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REPORT

Report on the 9th international workshop on the CCN family of genes, November 2–7, 2017, Saint-Malo, France

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Overview

Following requests by many colleagues, the 9th biennial International workshop on the CCN Family of Genes was held at the Palais du Grand Large of Saint-Malo France, November 2–7 2017. It was a return to Brittany where the first three enjoyable CCN workshops were held by the International CCN Society in the early 2000's. At the 2017 workshop the variety of topics covered in the eight sessions of the program was another indication of the wide range of functions played by the CCN proteins.

In an effort to widen the multidisciplinary exchanges that were initiated in previous meetings, **Joanne Murphy-Ulrich** and **Kim Midwood** were invited to participate in a special session on matricellular proteins. Their excellent presentations were very well received by all the participants at the meeting who were given the opportunity to get both a broad overview of the field and updates on immunomodulatory matricellular molecules in inflammatory diseases and thrombospondin-1 in the regulation of TGFβ activation in pathological conditions.

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The efforts deployed by the ICCNS and the distinguished representatives of ASMB (American Society for Matrix Biology) and BSMB (British Society for Matrix Biology) to foster better and wider connections should benefit the scientific community at large. **B. Perbal** presented an updated overview of the CCN family of proteins, with an emphasis on the complex series of spatio-temporal combinatorial events governed by the tetramodular organization of CCN proteins. This overview represents an example of the signaling coordination mechanisms that are required for balanced developmental processes to occur.

As a part of the educational sessions of the meeting, **Meenhard Herlyn**, who was the recipient of the 6th ICCNS-SPRINGER award, delivered a truly inspiring presentation on melanoma biology with a summary of both historical and prospective views of translational approaches that were now feasible due to the most advanced technologies and top level basic science. This presentation which concluded the educational sessions of the meeting, opened the path to a series of talks dealing with the involvement of CCN proteins in tumor angiogenesis and progression, oestrogen sensitization in breast cancers and metastasis in melanoma.

Special session on matricellular proteins

Chaired by Bernard Perbal

Kim Midwood presented new insights into the growing interest in innate immunity and how targeting of immunomodulatory matricellular molecules could be used for the treatment of inflammatory diseases. Emphasis was placed on matrix proteins like tenascin-C which is induced upon tissue injury and can directly activate toll-like receptor 4(TLR4) mediated sterile inflammation. Interestingly, other matricellular proteins can carry an inflammatory epitope that can be recognized by TLR4. Thus, identifying pathologically relevant forms of

matrix constituents will help us better understand inflammation. These findings promise to lead to new diagnostic tools and translation into novel therapeutics relevant to autoimmune and fibrotic diseases and the tumor microenvironment.

Joanne Murphy-Ullrich brought new ideas on the role of the matricellular protein thrombospondin-1(TSP-1) in regulation of latent TGF-beta activation in disease. The powerful roles of TGF-beta in fibrogenesis, metastasis, growth control and immune regulation make it a singular critical molecule. Since TGF-β is secreted by cells in a latent form (LAP) its mechanisms of activation involve proteolytic processing, binding to integrins, cytoskeletal contractility, post-translational processing by ROS and binding to TSP-1. The process can be manipulated and in the context Joanne discussed the peptide antagonist LSKL and newer derivative compounds as potential therapeutics for diabetic cardiomyopathy, nephropathy, and in osteolytic hematologic cancer and multiple myeloma. Other efforts are ongoing to develop molecule antagonists based on structure-based modeling.

Session II- Meenhard Herlyn: Springer awardee special lecture

Meenhard Herlyn, recipient of the Springer Award, has been recognized for major contributions to our understanding of melanoma and a leader in advancing therapy. At this meeting Meenhard brought attention to the observation that dysregulation of CCN3, is an early indicator of transformation of melanocytes into melanoma that escapes control by the epidermis and invades the dermis. This first step may lead only to benign nevi marked by a single mutation such as $BRAF^{V600E}$. Subsequent hijacking of developmental genes revealed through siRNA screens identified LPAR1-RAPA1A axis as a key signaling pathway essential for survival of the neural crest phenotype as well as melanoma cell growth, invasion and therapy resistance to MAPK kinase inhibitors, proliferation relevant. LPAR signaling governs survival through activation of Hippo/YAP and S6-kinase-PI3-kinase pathways. This therapeutic approach in fact gets at the heart of the stemness character of cancer and demonstrates how CCN proteins figure prominently in cell behavior and tissue organization.

Then the workshop went on to delve deeper into the normal and pathobiological roles and functions of the CCN proteins.

Session III-cancer

Chaired by Meenhard Herlyn and Sushanta Banerjee

Sushanta Banerjee continued with studies showing the anti-invasive role of CCN5 in various cancers and in particular breast cancer. CCN5 is well expressed in normal breast epithelium, and in the low-invasive and rarely metastasizing ER-α positive tumor cells. Using a genetically engineered mouse model to turn on CCN5 in ductal epithelial cells, up-regulated ER-α. In a variety of other models, mouse mammary gland culture, and human mammary epithelium and tumor cell lines this relationship holds, as well as after treatment of cells with recombinant CCN5 protein. Apparently CCN5 operates by interacting with integrin α6β1, suppressing Akt and activating FOXO3a. CCN5 treatment in combination with tamoxifen (sensitization) is also capable of restoring ER-α in TNBC (triple-negative breast cancer) cells offering a potential new therapeutic approach. Finally, an exciting regulatory connection between CCN5 and the control of leptin induced tumor progression (the obesity angle) offers a new avenue for therapeutic intervention. Thus we anticipate exciting new insights into the anti-tumor roles of CCN proteins.

In investigating further roles of CCN2, **Andrew Leask** presented studies on the cancer-associated fibroblasts (CAFs) in melanoma. Meenhard Herlyn had earlier presented how CCN3 might preserve the barrier to invasion, but here the focus falls on metastatic melanoma that portends an extremely poor prognosis. Drug resistance, not surprisingly, leads to poor clinical outcomes. In contrast, Leask et al. turned their attention to the stromal component and showed that CCN2 expression negatively correlated with survival and positively correlated with angiogenesis. In an animal model, loss of CCN2 from collagen type 1 expressing CAFs impaired metastasis. As CAFs are myofibroblastic their inactivation led to decreased periostin expression and thereby impaired angiogenesis and vasculogenic mimicry. CCN2 deficient B16(F10) cells showed impaired invasion and impaired tubulogenesis formation (mimicry component) and reduced type 1 collagen, VEGF and PDGF expression. Thus CAF-derived CCN2 is a viable therapeutic target yet to be realized for melanoma.

Curzio Ruegg further presented on breast cancer in terms of the role of CCN1 in promoting invasion and metastasis of cancers growing in a previously irradiated field (as after radiotherapy). The microenvironment becomes highly hypoxic, driving high expression of CCN1 and therein promoting tumor cell survival, migration, invasion and metastasis in a αVβ5- integrin dependent manner. Inhibiting the integrin can reverse the CCN1-mediated effects. The investigators generated Rip1Tag2Cyr double transgenic mice to assess Rip1Tag2 driven multistep pancreatic β-cell carcinogenesis. The tumors were larger and more vascularized, but CCN1 had no effect on development of angiogenesis. CCN1 effects could be reversed by blocking VEGFR2 function, thus highlighting VEGF dependency. CCN1 promoted β-cell invasiveness but not metastasis. They next examined the role of CCN1 in TNBC lung metastasis (xenograft modele) using MDA-MB-231 and SUM159 cell lines. Silencing CCN1 impaired cancer cell extravasation to lung and trans-endothelial

migration in vitro but not metastatic outgrowth in vivo. Interestingly, CCN1 suppressed anoikis via β1-integrin and in an AMPK-α dependent manner. Thus CCN1 supports the metastatic phenotype in the hypoxic microenvironment leaving open the question whether CCN1 supports tumor stem cells that flourish in such environments.

Ken-ichi Katsube noted that angiogenesis and Notch signaling can be modulated by proteins like Fringe and CCN3, leading them to study the regulation of the regenerative process after injury. Considering that cancer can be thought of as an injury that does not heal, angiogenesis was explored in a model of oral squamous cell carcinoma model in which Notch 3 expression was related to poor prognosis. Interestingly, Notch 3 expression was noted in the surrounding interstitial tissues. Co-culturing of squamous cell carcinoma cells (HO1-N-1 cell line) and normal human dermal fibroblasts showed significant upregulation of Notch 3 in the fibroblasts. When HUVEC cells were added to the cultures, significant tubular structures were promoted suggesting that tumor cells can exploit stromal cells to promote angiogenesis and thus tumor progression.

Herman Yeger turned attention to how homeostasis might be disrupted to tackle cancer progression and possibly earlier stages. The group reported on the potent anti-tumor properties of dietary components (in particular the Mediterranean diet) such as sulphoraphane (SFN). Using SFN to target growth and survival pathways (models of bronchial carcinoids and neuroblastoma) a combination with acetazolamide (AZ) that disrupts pH homeostasis proved highly effective in inhibiting growth in vitro, clonogenicity, and xenograft tumors in immuncompromised mouse models. A similar efficacy was also realized with bladder cancers. Since dietary molecules like SFN also function as epigenetic modulators (HDAC inhibitors) simultaneous targeting of multiple pathways and pH regulation (metastasis relevant via carbonic anhydrases) may prove effective irrespective of the oncogenic drives. Importantly, dietary agents are minimally non-toxic and AZ is already in clinical use so repurposed here.

Session IV-developmental biology

Chaired by Havard Attramadal and Stephen Twigg.

Taihao Quan has been investigating the deleterious changes to skin that occur during aging. Overall, alterations to the connective tissues and associated extracellular matrix leads to loss of structural integrity and function. CCN1, predominantly expressed in dermal fibroblasts, is elevated in aging skin. Subsequent thinning of the dermis via enzymatic destruction of the ECM components and increase in pro-inflammatory cytokines (termed inflammaging) accounts for the aging phenotype. This phenotype was reproduced in a transgenic mouse model where CCN1 was expressed in fibroblasts under the collagen type1α2 gene promoter and enhancer. In this model of accelerated dermal aging, there was increased susceptibility to skin cancer/papilloma development. Thus therapeutic targeting of CCN1 may be of benefit in the elderly. It would be interesting to uncover whether dysregulated CCN1 may also be responsible for aging and cancers in other organ sites with aging.

Brahim Chaqour has worked extensively on angiogenesis in normal and disease models. Here he explored the effector molecules involved in pathological intercellular signaling at the level of cell-matrix and endothelial cell-pericyte interactions. CCN1 is expressed dynamically in angiogenic endothelial cells (EC). Deleting CCN1 using a cre/lox strategy resulted in EC hyperplasia and caused blood vessels to coalesce into large flat hyperplastic sinuses suggesting loss of essential communication. This finding is consistent with a loss of VEGF receptor activation thus loss of CCN1-mediated feedback. In a model of oxygen-induced retinopathy, pericytes become the predominant CCN1 producers and deletion of CCN1 results in decreased pathological retinal neovascularization. CCN1 can induce non-canonical Wnt5a in percicytes while exogenous CCN5 inhibits CCN1 expression, which induces EC proliferation and hypersprouting. Treatment of mice with TNP470, an inhibitor of angiogenesis reestablished endothelial CCN1 expression and decreased pathological neovascularization. Thus the intimate communication between pericytes and endothelium may depend on CCN1 in a finely balanced manner to prevent pathogenesis while maintaining physiological angiogenesis. It is interesting to note that further work from the laboratory has identified abscisic acid as being able to protect against retinopathy pathology. Abscisic acid is critical for dropping of leaves indicating that nature may have other clever designs and molecules worth exploiting for CCN work.

Nira De La Vega Gallardo (Queen's University, Belfast) presented studies that followed the identification of CCN3 as a T-reg-derived mediator of oligodendrocyte differentiation and myelination in vitro. T-regs are also required for remyelination in vivo. In organotypic brain slices, CCN3 enhanced developmental remyelination and oligodendrocytic differentiation in primary mixed glial cultures. In the mouse, CCN3 is found in postnatal development of the CNS. It colocalizes with NeuN, a neuronal marker. Major sites of expression were found in layers II and V of the cerebral cortex, CA1 field in hippocampus, and suprachiasmatic nuclei. CCN3 was secreted from brain slices. In demyelinated mice fed cuprizone, CCN3 was expressed in septal nuclei during demyelination and early stages of remyelination. Further studies will determine if CCN3 responds to stress or is indeed an important factor in a possible field gradient effect. Promise is shown for development of regenerative therapeutics if CCN3 is essential for myelination/remyelination.

Kunimasa Ohta previously introduced the workshop to a new secreted molecule *Tsukushi* (TSK*)* that is a member of the family of small leucine rich proteoglycans that binds to nodal/Vg1, BMP4/chordin, FGF8, Frizzled 4, TGFβ1 and Delta modulating their downstream intracellular signaling. Thus TSK is yet another extracellular signaling mediator that is able to interact with CCN proteins. In the subventricular zone (SVZ) neurogenesis persists in the postnatal brain. TSK knockout (TSK−/−) mice showed aberrant cell proliferation and cell death in SVZ, resulting in expansion of the lateral ventricle (hydrocephalus). Since hydrocephalus in humans exhibits lateral ventricle changes, a genomic sequencing analysis of 12 hydrocephalus patients was performed, and found 2 heterozygous nucleotide changes were identified within the TSK coding region (missense mutations) suggesting a possible cause of hydrocephalus. In addition Kunimasa presented initial provocative findings that cellular reprogramming may involve ribosomes.

Session V-functional aspects

Chaired by Enrique Brandan and Bernard Perbal.

Stephanie Frost presented molecular biology studies on CCN2 to identify the finer aspects of transcription regulation, focusing on characterization of the DNA sequences proximal to the gene and its promoter region. An in silico approach exploited the ENCODE information to analyze the region 300 kb upstream of the CCN2 transcription start site. Taking into consideration enhancer associated histone modifications, DNase hypersensitivity sites, and inter-species conservation enabled selection of potential enhancer regions. Reporter constructs were created to drive lacZ via the silent Hsp68 minimal promoter and by microinjection of linearized constructs into B6CAF1 mouse embryos the transgene expression was assessed at E15.5. An enhancer, at 100 kb from the start site, drove expression in blood vessels, and an additional three enhancers facilitated expression in chondrocytes. The two enhancers at -198 kb and -229 kb drove expression primarily in chondrocytes and mesenchymal (dermal fibroblasts) and vascular tissues. Expression in periosteal tissues were associated with the -229 kb enhancer. A − 135 kb enhancer site drove expression in articular chondrocytes. Taken together with the finding of regulatory sequences 4 kb upstream there is potential for a broad range of tissue expressions. Further studies will attempt to unravel possible temporal spatial contexts for the enhancers. Whether such fine tuning exists for other CCN proteins has yet to be explored.

Satoshi Kubota emphasized the potential on molecular therapeutic properties of CCN proteins and undertook a search for small compounds that could induce CCN2 particularly to counteract osteoarthritis. Screening of an orphan ligand library identified harmine, a member of the β-carboline family. Harmine significantly enhanced chondrogenesis as well as CCN2 production (chondrocytic HCS-2/8 cells and osteoarthritic articular chondrocytes as models). The glucocorticoid fluocinoline acetonide (FA) was also identified as a novel synergistic factor for TGFβ3-associated chondrogenesis. Application of FA with TGF-β3 to damaged articular cartilage (mouse model) strikingly enhanced regeneration greater than TGF-β3 alone. In addition, monoiodo acetate (MIA- a toxic compound to a glycolytic enzyme) was found to enhance CCN3 expression via its proximal promoter. This brings attention to a novel metabolic regulatory system of CCN3 in chondrocytes.

Malini Sen commented on the mutations in the CCN6 coding region that were linked to Progressive Pseudo Rheumatoid Dysplasia (DPRD), a debilitating disease marked by progressive loss of cartilage and irregular bone growth. However, the molecular mechanism of this disease has remained elusive. Using the C28/12 chondrocyte line Sen et al. found that CCN6 localizes to mitochondria. siRNA knockdown resulted in ROS accumulation in mitochondria and calcium implying that CCN6 is important for mitochondrial functions. CCN6 depletion also leads to ROS-dependent PGC1α induction with increased mitochondrial mass and volume density and an altered morphology. The increased number of respiring mitochondria and enhanced rate of ATP synthesis indicate augmented electron transport chain activity. Using chromatin immunoprecipitation and a reporter luciferase assay it was shown that Nrf2 (a key member of the intrinsic defense system) binds to the CCN6 promoter and represses CCN6 transcription. The results suggest that CCN6 operates as a guardian of mitochondrial functions utilizing Nrf2 pathway as the mediator. Sen et al. are investigating whether CCN6 operates at the level of the electron transport chain via its multimodular architecture.

Gary Fisher presented further information on the role of CCN2 in skin fibroblasts. siRNA depletion of CCN2 coding or non-coding regions, inhibited fibroblast proliferation with accumulation of fibroblasts in G1/G0. CCN2 knockdown abrogated serum stimulated cyclin dependent cycle progression (inhibition of cdk2/cyclin E and Cdk4/cyclinD nuclear translocation). CCN2 expression was directly regulated by YAP transcription factor which is important for proliferation. Inhibition of fibroblast proliferation due to knockdown of YAP could be rescued by expression of CCN2 making the functional connection. The evidence points to CCN2 as a novel cell cycle regulator in addition to the well-known regulation of ECM in primary skin fibroblasts. Thus, CCN2 functionally links fibroblast proliferation, through cell cycle regulation, and fibrotic activity. This perspective adds one more aspect to the role of CCN proteins in the responses of skin to injury.

Havard Attramadal has explored how degradation products of CCN2 possess unique biological properties. This question has been of considerable interest in the field

for many years in light of the recognized bioactivities of certain smaller fragments of CCN proteins. A proteolytic fragment comprising the carboxy terminal domains III (TSP-1 repeat) and IV (cysteine knot) was shown to convey all biologically relevant activities of CCN2. A homodimer was 20-30× more potent than the monomer form. CCN2 could be cleaved with MMPs 7,8,12 and 13. Batismat, a MMP inhibitor, blocked this cleavage. The homo-dimer fragment of domains II and IV potently activated fibroblast migration, stimulated assembly of focal adhesion complexes, enhanced RANK-induced osteoclast differentiation of RAW264.7 cells and promoted mammosphere formation of human MCF-7 breast tumor cells. Interestingly, the N-terminal fragment of CCN2 showed no activity. These data highlight that the role of the N-terminal fragment requires further study, including whether it modulates the other domains of CCN2 in specific organ type cellular and tissue functions and whether it participates in biologically relevant protein-protein interactions.

Session VI-fibrosis 1

Chaired by Satoshi Kubota and Gary J Fisher.

The Fibrosis 1 session consisted of four presentations that focused on new insights into the pathophysiology of cancer and fibrosis.

Philip Trackman reviewed his long-standing work on the lysly oxidase family of enzymes. This family consists of five members that catalyze cross-linking of collagen and elastin, which is necessary to stabilize their structures and for their biological functions. The talk focused on lysyl oxidase like-2 (LOXL2) and its role in oral squamous cell cancer (SCC). LOXL2 is upregulated in oral SCC and serves as a marker of unfortunate outcome. Philip and his team investigated possible roles of LOXL2 in promoting oral SCC using proteomic techniques and discovered that LOXL2 oxidizes platelet derived growth factor receptor-beta (PDGFR-β) and integrin alpha V. In functional studies using oral fibroblasts the actions of LOXL2 were shown to enhance the activity of PDGFR-β and promote fibroblast adhesion. Importantly, a novel LOXL2 inhibitor reduced tumor growth in oral SCC mouse models, further implicating LOXL2 as a driver of oral SCC. In addition to presenting new mechanistic insights into the role of LOXL2 in oral SCC, this presentation highlighted the emerging, multifunctional role of fibroblasts in promoting epithelial tumor formation and progression.

Federica Accornero added to the well-discussed topic of fibrosis with a lively presentation on the role of CCN2 in injury-induced heart fibrosis. Using mice with cardiomyocyte-specific knockdown or overexpression of CCN2, she showed that expression of CCN2 in cardiomyocytes had no effect on cardiac fibrosis. In contract, knockdown of CCN2 in activated cardiac fibroblasts significantly reduced injury-induced cardiac fibrosis. Federica's data point out the cell-type specific nature of the actions of CCN2 in the fibrotic process.

Amy Horwell studying the role of CCN2 in fibrosis, shared her novel work on the role of CCN2 in bleomycin-induced lung fibrosis. Amy and co-workers examined the impact of fibroblast-specific versus ubiquitous cell-type knockout of CCN2 on bleomycin-induced lung fibrosis. In contrast to the results presented by Frederica in injury-induced cardiac fibrosis, fibroblast-specific knockout of CCN2 did not reduce bleomycin-induced lung fibrosis. Interestingly, ubiquitous cell-type knockout of CCN2 caused enhanced fibrosis. This surprising finding reveals that CCN2 can act to blunt fibrosis, as opposed to promote fibrosis, as has been shown in a variety of models. This potential duality of CCN2 function highlights the complexity of the fibrotic process, and the cell-type specific and organ-type specific actions of CCN2. The authors point out that general inhibition of CCN2 for the treatment of lung fibrosis may give rise to unexpected, detrimental consequences.

David Brigstock continued the theme of the role of CCN2 in fibrosis in the context of exosomes and liver damage. Exosomes are small sub-cellular vesicles that carry functional molecules (miRs, mRNA, protein) from one cell to another. Delivery of the exosomes' contents into the receiving cell may have profound effects on its function. David described his fascinating mouse studies showing that acute CCl_4 -induced liver damage was substantially inhibited by IP injection of exosomes that were purified from healthy cultured hepatocytes. Administration of exosomes inhibited expression of CCN2 and other markers of liver fibrosis and damage. Furthermore, IP injection of hepatocyte exosomes reversed liver fibrosis due to long-term CCl_4 administration. Using fluorescently-labelled exosomes, the target cells in mouse livers were identified as hepatocytes and hepatic stellate cells (HSC). Exosome treatment also reduced markers of cytotoxicity and fibrosis in cultured hepatocytes and HSC that were exposed to alcohol. The hepatocyte exosomes were found to contain several species of micro RNAs, and administration of mimics of each these micro-RNAs reduced expression of CCN2 and other fibrogenic mediators and markers of damage in cultured HSC and hepatocytes. These novel data reveal the importance of exosomes in the regulation of cellular function and the therapeutic potential of exosomes to prevent and treat liver fibrosis. Exosome technology is in the early stages and may hold great promise for both enriching our understanding of the pathophysiology of complex diseases, such as fibrosis, and future therapeutic applications.

Session VII-fibrosis 2

Chaired by Joanne Murphy-Ullrich and Lester Lau.

Mary Barbe discussed the hypothesis that tissue fibrosis plays a key role in motor dysfunction associated with overuse-

induced musculoskeletal disorders. Using a rat model in which tissue fibrosis is induced in a high repetition high force (HRHF) protocol, it was found that CCN2, interferon gamma (IFN-γ), and Substance P-Neurokinin 1 (NK-1) may play a role in tissue fibrosis and sensorimotor declines. Treatment with anti-CCN2 monoclonal antibodies (FG-3019) rescued HRHF-induced decline in grip strength and forepaw mechanical allodynia, and reduced HRHF-associated collagen deposition. Treatment with a NK-1 antagonist (L732138) also reduced HRHF-induced declines in grip strength and collagen deposition. Treatment targeting IFN-γ was less effective. These findings suggest that anti-CCN2 and NK-1 antagonist treatments may help to curtail overuse-induced nerve and musculotendinous fibrosis.

Enrique Brandan studies amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease characterized by upper and lower motoneurons degeneration. Although the majority of ALS cases are sporadic, about 5–10% of ALS are familial and 20% of familial ALS can be explained by mutations in superoxide dismutase 1 (SOD1). Transgenic mice carrying the human $SOD1^{G93A}$ gene serve as an animal model for ALS and exhibit enhanced TGF-β signaling. Treatment of these mice with the monoclonal neutralizing antibody (FG-3019) against CCN2 reduced fibrosis and muscle atrophy in skeletal muscle of $hSOD1^{G93A}$ mice. Anti-CCN2 treatment also improved neuromuscular junction (NMJ) innervation and muscle and locomotor performance. These results show that blocking CCN2 significantly improves ALS phenotypes in $hSOD1^{G93A}$ mice. The possibility that blocking CCN2 function may help alleviate ALS-associated symptoms was discussed.

Tonia Vincent presented a provocative hypothesis in which CCN2 plays a major role in the activation of TGF-β. It was shown that CCN2 is covalently bound to latent TBF-β in chondrocytes, and this complex is sequestered on heparan sulfate in the extracellular matrix and release upon injury. The released latent complex then binds to cell surface TGFβR3 in a heparan sulfate-dependent manner, allowing activation of the canonical TGFβ signaling pathway. Thus, CCN2 is proposed to be a new latent TGF-β binding protein whose major function is to control the matrix sequestration and activation of TGF-β through TGFβR3.

Masaharu Takigawa showed that several members of the CCN family are found in platelet-rich plasma (PRP), which has been used to enhance the regeneration of damaged joint tissues, such as osteoarthritic cartilage. Masaharu described the expression of all six members of the CCN family in platelets and megakaryoctyes. Application of a molecular mixture composed of the same CCN proteins as those found in PRP stimulated the expression of cartilage matrix components in human chondrocytes. In searching for a method to enhance production of endogenous CCN proteins, it was found that low intensity pulsed ultrasound (LIPUS) can induce the expression of CCN2 and major cartilage matrix genes. This effect is CCN2-dependent as matrix genes expression is not observed in CCN2-deficient chondrocytes. Furthermore, LIPUS has been used clinically for promoting bone fracture healing and is able to induce CCN2 expression in rat articular cartilage in vivo. Thus, the application of LIPUS may promote joint regeneration through the induction of CCN2.

Roel Goldschmeding showed that lymphangiogenesis, for which a major driver is VEGF-C, is correlated with the degree of renal interstitial fibrosis. It was found that lymphangiogenesis during tubulointerstitial fibrosis is associated with increased expression of CCN2 and VEGF-C in human obstructed nephropathy as well as in diabetic kidney disease. In mouse models of kidney disease, CCN2 knockout or knockdown significantly reduced lymphangiogenesis and VEGF-C expression. CCN2 induces VEGF-C production in HK-2 cells and CCN2 protein directly interacts with VEGF-C in vitro. Interestingly, full-length CCN2 inhibits VEGF-C induced capillary formation in human lymphatic endothelial cells. Therefore, CCN2 is significantly involved in fibrosis-associated renal lymphangiogenesis through direct interaction with VEGF-C and regulation of VEGF-C production.

Session VIII-inflammatory diseases

Chaired by David Brigstock and Philip Trackman.

Shu Wu reported recent data on the role of CCN2 in bronchopulmonary dysplasia (BPD), the most common and serious chronic lung disease of premature infants. Lungs from epithelial CCN2 transgenic mice showed enrichment for inflammatory pathway components related to cytokine-cytokine receptor interaction, toll-like receptor signaling, chemokine signaling and asthma. Alveolar macrophages (AM) from these mice showed increased expression of markers of alternatively activated macrophages (AAM) such as RELM-α and Ym1/2. Increased expression was also reported for β-catenin, IL-33, and its receptor, ST2L. RELM-α was also upregulated in experimental BPD and its adenoviral delivery caused a BPD-like pathology in mice. These results identify key pathways by which AAM is induced following increased epithelial CCN2 production and show that CCN2 induces BPD via RELM-α.

Stephen Twigg summarized our knowledge of CCN proteins in obesity and diabetes and its complications, both as potential markers and mediators of disease. He then presented data showing that in the mouse NASH model of high fat feeding with low dose STZ-induced diabetes, I.P. administration of anti-CCN2 antibody resulted in both anti-fibrotic and anti-inflammatory effects in the liver, as shown by,

respectively, reduced levels collagen I/III mRNA and/or protein and MCP-1 mRNA. Anti-CCN2 therapy was also associated with restoration of p-ERK or CCN3 levels, which are otherwise up- or down-regulated in this model. The involvement of p-ERK supports the concept that this MAPK pathway may mediate liver fibrosis in NASH. Follow-up in vivo studies will use hepatocyte-specific knockdown of CCN2 to directly implicate hepatocyte CCN2 as a key mediator of type 2 diabetes induced-NASH.

Michelle Naughton following up on their findings that regulatory T cells (Treg) produce CCN3 that regulates oligodendrocyte differentiation and myelination, described studies to investigate CCN3 in multiple sclerosis (MS). However, plasma CCN3 levels did not significantly differ between patients who were in disease relapse as compared to remission, although CCN3 levels were significantly higher in CSF compared to plasma in all patients. As assessed by IHC, CCN3 was present at higher levels in MS brain tissue versus controls and, further, CCN3 levels were increased in remyelinating lesions. These preliminary results lay the groundwork for studying the role of CCN3 in intrinsic CNS regeneration and therapy of chronic neuropathies.

Pauline Henrot presented data on the contribution of skin pigmentation to the regulation of CCN2 or CCN3 in systemic sclerosis (SSc). As assessed by IHC in comparison to healthy controls, SSc patients showed no change in dermal CCN3 expression but exhibited increased dermal and epidermal CCN2 expression that was strongly associated with fibrosis. CCN3 expression in dermal blood vessels was significantly decreased in SSc skin and correlated with a decreased superficial papillary dermis and decreased frequency of skin capillaries. Epidermal CCN3 was uniformly lower in patients without pigmentary disorders, and highly variable in SSc patients with pigmentary disorders. Thus CCN3 deficiency may contribute to vascular and pigmentary dysregulation in SSc patients and restoration of CCN3 levels may be a therapeutic option for restoring vascular integrity in SSc.

Session IX-pathology

Chaired by George Bou-Gharios and Andrew Leask.

Craig M. Keenan used a floxed CCN2 mouse strain to delete the CCN2 gene specifically in chondrocytes in male mice aged 8 weeks. Osteoarthritis was induced, at 10 weeks of age, by surgical destabilization of the medial meniscus (DMM), with joints being analysed 4 and 8 weeks post-surgery. Initial data suggest that CCN2 plays a role in limiting cartilage degeneration at 4 weeks, but does not modify bone changes in response to joint destabilization.

Abdellatif Elseoudi also discussed osteoarthritis. The effect of FGF-1 on CCN2 expression was evaluated using human chondrocytic HCS-2/8 cells. The cellular phenotype was estimated by the gene expression of chondrocytic markers. FGF-1 repressed CCN2, ACAN and COL2A1, but increased MMP-13 in HCS-2/8 cells. The effects of FGF-1 were also seen on a minimal CCN2 promoter/reporter construct.

Lester Lau examined the role of CCN1 in intestinal mucosal healing, notably in Crohn's disease (CD) and ulcerative colitis (UC). CCN1 (CYR61) is upregulated in human patients with CD and UC and Knock-in mice expressing a dominant -negative mutant CCN1 which is unable to bind an integrins $\alpha 6\beta 1$ and $\alpha M\beta 2$ showed increased mortality in experimental colitis including impaired epithelial regeneration and increased fibrosis. These results suggest that CCN1 may play a key role in limiting intestinal damage and promoting healing after injury.

As usual, the meeting format provided the attendees with the unique opportunity to meet with many of the leaders in the CCN field and discuss the most recent advances in topics related to CCN gene expression and CCN proteins functions in normal and pathological conditions.

Once more the communications were of high quality and all participants cheered the proposition to have the next meeting at Niagara Falls with Andrew Leask as a local organizer.

Thanks to **Annick Perbal** the quality of the social events and local organization was outstanding.