussion

Identification of the binding site on CD148 allows us to pursue this as a potential target for a new therapy to treat diabetic retinopathy, specifically to block vascular permeability and angiogenesis.

Forward genetics approach to identify modifiers for collagen type IV-related disorders in the nematode C. elegans

Anna Gatseva[1], *Iain Johnstone*[2], *Antony Page*[3], *Tom van Agtmael*[1]

[1] Institute of Cardiovascular and medical Sciences, University of Glasgow, Glasgow, G12 8QQ

[2] School of Life Sciences, University of Glasgow, Glasgow, G12 8QQ

[3] Institute of Biodiversity Animal Health & Comparative Medicine, University of Glasgow, Glasgow, G61 1QH

Introduction

Identifying genetic modifiers of disease increases our understanding of pathomolecular disease mechanisms and holds the promise of establishing novel therapeutic targets. Mutations in the extracellular matrix component collagen IV (Col4a1) cause cerebrovascular, eye and kidney disease, for which there are no treatments. Genetic modifiers can modulate Col4a1 disease outcome, but their identity remains unknown. To address this, we employed a mutagenesis approach in C. elegans carrying a Col4a1 mutation that displays the same disease mechanism mammalian Col4a1 mutations: matrix defects and intracellular collagen accumulation.

Materials and Methods

Col4a1 mutant C. elegans were treated with ethylmethanesulfonate (EMS) and offspring were selected for viability at the 20ºC, which is lethal for Col4a1 mutant strain. The viability, and morphology including length of modifier strains was determined.

Results

EMS mutagenesis was performed on 600 animals that produced 85 eggs (170 gametes), meaning a mutagenesis screen of 102,000 genomes was performed. As EMS induces 0.0004 loss of function mutations on average per genome, 5,000 genomes need to be mutagenized to achieve saturation. We therefore achieved ~20 mutant alleles of every gene.
We identified 40 modifier strains with an increase in survival of 10-70% at 20ºC. One of these modifiers strains partially rescued the dumpy mutant phenotype in the Col4a1 mutant strain. This rescued phenotype shows Mendelian inheritance indicating the modifier reflects a single mutation. We have selected four strains with the highest increase in survival, for mapping and identification of modifier mutation by whole genome sequencing.

Discussion

Using EMS mutagenesis, we successfully generated C. elegans strains in which the embryonic lethality is rescued to different extents confirming different modifier genes can modulate Col4a1 disease. Identification of these genetic modifiers of collagen IV disease represents the first step in delineating their molecular mechanism, and their "translation" to mammalian orthologs to determine if their efficacy is conserved in patient cell lines and mouse models of Col4a1 disease.

Mechanical stimulation for tissue engineering: characterising load-induced changes by the 'collagen barcode'

AJ Janvier[1], E Canty-Laird[1], JR Henstock[1]

[1] Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom

Introduction

Differentiated cells can be characterized by the composition of collagen isoforms that they produce in response to specific mechanical loads – effectively a 'collagen barcode' that functionally defines engineered tissues.

Collagen is one of the major structural proteins within the body, currently known to exist as 28 different isoforms that are each associated with specific functions in the tissue, such as mechanical resilience, structure, proteoglycan-binding and cell fate regulation. The combinations and ratios of collagen types vary across all tissues, e.g. Type I,III,V,XI,XII,XIV in tendon; Type II,IV,V,VI,IX,XI in cartilage.

Materials and Methods

We have used 3D printing to generate bespoke bioreactor components which apply mechanical stimulus to cells seeded within 3D constructs. We have customised our culture components around an EBERS TC3 bioreactor and replicated individual chambers at low cost.

The mechanical stimuli that have been applied at this stage is cyclic tensile force at 3, 5 and10% strain, 1Hz for 5 hours per day over 3 weeks. Western blot was used to quantify collagen isoforms produced at different stages of the loading regime. Supplementary data was collected using qPCR, histology, TEM and two-photon/second harmonic generation to visualize structure.

Results

Our early results show that in response to cyclic tensile loading hMSCs alter the collagen composition of the extracellular matrix they produce. Collagen I and III were upregulated, whilst V was suppressed, versus unloaded controls. Collagens XI, XII and XIV were detected in both loaded and controls, but at low concentrations – these will be investigated in more detail in future experiments.

Discussion

Defining 'optimal' loading conditions may help generate engineered tissues with comparable ratios of collagen types as found in healthy native tissue, and thus improved functionality and integration as implants. Using total collagen isoform expression to characterize these tissues in comparison to native, functional tissue is a novel approach which sheds light on developmental processes recapitulated in tissue engineering and allows us to better define successful bioengineered implants.

Endocytosis of extracellular molecules in respiratory health and disease

Maria Martina Meschis[a], Carsten Scavenius[b], Jan J. Enghild[b], Sonya Craig[c], George Bou-Gharios[a], Kazuhiro Yamamoto[a].

[a]Musculoskeletal Biology I, Institute of Ageing and Chronic Disease, University of Liverpool.

[b]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark.

[c]Liverpool Sleep and Ventilation Centre, Thoracic Department, University Hospital Aintree.

M.Meschis@liverpool.ac.uk

Introduction

Low-density lipoprotein receptor-related protein 1 (LRP1) is ubiquitously expressed and mediates endocytosis of various molecules. Recent large genome-wide association studies identified LRP1 as a gene relevant for lung function. However, little is known about LRP1 function in lung. Therefore, we investigated the effect of LRP1 blockade on primary lung cells and identified molecules regulated by LRP1.

Materials and Methods

LRP1 protein levels and endocytic capacity were measured by Western blotting in human primary lung fibroblasts (MRC-5), human primary bronchial epithelial cells (BEAS-2B), and cells isolated from mouse lung tissue. Cell viability in the presence of LRP1 blockers, receptor-associated protein or soluble form of LRP1 (sLRP1), was assessed by cell counting and colorimetric MTS assay. LRP1 ligands in conditioned media of the cells were isolated by sLRP1 and anti-sLRP1 magnetic beads and molecules bound to sLRP1 were identified by mass-spectrometry.

Results

LRP1 was highly expressed in the primary lung cells. Exogenously added ADAMTS-5, one of LRP1 ligands, was internalised by the cells with half-life of <8-hours. Importantly, we found that incubation with LRP1 blockers resulted in significant death of the cells. Furthermore, we identified total 67 ligand candidates including 50 previously unreported novel ligands.

Discussion

Cell death in lung tissue has been implicated in the pathogenesis of several pulmonary disorders. An increased level of soluble shed form of LRP1 was detected in bronchoalveolar lavage fluid from patients with acute respiratory distress syndrome and lung cancer. Furthermore, our proteomics study identified new LRP1 ligand candidates including molecules involved in cell survival, inflammation and extracellular matrix remodelling. We hypothesise that endocytic clearance of extracellular molecules is essential for lung tissue homeostasis and dysregulation of this clearance system is detrimental to the tissue. We are currently testing this hypothesis using recently established tissue-selective conditional LRP1 knockout mice.

Trafficking of JAM-C is regulated by cytoplasmic tail motifs and is essential to endothelial cell migration

Thomas P. Mitchell[1], Katja B. Kostelnik[1], Amy Barker[1], Christopher Schultz[1], Vinothini Rajeeve[2], Ian J. White[3], Michel Aurrand-Lions[4], Sussan Nourshargh[1], Pedro Cutillas[2] 5 and Thomas D. Nightingale[1]

1. Centre for Microvascular Research, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, UK.

2. Cell Signalling & Proteomics Group, Barts Cancer Institute, Queen Mary University of London, London, UK.

3. MRC Laboratory of Molecular Cell Biology, University College London, London, UK.

4. Aix Marseille University, CNRS, INSERM, Institut Paoli-Calmettes, CRCM, Marseille, France

Introduction

Junctional adhesion molecule C (JAM-C), is a component of endothelial cell tight junctions, contributing to the barrier function of the endothelium.

In the process of angiogenesis, endothelial cells must migrate necessitating the breakdown of cell contacts via the disassembly of cell junctions. We examined if motifs within the cytoplasmic tail of JAM-C regulate its internalisation and trafficking, contributing to the regulation of endothelial migration.

Materials and Methods

We created constructs of wild type and mutant JAM-C with peroxidase and fluorophore tags. Using these constructs we utilised techniques including proximity biotinylation, immunofluorescence labelling, knockdown/rescue and scratch wounds to elucidate the role of the JAM-C cytoplasmic domain in trafficking – at a biochemical and functional level.

Results

We show that a key point of regulation is the ubiquitylation of the JAM-C cytoplasmic domain, which is necessary for internalisation and endothelial cell migration. The role of the PDZ-binding domain is also examined.

Discussion

We show that trafficking is an essential regulatory component of JAM-C function, controlled by motifs within the cytoplasmic tail. Endothelial cell migration is necessary for angiogenesis and this can be regulated by junctional disassembly.

Title: Unravelling Extracellular Matrix Endocytosis in Breast Cancer

Keqian Nan[1], *Elena Rainero*[1]

[1] Department of Biomedical Science, The University of Sheffield, Sheffield, S10 2TN, United Kingdom

Introduction

Breast cancer is one of the three most common cancers worldwide and the leading cause of cancer-related death in women. The extracellular matrix (ECM) is a highly dynamic and complex meshwork of secreted proteins that regulates cellular behaviours through biochemical and biomechanical mechanisms. Deregulated ECM remodelling is one of the hallmarks of cancer. Preliminary data from our lab suggest that certain ECM components are preferentially internalized by invasive breast cancer cells through a β1 integrin-dependent pathway. Besides, ECM internalization contributes to the directional migration of invasive cancer cells. Hence, we aim to specifically illustrate which ECM components and β1 integrin heterodimers are involved in this process and how they promote invasive cancer cell migration.

Materials and Methods

We used invasive breast cancer cells extracted from a polyoma middle T-driven mouse mammary tumour (PyMT#1) and normal mouse mammary epithelial cells (NMuMG). To analyse ECM internalization, we used simple matrices (Matrigel, laminin and collagen I) as well as fibroblast-generated cell-derived matrices. The ECMs were fluorescently labelled, cells were plated on these for 12hr, fixed and stained. Z-stacks were acquired with confocal microscopy and analysed using Image J. The cells were synchronised with serum starvation and double-thymidine block.

Results

Here we shown that the internalization of Matrigel, laminin and cell-derived matrices, but not collagen I, is strongly upregulated in PyMT#1 cells compared to NMuMG cells. At the same time, ECM components internalization is cell cycle-dependent in PyMT#1 cells, but not in NMuMG cells. In particular, Matrigel, laminin and cell-derived matrices internalization, rather than collagen I, is significantly increased when PyMT#1 cells are synchronised in the G1/S phase of the cell cycle, compared to non-synchronised cells. Interestingly, there is no significant difference in the internalization of ECM components between synchronised and non-synchronised NMuMG cells.

Discussion

Altogether, these results suggest that invasive breast cancer cells specifically upregulate the internalization of laminin-rich networks. Additionally, the increased Matrigel and laminin internalization at G1/S phase in PyMT#1 might result from cell cycle-dependent changes in integrin trafficking and subsequently adhesion complex localization and turnover, which may promote invasive cancer cell migration.

Identifying disease mechanisms of vascular Ehlers Danlos Syndrome

Ramla Omar[1], Fransiska Malfait[2], Neil Bulleid[3], Tom Van Agtmael[1]

1. Institute of Cardiovascular and Medical Sciences, University of Glasgow
2. Centre for Medical Genetics, University of Ghent, Belgium
3. Institute of Molecular Cell and Systems Biology, University of Glasgow

Introduction

Vascular Ehlers Danlos Syndrome (vEDS) is the most serious subtype of EDS caused by *COL3A1* mutations**.** The majority of causal *COL3A1* mutations are glycine substitutions but the disease mechanism is largely unknown which is why current treatment is only symptomatic. Missense mutations in secreted proteins such as collagen can lead to the accumulation of misfolded protein in the endoplasmic reticulum (ER) and ER stress. For this project, we aim to investigate the disease mechanism of vEDS.

Materials and Methods

We established and investigated primary patient fibroblast cells harbouring *COL3A1* glycine mutations (G189S and G906R). ER stress markers and levels of intracellular collagen III were analysed. Sensitivity to trypsin digestion was used to test the triple helical stability of secreted collagen III as a measure of collagen folding quality. Cells were treated with chemical chaperones (PBA/TUDCA) or autophagy stimulator (CBZ) to investigate potential treatments.

Results

While both mutations caused intracellular collagen III retention, this was more severe in COL43A1 G906R mutant cells. The secreted collagen III of both mutant cells had increased sensitivity to trypsin digestion, indicating secretion of mutant misfolded collagen. The intracellular accumulation of collagen III in G906R cells caused activation of ER stress pathways (IRE1 and PERK) and apoptosis indicated by higher levels of CHOP. Interestingly, the G189S mutation resulted in an apparent milder level of ER stress without CHOP activation. We also observed that treatment using CBZ appeared to decrease mRNA levels of ER stress markers in G906R cells while TUDCA was able to target ER stress in G189S cells.

Discussion

We have identified that ER stress plays a role in the disease mechanism of vEDS, whereby individual *COL3A1* mutations have differential activation of ER stress with higher ER stress levels being associated with more C-terminal glycine mutations. We also establish that misfolded protein is being secreted to the extracellular matrix, which could affect correct fibril formation. Finally, our results demonstrate the need for specific treatment for individual mutations.

Exosites in Hypervariable Loops of ADAMTS Spacer Domains control Substrate Recognition and Proteolysis

Salvatore Santamaria[1], Kazuhiro Yamamoto[2], Adrienn Teraz-Orosz[1], Christopher Koch[3,4], Suneel S. Apte[3], Rens de Groot[1], David A. Lane[1] and Josefin Ahnström[1]

[1] Centre for Haematology, Imperial College London, Du Cane Road W12 0NN, London, UK; [2] Institute of Ageing and Chronic Disease, William Henry Duncan Building, University of Liverpool; [3] Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Cleveland OH; [4] Department of Chemistry, Cleveland State University, Cleveland OH

Introduction

ADAMTS (A Disintegrin-like and Metalloproteinase domain with Thrombospondin type 1 Motif)-1, -4 and -5 share the abilities to cleave large aggregating proteoglycans including versican and aggrecan, two molecules that exert an important structural role in cardiovascular tissues and cartilage, respectively. The proteoglycanase activity of these enzymes has been implicated in a variety of developmental processes as well as diseases such as cardiovascular disease, aneurysms and osteoarthritis. However, the interaction between ADAMTSs and proteoglycans has not been fully elucidated.

Materials and Methods

Various ADAMTSs and versican V1 variants were expressed and purified. ADAMTSs were quantified by active-site titration with tissue-inhibitor of metalloproteinase (TIMP)-3. Following incubation of ADAMTSs with versican, the reaction was stopped with EDTA. The cleavage product was then quantified using a newly-developed in house sandwich ELISA detecting the N-terminal fragment resulting from cleavage at the Glu441↓442Ala bond within the βGAG region. Aggrecanase activity was evaluated by western blot.

Results

We determined, for the first time, kinetic constants for versican proteolysis. ADAMTS-5 (k_{cat}/K_m 35 x 10^5 M^{-1} sec^{-1}) is a more potent (~18-fold) versicanase than ADAMTS-4 (k_{cat}/K_m 1.86 x 10^5 M^{-1} sec^{-1}), whereas ADAMTS-1 versicanase activity is comparatively low. Deletion of the spacer domain reduced versicanase activity of ADAMTS-5 19-fold and that of ADAMTS-4 167-fold. Co-deletion of the ADAMTS-5 cysteine-rich domain further reduced versicanase activity to a total 153-fold reduction. Substitution of two hypervariable loops in the spacer domain of ADAMTS-5 (residues 739-744 and 837-844) and ADAMTS-4 (residues 717-724 and 788-795) with those of ADAMTS-13, which does not cleave proteoglycans, caused spacer-dependent reductions in versicanase activities. Moreover, we found that the same loops are involved in aggrecan binding since the resulting chimeras showed decreased aggrecanase activity.

Discussion

Our results demonstrate that these loops contain exosites critical for interaction with and processing of versican and aggrecan. Together with previous work on ADAMTS-13 our results suggest that the spacer domain hypervariable loops may exercise significant control of ADAMTS proteolytic activity as a general principle. Identification of specific exosites also provides targets for selective inhibitors.

An innovative proteomic workflow identifies ECM-binding ADAM17 substrates

SD Scilabra[1-2], *M Calligaris*[1-2], *SA Mueller*[2] *and SF Lichtenthaler*[2-3].

[1] Fondazione Ri.MED, Department of Research, IRCCS-ISMETT, via Tricomi 5, 90127, Palermo, Italy.
[2] German Center for Neurodegenerative Diseases (DZNE), Feodor-Lynen Strasse 17, 81377, Munich, Germany.
[3] Neuroproteomics, School of Medicine, Klinikum rechts der Isar, and Institute for Advanced Science, Technische Universität München, 81675, Munich, Germany.

Introduction
ADAM17, a member of the "disintegrin and metalloproteinase" family, is responsible for the ectodomain shedding of over 80 transmembrane proteins, spanning from signalling molecules, such as cytokines and growth factors, to cell adhesion molecules. ADAM17 plays a crucial role in many biological processes, including inflammation and development; consequently, its dysregulated activity is linked to a number of pathological conditions. Two inactive cognates of rhomboid proteases, namely iRhom1 and 2, emerged as essential regulators of ADAM17, playing a key role in its maturation, activation and substrate-selectivity. The aim of this study is using shotgun proteomics to investigate function and regulation of ADAM17 in a broader manner, which includes identification of novel ADAM17 substrates and evaluation of iRhom-mediated substrate selectivity.

Materials and Methods
We established an advanced workflow for secretome analysis, which includes "filter-aided sample preparation" (FASP) and label-free quantification (LFQ), to identify ADAM17 substrates in a systematic manner. This method was complemented with the analysis of heparin-treated secretome followed by label-free quantification ("hep-sec analysis"), which allows the detection of proteins, including ADAM17 substrates, whose soluble levels in the secretome are low due to their high affinity for extracellular matrix (ECM) components or cell surface heparan sulfate proteoglycans.

Results
Together with known ADAM17 substrates, our proteomic approach allowed the identification of novel substrates, including matrix remodeling-associated protein 8 (Mxra8) and H2-D1, a class I major histocompatibility molecule. Shedding of Mxra8 and H2-D1 was further validated by immunoblotting as an orthogonal method. Furthermore, we took advantage of our proteomic method to investigate which substrates are released by ADAM17 in an iRhom1- or iRhom2-dependent manner. We found that either iRhom is sufficient to support shedding of the majority of ADAM17 substrates. However, shedding of some of these substrates was strictly dependent on iRhom1or iRhom2, without the other iRhom being able to compensate.

Discussion
In conclusion, we established a proteomic workflow for secretome analysis, called "hep-sec analysis", that is able to detect ECM-binding proteins with low levels in the conditioned media. Our study identified a number of novel ADAM17-substrates and investigated iRhom-mediated substrate-selectivity of ADAM17 at an omic level, thus providing more insights into the biology of the protease.

Development and validation of an inducible LaNt α31 overexpressing mouse model

CJ Sugden[1], LD Troughton[1], K Liu[2], G Bou-Gharios[2], KJ Hamill1[1]

[1] Department of Eye and Vision Science, University of Liverpool, UK

[2] Department of Musculoskeletal Biology, University of Liverpool, UK

Introduction

LaNt a31 comprises a laminin N terminal domain and short stretch of laminin-type epidermal growth factor like repeats. This structure is similar to the netrin family of proteins and suggests a role in signalling or influencing laminin network assembly. Moreover, in vitro studies suggest a role in wound repair/cell migration and matrix deposition, while IHC staining has revealed that LaNt α31 is redistributed during the wound healing process, and is upregulated in some cancers. However, no direct functional studies have been performed in living animals

In order to investigate the function that LaNt α31 plays in vivo, we have developed a tamoxifen-inducible, LaNt α31 overexpressing mouse model.

Materials and Methods

A UbC-LoxP-LaNt α31-T2A-tdTomato construct was generated and validated by transfecting along with pCAG-Cre:GFP into HEK293A cells. Expression was confirmed by western blotting and imaging of tdTomato fluorescence. Transgenic mice lines were generated by pronuclear microinjection. To confirm transgene expression, fibroblasts were isolated from E13.5 embryos, and expression induced using an adenovirus coding for Cre recombinase. Expressing mouse lines were mated with R26CreER mice. To confirm transgene could be activated in vivo, pregnant mice were given tamoxifen at E13.5, and cells isolated from embryos at E19.5, then PCR, western blot, and fluorescence microscopy, performed.

Results

Establishment of a UbC-LoxP- LaNt α31-T2A-tdTomato transgenic mouse line was confirmed via PCR, fluorescence microscopy, and western blot. Functional implications of LaNt overexpression was investigated using scratch wound assays in vitro, these data revealed that primary dermal fibroblasts from mice expressing the transgene have a reduced capacity to close a wound in vitro.

Discussion

This model allows us to overexpress LaNt α31 in any tissue, and at any stage of development. This will become a valuable model to understand the biological roles of LaNt α31 in their true physiological context, and how this protein interacts with its environment to influence physiological processes.