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ARTICLE

Report on the 11th international workshop on the CCN family of genes, Nice, October 20–24, 2022

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Abstract
In celebration of the twentieth anniversary of the inception of the CCN society, and of the first post-Covid-19 live meeting, the executive board of the ICCNS had chosen Nice as the venue for the 11th International workshop on the CCN family of genes. On this occasion participation in the meeting was extended to colleagues from other cell signaling fields who were invited to present both an overview of their work and the future directions of their laboratory. Also, for the first time, the members of the JCCS Editorial Board were invited to participate in a JCCS special session during which all aspects of the journal « life » were addressed and opened to free critical discussion. The scientific presentations and the discussions that followed showed once more that an expansion of the session topics was beneficial to the quality of the meeting and confirmed that the ARBIOCOM project discussed last April in Nice was now on track to be launched in 2023. The participants unanimously welcomed Professor Attramadal's proposition to organize the 2024, 12th International CCN workshop in Oslo, Norway.

Keywords International CCN Society (ICCNS) · Journal of Cell Communication and Signaling (JCCS) · CCN proteins (CCN1-6) · ICCNS award · Spatiotemporal combinatorial model · Transcriptomics · Genome wide association · Studies (GWAS) · Cancer

JCCS editorial board meeting

The general feeling was that the Journal is now recognized as a reliable source of high quality science in competitive fields. With a yearly average of 1 submission per day and 143,747 downloads (34% increase over 2022, and 100% over 2021) the JCCS audience is significantly increasing. All the editors expressed their willingness to help the reinforcement of the JCCS position in a realm where a considerable number of unreliable articles are being published online by predatory journals. Among the various topics that were addressed, the manuscript submission flow, rate of acceptance, relative origins of the published manuscripts, and monthly numbers of downloads were critically reviewed in the context of the Covid-19 pandemic and of the reorganization of the Editorial Board that had been planned for several years but never really achieved.

All other JCCS aspects including the support from Springer were also discussed in terms of the future needs of JCCS.

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Most high rank journals benefit from a dedicated group of scientists interested in increasing the reputation of journals. In spite of the JCCS impressive increase in the download rates, and its marked progression in the Impact Factor over the past few years, the Board felt that the journal would benefit from better logistic support. The Board also recognized the need for a more aggressive publicity and a better distribution of JCCS information at international meetings.

ICCNS award and scientific sessions

The 2022 Awardee scientific presentation: CCN1 in wound healing and tissue regeneration by **Lester Lau**.

Whereas CCN1 is critical for placental angiogenesis and heart development during embryogenesis, it plays diverse roles in wound healing and tissue regeneration. CCN1 promotes wound healing by functioning as an opsonin for the removal of bacteria and apoptotic neutrophils. This endocytic process leads to macrophage expression of TGF-β1, which induces the differentiation of precursor cells into myofibroblasts and the production of extracellular matrix to support healing. Finally, CCN1 directly induces myofibroblast senescence to accelerate matrix remodeling and curb fibrosis. In liver regeneration after partial hepatectomy, CCN1 promotes hepatocyte proliferation by inducing senescence in hepatic stellate cells, leading to the secretion of mitogenic cytokines including IL-6 and CXCR2 ligands. Moreover, CCN1 is required for the regeneration of bile ducts after cholestasis by activating Notch signaling. In the intestinal epithelium, CCN1 coordinately regulates the proliferation and differentiation of Lgr5 + stem cells through the control of Wnt and Notch pathways. In each of these biological contexts, the functions of CCN1 can be attributed to its direct binding to either integrins αvβ3/αvβ5 (epithelial or myeloid cells) or α6β1 (fibroblastic cells) to activate integrin-mediated signaling with cell-type specific consequences. The roles of specific integrins were established by the use of CCN1 mutant proteins that cannot bind specific integrins, knock-in mice that express integrin-binding defective mutants, and the effects of specific integrin inhibitors in vitro and in vivo.

Scientific sessions

Session 1-regulation of cell growth and differentiation

Brahim Chaqour presented new findings about the involvement of the matricellular protein CCN2 in the regulation of progenitor cell growth and lineage specification during embryonic development. The presented work focused on the development of the retina, a neurovascular tissue commonly viewed as an extension of and "window to" the brain by virtue of their shared structural and functional properties. CCN2 expression is crucial for not only angiogenic growth and expansion of the mouse retinal vasculature during early postnatal stages of life, but also for the formation and maintenance of the blood retina and brain barrier. However, mining transcriptomic data of embryonic mouse retina showed that retinal progenitor cells are also major source of CCN2 in embryonic retina. Loss of CCN2 function in these cells resulted in major neuronal and glial defects that culminate into retinal hypocellularity. This study highlighted the broad range of fetal and embryonic genes that are regulated by CCN2 during the process of neurogenesis and gliogenesis. These developmental processes are dependent on the functional interaction between CCN2 and the hippo YAP signaling pathway. These findings suggest a potential implication of CCN2, in neurodegenerative diseases affecting the retina and brain.

Stephen Twigg's presentation highlighted a potential endocrine function of CCN1 and CCN2 in the metabolic syndrome wherein type 2 diabetes is a confounder. The presented study characterized liver phenotype and systemic changes in a preclinical model of non-alcoholic steatohepatitis (NASH) with targeted deletion of either CCN1 or CCN2. Findings showed that liver fibrosis characteristic of NASH was reduced in the CCN1 and CCN2 knockout models. The ERK signaling pathway was implicated in this regulation. However, a systemic metabolic phenotype was also observed in the CCN1 knockout model whilst the CCN2 knockout model did not exhibit such phenotype. These studies attributed endocrine function to CCN1 distinguishing it from CCN2 properties.

Kunimasa Ohta presented his most recent data on the extracellular secreted protein referred to as Akhirin (AKH). Structurally, AKH comprises two von Willebrand factor-A (vWF-A) domains and one Limulus factor C, Coch-5b2 and Lgl1 (LCCL) domain. The Ohta's laboratory has pioneered studies investigating the tissue distribution of AKH and showed that it was specifically expressed in the central nervous system (CNS). AKH is expressed in the neural stem cell niche of the CNS including the retina, brain and spinal cord although its expression changes during development. In the eye, AKH localizes in the head ectoderm overlying the lens vesicle at stage 17 and in the retinal pigment epithelial layer at stage 22 and accumulates in neuronal stem cell niche found in the ciliary marginal zone at the postnatal stage. In the spinal cord of developing mouse embryos, AKH localized in ependymal cells of the spinal cord. In the adult, the expression of AKH was very low but wa substantially increased in ependymal cells after spinal cord injury.

In the developing brain, Ohta's group has reported a transient expression of AKH in the subventricular zone of the brain and hippocampus and a more permanent expression in the hippocampal CA2 region after birth. The presented data further showed that, in addition to the neuronal ependymal cell layer, AKH is also expressed in choroid plexus ependymal cell layer and cerebral spinal fluid during embryonic development. AKH knockout in mice led to suppression of neuronal stem cell proliferation and aberrant expansion of the left ventricles together with blood vessel and behavioral abnormalities. Thus, AKH seems to be critical for neurogenesis and normal brain development.

Gary Fisher presented studies from his group on the organization of the extracellular matrix (ECM) of the dermis and the role of YAP/ TAZ in the maturation process of the dermal matrix in mice during development. The group has shown that the number of fibroblasts, the main cellular sources of collagen in the dermis, remained the same at P0 and P20 even though collagen fibrils were produced and accumulated in the mouse skin during the first three weeks after birth. Targeted deletion of YAP in fibroblasts resulted in reduced both expression and accumulation of collagen fibrils in the dermal matrix. Transcriptomic analyses of the dermis revealed that the expression of numerous matrix genes was altered by YAP deletion in fibroblasts as well. The presented studies indicated that YAP plays an important role in the control of ECM synthesis and organization in the developing skin. These findings are consistent with the role of YAP dysregulation in various human diseases linked to the matrisome.

David Roberts presented analyses of genetic studies comparing phenotypes associated with CCN1 or thrombospondin 1 (THBS1) deficiency and how loss of function of these genes and exposure to stresses may reveal unsuspected roles of these genes in viability. By mining the human Genome Aggregation Databases for loss of function mutants for these genes, the probability of the loss of function intolerance (pLI) to gene deficiency and missense mutations in these genes was determined.

Pekovic-Vaughan Vanja's presentation was focused on the remodeling of ECM proteins by the circadian clock during skeletal muscle cell differentiation. These studies are based on previous observations linking skeletal muscle secretome to ECM protein secretion and a potential important role of a muscle-specific circadian timing regulation of this process. The presented data showed that secreted proteins by differentiating skeletal muscle cells were in part regulated in a time-of-day-dependent fashion. Several proteins of the CCN family were among the ECM molecules to be under the control of the circadian clock both in in vitro muscle cell cultures and in vivo. A molecular crosstalk was evident between the molecular clock and transcriptional regulation of matrix genes including those encoding the CCN proteins. Moreover, as circadian rhythms become altered with age at both the behavioral and molecular levels, this further alters the transcription of ECM genes regulated by the circadian clock.

Ulf Smith's last presentation in this session was focused on the role of cell senescence in metabolic syndrome and the prospect of using senolytic agents to eliminate senescent cells as a therapeutic approach. Ulf Smith described results of his studies showing increased senescence markers and cell cycle regulators in adipose cells from obese and T2D individuals. Genetic predisposition to T2D and obesity increased differentiated adipose cells' risk of becoming senescent. His studies also suggested a role of hepatocyte cell senescence in the development of hepatic steatosis as treatment of hepatocyte cell line HepG2 with senescence-inducing drugs upregulated senescence markers and increased insulin signaling. Reversibly, hyperinsulinemia promotes the senescence of hepatocytes and adipose cells. Thus, cell senescence could be critical in the pathogenesis of cardiovascular and liver diseases independently of aging.

Session II-cancer development and therapy

Taihao Quan explained that CCN1 overexpression in dermal fibroblasts might accelerate dermal aging and promote skin cancer in a mouse model in which human CCN1 (Col1a2-CCN1) is conditionally overexpressed. Their studies suggested that age-related alteration of the dermal microenvironment is necessary for developing keratinocyte cancer, which partially explains the prevalence of skin cancer in the elderly.

Kathryn E. Meier discussed how CCN1 potentially plays a role in downstream signal transduction events required for LPA-induced cell proliferation. Specifically, in cancer cells, CCN1 secreted into the extracellular space can facilitate the activation of additional receptors and signal transduction pathways, contributing to the biphasic responses typically seen in response to growth factors.

Sushanta Banerjee reported that overexpression of CCN1 has been associated with mutant K-Ras dependent pancreatic cancer growth and metastasis in lungs of a genetically engineered mouse model in which pancreatic tumors were generated by introducing mutant K-Ras and mutant p53 genes. Findings from this study suggest that since mutant K-Ras is still a non-druggable target, CCN1 could be used as a potential target for treatment of pancreatic ductal carcinoma.

Veronica Giusti recognizes the value of IGF2BP3, an insulin-like growth factor 2 mRNA binding protein 3, in the regulation of exosomal microRNA that affects the invasive

fronts in Ewing sarcoma cell lines. This event could be regulated through the PI3K/AKT pathway.

Herman Yeger presented evidence in support of the Mediterranean diet receiving increasing attention as it significantly lowers the incidence of many major cancers that were discussed. Phytochemicals could also target the critical tumor stem cells and the tumor microenvironmental role in the malignant progression of cancer. Thus, chemoprevention seems to be a feasible goal for reducing the global burden of cancers. How are we still missing the mark?

Session III - chondrogenesis and osteoarthritis

Ali Mobasheri presented work on investigation of the clusterin connectome in chondrocyte biology and osteoarthritis. Clusterin is a holdase chaperone present both intracellularly and extracellularly that has been implicated in a multitude of functions including cytoprotection, cell differentiation, regulation of apoptosis, and clearance of cellular debris. Thus, the authors hypothesized that clusterin and its connectome might have significant potential as biomarkers of osteoarthritis. Using bioinformatics tools (the STRING database, Cytoscape, and the QIAGEN Ingenuity Pathway Analysis) the authors analyzed the clusterin connectome, in particular in an inflammatory environment. The authors identified several interacting partners among intracellular chaperones, aggregate-forming proteins, regulators of apoptosis, and complement proteins. The authors predicted several novel potential components of the clusterin connectome, including selenoprotein R, semaphorins, and meprins, which will enable design of putative diagnostic biomarker panels to be investigated in osteoarthritis.

Csaba Matta, presented data from transcriptome and proteome analysis of micromass cultures of differentiating embryonic limb bud-derived progenitor cells. The differentiation process was monitored for 15 days by RNA sequencing and high-throughput mass spectrometry (MS). The authors found that CCN2 and CCN1 had the highest transcript number in differentiating embryonic limb bud-derived progenitor cells, particularly towards the later stages of the process, consistent with CCN2 and CCN1 expression during the process of endochondral ossification. CCN3 was also found to be expressed peaking on day 6 of culturing. However, only CCN2 could be identified in the proteome analyzed by MS. These studies highlight that CCN proteins are expressed in differentiating chondrocytes and may play important roles in early chondrogenesis. However, more targeted approaches are required to uncover the specific roles of each member of the CCN family in chondrogenesis.

Satoshi Kubota presented work focusing on the specific role of CCN3 in cartilage. His group reported that CCN3 is subject to induction by starvation and impaired glycolysis and that the function of CCN3 is to protect chondrocytes by repressing cell proliferation and directing cells towards quiescence in order to restrict energy consumption. Thus, CCN3 appears to be a survival factor involved in cytoprotection of chondrocytes in developing osteoarthritis. The authors also delineated the DNA element and transcription factor RFX1 involved in metabolic regulation of CCN3.

Roland Takacs presented new analyses of the connections between the transcriptional regulation of clusterin and CCN genes in normal and osteoarthritic articular cartilage, using existing global-RNA-sequencing gene expression data sets. Of the six CCN genes, CCN1, CCN2, and CCN4 were found to have the highest expression levels in the data sets that were examined. Interestingly, the enhancer regions of all three of these CCN genes and clusterin were found to have significant overlap with respect to transcription factor binding motifs. Furthermore, clusterin and all three of the CCN genes were upregulated in samples from osteoarthritic compared to normal cartilage. Common enhancer motifs for the transcription factors ZFHX2, NR2F2, and EGR2 were found to be present in clusterin and each of the three CCN genes. These data highlight the possibility that clusterin and CCN1, CCN2, and CCN4 may be coordinately regulated by transcriptional pathways in normal and osteoarthritic cartilage. Future research will focus on functional studies aimed at unraveling these potential transcriptional pathways.

Blandine Poulet presented data on the impact of genetic deletion of Fibrillin-1 in mouse limbs on the development of osteoarthritis. The studies utilized a repetitive traumatic joint injury model of osteoarthritis. Histological imaging revealed that deletion of Fibrillin-1 leads to increased osteoarthritis development at later time points, suggesting that lack of Fibrillin-1 is involved in the progression of osteoarthritis. These data are consistent with the observed decreased levels of Fibrillin-1 in human osteoarthritic tissues. Interestingly, increased osteoarthritis was observed in male, but not female, knockout mice, suggesting a sex-dependent role for Fibrillin-1 in musculoskeletal health. Pathway analysis from RNAseq data revealed modified cellular responses in the TGF-beta and PI3K/ATK pathways in Fibrillin-1 knockout mice. The data support the role of Fibrillin-1 loss in promoting osteoarthritis progression. Future research will focus on the pathways by which decreased Fibrillin-1 enhances the development of osteoarthritis.

Sima Zolfaghari reported on studies that investigated the functionality of the C-terminal TSP-1 homology domain in CCN5, which lacks the C-terminal cystine knot domain that is present in other members of the CCN family. The CCN5 TSP-1 homology domain was produced in cell culture as secreted fusion proteins attached to His-Halo-Sumo or the amino-terminal region of albumin. The purified TSP-1 homology fusion proteins inhibited both the AKT

and ERK1/2 kinase pathways, as well as CCN1/2-induced fibroblast migration in a scratch wound assay. Interestingly, CCN3 TSP-1 homology domain fusion proteins displayed similar inhibitory activities as the TSP-1 homology domain fusion proteins of CCN5. The TSP-1 homology domain fusion proteins also exerted regulatory effects on two different mammary adenocarcinoma cell lines. These data support the concept that the TSP-1 homology domain of CCN5, which is released from full-length CCN5 by proteolytic processing, may exert multiple inhibitory and stimulatory biological functions. Future studies are aimed at investigating possible therapeutic applications of TSP-1 homology fusion proteins.

George Bou-Gharios presented data on the role of low-density lipoprotein receptor-related protein 1 (LRP1) on lung tissue homeostasis. Proteins that bound to the soluble form of LRP1, which blocks the binding of LRP1 ligands to the membrane-bound LRP1, were identified by amino precipitation coupled mass spectrometry. Sixty-seven ligand candidates including 50 previously unreported potential ligands were identified. These potential LRP1 ligands included proteins that are involved in several biological processes including cell survival, inflammation, and extracellular matrix remodeling. Given that LRP mediates endocytosis, the binding of LRP1 ligands to LRP1 could inhibit endocytosis and thereby be detrimental to cell function. Future studies will test this hypothesis in a tissue-specific conditional LRP1 knockout mouse model.

Educational session

INVITED SPEAKER: **Jean-Claude Chermann**.

Because of the COVID-19 pandemics impact on the mobility of scientists from abroad who were initially contacted to give a thematic educational lecture at the end of the Workshop, the organizers invited Professor Jean-Claude Chermann, from Nice, to give a lecture on his own perception of the HIV story. We also asked J-C Chermann to present his views and opinions regarding the future of therapeutical approaches which are presently developed to fight this scourge which was recently said to be responsible for more than 35 millions death worldwide.

Professor Chermann quickly reviewed the scientific steps that have led to the identification and first molecular cloning of HIV and the subsequent controversies which arose after following the attribution of the Nobel Prize in Physiology or Medicine to H. zur Hausen, F. Barré-Sinoussi and L. Montagnier[1].

Many articles have discussed the fact that Professor Chermann had not been rewarded for the direction of his team, of which F B-S was a member.

For those who were not aware of the details, J-C Chermann recalled that there is driving "rule" for authors' orders in the academic educated scientific publishing. We generally put at the first position in the list of co-authors, the student who needs credentials to get a position, and at the last position the name of the Head of the Laboratory or Department in which the work was performed. This custom applied to the 1983 Science paper describing the isolation of HIV[2], resulted in putting F. B.-S. first, J.-C. C. second and L. M. last.

Based on the fact that the student would not have been able to lead the project on her own, without her mentor, the decision of the Nobel Committee was claimed by many researchers to be vitiated by a manifest error of assessment, tainted by inglorious behavior.

In spite of the profound moral shock and distress that Professor Chermann felt when he heard about the decision of the Nobel Committee, his presentation at the CCN Workshop was tinted with a taste of humor and sarcasm that was appreciated by all participants.

[1] An overview of the HIV molecular cloning saga can be found in the 2008 issue of lancet that followed the attribution of the Nobel Prize that year.

[2] Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vézinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L. (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science. 220 :868–871.