

British Society for Matrix Biology Meeting, Spring 2010

Vascular matrix in health and disease

Gareth Hyde¹, Andrew Marsen² and Megan Murray³

¹The Cardiovascular Research Group & Wellcome Trust Centre for Cell-Matrix Research, Faculty of Medical and Human Sciences, The University of Manchester, Manchester, UK;

²Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, The University of Manchester, Manchester, UK; ³School of Biological Sciences, University of East Anglia, Norwich, UK.

The BSMB spring meeting of 2010 was held at Hulme Hall, The University of Manchester on the 29th and 30th of March. The Meeting was organised by Dr Ann Canfield, who was ably assisted by Miss Anna Fildes. The programme included talks from many international and national leaders in the fields of vascular development, stem cells and regeneration, angiogenesis and vascular pathology. In addition, there was the BSMB Open session, a presentation on funding opportunities from The British Heart Foundation by Professor Jeremy Pearson (Associate Medical Director, The British Heart Foundation) and the Fell Muir Award lecture. This year the Fell Muir Award was presented to Professor Gillian Murphy for her seminal work on Matrix Metalloproteinases.

Over 160 delegates attended the meeting and 74 abstracts were submitted; of which 12 were selected for oral presentation. The BSMB awarded Presenter Bursaries to Jennifer Barra, Andrew Hamilton, Elena Okina, Amit Patel, Blandine Poulet, Jennifer Veevers and Kate Williamson; Gareth Hyde, Andrew Marsen and Megan Murray were awarded Reporter Bursaries. All of the posters were of an extremely high quality, but following extensive deliberations the invited speakers awarded prizes to Jennifer Veevers, Szymon Manka and Anna Woskowicz. Prizes were generously sponsored by The International Journal of Experimental Pathology. Financial support was also gratefully received from The Company of Biologists, The British Heart Foundation, (gold sponsors), The Wellcome Trust Centre for Cell Matrix Research (silver sponsor), Olympus, Roche, R&D Systems, Merck, Applied Biosystems, Invitrogen and Appleton Woods (bronze sponsors).

Session 1 – Matrix Proteins and Vascular Development

Co-chaired by Dr Clair Baldock and Dr Stuart Cain from the University of Manchester.

The first session was opened by **Professor Charlie Little** (University of Kansas, USA) with a presentation entitled 'Computational analysis of cellular and ECM motion during early vascular morphogenesis'. Professor Little started his talk by suggesting that one of the mechanisms that allows increased complexity in higher mammals is biomechanics and that one area of biomechanics that is commonly overlooked is motion relativity. Motion relativity is a concept that was first described by Galileo in 1632, however biologists have often failed to account for it when analysing cell "migration". As a result, the concept that cells move in an autonomous fashion is a common one and the extracellular matrix scaffold is largely ignored. To analyse relative motion during avian embryo development, Professor Little's laboratory label both the cells and the endogenous extracellular matrix fibres and monitor their trajectories using time-lapse imaging. By subtracting ECM displacement from the total cellular displacement it is possible to determine the actual cell autonomous motion that is taking place. The results of this analysis demonstrated that during avian embryo primitive streak formation there is little cell motion relative to the ECM and that the majority of morphogenesis is due to the ECM and the cells moving together as a tissue. Professor Little went on to present data using transgenic quail embryos in which endothelial cells express YFP under the

control of the Tie1 promoter. Analysis of these avian embryos showed that the endothelial cells are in constant motion during early vascular patterning. However, in addition to this cellular motion there is a higher level of motion where the entire endothelial tube and ECM is moving within the developing embryo. In a final example, he showed that during the early stages of heart formation major tissue deformations take place that results in the large scale passive movement of the cardiogenic cells. Professor Little concluded by saying that these time lapse images show that the extracellular scaffold is in constant motion and that this has major implications for our understanding of organ morphogenesis. If the ECM is in constant motion then ECM bound morphogen gradients - widely thought to guide cell migration and regulate morphogenesis – must also be in constant motion. Future models of embryo and organo-genesis must take into account ECM motion.

The second presentation of this session was given by **Dr Lynda Harris** (University of Manchester, UK) and was entitled 'Matrix metalloproteinase-12 mediates elastolysis in remodelling human uterine spiral arteries'. Dr Harris began by describing how the uterine spiral arteries remodel during pregnancy and how this is required for optimal blood flow to the placenta and nutrient exchange. The remodelling that takes place requires degradation of the elastin fibres within the arterial wall and Dr Harris then went on to show that placental derived cytotrophoblasts (CTB) can degrade elastin and that this degradation can be prevented with a MMP inhibitor. Next, micro-array data was presented demonstrating that CTBs express high levels of MMP12 and that a specific MMP12 inhibitor could reduce the elastolysis seen in both CTB and VSMC extracts. Immunohistochemistry was then presented confirming that MMP12 is expressed by interstitial and endovascular trophoblasts in the first trimester placental bed and by VSMC in the remodelling spiral arteries. Finally, it was shown that perfusion of unremodelled spiral artery segments with CTB conditioned media resulted in the expression of MMP12 by the VSMC in those arteries, indicating that CTBs secrete a factor that drives VSMC MMP12 production. Dr Harris concluded her talk by suggesting that it is the coordinated actions of trophoblasts and spiral artery VSMC that mediates elastin degradation and spiral artery remodelling.

The third presentation of the initial session was given by **Dr Athanasios Didangelos** (King's College, UK). Dr Didangelos gave a talk entitled 'Proteomic characterisation of extracellular space components in the human aorta' and began by stressing that the vascular extracellular environment consists not only of secreted signalling molecules but also the extracellular matrix. Few proteomic studies have targeted vascular extracellular matrix proteins and Dr Didangelos went on to describe the methodology he had optimised for the extraction and enrichment of extracellular matrix proteins from human aortas. This procedure included a 3-step extraction process. The first extraction was with 0.5M NaCl, the second with 0.08% SDS and the third with 4M guanidine-HCL. The extracted protein was then separated on 1D gels and fractions analysed by liquid chromatography tandem mass spectrometry on a LTQ Orbitrap. This process identified 103 extracellular space proteins, one third of which were not known to be present in the vascular extracellular environment. Four of these novel vascular extracellular space components were: podocan, sclerostin, agrin and asporin and immunohistochemical data was presented confirming the presence of these proteins in the vascular extracellular matrix. Dr Didangelos concluded by stating that this proteomic methodology was a powerful tool for the analysis of the extracellular environment, and that it could now be used to analyse ECM remodelling and degradation in important pathological processes.

The final talk of this session was given by **Professor Bob Mecham** (Washington University, St Louis, USA) and was entitled 'Vascular extracellular matrix and vessel wall development'. Professor Mecham began by showing an electronmicrograph of a cell within its extracellular matrix demonstrating that cell-matrix contacts occur every 20nm along the cell membrane and emphasising how much cells and the ECM are connected. Next, Professor Mecham went on to discuss the evolution of blood vessels. In invertebrates, blood vessels are stiff tubes

with a collagen extracellular matrix. Invertebrates have an open or partially closed circulatory system with no pulsatile flow and low blood pressure. As you move to higher invertebrates and lower vertebrates, fibrillin appears in the vessel wall giving the vessel slight elastic properties. Higher vertebrates possess a closed circulatory system with a chambered heart that generates pulsatile flow through the vascular system. In these animals the vessel wall also contains elastin which is produced by a layer of vascular smooth muscle cells. It is this elastic network that allows the large arteries to store and release energy meaning the vasculature can cope with the high blood pressure and pulsatile flow generated by the heart. To investigate the role of elastic fibres in cardiovascular development and function, mice with either reduced elastin (Eln+/-) or no elastin (Eln-/-) were studied. Compared to wild-type mice, Eln-/- mice have thicker vessel walls due to smooth muscle cell overproliferation, reduced compliance, 2-fold higher blood pressure in the left ventricle and impaired heart function. Mice with reduced elastin (Eln+/-) also have increased smooth muscle layers but these do not appear until just before birth. Eln+/- mice have 25% increased blood pressure in the left ventricle, but normal heart function and a normal life span. Professor Mecham suggested that these results show that reduced elastin leads to adaptive remodelling of the vessel wall which in turn allows normal heart function and life span in these mice. However, a complete absence of elastin leads to a level of vascular remodelling that is pathological, fails to compensate for the lack of elastin and results in death. Professor Mecham went on to discuss the implications of this work for supravalvular aortic stenosis (SVAS) a disease caused by haploinsufficiency of elastin. His data suggests that haploinsufficiency of elastin affects vessel wall structure and blood pressure during late embryogenesis and that this is when you would have to intervene to treat SVAS. Professor Mecham suggested this could be done by modulating the blood pressure of the mother to influence vessel wall development. In an attempt to do this, mice pregnant with Eln+/- embryos were treated with one of two ACE inhibitors, Candesartan or Captopril. Candesartan caused a reduction in blood pressure but failed to rescue the vessel wall abnormalities. However, Captopril did appear to rescue the vessel wall abnormalities which preliminary data suggested was through an angiotensin receptor 2 pathway.

Session 2 – Stem Cells and Vascular Regeneration

Co-chaired by Prof Martin Humphries and Dr Colette Inkson from the University of Manchester.

Session two began with an in-depth review of work from the lab of **Professor Cay Kielty** (University of Manchester, UK) entitled '*Mesenchymal stem cells in vascular remodelling: pivotal importance of Platelet Derived Growth Factor Receptor (PDGFR) signalling*'. Professor Kielty focused on the expression of PDGFR α and β , neuropilin-1, and $\alpha 5$ integrins in mesenchymal stem cells (MSCs). MSCs contribute to neovascularisation at sites of tumourigenesis and ischaemic injury, which is controlled by a complex signalling and crosstalk mechanism, as elucidated by Professor Kielty's group. Professor Kielty began by introducing neuropilin-1, a pro-angiogenic membrane-bound VEGF receptor, describing its co-localisation with sites of PDGFR α phosphorylation and its role in controlling MSC migration and proliferation. Professor Kielty also revealed that MSC adhesion to fibronectin induces $\alpha 5 \beta 1$ integrin-dependant tyrosine phosphorylation of PDGFR β . This in turn regulates MSC migration in a PI3-kinase dependant manner. In fact, phosphorylated PDGFR β was shown to co-immunoprecipitate with the $\alpha 5$ subunit and also co-localise with focal adhesions containing $\alpha 5 \beta 1$. Professor Kielty concluded by highlighting the importance of integrin – matrix crosstalk in neovascularisation and explained how this work has contributed to understanding the role of the PDGFRs in vascular development.

The second talk of this session was given by **Dr Lin Cooley** (University of East Anglia, UK). Dr Cooley reviewed her work on the '*Reversible trans-differentiation of blood vascular endothelial cells to a lymphatic-like phenotype in vitro*' and described an unexpected plasticity in the endothelial cell phenotype. Previously the blood vascular and the lymphatic systems have been thought of as comprising functionally distinct cell lineages, despite their structural similarities. Dr Cooley's work, however, has shown that lymphatic vessel markers such as LYVE-1 and Prox-1, the master regulator of lymphatic cell phenotype, are expressed in a

model of three-dimensional (3D) vascular tubulogenesis; human umbilical vein endothelial cells (HUVECs) cultured in type 1 collagen. Dr Cooley also described the down-regulation of blood vascular genes, including Laminin β 1, VEGF-C and CD44, suggesting that HUVECs in a 3D environment adopt a lymphatic phenotype. In order to further investigate this unanticipated expression pattern, HUVECs were cultured in 3D fibrin and Matrigel, which revealed the same lymphatic expression signature. Surprisingly, even when tubulogenesis was inhibited by culturing HUVECs either in '2D' on top of matrigel or in the presence of a broad-spectrum metalloproteinase inhibitor, the lymphatic phenotype remained. Dr Cooley also found that adult human aortic endothelial cells (HAEC) displayed the same phenotypic plasticity, expressing increased lymphatic markers during 3D tubulogenesis. Dr Cooley explained that, aside from culturing HUVECs in a monolayer, the only way to induce a more vascular phenotype in these cells was to co-culture with pericytes, cells that form the outer layer of blood vessel walls. Finally, Dr Cooley concluded that the extracellular environment is perhaps the most important factor in vascular or lymphatic lineage specification and also challenged the use of HUVECs as a strictly endothelial model system.

BSMB Fell Muir Award

Chaired by Professor John Couchman from the University of Copenhagen

The session ended with the presentation of the 2010 BSMB Fell-Muir Award, sponsored by The International Journal of Experimental Pathology, to **Professor Gill Murphy** (University of Cambridge, UK). Professor Murphy began her acceptance with some heartfelt thanks to members of the matrix community and told of how proud she felt to be a part of growing field of research that now 'even has it's own Gordon conference!' Professor Murphy then went on to give a brief history of her involvement in matrix research, in a talk entitled '*Metalloproteinases – from demolition squad to master regulator*'. This presentation touched on the somewhat troubled history of the small molecule inhibitors of matrix metalloproteinases (MMPs) and discussed the move towards a focus on the membrane type-MMPs (MT-MMP) and the ADAMs (**a** disintegrin and metalloproteinase). The audience was brought up to date with a review of Professor Murphy's latest work; investigating the role of metalloproteinases in three-dimensional angiogenic sprouting. For this, Professor Murphy explained, her laboratory uses a 'mini-tumour model' that involves the co-culture of endothelial cells, dermal fibroblasts and breast cancer cells. Together these cells form a multi-cellular spheroid thereby enabling the quantification of angiogenic sprouting without the need for exogenous growth factors. Through the use of this model her group has discovered that expression of MT1-MMP by the fibroblasts and endothelial cells is vital for normal sprouting to occur. Interestingly, however, Professor Murphy explained that knocking down the expression of MT1-MMP in the breast cancer cell component had no effect on sprouting in this model. Professor Murphy also discussed an exciting new role for ADAM10 in the 'mini-tumour model'. ADAM10 is a sheddase known to regulate Eph/Ephrin cell-cell signalling by cleavage of Ephrin and is also understood to induce proteolysis of the ligand-bound Notch receptor. Both Eph/Ephrin and Notch signalling are acknowledged to play a role in angiogenesis, with ADAM17 known to cleave ligand-bound Notch triggering pro-angiogenic intracellular signal transduction to the nucleus. In the 'mini-tumour model', ADAM10^{-/-} fibroblasts stimulate increased sprouting, which serves to emphasise the importance of specific metalloproteinase inhibitors in this process. Professor Murphy concluded her presentation by highlighting the importance of dissecting the relationships between metalloproteinase structure and function in order to fully understand their roles in disease and thereby pave the way for novel therapies.

Session 3 – Angiogenesis

Co-chaired by Professor Gillian Murphy (University of Cambridge) and Miss Jennifer Bara (RJA Orthopaedic hospital, Oswestry).

Session three began with a plenary presentation by **Professor Bjorn Olsen** (Harvard, USA) who gave a talk entitled '*Molecular mechanisms of infantile haemangioma*'. Professor Olsen described the roles of vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) in infantile haemangioma, the most common tumours observed in infancy. In 80% of cases these tumours are small and do not require treatment however 20% of cases are associated with aggressive tumours which require treatment. Current therapeutic options are associated with variable levels of success and side effects. The work of Professor Olsen's group aims to elucidate the molecular mechanism of this disease and identify novel therapeutic approaches for its treatment. Data was presented to highlight how the proliferative phase of haemangioma was due to reduced levels of the VEGFA "decoy receptor", VEGFR1, which causes constitutive VEGF-mediated VEGFR2 phosphorylation in haemangioma endothelium. This increased VEGFR2 signalling is associated with increased endothelial proliferation and migration. Professor Olsen explained how reduced expression of VEGFR1 was found to be due to abnormalities in a VEGFR2/ β 1 integrin/ tumour endothelial marker 8 (TEM8) complex which would ordinarily stimulate expression of VEGFR. This was determined by the screening of endothelial cells from lesions of haemangioma patients for somatic/germline mutations. Further evidence was presented to show how bone marrow derived stem cells accumulate in the proliferating haemangioma endothelium over time in response to an associated transcription of recruitment molecules. It was shown that mechanisms involved in proliferation also contribute to bringing it to an end. That is, proliferation of haemangioma endothelium is associated with an increased expression of VEGFR1 which is believed to signal the onset of haemangioma involution. Based on these studies it is thought that the administration of neutralising antibodies against VEGFA may alleviate symptoms in the absence of VEGFR1 expression by reducing VEGFR2 activation.

Mr Andrew Hamilton (University of Manchester) then gave a short presentation entitled '*A critical requirement for heparan sulphate activation of vascular endothelial growth factor to mediate angiogenesis*'. The interaction of VEGF with heparan sulphate (HS) is known to regulate the spatial distribution of VEGF and influence endothelial cell activation; however this feature appears to be lost in diseases such as cancer and cardiac ischaemia. Mr Hamilton described studies investigating the requirement of HS for VEGF-induced angiogenesis to test the hypothesis that the addition of short HS fragments might rescue VEGF regulation in these conditions. Angiogenesis was investigated in zebrafish injected with plasmids encoding wild-type VEGF₁₆₅ or VEGF₁₆₅ containing mutations in the HS binding domain. The effect of the addition of exogenous synthetic HS and heparin oligosaccharides (of different fragment lengths and composition) at the onset of angiogenesis (20hbf) was studied. Mr Hamilton explained how the addition of heparin fragments altered vessel length and that these effects were glycosaminoglycan (GAG) length and composition dependent. The addition of 6-10kDa heparin chains reduced vessel growth (by 20%) whereas the addition of 13-15kDa oligosaccharides increased vessel growth (by 20-35%). Sequence specificity was also shown to be important, with the demonstration that oligosaccharides composed of repeating IdoA(2S)-GlcNAc(2S) units increased angiogenesis, whereas repeating units of IdoA-GlcNS(6S) decreased angiogenesis. These effects were both in contrast to large increases in vessel length induced by VEGF over-expression in the absence of GAG which showed increased angiogenesis, whereas the VEGF mutants did not respond to VEGF. These studies thus highlighted the importance of the HS:VEGF interaction for regulating angiogenesis.

The third talk of the session was given by **Dr Magali Le Goff** (University of Manchester) who presented her work entitled '*The glycoprotein opticin inhibits collagen-induced angiogenesis*'. Dr Le Goff talked about the function of opticin, a class 3 small leucine-rich repeat protein which is almost exclusively present in the vitreous of the eye. The protein was initially discovered associated with collagen fibrils by Professor Paul Bishop's group at Manchester. As this protein is present in an avascular ECM, her research has focused on the potential anti-angiogenic role for opticin in the vitreous. She first described the hyaloid and retinal vasculatures in wild-type and opticin null mice and demonstrated that there was a delay in the hyaloid vessel regression which was associated with a delay in retinal vascularisation.

Studies on preretinal vascularisation, a characteristic associated with several ocular disorders, were performed using the oxygen-induced retinopathy (OIR) model. These studies showed that the opticin null mice develop preretinal neovascularisation. Therefore, opticin was shown to inhibit neovascularisation in the OIR model. Dr Le Goff also explained that opticin disrupts collagen-induced endothelial cell morphology by binding to collagen and blocking integrin binding sites, a finding which was described as a novel mechanism of action for this molecule which is now believed to have great potential as an anti-angiogenic therapeutic.

The final talk of this session was given by **Dr Holger Gerhardt** (London Research Institute, CRUK) who discussed '*Social cell behaviour in the angiogenic sprout*'. In this presentation, data from computational modelling and genetic mosaic sprouting assays (*in vitro* and *in vivo*) were presented to emphasise the regulation of endothelial cell behaviour during angiogenic sprouting and tip formation. Dr Gerhardt explained that dynamic endothelial cells compete for the tip position in vessel branches during angiogenesis and demonstrated that this behaviour is regulated by Notch and VEGF Receptor (VEGFR) signalling. It was shown that tips form during sprouting angiogenesis in response to VEGF which stimulates endothelial cell proliferation. This process can be regulated by matrix interactions e.g. between endothelial cells and fibronectin (FN). Blocking VEGF binding to FN reduces endothelial cell migration, highlighting the importance of this interaction. Furthermore it was shown that VEGFR levels control Notch activation which decreases filopodia expression at the leading face of the cell. This response prevents the cell becoming the tip cell and makes it resign to being a "stock cell", thus demonstrating how group dynamics determine which cells become tip cells in sprouting angiogenesis.

Session 4 – BSMB Open Session

Co-chaired by Professor Ray Boot-Handford (University of Manchester) and Dr Emma Blain (Cardiff University).

The BSMB Open Session was opened by **Professor Charles Streuli** (University of Manchester) with a presentation entitled '*How integrins control breast development and function*'. In this talk, Professor Streuli described recent work performed within his group using genetic analysis *in vivo* and primary cell culture to investigate how the ECM directs cell phenotype in breast epithelium. Specifically, he presented data highlighting how integrins control cell fate in the breast, focusing on the $\beta 1$ subunit which they found to be of crucial importance for mammary gland development. Data generated using a conditional knock out mouse model showed that $\beta 1$ integrins were vital for normal breast development and that $\beta 1$ knock out caused reduced cell proliferation and altered morphogenesis. He also explained how a time course study of lumen formation had revealed that apical polarity is disrupted in cells extracted from $\beta 1$ knock out mice with cells becoming "blind" to the ECM and formed inappropriate polarised surfaces which results in a disorganised lumen. Professor Streuli proposed a mechanism to describe the molecular basis of integrin $\beta 1$ control of cell polarity based on genetic analysis and summarised that $\beta 1$ integrins establish endothelial cell polarity via microtubules and that this is maintained via endosomal trafficking. The integrin signals via ILK, a lack of which is associated with a lack of microtubule organisation and defective endocytosis.

The second presentation in this session was given by Professor Andrew Leask (University of Western Ontario, Canada), who gave a talk entitled '*Integrin $\beta 1$ expression by fibroblasts is required for tissue repair in vivo*'. Professor Leask outlined studies carried out to investigate the role of fibroblasts in wound healing which is poorly understood at present. Work in his group has focused on the myofibroblasts, a specialised form of fibroblasts which promote tissue repair and scarring. He explained how they had investigated the role of the $\beta 1$ integrin in these cells, which is known to mediate cell attachment to numerous ECM molecules

including FN, laminin and collagens I, II and IV. Data was presented showing analysis of tissue collected from wound studies using fibroblast specific integrin $\beta 1$ knock out mice. Tissues were analysed by histology, immunohistochemistry, real time PCR and Western blot analysis. The deletion of the $\beta 1$ integrin subunit was found to be associated with reduced wound closure and ECM production. Reduced numbers of myoblasts were associated with $\beta 1$ knock out along with a reduction in cell spreading and cell adhesion. Professor Leask also showed data which highlighted how $\beta 1$ deficient fibroblasts displayed reduced levels of TGF- β activation. He went on to describe how the addition of active TGF- β alleviated the phenotypes of $\beta 1$ knock out mice, highlighting a crucial role for this integrin subunit in TGF- β mediated wound healing and a potential new method for controlling tissue repair *in vivo*.

Dr Jennifer Potts (University of York, UK) then gave a short talk entitled '*What do bacterial proteins tell us about the structure of human fibronectin?*' In her presentation, Dr Potts described studies investigating interactions between the cell binding domain of proteins produced by *Streptococcus pyogenes* and *Staphylococcus aureus* with human plasma/ECM FN. FN-binding proteins (FnBPs) produced by these bacteria are important as they facilitate the uptake of bacteria into human epithelial and endothelial cells. These studies were designed to elucidate how the protein recognises FN and why they use FN to invade cells. Data collected using X-ray crystallography, nuclear magnetic resonance and isothermal titration calorimetry revealed that the Streptococcal FnBP contains binding sites for at least 6 F1 modules identified within the N-terminal region of FN. Dr Potts described how the FnBP forms a tandem β -zipper along these sequential F1 domains to bind to FN. The protein also exploits its own intrinsic disorder to form high affinity interactions with different regions of FN, allowing high affinity binding of FnBPs to numerous FN molecules. Bacteria are then able to exploit FN internalisation, which occurs routinely during ECM turn-over via endocytosis, to invade human cells. Dr Potts proposed that bacteria FnBPs may disrupt polymerisation of plasma FN and subsequently exploit host cell activity to invade human cells in processes likely to be associated with infection.

The fourth presentation in this session was given by **Dr Clair Baldock** (University of Manchester, UK), who gave a talk entitled '*3D structure of collagen VI microfibrils and the role of metal ions in their conformation and assembly*'. Dr Baldock presented new data on the structure and function of collagen VI microfibrils which are found in skin, cornea, large blood vessels and cartilage. Mutations in this collagen have previously been linked to the heritable conditions Ullrich congenital muscular dystrophy and Bethlem myopathy. Collagen VI microfibrils are thought to link cells to the ECM although their precise role remains unknown. To this end, Dr Baldock described studies investigating the structure and function of the 10 consecutive von Willebrand factor (vWf) A-domains located in the extended N-terminal globular region of the $\alpha 3$ chain of the protein. Microfibrils extracted from bovine cornea were analysed by transmission electron microscopy using negative staining. A recombinant protein consisting of nine contiguous vWf domains was also expressed and analysed by small angle X-ray scattering. Data collected using these two techniques showed that the microfibrils formed a compact 'C Shaped' stable conformation. The results of studies investigating the function of these vWF domains within the protein were then presented highlighting that they interacted with the divalent cation Zn^{2+} . Data collected using quartz crystal microbalance with dissipation monitoring, analytical ultracentrifugation and multi angle laser light scattering showed that the protein was monomeric in solution. However, upon binding of Zn^{2+} a conformational change and oligomerisation was induced revealing that these domains within the $\alpha 3$ chain may play a crucial role in the function of collagen VI microfibrils.

Next, **Dr Brigit Leitinger** gave a talk entitled '*Trafficking defects and loss of ligand binding are the underlying causes of all reported DDR2 missense mutations found in Spondylo-metaepiphyseal dysplasia with short limbs and abnormal calcifications [SMED-SL] patients*'. SMED-SL is an autosomal recessive disorder characterised by short stature, short limbs, abnormal metaphyses and epiphyses and premature calcifications. Recently it was reported

that the cause of SMED-SL is missense mutations in the DDR2 gene. The DDR2 gene encodes discoidin domain receptor 2 which is a transmembrane tyrosine kinase that acts as a collagen receptor. To identify novel disease causing mutations in the DDR2 gene Dr Leitinger and colleagues isolated genomic DNA from patient peripheral blood leucocytes and sequenced through the gene exons and the gene flanking regions. This approach identified a novel missense mutation in the DDR2 gene, p.E113K, in two siblings. A second family with SMED-SL was found to have a previously reported mutation, p.R752C. To examine the molecular and cellular mechanisms of SMED-SL, DDR2 constructs containing the previously identified mutations, p.R752C, p.I726R and p.T713I and the novel missense mutation, p.E113K were generated and expressed in human cell lines. Western blot analysis demonstrated that all mutations resulted in a loss of collagen induced receptor activation. Immunocytochemical sub-cellular localisation of the mutant DDR2 showed that all the previously reported DDR2 mutations resulted in the retention of the receptor in the endoplasmic reticulum. However, the novel p.E113K mutant DDR2 appeared to be trafficked normally. To further analyse the consequence of the p.E113K mutation, recombinant DDR2 ectodomain was produced and its ability to bind collagen was investigated using solid phase assays. This study demonstrated that although p.E113K DDR2 is trafficked normally, it can not bind either collagen I or II and therefore fails to activate. Finally, analysis of the crystal structure of DDR2 confirmed that E113 is one of the residues that makes contact with collagen. Dr Leitinger concluded by saying that this work demonstrates that SMED-SL can result from at least two different 'loss of function' mechanisms: failure to traffic the DDR2 receptor to the plasma membrane and loss of the ability to bind ligand.

The penultimate talk in session 4 was given by **Dr Mark Morgan** (University of Manchester, UK) and was entitled '*Syndecan-4 phosphorylation: a molecular switch regulating integrin trafficking and focal adhesion dynamics*'. Dr Morgan began his presentation by discussing how the precise regulation of focal adhesion dynamics is essential for cell migration during both development and repair, as demonstrated by the fact that disruption of some integrin family members or syndecan 4 receptor engagement leads to wound healing defects. Dr Morgan went on to present data demonstrating that Src tyrosine kinase can directly phosphorylate tyrosine 180 in the cytoplasmic domain of syndecan 4. To investigate the role of phosphorylation at this site, phosphomimetic or phospho-null syndecan 4 mutants were expressed in syndecan 4 null fibroblasts, and focal adhesion turnover and cell migration were assessed by FRAP and scratch assay, respectively. This study demonstrated that phosphorylation of tyrosine 180 stabilised focal adhesions and disrupted cell migration. In addition, it was shown that Src dependent phosphorylation of tyrosine 180 in syndecan 4 reduced the activity of Arf6, and thereby suppressed the delivery of $\alpha 5\beta 1$ to the cell surface. Preventing the phosphorylation of tyrosine 180 activated Arf6 promoted the trafficking of $\alpha 5\beta 1$ to the cell surface, reduced trafficking of $\alpha v\beta 3$ and accelerated focal adhesion turnover. Dr Morgan concluded by suggesting that Src phosphorylation was acting as a molecular switch to target different integrin heterodimers to the cell surface in order to modulate focal adhesion stability and cell migration and that this may be an important regulatory event in wound healing and morphogenesis.

Professor Mark Ginsberg (University of California, San Diego, USA) closed the session with a presentation entitled '*The nexus of extracellular matrix and signal transduction*'. Professor Ginsberg began by discussing recent work from his laboratory in which physiological integrin activation had been recreated in vitro using purified $\alpha 11\beta 3$ integrin and liposomes or nanodiscs. This approach demonstrated that the integrin had to be embedded within a lipid bilayer for talin to be able to activate it. Moreover, when the integrin is embedded within a lipid bilayer, talin binding is sufficient to activate $\alpha 11\beta 3$ and neither integrin clustering nor kindlins are required. Professor Ginsberg went on to discuss the role of CD98 heavy chain in adaptive immunity. CD98 heavy chain is an 80KDa transmembrane protein that combines with one of several light chains to form a heterodimer. CD98 is known to have at least two functions within the cell membrane, amino acid transport and mediating integrin signalling. To determine the role of CD98hc in adaptive immunity it was conditionally deleted from B cells

using a CD19 cre mouse line. This deletion resulted in a reduced antibody response due to a loss of B cell proliferation and plasma cell formation. Rescue experiments using CD98hc mutants demonstrated that it was the integrin binding domain that was required for the normal antibody response and that the amino acid transport function of the protein was dispensable. Finally Professor Ginsberg presented data on the role of CD98 in proliferative vascular smooth muscle cells. Analysis of the expression of CD98 in the vasculature showed that it was present only at very low levels in normal vessels; however it was upregulated in the neointima of injured murine carotid arteries. To determine what function CD98 has in the vasculature it was conditionally ablated using a vascular smooth muscle cell (VSMC) specific cre recombinase (SM22alpha). This deletion did not effect normal vessel development or morphology; however neointima formation following injury was significantly reduced. Furthermore, loss of CD98 suppressed VSMC proliferation and induced apoptosis *in vitro* and *ex vivo*. As in B cell dependent adaptive immunity, rescue experiments with CD98 heavy chain mutants demonstrated that it is CD98 - integrin interactions that are required for VSMC survival. As the loss of CD98 selectively inhibits the proliferation of activated VSMCs in injury - and does not affect normal vessel morphology – it is a potential therapeutic target in vaso-occlusive disorders such as stent restenosis. Professor Ginsberg concluded by stating that the link between B cell adaptive immunity and injured vessel neointima formation is rapid cellular proliferation and that perhaps CD98s main biological role is integrin-dependent signalling promoting rapid cellular proliferation.

Session 5 – Vascular matrix in Pathology

Co-chaired by Dr Ann Canfield and Dr Gareth Hyde from the University of Manchester.

Session five opened with a talk from **Dr Sarah George** (University of Bristol, UK) in which she discussed how '*Matrix degrading metalloproteinases influence vascular smooth muscle cell behaviour and atherosclerosis*', with reference to novel animal models of plaque stability. Expression of the MMPs has been linked to rupture of atherosclerotic plaques due to destabilisation following MMP-driven degradation of ECM proteins. However, Dr George explained that broad spectrum MMP inhibitors fail to decrease plaque size *in vivo*. In a vascular setting it is the vascular smooth muscle cells (VSMCs) that secrete the bulk of the ECM proteins. It follows, therefore, that VSMC apoptosis is detrimental for plaque stability and, as Dr George went on to discuss; this process appears to be mediated by the cadherins (calcium dependant adhesion molecules). During VSMC apoptosis, N-cadherin fragments accumulate. Use of BB94, an MMP inhibitor, decreases this fragmentation suggesting the extracellular portion of N-cadherin is cleaved and inactivated by MMPs leading to loss of cell-cell junctions and apoptosis. Dr George's work has shown a role for MMPs -7, -9 and -12 in control of this process. The cleavage of N-cadherin and subsequent apoptosis is driven by MMP-7, with MMP-7 null mice maintaining full length N-cadherin and a decreased susceptibility to apoptosis in atherosclerotic plaques. Conversely, MMP-9 and -12 induce VSMC proliferation, also by cleaving N-cadherin. Dr George explained that this anti-apoptotic role for MMP-9 and -12 appears to be controlled via downstream signalling through the Wnt/ β -catenin pathway with a decrease in nuclear β -catenin observed in MMP-9 and -12 deficient cells. Dr George concluded her talk by reflecting on the divergent role of MMPs in atherosclerosis and stressed the importance of developing selective MMP inhibitors; a common sentiment throughout the meeting.

The second talk of the session was presented by **Dr David Abraham** (UCL, UK) who discussed '*The role of Nkx2-5 in atherosclerosis*'. Nkx2-5 is a transcription factor that plays a vital role in the development of the cardiovascular system by activating Col1 α 2 expression in vascular smooth muscle cells (VSMC). Nkx2-5 appears to play a role in the generation of atherosclerotic plaques, as evidenced by the use of a mouse model of atherogenesis. Dr Abraham described the generation of an ApoE^{-/-}/Col1 α 2-LacZ-Tg mouse which revealed an increase of Nkx2-5 in atherosclerotic lesions along with an overall increase in complex lesions. Through the use of this mouse, his group has shown that Nkx2-5 is almost exclusively expressed in type 1 collagen producing cells *in vivo*. Nkx2-5 appears to trigger a VSMC phenotype in these cells, including the expression of α -smooth muscle actin (α SMA), smoothelin and smooth muscle-myosin heavy chain. Dr Abraham explained that Nkx2-5 also

co-localises with type 1 collagen and α SMA, as predicted. Interestingly Nkx2-5 does not co-localise with macrophage markers, despite macrophage foam cells forming a major component of lesions. Nkx2-5 expression in human coronary artery atherosclerosis was also examined, revealing significant expression in the most advanced lesions. Dr Abraham went on to discuss Nkx2-5 expression in VSMC isolated from mouse aortas. Targeting Nkx2-5 with RNAi revealed a concomitant down-regulation of type 1 collagen in these primary cells, further strengthening the link between collagen expression and this transcription factor. Finally, Dr Abraham commented on the potential use of Nkx2-5 as a biomarker of plaque development and its dependant pathways as a rich repository of possible therapeutic targets.

The third talk of the afternoon came from **Professor Elena Aikawa** (Harvard, USA), titled '*Pathologic matrix calcification: insights from molecular imaging*' and focused on the clinical significance of cardiovascular calcification. Cardiovascular calcification is linked to atherosclerosis, dislipidemia, diabetes, chronic kidney disease and inflammation. Professor Aikawa explained that conventional clinical imaging techniques are only able to identify late stage cardiovascular calcification, by which time these lesions can become unstable increasing the risk of myocardial infarction and other clinically significant complications. With the use of NIR (near infrared) optical molecular imaging, however, she has been able to detect tissue mineralisation at the earliest stages of the disease. For example, NIR activity co-localises with Runx2 activity; a key transcription factor in osteoblast differentiation, and alkaline phosphatase; an indicator of osteoblast activity and bone deposition. By using a mouse model of hypercholesterolemia, early calcification was also shown to co-localise to areas of inflammation in the aorta. Professor Aikawa described further work using samples of human aortic valve stenosis which reveal co-localisation of calcification with inflammation and she suggested that these develop in parallel. She hypothesised that the presence of macrophages in these areas of inflammation may increase expression of cathepsin S, a protease responsible for the cleavage of elastin, releasing biologically active elastin fragments. Professor Aikawa finished her talk by describing some recent work quantifying the relationship between cardiovascular calcification and osteoporosis, which paradoxically appear to progress in parallel. It appears that early detection of calcification and the modulation of inflammatory pathways is vital for any potential treatment of either disease. Professor Aikawa concluded by revealing that this method of molecular imaging had been approved for use in humans, which will no doubt lead to more exciting work in this field.

Next, we heard from **Dr Tom Van Agtmael** (University of Glasgow, UK) who discussed his research revealing that a '*Col4a1 mutation in mice leads to defects in vascular function and blood pressure regulation associated with reduced blood volume and UPR activation*'. Type IV collagen forms a major component of the basement membrane, and mutations in the Col4 α 1 gene have been shown to cause a spectrum of eye, kidney and vascular defects including blood pressure deregulation, known as HANAC (**h**ereditary **a**ngiopathy with **n**ephropathy, **a**neurysms, and muscle **c**ramps) syndrome. Dr Van Agtmael described his use of the Col4 α 1^{+/-}Raw mouse, with a glycine to aspartic acid mutation, to study the effect of loss of Col4 α 1 *in vivo*. These mice are characterised by basement membrane defects in the descending aorta, cerebral haemorrhage at birth and abnormalities in nitric oxide-mediated vasodilation, causing hypotension. Interestingly, Dr Van Agtmael revealed that the phenotype could be rescued by the inhibition of nitric oxide synthase using L-NAME, reducing vasodilation and the potential for haemorrhage. He also touched on the possibility that mutant type IV collagen may induce the unfolded protein response (UPR) and revealed increased BiP, a marker of UPR, is expressed by Col4 α 1-mutant cells. Dr Van Agtmael concluded by suggesting that UPR may be implicated in other vascular function defects due to the variety of basement membrane proteins, like type 4 collagen, with a role in vascular biology.

The final talk of the session, and of the meeting as a whole, was from **Professor Francesco Ramirez** (Mount Sinai School of Medicine, USA) on '*Elastic fibres in aorta development, compliance and disease*'. Professor Ramirez discussed the role of the large glycoproteins fibrillin-1 and -2 in tissue integrity and the regulation of cell signalling. Fibrillins contribute to tissue integrity by forming the major component of microfibrils, which in turn form a sheath around elastin fibres. Their role in control of signalling events centres on the ability of fibrillins to sequester TGF β and BMP in the extracellular matrix. Professor Ramirez reviewed his previous work describing the characterisation of fibrillin mutations in both human patients and genetically engineered mice. Loss of fibrillin function leads to disease; for example, the

fibrillin-1 mutant mouse suffers impaired lung development, muscle hypoplasia, aortic aneurysm and mitral valve prolapse due to deregulated TGF β signalling. This mouse serves as a model for Marfan syndrome, a genetic disorder of the connective tissue. More recently, a mouse model of neonatal lethal Marfan syndrome has implicated MAPK signalling in disease progression also. Specifically, in the fibrillin-1 null mouse phosphorylated p38 MAPK accumulates before phosphorylated Smad2/3, the downstream signal transducers of TGF β receptor activation. Professor Ramirez explained that this event suggests signalling is first perturbed by improper cell-matrix interactions, triggering MAPK signalling, before being influenced by abnormal TGF β activation. Finally, Professor Ramirez described some recent work that further focuses on the role of fibrillin in matrix structure, revealing that, despite its role in mediating signalling events, fibrillin-1 is not vital for the cross-linking of elastin fibres. This work demonstrates additional discrete roles for components of elastin fibres, hinting at a further level of complexity in the control of vessel wall structure and integrity.