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MEETING REPORT

Report on the 7th international workshop on the CCN family of genes

October 16–19, 2013–Nice, France

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Abstract In this report, chairs of the 7th International Workshop on the CCN family of Genes, review the progress made in understanding the biological functions of CCN proteins (CCN1, CCN2, CCN3, CCN4, CCN5 and CCN6) with a particular focus on their implications in various pathological conditions, including cancer, fibrosis, diabetes, and cardiovascular diseases.

Keywords CCN proteins · ICCNS · Cancers · Differentiation · Development · Inflammation · miRNAs · Fibrosis · Diabetes · Cardiovascular diseases · Dermatology · Gene expression

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The 7th International Workshop on the CCN family of genes was held in Nice in splendid weather that made this meeting even more pleasant. Despite the attractive venue and the magnificent scenery of the coastline most of the speakers and participants resisted the temptation to walk along the beach. As in previous workshops, thanks to the amazing work performed by Annick in a very short period of time the social organization on site was outstanding. All were appreciative of the high quality of the local food and wines and the continuation of the very good moments that we all previously shared around the world.

The excellent science that was presented in Nice made the program quite intense. The very good response from all leaders in the field, including newcomers and the core of scientists who make these meetings true family reunions, provided the proof that the Workshop was reaching its goals and that the ICCNS Workshop is THE forum for scientific exchange in the CCN field.

Upon the request of B. Perbal, the poster board presentations were replaced on site, for the first time in this meeting, by presentations of oral posters. In spite of the short notice given to the young investigators who had brought posters, the presentations that they produced were excellent and very much appreciated by the participants.

For the first time since our first workshop in Saint-Malo, the topics discussed at the meeting provided the material for a half-page article in the local newspaper Nice Matin. The meeting was also the occasion to discuss several important changes proposed by B. Perbal, regarding the journal of Cell Communication and Signaling and the Scientific Committee of the International CCN Society. All these are presented in the editorial of the present JCCS issue

The scientific part of the meeting began with a presentation by **B. Perbal** who reviewed the roles and functions of CCN proteins in health and disease. B. Perbal presented structural considerations that led to the model already discussed in a previous review, in which the multimodular organization of the CCN proteins is the basis for homo and heterotypic interactions with other regulatory proteins, from either within the CCN family, or from other families of regulators. Indeed, such interactions have been reported. The distribution of CCN3 in normal and cancer tissues was used to exemplify the spatiotemporal regulation of CCN proteins as the major reason for the complex biological activities of CCN proteins whose functions most probably result from combinatorial events with interacting proteins and ligands. The multiplicity of pathways involving CCN proteins was also discussed to introduce future directions for research in the field and to point out questions that have been overlooked and remain open in spite of their considerable importance.

For the first time in this series of meetings, one chair of each session was asked to provide a short introduction of the topic and contribute to the writing of the present report. Thanks to all co-authors for their participation.

The topic of session 1 was Pathobiology of CCN proteins with a focus on cancer.

In the American women breast cancers are the second most common cause of cancer death (15 %) after lung and before colon and rectal cancers (26 and 9 % respectively). Three presentations dealt with the roles and functions of CCN proteins in breast cancers.

CCN5 is known to block migration and motility of cancer cells. Indeed, it reverses the epithelial mesenchyme transition (EMT) and stemness of tumor cells and acts as an anti-invasive effector for breast tumor cells. Previous work published by S. Banerjee's group on inhibition of the HIF1alpha–Twist–miR10B axis showed inhibition of migration, invasion and metastasis. After a very thorough discussion of p27 and CCN5 expression dynamics in non-invasive and invasive breast cancer tissues, **S. Banerjee** presented the effects of P27 silencing on CCN5-induced inhibition of MDA-MB-231 cell proliferation, and the effects of CCN5 on p27 expression, distribution and stability. In his interesting working model, S. Banerjee proposed that the CCN5-alpha6beta1 complex inhibits the cytoplasmic-nuclear translocation of P27 and inhibits pAKT which in turn results in increased transcriptional activation via Foxo3 and stabilization of the nuclear form of p27, leading to growth arrest at the G1/S transition of the cell cycle.

In her presentation on the role of CCN6 as a regulator of breast cancer cells stemness, **C. Kleer** first recalled that CCN6 expression is lost in 79 % of inflammatory breast cancers and that interaction of CCN6 with IGF-I signaling has also been established. CCN6 knock down (KD) triggers EMT and invasion of mammary epithelial cells, increases the resistance of tumor cells to anoikis, and increases cell survival. Use of rhCCN6 rescues EMT and the invasive potential associated with CCN6 KD. This is accompanied by an up regulation of BMP4 and activation of the PAK1-p38 pathway.

On the other hand, CCN6 overexpression induces EMT and reduces both invasion and motility of MDA-MB 231 cancer cells. C. Kleer presented and discussed a very exciting molecular model in which it is hypothesized that the tumor inhibitory function of CCN6 is directly linked to its ability to regulate cell stemness and plasticity via Slug. In favor of this model, C. Kleer presented results showing that in breast cancer cells, CCN6 overexpression induces MET and decreases Slug expression which is known to cooperate with Sox9 and other factors to induce mammary stem cells.

Among the 226,000 American women who have been diagnosed with beast cancers in 2012, about half are expected to develop ductal carcinoma, which affects milk ducts. Staining for Estrogen and Progesterone receptors, and for human growth epidermal receptors (HER1 and HER2) have routinely been used for the classification of the invasive ductal carcinomas.

At early stages of tumor development, the tumor cells that stain positive for ER respond to anti-estrogen therapy with a good prognosis. However, at late stages of tumor development, the tumors become unresponsive to hormonal therapy, contain cells which do not stain for ER, and show a poor prognosis.

Previous work presented by **R. Lupu** had established a correlation between high levels of CCN1 and aggressiveness of tumor cells. In the MCF7 cell line, CCN1 induced resistance to anti-estrogen treatment that is associated with the induction of hormone independent tumors in vivo which metastasize to the lungs. These observations suggested that the expression of CCN1 induces breast cancer progression, with the acquisition of a CCN1-induced advanced tumor phenotype characterized by anti-estrogen resistance, constitutive activation of receptor estrogen and acquisition of a metastatic potential. The use of several mutants had confirmed that the disruption of CCN1 interaction with integrin alpha6beta1 interferes with the induction of the advanced cancer phenotype by CCN1. Interestingly, CCN1 was found to bind and co-localize with ERalpha in the nucleus of MCF7 tumor cells. R. Lupu presented a working model in which tumor progression induced by CCN1 partially results from an interaction of CCN1 with FOXO3a and modulation of estrogen receptor transcription activity. Additional results indicated that targeting the interaction of CCN1 with integrin alphavbeta3 might allow access to the angiogenic and tumor compartments. The methodological basis of a dual target phase I assay were presented.

The role of caprin-1 in osteosarcoma tumor growth and lung metastasis in mice was presented and discussed by **A. Sabile**. Osteosarcoma (OS) is the most frequent pediatric

tumor that can be diagnosed as either, localized and non-metastatic with a 70 % 5-year survival rate, or as a metastatic disease with 20 % 5-year survival rate. In addition to ezrin, which is a well-known prognostic and diagnostic marker of OS metastasis, CCN3 and CCN1 were previously found to be markers of poor prognosis for pediatric OS. Caprin (cytoplasmic activationproliferation-associated protein1), a new CCN1-interacting protein was reported to co-localize with CCN1 in stress granules, inhibit cisplatin-induced apoptosis and promote tumor growth in primary tumors. Interestingly, overexpression of Caprin-1 in OS activates the Akt and ERK1/2 signaling pathways and promotes lung metastasis in mice. A. Sabile indicated that patients whose tumors co-express Caprin-1 and CCN1 show a much shorter overall survival.

In the last talk of the session, **G. Shafer** presented data showing that in co-cultured fibroblasts CCN2 expression is down regulated in a Smad7 and ERK-dependent manner. In the model presented, human breast tumor cells (MDA-MB-231) and human breast fibroblasts (CCD-1068SK) were set up in an indirect co-culture system allowing measurements of RNA, proteins, and performing microarray analysis, Quantitative Polymerase Chain Reaction (QPCR), and western blotting (WB).

The results obtained indicated that Smad7 knockdown leads to the up regulation of both CCN2 and type 1 collagen, whereas overexpression of Smad7 had the opposite effects. Similarly, CCN2 KD leads to decreased collagen type I expression. In addition, it was shown that Smad7 negatively regulates ERK signaling in direct co-cultures.

The afternoon cancer session provided a unique opportunity to hear professor **C. Croce**, recipient of the 4th ICCNS Springer Award, present and discuss in a critical and thorough manner the problems that needed to be solved to establish the genetic basis of cancer and the birth of new approaches that led to the discovery of miRNA implication in cancers.

In his very inspiring talk about the causes and consequences of microRNA dysregulation in cancer, C. Croce reviewed his considerable contribution to the identification of the chromosomal abnormalities that occur in human hematopoietic tumors. Deciphering the molecular mechanisms involved in B-CLL and TCLL/T-PLL under TCL1 deregulation, either in B-cells or T-cells was described in depth by C. Croce who recalled that without perseverance, he and his team would have not discovered the first example of a microRNA involved in the genesis of cancer. This discovery has open the door for many other examples and the number of microRNAs mapping in regions involved in human cancer has expanded at an incredible pace. MicroRNAs are now well known as either oncogenes or tumor suppressors.

C. Croce then discussed the implication of mir-29 in indolent CLL as compared to the implication of TCL1 overexpression in aggressive CLL, both in relation with inactivation of DNMT3A/DNMT3B.

The balanced action of miRs in the regulation of proliferation, apoptosis, invasion and angiogenesis was also pointed out by C. Croce, who focused on the interesting example of mir155 in the context of modulation of mismatch repair and genomic stability. The mutator activity induced by miR 155 being linked with inflammation and cancer. The role of miRs in signaling the tumor microenvironment, with the implication of miR21 being transported between the cancer cells and immune cells, was another very exciting topic covered by C. Croce.

In the second part of this session, the regulation of CCN functions by microRNAs was the subject of three presentations.

S. Irvine first provided evidence indicating that inhibiting miR-130A/B restores CCN3 expression and induces apoptosis and cell cycle regulation in CML (chronic myeloid leukemia) cells. Inhibition of CCN3 by p210 Bcr Abl reduce proliferation, induce apoptosis, promote differentiation, and increase adhesion. The data presented established that BCR-ABL reduction increased CCN3 expression and deregulated miRNAs expression in CML. The expression of mir17-92 cluster was overexpressed in CML, whereas the expression of the miR328 cluster was decreased. The expression of miR 130a and 130b, predicted to target CCN3, were found to be strongly decreased with BCR-ABL reduction, and with Imatinib treatment of K562 cells. Experiments performed with HL60 and K562 cells indicated that miR-130a/b KD with antimiRs resulted in increased CCN3 protein expression. Decreased mir130a/b expression reduced mitogenic signaling, cell viability and induced both PARP with caspase-3 cleavage and P16/P27 expression.

The regulation of migration and stemness of glioma stem cells by miR145 via CCN2 targeting was presented by **C. Brodie**. Starting from the observation that miR145 inhibits cell invasion, regulates migration and podosome formation in glioma cells, it was shown that CCN2 is a target of miR145 and that silencing CCN2 mediates the inhibitory effects of MiR145 on cell migration and motility.

Interestingly, CCN2 was reported to promote stemness of glioma stem cells (GSCs) and delivery of miR145 by MSCs was shown to inhibit self-renewal of GSCs.

As a conclusion, CCN2 promotes glioma cell migration, GSC stemness and mesenchymal transformation. Targeted therapeutic miRNA delivery in vivo was proposed to be facilitated by use of MSCs that may provide an efficient route to antitumor activity.

The last talk of the session presented by D. Brigstock addressed the regulation of CCN2 fibrogenic activity by exosomal miRNAs. Background information included the inhibitory effect of mir214 on CCN2 expression, and the identification of CCN2 in hepatic stellate cells.

In his very lively presentation, **D. Brigstock** first introduced the « world of exosomes » and their considerable

potential biological interest. Starting from the observation that exosomal miR214 inhibits CCN2 in a dose dependent manner in HSC, D. Brigstock then showed that it is transfered between activated HSC by exosomes, and targets CCN2. The proposed action of exosomal miR214 includes its delivery from quiescent to fully activated HSC that could modulate the level of the fibrotic response. Of course, as pointed out by D. Brigstock, the exosomal system is complex, but might represent an original and powerful intercellular communication system opening many targeted therapeutic applications.

In Session III the role of CCN proteins in metabolism and differentiation was examined in the pathobiology context .

M. Goppelt-Struebe reported on the study of CCN2 synthesis and secretion in polarized primary renal epithelial cells, obtained from proximal and distal human tubules, respectively. Synthesis of CCN2 was differentially regulated depending on the type and application of the stimuli: lysophosphatidic acid or TGFβ. Most interestingly, vectorial secretion of CCN2 to the apical and/or basolateral side of the polarized cells was observed while the molecular mechanisms are still under investigation.

J. Buteau reported that CCN3 is increased in obesity and insulin resistance. To make matters worse, CCN3 was found to impair pancreatic beta-cell function. CCN3 may, therefore, serve as a potential molecular target for diabetes treatment.

Also related to diabetes and obesity, **U. Smith** presented data showing that CCN5 is a commitment factor regulating the development of mesenchymal precursor cells into the adipogenic lineage. This is induced by BMP 4 which dissociates the cytosolic complex of CCN 5 and the PPARgamma transcriptional activator ZFP 423. This allows nuclear entry of ZFP 423 and PPARgamma transcriptional activation promoting adipogenic development.

B. Chaqour presented work on another diabetic complication, and reported on the role of CCN2 in retinal vessel development and repair of damaged retinal vessels following hyperoxic injury. Data showed that CCN2 expression was required for normal retinal vessel development but, under ischemic conditions, CCN2 was upregulated and exacerbated the formation of abnormal blood vessels. CCN2 promoted aberrant formation of retinal vessels through p53-dependent upregulation of the expression and activity of matrix metalloproteinase 2.

J. Castellot discussed the opposite regulation of CCN5 as a function of hyperoxia in vivo in lung type I alveolar epithelial cells (high CCN5, high proliferation under normoxia; opposite hyperoxia) and type II alveolar epithelial cells (low CCN5, low proliferation under normoxia, opposite under hypoxia). These findings are different from mesenchymal cells, and indicate that context-dependent effects are important in CCN5 biology.

M. Ono has found that CCN4 expression is elevated in dermal wound healing, influencing the proliferation and migration of dermal fibroblasts. Studies were performed in a CCN4 knockout model, and in human adult dermal fibroblasts as a function of CCN4 siRNA knockdown.

M. Cario-André and co-workers have uncovered interesting relationships between CCN2 and epidermal pigmentation that could have relevance to resistance to melanoma development. In scleroderma patients, a correlation was found between expressions of CCNs, FGF-2, and pigmentation, suggesting that under certain conditions CCNs contribute to development of pigmentation.

Session IV of pathobiology of CCN proteins dealt with fibrosis.

Although the focus of this session was on CCN proteins and fibrosis, the role of fibrosis is so complex and wide-ranging that a rather diverse and fascinating set of talks were presented. The role of CCN proteins in the pathogenesis of fibrosis in different organs such as kidney and skin was the subject of several talks, while other presentations involved model systems for muscular dystrophies and repetitive motion injuries in the workplace. A common theme in all of these presentations was the identification of potential therapeutic targets for treating fibrosis.

L. Lau led off this session by presenting data indicating the existence of a CCN1-mediated novel integrin-dependent mechanism of fibrosis resolution, suggesting that the CCN1 signaling pathway may contain useful therapeutic targets.

R. Goldschmeding discussed CCN2 as a potential therapeutic for use in kidney fibrosis. Currently, no effective treatment exists for this disease. Noting that CCN2 occupies a central role in the signaling processes that are dysregulated in kidney fibrosis, he presented data on CCN2 expression and bioavailability suggesting a complex interplay of the different cell types that make up the kidney with respect to this member of the CCN family.

E. Brandan presented data from a dystrophic mouse model that examined the muscular phenotype following inhibition of CCN2 or stimulation by angiotensins. Muscular dystrophies are characterized by a decrease of skeletal muscle mass and force and an increase in fibrosis. When CTGF (CCN2) was inhibited, or when exogenous Angiotensin 1–7 was administered, a better skeletal muscular phenotype was observed. These results are promising as possible therapeutic approaches to improve quality of life in individuals with skeletal muscular dystrophies.

R. Stratton reported results from his group showing that the MRTF-A SRF pathway is a key inducer of fibrosis in Systemic Sclerosis and is responsible for feed-forward

enhancement of fibrosis by the stiff extracellular matrix in Systemic Sclerosis. This opens up the possibility that targeting the MRTF-A pathway might lead to anti-fibrotic treatments for Systemic Sclerosis.

J. Nikitorowicz-Buniak test their working hypothesis that environment induced damage to epithelial cells is promoting this disease through aberrant epithelial-mesenchyme cross-talk, and that CCN2 has an essential role in this process. Her data indicate that abnormal Systemic Sclerosis epidermis is releasing profibrotic and proinflammatory mediators, including both CCN2 and S100A9. This group posits that these changes in the epithelial layer have a role in the pathogenesis of Systemic Sclerosis and contribute to the inflammation and fibrosis observed.

M. Barbe and her colleagues examined serum and muscle CCN2 and its relationship to fibrosis and declining grip strength in a unique rat model of work-related (eg, repetitive motion injury) musculoskeletal disorders. Grip strength declined significantly in these rats, a decline that correlated significantly with increased CCN2 in muscle and serum. Thus fibrosis appears to be a critical component in repetitive motion injuries that deserves further study as a potential target for therapeutic intervention, and serum CCN2 may serve as a serum biomarker of fibrosis progression in these disorders.

Aspects of CCN biology from "genes to proteins" were covered in session V.

To better understand the etiology of phenytoin-induced gingival overgrowth, **P. Trackman** described studies of the role of lysyl oxidases (LOXL), which are required for the biosynthetic maturation and cross-linking of collagens and elastin in the extracellular matrix, acting downstream of CCN2 to stimulate collagen accumulation in human gingival fibroblasts. LOXL2, but not LOXL were upregulated by CCN2 while knockdown of LOXL2 reduced both basal and CCN2-stimulated collagen accumulation, and surprisingly dramatically reduced cell proliferation. Overall these data suggest that gingival overgrowth and fibrosis reflect the ability of CCN2-stimulated LOXL2 to stimulate proliferation of fibrogenic cells and stabilize the extracellular matrix.

CCN expression and protein fragmentation in diseases such as lung cancer and chronic obstructive pulmonary disease (COPD), both of which can be caused by chronic smoke exposure, was discussed by **F. Gueugnon**. Human lung cancer samples showed suppressed expression of CCN1, 2 or 3 whereas expression of CCN1 was enhanced in COPD versus control smokers. In mouse models, pulmonary CCN3 expression was decreased by acute smoke exposure and expression of CCN 1, 3 or 4 were decreased by chronic smoke exposure. Interestingly, whereas CCN proteins were mainly intact in the lungs, they were highly fragmented by bronchial and/or inflammatory proteases in sputum and bronchial aspirates from smokers and patients with COPD suggesting that, in addition to differential gene regulation, CCN proteolysis could be an important mechanism controlling CCN function in the lungs.

In investigating the role of the CCN2-binding partner, low-density lipoprotein receptor-related protein 1 (LRP-1), in growth plate chrondrocytes, **S. Kubota** showed that CCN2 was transported across the chondrocyte layer following its binding to LRP-1 which resulted in its clathrin-dependent internalization and transcytosis. This novel function for LRP-1 may account for the broad distribution of CCN2 protein in the proliferating to late hypertrophic layers as compared to CCN2 mRNA which is restricted to chondrocytes in the pre-hypertrophic layer. Also, platelet-derived growth factor receptor-like (PDGFRL) protein was shown to be as a new binding partner for CCN2 and studies are underway to establish the relationship, if any, between putative tumor suppressive properties of PDGFRL and its ability to act as a molecular decoy for CCN2.

CCN1 expression is induced during cardiac ischemia in humans and mouse models and **F. Mo** discussed his investigations of the functional role of CCN1 in heart injury. Unlike their wild-counterparts, mice carrying the apoptosis-defective mutant allele Ccn1-dm, which encodes an integrin $\alpha6\beta1$/HSPG-binding defective mutant form of CCN1, were resistant to isoprotenerol-induced cardiac injury or apoptosis. Functionally, CCN1 was shown to sensitize cardiomyocytes to Fas ligand (FasL)-mediated apoptosis by engaging cell-surface integrin $\alpha6\beta1$ and upregulating reactive oxygen species which in turn activated MAPK p38 and cell surface Fas expression. Since FasL and CCN1 are often induced by similar injuries in the heart, the ability of CCN1 to drive susceptibility to stress-induced cardiomyocyte apoptosis may represent a common and critical pathophysiological response in a broad range of cardiac diseases.

Whereas numerous studies of CCN2 have focused on its potential involvement in fibrosis and ensuing deleterious effects on organ function, **H. Attramadal** presented intriguing evidence that CCN2 functions as a protective factor in heart injury models. CCN2 increased tolerance towards ischemia-reperfusion injury in transgenic mice with cardiac-restricted overexpression of CCN2 or in ex vivo perfused hearts exposed to recombinant human CCN2. The cardioprotective effect of CCN2 was attributed to CCN2-stimulated PI3 kinase/Akt/GSK-3β signaling in cardiac myocytes. CCN2 was also found to enhance healing of myocardial infarction by enhancing collagen synthesis in the differentiating scar tissue replacing necrotic myocardial tissue. The enhanced healing of the myocardial infarction resulted in reduced dilatation of the left ventricle and less deterioration of myocardial function. Furthermore, myocardial overexpression of CCN2 did not lead to detrimental myocardial fibrosis of non-ischemic myocardial tissue. These findings demonstrate that CCN2 can activate pathways that protect and preserve myocardial function during injury and highlight important

functional differences as compared to many previously documented fibrosis studies.

Finally, **A. Leask** reported that his group has determined that the critical cell type mediating bleomycin-induced skin fibrosis is the skin progenitor cell (SKP). In the absence of CCN2 expression by either mesenchymal cells or SKPs, bleomycin is not able to induce fibrosis and SKPs are not recruited to the fibrotic lesion. Moreover, CCN2-deficient SKPs are defective in myofibroblasts differentiation. Thus CCN2 contributes to skin fibrosis by being required for the ability of SKPs to differentiate into myofibroblasts. These exciting results suggest that the process of stem cell recruitment represents a potentially useful target, either alone or in combination with anti-CCN2 strategies, for combating fibrotic skin disease.

Session VI was focused on the biological functions of CCN proteins in development and was the time for the Springer scholarship awardees to present their work.

M. Takigawa reported that cartilage-specific overexpression of Ccn2 in transgenic mice stimulated the proliferation and differentiation of growth-plate chondrocytes, promoting endochondral ossification. Furthermore, these mice are protected against the development of osteoarthritic changes in aging articular cartilage. CCN2 can bind several ECM proteins, growth factors, and growth factor receptors such as FGFR1 and FGFR2, and it can homodimerize with CCN2 or heterodimerize with CCN3. Thus, the distinct and diverse biological actions of CCN2 may depend on its specific binding partners in the microenvironment of the target cell types.

T. Hattori reported that mutant mice in which sequence encoding the 3rd domain of CCN3 is deleted showed abnormal endochondral ossification. Cartilage-specific overexpression of Ccn3 in transgenic mice resulted in delayed endochondral ossification and dwarfism. Accumulating data indicate that overexpression of CCN3 in cartilage may inhibit vascular invasion and impair osteogenesis by reducing osteoblastogenesis.

A. Yamaguchi found that ccn3 gene was identified as a highly expressed gene at the early phase of bone regeneration in a mouse bone regeneration model by microarray analysis. Although the skeleton developed normally in Ccn3 knockout mice, bone regeneration was accelerated in these mice. Thus, CCN3 is upregulated in the early phase of bone regeneration and acts as a negative regulator for bone regeneration.

There were three Springer Scholarships Award presentations given by the awardees.

First, **W. Si** demonstrated that CCN1 is a direct target of β-catenin signaling. In the first part of her talk, W. Si recalled that hepatic stellate cells are key factor in the development of hepatic fibrosis and that the activation of HSCs promotes the development of hepatocellular carcinoma. CCN1 enhances the function of hepatic stellate cells in driving the progression of live fibrosis–hepatic cirrhosis-hepatocellular carcinoma, as shown by the increase of migratory and invasive potential of HCC cells in vitro. Studies performed with SCID mice established that, CCN1 also increase proliferation of HCC cells in vivo. As a conclusion, it was proposed that CCN1 modulates the microenvironment of activated HSCs by acting on fibrogenesis and angiogenesis.

This study lays the foundation for providing a new diagnostic marker for liver fibrosis and treatment target for hepatocellular carcinoma.

Second, **M. Howlett** reported that CCN2 is expressed at significantly higher levels in approximately 75 % of pre-B Acute Lymphoblastic Leukemia (ALL) specimens compared to normal cells. They demonstrate in vivo that CCN2 accelerates leukemic development while knocking it down significantly reduces leukemic cell growth and engraftment. CCN2 accelerates growth of bone marrow stromal cells and only enhances leukemic cell growth in the bone marrow late in disease progression, suggesting that CCN2 acts via the bone marrow microenvironment.

Finally, to better understand the role of extracellular signaling in mouse hepatic stellate cells (HSC), **A. Charrier** demonstrated that exosomes produced by HSC contain both CCN2 mRNA and protein. The activation of HSC into CCN2-expressing pro-fibrogenic myofibroblasts was associated with increased levels of exosomal CCN2 mRNA. Exosomes from donor CCN2-GFP-transfected HSC or human HSC (LX2) cells were taken up by recipient HSC or LX2 cells, respectively, as evidenced by the presence of GFP in the recipient cells either after the incubation of recipient cells either with isolated exosomes or with co-cultured donor cells. This uptake was prevented in the presence of a chemical exosome inhibitor. These results suggest that the pro-fibrogenic function of HSC is likely regulated by exosomal cargo such as CCN2; exosomes would appear to provide a protective environment for CCN2 protein and/or mRNA, allowing them to avoid degradation as they traverse the extracellular space.

The meeting ended with a banquet during which participants could share in the pre-retirement celebration honoring Professor Masaharu Takigawa who will retire later this year.

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