CCN workshop

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Report on the Third International Workshop on the CCN Family of Genes

The CCN family currently comprises six extracellular matrix associated proteins (CCN1–6) that regulate diverse cell functions. Although CCN molecules regulate vital processes in vivo (for example, chondrogenesis, angiogenesis, and matrix remodelling) and are associated with several pathophysiological disorders (such as fibrosis and tumorigenesis), it has been a challenge to define the underlying biological mechanisms involved. Progress has been hampered by the disparate in vivo and in vitro models used by investigators in the field, and by the difficulty in obtaining validated reagents (particularly recombinant CCN proteins) for experimental use. In spite of these drawbacks, there have been many exciting developments in the field over the past few years, and it was against this backdrop that investigators convened for the Third International Workshop on the CCN Family of Genes in St Malo, France on 20–23 October 2004.

GENE EXPRESSION

Although studies in the early 1990s showed that CCN1 and CCN2 were encoded by immediate early genes, current research in the field highlights the diverse mechanisms that influence the regulation of CCN gene expression. M Goppelt-Struebe (Germany) reported that CCN2 expression in endothelial cells or fibroblasts is inhibited by monomeric G actin, but it is stimulated by F actin stress fibres, emphasising the importance of changes in cell morphology as a determinant of CCN2 production. R O'Leary (Ireland) reported the downregulation and disassembly of several cytoskeletal proteins in human glomerular mesangial cells in response to CCN2, suggesting that actin reorganisation is among the pathophysiological effects of CCN2 in diabetic nephropathy.

CCN2 is a well characterised regulator of chondrogenesis and S Kubota (Japan) reported that CCN2 expression was enhanced during chick chondrocyte differentiation as a result of enhanced gene transcription and mRNA stabilisation, the latter of which was attributed to a novel regulatory element in the 3′-untranslated region of the chick ccn2 gene, which is distinct from ones found in mouse or human ccn2 genes. In addition to promoting chondrogenesis, ccn2 is expressed by breast cancer cells and may be involved in their metastasis to bone. In studies designed to address aspects of gene regulation in these scenarios, T Eguchi (Japan) reported that ccn2 expression is principally activated by cis-elements, including TRENDIC (transcription enhancer dominant in chondrocytes) in the human HCS-2/8 chondrocyte cell line, whereas it is mainly Smad regulated in MDA231 breast cancer cells.

> "Variations in tissue microenvironment can have important effects on ccn2 expression"

Another major property of CCN2 is as a stimulator of fibrogenic pathways, and much attention has previously focused on its action as a downstream mediator of transforming growth factor β (TGFβ) through Smad dependent pathways. Nonetheless, additional mechanisms of regulating ccn2 expression have been identified using cells from fibrotic lesions as compared with their normal counterparts. V Haydont (France) reported that, in smooth muscle cells from radiation enteritis, pharmacological inhibitors of the Rho/Rock pathway were effective, at early time points, in blocking expression of ccn2 but not of TGFβ or collagen. G Yang (USA) reported that basal or serum stimulated expression of ccn2 is more exaggerated in keloid fibroblasts than in normal fibroblasts. Although Smads were involved in both cell types, raised ccn2 expression by keloid fibroblasts was associated with increased activation of AP-1 and c-Jun. X. Shi-wen (UK) reported that endothelin 1, which is raised in fibrotic diseases including those of the lung, stimulated ccn2 expression in lung fibroblasts via Erk1/2 and transcriptional activation of the BCE-1 element, but not the Smad element, in the ccn2 promoter. P Trackman (USA) reported that prostaglandin E_2 (PGE_2) inhibited TGFβ induced CCN2 production in IMR-90 cells via p42/p44 mitogen activated protein kinase, yet in gingival fibroblasts ccn2 expression was less sensitive to inhibition by PGE_2 as a result of a p38 mitogen activated protein kinase mechanism. These data may explain why fibrotic gingival outgrowths overexpress TGFβ and CCN2 in the presence of PGE_2. Overall, these presentations highlighted how variations in tissue microenvironment can have important effects on ccn2 expression.

RECEPTORS/SIGNALLING

In the past few years, cell surface integrins have emerged as signalling receptors for CCN proteins. L Lau (USA) showed that integrins αvβ3 and α6β1 mediate the distinct functions of CCN1 in mesenchymal cells, and identified specific binding sites for each integrin. Moreover, CCN1 mutant proteins disrupted at specific integrin binding sites block CCN1 functions mediated through the cognate integrins. D Brigstock (USA) showed that in hepatic or pancreatic stellate cells, CCN2 stimulated adhesion, migration, or gene expression involved interactions of integrin αvβ3 or integrin α5β1, respectively, with discrete novel regions in module 4 of the CCN2 protein. A Leask (UK) showed that endogenous CCN2 facilitates fibroblast spreading on fibronectin substrate through its ability to bind directly to fibronectin, integrins, and synedcan 4, suggesting that CCN2 may function principally by regulating receptor–ligand interactions.

Although many of the biological properties of the CCN proteins are attributable to their interactions with integrins and heparin sulfate proteoglycans, other lines of investigation were reported that may help to define additional receptor molecules. Potentially promising approaches included the yeast two hybrid system for CCN1 discussed by P Robertson (USA) and CCN2 crosslinking to mesangial cells reported by N Wahab (UK).

DIFFERENTIATION AND DEVELOPMENT

Several CCN proteins have been recognised as major regulators of skeletal development. M Takigawa (Japan) reported a role for CCN2 in cartilage repair and osteogenesis, proposed that mesenchymal cell differentiation in wounded vascularised tissues may be promoted by CCN2 from platelets, and showed differential biological activities of the individual modules of CCN2. L Dornbach (USA) showed that ccn1

null mice that survive to birth exhibit skeletal abnormalities involving the radius, ribs, sternum, and vertebrae. Importantly, some skeletal defects were more exacerbated in mice that were null for both ccn2 and ccn1 than in those that were null for ccn2 or ccn1 alone, suggesting that these molecules have both interacting and independent functions in regulating skeletal development. P Francis-West (UK) reported that ccn4 and ccn6 are expressed in the joints and chondrocytes of the developing chick long bones and in differentiating ATDC5 cells, in which they act downstream of β catenin but have opposing effects on matrix production. Based on transfection studies and a fracture repair model, L Desnoyers (USA) showed that CCN4 acts to promote mesenchymal cell proliferation and osteoblastic differentiation yet represses chondrocyte differentiation.

Data presented by K Katsube (Japan) showed that in the Kusa bone marrow derived stem cell line, ccn3 expression was upregulated by Notch, which is associated with the ability of the cells to follow a neurogenic rather than osteogenic pathway. These results suggest that CCN3 may mediate specific Notch dependent cell lineage development. Effects of CCN3 on gene expression were further discussed by N Planque (France), who showed that module 4 of CCN3 has DNA and nuclear binding activity, and that this property is responsible for the association of CCN3 with the nucleus and its regulation of gene transcription.

PATHOBIOLOGY

Studies of CCN2 in fibrotic diseases continue to be a major research focus of many investigators. E Brandan (Chile) reported that ccn2 was overexpressed in the fibrotic mdx mouse model of Duchene muscular dystrophy and that in C2C12 mouse myofibroblasts, ccn2 was induced by TGFβ or lysophophatidic acid and, when added exogenously, promoted cell differentiation and proteoglycan synthesis, in addition to complexing with proteoglycans such as decorin. S Ahmed (Norway) showed that, in a model of lung fibrosis secondary to cardiac ischaemia, ccn2 was overexpressed in the lung and attributed to production by endothelial cells and macrophages. In a study of atherosclerosis by I Cicha (Germany), CCN2 protein concentrations were greatly increased in lipid rich plaques compared with fibrous plaques, and it was suggested that it acts as a monocyte chemoattractant in diseased tissue.

Diabetic nephropathy is an important cause of morbidity and mortality and is a fibrotic disorder that is strongly associated with overexpression of ccn2. S Twigg (Australia) reported that CCN2 contributes to high glucose induced matrix production by increasing the expression of TIMP-1 and thus decreasing the rate of matrix breakdown. B Riser (USA) showed that urinary CCN2 concentrations assessed by enzyme linked immunoabsorbent assay were increased six to sevenfold in an animal model of diabetic nephropathy and in human subjects with diabetes and renal disease. Some individuals with diabetes but without renal disease had raised CCN2 concentrations, although longitudinal studies to examine whether this is predictive of diabetic nephropathy have yet to be conducted. Urinary CCN2 from most diseased patients comprised intact 38 kDa CCN2 in addition to unidentified 12 kDa and 200 kDa moieties. Because urinary CCN2 may reflect a failure of resorption and/or local production in the kidney, examination of circulating CCN2 may be more appropriate. To this end, R Goldschmeding (the Netherlands) reported that plasma CCN2 values in patents with diabetic nephropathy were raised and correlated with plasma creatinine concentrations and albuminuria.

CCN5 inhibits the proliferation and motility of smooth muscle cells. J Castellot (USA) reported that CCN5 is widely expressed in many embryonic and adult tissues, but that values are low at sites of smooth muscle cell hyperplasia, such as leiomyomas or restenosis. Preliminary data using Ad-CCN5 suggest that CCN5 may have therapeutic benefit in the treatment of smooth muscle cell proliferative disorders.

TUMORIGENESIS

Since the discovery of CCN3 as an antiproliferative protein whose truncation reveals oncogenic potential, a growing body of evidence supports the concept that alterations of CCN protein structure or expression are associated with cancer development. The first evidence for CCN3 being involved in non-solid tumorigenesis was presented by L Gilmour (Ireland), who reported that ccn3 is downregulated in human chronic myeloid leukaemia (CML) cell lines and primary CML cells compared with normal bone marrow cells. In CML cells, ccn3 expression is regulated by the Bcr–Abl kinase. The reduced concentrations of CCN3 in patients with CML were in agreement with high expression of the Bcr–Abl kinase. Interestingly, patients undergoing remission showed recovery of normal CCN3 expression. Adding to the variety of proteins with which CCN3 combines, C Naus (Canada) presented results establishing that CCN3 physically interacts with the C-terminus of Connexin 43 (Cx43). This observation provides a possible explanation for the association between Cx43 expression and reduced tumorigenicity, because expression of full length CCN3 correlates with a growth suppressed phenotype in several cell systems including glioblastoma, choriocarcinoma, and Ewing sarcoma. In growth suppressed glioblastoma cells, Cx43 and CCN3 were shown to colocalise at the plasma membrane.

> ''P Koeffler presented results confirming that CCN proteins can indeed act either as oncogenes or tumour suppressors, depending upon the type of cancer considered''

R Lupu (USA) reported that expression of ccn1 in MCF7 breast cancer cells results in upregulation of the CCN1 receptor integrin αvβ3, which in turn promotes breast cancer cell proliferation, survival, and Taxol resistance. P Koeffler (USA) presented results confirming that CCN proteins can indeed act either as oncogenes or tumour suppressors, depending upon the type of cancer considered. For example, the expression of ccn1 in cancer cells of the breast, brain, and ovary promotes their growth in vitro and tumorigenicity in immunodeficient mice. In contrast, overexpression of ccn1 inhibits the growth of endometrial or lung cancer cells. Divergent proproliferative and antiproliferative effects of CCN1 were suggested to involve a balance between β catenin induced stimulation of p53 via c-myc and integrin linked kinase (ILK) inhibition of p53.

CONCLUSIONS AND PERSPECTIVES

Most of the data presented at this excellent meeting confirms the major involvement of CCN proteins in fundamental biological processes. As discussed by B Perbal (France), the variety of functions attributed to the CCN proteins makes them resemble an intriguing group of ''moonlighting proteins'', which play distinct roles in various biological contexts. Many complicated signalling networks governing cell fate and biology are interconnected via a relatively small number of regulatory proteins. A fascinating aspect of signalling regulation and cell communication centres around understanding how limited numbers of similar or identical regulators are assembled in different ways to provide combinatorial events that permit the control of cellular responses to complex environments. The

variety of biological functions assigned to the CCN proteins, together with their multiple sites of action, highlight them as potential key factors in the assembly of integrative circuitry needed for efficient cellular crosstalk and the adaptation of living organisms to their surrounding medium.

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ECHO

Study clarifies PCR results for viral CNS infections

Please visit the *Journal of Clinical Pathology* website [www.jclinpath.com] for a link to the full text of this article.

The diagnostic value of PCR assay for viral infections of the CNS will be improved now that a study has identified factors affecting test performance.

The positive predictive value was only 54% for infections judged clinically as likely CNS infections versus possible or unlikely infections when multiplex PCR results for various viral nucleic acids in patients' CSF were matched with illness episodes. Twenty seven CSF samples for possible or unlikely CNS infections were positive for Epstein Barr virus. Most samples from episodes most likely to be CNS infections were positive for herpes simplex virus (HSV) and enterovirus. The negative predictive value was 83%, meaning that negative results can be interpreted as discounting viral infection with only moderate confidence. CSF samples obtained between three and 14 days after onset of neurological symptoms had the best chance of detecting viruses, as did those in which white cell count was raised.

PCR tests were done on 787 frozen samples collected over four years in a teaching hospital. Comparing results with episodes of possible viral CNS infections was possible for 494 episodes in 483 (66%) patients with detailed clinical data; 15% of patients were aged <1 year and 26% up to 16 years. Each sample was tested for human herpes virus types 1–6, human polyoma virus JC, enteroviruses, and Epstein Barr virus.

PCR assays have established that several known viral infections can include involvement of the CNS, but many have not been validated for CNS infections, and doctors are often unsure what positive or negative findings mean for patient management.

▲ Davies NWS, *et al. Journal of Neurology, Neurosurgery, and Psychiatry* 2005;**76**:82–87.