



## British Society for Matrix Biology - Autumn 2010 Meeting Report

September 6-7<sup>th</sup>: Inflammation Meets Matrix Biology  
Organised by Jelena Gavrilovic, University of East Anglia, Norwich

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### **Inflammation Meets Matrix Biology**

The main Autumn meeting of the BSMB was organized by Jelena Gavrilovic and held in the Thomas Paine Centre at the University of East Anglia (UEA) on 6<sup>th</sup>-7<sup>th</sup> September 2010. The meeting attracted 120 delegates and financial support was gratefully received from The Company of Biologists and the British Heart Foundation (Gold Sponsors); from the School of Biological Sciences at UEA, Roche and the International Journal of Experimental Pathology (Silver sponsors) and from AbCam, Exiqon, Lonza, Lavisio Biotec, Promega, R&D Systems, Qiagen and Sartorius Stedim (Bronze sponsors). The programme featured eleven plenary lectures and eleven short talks from submitted abstracts as well as two poster sessions. The BSMB Young Investigator Lecture was given by Adam Byron. Mark Fidock from Pfizer also gave an informative short talk about "Careers in Big Pharma". The meeting focused on the interplay between inflammation and matrix biology with expert speakers from the USA, Europe and the UK sharing new data emerging from cross-talk between these areas of interest. The organiser is very grateful to BSMB Treasurer, David Young, for his tremendous support as well as to Graham Riley, John Couchman, Jane Lohmann and other members of the BSMB committee for their input. Nicki Stead of UEA conference services also made an invaluable contribution both prior to and during the meeting.

The International Journal of Experimental Pathology generously sponsored three prizes of £150 and the posters were judged by the Plenary Speakers. The prize recipients were Ching-Yan Chloe Yeung (Manchester, Richard Kelwick (UEA) and Thomas Duncan (Cardiff).

#### Session One: Inflammation overview and markers of inflammation

The session was opened by **Professor Luke O'Neill** (Trinity College, Dublin) who provided an electric start to the conference with a presentation on 'New targets and processes in Toll-like receptor and NOD-like receptor signalling for anti-inflammatory therapeutics'. He argued that the revealing of multiple pathways involved in innate immunity had been the most important advance in the field of inflammation research since the turn of the millennium. Seven distinct receptor families have been identified and implicated in triggering the innate immune response. These receptor families include Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-I-like

receptors (RLRs). A number have been implicated in disease as our understanding of their structure and function has improved. For instance, contact nickel allergy is mediated by direct activation of TLR4 signalling independent of the LPS-binding domain. This raises the possibility of targeted therapy that does not interfere with LPS responses. Nalp3, of the NLR family has been implicated as a key receptor in Type 1 and 2 diabetes mellitus as well as atherosclerosis. As a result there has been resurgence of interest in the IL-1 system as a key driver of inflammation. The use of IL-1 receptor family members is providing information regarding the role of Th1, Th2 and Th17 cells in inflammatory disease.

In addition to the discovery of various receptors, there is now a good understanding of the major components activated by TLRs. This has led to the revelation that receptor-signalling systems are finally balanced with MicroRNAs emerging as key negative regulators. Prof. O'Neill went on to discuss miR-155 and miR-21 as key regulators of TLR4 signalling. MicroRNAs may provide new therapeutic targets and have the potential to be biomarkers of disease.

The second presentation was by **Dr. Charlotte Lawson** (Royal Veterinary College, London) on 'Identification of agnostic anti-ICAM-1 antibodies in serum from scleroderma patients'. Scleroderma (SSc) is characterised by circulating antibodies, including ones to endothelial cell antigens such as the intercellular adhesion molecule (ICAM)-1.

To establish whether SSc patients make antibodies directed against ICAM-1 she developed an ELISA to measure ICAM-1 antibodies in sera. She collected serum from 31 patients with diffuse SSc, 36 patients with limited SSc and 25 control patients. She used a human umbilical vein endothelial cell (HUVEC) system to assess whether these antibodies are pro-inflammatory. This was determined by the generation of reactive oxygen species (ROS), phosphorylation of MAPK Erk-1/-2 and cell surface expression of VCAM-1.

Dr. Lawson demonstrated that patients with diffuse and limited SSc had significantly elevated levels of anti-ICAM-1 antibodies in their serum. Anti-ICAM-1 antibodies from SSc patients caused cross-linking of ICAM-1 in the HUVEC system with significant increased ROS, Erk-1/-2 phosphorylation and VCAM-1 expression. Anti-endothelial cell antibodies targeting endothelial antigens, such as ICAM-1, is likely to contribute to disease progression and may serve as a biomarker of disease.

The third presentation was by **Dr. Thomas Cox** (Institute of Cancer Research, London) on 'Matrix remodelling and the pre-metastatic niche: Implications for lysyl oxidase'. Metastasis of a solid tumour to a specific secondary site requires preparation of that site for colonisation. Recruitment of non-malignant haemopoietic cells is key to the development of this pre-metastatic niche. Lysyl oxidase (LOX) catalyses the crosslinking of collagens and elastin in the ECM. Dr. Cox and colleagues used a combination of in vitro and in vivo models to study the role of LOX on CD11b<sup>+</sup> Bone Marrow Derived Cell (BMDC) mobilisation and recruitment to the pre-metastatic lung. In fibrotic lung tissue, as a result of bleomycin treatment, LOX expression was increased and this was found to be a pre-requisite for CD11b<sup>+</sup> BMDC mobilisation. The increased numbers of CD11b<sup>+</sup> cells resulted in upregulation of matrix metalloproteinase-2 (MMP-2) and further ECM remodelling. Inhibition of LOX reduces mobilisation, pulmonary recruitment and metastasis in vivo. Finally, he raised the possibility that LOX may be a therapeutic target for preventing cancer metastasis.

**Dr. Mark Fidock** (Pfizer Ltd., Sandwich) finished the session with a talk on 'Preclinical translational biomarkers for immune modulation'. He started by outlining the steps of drug development from basic science research through to clinical trials. The emphasis was on the length of time it took and attrition rate of products, which meant that most never made it to market (ratio 25:1). The cost of bringing a drug to

market currently stands at \$1Bn. Key issues are the interspecies difference during pre-clinical testing, toxicity and efficacy.

Biomarkers have an important role in this process. They have the potential to demonstrate proof of mechanism, drug activity, and disease burden. Biomarkers of pharmacology can demonstrate exposure of the site of action, binding to the target and be an expression of pharmacological action.

He then went on to provide two examples of biomarkers. The first used flow cytometry to identify a human eosinophil shape change used to monitor a histamine receptor. The second used 2' 5' oligoadenylate synthetase (OAS) to monitor viral load in patients with hepatitis C. OAS increases as viral load decreases.

Overall the use of biomarkers in drug development prevents late drug trial failures and stops ineffective drugs earlier. It also improves the public's faith in pharmaceutical companies.

#### Session two: ECM, Metalloproteinases & Inflammation

The afternoon session was opened by **Professor Bill Parks** (University of Washington, Seattle) who gave an excellent lecture entitled 'MMPs: Effectors of Immunity'. Prof. Parks highlighted the importance of MMPs as key effector enzymes with the ability to moderate a variety of immune responses in connective tissues, emphasising the crucial role of MMP knockouts in advancing our understanding of disease mechanisms. Matrilysin (MMP7) has an important role in the acute stage of the inflammatory response, with crucial roles in antibacterial defence and controlling transepithelial advancement and activation of neutrophils, with the shedding of syndecan/KC complex acting as a 'check point' for neutrophils activation. In contrast Stromelysin (MMP10) functions to moderate inflammation and immune functions via controlling the activated state of infiltrating macrophages. MMP10<sup>-/-</sup> mice exhibit greater macrophage influx to damaged pulmonary epithelium with an exacerbated immune response as the altered activation of macrophages in these knockout mice favours M1 (pro-inflammatory) macrophage polarity. The use of these disease models has greatly enhanced our understanding of such immune processes, showing MMP10 functions to promote M2 macrophage bias in M1 activated cells. In conclusion, both MMP7 & 10 function to control distinct opposing immune responses to both injury and infection.

The second presentation was given by **Dr. Ernst Poeschl** (University of East Anglia, UK) on the topic of expression of the Anxa5 gene, a novel marker for perivascular cells (PVC). The A5 gene modulates immune reactions and in high concentrations it has the ability to block macrophage phagocytosis and roles in chronic auto-immunity. The results of the group's in vitro study demonstrated the presence of PVC in 3D co-culture systems with HUVEC stimulated both cell survival and alignment, with PVC promoting the formation of neotubes in addition to changes in gene expression patterns in PVC. Hence the mutual communication of both cell types is central to cell survival and structural maturation of vascular structures.

The third presentation was by **Dr. Emmanuel Pinteaux** (University of Manchester, UK), reporting on the role of the ECM in neuronal injury and inflammation in an in vitro model of injury. The aim of the study was to investigate how adhesion of key ECM molecules regulates IL-1 induced astrogliosis in vitro. She highlighted the importance of Interleukin-1 which is capable of inducing both astrogliosis and neurotoxicity in brain ECM via MAPK, ERK and ROCK/ RhoA signalling cascades. The study concluded that the mechanism of IL-1 mediated neurotoxicity involved ERK 1 / 2 activity, RhoA de-activation and astrogliosis, which are all regulated by the ECM. Future work in this field should focus on the development of an in vivo mouse knockout model.

The session concluded with **Professor Sussan Nourshargh** (Queen Mary University of London, UK) discussing leucocyte transmission through venular walls. Our understanding of the mechanisms mediating leukocyte migration is currently limited. The present study reported areas within endothelial cell basement membrane where expression of Laminin 10 and Collagen IV were reduced and found to be associated with sites of IL-1 induced neutrophil migration. She concluded that monocytes utilise alternative transmigration mechanisms to neutrophils, giving us an insight into the complexity of leukocyte emigration through the venular basement membrane.

#### BSMB Young Investigator Award

The Young Investigator award lecture was given by **Dr. Adam Byron** (Wellcome Trust for Cell-Matrix Research, University of Manchester, UK) and was entitled "Quantitative proteomic profiling of adhesion receptor signaling".

Adhesion to the extracellular matrix is essential for a multicellular existence. Integrin adhesion receptors on the cell surface transduce signals that control cell shape, movement and differentiation and tissue integrity in various developmental, homeostatic and disease processes. Integrin-ligand binding is orchestrated by a combination of membrane-associated signalling complexes and mechanosensitive connections to the actin cytoskeleton. The binding of extracellular ligands to integrins causes allosteric changes in the receptors, integrin clustering and accumulation of membrane-associated signalling complexes. However, the extent to which different ligands stabilise different integrin conformations and recruit different adhesion complexes to transduce specific signals is not known. A major challenge in cell-adhesion research is to understand how ligand engagement of integrins can elicit a specific cellular response and how this is controlled by the composition and dynamics of adhesion complexes. Such an understanding will provide important insights into the molecular mechanisms that regulate cell morphology, migration, differentiation and survival.

The ligand-dependent, allosteric nature of integrin activation and signalling-complex recruitment makes isolation of adhesion complexes difficult. In addition, like other membrane receptor-associated signalling complexes, adhesion complexes have been refractory to proteomic analysis due to their lability and inaccessibility. To study at a global level the signalling events mediated by integrins, Dr. Byron and colleagues developed a methodology for the isolation and mass-spectrometric analysis of stabilised, ligand-induced integrin adhesion complexes. Using a bead-based affinity-purification approach, they specifically and efficiently isolated adhesion complexes, which were then catalogued and quantified by mass spectrometry-based proteomics.

Initial studies were focussed on protein complexes induced by two structurally related integrins,  $\alpha_4\beta_1$  and  $\alpha_5\beta_1$ , and their ligands, vascular cell adhesion molecule-1 (VCAM-1) and fibronectin (FN), respectively. In contrast to previous reports of up to 180 molecules associated with sites of integrin-mediated adhesion in a hypothetical "adhesome", Dr. Byron identified 185 proteins specifically enriched to ligand-engaged  $\alpha_4\beta_1$  and 406 proteins specifically enriched to ligand-engaged  $\alpha_5\beta_1$ . Bioinformatic analysis revealed distinct "flavours" of heterodimer-specific  $\beta_1$ -integrin complexes induced by different ligands and a surprising difference in scale and composition. Moreover, regulator of chromosome condensation-2 (RCC2), a molecule not previously linked to integrin signalling, was identified in  $\alpha_5\beta_1$ -FN complexes but not  $\alpha_4\beta_1$ -VCAM-1 complexes. Interaction network analysis highlighted RCC2 at a position of intersection between  $\beta_1$ -integrin, Rac1 and Arf6 sub-networks. Intriguingly, reduction of expression of RCC2 by RNA interference enhanced Rac1 and Arf6 activity during cell spreading on FN, suggesting that RCC2 acts as an "activity limiter"

for these small guanosine triphosphatases. Importantly, depletion of RCC2 also reduced persistent cell migration along FN fibres, demonstrating a regulatory role for RCC2 in directional cell movement.

Summarising more recent work, Dr. Byron reported the isolation of complexes associated with multiple integrin receptor–ligand pairs and the cataloguing of their proteomes at multiple time points. Quantitative, comparative analyses defined the temporal profiles of a core integrin-associated network and several receptor-specific sub-networks during the initial stages of cell adhesion. Bioinformatic interrogation of hierarchically clustered proteins revealed distinct temporal dynamics of protein modules relevant to cell-adhesion processes. Integrin subunits increased in abundance in adhesion complexes over a 30-minute time course and were grouped by clustering analysis with many known integrin-associated proteins and some components not previously linked to integrin signalling. The development of this proteomic workflow now allows the molecular dynamics of adhesion complexes to be measured directly and presents an entry point for quantitative, systems-level analyses of adhesion signalling in health and disease.

### Session three: Novel aspects of cell adhesion/migration in inflammation

This session started with a talk from **Professor John Couchman** (Copenhagen, Denmark) on 'Inside-Out Activation: A Look Beneath the Surface'. Professor Couchman initially introduced his subject by highlighting the importance of regulation of integrin activation for cell adhesion and migration. These processes are of particular relevance to a functional immune system where inside-out activation of integrins plays a vital role. He next introduced us specifically to RhoA through its requirement in cytokine and protein kinase C mediated integrin activation. This interaction of cytokines with their receptors is an example of this inside-out activation of integrins.

In the study discussed by Professor Couchman, K562 cells were used to examine the mechanisms behind short-term phorbol ester treatment activating the sole fibronectin receptor in these cells ( $\alpha 5\beta 1$ ). This specific stimulator thus mediated adhesion and spreading on fibronectin by K562 cells. In these cells integrin activation is under strict regulation that has been linked to protein kinase C and RhoA in downstream processes.

Professor Couchman clarified how RhoA has been associated through both siRNA studies and also in conditional knockout mice, in which adhesion of cytokine activated thymocytes is compromised. Levels of RhoB in these cells were increased, indicating RhoB cannot compensate for the absence of RhoA. Consequently he then explained how it has become important to investigate the specific connections between protein kinase C and RhoA.

RhoGDI, which may target and sequester Rho GTPases, was initially identified as being targeted by conventional isoforms of protein kinase C. The serine 34 residue of RhoGDI, which faces onto the switch region of RhoA, was defined as the key residue and can be phosphorylated when bound to RhoA, Rac1 or cdc42. Indeed this residue was found to have selectively increased affinity for RhoA, in comparison to other GTPases, when phosphorylated. In vivo FRET confirmed this in a neat experiment where by mutating the serine 34 residue to form a phosphomimetic form of RhoGDI, resulted in a decrease in affinity for RhoA, thus increasing the GTP loading of RhoA. Thus, Professor Couchman concluded that this study demonstrates that RhoA and protein kinase C pathways are indeed linked by a protein that is known to regulate the availability and function of GTPases.

The second talk of the session was given by **Dr. Alicia Arroyo** (Madrid, Spain) entitled 'Beyond Extracellular Matrix Degradation: New Functions of MT-MMPs in Leukocyte Biology'. Dr Arroyo began her presentation by explaining that not only do

MMPs degrade extracellular matrix (ECM) components but also modulate the bioactivity of soluble factors and transmembrane receptors. She then highlighted two such examples, MT1-MMP anchored to the plasma membrane by a transmembrane and also MT4-MMP which is anchored via a GPI link. Dr Arroyo explained that these two MT-MMPs have been shown to be involved in tumor invasion and metastasis. However, recently her group has linked the two to inflammatory contexts such as monocyte transmigration and angiogenesis induced by agents such as MCP-1/CCL2, nitric oxide and PGE2.

Their group had used proteomic methods in combination with wider ranging studies to understand the further functions of these two MT-MMPs in monocyte and macrophages. As a result Dr. Arroyo has identified possible MT1-MMP cellular substrates in myeloid lineage cells, specifically examining MT1-MMP function, in regulating macrophage motility along their fusion into osteoclasts or into inflammatory giant cells. She links this activity to the cytosolic tail of MT1-MMP to provide optimal Rac1 activity, mediated via interaction with the adaptor protein p130Cas. Further to this Dr. Arroyo discussed MT4-MMP, which is a poor degrading agent, in mice where the gene is knocked out the macrophages were found to be larger and more elongated.

In conclusion Dr. Arroyo suggested that her findings are indicative of MT1-MMP playing a role in inflammatory processes, a function which is independent of any role in degrading ECM.

The final talk of this session came from **Dr. Mikala Egeblad** (Cold Springs Harbor Laboratories, USA) whose talk was entitled 'Matrix metalloproteinases as Stromal Regulators of Cancer Invasion and Therapy Responses'. Dr Egeblad started by putting us in the picture in terms of communication between cancer cells, stromal cells and extracellular matrix, which make up solid tumors, contributing to progression. She then outlined how her studies involved using intravital microscopy to study tumor micro-environment in live mice.

Dr. Egeblad further discussed how type 1 collagen expression in metastatic cancer becomes dramatically increased. Indeed myeloid cells were found to migrate the most through tumor areas with dense collagen, Col1A1 increasing the metastasis risk, and linking Col1A1 to cancer progression. She also demonstrated using some fantastic imagery how MMP14 (MT1-MMP) is expressed on multiple slow migratory stromal cells at the front of invading epithelia in developing mammary glands. Even more interestingly she managed to show how her group have linked this expression to the ability of MT1-MMP mediated collagen cleavage to control epithelial invasion in mammary gland development.

To further assess this function a mouse model of metastatic mammary carcinoma, MMTV-PyMT, was crossed with mice with MMP-resistant collagen. Intriguingly the results of this demonstrated that metastasis to the lungs was lowered however tumour size was not affected. This suggested that MMP-specific remodeling of the collagen scaffold being important in regulating epithelial invasion in mammary development and cancer.

Following on from this Dr. Egeblad discussed treating the MMTv-PyMT mouse at different points of tumour progression with doxorubicin, a chemotherapeutic drug. Despite this being a drug that targets proliferating cells the difference between response to doxorubicin in early and late carcinoma stages did not correlate with cancer cell proliferation. In contrast from live imaging she could see that vascular leakage of injected drugs into tumor tissue was elevated during the carcinoma stages in comparison to hyperplasia. Additionally increased myeloid cell infiltration was associated with this effect regulated by MMP9 expressed by these cells. MMP-9 has the ability to limit the bioavailability of TGF- $\beta$  thus increasing how leaky the vasculature was. Finally she showed how high numbers of myeloid cells had been recruited to tumors, which responded to treatment.

Dr. Egebald therefore concluded that due to the wide-ranging roles of myeloid cells in tumor progression, the interactions described in her study between chemotherapy and innate immune cells could feasibly have implications in the clinical treatments.

#### Session Four: BSMB Open Session

The first talk of session four was delivered by **Dr. Andrew Doyle** (Laboratory of Cell and Developmental Biology, Bethesda USA) in which he addressed the stimulating debate regarding the use of 2-dimensional cell culture systems in comparison to 3-dimensional model systems, which arguably may be more representative of in vivo scenarios. He addressed these points by bringing a further complexity to the debate by examining a 1-dimensional cell migration system. His aims were to further investigate the role of matrix topography for migrating cells in a 3-dimensional ECM.

Dr. Doyle initially hypothesized that the distinctive characteristics of 3D cell migration may be explained by fibrillar ECM topography providing a physical basis for migration.

Along with his fellow authors he managed to develop microphotopatterning ( $\mu$ PP), which can be used to create thin films that in turn produce single micropatterned lines which are nearly one-dimensional. Using these films he managed to re-create the cellular phenotypes induced by 3-D cell derived matrices. Additionally cell migration was shown to be rapid, uniaxial, myosin II contractility dependent and independent of ECM ligand density in 1D and 3D systems.

From the talk Dr. Doyle depicted how the migration dynamics of the leading edge of fibroblasts can be enhanced by the 1D fibrillar micropatterns. These dynamics are also influenced by the spatial positioning of cell adhesions in both the lateral spacing and proximity to the leading edge. Furthermore these 1D fibrillar patterns have a novel focal adhesion component arrangement, which is also vital for efficient cell migration.

The overall finding led to the idea that the migrating cells in 3D fibrillar matrices are doing so through a 1D mechanism, which cannot be mimicked, by 2D matrices. Overall Dr Doyle's findings suggest that the physical structure of ECM is important in directing cell migration whilst orientated 3D matrices allow fibroblasts to align, polarize and migrate directionally. Mechanistically this involves using 1D fibril regulation of actomyosin networks. This regulation is thought to modify cellular axial phenotype and promote proficient directional protrusion.

The first short talk of session four was presented by **Katarzyna Gawel** from Cardiff University who gave a fantastically engaging talk on TMEFF2 is a novel substrate for type 2 transmembrane serine proteases. Katarzyna informed us how TMEFF2 (transmembrane protein with EGF-like and two follistatin motifs 2) could be a potential target in prostate cancer due to its presence in normal prostate gland and prostate cancer cells. She introduced us to the relevance of this target by explaining that although its role in prostate cancer development is unknown, previous studies have demonstrated that its ectodomain is released from the cell surface. This release has been linked to the action of the ADAM family (10 and 17) whilst the retained fragment can be processed by  $\gamma$ -secretases. She therefore hypothesised at the start of her investigation that TMEFF2 may be enzymatically regulated, particularly those involved in prostate cancer. The specific ones, which she proposed to investigate, were type 2 transmembrane serine proteases (TTSPs), GPI anchored serine protease prostasin and further ADAMs (ADAM9, 12 and 15).

Through her studies we could see that matrilysin and hepsin can significantly increase AP-TMEFF2\_ECD shedding from HEK293 cells over-expressing AP/V5 tagged TMEFF2. Subsequent western blots of cell lysates demonstrated that TTSPs generate different TMEFF2 fragments when compared to ADAM mediated cleavage, when these cells were transfected with these enzymes. She hypothesized that this may be indicative of a diverse biological activity of these two enzymes due to

different cleavage sites in TMEFF2. Indeed when matrilysin-2 is over-expressed TMEFF2\_ECD release is increased in a process which can be down regulated by inhibitors of metalloproteinases. However Katarzyna suggested this may be due to activation of G protein-coupled receptors, e.g. protease-activated receptors (PARs) and ADAMs and not due to direct TMEFF2 cleavage. Cells transfected with ADAM9 and ADAM12 also displayed increased TMEFF2 cleavage.

She concluded with the further aim of understanding the biological activity of TMEFF2-ECD fragments in combination with the cellular location of the cytoplasmic fragment.

Next, **Dr. Simon Tew** (Faculty of Veterinary Sciences, University of Liverpool, Cheshire, UK) discussed the regulation of SOX9 by chondrocytes. SOX9 is an important transcription factor whose expression by chondrocytes is essential for the function of the cell and the regulation of the extracellular matrix genes. The aim of the study was to show whether the turnover of SOX9 mRNA was also controlled by chondrocyte phenotype and to identify the mechanisms regulating this process. Human Articular Chondrocytes (HAC) or human bone marrow derived stem cells (BMSCs) were cultured in standard 14 day chondrogenic pellet conditions containing TGF $\beta$ 1+/-BMP7. All mRNA quantification was performed using Real Time qRT-PCR (quantitative Reverse Transcription -Polymerase Chain Reaction). Small interfering RNA (siRNAs) targeting RNA binding proteins (RNBP) were transfected into SW1353 chondrosarcoma cells and effective protein knockdown assessed by western blotting before SOX9 mRNA and protein levels were measured. The results showed that there was an increment in the  $t_{1/2}$  of SOX9 mRNA in HACs (level decreased) in comparison to BMS cells which haven't been affected. In addition the siRNA knockdown of the RNBP tristetruprolin (TTP) caused SOX9 mRNA and protein levels to increase in SW1353 cells, which give evidence that SOX9  $t_{1/2}$  can be controlled by the RNBP TTP. These findings represent a novel means for chondrocyte ECM regulation via post transcriptional control of SOX9.

The third presentation came from **Dr. Tracey Swingler** (Biological Sciences, University of East Anglia, Norwich, UK). She started with overview knowledge of endochondral ossification in skeletal bone with a description about the mesenchymal cells aggregation and differentiation, and the key regulators controlling both processes including gene expression regulators MicroRNAs (miRNAs). The aim was to profile the expression of miRNAs in a cell model of chondrocyte differentiation, verify expression of key miRNAs in vivo and investigate functions.

Murine embryonic carcinoma cell line (ATDC5) was induced to differentiate through chondrogenesis in vitro, and the expression of the miRNAs and its regulator were verified using in situ hybridisation. Cell differentiation was verified via GAG staining and measurement of markers (e.g. type III X collagens) was done using the qRT-PCR. The results suggest that some microRNAs -23, were strongly regulated during chondrocyte differentiation. Interestingly, cartilage specific genes (miR-140 and miR-455) did have the potential to modulate Smad signalling (signal transduction molecules) in the growth plate and to function as regulators of chondrocyte proliferation and hypertrophy during endochondral ossification. The increase in expression of miR-140 and miR-455 in human osteoarthritic cartilage may reflect or regulate the recapitulation of the chondrocyte developmental programme which may contribute to pathogenesis of osteoarthritis.

A very interesting presentation was made by **Louise Kung** (Trust Centre for Cell Matrix Research, University of Manchester, Manchester, UK), who started explaining the role and effect of mutated extracellular matrix (ECM) proteins on the intracellular

processes in a number of connective tissue diseases. In some of these diseases (e.g. chondrodysplasias), endoplasmic reticulum (ER) undergoes stress leading to the activation of the unfolded protein response (UPR). This project was design to investigate the generic role of the ER stress and the UPR in the pathogenesis of these diseases models. The expression of Tg<sup>co9</sup> (the cog mutant form of thyroglobulin) in newborn transgenic mouse line was measured using immunohistochemistry technique. The results show that Tg<sup>co9</sup> expression did induced ER stress leading to the activation of the UPR by increasing the luminal binding protein (BiP) expression (i.e. key marker of the UPR activation), and this did play a vital role in proliferative zone defects in mice with the transgene display causing mild chondrodysplasia.

**Professor Andrew Newby** (Bristol Heart Institute, UK) started by giving an epidemiological statistics data about the prevalence of cardiovascular related death in the UK, addressing that more than a quarter of population had been suffering from Myocardial Infarction (MI) in the last decade. MI considered being one of the leading causes of death in the developed world prior even to Cancer. He explained how MMPs (Matrix Metalloproteases) and TIMPs (Tissue Inhibitors of Metalloproteinases), (type 3 in particular) play their role in the pathogenesis of cardiovascular diseases by inhibiting the development of plaque, promote fibrosis and encourage cell apoptosis (e.g. smooth muscle cells)i.e. they got an anti-inflammatory stimulation.

Different strategies have been used to minimize those effects. One of them was the usage of selective inhibitors of MMPs, influencing a variety of MMP and TIMP expression, leading to slow down the progression of plaques, by preventing apoptosis of foam cells and reducing calcification. Foam cells are derived from both macrophages and smooth muscle cells. They are not dangerous as such, but can become a problem when they accumulate at particular foci thus creating a necrotic centre of the atherosclerosis, result in ischemia, and contribute to myocardial infarction. There is a high selective subgroup of MMPs (e.g. MMP-14) that could be associated with the activation of monocytes and macrophages by inflammatory mediators.

With a percentage of high MMP14, and low TIMP-3 (MMP14hiTIMP-3lo), foam cells that are derived from macrophages, turned to be highly invasive, more proliferative, extremely harmful to the matrix and prone to high rate of apoptosis. The data from Newby Lab. did show that the prevalence of MMP14hiTIMP-3lo macrophages in human plaques characterises plaques vulnerability to rupture, leading to showering of emboli in the blood stream. With a percentage as high as 45% of MMP-14, and as low as 45% of TIMP-3, patients would have the liability to suffer from adverse cardiovascular events including death. In conclusion, the balance between MMP-14 and TIMP-3 is clearly a matter of life or death.

#### Session five: Cell Signalling in inflammation

Session 5 was opened by a presentation from **Professor Liliana Schaefer** (University of Frankfurt, Germany). In this lecture, Professor Schaefer discussed how the ECM could convey pro inflammatory signalling pathway. It was very interesting to show how the tissue stress or injury is monitored by the innate immune receptors, detecting the release of a small leucine-rich proteoglycan called biglycan and turning this signal into an a robust inflammatory response. Biglycan could trigger the innate immunity through the interaction with Toll like receptor (2 and 4), and

purinergic P2X<sub>4</sub>/P2X<sub>7</sub>, leading to the activation of the inflammasome and IL-1  $\beta$ . This results in rapid activation of p38, Erk and NF- $\kappa$ B, thereby stimulating the expression of TNF- $\alpha$  and macrophage inflammatory protein-2 (MIP-2). The macrophage does not normally express biglycan, but the expression could happen as result of the pro inflammatory factors like IL-1, with the production of IL-1 could happen as a result of dual triggering for both toll like receptors ( 2 and 4 This leads to the activation of IL-1 beta gene transcription and the nucleotide –binding oligomerization domain (NOD) like receptor for the activation of inflammasome. It is a very complex process. There was a very nice conclusions shown at the end, with a diagram illustrating all pathways concluded form these experiments.

Lecture 2 of the session was presented by PhD student **Laura Evan** (Cardiff University, UK). In this talk, Pre-B Cell Colony-Enhancing factor (PBEF) was discussed as a possible therapeutic target in treating patients with rheumatoid arthritis (RA). She showed how the effect of blocking PBEF factor on the progression of RA and found that the use of APO866 (PBEF blocker) as a therapy to prevent cartilage destruction in RA. Experiments were performed in vitro, ex vivo and in vivo. Western blotting and qPCR images shown explained the effect of pro-inflammatory cytokines (IL-1 $\beta$ , TNF- $\alpha$  and OSM) on the PBEF expression in a time and dose dependent manner. There was an upregulation of MMP-3 in fibroblast, an effect counteracted by pre treating cells with APO866. EX vivo studies explained the effect of APO866 and IL-1 beta treatment on bovine cartilage explants cultures and showed a reduction in MMP activity using the zymography technique and prevention of proteoglycan loss in cartilage, which was assessed by Safranin-O staining and DMMB activity. Using the arthritis induced model (collagen induced arthritis) in mouse paws, there was a an increase in m RNA expression of MMP mRNA (1a, 3, 9 and 13) and MMP Sense 750 flourescence compared to non arthritic models. In pilot experiments, animals treated with APO866 showed significantly reduced MMP m RNA expression and retarded proteoglycan loss. Images of cartilage induced arthritis model displayed in the presentation explained the protective effect of APO866 using MMPsense 750 probe fluorescent imaging experiments.

The third talk was presented by **Dr. James Whiteford** (Queen Mary School of Medicine, UK), with a title Syndecan -2core protein directly interacts with the protein tyrosine phosphatase CD148/PTPRJ to promote integrin mediated adhesion. Syndecans are multifunctional heparin sulphate proteoglycans that are involved in many cellular processes, such as migration, cell adhesion and growth factor interaction. The heparin sulphate chain of syndecan interacts with growth factor, ECM matrix and inflammatory mediators, while it has been found that the extracellular parts of these molecules possess adhesion regulatory domains, which are involved in cell migration and angiogenesis. These latter processes are characteristic of cells of mesenchymal origin as wells as leukocytes and  $\beta$ 1 integrin is essential in this process. However, the exact mechanism and the molecular requirements were discussed in this presentation. Protein tyrosine phosphatase CD148/PTPRJ is involved in this process and found that this signalling pathway is mediated by PI3K kinase. It was also possible to show that p85 subunit of PI3Kinase is a substrate of protein tyrosine phosphatase CD148/PTPRJ. It was nicely shown how protein tyrosine phosphatase CD148/PTPRJ distinguish between syndecan -2 and 4, with competitive inhibition experiments were explained the importance of N-glycosylation of protein tyrosine phosphatase CD148/PTPRJ is important to discriminate between syndecan-2 and -4. Solid phase binding assay and immunoprecipitation elucidated the protein to protein interactions.

There was a nice presentation given by PhD student **Douglas Dyer** (Manchester University), entitled TSG-6 binds to CXCL8 and modulates its activity. TSG-6 is expressed during inflammation and especially in the synovial fluids of patients with RA. There was a nice hypothesis put for this, explained that there are a lot of literature search on the involvement of TSG-6 in the protease activity, inhibition of neutrophils extravasation, with a reduction in cartilage damage together with decreased MMP activity in mice model with antigen induced arthritis, but the potential mechanisms behind this protective response is not clear. There was a new technique used called plate based assays and found that CXCL8 interacts with TSG-6 via its link module at different PH levels. The measurements obtained from Surface plasmon resonance technique further supported this interaction. Endothelial cell line EA were used and when these cells treated with CXCL8, there was a 50% increase in the production of MMP-9 activity, which is involved in the angiogenesis and the cartilage damage of RA. This effect was reversed when endothelial cells were pretreated with both Link\_TSG6 the full-length protein, which may suggest its protective action in RA.

This session was concluded by **Professor Drew Rowan** (Musculoskeletal Research Group, Newcastle University). He talked about inflammatory signals in arthritis-the perils of cross-talk. He introduced his talk by explaining the amazing ECM of the cartilage and all work done in cartilage research showed that aggrecan and collagen molecules are the known possible targets for treating patients with RA and OA. There was a nice table presented explained the differences in both OA and RA. One of these differences is that in OA, the progression of the disease condition is slow while in RA, the process is rapid. There are proteolytic degradation of cartilaginous collagen in both OA and RA. The key elements in this process is to tackle the cytokines, such as IL-1 $\beta$  and TNF- $\alpha$ . In this study, oncostatin and IL-1 $\beta$  was used as a model to induce the expression of matrix metalloproteinases and collagenases, which are responsible for cartilage degradation. Data presented showed that protein kinase pathway is important in this process and different isoforms of PKC are involved depending whether the stimulus is simple or combined. This may indicate that through understanding of the cell signalling pathway associated with inflammation in any disease condition is quite useful before new therapies will be approached. The presenter was able to show that Akt signalling pathway is also involved and he supported his conclusions by silencing experiment done in human articular chondrocytes and using different inhibitors for these processes. At the end there was a nice diagram illustrating the differential expression of MMP1 and 13 and different isoforms account for this.