

## Workshop A: Vascular biology and angiogenesis

**Arnaud Robinet** (Reims, France) introduced the workshop by talking about the cardio-protective effects of elastin-derived peptides. These peptides increased angiogenesis in the chick chorioallantoic membrane *in vivo* model and stimulated tube formation and migration in *in vitro* assays. These peptides were investigated in cardiovascular models such as the rat heart ischemia reperfusion model and all tested parameters such as rate pressure product, CK release and necrosis area demonstrated cardioprotective effects of these elastin-derived peptides. He concluded by highlighting the importance of both NO and RISK pathways in this cardio-protection.

**Masayuki Shimoda** (Tokyo, Japan) talked about the involvement of ADAM-28 in the endothelial cell-leukocyte interaction. His group previously showed that ADAM28 enhanced leukocyte adhesion to endothelial cells through interaction with P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes. Here he showed that both the secreted and membrane-bound forms of ADAM28 were expressed on normal and bronchopneumonia lung tissues. However, only the secreted form (ADAM28s) was expressed in the diseased samples. Treatment of human umbilical vein endothelial cells with inflammatory mediators such as TNF- $\alpha$ , IL1 $\alpha$  or PMA induced expression of ADAM28s through protein kinase C. All together, his findings indicated that induction of ADAM28s expression by endothelial cells under inflammatory conditions regulates leukocyte adhesion to endothelial cells.

**Zhigang Zhou** (Norwich, UK) showed a detailed characterisation of perivascular cells isolated from embryonic and adult mice with a targeted allele of the annexin A5 gene. He showed that this cell population maintain their perivascular markers *in vitro*. These cells were used in a co-culture system with human umbilical vein endothelial cells and results showed that in a 2D system, they promote angiogenesis. In a 3D model, they observed a slower regression of the endothelial cell network and a more efficient deposition of basement membrane components, contributing to the stabilisation and maturation of the neovessels. In conclusion, he highlighted that his novel co-culture system produced more stable and mature neovessels and could be used for further understanding the angiogenic process.

**Ritva Heljasvaara** (Oulu, Finland) presented a chemically-induced skin cancer mouse model of their transgenic J4 strain which overexpresses endostatin, a proteolytic fragment of collagen XVIII. She showed that no significant differences were observed between the wildtype and transgenic mice in tumour size and ratio between benign and malignant phenotypes. However, a reduction in the lymphatic network as well as in the lymph node metastasis was observed in the J4 mice. Further analysis of lymphangiogenic process in these mice revealed a reduction in expression of VEGF-C and its receptor VEGF-R3. A decrease in the mast cell accumulation in tumours of these mice was also observed. Furthermore, endostatin inhibited mast cells adhesion and migration to fibronectin, suggesting that it may regulate VEGF-C producing mast cell adhesion to tumour associated extracellular matrix.

**Aikio Mari** (Oulu, Finland) gave an overview regarding the collagen XVIII variants. Whereas the non-collagenous C-terminal (NC1) domain and endostatin are extensively studied, the functions of the other collagen XVIII variants and their N-terminal NC11 domains remain poorly characterised. Therefore, variable NC11 domains were expressed recombinantly. The three expressed NC11 variants showed extensive glycosylation with the short variant being a hybrid heparan/chondroitin sulphate proteoglycan. The glycosylated form of this fragment increased FGF-induced endothelial cell migration and bound to this growth factor. However, the unglycosylated form did not bind to either FGF or VEGF. Therefore, the glycosaminoglycans of this short variant may participate in presenting growth factors to endothelial cells by directly binding to them.

**Alain Colige** (Liège, Belgium) talked about VEGF111, a VEGF-A isoform lacking exon 5. This newly identified isoform is expressed by various human cell lines after chemotherapeutic agents or UV irradiation induction. He showed that contrary to other VEGF-A isoforms such as VEGF121 and VEGF165, VEGF111 was resistant to proteolytic cleavage by plasmin and fluids from chronic wound. VEGF111 induced VEGF-R2 and ERK1/2 phosphorylation, promoting endothelial cell proliferation and migration. Recombinantly expressed VEGF111 injected in nude mice revealed higher vessel density compared to VEGF121 or

VEGF165 injection. Use of VEGF111 in a mouse model of heart infarction showed improved survival. The speaker focused on the VEGF111 resistance to degradation and high diffusion as main contributors to its function.

### **Workshop C: From molecules to extracellular machines. Dynamics of the extracellular matrix.**

**Dieter Reinhardt** (Montreal, Canada). The presentation discussed work focussed on understanding the role of fibronectin in the assembly mechanism of fibrillin-1. Fibrillin-1 is a major component of microfibrils and mutations in fibrillin-1 cause Marfan syndrome. siRNA experiments were performed and it was concluded that fibrillin-1 assembly is dependent on the presence of fibronectin fibres in extracellular layers. Further experiments examined the molecular interactions between these proteins and it was found that fibronectin interacts with the C-terminal region of fibrillin-1.

**Uwe Hansen** (Munster, Germany) presented work on the small leucine-rich repeat protein, opticin, and its interaction with type II collagen containing fibrils. Data was presented showing opticin localises to the overlap region of the D-period of vitreous collagen fibrils. Furthermore, opticin bound collagen II and collagen XI fibrils *in vitro* with high affinity. This interaction was not reduced in the presence of high salt concentrations. Although binding took place, the rate of fibrillogenesis and the final fibre diameter was not found to be altered by opticin. Furthermore, opticin does not alter the final fibre morphology.

**Emilie Chautard** (Lyon, France) discussed the development of a new database, MatrixDB (<http://matrixdb.ibcp.fr>). The database contains information regarding experimentally determined interactions between 238 extracellular molecules totalling 1784 interactions. The database can be queried to retrieve the binding partners of a molecule or subset of molecules and restrictions can be placed on the search to limit the data to individual species. Analysis of the interaction network has shown sensitivity against the most connected biomolecules such as MMP-2, MMP-9 and collagen I. Ongoing work involves the creation of a model for the extracellular processes involved in angiogenesis.

**Anthony Weiss** (Sydney, Australia) described a model for human tropoelastin assembly. Elastin is composed of many tropoelastin molecules and it is the elastic protein that allows tissues to revert to their original shape. Significant assembly of tropoelastin was reported to begin above 32°C by reversible biomolecular interactions. The equilibrium dissociation constant for this process was reported to be 23±3 nM. Further, initiation of the assembly is not dependent on a single part of the molecule. The best model of association was reported to be a head-to-tail configuration of the tropoelastin monomers.

**Elena Makareeva** (Bethesda, USA) discussed the presence of osteogenesis imperfecta (OI) mutations in collagen type I and the effect the mutations had on the stability of the collagen helix. Forty eight glycine substitutions were analysed from a variety of OI patients. Differential scanning calorimetry and circular dichroism were used to map changes in the collagen melting temperature. The study found variations in the melting temperature depending on the location of the substitution in the triple helical region. No correlation between the melting temperature and the identity of the substituting residue was observed.

**Emmanuel Belamie** (Paris, France) presented the final talk of the workshop on the properties and self-assembly of collagen in concentrated solutions and biomimetic gels. The generation of fibrillar materials has important consequences for tissue engineering as fibrillar structures form major components of the bone, cornea, tendon and skin. The ordered density of collagen type I matrices were adjusted by altering the electrostatic interactions. Further, it was shown that fibrillar aggregates were formed in acidic solution when collagen concentrations are greater than 150 mg/mL. Analysis of these fibres was performed by transmission electron microscopy and SAXS. The collagen concentration was reported to greatly influence the appearance of the gels and the morphology and structure of the collagen fibrils

## **Workshop D: ECM: sources and reservoir of biomolecules, and ECM: from inside the cells to the matrix**

**Clemence Chomel** (Paris, France) was the first to present, introducing her work on Angiopoietin-like 4 (ANGPTL4), a secreted protein of the angiopoietin family. Following on from previous work showing an induction of ANGPTL4 expression in endothelial cells in response to hypoxia and in human ischemic tissues from peripheral artery disease, she investigated the structural basis underlying the previously reported anti-angiogenic role of ANGPTL4 and its interaction with the subendothelial ECM. The full-length protein contains two domains, a coiled-coil domain (CCD) and a fibrinogen-like domain (FLD), bound by a linker region, and four deletion mutants were produced to investigate the function of the CCD and FLD both with and without the linker. She showed that FLD was secreted into the medium whilst CCD interacted with the ECM, likely through the ability of CCD and full-length ANGPTL4 to bind to heparan sulphate and dermatan sulphate, as demonstrated using Surface Plasmon Resonance binding assays. CCD also decreased the adhesion and motility of human umbilical vein endothelial cells and affected tube formation by human microvascular endothelial cells on matrigel. Proteolytic cleavage at the identified  $_{161}\text{RRKR}_{164}$  site generates CCD and FLD fragments, though they are yet to identify the proteases that cleave here and may therefore regulate the bioavailability of ANGPTL4.

**Ismail Hendaoui** (Rennes, France), presenting his work on the *frizzled* module (FZC18) of the third amino terminal end variant of collagen XVIII (V3C18). They have demonstrated a 100% probability that the 3-dimensional model of FZC18 matches the crystal structure of the cysteine-rich domain of secreted *frizzled* related protein inhibitor-3 (SFRP3) and *frizzled-8*. *In vivo*, V3C18 was proteolytically processed into a FZC18-containing 50kD glycoprotein precursor that, *in vitro*, bound Wnt3a through FZC18 and suppressed Wnt3a-induced stabilisation of  $\beta$ -catenin. FZC18 suppressed Wnt/ $\beta$ -catenin signalling in colorectal and liver cancer cell lines and reduced *in vitro* cell growth, whilst full-length V3C18 increased Wnt signalling. Immunostaining in conjunction with confocal microscopy showed FZC18 was associated with the cell membrane, which was confirmed by cell fractionation experiments. Work by this group is the first to demonstrate extracellular matrix control of Wnt signalling through a collagen-embedded *frizzled* molecule.

**Zuzana Saidak** (Amiens, France) introduced her work on the role of the Calcium Sensing Receptor (CaSR) in the migration of breast cancer cells, reporting that approximately 75% of breast cancer patients develop bone metastases. CaSR is expressed in both healthy and cancerous breast cells, in addition to cells involved in mineral homeostasis such as in bone and intestines. In Boyden Chamber and Scratch Wound migration assays, she showed an increase in breast cancer cell migration with increasing  $\text{Ca}^{2+}$  concentration, demonstrating a significantly greater effect of  $\text{Ca}^{2+}$  concentration on the more metastatic breast cancer cell lines. Inhibition of ERK1/2 phosphorylation or phospholipase C $\beta$  removed the migratory response to  $\text{Ca}^{2+}$ . Extracellular  $\text{Ca}^{2+}$  was shown to activate ERK 1/2 in all four cell lines, with the greatest extent of phosphorylation in the more metastatic ones. CaSR-siRNA abolished the  $\text{Ca}^{2+}$ -induced ERK1/2 activation and removed the migratory response to  $\text{Ca}^{2+}$ . Her work has shown that CaSR is involved in and required for the migratory effect of  $\text{Ca}^{2+}$  on breast cancer cells, making it a potential new target for the treatment of secondary metastatic breast cancer.

**Farhana Suleman** (Manchester, UK), introducing their mouse model of Pseudoachondroplasia (PSACH), a genetic disease that results exclusively from mutations in cartilage oligomeric matrix protein (COMP). The model was generated through the introduction of the most common COMP mutation ( $\Delta\text{D469}$ ), and mice display a 61.9% increase in the degree of hip flexion and, though normal at birth, develop short limb dwarfism due to disorganisation of the growth plate. There was little extracellular COMP staining in the growth plate of mutant mice and at the cellular level, hypertrophic chondrocytes contained enlarged endoplasmic reticulum due to retention of the mutant COMP. Compared to the growth plate of wildtype mice, mutant mice demonstrated a 17.5% decrease in chondrocyte proliferation by three weeks of age and apoptosis was increased and spatially dysregulated in the growth plate. This work has begun to investigate a model that will be a useful tool for understanding disease pathways in PSACH.

**Judith Seul** (Cologne, Germany) was next to present her work analysing the role of testicans (testican-1, -2 and -3), a family of secreted proteoglycans structurally related to the BM-40/SPARC/Osteonectin family of extracellular  $\text{Ca}^{2+}$ -binding proteins. They show broad expression during embryonic development and in adult

mice their expression is restricted mainly to the nervous system, with testican-2 also strongly expressed in endocrine organs and the lungs. Testicans have also been shown to modulate MMP activation *in vitro*. The group generated knockout mice deficient for all three testicans to exclude a potential functional compensation as mice deficient for each of the testicans showed no obvious phenotype compared to wildtype litter mates. Testican triple knockout mice were viable and fertile and showed no obvious brain phenotype except for a reduction in brain ventricle size. However, knockout mice were significantly fatter than wildtype litter mates, being consistently and significantly heavier after 5 weeks of age, but also possessing an increased body fat content relative to body weight. Knockout mice had a decreased food intake compared to wildtype mice, despite their increased weight. This is likely due to the decreased energy expenditure demonstrated for knockout mice which the group proposed is due to either a lowered basal metabolic rate or a decreased physical activity. In addition, in wildtype mice testicans were detected in the hypothalamus, the site of body weight control. This work suggests a role for testicans in the control of body weight.

**Barbara Smith** (Boston, USA), presented studies of a transcriptional complex that mediates interferon-gamma (IFN- $\gamma$ ) induced repression of collagen and activation of major histocompatibility complex class II (MHC II). Class II transactivator (CIITA) is a transcriptional regulator expressed by B cells and dendritic cells and up-regulated by IFN- $\gamma$ . Through an increase in CIITA, IFN- $\gamma$  up-regulates MHC II and down-regulates type I collagen expression. Down-regulation of type I collagen was dependant on phosphorylation of CIITA and the co-repressor molecules Sin3B and HDAC2 co-precipitated with CIITA in a phosphorylation-dependant manner. Phosphorylation appeared to be GSK3/CK1 mediated as inhibitors of each kinase abrogated IFN- $\gamma$  induced repression of type I collagen expression. Her group has identified a potential GSK3 site in the proline/serine/threonine domain of CIITA, showing that a serine mutation (S373A) blocks type I collagen repression. Other work presented looked at a role of A2b adenosine receptor (A2bAR) in the modulation of inflammation in the vasculature. Activation of A2bAR by agonists decreases IFN- $\gamma$  induction of CIITA expression in human aortic smooth muscle cells (SMCs) and CIITA is up-regulated in the SMCs of A2bAR knockout mice. Increased CIITA expression therefore resulted in increased MHC II and decreased type I collagen levels in the knockout mice. Finally, studies of peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) showed it co-precipitates with CIITA and a PPAR $\gamma$  agonist sensitises cells to IFN- $\gamma$  treatment by increasing recruitment of PPAR- $\gamma$  to collagen and MHC II genes. These studies have identified some critical targets for investigating diseases involving the immune response and extracellular matrix remodelling.

### **Workshop E: Extracellular matrix diseases and therapies**

**Joan Marini** (Bethesda, USA). The workshop was opened with a talk on two recently identified autosomal recessive forms of osteogenesis imperfecta (OI). Mutations have been identified in two genes, cartilage-associated protein (*CRTAP*) and prolyl 3-hydroxylase 1 (*LEPRE1*) respectively. The mutation identified in *LEPRE1* (IVS5+1G>T) was found to be common amongst West-African and African-American descent individuals and it was suggested that the mutant allele was stable in this population. The carrier frequency of the IVS5+1G>T allele was determined from African-American newborns from Pennsylvania and it was estimated that 1 in 330,000 African-Americans die from OI caused by homozygosity of the IVS5+1G>T mutant allele.

**Fransiska Malfait** (Ghent, Belgium) began her talk by indicating that most mutations in the two genes coding for type I collagen (*COL1A1* and *COL1A2*) are the result of an amino acid substitution from glycine to a bulkier residue, resulting in the disorder osteogenesis imperfecta (OI). Mutations in these genes can also lead to Ehlers-Danlos syndrome arthrochalasia type (EDS VIIA&B). Recently type I collagen mutations have been found to result in additional phenotypes distinct from OI and EDS VII A&B. The range of different phenotypes were discussed and it was suggested an awareness of these features would aid a molecular diagnosis and long term follow-up treatment.

**Ray Boot-Handford** (Manchester, UK) discussed a mouse model used to study metaphyseal chondrodysplasia type Schmid (MCDS). The mutant mouse contained a mutation in the *collagen X* gene equivalent to the human N617K mutation. Mutant collagen X was found to be retained intracellularly and resulted in upregulation of the unfolded protein response (UPR), a feature of endoplasmic reticulum (ER)

stress. The growth plate hypertrophic zone was increased and the rate of bone growth was decreased in mutant animals. To further investigate whether this effect was caused by ER stress a second mouse line containing a mutant form of thyroglobulin ( $Tg^{cog}$ ), which is unable to fold correctly, was expressed in the growth plate. The resulting mice mirrored the phenotype of the MCDS mutant mice and thus provided evidence that ER stress is instrumental in the MCDS disease mechanism.

**Luciano Merlini** (Ferrara, Italy) talked about Ullrich congenital muscular dystrophy (UCMD) and Bethlem myopathy, skeletal muscle disorders caused by collagen VI mutations. A mouse model containing a targeted disruption in the *col6a1* gene was generated and found to have mitochondrial defects and increased apoptosis. Homozygous mutant mice were cured using cyclosporine A; this acts as an immunosuppressant which desensitizes the mitochondrial permeability transition pore. Treated mice showed improved recovery of muscle fibre apoptosis and ultrastructural lesions. *In vitro* treatment of human UCMD skeletal muscle cells showed improvement and led to a trial of five patients who had collagen VI myopathies. Cyclosporin A was administered orally for 1 month. Results following treatment demonstrated improved muscle regeneration.

**Sudheer Kumar Gara** (Cologne, Germany) described three novel collagen VI chains that are highly homologous to the collagen VI alpha 3 chain. Previously three collagen VI chains have been described and designated  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 3$  chains. The novel collagen VI genes,  $\alpha 4$ ,  $\alpha 5$  and  $\alpha 6$  are arranged consecutively on chromosome 9 in the mouse genome. Localisation studies have determined collagen VI chains  $\alpha 4$ ,  $\alpha 5$  and  $\alpha 6$  chains lie close to basement membranes. Interestingly the gene encoding the collagen VI  $\alpha 4$  chain in humans was found to be a non-processed pseudogene due to a large-scale pericentric inversion of the region. Furthermore, collagen VI  $\alpha 5$  was recently linked to atopic dermatitis and named *col29a1*.

**Richard Wilson** (Melbourne, Australia) received a travel fellowship awarded by the International Society of Matrix Biology for his presentation on proteomic profiling of cartilage degradation *in vitro*. Cartilage explants from the femoral head of 3 week old mice were cultured in the presence of catabolic reagents. The conditioned media was subsequently analysed by SDS-PAGE and 2-D PAGE and the proteins identified were compared to control, non stimulated explants. The differential abundance of 20 proteins was identified between the control and stimulated media. An example of a differentially regulated protein includes gelsolin which is involved in cytoskeletal reorganisation of arthritic synovial fibroblasts and may therefore be a novel pathological mediator in cartilage.

#### **Workshop F: ECM and fibroproliferative diseases – Maturation and aging of the ECM**

**Gilles Faury** (Grenoble, France) explained how elastin (eln) is the main component of elastic fibres and the progressive degradation during aging results in the loss of arterial integrity. He generated eln mutant mice to study whether early eln deficiency could also influence the aging process. Eln heterozygous mutant mice have a normal lifespan. However, they contain longer arteries with smaller diameter and rigid walls with additional but thinner elastic lamellas resulting in high blood pressure. Juvenile eln heterozygous mice resemble older wildtype mice with vascular aging e.g. cardiac hypertrophy, enhanced fragmentation of elastic fibres resulting in loss of elasticity of the arterial wall and ECM accumulation in the aortic wall. Modifications were also seen in the renal artery. Data suggested that eln is involved in normal physiology and arterial aging.

**Sabine Ratzinger** (Regensburg, Germany) used sub epithelial myofibroblasts (SEMF) from normal and diseased tissue from patients with Crohn disease to study the cell matrix interaction at various stages of the disease. Myofibroblast from the lamina propria showed increased collagen XVI in diseased tissue by western blotting and fluorescent microscopy. When collagen XVI was used in a proliferation capacity assay cell spreading was decreased when compared to normal cells. This could indicate a role for collagen XVI in disease progression. They noted that there was an increased level of  $\alpha 1$  integrin which is known to bind to collagen XVI resulting in cell adhesion to the ECM and may keep SEMF at the inflammation site.

**Nadia Ninfa Albanese** (Palermo, Italy) presented data on the effects of micro environmental factors on neoplastic cell behaviour using a well characterised breast cancer cell line, 8701-BC. The cells were grown in either a transwell co-culture system or incubated with normal fibroblast conditioned medium collected after 48 hrs. The results showed that the cancer cell growth increased by 58 % and also increased the rising and

migration properties. They produced a proteomic map using MALDI TOF and found 30 % of proteins were affected by the fibroblast co-culture. The results showed that the fibroblast may have a beneficial effect on cancer cell growth rather than be host defenders.

**Marie-Claire Schanne-Klein** (Palaiseau, France) reported on the use of multiphoton microscopy based on the second harmonic generation (SHG) from collagen fibres to examine the three-dimensional heterogeneous accumulation and structure of collagen fibres during fibrotic pathologies. They showed that SHG is able to distinguish between fibrillar and non-fibrillar collagen and provide sensitive quantitative measurements in the absence of any staining of the tissue. They used a murine model of hypertensive renal fibrosis using angiotensin II (Ang2) and showed how cross linking of collagen by transglutaminase-2 may influence tissue remodelling. SHG was able to consistently distinguish between TG2 deficient mice compared to wildtype and that this procedure should help pathological studies of tissue fibrosis.

**Heli Ruotsalainen** (Oulu, Finland) discussed how formation of glycosylated hydroxylysines *in vivo* is catalysed by the multifunctional LH3 having lysyl hydroxylase and collagen galactosyltransferase and glucosyltransferase activities. They produced LH3 KO mice that lack the LH3 protein and thus all 3 LH3 activities and LH mutants that contain a point mutation that destroys the LH activity leaving the glucosyltransferase activity intact. At E9.5 LH3 mutants possessed collagen IV that was fragmented and intracellular leading to lack of basement membrane. LH mutants caused under-glycosylated collagen type IV and VI leading to alterations in the structure of the basement membrane. These results demonstrate that glycosylation of hydroxylysine is essential for the structure of ECM and basement membrane.

**Heather Yeowell** (Durham, USA) discussed how the alternatively spliced form of lysyl hydroxylase 2 (LH2) includes an extra exon 13A and is suggested to have telopeptide LH activity and since LH2 is over expressed in scleroderma fibroblasts may play an important role in controlling fibrosis. They showed that RNA-binding splicing proteins T-cell intracellular antigens (TIA-1 and TIAL2) and polypyrimidine tract binding (PTB) protein regulate LH2 (long) differently. A 5 fold increase was shown with over expression of TIAs whereas over expression of PTB it was decreased. The results suggested that TIA and PTB are two potential targets for the regulation of LH2 alternative splicing and provide a possibility of therapeutic target of scleroderma.

## **Workshop G: Stem cells**

**Michael Stock** (Erlangen, Germany) described the structure, expression and function of 'unique cartilage matrix associated protein' (Ucma). The protein is comprised of 138 amino acids and is almost exclusively expressed in cartilage. Upon secretion into the ECM the protein is processed by a furin-like enzyme to produce a 37 amino acid N-terminal peptide and a 74 amino acid C-terminal peptide. Michael described his interest in Ucma mRNA and protein expression patterns and how this led to the generation of antibodies to the uncleaved precursor and the processed C-terminal peptide. Studies using these antibodies highlighted differential patterns of distribution of the precursor protein and the processed C-terminal peptide. The uncleaved precursor protein displayed a similar restricted distribution pattern in cartilage as Ucma mRNA whereas the processed C-terminal peptide was identified in the entire cartilage matrix. Recent data were also presented relating to the function of the protein. Michael hypothesised that the protein may have a role regulating the architecture of the cartilage matrix.

**Thomas Schreiber** (Tuebingen, Germany) discussed his studies investigating how the microenvironment of stem cells affects their behaviour. He explained how stem cells interact with their local environment via integrins and that previous work had suggested roles for cell surface integrins  $\alpha4$  and  $\alpha6$  in the retention of progenitor cells in the bone marrow. He described his work looking at the expression and functions of the integrin subunits  $\alpha7$  and  $\alpha9$  within the osteoblastic bone marrow niche. Studies using cell-cell adhesion assays showed that progenitor cell binding to osteoblasts was markedly reduced (by ~70%) following incubation with function blocking antibodies against integrin subunit  $\alpha9$ . Other studies highlighted that addition of anti  $\alpha9$  antibodies significantly reduced progenitor cell proliferation and differentiation. These data led Thomas to propose two potential functions for integrin subunit  $\alpha9$  in the osteoblastic stem cell niche,

one involving the regulation of progenitor cell differentiation and one involving the linkage of progenitor cells with the ECM.

**Svetlana Popova** (Bergen, Norway) described her work investigating the function of the recently identified integrin  $\alpha 11\beta 1$ . Previous work has looked at the expression and potential function of integrin  $\alpha 11\beta 1$  in diseased tissue where levels were shown to be increased in certain cancers such as non-small-cell lung adenocarcinoma. In this regard, the integrin  $\alpha 11\beta 1$  could potentially be a target for cancer therapy, however little is known about normal expression of the integrin. Svetlana presented data examining the expression patterns of  $\alpha 11$  mRNA and protein in adult mouse tissues. This work highlighted the restricted expression of the integrin chain and identified it as a good marker for a subset of fibroblasts in adult tissue. Svetlana explained that future work will aim to explore the potential for the integrin to be used as a marker for activated fibroblasts in pathological conditions.

**Alain-Pierre Gadeau** (Toulouse, France) described his recent work investigating a potential role for the multipotent protein osteopontin (OPN) in vascular repair and endothelial remodeling (also known as re-endothelialisation). Previous studies have shown that estrogens ( $E_2$ ) can accelerate re-endothelialisation although the mechanism by which this is achieved is still unknown. Alain-Pierre presented data which gave clues to this mechanism. His studies using wildtype and OPN knockout mice identified OPN as a mediator of the  $E_2$  induced re-endothelialisation. He explained how his mouse work had shown that  $E_2$  were unable to trigger endothelial repair in the absence of OPN and how his research using a graft strategy had highlighted that OPN was required for the adhesion of bone marrow (BM) cells to epithelial cells, a prerequisite event for  $E_2$  mediated endothelial healing. Alain-Pierre concluded that these studies suggested that  $E_2$  accelerate re-endothelialisation via BM-derived cell synthesis of OPN. The production of OPN is crucial for the homing of BM-derived cells to sites of injury and for their ultimate adhesion upon the endothelial cells of the regenerating endothelium.

**Katrin Blumbach** (Cologne, Germany) described her work investigating the function of the hyaline cartilage component collagen IX in matrix organisation and bone development. It is believed that the protein, due to its periodical localisation along the collagen fibril, acts as a macromolecular bridge linking collagen fibrils and ECM molecules but this remains unconfirmed at present. Her studies using tissue from new born collagen IX deficient mice suggested that collagen IX was required for the cellular incorporation of the cartilage specific ECM protein matrilin-3. In these tissues matrilin-3 biosynthesis was shown to be unaffected; however large amounts were released into the medium of cultured chondrocytes as opposed to being integrated into the cell layer of cultured cells as in wildtype cells. Data were also presented which showed that collagen IX and COMP double knockout mice displayed shortened and widened long bones and severe growth plate abnormalities. As this phenotype was also presented in collagen IX single knockout mice it suggested a role for collagen IX in bone development.

**Christine Chuang** (Sydney, Australia) described her recent work investigating the role of perlecan GAG chains in development. Previous studies have shown that mice lacking perlecan or fibroblast growth factor (FGF) or FGF receptor (FGFR) all display severe chondrodysplasias due to defective endochondral ossification. This pathological phenotype highlights the importance of the three molecules for cartilage formation and bone development and suggests they may share signalling pathways. Christine's initial studies identified the GAGs which decorated the chondrocyte perlecan protein core to be heparan sulphate (HS), chondroitin 4-sulphate (C4S), chondroitin 6-sulphate (C6S) and keratan sulphate (KS). She went on to describe GAG digestion experiments which highlighted that HS was crucial for the formation of tertiary complexes incorporating perlecan, FGF-2 and FGFR1 or perlecan with FGF-18 and FGFR3. Further studies were described, the results of which suggested functions for the specific perlecan GAG chains. Data generated using a ligand and carbohydrate engagement (LACE) assay demonstrated that HS controlled signalling induced by the formation perlecan-FGF-FGFR tertiary complexes whilst data from proliferation experiments highlighted the influence of C4S, C6S and KS in perlecan localisation.

## **Workshop H: Tissue engineering: from the lab to the patient**

**Hélène Janin-Manificat** (Lyon, France) reported on the reconstruction of human corneas using magnetically orientated collagen scaffolds. The speaker stressed that the shortage of donor's corneas and the risk of human transmitted diseases make the development of tissue engineered corneas highly desirable. Thus orthogonal collagen fibril stacks resembling the organisation of native corneal stroma were developed using magnetic techniques. These stacks were populated by human corneal keratocytes which followed the orientation of collagen fibrils. The analysis of specific markers confirmed formation of epithelium and neosynthesis of different collagen types in this tissue engineered construct. Magnetically orientated collagen scaffolds were also implanted in rabbit corneas. After 2 months, the animals were sacrificed and their corneas were examined by histology and electron microscopy. The only rabbit (out of four) which had retained the scaffold implant, demonstrated a well-organised lamellar structure in stroma. It was suggested that microtearing during suturing due to the brittleness of the scaffold was the main cause of unsuccessful implantations. The speaker proposed to decrease scaffold crosslinking to make the implants more flexible.

**K.M. Abberton** (Melbourne, Australia) reported on research into tissue-engineered adipose tissue for repairing soft tissue defects such as breast replacement after mastectomy. The speaker focused on the refinement of a previously reported matrix obtained from skeletal muscles (Myogel) which is capable of supporting adipogenesis. He discussed its use in tissue-engineering chamber models and its advantages over the commercially available Matrigel. Myogels were extracted from rat, porcine and human skeletal muscles. All provide a matrix which is similar to Matrigel in total protein level assessed by colorimetric assay and also have a high content of growth factors. In their tissue-engineering model, adipose tissue was successfully developed in the chambers containing Myogel but not in chambers containing Matrigel. In a dorsal subcutaneous implant model, modified Myogels were assed for cytotoxicity by the MMT assay. All findings reported to date make refined Myogels promising scaffolds for adipose tissue engineering.

**Anne Gigout** (Montreal, Canada) proposed the approach of using stirred bioreactors for culturing bovine chondrocytes. Shear stress in the stirred environment limited the size of cell aggregates in comparison with static conditions; this size limitation was important for maintaining of cell nutrient supply in the centre of aggregates and also helped avoid the loss of cell homogeneity. Chondrocytes in stirred aggregates maintained their original round phenotype and expressed collagen type II, VI and IX, fibronectin, COMP and glycosaminoglycans typical for the pericellular matrix of cartilage. It was found that the also chondrocytes expressed integrin receptors. Chondrocytes in aggregates proliferated in presence of Pluronic F-68 although the rate of proliferation was around 2.5 times lower than that in monolayer culture. Thus the proposed bioreactor can help in the simultaneous achievement of two of the main goals in cartilage tissue engineering which usually are difficult to solve: maintenance of the original chondrocyte phenotype and cell proliferation.

**Sylvain Vigier** (Paris, France) reported the results found when comparing two new collagen scaffolds developed for bone healing with freeze-dried collagen sponges. Scaffolds were obtained from self organised acid-soluble collagen solutions at 5 mg/mL (5MG) and 40 mg/mL (40MG). TEM analysis demonstrated a homogeneous network of fibrillar collagen organised in bundles, whereas the walls of collagen sponge consisted of undefined thin microfibrils. 5MG and 40MG were populated by transformed human osteoblasts (FHSO-6). The cells adhered to both scaffolds and survived for one month at least. The speaker indicated the difference in morphology between cells cultured in those two tissue engineered constructs: cells on the surface of 5MG demonstrated typical square-shape osteoblast morphology and were arranged in layer as in bone; cells on 40MG (and collagen sponges) exhibited flat and an elongated resting phenotype. After one month's culture, the calcification of both constructs was demonstrated by von Kossa staining. 5MG and 40MG were used to fill 5mm cranial defects in rats and compared to results with spontaneous healing. 40MG scaffold demonstrated better healing properties than 5MG, with a higher degree of mineralization than spontaneous healing and a progressive transformation into the bone.

**Dr. Michele Spina** (Padova, Italy) described the analysis of the composition of porcine aortic (AR) and pulmonary (PR) valve roots before and after detergent-based decellularisation. This is of interest as the composition determines mechanical properties of the tissues. The research focussed on preparation of decellularised scaffolds for tissue engineering. The valves consist of leaflet, sinus and a proximal wall

segment; all three structures were analysed for water and lipid content and the distribution of dry mass. Decellularisation did not affect mass distribution in AR but for PR it resulted in loss of tissue fractions comprising collagen. It was concluded that nevertheless, the thickness differences between aortic and pulmonary leaflets arise mostly because of differences in hydration rather than because of differences in mass of their structural components. The speaker stressed that these findings emphasize that the importance of tissue water content for dynamics of heart valves, and pointed out that it could be routinely underestimated.

### **Workshop Bii: Cell-matrix interactions & signalling II'**

**Aurelia Raducanu** (Martinsreid, Germany) discussed how  $\beta 1$  integrin regulates mitotic spindle orientation in chondrocytes via modulation of the cell shape. She found that wildtype chondrocytes spread and orient their spindle parallel when cultured on fibronectin or vitronectin, but being round and randomly orienting their spindle relative to the substrate when cultured on collagen and poly-L-lysine. However,  $\beta 1$  integrin deficient chondrocytes do not spread on fibronectin, collagen or poly-L-lysine and orient their spindles randomly. These results indicate that  $\beta 1$  integrin-dependent chondrocyte-matrix interactions are pivotal for cellular geometry, which in turn orients the mitotic spindle and determines the division axis.

**Alexandre Carisey** (Lyon, France) gave a talk about how Tenascin-X (TNX) modulates cell spreading on collagen I and acts as a regulator in FAK-dependent pathway. TNX is a large ECM protein involved in cell adhesion and spreading. Using fibrosarcoma, osteosarcoma and glioblastoma cell lines and human primary fibroblasts, they found that there is less cell spreading and an increased number of filopodia containing fascin when cells are plated on TNX +collagen. In addition, focal complex maturation is delayed within cells cultured in the presence of TNX. All these data suggest that TNX regulates cell-matrix induced signalling and modifies cell spreading.

**Maurizio Mongiat** (Aviano, Italy) gave a talk entitled 'An N-terminal region is responsible for EMILIN-2 induced extrinsic apoptosis'. Elastin Microfibril Interface Located Proteins (EMILIN) are a family of ECM glycoproteins involved in elastogenesis and hypertension. EMILIN2 inhibits the growth of cancer cell line through an apoptosis pathway dependent on caspase-8 and caspase-10 activation. To further investigate the mechanism of this inhibition, EMILIN2 deletion mutants were developed and subsequently identified that the N-terminal fragment which can bind to death receptors, activate caspase-8 and induce apoptosis in tumour cells. In addition, it was found that the growth of different cell lines in 3D matrices and the colony formation in soft agar were significantly impaired in the presence of EMILIN2. Therefore, EMILIN2 fragments are a potential anti-neoplastic tool for cancer treatment.

**Frédéric André** (Marseille, France) described how he investigated the mechanism by which  $\alpha V$  integrin and E-cadherin/catenin complex can modulate insulin-like growth factor I (IGF-I)-induced cell migration. They found that  $\alpha V$  integrin, E-cadherin and IGF-IR form a multimeric complex in cancer cells, and interact at cell-cell contact sites. IGF-IR ligation by IGF-I induced the disruption of the complex and the relocalisation of  $\alpha V$  integrin from cell-cell contacts to focal contact sites. This perturbation is correlated with the observed increase in cell migration and cell invasion. His results suggest that regulation of the scaffolding of the  $\alpha V$  integrin/E-cadherin/IGF-IR complex is essential for the modulation of cell mobility.

**Elisabeth Georges-Labouesse** (Strasbourg, France) told us that integrin  $\alpha 6\beta 4$  is a laminin receptor which provides stable adhesion of epithelial cells to the underlying basement membrane and is essential for skin integrity. To further investigate the roles of  $\alpha 6\beta 4$  integrin in the intestinal epithelium, a mouse cell line was generated in which ablation of the  $\alpha 6$  integrin chain was induced specifically in the intestinal epithelium. It was found that there are serial defects in this mouse including epidermal detachment, absence of hemidesmosomes, enlarged intestines associated with thickening of both the mucosa and muscle layers. All animals also had chronic inflammation with superficial erosions and inflammatory cells infiltrations resembling inflammatory bowel diseases (IBD). Therefore, this mouse line will constitute a model to study the pathways implicated in intestinal tumorigenesis.

**B.Sulka** (Lyon, France) gave the last talk named 'A role for syntenin in syndecan-1 formation of filopodia'. Syndecan-1 participates in cell adhesion to the precursor form of laminin332 through an interaction with its

carboxy-terminal LG4/5 domain, and this interaction promotes cell spreading and contributes. It is found that syntenin-1 which has been suggested to couple syndecans to cytoskeletal proteins, is recruited by syndecan-1 in cells plated on the LG4/5 fragment. Meanwhile, syntenin-1 binding to syndecan-1 is prevented when cells are treated with orthovanadate which inhibits tyrosine dephosphorylation. Therefore, syntenin binding to syndecan-1 appears directly correlated with the dephosphorylation level of syndecan-1. Furthermore, it is found that binding of syntenin-1 to syndecan requires dephosphorylation of the tyrosine residue Y309 within the EYFA sequence, and the phosphorylation of this tyrosine residue prevents binding of syntenin. All of these results indicated that syntenin-1 can act as a molecular switch essential in the formation of syndecan-1 mediated filopodia.

### **Workshop J: MMPs – Extracellular matrix and cell surface remodelling**

**Nami Sugiyama** (Helsinki, Finland) opened the workshop by describing how MT1-MMP (membrane-type 1 matrix metalloproteinase) is responsible for ECM remodelling during both physiological and pathological events, and has been found to have increased expression in a number of cancers. To understand the upstream signalling events that lead to production and activation of this enzyme in cancer they expressed a library of 90% of all human protein kinases in HT1080 fibrosarcoma cells and measured the change in MMP2 activity, via zymography, as an indirect measure of MT1-MMP activity. This identified 12 kinases as positive regulators of the MT1-MMP/MMP2 cascade, all belonged to PKC, IL-1 $\beta$ /TNF- $\alpha$ , TGF- $\beta$  and receptor tyrosine kinase (RTK) signalling pathways. Validation of the 12 kinases via siRNA and inactive kinases identified RTKs as novel effectors of MT1-MMP activity, possibly via their role in IL-1 $\beta$ /TNF- $\alpha$  and TGF- $\beta$  signalling. Kinase induction of MT1-MMP resulted in collagen degradation and 3D cell invasion. These effects were thought to be mediated by changes in MT1-MMP subcellular localisation rather than changes in transcription. IL-1 $\beta$  pathway kinase increased levels of active MT1-MMP on the cell surface and RTK interaction with MT1-MMP resulted in recycling of the enzyme to the invasive front of cancer cells.

**Yasunori Okada** (Tokyo, Japan) spoke on prototype membrane anchored ADAM12 (a disintegrin and metalloproteinase: ADAM12m) and how they found it to be highly expressed in osteoarthritic cartilage compared to normal in their quantitative RT-PCR ADAM screen. In situ hybridisation and immunolocalisation showed that the enzyme is expressed and localised to the cell membrane of osteoarthritic chondrocytes. Immunolocalisation also revealed that immuno-reactivity directly correlated to Mankin scores, chondrocyte cloning and chondrocyte proliferation. Addition of TGF- $\beta$  to OA chondrocytes resulted in increased ADAM12m expression, chondrocyte proliferation and degradation of insulin growth factor binding protein-5 (IGFBP-5). TGF- $\beta$ -induced chondrocyte proliferation was inhibited by suppression of IGF-I signalling. Agarose gel culture of OA chondrocytes showed the ADAM12m siRNA, ADAM inhibitor and anti-ADAM12 antibody could all block TGF- $\beta$ -induced chondrocyte proliferation and clustering and IGFBP-5 degradation. He concluded that ADAM12m may be involved in OA chondrocyte cloning via increased bioavailability of IGF-I after IGFBP-5 degradation.

**Yoshifumi Itoh** (London, UK) presented data which indicates that MT-MMP1 (membrane-type 1 matrix metalloproteinase) is essential for synovial cell invasion of cartilage in human rheumatoid arthritis (RA). He described how pannus invasion of the cartilage is dependent on collagenolytic enzymes and how MT1-MMP, which is a membrane bound collagenolytic enzyme, is already known to promote cell invasion of a number of cell types. Dr Yoshifumi found high levels of MT1-MMP expression in RA synovial fibroblasts and at the invasion front of the pannus in the RA joint. Freshly isolated RA pannus and synovial fibroblasts were also found to invade 3D collagen matrix. This was determined to be MT1-MMP dependent as invasion was sensitive to TIMP-2 and the broad spectrum MMP inhibitor GM6001, but not TIMP-1. In concluding his presentation, Dr Itoh suggested that MT1-MMP-dependent invasion could be a potential therapeutic target for the use in RA treatment.

**Satsuki Mochizuki** (Tokyo, Japan) discussed the role of ADAM28 (a disintegrin and metalloproteinase) in cancer cell invasion and metastasis. They have previously reported that increased expression of membrane and secreted forms of ADAM28 (ADAM28m and ADAM28s) in cancer has a positive correlation to cell proliferation and lymph node metastasis. To further this they used the yeast two-hybrid system to identify ADAM28s interacting proteins and found von Willebrand factor (vWF), which is a known anti-metastatic

factor. Further binding assays with immobilised vWF found it bound to <sup>125</sup>I-labelled proADAM28s in a dose dependent manner, and this could be reduced by the addition of anti-vWF antibody and with unlabelled forms of proADAM28s. Dr Mochizuki then described how incubation of vWF with active ADAM28 resulted in cleavage of the vWF multimer. vWF is a known substrate of ADAMTS13, however ADAM28 was found to cleave the factor in a different location through the use of fluorogenic peptide substrate FRETs-VF73. Addition of vWF to ADAM28 non expressing cell line A459 resulted in cell apoptosis, but addition to ADAM28 high expressing cell line PC9 did not have any effect. Dr Mochizuki concluded that ADAM28 may play a role in cancer metastasis by cleaving vWF and preventing apoptosis.

**Graham Riley** (Norwich, UK) described how chronically painful tendon and ‘spontaneous’ rupture of tendon can be related to degradation and changes in tendon matrix. Ruptured, chronically painful and normal Achilles tendon were collected and ADAMTS-4 (A disintegrin and metalloproteinase with thrombospondin motifs) mRNA was found to be increased 8-fold in ruptured Achilles tendon compared to normal tendon. Western blots were performed on tendon tissue extracts and revealed that mature ADAMTS-4 (68 kDa) could only be detected in ruptured tendon, whereas processed ADAMTS-4 (53 kDa) was found in both chronic painful tendinopathy and normal tendon. Achilles tendon cells were cultured both in monolayer and 3D collagen gels in the presence of cytokines to investigate the regulation of ADAMTS-4. The addition of TGF-β stimulated ADAMTS-4 mRNA expression (~20 fold increase after 24 hours) in the cultured cells, and this induction could be further increased synergistically with IL-1. After TGF-β stimulation increased levels of immunoreactive proteins were detected in cells consistent with processed ADAMTS-4, and a smaller form of the protein (40-45 kDa) was detected in conditioned medium. ADAMTS-4 expression increased in cells cultured in 3D collagen gels compared to monolayer cultures, whilst ADAMTS-1 and -5 mRNA was lower in 3D cultures when compared to monolayer. Both ADAMTS-1 and -5 showed opposite regulation to TGF-β and IL-1 treatment in comparison to ADAMTS-4. Dr Riley concluded that ADAMTS-4 could therefore be implicated in tendon matrix proteoglycan turnover.

**Catherine Moali** (Lyon, France) discussed how the over-expression of tolloid proteinase BMP-1 can lead to loss of cell adhesion and changes in cell-matrix interactions in epithelial cell line HEK293. This initial observation led to numerous cell adhesion assays with different matrix components. These assays revealed that the likely substrate was a matrix component rather than a cellular receptor. The conditioned matrix and medium collected from BMP-1 expressing cells contained a C-terminal fragment of thrombospondin-1 (TSP-1) that was not seen with vector only cells. Further *in vitro* experiments confirmed TSP-1 as a substrate of BMP-1 and the cleavage site was determined. The cleavage of TSP-1 was not very efficient *in vitro* indicating that other factors are required for efficient cleavage and/or other cleavage events happen simultaneously. She concluded the talk by describing iTRAQ™ (Isobaric Tags for Relative and Absolute Quantification) which they are currently using to identify changes in the ECM deposited by BMP-1 producing cells.