Title

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Journal

Journal of Cell Communication and Signaling, 10(1)

ISSN

1873-9601

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Publication Date

2016-03-01

DOI

10.1007/s12079-016-0317-y

Peer reviewed

J. Cell Commun. Signal. (2016) 10:77–86
DOI 10.1007/s12079-016-0317-y

EDITORIAL

Report on the 8th international workshop on the CCN family of genes – Nice November 3–8, 2015

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Received: 29 January 2016 / Accepted: 29 January 2016 / Published online: 26 February 2016

The 8th international workshop on the CCN family of genes was held for a second time in Nice, from November 3rd to 8th, 2015. Under particularly sunny and warm weather everyone enjoyed the charms of the Mediterranean coastline.

Thanks to Annick Perbal, the social events during the meeting were a real success and were truly well appreciated by all. The choice of the Westminster hotel was an excellent one.

This year the ICCNS honored and warmly welcomed **Judith Campisi**, the recipient of the 2015 ICCNS-Springer award, who accepted to present a conference on senescence and cancer. We are grateful to Judith Campisi who came a long way from San Francisco, despite being under the weather with a bad cold and sore throat. We also appreciate that she was able and enthusiastic about participating with us in the full sessions of the meeting. It is always a unique and fruitful opportunity for all participants, either young or established scientists, to share time with the awardees.

This year we also had the pleasure to welcome **Robert Baxter**, a former ICCNS-Springer awardee, who flew in from Australia to join us and present a special conference on the role of IGFBP3 in the breast cancer response to DNA damaging therapy.

Those who did not have the opportunity to make it at the time Robert Baxter received the award in Sydney, could then enjoy sharing experiences and discuss possible levels of interactions between the ICCNS and IGF societies.

All attendees agreed that the quality of the science presented at the meeting was excellent. All speakers made the effort to include in their presentation a concise overview of their topic, which made their talk appreciable to all, including newcomers to the CCN field.

Several participants who were attending the meeting for the first time informed us, to our great satisfaction, that they would make sure to come back for the 9th edition !

In his scientific introduction to the meeting, **Bernard Perbal** addressed questions regarding the progress made over the past decade in the understanding of the variety and complexity of functions assigned to the CCN proteins.

The bulk of data obtained over a few years period following the discovery of the three founding members of the CCN family [(CCN1/cyr61; CCN2/ctgf and CCN3/nov), and the Wnt-induced secreted proteins that were later identified as CCN proteins (CCN4-6)], set the stage for a new scientific era that proved rich in exciting discoveries on CCN proteins with promise for contributing to the betterment of human welfare.

Results obtained in the early 90s established the bipartite functionality of the CCN family of proteins, with different

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members involved in either the stimulation or in the inhibition of cell proliferation.

From one point of view past observations, can be considered either negatively as what has been wiped off by the action of time, or positively as the bulk of what remains and on which future efforts are to be built. New « pioneers » cannot just wipe out the past and then present models that are in fact based on previously published concepts. As stated by B. Perbal, it is quite disturbing that the bibliographic citations of many newly published manuscripts do not extend more than a decade ago, at the most. In effect, the field needs to move forward and avoid conceptual stalling.

That the CCN proteins act in concert, either as agonists or antagonists has been established since the early 90s. What remains to be understood are the molecular bases for their opposite or synergistic actions, and non-redundant functions.

B. Perbal also stressed that surprisingly, very little information has stemmed from a structure-function analysis of the various CCN proteins. There is no doubt that the field suffers from the lack of information regarding the functional specificity of the four constitutive modules that make up the biologically active CCN proteins.

Swapping of the four protein domains as was performed in the case of IGFBP3 and CCN3, would certainly help to better understand a fascinating paradox and pinpoint the essential components that are responsible for the highly specific functions of CCN proteins that share a high degree of structural conservation.

For example, introducing the CT module of a CCN proteins that belong to either the positive or negative group of regulators, into CCN5 which is deprived of a CT module, would certainly allow one to grasp some of the functions of this domain in different structural contexts.

Likewise, directed mutagenesis of cysteine in CCN proteins is expected to be informative, since it is widely accepted, without any kind of experimental demonstration, that the pairwise association of the 38 conserved cysteine residues in CCN proteins is responsible for the function of each domain.

However, a close examination of predicted secondary structures reveals that in spite of the high degree of conservation of cysteine residues, both in place and number, in the different CCN proteins, the presence of these residues in slightly different primary structure environments, does not appear to impact the secondary structure in a similar way.

Understanding these spatial interactions is critical to a better knowledge of the structural bases for CCN biological functions.

On another standpoint, and in the light of new recent results, B. Perbal came back to a model that he proposed in 2001 that might account for the striking functional diversity of the CCN proteins. This model is based on the observations that i) the expression of the CCN proteins is subject to a tight spatial and temporal regulation, and ii) each domain of the CCN proteins has been reported to interact physically with several key components of cellular regulatory pathways.

In this model, the bioavailability of each ligand and partner that interact with the various CCN modules dictates potentially the activation or inhibition of regulatory signaling pathways. Tissue specific expression of ligands, partners and CCN proteins coupled to their temporal regulation of expression provides the basis for a complex network of combinatorial events, the existence of which being critical to the coupling and coordination of different regulatory pathways in which CCN proteins are known to participate.

Deciphering novel aspects of **CCN proteins expression and functions** was the topic of **session** I. **George Bou-Gharios** reported the identification of new enhancer sequences that are involved in the control of CCN2 tissue-specific expression. **Takashi Nishida** presented data supporting the idea that activation of RhoA and MAPK signaling induced by low intensity pulsed ultrasound (LIPUS)-stimulated Ca2+ influx resulted in an increased CCN2 production which in turn induced chondrocyte differentiation, and **Koichiro Muromachi** reported data showing regulation of CCN2 expression and membrane glycosylation in the human dental pulp, by the bone morphogenetic protein BMP-1. A new role for CCN2 in maintaining both energy supply and balanced aminoacid metabolism in chondrocytes was discussed in the presentation given by **Satoshi Kubota**.

Stimulation of osteoblast and osteoclast differentiation by lysyl oxidase propeptides, with a possible role for CCN2 was presented by **Philip Trackman**, whereas data presented by **Mitsushiro Hoshijima** suggested novel functions of a CCN2-rab14 association in proteoglycan synthesis by chondrocytes.

The regulation of gene expression is known to involve a whole series of specific DNA sequences and structural modifications of histones, as established by the identification of DNAse hypersensitive regions in the vicinity of coding sequences.

Historically, proximal elements were first identified upstream of the core promoter sequences at which initiation of transcription by RNA polymerase begins.

Histone modifications associated with the regulation of gene transcription were identified as part of downstream, upstream and distal regulatory elements.

As nicely reviewed by G. Bou Gharios, the tissue specific regulation of CCN2 expression involves several regulatory elements localized upstream and downstream of the coding sequences.

Early work from Grotendorst's group identified TGF beta response elements in the −134–169 region upstream to the CCN2 transcription start in addition to several other canonical regulatory sites. Additional data obtained by other groups established that regulation of CCN2 gene transcription by TGF beta is SMAD-dependent. Induction of CCN2 by TGF beta in fibroblasts requires both SMADs and TGF beta elements.

A set of DNA sequences localized between −202 and −180 upstream of the CCN2 transcription start site was later found

to function as a dominant transcription enhancer in chondrocytes. Based on the previous identification of stage-specific elements at −433 bp that involve either repressor complexes comprising Sox9 in proliferating chondrocytes or stimulating complexes involving beta catenin and TCF/LEF in hypertrophic chondrocytes, G. Bou Gharios' group undertook a search for possible tissue specific CCN2 enhancers. The use of ENCODE, the Encyclopedia of DNA Elements, and the analysis of sequence conservation accross phylogenetic tree, acetylation and methylation sites, identified four potential enhancers among which the −100 kb is active in endothelial cells and veins, and the −200 kb region is strongly active in skin and in hypertrophic chondrocytes. The factors involved in the tissue specific activation of these cis-acting elements is currently underway.

Application of low intensity pulsed ultrasound (LIPUS) is presently used as a means to heal bone fractures and as a non-invasive therapy for osteoarthritis (OA).

Because it shows non-thermogenic and non-destructive actions, it is also included in therapeutic osteointegration for implants in dentistry.

Since CCN2 has been previously identified as a potential molecule for OA therapy, T. Nishida et al. investigated the effects of LIPUS on CCN2 production levels on signaling in chondrocytes from human and rat chondrocytic cell lines and from rat primary articular cartilage.

In human and rat chondrocytic cells, the expression of CCN2, as measured by RTPCR was found to increase upon a 30 mW/cm^2 treatment but did not seem to further increase when higher intensities were applied.

LIPUS also induced Ca^{2+} channels whose expression was decreased by CCN2 siRNAs. This observation might be of significance in the light of the previously reported effects of CCN2 on Ca^{2+} flux in several different cells types.

The bulk of results presented by T. Nishida suggested that, based on the CCN2 production and subsequent chondrocyte differentiation resulting from LIPUS-activation of RhoA and MAPK signaling, LIPUS might be recommended as an OA therapy.

However, it would be interesting to evaluate whether LIPUS induced the same effects in vivo within a longer term range.

In a very lively presentation of data regarding the effects of bone morphogenetic protein-1 (BMP-1) on CCN2 and cell surface glycosylation, in human dental pulp, K. Muromachi first pointed out that BMP-1 is a particular type of BMP, as it belongs to the astacin family of metallo-endopeptidases, whose functions include activation of growth factors and processing of the extracellular proteins.

During tooth development, dentin specific proteins, DMP1 (dentin matrix protein 1) and DSPP (dentin sialophosphoprotein), are degraded by BMP-1.

Because of the downstream role of CCN2 in osteogenesis and chondrogenesis, key steps in tooth development, Muromachi et al. aimed to clarify the relative involvement of BMP-1 and CCN2 in the pathophysiology of dental pulp.

These authors first established that CCN2 expression was induced in dental pulp cells, independently of the proteolytic activity of BMP-1.

In reparative dentin subjacent dental caries, CCN2 and BMP-1 were increased.

Fluorescent BMP-1 was shown to internalize into the cytoplasm of dental pulp cells, and the BMP-1 increased production of CCN2 was dependent upon dynamin-related endocytosis (cellular internalization).

T. Muromachi also presented data indicative of a modulation of glycosyl transferase gene expression by BMP-1.

Therefore, the authors concluded that the novel roles of BMP-1, which alters o-linked glycosylation and modulated glycosyltransferase expression, should help elucidate the mechanisms of dental pulp repair.

In their model, the production of BMP-1 by ondotoblasts affects cell surface glycosylation, and induces CCN2, which targets odontoblast-like cells at the level of reparative dentin.

A transcriptome analysis of CCN2 KO chondrocytes revealed that protein synthesis levels were increased, as shown by an up regulation of ribosomal gene expression and total protein quantification in these cells.

In an attempt to gain a better insight into the biochemical mechanisms that account for the promotion of both proliferation and differentiation, considered as two apparently opposite biological events, S. Kubota and his group undertook a metabolomic analysis aiming to provide a quantification of CCN2 effects on energy and amino acid metabolism.

Measurements of nucleotide triphosphates (NT) in CCN2 KO cells indicated a stable reduction of all NTs that could be reversed by the addition of exogenous CCN2. Along the same line, silencing of CCN2 expression decreased ATP levels by 50 %.

In contrast, the aerobic production of ATP in CCN2 deficient cells was not altered, suggesting that the decrease of ATP synthesis observed in the absence of CCN2 might result from impaired glycolysis.

Indeed, the analysis of metabolism-related genes expressed in CCN2 null cells revealed that the levels of three enzymes involved in glyceraldehyde 3-phospate production (Phosphoglycerate Kinase 1, phosphoglycerate kinase, and alpha enolase) were significantly decreased (50 %).

As emphasized by S. Kubota, these observations suggested that energy production in CCN2-deficient cells might involve aminoacid catabolism.

Indeed, a 50–60 % reduction in the essential aminoacid load was observed, in CCN2 null mice, independent of aminoacid transporter modifications.

Interestingly, the uptake of aminoacids was increased in CCN2 KO murine chondrocytes and reduced upon CCN2 addition in HCS 2/8 cells.

Whether this observation is related to the capacity of recombinant CCN2 to bind free aminoacids in vitro remains to be assessed.

Collectively, the data presented by S. Kubota open new avenues in the field and argues for a new role for CCN2 as a regulator of aminoacid metabolism and cellular energy supply.

Concerning the biological activities of lysyl oxidase (LOX) on osteoblasts and osteoclast differentiation, P. Trackman reported on experiments that were aimed to assess a possible therapeutic strategy for bone metastasis, based on the use of LOX pro-peptide LOX-PP.

The enzymatic conversion of peptidyl lysine to peptidyl allysine by lysyl oxydase is an essential step in collagen and elastin crosslinking.

The active lysyl oxidase is produced from an extracellular secreted pro-enzyme that is cleaved into a mature 30 KDa LOX enzyme and a smaller 18 KDA LOX-PP pro-peptide.

Inhibition of LOX by beta aminopropionitrile (BAPN) that is contained in Lathyrus odoratus seeds (sweet peas) results in osteolathyrism, a connective tissue disease due to collagen cross-linking deficiency.

In mice, rLOX-PP was reported to inhibit growth and promote apoptosis of pre-existing breast cancer tumor NF639 xenografts. It is also known to inhibit ras signaling by different mechanisms.

LOX-PP was reported to inhibit terminal differentiation of primary calvaria osteoblasts at early stages of culture, and inhibits cell growth of normal MC3T3-E1 pre-osteoblasts.

Interestingly, LOX-PP enhances the radio sensitivity of cancer cell lines and in irradiated cells, it co-localizes with MRE11 a nuclear DNA repair regulatory system for double strand breaks.

The question asked here was to determine whether LOX-PP interferes with signaling networks in bone metastasis context.

First, rLOX-PP was shown to inhibit the proliferation of osteoblasts stimulated by conditioned media from two different prostate cancer cells (DU145 and PC3) and to promote osteoblast mineralization of primary bone marrow stem cells (BMSCs) in the presence of DU145- or PC3- conditioned medium.

Primary BMSCs osteoblastic differentiation was also stimulated by rLOX PP. Osteoclast development from primary BMSCs was also promoted by rLOX-PP in the absence of added M-CSF and RANKL, by inhibiting osteoprotegerin (OPG) expression, stimulating CCN2 expression, and increasing osteoclast fusion.

Finally, the results of in vivo studies presented by P. Trackman established that rLOX-PP produced by PC3 cells implanted in tibia of mice enhanced the resorption of bone induced by PC3 prostate cancer cells.

It therefore appears that LOX-PP might be a key factor in the maintenance of normal bone turnover, by coupling interactions between osteoclasts and osteoblasts.

In the last presentation of this session M. Hoshijima reported in vitro data showing that the physical interaction of CCN2 with RAB14, which was detected in a two hybrid system, also occurs when the two proteins are ectopically over-expressed in COS 7 monkey cells.

Rab14 is involved in vesicle trafficking. After establishing that the interaction involved the IGFBP module of CCN2, Hoshikima et al. showed that siRNA inhibition of either CCN2 or Rab14 resulted in increased RNA expression of stress proteins including the CCAAT-enhancer-binding protein homologous protein (CHOP), binding immunoglobulin protein [(BiP) also known as heat shock 70 kDa protein], and the ST3GAL1 membrane protein (also known as SIAT4A), that transfers sialic acid to galactose-containing substrates.

In contrast to chondrocytes treated with CCN2 siRNA, which expressed a lower level of aggrecan mRNA, chondrocytes treated with Rab14 siRNA did not show any decrease of aggrecan mRNA expression.

Establishing whether the physical interaction of Rab14 and CCN2 plays a role in proteoglycan synthesis by chondrocytes, requires the demonstration that both Rab14 and CCN2 co-localize in vivo, within the same cells and at the same time. Also, it would be interesting to establish whether IGFBP modules of other CCN proteins also physically interact in vivo with Rab14.

Session II was devoted to developmental biology

Investigations into the developmental functions of CCN proteins have been greatly facilitated by the availability of genetic models in mice. Knockouts or conditional deletions of all members of the *Ccn* gene family have now been accomplished, and studies using these models have yielded a wealth of information on the diverse roles of CCNs in developmental and pathological processes.

In her introduction of the session, Karen Lyons reported that mutant mice lacking *Ccn1* or *Ccn4*, but not *Ccn5*-null mice, exhibit a profound low bone mass phenotype. CCN1 and CCN4 are found to be important for bone marrow angiogenesis and the commitment of osteoprogenitors to the osteogenic lineage. Further analysis revealed that CCN1 is essential for suppressing the expression of sclerostin, which inhibits *Wnt* signaling by binding LRP5 and LRP6, thus allowing *Wnt*-mediated signaling during mechanical loading to exert a protective effect on bone mass.

Joseph Tarr observed that *Ccn2* knockout mice display numerous craniofacial defects including defects in bony palate formation. Growth of the palatal shelves is arrested at an early stage in the process of palatogenesis, and palate organ culture reveals that CCN2 deficiency causes inhibition of palatal shelf fusion. Mesenchyme-derived pre-osteoblasts from crania of

CCN2 KO mice showed decreased proliferation and adhesive signaling, including cell adhesion, cell spreading, cytoskeletal organization, focal adhesion formation, which may contribute to the failure of the palatal shelves to form and grow in *Ccn2*-null mice.

Tsukushi (TSK) is a member of the small leucine-rich repeat proteoglycan family and plays diverse roles during development. **Kunimasa Ohta** reported that $TSK^{-/-}$ mice show elevated apoptosis and aberrant proliferation in the subventricular zone of the brain, defects that can be rescued by transgenic expression of TSK. These results support a role for TSK in neurogenesis. In another project, Dr. Ohta showed that human dermal fibroblasts infected with lactic acid bacteria are reprogrammed into multipotent progenitor cells, forming cell clusters that can differentiate into endodermal, mesodermal and ectodermal cells. These findings support a potential mechanism in which bacteria may usurp the plasticity of their cellular niche to promote the dissemination of infection.

CCN1 is known to play important roles in angiogenesis and *Ccn1* knockout mice are embryonic lethal due to severe cardiovascular defects. **Brahim Chaqour** found that conditional deletion of *Ccn1* in the retina leads to formation of a dense retinal vascular network that lack the normal hierarchical arrangement of arterioles, capillaries and venules. CCN1 induces the expression of Src homology region 2 domain-containing phosphatase-1 (SHP-1), which dephosphorylates VEGFR2 at tyrosine residues, thus preventing endothelial cell hyperproliferation. CCN1 reduces pathological neovascularization by protecting against VEGFR2 overactivation under conditions such as ischemia, indicating that CCN1 safeguards against aberrant angiogenic responses.

David Brigstock showed that hepatic stellate cells and hepatocytes communicate with each other in part through exosomes, which are nanovesicles that carry numerous cellular components including mRNA, miRNA, and proteins as cargo. In particular, exosomes secreted from injured or fibrotic livers contain pro-fibrotic molecules including *Ccn2* mRNA, CCN2 itself, and miR-214, which regulates *Ccn2* expression. Capturing or manipulating exosomal payloads may provide novel diagnostic or therapeutic opportunities in liver fibrosis.

The **Session III on CCN proteins in wound healing and tissue regeneration** reported new in vivo sites of action for CCN proteins, demonstrated contrasting and overlapping functions for members of the CCN family, and provided ample illustration of the context-specific effects of CCNs. Some presentations demonstrated the distinct effects of distinct CCN proteins in a specific tissue. Others included comparisons of the effects of CCN proteins in the context of regenerative repair and fibrosis in a specific tissue, revealing that CCN proteins exert multiple functions by activating distinct pathways in distinct cell types in these models. Several of the presentations investigated the contributions of distinct CCN domains in mediating these diverse activities. A theme that emerges is that the diverse actions of CCN proteins suggest diverse therapeutic opportunities.

Andrew Leask reported on in vivo studies in his lab that have provided new insights into the role of CCN2 in the context of skin tissue repair and fibrosis. CCN2 is essential for myofibroblast differentiation in fibrosis in many contexts, including bleomycin-induced skin fibrosis. The role of CCN2 during normal skin wound repair is far less clear. Lineage tracing studies performed by Dr. Leask and colleagues revealed that CCN2 expression is restricted to Sox2-expressing dermal progenitor cells. Data were presented showing that loss of CCN2 from these cells led to the failure of these cells to be recruited to the wound site. A striking finding was that loss of CCN2 in these dermal progenitors led to no apparent defect in wound healing. What then is the role of CCN2 in Sox2-expressing progenitor cells? These cells are normally localized in the dermal papilla of the hair follicle. Dr. Leask presented data showing that loss of CCN2 in these cells results in an increase in hair follicle cycling as a consequence of increased Wnt activity. Overall, these data indicate that CCN2 is not essential for cutaneous wound repair, but is a specific anti-fibrotic target.

The theme that CCN proteins have distinct functions in the context of tissue regeneration and fibrosis was further established by **Lester Lau**. Dr. Lau presented elegant work from his laboratory showing that CCN1 plays multiple roles in hepatobiliary injury repair via distinct mechanisms. The role of CCN1 was explored in various models of liver regeneration and fibrosis. Using mouse models and in vitro studies, he showed that CCN1 enhances bile duct regeneration by activating the NF-kB pathway via integrins, leading to enhanced Jag1 expression and Jag/Notch signaling. In the context of liver fibrosis, CCN1 limits liver fibrogenesis by triggering senescence via integrin $\alpha6\beta1$-mediated ROS accumulation. A highlight of these studies was the use of CCN1-deficient mice as well as knockin strains harboring alleles of CCN1 that are defective for engagement of $\alpha v\beta5/\alpha v\beta3$ or $\alpha6\beta1$ integrins. These unique mouse models provided one of the clearest demonstrations to date of the importance of integrin-mediated events in CCN action in vivo, and provided vital insights into the identities of the distinct pathways regulated by the engagement of integrins with distinct domains of CCN1.

Work presented by **Masaharu Takigawa** also illustrated the in vivo effects of distinct CCN protein domains. The problem of regeneration of articular cartilage was the focus of the presented studies. Dr Takigawa and colleagues have demonstrated previously that CCN2 is a potent stimulator of cartilage regeneration in vivo. The presented studies investigated the effects of the 4 distinct modules of CCN2 on the ability of chondrocytes to produce cartilage matrix. These studies showed that the TSP1 domain exerts the most potent

chondrogenic potential in vitro. Comparisons of the effects of this domain to full length CCN2 in an in vivo rat osteoarthritis model verified the efficacy of the TSP1 domain, and unexpectedly revealed that it exhibits more prominent regenerative effects than full length CCN2. The mechanistic basis for these differences is as yet unclear but of considerable interest. In additional studies, it was shown that CCN3 promoted articular cartilage matrix expression in vitro and protected against cartilage degeneration in vivo. This is of interest because a number of studies have reported that CCN2 and CCN3 exert distinct activities. In summary, these studies provide additional evidence for the distinct roles of specific CCN domains in vivo. Therapeutically, these studies raise the possibility that combined administration of the CCN2 TSP1 domain in conjunction with CCN3 could lead to superior cartilage regeneration.

The above presentations focused on animal models. There is far less information about CCN function in humans. Thus **Stephen Twigg**'s presentation on the potential role of CCN2 in human diabetic foot ulcers provided an important perspective. Wounds in diabetic patients typically show abnormal healing, characterized by persistent inflammation and inability to resolve the wound beyond a granulomatous phase. Data from human patients revealed that increased CCN2 levels in ulcer fluid correlated with an increased rate of wound healing. In an animal model of foot wound healing, Dr Twigg and colleagues have found that exogenous CCN2 had minimal effect on normal mice, but accelerated healing in diabetic animals. Thus, while the profibrotic actions of CCN2 are generally considered to be deleterious, the work from Stephen Twigg and colleagues indicates that this property of CCN2 might be beneficial as a therapy to promote healing of wounds in a pro-inflammatory environment.

The 2016 **ICCNS Springer Award** was presented to Judith Campisi, who delivered a prospective review on "Cancer and aging: Rival demons and signaling mechanisms".

As pointed out by J. Campisi, in the summary of her presentation "aging is the single largest risk factor for developing a panoply of diseases, including diseases as diverse as neurodegeneration leading to Parkinson's or Alzheimer's diseases and cancer.

Aging is also accompanied by inappropriate hyperplastic proliferation of cells.

Recent progress in the common signaling mechanisms and cell fate responses that drive disparate age-related diseases were discussed. At the heart of this convergence is the cell fate decision termed cellular senescence.

The pleiotropic senescence response entails a complex signaling cascade that ultimately determines important physiological responses ranging from tumor suppression to wound healing."

Senescent cells lose their ability to grow and become quiescent. They are found at all sites of age-related pathologies including arthritic joints, brain of patients with Alzheimer, atherosclerotic plaques and pre-neoplastic lesions.

Senescent cells are detected throughout evolution, from zebrafish to human and in many various tissues.

Cellular senescence is maintained by the action of tumor suppressor genes whose alterations may trigger an abnormal proliferation of senescent cells.

Senescent cells show paracrine activities that result in the disruption of normal tissues, alteration of stem cell functions and promotion of malignant phenotypes.

Work performed in J. Campisi's group has identified a group of about 50 proteins, including proinflammatory cytokines, growth factors, and chemokines that are produced by senescent cells.

The senescence-associated secretory phenotype (SASP) provides the basis for a better understanding of the apparently opposite biological roles of senescent cells in normal and pathological conditions.

In her conference, J. Campisi presented different strategies to fight against pathologies induced by senescent cells.

These include either acting at the level of signaling pathways, such as DNA damage response, p38MAPK-NF-kB, and mTOR that are known to drive the SASP, or destroy the senescent cells in vivo.

Along this line, J. Campisi discussed the advantages and disadvantages of the various approaches, and pointed out that DNA damaging therapies induced the generation of persistent senescent cells that fuel cancer progression.

After a critical analysis of cellular senescence in the context of chemotherapy, Parkinson's disease and wound healing, J. Campisi concluded by commenting on ways to either select or replace SASP factors to have a grip on tumor suppression and tissue repair or to eliminate senescent cells in order to fight against age related degeneration and cancer.

In his **Special ICCNS Springer conference, Robert Baxter** discussed the role of insulin-like growth factor binding protein-3 (IGFBP-3) in the breast cancer response to DNA damaging chemotherapy.

About 15 % of breast cancers are triple-negative for estrogen and progesterone receptors, and HER2 overexpression. These typically aggressive cancers are generally treated with DNA-damaging chemotherapy drugs that induce DNA double strand breaks (DSB). The ability to repair DSB damage allows breast cancer cells to develop treatment resistance. R. Baxter's group previously reported that IGFBP-3 interacts in the nucleus of basal-like triple-negative breast cancer cells with the epidermal growth factor receptor (EGFR) and DNA-dependent protein kinase (DNA-PKcs) to modulate DSB repair by non-homologous end-joining. Nuclear localization of EGFR and IGFBP-3, and of their complex measured by coimmunoprecipitation or proximity ligation assay, was enhanced by etoposide or doxorubicin, and inhibited by EGFR kinase inhibition. Nuclear DNA-PKcs-IGFBP-3

interaction peaked 4 h after treatment. IGFBP-3 downregulation by siRNA attenuated the stimulation of nuclear DNA-PKcs-EGFR complexes and of DNA repair activity in a NHEJ assay. An unbiased proteomic analysis of IGFBP-3-interacting proteins in basal-like breast cancer cell lysates has revealed potential new binding partners, with known DNA- and RNA-binding activity, that may be involved, together with IGFBP-3, in the DNA damage response in basal-like breast cancer. Interaction of IGFBP-3 with these proteins in response to chemotherapy is prevented by poly ADP-ribose polymerase (PARP) inhibition, suggesting a role for PARP in IGFBP-3-dependent DSB repair. R. Baxter presented and discussed new unpublished data supporting his proposal that targeting the DNA repair function of IGFBP-3 may sensitize basal-like triple-negative breast cancers to chemo- or radiotherapy.

The implication of **CCN proteins in cancer** was discussed in **Session IV** of the meeting.

Cancer research is one of the major research fields that constitute CCN family research, since every CCN family member is shown to be associated with certain malignancies. Indeed, association with all of the 6 CCN family members is reported in the case of breast cancers. In the Cancer session of this workshop, critical new findings were presented not only from a viewpoint in relation to CCN family, but also from a wider point of view, which would help the development of comprehensive knowledge in the audience.

Insulin-like growth factor-binding protein 1 (IGFBP-1), which shares significant structural homology with CCN family proteins, is known to regulate the bioavailability of IGF-I via direct molecular interaction. IGFBP-1 hyperphophorylation caused by impaired maternal-fetal amino acid transfer is closely associated with fetal growth restriction via increased affinity to IGF-I. **Madhulika Gupta** unveiled the intracellular signaling pathway from leucine deprivation to IGFBP-1 hyperphosphorylation via sophisticated approaches. Phosphorylation of IGFBP-1 in human hepatocytic cells was enhanced by leucine deprivation, resulting in decreased activity of IGF-I, which was inhibited either by calmodulin kinase 2 (CK2) or protein kinase C (PKC) inhibitor. Of note, activation of CK2 by leucine deficiency was prevented by the PKC inhibitor. Therefore, the role of PKC as a mediator of the signaling pathway from amino acid deprivation to IGFBP-1 phosphorylation by CK2 was indicated.

Although CCN6 has been shown to counteract malignant phenotypic changes in breast cancers, a physiological role of CCN6 in mammary gland development had remained unclear. **Celina Kleer**'s presentation provided novel findings that suggest a requirement of CCN6 for normal development of mammary gland. By creating and analyzing conditional knockout mice with specific deletion of CCN6 in mammary gland, her group found significant hypoplasia during the development. Preliminary studies suggest a role for CCN6 in breast tumor development. Considering the clinical impact, further advance in research on this point is expected.

The association of CCN2 and melanoma had been already recognized; however, little information was available to account for the role of this CCN family representative therein. By a series of experiments using a CCN2-deficient cell line and syngeneic mice, **Andrew Leask**'s group could discriminatively analyze the role of CCN2 either from tumors, or from tumor stroma in tumor progression. In his talk, it was indicated that, regardless of the producers, CCN2 promotes the invasion and metastasis of melanoma cells in vitro and in vivo. It should be noted that CCN2 did not show significant impact on the cell proliferation and growth of the melanoma, as reported occasionally in other tumor cells. This finding is consistent with the fact that no correlation was observed between CCN2 levels and BRAF mutations, which is associated with cell growth, in human cases. Most interestingly, periostin, another matricellular protein, was rediscovered as a downstream mediator of CCN2 function in these melanomas.

Obesity is a common systemic status that induces and/or promotes a number of complications represented by cancers. **Stephany Barreto** provided a comprehensive review on the role of matricellular proteins including CCN family members in obesity-related disorders leading to cancer.

Recent research in cancer biology revealed the critical role of cancer stem cells (CSCs) with self-renewal capacity in the development of malignancies and their drug resistance. Therefore, a new chemotherapy targeting CSCs are desired for cancer treatment. Additionally, most of current chemotherapeutic protocols, as represented by cisplatin-based ones, inevitably cause severe side effects. From these points of view, H**erman Yeger** proposed a promising strategy for cancer treatment. He provided evidence showing that a combination of two small molecules potently suppressed cancer cell growth, survival and self-renewal capability in neuroblastoma, lung carcinoid and bladder cancer cells. These compounds are a carbonic anhydrase inhibitor, acetazolamide, and a survival pathway targeting isothiocyanate, sulforaphane that are also found in cabbage, cauliflower and broccoli. Since these molecules are of minimal toxicity, clinical application of such compounds is highly expected.

In his introduction to Session V on Skin Biology and Fibrosis, **Herman Yeger** reviewed the somewhat eclectic subject areas with an overview of key elements that constituted the subject material in each presentation. The overall message was that even though a particular field could be mature in its elaboration of factors and mechanisms, yet the role of CCN proteins was minimally investigated even with strong suggestive evidence of participation. Thus Session V proved valuable in demonstrating how CCN proteins could figure into a variety of biological processes and pathobiological scenarios.

Gary Fisher started off the session, with a study on Yes-associated protein (YAP), a transcriptional co-activator, activated through the Hippo signaling pathway. Following Andrew Leask's first report that YAP targets CCN1 and CCN2, Dr Fisher proceeded to investigate YAP in skin. First he showed specific expression of YAP in the proliferative layers of normal skin epithelium and that there is substantial elevation of YAP in transformed epithelium in cutaneous basal cell carcinoma, the most prevalent form of human cancer. CCN1 and CCN2 expression were substantially elevated, and all three were co-expressed in transformed epithelium in cutaneous basal cell carcinoma. As might be expected from elevated CCN2 there was increased production matrix of type 1 collagen, fibronectin, and alpha smooth muscle actin in the stroma resulting in increased mechanical stiffness of the surrounding extracellular matrix (ECM) as shown by Atomic Force Microscopy. The basal cell tumor islands that invade the lower compartment of skin maintained this stiffness. Knockdown of YAP or CCN1, but not CCN2, inhibits proliferation of skin epithelial cells (keratinocytes). Restoration of CCN1 overcomes YAP inhibition of keratinocyte growth. Knockdown of YAP or CCN2, but not CCN1, inhibits skin fibroblast proliferation and ECM production. Restoration of CCN2 overcomes YAP inhibition of fibroblast growth and ECM production.

Taken together above data support the hypothesis that YAP is a key regulator of aberrant keratinocyte growth and fibroblast activation, which are mediated by enhanced expression of YAP target genes CCN1 and CCN2, respectively, in cutaneous basal cell carcinoma. This then supports the proposal of therapeutic targeting of these proteins in BCC.

Bethan Monk gave an excellent presentation on the subject of atherosclerosis. Cardiovascular diseases remain the leading cause of global mortality. Atherosclerosis is the underlying cause of cardiovascular disease and is characterized by lipid-filled inflammatory plaques within the blood vessel wall. Viability of vascular smooth muscle cells (VSMCs) within the fibrous cap of atherosclerotic plaques is pivotal to preventing plaque rupture, subsequent thrombosis and myocardial infarction. The group had previously demonstrated that oxidative stress induced VSMC apoptosis is reduced by Wnt5a via induction of CCN4 via a β-catenin/CREB dependent, TCF independent, signaling pathway. The aims were to 1) investigate whether this anti-apoptotic effect was specific to Wnt5a or shared by two other members of the Wnt family-Wnt3a and Wnt4 and 2) identify the signaling pathway involved in any pro-survival effects observed. Wnt3a could rescue H_2O_2 induced VSMC apoptosis, however, Wnt4 produced no anti-apoptotic effect. Wnt3a activated β-catenin/TCF signaling, even in the presence of oxidative stress, and did not activate CREB. TCF was at least in part necessary for the anti-apoptotic effect of Wnt3a as inhibition of β-catenin/TCF signaling using CCT031374 hydrobromide significantly reduced Wnt3a-mediated rescue. Although Wnt3a induced up-regulation of three pro-survival genes, IGF-1, CCN4 and CCN5, only CCN5 was at least in part necessary for Wnt3a-mediated rescue of H_2O_2 induced VSMC apoptosis. Wnt5a and Wnt3a can rescue oxidative stress induced VSMC apoptosis, however, Wnt4 has no anti-apoptotic effect. Wnt5a signals through β-catenin and CREB to induce CCN4 expression to promote VSMC survival, whereas, Wnt3a activates β-catenin and TCF to induce CCN5 expression and promote survival.

Enrique Brandan brought us into the severe disorder of amyotrophic lateral sclerosis (ALS) an essentially fatal disease. He evaluated skeletal muscle markers and the role of CCN2 and hypoxia inducible factor, HIF1α, associated to mouse sciatic denervation and to a mouse model of ALS, the broadly investigated hSOD1 G93A transgenic mouse. As an aside, it should ne noted that a recent review reported that literally thousands of studies using this mouse model have been conducted; yet it was difficult to find one asking about CCN proteins. Thus it was enlightening to hear about this work. CCN2 is upregulated by HIF-1α which is the pathognomonic responder to hypoxia. He was then able to show that: Muscular fibrosis corresponds to an excessive accumulation of extracellular matrix (ECM) replacing functional tissue, a characteristic found in several myopathies and neuropathies. Chronic muscular damage reduces vascularization and adequate blood flow, challenging the maintenance of adequate oxygen availability. This triggers ischemic foci and activate Hypoxia-inducible pathways. CCN2, a key factor promoting fibrosis is upregulated by HIF-1α. In models of sciatic denervation and symptomatic ALS an increase of TGF-β signaling and CTGF were increased together with ECM molecules. The increment in fibrotic markers paralleled an increased expression and nuclear localization of HIF-1α. When the same parameters were evaluated in CTGF deficient mice, the extent of fibronectin and collagen deposition was reduced compared to control mice. Pharmacological stabilization of HIF-1α increases CTGF and ECM deposition in both healthy and damaged (denervated) skeletal muscle. These observations suggest that ALS skeletal muscle fibrosis might be related to muscle denervation and not necessarily to the disease itself (Transgene expression of SOD1). HIF-1α activation could be a compensatory muscle response leading to improved oxygen availability upon damage and under pathological conditions; it might also be participating in the development of muscular fibrosis. Thus the HIF-1α pathway utilizes CCN2 to fully exert its fibrotic role.

Muriel Cario-Andre focused on the role of CCN3 in the hypopigmentary disorder, vitilago, and in system sclerosis, a dermal disease, staying within the skin biology theme. As CCN3 has surfaced as a CCN protein with opposite effects to other CCN proteins it was interesting to learn that CCN3 is prominently expressed in certain dermal phototypes as well as in the epidermis. Exploiting a mouse model of caucasion-

negroid switch and melanocytes, keratinocytes and fibroblasts where she transduced lentiviral shRNA to CCN3 the results were surprising. Melanocytes did not survive while keratinocytes and fibroblasts proliferated. It was found that in melanocytes DDR1 was inhibited forcing detachment ands anoikis while in keratinocytes and fibroblasts the transient suppression of CCN3 was resolved permitting further growth. Fibroblasts were cultured form a scleroderma patient with hyperpigmentation. CCN3 was expressed. Normal epidermis incubated with sclerodermal fibroblast conditioned medium showed multiple isoforms of CCN3 being expressed and in addition perturbation of melanocytic homeostasis. Overall, CCN3 is in balance with skin homeostasis and tightly regulated. Downregulation of CCN3 disturbed this homeostasis. How this could affect other CCN proteins might reveal new insights into the required CCN protein balance.

Finally, **Joseph Choukroun** took the session far afield but did present some provocative data around his use of platelet concentrates and low speed isolated mesenchymal cell fractions for regeneration of wounds and injured cartilage. The images presented of responding patients (before and after) were indeed impressive. We await further controlled studies to determine the extent to which this treatment could be more widely adopted as a front line therapy. Of course it would be interesting to analyze the status of CCN proteins in these reparative processes given that CCN2 figures prominently in wound repair as well as other CCN proteins that could provide the balance needed for a non-conflicted wound repair process as suggested by the images.

The **Pathobiology Session** highlighted many new discoveries regarding the importance of CCN proteins in a large range of human diseases. Two common themes emerged from the presentations: 1) aberrant expression of CCN proteins plays major roles in the pathophysiology of many diseases, and 2) the promise of directly or indirectly modulating CCN protein levels for therapeutic benefit.

Four presentations focused on the role of CCN proteins in lung pathology.

Gustavo Matute-Bello, described the role of CCN1 in acute lung injury in three different mouse models. The research of Dr. Matute-Bello and his colleagues focused on the role of CCN1 in the fibroproliferative repair phase, following acute injury. In each of the three mouse models, injury led to induction of CCN1 within the injured alveoli. This induction occurred prior to and coincident with fibroplasia and fibrosis. Interestingly, addition of exogenous CCN1 in the context of acute lung injury further exacerbated abnormal collagen production. These data provide further evidence for the key role of CCN1 in fibrosis that occurs following injury.

Continuing the theme of the role of CCN2 in pulmonary fibrosis, **Shu Wu** described her important studies on the pathophysiology of bronchopulmonary dysplasia (BPD). BPD is the most common and serious chronic lung disease of premature infants. CCN2 has been found to be elevated in BPD, and to understand its role Dr. Wu and her colleagues generated a novel inducible transgenic mouse model with overexpression of CCN2 in type II alveolar epithelial cells. Importantly, induction of CCN2 in these mice recapitulated the major pathological features of BPD. Furthermore, Dr. Wu reported that many of these features are associated with beta-catenin signaling. Importantly, antibody neutralization of CCN2 reduced beta-catenin signaling and lung damage in a neonatal rat model of BPD. These findings provide exciting proof of concept for targeting CCN2 beta-catenin signaling pathway to prevent BPD in premature infants.

Expanding on the critical role of the CCN family of genes in lung fibrosis, **Stephan Klee**, reported on the involvement of CCN4 in idiopathic pulmonary fibrosis (IPF). Dr. Klee and his colleagues demonstrated that CCN4 increases alveolar epithelial type II cell hyperproliferation and induces myofibroblast, differentiation, and collagen production in lung fibroblasts. Using primary human lung fibroblasts, Dr. Klee and his colleagues found that CCN4 is significantly induced by pro-fibrotic cytokines TGF-beta and TNF alpha, and that CCN4 acted in concert with these cytokines to induce the proinflammatory cytokine interleukin-6 (IL-6). Importantly, this induction of IL-6 was shown to promote lung fibroblast proliferation. Thus, CCN4 is critically involved in a cytokine network that drives proliferation and pro-fibrotic transformation in lung fibroblasts. These data support the concept that targeting CCN may provide therapeutic benefit in IPF.

Rounding out the group of talks on lung pathology, **Yves Courty** reported on the roles of CCN family genes in smoking-related infectious lung diseases. Dr. Courty and his colleagues demonstrated changes in expression of CCN family members in various mouse models of lung infection, including exposure to cigarette smoke, influenza A virus infection, and bacterial infection. The data revealed variable patterns of expression of CCN1, CCN2, CCN3, CCN4, and CCN5 depending on the type of infection. Furthermore, it was shown that CCN3 and CCN4 were significantly decreased in lungs of smokers, while in chronic obstructive pulmonary disease CCN1, was significantly increased. Thus, expression of CCN family members is altered in several lung diseases, and the pattern of expression varies depending on the nature of the disease.

The remaining four talks in the session exemplified the diverse roles of CCN family genes in pathophysiology, ranging from the central nervous system, the musculoskeletal system, and the vasculature.

Jake Bedore, described the role of CCN2 in intervertebral disc degeneration and associated back pain, which commonly occur with aging. Dr. Bedore and his colleagues found that mice with targeted deletion of CCN2 in the inner core of the vertebral disc developed disc degeneration by 9 months of age. This degeneration was associated with molecular markers

of inflammation and extracellular matrix degradation as well as clinical symptoms indicative of reduced mobility and discomfort. These elegant studies demonstrate the importance of CCN2 in the maintenance of vertebral health, and provide proof of concept that CCN2 therapy may be beneficial for treatment of age-associated back pain.

Helen Williams described the role of CCN4 in the progression of atherosclerosis and plaque composition. Using a mouse model of atherosclerosis, Dr. Williams and her colleagues found that deletion of CCN4 significantly increased both the size and fragility of atherosclerotic lesions. Thus, it appears that CCN4 may be protective against both vascular occlusion and aneurysm formation. These results provide further support for an important role of CCN4 in vascular smooth muscle biology, and identify the potential of CCN4 to treat cardiovascular diseases.

In the final presentation, **Zhiyong Lin** described his exciting research on the role of CCN3 in formation of abdominal aortic aneurysm. Dr. Lin and his colleagues found that CCN3 is significantly reduced in rodent models of aortic aneurysm and that CCN3 knockout mice display severe aortic aneurysm. Interestingly, overexpression of CCN3 mitigated aneurysm progression. While the mechanism for the actions of CCN3 in aneurysm formation remains incompletely understood, Dr. Lin presented data indicating involvement of the ERK1/2 pathway. The findings from this study are important for at least two reasons: 1) demonstrating a novel role of CCN3 in vascular biology and disease, and 2) illustrating the potential of a new CCN3 knockout model to reveal unknown aspects of CCN3 functions and mechanisms of action.

Fig. 1 2015 Springer Scholarships Awardees. From left to right : Jake Bedore, Bethan Monk, Joseph Tarr

In summary, the excellent presentations in the Pathobiology Session illustrated the remarkably broad reach of CCN family genes in mammalian biology, and the substantial deleterious impact that dis-regulation of CCN family proteins functions can impart on health. Aberrant expression levels appear to be the primary mechanism by which CCN family proteins promote disease. Thus, development of therapeutic strategies aimed at enhancing or limiting the activities of specific CCN proteins is an exciting future direction for CCN family research, and holds great promise for advancing medical science.

As a scientific conclusion to the meeting, and after deliberations of the ICCNS Council's members with the President of the ICCNS, three Springer scholarships have been offered to young scientists who presented an excellent oral communication during the meeting. The scholarships are offered to the recipients by Springer in order to help covering their meeting fees. The recipients were Bethan Monk (School of Clinical Sciences, University of Bristol, UK), Jake Bedore (University, London, Ontario, Canada), and Joseph Tarr (Temple University School of Medicine, Philadelphia, USA).

Our renewed congratulations to these young talented scientists (Fig. 1).

Not only the 8th international Workshop on the CCN family of genes brought together worldwide leaders in the CCN field and younger scientists joining the field, it was also the place to hear outstanding communications in many new aspects of CCN proteins biology. The high quality of the presentations that builds up at each meeting, is a very encouraging sign. The CCN field is getting stronger and wider.

The participation of worldwide specialists in the fields of Senescence and Cancer with J. Campisi, and IGF Binding Proteins with R. Baxter is another testimony that the CCN field is of interest outside of our scientific community and is becoming recognized as a major one in Cell Biology.

We wish to thank all participants for their valuable input.

Acknowledgments The various parts of this report are from colleagues who were invited to act as the main chairs of meeting sessions which did not necessarily correspond to the topic in which they specialized. The organizers of the meeting are grateful to all of them for "playing the game" and providing an outsider-type of view.