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EDITORIAL

The CCN family of genes: a perspective on CCN biology and therapeutic potential

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Abstract The CCN family of genes currently comprises six secreted proteins (designated CCN1–6 after Cyr61/CCN1; ctgf/CCN2; Nov/CCN3; WISP1/CCN4; WISP2/CCN5, WISP3/CCN6) with a similar mosaic primary structure. It is now well accepted that CCN proteins are not growth factors but matricellular proteins that modify signaling of other molecules, in particular those associated with the extracellular matrix. CCN proteins are involved in mitosis, adhesion, apoptosis, extracellular matrix production, growth arrest and migration of multiple cell types. Since their first identification as matricellular factors, the CCN proteins now figure prominently in a variety of major diseases and are now considered valid candidates for therapeutic targeting. Dissection of the molecular mechanisms governing the biological properties of these proteins is being actively pursued by an expanding network of scientists around the globe who will meet this year at the 5th International Workshop on the CCN family of Genes, organized by the International CCN Society (http://ccnsociety.com), home for an international cadre of collaborators working in the CCN field.

Keywords CCN family of genes · Biology · Therapeutic potential · CCN Society · CCN Workshop · CCN1 · CCN2 · CCN3 · CCN4 · CCN5 · CCN6

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Introduction

There is a growing recognition of the importance of cell–matrix interactions in normal biological processes and in the pathogenesis of disease. The CCN family and their cognate proteins are gaining increasing attention for a wide spectrum of biological events that underlie development and physiological functions of all organs in the body. Strikingly, differential and inter-connected expression of the CCN family members may determine the ultimate behavior of normal, diseased and cancerous cell types (Table 1 from Kubota and Takigawa 2007).

The International CCN Society (ICCNS) was founded in France to stimulate collaborations and provide a home for the international cadre of researchers working in the CCN field. A biennial International Workshop on the CCN family of Genes has been organized since 2000 and its success has sparked greater interest and recruitment of new investigators into the field.

On the occasion of the 5th International Workshop that will be held in Toronto this year (see http://ccnsociety.com), we present in this short review an updated Perspective on CCN Biology and Therapeutic Potential.

CCN family of genes and proteins

Nomenclature of the CCN family of genes is based on cysteine-rich protein 61 (Cyr61; CCN1), connective tissue growth factor (CTGF;CCN2) and nephroblastoma overexpressed protein (Nov; CCN3). The CCN family currently comprises six secreted proteins with a similar modular secondary structure (Fig. 1 from Perbal and Perbal 2007; Fig. 2 and 3 from Leask and Abraham 2006; Fig. 4 from Kubota and Takigawa 2007). CCN proteins comprise four

Table 1 Angiogenic properties of the CCN family members and their molecular counterparts

Member	Effects on endothelial cells and smooth muscle cells					Interacting molecules	Relevance to malignancies
	In vitro				In Vivo		
	Migration	Proliferation	Adhesion	Survival			
CCN1	+(E[a])(SM[b])	(E)(SM)	(E)(SM)	(E)(SM)	+	$\alpha_v\beta_3$, $\alpha_6\beta_1$ $\alpha_{IIb}\beta_3$, $\alpha_v\beta_5$, $\alpha_M\beta_2$	+&−
CCN2	+(E)	+(E)	+(E)	+(E)	+	$\alpha_v\beta_3$, $\alpha_6\beta_1$ $\alpha_{IIb}\beta_3$, $\alpha_v\beta_5$, $\alpha_M\beta_2$, $\alpha_5\beta_1$, LRP1, fibronectin, TrK A, perlecan, BMP-4, TGF-β< VEGF	+&−
CCN3	+(E)	N.D.	+(E)	+(E)	+	$\alpha_v\beta_3$, $\alpha_6\beta_1$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, Cx43, Notch	+&−
CCN4	N.D.[c]	N.D.	N.D.	N.D.	N.D.	biglycan, decorin	+&−
CCN5	N.D.	−(SM)	N.D.	N.D.	N.D.	(unknown)	+&−
CCN6	N.D.	N.D.	N.D.	N.D.	N.D.	(unknown)	+&−

[a] Vascular endothelial cells
[b] Vascular smooth muscle cells
[c] Not determined

domains: an insulin-like growth factor binding protein (IGFBP) domain (domain I), a Von Willebrand factor domain (domain II), a thrombospondin-homology domain (domain III), and a cysteine knot, heparin-binding domain (domain IV). An N-terminal located signal sequence and a hinge region between domains II and III govern secretion and susceptibility to proteinase cleavage, respectively. Cleaved fragments may possess unique biological functions, yet to be determined.

Since their first discovery 15 years ago much has been learned about the biochemistry of CCN proteins and their expression during development, in normal adult tissues and in disease. In vitro studies and more recently CCN transgenic and knockout mice have yielded further insights and a better understanding of the diversity and complexity of CCN activities. It is now well accepted that CCN proteins are not growth factors but matricellular proteins that modify signaling of other molecules, in particular those associated with the extracellular matrix. CCN proteins are involved in mitosis, adhesion, apoptosis, extracellular matrix production, growth arrest and migration of multiple cell types. In fact they are expressed early in development and then are differentially recruited by cells to facilitate multiple tissue/organ functions, and critically during wound healing and disease. The field is comprehensively covered in a recent monograph by Perbal and Takigawa (2005).

The current state of knowledge on the CCN proteins and interacting protein partners suggest that the CCN proteins integrate communication between the extracellular matrix and the cell surface (Fig 1; Perbal and Perbal 2007). As depicted in Fig. 2 from Leask & Abrahams review (2006)

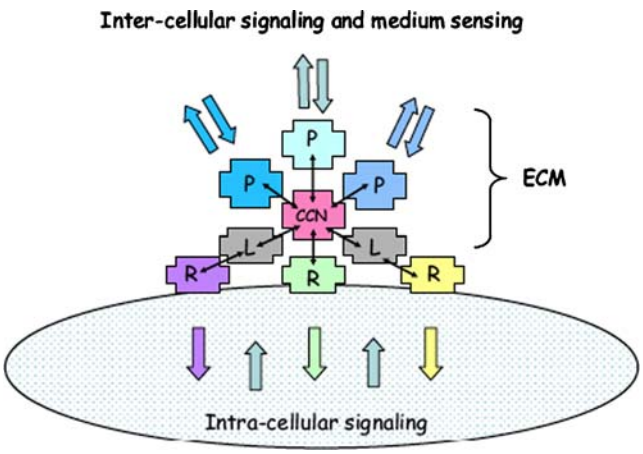

Fig. 1 Schematic representation of the multiple types of interactions in which CCN proteins may be involved for signaling cell functions. *CCN* CCN proteins, *L* ligands of receptors, *R* receptor. *ECM* extracellular matrix, *P* potential partners in the extracellular matrix. From Perbal and Perbal, JCCS 1:1, 2007 (reproduced with permission)

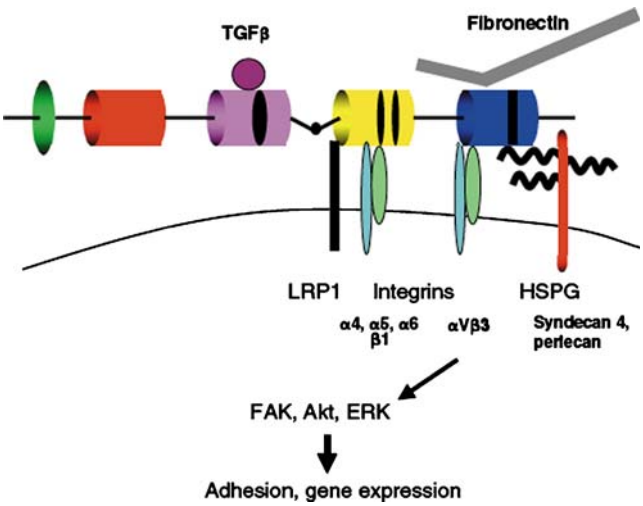

Fig. 2 Signaling by CCN family members, CCN1, CCN2 and CCN3 bind TGFβ, fibronectin, integrins, LRP1 and HSPGs as indicated. CCN proteins appear to signal principally through the C-terminal quarter (domain IV) to activate adhesive signaling pathways and hence amplify responses to TGFβ or fibronectin. From Leask and Abraham, J Cell Science 109: 4803–10, 2006 (reproduced with permission)

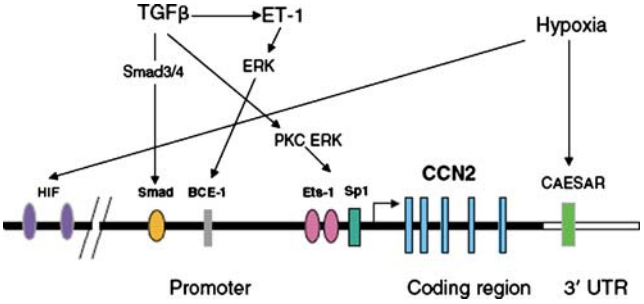

Fig. 3 Regulation of the *CCN2* promoter and 3′ untranslated region (3′ UTR). The *CCN2* promoter contains recognition sequences for HIF, Smad, BCE-1, Ets-1, Sp1, as indicated. The 3′ UTR of the gene (white rectangle) contains a cis-acting element of structure-anchored repression (CAESAR). Hyoxia, TGFβ and endothelin-1 induce *CCN2* as indicated. From Leask and Abraham, J Cell Science 109: 4803–10, 2006 (reproduced with permission)

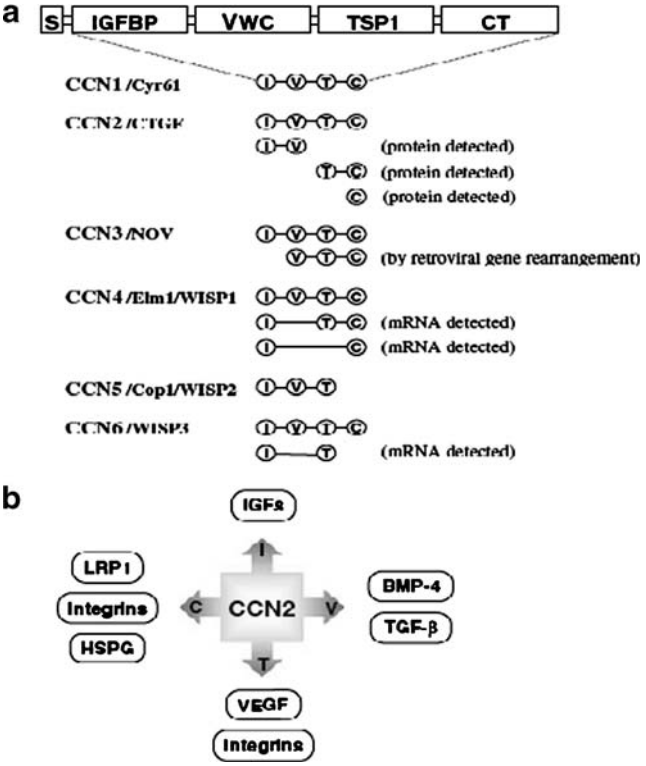

Fig. 4 **A** The primary structure of the CCN family proteins and their reported variants. The general structure is composed of four conserved modules is illustrated as a nascent translation product with a signal peptide for secretion (S) at the top. The module names abbreviated here are fully describe in the text. The modules are then further abbreviated into a single letter when describing the structure of each member or its variant. Namely, "I", "V", "T" and "C" represent IGFBP, VWC, TSP1 and CT modules, respectively. In addition to the names under the unified nomenclature, a few classical and well-known names are also presented. As briefly noted in the figure, CCN2 variant proteins were reported by Brigstock et al. [52] Kubota et al. [53], Hinton et al. [54] and Boes et al. [55]. The N-terminal truncated form of CCN3 was identified in nephroblastomas by Joliot et al. [11]. In the case of CCN4 and CCN6, variants were confirmed at mRNA level, which were generated through the alternative mRNA splicing [40–42]. **B** Multiple molecular interactions by the CCN proteins as exemplified by CCN2. In this panel also, each module is indicated by a single letter abbreviation, as explained above. The abbreviation HSPG stands for heparan sulfate proteoglycans. All of the other abbreviations are explained in the text. From Kubota and Takigawa, Angiogenesis 10:1–11, 2007 (produced with permission)

domains III and IV bind integrins, LRP1 receptor and HSPG to effect intracellular signaling of key pathways. CCN proteins mitigate activities of the ECM and associated growth factors like TGFβ, BMP4, IGFs, and VEGF. This positions the CCN proteins in the matricellular sphere and as controllers of cell–matrix communications.

Interestingly, truncated forms of the CCN proteins appear to translocate to the nucleus where they are postulated to be involved in transcriptional regulation. Theoretically, this may serve as the means to transmit environmental information to central control. In support of this notion that CCN genes are sensitive to environmental conditions, Fig. 3 from Leask and Abrahams review (2006) shows how CCN2 can be regulated by hypoxia and injury/inflammatory mediators.

Figures 5 and 6 from Kubota and Takigawa review (2007) depicts how CCN proteins figure prominently in angiogenesis during normal development of tissues and organs and during tumor angiogenesis. CCN2 is a well established promoter and inducer of the chondrogenic and osteogenic lineages placing it in the centre of skeletal formation and deformation.

Looking at the structural similarities amongst the CCN proteins it was at first not easily understandable what functional differences could exist. It now turns out that the CCN proteins likely comprise a homeostatic regulatory system where one member drives a process while a closely related member can inhibit the same process. Although the molecular details of this regulation are not yet well understood the concept has stirred great interest given that it may open up new means for therapeutic manipulation of disease processes. Thus in this battle between the CCN family members the relative expression levels of individual CCN proteins, full length and shorter versions, (often multiple in one site) may ultimately decide the character of physiological and pathological processes. Figure 7 from Chaqour and Goppelt-Struebe review (2006) illustrates another key component in the biology of CCN proteins, that is, modulation by dynamic forces that act upon cells. In pathological conditions like hypertension, obstruction and hemodynamic overload, altered signaling induced by stretching and compression forces on cells modulates CCN expression and can lead to either positive compensatory responses or to aberrant outcomes such as fibrosis. Thus CCN2 has attracted considerable attention as a fibrosis inducing CCN protein when overexpressed or when prolonged in its activity. Recent studies have demonstrated how downregulating CCN2 expression and function ameliorates the pathological process. Importantly,

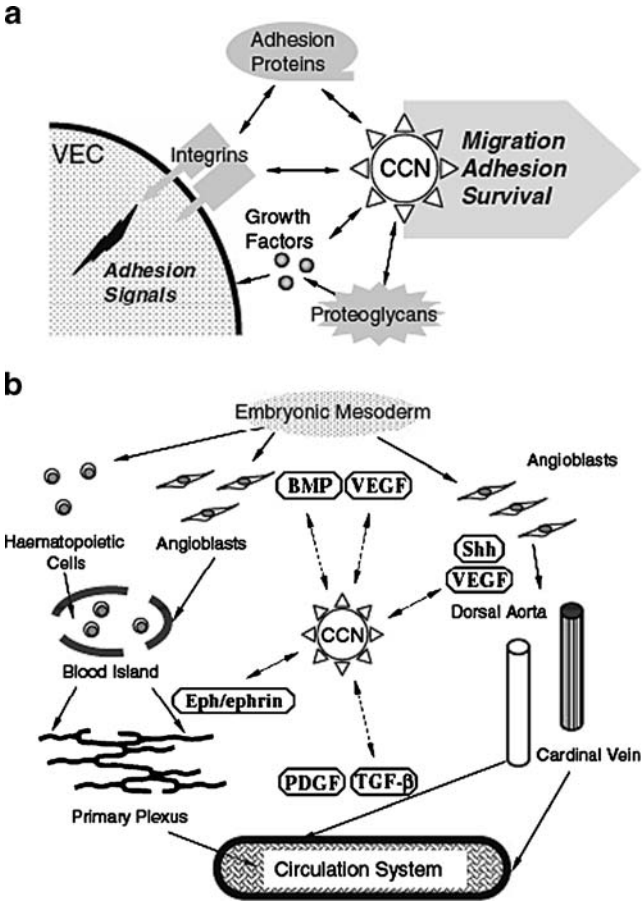

Fig. 5 **A** The general mechanism of angiogenic action of the CCN proteins. Through the interaction with integrins, angiogenic CCN proteins, such as CCN1, CCN2 and CCN3, promote the migration, adhesion and survival of vascular endothelial cells. CCN proteins are also known to bind to several ECM components including proteoglycans and adhesion proteins which furnishes microenvironmental basement for the neovas-cularization by modulating the ECM architecture. The function of other growth factors is occasionally modulated by the direct interaction with these CCN proteins. **B** The hypothetical roles of CCN proteins in the regulation of the development of embryonic cardiovascular systems at early stages. Based on the findings obtained in vitro, the possible interactions of CCN proteins with the signaling molecules involved are illustrated. All of the full designations of the abbreviated names can be found in the text. Eph is the receptor of ephrin. From Kubota and Takigawa, Angiogenesis 10:1–11, 2007 (produced with permission)

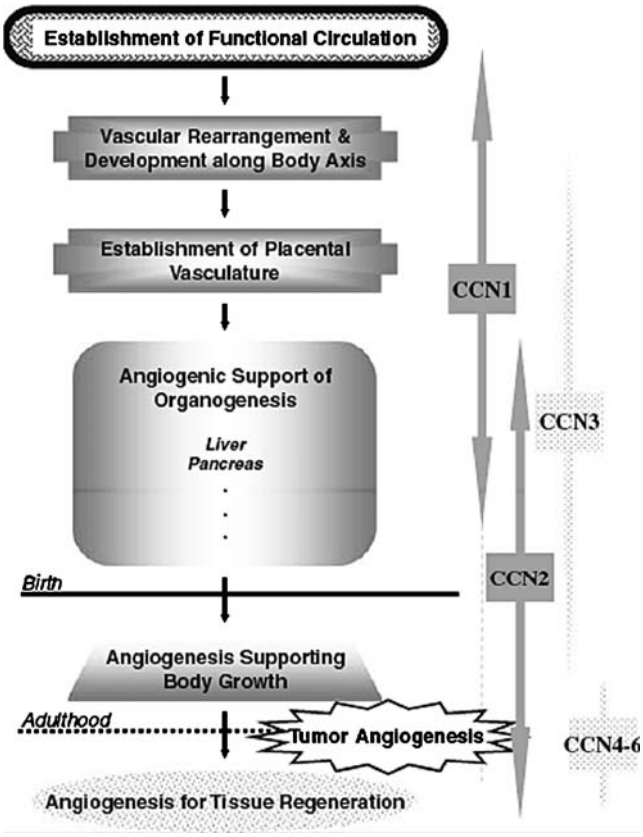

Fig. 6 Assignment of the functioning stages for angiogenic CCN proteins in the angiogenic events throughout the life. According to the accumulating findings, CCN1 appears to play a critical role in the earlier stages of embryonic vascular development, while its function at later stages, such as tissue regeneration, may not be ruled out. In contrast, CCN2 is required for the angiogenic events at later stages of development and it is obviously involved in the regeneration of various tissues. Although no strict requirement of CCN3 in particular angiogenic events has yet been specified, it is plausible that CCN3 supports the CCN1 and CCN2 function in a certain aspects of angiogenic events during the development and maintenance of the life. The angiogenic roles of CCN4, 5 and 6 still remain to be investigated further, since an association of these proteins with malignancies has bee suggested. From Kubota and Takigawa, Angiogenesis 10:1–11, 2007 (produced with permission)

due to their secretory status, CCN proteins are also biomarkers of the pathological process.

Experimental resources in the CCN field

In order to dissect and delineate the biological functions of CCN proteins both in health and disease it is crucial that there are appropriate and informative experimental models and reagents. Similar to other fields, the CCN field has started to more effectively develop its experimental tools. Transgenic mouse models, in vitro cell line models, and generation of domain and isoform specific antibodies constitute a rich source of tools for the studies. Knockout mouse models of CCN have uncovered a broader role for CCN proteins in normal and pathological processes [Kawaki et al. 2008; Heath et al. 2008; Canalis 2007; Kuiper et al. 2007; Kutz et al. 2005; Ivkovic et al. 2003; Brigstock 2002]. Cell line models, especially those capable of transitioning developmental states, like stem cells, are proving effective in exploring phenotype/function relationships [Katsuki et al. 2008; Schutze et al. 2007; Djoua et al. 2007; Si et al. 2006]. More recently, domain specific antibodies for CCN3 have revealed some interesting and somewhat unexpected aspects of CCN variant localization and potential functions [Lazar et al. 2007, Subramaniam et al. 2008]. The recognized CCN protein fragments generated by still uncharacterized pro-

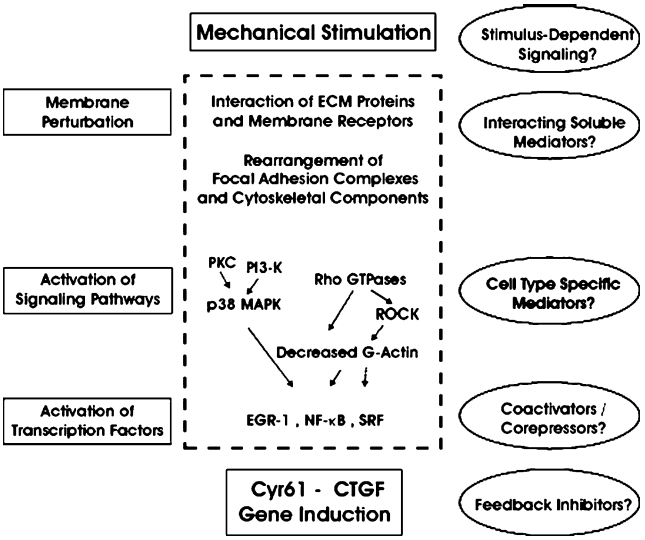

Fig. 7 Schematic model of the mechanical regulation of Cyr61 and CTGF indicating different regulatory levels and open questions (see Conclusion). Mediators, which have been related to mechanical stimulation of *Cr61* or *CTGF* gene induction, are shown in the middle panel; details are outlined in the text. From Chaqour and Goppelt-Struebe FEBS J 273:3639, 2006 (reproduced with permission)

teases add further complexity, as does the revelation of a growing number of different interacting protein partners and modifiers [see reviews]. There is always the risk that it may become more difficult to discriminate the trees from the forest. Nevertheless, having the means to accurately mark the 'trees' will hopefully mitigate against this problem. The ICCNS workshops in concert with JCCS will thus provide the means and opportunities to address all such issues and explore the depth of the CCN field within a highly interactive and open forum.

CCN family as potential therapeutic targets

Given the putative roles that CCN proteins play in multiple biological processes it is not surprising that aberrant expression could have pathogenic consequences. In this context the potential clinical applications for CCN are now surfacing. A discussion on the applications for CCN3 has been reported (Perbal 2003) and those for CCN2 have stemmed from its involvement in a number of vital functions. Angiogenesis has become a key target for chemotherapeutic intervention in the process of cancer. Evidence suggests that this VEGF driven mechanism focuses on deregulated CCN2.

Overexpression of CCN proteins in adult tissues is a pathological event so targeting CCN proteins and normalizing levels would be beneficial and introduce few adverse effects. Although most of the attention in the field has concentrated on CCN2, being prominent in angiogenesis, atherosclerosis, cardiac fibrosis, diabetic nephropathy, systemic sclerosis, and potentially asthma (the major disease processes in the western hemisphere and now a growing concern in the eastern hemisphere) other CCN proteins are now starting to gain attention as their roles become defined and, as indicated above, their relationship as a family of homeostasis modulators. Thus for millions of patients, CCN research may one day reap new therapies for these global burdens.

Current clinical trial perspectives in the CCN field

The major focus of the biopharmaceutical industry has been thus far on CCN2 because this CCN protein has been studied in greatest detail and due to its strong clinical association with fibrosis. A small number of clinical trials have been conducted using monoclonal antibodies to CCN2, eg, FibroGen's FG-3019 in idiopathic pulmonary fibrosis, incipient diabetic nephropathy and other syndromes; for example, CCN2 levels can be measured in urine after treatment of hypertensive type 1diabetic nephropathy patients with Losartan. Other diseases seen as targets for CCN2 are degenerative disc and cancers such as pancreatic (positively correlated) and lung cancer where CCN2 may actually interfere with metastasis. Interim reports on these efforts suggest good progress. Much more has to be learned about other CCN proteins from all disease perspectives before therapeutic targeting is considered.

The strong commitment of several private companies to development of effective molecular therapeutics for cancer, inflammation, metabolic and neurological problems, also falls within the scope of the CCN field where CCN proteins have been identified as key players in similar processes. In one example, CCN 1 and CCN3 are involved in growth promotion or inhibition of a variety of cancers (including solid and hematopoietic related tumors) and can serve as prognostic indicators in some cancers. Evidence suggests that CCN proteins function through cell membrane receptors, partially identified, including integrins and can also modulate activities of the IGF family of growth factors. Thus targeting of CCN proteins with antibodies or peptides are viable possibilities. In a second example, CCN2 figures prominently in chondrogenesis and osteogenesis, relating to musculoskeletal issues, and importantly in fibrosis, the consequence of tissue injury and inflammation. As indicated, CCN2 is already a therapeutic target under active investigation. Since much more has yet to be explored on how CCN proteins modulate homeostatic functions there are multiple CCN avenues to be opened that will yield new mechanistic insights and thereby possibilities for development of novel therapeutics.

Conclusion

The emerging CCN field has now uncovered a unique biological niche in the way cells interact with each other, with the external matrix and the factors that modulate critical biological processes of organ development, functional homeostasis, and disease. There is no longer doubt that the CCN proteins participate fully in governing skeletal development, the vascular supply, and the architecture of organs. Manipulating CCN expression changes how biological systems operate. Abnormal or deregulated expression of CCN proteins contributes significantly to the biology of cancer, fibrosis, and inflammatory sequelae. CCN proteins are therefore seen as obvious targets for therapeutic intervention. The 5th International Workshop on the CCN family of Genes will serve again as the main venue for bringing together the investigators and facilitators for future development of clinically effective therapeutics based on the essential biological properties of the CCN proteins.

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