

## COMMENTARY

# The CCN family: a new stimulus package

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### Abstract

The CCN family comprises cysteine-rich 61 (CYR61/CCN1), connective tissue growth factor (CTGF/CCN2), nephroblastoma overexpressed (NOV/CCN3), and Wnt-induced secreted proteins-1 (WISP-1/CCN4), -2 (WISP-2/CCN5) and -3 (WISP-3/CCN6). These proteins stimulate mitosis, adhesion, apoptosis, extracellular matrix production, growth arrest and migration of multiple cell types. Many of these activities probably occur through the ability of CCN proteins to bind and activate cell surface

integrins. Accumulating evidence supports a role for these factors in endocrine pathways and endocrine-related processes. To illustrate the broad role played by the CCN family in basic and clinical endocrinology, this article highlights the relationship between CCN proteins and hormone action, skeletal growth, placental angiogenesis, IGF-binding proteins and diabetes-induced fibrosis.

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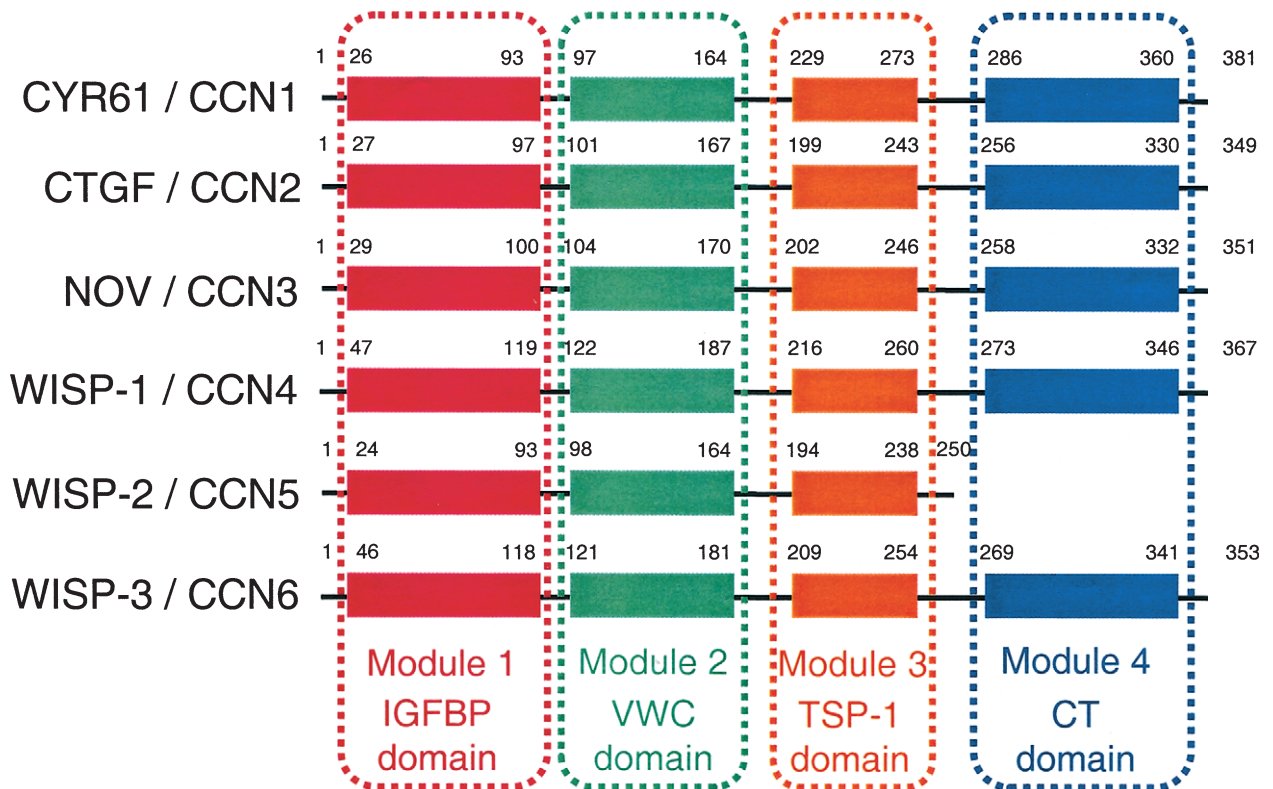
### Introduction

Over the last few years, articles on members of the new 'CCN' family – which includes cysteine-rich 61 (CYR61/CCN1), connective tissue growth factor (CTGF/CCN2), nephroblastoma overexpressed (NOV/CCN3), and Wnt-induced secreted proteins-1 (WISP-1/CCN4), -2 (WISP-2/CCN5) and -3 (WISP-3/CCN6) – have started to be published in this and other endocrinology journals. This trend reflects the finding that a variety of endocrine functions are impacted directly and indirectly by individual CCN proteins and that they probably are important modulators of hormonal pathways and hormonally regulated processes.

Originally discovered in the early to mid 1990s, members of the CCN family are 30–40 kDa proteins that are extremely cysteine-rich (10% by mass) (Brigstock 1999, Lau & Lam 1999, Perbal 2001). Collectively, these proteins stimulate mitosis, adhesion, apoptosis, extracellular matrix (ECM) production, growth arrest and migration, and regulate angiogenesis, tumor growth, placentation, implantation, embryogenesis and endochondral ossification. Target cells include fibroblasts, epithelial cells, endothelial cells, smooth muscle cells and neuronal cells (Moussad & Brigstock 2000). One of the most important

features of CCN proteins – yet one of the least understood – is that they are multi-modular mosaic proteins containing four conserved modules which are present in other unrelated extracellular proteins (Bork 1993, Brigstock 1999, Lau & Lam 1999, Perbal 2001). Module 1 is an insulin-like growth factor (IGF)-binding domain, module 2 is a von Willebrand type C domain, module 3 is a thrombospondin-1 domain, and module 4 is a C-terminal domain containing a putative cystine knot (Fig. 1). CCN proteins may exhibit biological activities that are consistent with the known properties of each constituent module, although data have started to emerge showing that certain modules can also act inter-dependently. The modular configuration may not only dictate direct actions of CCN proteins on target cells, but also their bioavailability, half-life, binding of other protein moieties, and regulation in time and space (Bork 1993, Brigstock 1999, Perbal 2001). Diversity in CCN functions may arise due to structural heterogeneity in the modular configuration. For example module 4 is absent in WISP-2/CCN5 (Pennica *et al.* 1998, Zhang *et al.* 1998) and modules 1–3 are absent in products of CTGF/CCN2 proteolysis (Brigstock *et al.* 1997, Ball *et al.* 1998).

The underlying mechanisms of action of CCN proteins have proven elusive, perhaps because the earlier (and



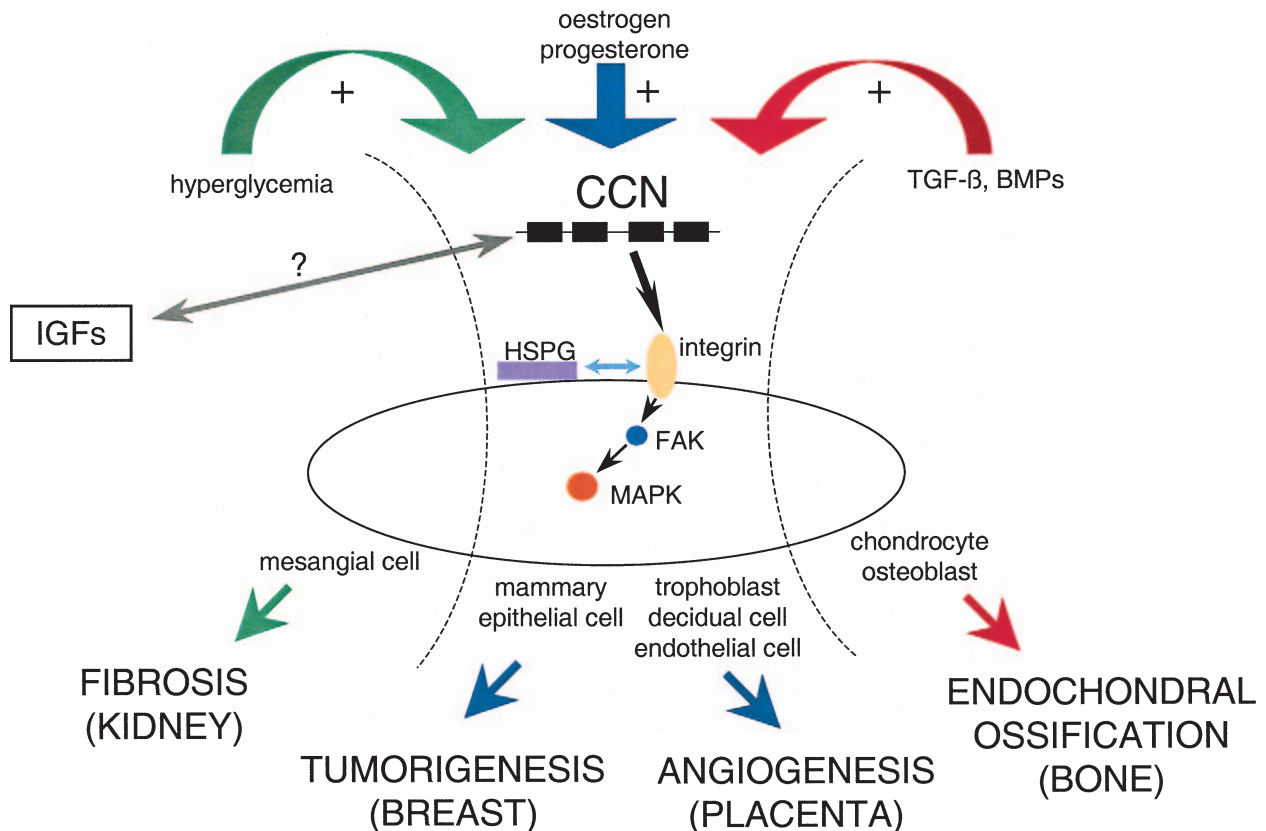
**Figure 1** Modular structure of the CCN protein family. Residues are numbered according to the human orthologs of each protein. Amino acid sequences within individual modules are conserved 38–98%. There is absolute conservation of 38 cysteine residues except in WISP-1/CCN4, which lacks four cysteines in module 2. Note the absence of module 4 in WISP-2/CCN5. Bioactive forms of CTGF/CCN2 lacking modules 1 and 2 or 1–3 and of NOV/CCN3 lacking module 1 have been reported. There are 25- to 30-residue signal peptide sequences preceding module 1 in each protein.

naive) view was that they acted in a similar fashion to classic growth factors. Attempts to identify unique specific high-affinity signal-transducing receptors have been fruitless. It is now felt that these molecules may act as matricellular proteins that bridge the functional and physical gap between ECM-associated proteins and cell surface molecules (Lau & Lam 1999). Recent data have shown that CYR61/CCN1, CTGF/CCN2 and NOV/CCN3 bind to cell surface integrins and thereby induce intracellular signaling events that include kinase activation and gene transcription (Lau & Lam 1999, C C Chen *et al.* 2000, 2001, N Chen *et al.* 2000, Grzeszkiewicz *et al.* 2001, 2002, Leu *et al.* 2002, Schober *et al.* 2002). Additionally, CCN proteins exhibit strong affinity for heparin and are localized on cell surfaces or in ECM in association with heparan sulfate proteoglycans (HSPGs). The diverse biological functions of CCN proteins may reside in the unique interactions of individual CCN proteins with specific integrin subtypes expressed by a given target cell, the utilization of cell surface HSPGs as co-receptors, and the cross talk of these binding moieties with other signaling molecules such as growth factor receptors.

Below, I briefly summarize five aspects of CCN biology that are of interest to endocrinologists. While this list is not exhaustive, it serves to highlight the broad and versatile endocrinological aspects of this new gene family. A conceptual framework is presented in Fig. 2.

### Hormone action

CYR61/CCN1 and CTGF/CCN2 are both estrogen-inducible and overexpressed in steroid-dependent breast or uterine tumors (Tsai *et al.* 2000, 2002b, Sampath *et al.* 2001a,b, 2002, Xie *et al.* 2001a,b). CYR61/CCN1 promotes breast cancer progression and is associated with more advanced disease (Xie *et al.* 2001a, Tsai *et al.* 2002a). Proliferation of breast cancer cell lines *in vitro* is antagonized by anti-CYR61/CCN1 antibodies and the production of CYR61/CCN1 by these cells is antagonized by anti-estrogens (Sampath *et al.* 2001a, Tsai *et al.* 2002b). WISP-2/CCN5 is also induced by estrogen in breast cancer cells (Inadera *et al.* 2000, 2002). Anti-progestins are effective in antagonizing CYR61/CCN1



**Figure 2** Key pathways of CCN protein regulation and function. Some of the major pathways of CCN action described in the text are shown. Note that evidence for CCN protein interactions on cell surfaces has not been verified for all cell types depicted nor for all CCN proteins. To date, activation of focal adhesion kinase (FAK) and mitogen-activated kinase (MAPK) have been demonstrated principally in fibroblasts or endothelial cells treated with CYR61/CCN1, CTGF/CCN2 or NOV/CCN3. TGF- $\beta$ , transforming growth factor-beta; BMPs, bone morphogenetic proteins.

production by breast cancer cells (Sampath *et al.* 2002). Collectively, these data point to the CCN family members as important downstream mediators of estrogen- and progesterone-regulated cell growth. However, CCN proteins may also impact other growth regulatory pathways in breast cancer cells. WISP-3/CCN6, for example, is a tumor suppressor that exhibits reduced expression in inflammatory breast cancer (Kleer *et al.* 2002).

In addition to their roles in steroid-dependent tumor growth, CCN proteins are involved as downstream mediators of normal physiological hormone action in organs such as the uterus or ovary. Uterine CTGF/CCN2 is regulated by both estrogen and progesterone and appears to be important for maintenance or remodeling of stromal ECM (Rageh *et al.* 2001, Cheon *et al.* 2002). In the ovary, CTGF/CCN2 production is regulated by gonadotropins or transforming growth factor-beta (TGF- $\beta$ ) and is associated with thecal cell recruitment and mitosis, and maintenance of the corpus luteum (Wandji *et al.* 2000, Slee *et al.* 2001, Harlow & Hillier 2002, Harlow *et al.* 2002, Liu *et al.* 2002).

### Skeletal growth

CTGF/CCN2 has emerged as an important player in chondrogenesis and endochondral ossification as shown by its ability to promote proliferation, maturation and hypertrophy of cultured growth or articular cartilage cells (Takigawa *et al.* 2003). During embryogenesis, CTGF/CCN2 is expressed in zones containing hypertrophic chondrocytes or calcifying cartilage of long bones, ribs, vertebral column and phalanges. It is also produced by osteoblasts in primary spongiosa and ameloblasts or odontoblasts in incisor teeth (Takigawa *et al.* 2003). CTGF/CCN2 is expressed in hypertrophic chondrocytes, proliferating chondrocytes and proliferating periosteal cells during healing of fractured ribs (Nakata *et al.* 2002). During limb regeneration in newts, the expression of CTGF/CCN2 is consistent with a role in osteoclast production and endochondral ossification of the hypertrophied cartilaginous limb skeleton (Cash *et al.* 1998).

*In vitro*, CTGF/CCN2 promotes chondrocyte cell growth and hypertrophy, as well as increased expression of

proteoglycans, aggrecan and collagens (Nakanishi *et al.* 2000, Nishida *et al.* 2000, 2002). CTGF/CCN2 levels in skeletal cells are stimulated by TGF- $\beta$  and bone morphogenic protein-2, consistent with the known role of these proteins in skeletal growth as well as the observation that CTGF/CCN2 is an immediate early gene for TGF- $\beta$  (Nakanishi *et al.* 1997). Finally, CTGF/CCN2-null mice demonstrate multiple skeletal defects including expanded hypertrophic zones of long bones, possibly due to decreases in growth plate angiogenesis, chondrocyte proliferation and matrix-degrading enzymes. (Ivkovic *et al.* 2003).

CYR61/CCN1 promotes collagen and cartilage production in limb bud micromass cultures and stimulates chondrogenesis, mitogenesis and adhesion in limb mesenchymal cells (O'Brien & Lau 1992, Wong *et al.* 1997). CYR61/CCN1 production in fetal osteoblasts is regulated by vitamin D3 (Schutze *et al.* 1998) while NOV/CCN3 is involved in calcium signaling (Li *et al.* 2002), which may pertain to a role in bone metabolism. Mutations in the WISP-3/CCN6 gene are associated with progressive pseudorheumatoid dysplasia, an autosomal recessive skeletal disorder characterized by continued cartilage loss and bone destruction (Hurvitz *et al.* 1999). WISP-3/CCN6-null mice have more mature vertebral endplates, a phenomenon that may be due to accelerated endochondral ossification (see Perbal *et al.* 2003).

### Placental angiogenesis

Production of CTGF/CCN2 by mouse uterine stromal cells increases dramatically as the cells differentiate into decidual cells following implantation (Surveyor *et al.* 1998). At later stages, CYR61/CCN1 and CTGF/CCN2 are present in trophoblast giant cells (Kireeva *et al.* 1997). In the non-invasive placenta of the pig, CTGF/CCN2 production by uterine epithelium is markedly reduced during the attachment phase and may facilitate sub-epithelial matrix reorganization and angiogenesis (Moussad *et al.* 2002). Thereafter many cells in the utero-placental unit produce high levels of CTGF/CCN2, which may promote matrix stabilization and angiogenesis within it. An essential role for CYR61/CCN1 during embryogenesis has recently been shown in knockout mice, which failed to develop *in utero* due to vascular defects in the placenta (Mo *et al.* 2002). The defect appeared to be related to the inability of new capillaries to bifurcate, suggesting that CYR61/CCN1 facilitates the development of a branching capillary network. Indeed, the ability of CYR61/CCN1 and CTGF/CCN2 to regulate endothelial cell proliferation and angiogenesis is well established (Brigstock 2002). Recently, NOV/CCN3 was shown to be angiogenic (Lin *et al.* 2003), although WISP-3/CCN6 appears to be anti-angiogenic (Perbal *et al.* 2003).

### IGF binding

Module 1 exhibits similarity to IGF-binding proteins (IGFBPs) and speculation has arisen regarding a role for CCN proteins in IGF transport and delivery (Bork 1993, Kim *et al.* 1997, Burren *et al.* 1999). However, rather than exhibiting the high-affinity IGF binding ( $K_d=10^{-10}$ – $10^{-11}$  M) that is characteristic of IGFBP-1 to -6, the binding affinity of CCN proteins for IGFs is 2 or 3 orders of magnitude less. While IGF binding *in vitro* has been reported for CTGF/CCN2, NOV/CCN3 and WISP-3/CCN6 (Kim *et al.* 1997, Burren *et al.* 1999, Kleer *et al.* 2003), this interaction has not been definitively attributed to sequences in module 1 and classic IGF ligand blots with NOV/CCN3 proved unsuccessful (Chevalier *et al.* 1998). Although investigators have proposed that the CCN family is part of an IGFBP superfamily (Kim *et al.* 1997, Baxter *et al.* 1998, Rosenfeld 2001), the physiological importance of CCN-IGF interactions will remain controversial until the appropriate experiments are performed (Collet & Candy 1998, Grotendorst *et al.* 2000). It is noteworthy that some CTGF/CCN2 isoforms are active in the complete absence of module 1 (Brigstock *et al.* 1997, Ball *et al.* 1998), although a regulatory role of module 1 on these actions cannot be formally excluded. Interestingly, the IGF-binding property of WISP-3/CCN6 has been linked to IGF signaling in inflammatory breast cancer (Kleer *et al.* 2003).

### Diabetes-induced fibrosis

Diabetes mellitus type 1 leads to fibrotic pathology in multiple organs including the kidney, arteries and heart. In early stages of diabetic nephropathy, high glucose levels are associated with mesangial cell hypertrophy and matrix production and this effect is mimicked by exogenous stimulation with CTGF/CCN2 (Riser *et al.* 2000, Wahab *et al.* 2002). In addition, high glucose conditions, TGF- $\beta$ , mechanical strain or CTGF/CCN2 itself promote CTGF/CCN2 expression by mesangial cells (Wahab *et al.* 1996, Murphy *et al.* 1999, Riser *et al.* 2000). As in other fibrotic diseases, CTGF/CCN2 acts downstream of TGF- $\beta$  and has been linked to chronic tubulointerstitial fibrosis (Wang *et al.* 2001, Yokoi *et al.* 2001, 2002) suggesting that CTGF/CCN2 may be a novel therapeutic target in renal fibrosis (Crean *et al.* 2001). CTGF/CCN2 expression is inhibited in the diabetic kidney by aminoguanidine, an inhibitor of advanced glycosylation end products (AGEs), which are pro-fibrotic and cause induction of CTGF/CCN2 and fibronectin (Twigg *et al.* 2001, 2002a,b). CTGF/CCN2 levels are also elevated in diabetes-induced cardiac fibrosis and atherosclerosis and these can be inhibited by, respectively, inhibitors of either AGE or angiotensin-converting enzyme (Candido *et al.* 2002, 2003, Way *et al.* 2002).



## Concluding comments

Although this article has focused on endocrinological aspects, research into all areas of the CCN family continues to gain pace. In the next few years, we can expect to learn much more about aspects of CCN structure–function as well as more detailed studies of the production, regulation and action of CCN molecules in normal and diseased tissues. There is guarded optimism that at least some of these factors will have useful prognostic or diagnostic applications and that some may even be viable therapeutic targets. In the current era of gene–array technology, we can anticipate that CCN proteins will be implicated in additional endocrine–related processes and that new functions for CCN proteins will be unraveled.

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