

The Joint BSMB/Biochemical Society Focused Meeting, “Matrix Turnover – Mechanisms and Common Denominators” took place at the Atrium and Pennine Lecture Theatre, Sheffield Hallam University, on April 2<sup>nd</sup>/3<sup>rd</sup>, 2007. The meeting was organised by Dave Buttle with assistance from the Biochemical Society secretariat, in particular Sharon Vivers. Sponsorship for the meeting was gratefully received from AstraZeneca, The Ipsen Fund, British Heart Foundation, VWR International, BMG Labtech, International Journal of Experimental Pathology and Biochemical Society Transactions. There were 7 recipients of BSMB bursaries. Reporter Bursaries went to Martin Reid (Sheffield Hallam University), Gavin Jones (Cambridge) and Chidi Molokwu (University of Sheffield). Presenter Bursaries were received by Jumi Adeniji (University of Sheffield), Helen Fielder (Oxford), Darren Plumb (Manchester) and Siyuan Li (Cardiff) After a vote by the delegates, three IJEP-sponsored poster prizes were won by Claire Clarkin and Ben Wheeler (both from the Royal Veterinary College) and Darren Plumb (Manchester).

Just over one hundred delegates attended, providing between them 44 posters and 19 oral presentations. The meeting got under way with a session on General Topics, chaired by Bruce Caterson (Cardiff). **Geoff Laurent** (UCL) kicked off the meeting with a lively presentation entitled “The regulation of matrix turnover: fibroblasts, forces, factors and fibrosis”, addressing the regulation of ECM turnover and focusing upon the formation of fibroses. He began by stating his belief that mechanisms of matrix turnover should be re-addressed. Identifying hypoxia, cytokines, coagulation and mechanosensing cascades as factors driving fibrosis, he suggested that each of these cascades activated a common final pathway involving myofibroblasts. TGF $\beta$  was identified as a central pro-fibrotic cytokine. The induction of TGF $\beta$  by the mechanical stretching of fibroblasts was described. He then explained how, in a bleomycin-induced mouse fibrosis model, the induction of TGF $\beta$  and collagen deposition were attenuated in neutrophil elastase and  $\alpha_v\beta_6$  integrin null mice. The neutrophil elastase-induced  $\alpha_v\beta_6$ -mediated activation of TGF $\beta$  in mouse epithelial cells was described and a role for both in an inflammation-mediated fibrosis was suggested. He then discussed coagulation cascades, identifying components such as thrombin and fibrinopeptides as promoters of fibroblast proliferation, and reporting that thrombin inhibitors blocked fibrosis in the mouse bleomycin model. He also showed that mice deficient in PAR (protease activated receptor)-1, a substrate of coagulation proteases, were also protected in the bleomycin model and possessed a reduced level of TGF $\beta$ . Furthermore Dr. Laurent showed that a PAR-1 agonist could induce  $\alpha_v\beta_6$ -mediated activation of TGF $\beta$  only in  $\beta_6$ -positive epithelial cells. He concluded that both inflammatory and coagulation cascades could initiate tissue damage and remodelling via the activation of TGF $\beta$ .

**Ann Canfield** (Manchester) (The serine protease HtrA1 regulates physiological and pathological matrix mineralization) described her work on this protease and its identification in a screen of cells undergoing osteogenic differentiation, its serine protease activity and its ability to bind and inhibit the signalling of TGF $\beta$ -family members. She then proposed that HtrA1 regulated matrix mineralization, referring to the expression of HtrA1 at sites of mineralization in human femoral arteries, the up-regulation of HtrA1 during vascular smooth muscle cell, pericyte and osteogenic differentiation and its marked down-regulation following mineralization and how, in osteoblasts, HtrA1 overexpression inhibited, and RNAi knockdown enhanced, mineralization *in vitro*. Dr. Canfield described experiments using recombinant HtrA1 possessing or lacking the C-terminal PDZ domain. She reported that HtrA1 attenuated vascular smooth muscle cell and osteoblast mineralization, an activity that required the PDZ domain, and that overexpression of HtrA1 inhibited BMP1-induced mineralization of osteoblasts. She also reported that HtrA1 can cleave decorin, fibronectin and matrix glial protein (MGP) and that of these only MGP cleavage required the PDZ domain.

**Anthony Day** (Manchester) began his talk on “The molecular basis of inter- $\alpha$ -inhibitor heavy chain transfer onto hyaluronan” by stating that most of the functions of hyaluronan (HA) arose from its interactions with protein ligands. He then used the expansion of the cumulus cell-oocyte complex (COC) during ovulation – a process driven by HA synthesis – as an example of the importance of HA-adherin interactions. Referring to gene knockout experiments that indicated that the absence of tumour necrosis factor-stimulated gene-6 (TSG-6), pentaxin-3 (PTX-3) or bikunin [a component of inter- $\alpha$ -inhibitor (I $\alpha$ I)] prevented COC expansion, he identified TSG-6 as having a central role in cumulus matrix stabilisation. Dr. Day explained how the binding of TSG-6 to both PTX-3 and HA enabled the formation of large HA aggregates and how the covalent attachment of heavy chains (HC) of I $\alpha$ I to HA enabled HA cross-linking via HC-HC interactions. He proposed that TSG-6 acted as a cofactor and catalyst in the transfer of HC to HA, acting as an intermediate by accepting a HC then transferring it to HA in a series of transesterification reactions. Data was presented that the predicted intermediates could be visualised during *in vitro* reactions and that in long-term reactions the amount of HC-HA was approximately 20-fold greater than the amount of TSG-6 present, suggesting it acted as a true catalyst. Dr. Day then described mutagenesis studies indicating that the HA binding site within the TSG-6-HC complex was distinct from that of TSG-6 alone, and suggested that a composite binding/transfer site is formed in the TSG-6-HC complex. Finally, the requirement of Mg<sup>2+</sup>/Mn<sup>2+</sup> in the formation of TSG-6-HC and HC-HA and identification of two Mg<sup>2+</sup> binding sites within the CUB module of TSG-6 were described, one of which Dr. Day reported to be involved in the formation of TSG-6-HC complexes.

**Marie Plainfosse** (Sheffield) talked about the “Influence of the extracellular matrix on the mechanical properties of tissue engineered cartilage” and began by discussing the potential uses of engineered cartilage and the current limitations of such constructs. She then stressed the importance of characterising the tribological properties (friction, lubrication, wear) of such constructs when considering their potential usefulness as a replacement for biological tissue. She highlighted the importance of the architecture and composition of tissue to its mechanical properties, and then described how indentation and friction tests indicated that, despite containing type II collagen and similar amounts of proteoglycan to native cartilage, engineered cartilage appeared immature, possessing less resistance to deformation and low frictional properties. Dr. Plainfosse concluded that advanced constructs with properties closer to native cartilage must be developed before clinical application.

The second session focussed on the Intervertebral Disc and was chaired by Tony Freemont (Manchester). **Judith Hoyland** (Manchester), in her presentation “Matrix synthesis and degradation in human intervertebral disc degeneration” summarised the economic and clinical cost of chronic lower back pain and suggested that, whilst causes of chronic lower back pain were multifactorial, the majority of cases involve the intervertebral disc (IVD). This was followed by an overview of the IVD structure, highlighting the low tissue cellularity and the importance of type II collagen and aggrecan within the nucleus pulposus (NP) to proper disc function. Dr. Hoyland stated that IVD degeneration commenced from the second decade of life and was generally characterised by changes in the NP such as reduced swelling pressure, dehydration and fissures, resulting in a reduced ability to dissipate load. She reported that the biochemical changes associated with such degeneration included decreased aggrecan and type II collagen, increased type I collagen, versican, low molecular weight proteoglycans (eg decorin and biglycan) and an increased expression of matrix degrading enzymes such as MMPs and ADAMTS aggrecanases. She presented data indicating that in IVD degeneration there was a net increase in these enzymes over their natural inhibitors (TIMPs) and suggested that this

represented a dysregulation of the normal homeostatic mechanism. Investigations on the role of IL-1 in IVD degeneration were shown, and Dr. Hoyland demonstrated that IL-1 $\alpha$  and  $\beta$  and IL-1 receptor, but not IL-1 receptor antagonist (IL-1ra) were increased in the NP of degenerate samples. She also described how addition of IL-1 to IVD cells resulted in decreased aggrecan and type II collagen expression and an increase in the expression of MMP-3, -13 and ADAMTS-4. Dr. Hoyland concluded by proposing that IVD degeneration could be prevented by the use of anti-catabolic agents such as TIMPs, metalloproteinase inhibitors or IL-1ra and presented evidence that application of IL-1ra to degenerate IVD cells, both in monolayer and in explants, could reduce the expression of MMPs and enzyme activity.

The next presentation, on “The influence of nutrient supply on the growth kinetics of intervertebral disc cells” was given by **Eustace Johnson** (Oswestry and Keele). He reported the effects of serum, glucose and oxygen availability upon the morphology, proliferation and senescence of bovine IVD cells in monolayer and alginate cultures. He emphasised the avascular nature of the IVD and highlighted the importance of nutrient supply to the growth and survival of IVD cells, stating that 50-70% of cells within the IVD may be dead, with the proportion of dead cells increasing with age. In monolayer cultures, Dr. Johnson reported that the absence of serum induced a stellate cell morphology and senescence, but had little effect on cell viability. In contrast, in the absence of glucose, cells adopted a polar morphology and displayed increased proliferation but decreased cell viability. He stated that there was no effect of oxygen deprivation upon cells in monolayer culture. In cells cultured in alginate an absence of serum resulted in increased senescence and decreased cell viability, whereas glucose deprivation increased cell proliferation, resulting in clusters of cells, an affect that was reduced if cells were also deprived of serum. Type I and II collagen within the alginate were decreased under conditions of glucose deprivation. Dr. Johnson described how, in explant cultures, serum deprivation induced a more stellate cell morphology and loss of proteoglycans. He concluded by proposing that changes described in these experiments mimic the *in vivo* situation in humans and suggested that serum-derived factors have a major influence on IVD cells.

**Siyuan Li** (Cardiff) presented work on “Age-related changes of cytoskeletal composition: a preliminary study of the role of cytoskeleton in intervertebral disc degeneration”, and stressed the importance of the cytoskeleton in the transduction of mechanical stimulation. He presented data on mRNA expression and protein localisation of five cytoskeletal proteins ( $\beta$ -actin,  $\beta$ -tubulin, vimentin, ezrin and vinculin) within the nucleus pulposus (NP), inner annulus fibrosus (IAF), and outer annulus fibrosus (OAF) of both young and mature bovine tail IVD. He identified zonal variation in the organisation of F-actin, which was punctate within the cytoplasm of NP cells but localised predominantly at the processes of IAF and OAF cells. Dr. Li described an age-related increase in ezrin mRNA in OAF cells and identified zonal variations in the mRNA level of  $\beta$ -actin, vimentin and ezrin, which were higher in the OAF than the NP, and  $\beta$ -tubulin, which was higher in the NP than OAF. An age-dependent decrease in the level of  $\beta$ -actin,  $\beta$ -tubulin and vimentin proteins within the OAF was identified, and compared to the absence of age-related changes in the NP. Dr. Li concluded that expression of  $\beta$ -actin,  $\beta$ -tubulin and vimentin is higher in the young bovine OAF than in the mature tissue and suggested that loss of cytoskeletal elements from the ageing IVD may affect mechanical signal transduction.

The delegates were enthusiastic in their welcome for the first ever recipient of the BSMB **Fell-Muir Prize** for outstanding contributions to the field and to the Society. In his lecture “From collagen chemistry towards cell therapy – a personal journey”, **Mike Grant** (Manchester) gave a personal account of his scientific career and described the development of collagen biochemistry. Shortly after his appointment as Lecturer in Medical Biochemistry at Manchester in 1966 he

identified the problem of how cells could synthesize molecules that spontaneously formed fibres at 37°C and which were longer than the cell as the focus of his research. He described how studies of collagen synthesis in the chick lens capsule led to the realisation that distinct collagen molecules existed. He also recounted how cell fractionation studies revealed that collagen was synthesized on membrane-bound ribosomes and how the observation that collagen synthesized in cell-free systems was larger than that secreted from cells led to the identification of collagen signal peptides. Dr. Grant went on to describe how cell cultures on collagen matrices with or without fibronectin resulted in the identification of type IX and X collagen and his subsequent work characterising type X collagen and the *coll10A1* gene. He then discussed the role of type X collagen in metaphysial chondroplasia- type Schmid, and the identification of a number of mutations within the *coll10A1* gene, particularly within the C-terminal domain. His studies on a mouse Schmid model in which one of the human C-terminal collagen X mutations had been introduced led to the identification of an unfolded protein response in hypertrophic chondrocytes. Dr. Grant commented that a similar cellular phenotype was observed in thalassaemia and cystic fibrosis and suggested that ER stress, due to an unfolded protein response, may be involved in a number of diseases. He concluded his presentation by acknowledging the work of Honor Fell.

The third session, Turnover in the CNS, was chaired by Nicola Woodroffe (Sheffield Hallam University). In the first presentation on “Brain matrix structure, turnover and necessity” **Uwe Rauch** (Lund) explained that the ECM constitutes 20% of the total volume of the brain. He described the brain matrix as being similar to that found in cartilage with filamentous ternary complexes of hyaluronan, link proteins and lecticans, but with a lack of interstitial collagens. In fact the cerebral ECM contains the widest variety of hyaluronan-binding proteoglycans including aggrecan, versican, brevican and neurocan. Dr. Rauch focussed his talk on neurocan, which is co-expressed with link protein in the developing brain. Neurocan-AP fusion proteins were utilised for hyaluronan binding studies. It was shown that neurocan bound to immobilised hyaluronan and that it directly interacted with cartilage link protein. Furthermore, it has been shown that the C-terminal domains of lecticans interact with tenascins, to form superstructures. In mice lacking tenascin C, tenascin R, neurocan and brevican he described the up-regulation of fibulin-1 and fibulin-2 as well as alterations in protein distribution patterns. This suggests that the cerebral ECM undergoes remodelling in response to loss of proteins from the matrix superstructure, and Dr Rauch stated that about 90% of proteoglycans are turned over in the developing rat brain every 24 hours. To further investigate this remodelling, knock-out mice were utilised. In brevican<sup>-/-</sup>, neurocan protein expression was up-regulated because there were more HA/link protein binding sites available to it. In wild type animals it was postulated that neurocan molecules that do not find a place to bind are internalised by the cells. Dr. Rauch described how he sees a major role for MMP-2 in the processing of non-integrated proteoglycans. Antibodies against neurocan and its cleavage site neopeptide were utilised to detect proteolytically processed (to C-terminal fragments) neurocan. It was postulated that there was a link between the presence of MMP-2 in the cerebral spinal fluid (CSF) and an increase in neurocan in the remodelling process. However, no neurocan fragments were detected in the CSF with the neopeptide antibody. This is surprising considering the presence of MMP-2 in the CSF, and suggests that other proteases are involved in this remodelling process.

**Jon Friedland** (Imperial College London) then talked about his work on “Astrocyte-leucocyte interactions and the mechanisms regulating matrix degradation in CNS tuberculosis”. He stated that CNS tuberculosis occurs in approximately 1% of all patients infected with *M. tuberculosis*. Destruction of the brain is not thought to be a direct effect of the bacilli but is believed to be the result of the host’s overactive inflammatory response, which if left untreated usually results in

death. It was hypothesised that an imbalance of MMPs and their inhibitors and the resulting compromise of blood-brain barrier integrity contributed to CNS TB. In patient samples it has been shown that MMP-9 is increased in TB meningitis when compared to viral/bacterial meningitis as well as in cerebral injury. Dr. Friedland explained experiments utilising conditioned medium from *M. tuberculosis*-infected monocytes (CoMTB) to stimulate MMP gene expression in a human astrocytic cell line. It was shown that MMP-1, -2, -3, -7 and -9 were up-regulated in a dose dependent manner whereas tissue inhibitors of metalloproteinases (TIMP)-3 levels were unchanged. The MMP-9 response was characterised further, showing that secretion was NF $\kappa$ B p65- and MAPK-dependent. Dr. Friedland continued his talk by discussing in detail the regulation of MMP-9 CoMTB-induced secretion by cytokines in astrocytes. IL-1 $\beta$ , TNF and INF- $\gamma$  were shown to upregulate MMP-9. Furthermore, INF- $\gamma$  and CoMTB synergistically increased MMP-9 activity, and a similar response was observed synergistically with IL-1 $\beta$  and INF $\gamma$ . The pathways by which these responses were induced were also discussed. In the final part of the talk, recent data in microglial cells was presented. In these cells CoMTB but not *M. tuberculosis* drove MMP-1 and MMP-3 secretion.

**Elena Ganea** (Bucharest) opened her presentation entitled “Bovine brain matrix metalloproteinases; detection and inhibition” with an overview of the MMP family, before detailing her work into the inhibitory effects of three small hydroxamate-based molecules on MMP-2 isolated from normal bovine brain homogenate. MMPs and their endogenous inhibitors (TIMPs) are essential for numerous physiological processes, but when an imbalance occurs various disorders can ensue, including cancers and various diseases of the CNS. Currently there are few MMP specific inhibitors in clinical use or remaining in trial, and there is a need for new, clinically effective compounds. Dr Ganea described enzyme kinetic studies on protein fractions of normal bovine brain designed to elucidate the inhibitory effects of the hydroxamate-based molecules on MMP-2 activity. The results demonstrated that all three compounds inhibited MMP-2. Additional enzyme kinetic studies using the same synthetic compounds on leucine aminopepsidase (LAP) also indicated inhibition of this enzyme, with the compounds showing competitive or mixed inhibition. It remains to be seen if these synthetic inhibitors are capable of crossing the blood brain barrier.

The final talk of the session was given by **Gail Haddock** (Sheffield Hallam University), on “Changes in brain expression of chondroitin sulphate proteoglycans (CSPGs) following transient middle cerebral artery occlusion in the rat”. Dr. Haddock presented her recent work examining changes in expression of the brain-specific CSPGs, brevican and phosphacan, following transient middle artery occlusion (tMCAO), a model of stroke. Both brevican and phosphacan were constitutively expressed at the mRNA and protein levels, and their mRNA levels remained unchanged in tMCAO tissue compared to sham operated at 24 hours and 5 days although there appeared to be a reduction in brevican mRNA at 6 hours post tMCAO. Brevican protein levels decreased in a small number of animals post tMCAO, but overall no significant changes were observed in the protein levels of brevican or phosphacan. Immunohistochemistry showed brevican to be associated with von Willebrand Factor (VWF)-positive endothelial cells, and GFAP-positive astrocytes. Phosphacan staining had a diffuse punctate pattern with some apparently associated with GFAP positive astrocytes. Brevican and phosphacan were co-localised with ADAMTS-4 on astrocytes. A decrease in phosphacan immunostaining was observed following tMCAO, accompanied by an increase in ADAMTS-4. Furthermore, there were localised dense areas of brevican immunostaining observed in the ipsilateral hemisphere, 5 days post occlusion. This was not seen throughout the whole tissue and only in small areas. Dr. Haddock suggested that the changes observed in CSPG expression are confined to specific areas within the affected hemisphere and are masked when protein is extracted from the whole

hemisphere and compared to the contralateral hemisphere. Dr. Haddock concluded her talk by stating that it remains to be determined whether the increase in ADAMTS expression is beneficial or detrimental to the healing process via effects on CSPG levels.

The session on Fibroses was chaired by Cay Kielty (Manchester). The session got off to a lively start with the presentation by **Jack Gauldie** (Hamilton, Ontario) on “TGF $\beta$  and Smad3 link matrix expression and progressive fibrosis”. Dr. Gauldie efficiently summarised 15 years’ work into progressive pulmonary fibrosis. In order to elucidate which factors mediate initiation and/or progression of lung fibrosis, he has subjected numerous genes individually to transient adenovirus-mediated gene transfer into rodent lung. The transfer of active TGF- $\beta$ 1, a key cytokine in fibrosis (see Geoff Laurent’s presentation), resulted in the induction of severe and progressive fibrosis without the presence of inflammation. Conversely, IL-1 $\beta$  transfer caused marked tissue damage and inflammation, which subsequently developed into progressive fibrosis associated with increased TGF- $\beta$ 1 concentrations observed in lung fluid and tissue. The fibrotic responses of both vector treatments were spatially dependent, as demonstrated by the administration of the TGF-  $\beta$ 1 vector to the plural space. Progressive plural fibrosis was induced, with a thickening of the mesothelium as new collagen was deposited. This fibrosis remained localised to the site of administration, showing only minimal extension into the lung parenchyma. Additionally, both vector treatments were time-dependent, as TGF- $\beta$ 1 and IL-1 $\beta$  adhered to the ECM, which effectively limits the migration potential of these factors. Furthermore, TGF- $\beta$ 1 is known to use the intracellular signalling molecule Smad3 during the fibrotic process, and Dr. Gauldie demonstrated that progressive fibrosis is not stimulated upon transient gene transfer of either TGF- $\beta$ 1 or IL-1 $\beta$  when Smad3 null mice are used, providing yet more compelling evidence for a major role of TGF- $\beta$  in the process of fibrosis.

**Shazia Chaudhry** (Manchester) continued the session with a presentation entitled “Fibrillin-1 regulates the bioavailability of TGF- $\beta$ 1”. Fibrillin-1 normally forms extracellular microfibrils, but mutations in the gene can lead to Marfan syndrome and related disorders. Additionally, the dysregulation of TGF- $\beta$  signalling also contributes to the pathology of Marfan syndrome. Dr. Chaudhry has established that specific overlapping fragments of fibrillin-1, PF10 and PF11, cause the release of endogenous TGF- $\beta$ 1, which stimulates TGF- $\beta$  receptor-mediated Smad2 signalling in HDF cells. Of the two fibrillin fragments, PF10 was the more potent at activating TGF- $\beta$ 1, as determined by immunoassays. A number of mechanisms have been suggested for fibrillin-1 regulation of TGF- $\beta$ 1, including the involvement of integrins, syndecans, thrombospondins and proteases. However, Dr. Chaudhry has eliminated their involvement and demonstrated that the fibrillin-1 fragments containing the TGF- $\beta$ 1-releasing sequence (PF10 and PF11) associate strongly with N-terminal fibrillin-1, effectively displacing and inhibiting the binding of latent TGF- $\beta$ -binding protein 1, a component of the large latent TGF- $\beta$  complex. This release of large latent TGF- $\beta$  complex from microfibrils can contribute to the pathology of Marfan syndrome and related disorders. Dr Chaudhry is currently investigating whether PF10 can regulate the bioavailability of the other two TGF- $\beta$  isoforms, TGF- $\beta$ 2 and TGF- $\beta$ 3.

**Kim Midwood**’s (Imperial College London) talk “Tenascin-C regulates fibroblast migration within 3Dfibrin-fibronectin matrices” focused on the role of tenascin-C in regulating fibroblast migration during tissue repair. Fibroblast migration from surrounding normal tissues into a wound is an important step in wound healing. The migratory process is normally tightly regulated, and insufficient fibroblast migration would lead to a non-healing wound, while excessive migration may contribute to a fibrotic response. Tenascin-C is an extracelullar matrix glycoprotein which is transiently expressed and localised within the interface between damaged

tissue and the surrounding normal tissue. Dr. Midwood showed that full length tenascin-C promotes fibroblast migration, and was able to map the domains of tenascin-C that were responsible for promoting fibroblast migration and also some fragments that had the opposite effect and inhibited migration. This may be a key to the regulation of fibroblast migration, as induction of tenascin-C recruits fibroblasts into the wound, and fragments resulting from its breakdown would prevent excessive fibroblast infiltration.

The final session of the meeting was on Tumour/stroma interactions, and was chaired by Colby Eaton (University of Sheffield). The first presentation on “3D extracellular matrix culture models of EGFR signalling and drug response” was delivered by **Paraic Kenny** (Berkeley, California). He gave an overview of the structure of the mammary ducts and acini and the histology of the acinar epithelial cells, and noted that breast epithelial cells grown in 2D cultures (monolayers) lost their normal phenotype, which is characterised by the formation of acini. However cells grown in 3D in basement membrane matrix had a phenotype more closely replicating the natural state, with acinar organisation and milk production. In contrast to normal epithelium, breast cancer cells formed disorganised clumps in 3D culture. Using this 3D culture model with a large panel of breast cancer cell lines, Dr Kenny was able to identify TACE/ADAM 17 as a new drug target in breast cancer. Suppression of ADAM 17 activity prevented the cleavage and mobilisation of two EGFR ligands, amphiregulin and TGF- $\alpha$ . Down-regulation of ADAM 17 induced malignant cells to revert to a pre-malignant phenotype. He also reported that breast tumours with higher expression levels of ADAM 17 and TGF- $\alpha$  have a poor prognosis, suggesting that targeting ADAM 17 may be of clinical utility in this subset of patients.

**Neil Cross** (University of Sheffield) continued the session with a talk entitled “Bone marrow stromal cell-derived insulin-like growth factor (IGF) II enhances growth and survival of prostate cancer cells and potentiates androgen action”. He presented a summary of the epidemiology of prostate cancer and pathophysiology of prostate cancer metastasis to bone, and went on to demonstrate that prostate cancer cell proliferation was induced by human bone marrow stromal cell (hBMSC) conditioned medium. This positive effect on prostate cancer cell proliferation was removed when IGFs were precipitated from the medium. Treatment with hBMSC conditioned medium increased prostate-specific PSA (hK3) expression (a marker for prostate cancer) from an androgen-responsive prostate cancer cell line. Combined treatment with androgens and hBMSC conditioned medium synergistically increased PSA expression. Further experiments confirmed that IGF II, but not IGF I, was the major factor in hBMSC conditioned medium. These results show that IGF II produced by hBMSC is an important factor favouring prostate cancer cell proliferation in bone. In addition, IGF II may be responsible for enhancing the effect of residual circulating androgens on prostate cancer cells following androgen-deprivation therapy.

**Izabela Podgorski** (Wayne State, Detroit) described her work “Exploring new roles for Cathepsin-K in prostate cancer”. Prostate cancer cells metastasise preferentially to areas of active bone remodelling, a process mediated by cathepsin K. Co-cultures of prostate cancer cells with bone marrow cells in 3D collagen I gels increased prostate cancer expression of cathepsin-K. Two proteins cleaved by cathepsin-K, SPARC and VEGF, were also up-regulated in the prostate cancer cells. Up-regulation of these proteins in obese subjects correlates with decreased levels of adiponectin, an adipocyte hormone with anti-inflammatory, anti-angiogenic and anti-tumourigenic properties. Co-culture of adipocytes with PC3 prostate cancer cells increased expression of cathepsin-K in the cancer cells while decreasing the expression of adiponectin in the adipocytes. Her data showed that cathepsin-K cleaves adiponectin and thereby potentially

modulates its activity. Further studies are ongoing to determine the role of adipocyte cathepsin-K on aggressive prostate cancer.

In the final talk of the conference, the theme of collagen and proteases was continued by **Jaro Sodek** (Toronto) (“Phagocytosis of collagen by fibroblasts and invading cancer cells is mediated by MT1-MMP”). He focussed on the role of MT1-MMP in remodelling of soft connective tissues, where a phagocytic pathway is used by fibroblasts. For this process to occur, the cross-linked collagen fibrils (such as type I) must be fragmented to allow internalisation. His work demonstrated that degradation of collagen substrates by fibroblasts correlated with the expression of MT1-MMP. MT1-MMP was localised to sites of collagen cleavage on the cell surface and also within cells. He went on to demonstrate that phagocytosis and degradation of collagen occurred without the action of MMP-2 and -9. Similar analyses of various cancer cells (ovarian, breast, fibrosarcoma) utilise a similar if not identical pathway of collagen degradation. More invasive ovarian cancer cell lines expressed more MT1-MMP and confocal microscopy showed that invading cells had phagocytosed surrounding biotinylated collagen. These studies demonstrate a pivotal role for catalytically active MT1-MMP in preparing collagen fibrils for phagocytic degradation by normal and transformed cells.