Emerging role of CCN family proteins in tumorigenesis and cancer metastasis (Review)

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Abstract. The CCN family of proteins comprises the members CCN1, CCN2, CCN3, CCN4, CCN5 and CCN6. They share four evolutionarily conserved functional domains, and usually interact with various cytokines to elicit different biological functions including cell proliferation, adhesion, invasion, migration, embryonic development, angiogenesis, wound healing, fibrosis and inflammation through a variety of signalling pathways. In the past two decades, emerging functions for the CCN proteins (CCNs) have been identified in various types of cancer. Perturbed expression of CCNs has been observed in a variety of malignancies. The aberrant expression of certain CCNs is associated with disease progression and poor prognosis. Insight into the detailed mechanisms involved in CCN-mediated regulation may be useful in understanding their roles and functions in tumorigenesis and cancer metastasis. In this review, we briefly introduced the functions of CCNs, especially in cancer.

Contents

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Abbreviations: ADAM, a disintegrin and metalloprotease; BSP, bone sialic protein; COMP, cartilage oligomeric matrix protein; L1, CD171; LAP-TGF-β, TGF-β latency-associated peptide; iC3b, inactivated complement component 3; PECAM-1, platelet and endothelial cell adhesion molecule 1; uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor; VEGF, vascular endothelial growth factor

Key words: CCN family proteins, receptors, signalling pathways, cell functions, cancers

1. Introduction

The CCN family of proteins is an acronym for cysteine-rich protein 61 (CYR61), connective tissue growth factor (CTGF) and nephroblastoma overexpressed (NOV), which were first identified in mouse, human and chicken in the early 1990s (1-3). Another three family members exhibiting the same basic structure domains of the first three CCN members have since been identified. The latter three members are involved in the Wnt-1 inducible signalling pathway and consist of Wnt-1-induced secreted protein-1 (WISP-1), WISP-2, and WISP-3 (4). As each CCN family member has several names associated with its structures or functions, the official nomenclature has been recommended (Table I).

CCNs are present in vertebrates, including zebrafish, poultry such as chickens, rodents including mice and rats, as well as humans and have been conserved during evolution. CCNs, with the exception of CCN5, which lacks a cysteine knot domain (CT) module, comprise an N-terminal secretory signal peptide and four functional domains: an insulin-like growth factor-binding protein domain (IGFBP), a Von Willebrand factor domain (VWC), a thrombospondin type-1 repeat module (TSP-1), and a CT (Fig. 1A). The two N-terminal domains are separated from the two C-domains by a variable linking sequence of amino acids (5). According to the domains CCNs, except CCN5, share five common exons, the first of which codes the signal sequence, while the other CCNs sequentially code the four functional domains with corresponding numbers of amino acids ranging from 349 to 381 (6).

The four discrete functional domains have different molecular structures that determine the types of binding partners and ligands with which they interact, resulting in a variety of biological functions. The known binding partners of each domain are different: insulin-like growth

factors (IGFs) bind with IGFBP; transforming growth factor β (TGF-β), bone morphogenic proteins (BMPs) and integrins bind with VWC; vascular endothelial growth factor (VEGF), LDL receptor proteins (LRPs), heparan sulphate proteoglycans (HSPGs) and integrins bind with TSP-1; and VEGF, LRPs, integrins, neurogenic locus notch homolog protein 1 (Notch1), fibulin C1, HSPGs and integrins bind with CT (7,8) (Fig. 1B).

2. Expression profiles and subcellular localization

CCNs exhibit different expression profiles and transcript levels in different tissues, organs and tumors (Tables II and III). The different expression levels of CCNs observed in embryonic tissues compared with that of adult organs indicates a potential role in development (Table IV). The changing transcript levels in tumors mean that CCNs may also be important during tumorigenesis.

The subcellular localization of each of the CCNs is also different. Immunohistochemical localization of CCN1 protein has indicated that invasive carcinoma cells show significant cytoplasmic and perinuclear protein overexpression compared to non-neoplastic ductal epithelium in invasive ductal carcinoma, whereas in ductal carcinoma *in situ* and lobular carcinoma *in situ*, CCN1 expression was weaker and heterogeneous (9). Previous findings have shown that CCN1 was detected, albeit not abundantly, in culture medium (10). CCN2 protein was detected in the nuclei of B16 (F10) cells and at the cell membrane, but was rarely detectable in the cytoplasm and the cell culture medium (10,11). CCN3 was detected in the medium, extracellular matrix (ECM) and at the cell membrane (12-14). A previous study revealed strong immunohistochemical staining of CCN4, CCN5 and CCN6 in normal colorectal epithelial cells, which was confined primarily to the cell membrane with slight staining of stromal tissue. In colorectal cancer (CRC) tissues, cell membrane and cytoplasmic staining were assessed. Membrane staining showed a reduction in CCN4, CCN5 and CCN6, whereas cytoplasmic staining showed a reduction in CCN5 but an increase in CCN4 and CCN6 (15). Furthermore, CCN5 is mainly localized to the nucleus in rat and human tissues (16).

3. CCN receptors

Similar to some ECM proteins, CCNs mediate cell functions, embryonic development, angiogenesis, wound healing, fibrosis, inflammation, tumorigenesis and development primarily through binding and interacting with well-known receptors, including integrins, HSPGs, IGFs, and lipoprotein receptor-related proteins (LRPs). Signalling pathways, such as Wnts, TGF-β, insulin receptor signalling (IRS) and Notch, are involved in the regulation of these cell functions. The interaction of CCNs with receptors and other main cytokines has been briefly summarised (Fig. 2).

Role of integrins in CCN functions. Integrins, found as heterodimers consisting of α- and β-subunits are common transmembrane receptors that mediate cell-to-cell and cell-to-ECM adhesive interactions while also transducing signals from the ECM to the cell interior and vice versa.

Table I. Nomenclature of the CCN family of proteins.

Official name	Alternative names
CCN1	CYR61, CTGF-2, IGFBP10, IGFBP-rP4, CEF10
CCN2	CTGF, IGFBP8, IGFBP-rP2, HBGF-0.8, HCS24, ecogenin
CCN3	NOV, NOVH, IGFBP9, IGFBP-rP3
CCN4	WISP-1, Elm-1, IGFBP-rP8
CCN5	WISP-2, CTGF-L, CTGF-3, HICP, Cop-1, IGFBP-rP7
CCN6	WISP-3, IGFBP-rP9

CYR61, cysteine-rich protein 61; IGFBP, IGFBP-related protein; HBGF, heparin-binding growth factor; Hcs, human chondrosarcoma; Elm-1, expressed in low metastatic cells; HICP, haparin-induced CCN-like protein; Cop-1, card-only protein 1; WISP-1, Wnt-1 induced secreted protein-1.

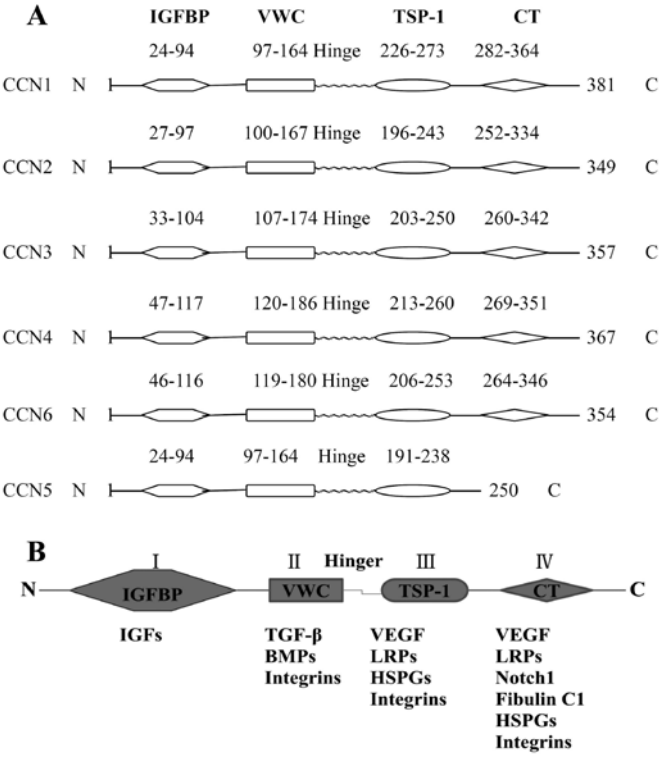

Figure 1. (A) Structure of CCN proteins. The locations of the four structural domains (IGFBP, VWC, TSP-1 and CT) are compared. The two N-terminal domains are separated from the two C-domains (except CCN5) by hinge regions, susceptible to protease cleavage. The arabic numerals display the size of each domain and their relative positions. (B) Four domains of CCNs and the known binding partners. Integrins can bind with VWC, TSP-1 and CT domain, while VEGF, LRPs and HSPGs can bind with TSP-1 and CT domain. IGFBP, insulin-like growth factor-binding protein; VWC, Von Willebrand factor; TSP-1, thrombospondin type-1; CT, cysteine knot; VEGF, vascular endothelial growth factor; LRPs, lipoprotein receptor-related proteins; HSPGs, heparan sulphate proteoglycans.

Currently, there are 24 members in the integrin family that have been identified to have 18 α-subunits and 8 β-subunits in their structures. In previous decades, it has been shown

Table II. Expression profiles of CCN family members: Breakdown by body sites.

Breakdown by body sites	CCN1 (TPM)	CCN2 (TPM)	CCN3 (TPM)	CCN4 (TPM)	CCN5 (TPM)	CCN6 (TPM)
Adipose tissue	1243	621	0	77	0	0
Adrenal gland	212	30	1548	30	0	0
Ascites	75	75	0	0	0	0
Bladder	33	66	0	0	0	0
Blood	0	8	8	0	0	0
Bone	474	1396	69	41	0	0
Bone marrow	61	964	0	0	0	0
Brain	104	106	64	0	2	3
Cervix	123	20	144	0	0	20
Connective tissue	462	1730	33	53	20	0
Ear	310	1677	931	62	0	0
Embryonic tissue	98	244	0	28	0	9
Eye	100	277	4	33	0	0
Heart	122	22	11	0	0	33
Intestine	120	250	4	12	4	0
Kidney	208	521	23	4	9	4
Larynx	170	213	0	0	0	0
Liver	238	107	24	4	0	0
Lung	131	191	8	5	17	0
Lymph	0	0	0	0	0	0
Lymph node	44	33	0	0	0	11
Mammary gland	85	66	13	6	0	6
Mouth	45	513	30	0	0	0
Muscle	37	28	18	28	0	0
Nerve	1223	450	64	0	0	0
Oesophagus	198	49	0	0	0	0
Ovary	167	177	19	9	0	0
Pancreas	168	281	9	14	0	0
Parathyroid	0	0	0	0	0	48
Pharynx	24	24	0	0	0	0
Pituitary gland	0	0	60	0	0	0
Placenta	215	77	0	0	250	0
Prostate	73	121	10	0	10	0
Salivary gland	0	0	0	0	98	0
Skin	227	389	33	0	0	4
Spleen	393	711	0	37	18	0
Stomach	282	553	62	0	0	73
Testis	43	96	2	0	6	6
Thymus	75	100	0	0	0	0
Thyroid	364	472	21	0	0	0
Tonsil	0	0	0	0	0	0
Trachea	308	849	19	0	0	0
Umbilical cord	581	1089	0	0	0	0
Uterus	348	400	21	51	12	0
Vascular	1219	3814	309	0	0	0

TPM, transcripts per million.

that integrins are associated with the different functions of CCNs (8,17) (Table V).

HSPGs. HSPGs are known to serve as co-receptors with integrins under certain circumstances (18). Heparin and

Table III. Expression profiles of CCN family members: Breakdown by health state.

Breakdown by pathophysiology	CCN1 (TPM)	CCN2 (TPM)	CCN3 (TPM)	CCN4 (TPM)	CCN5 (TPM)	CCN6 (TPM)
Adrenal tumor	158	0	948	0	0	0
Bladder carcinoma	113	284	0	0	0	0
Breast (mammary gland) tumor	53	42	0	10	0	10
Cervical tumor	57	0	57	0	0	28
Chondrosarcoma	676	1424	108	72	12	0
Colorectal tumor	79	35	0	0	8	0
Oesophageal tumor	231	57	0	0	0	0
Gastrointestinal tumor	278	641	50	0	0	59
Germ cell tumor	87	501	11	18	0	15
Glioma	55	121	37	0	0	0
Head and neck tumor	141	186	14	0	14	0
Kidney tumor	101	188	29	14	29	0
Leukemia	0	31	10	0	0	21
Liver tumor	229	156	52	0	0	0
Lung tumor	58	9	0	0	0	0
Lymphoma	27	0	0	0	0	0
Non-neoplasia	341	1407	0	10	0	0
Normal	187	319	45	10	32	3
Ovarian tumor	183	249	39	13	0	0
Pancreatic tumor	171	419	9	28	0	0
Primitive neuroectodermal tumor	23	0	0	0	0	0
Prostate cancer	48	19	9	0	0	0
Retinoblastoma	0	0	0	0	0	0
Skin tumor	31	71	31	0	0	7
Soft tissue/muscle tissue tumor	526	47	0	0	0	0
Uterine tumor	321	355	44	11	22	0

TPM, transcripts per million.

HSPGs play important roles in modulating cell adhesion and fibrosis through TSP or CT domains (19,20). CCNs are also capable of binding to HSPGs and mediate cell adhesion and Wnt signalling in some cell types (21-23). Furthermore, it has been previously demonstrated that CCN2 binds to fibronectin (HSPG2) through the CT domain and regulates cell functions (24,25).

IGFs. The IGF family, which includes the polypeptide ligands IGF-I and IGF-II, two types of cell membrane receptors (IGF-IR and IGF-IIR), six binding proteins (IGFBP-1 to IGFBP-6) and IGFBP proteases play an important role in various types of cancer (26). The IGFs have interactions with various molecules that are known to be involved in cancer development and progression. CCNs may bind IGFs with low affinity (27), however, the impact on several cell functions needs to be examined. In previous decades, the regulative role of CCNs in co-ordinating cell functions has been a major research focus. Overexpression of CCN2 in chondrocytes elevates the mRNA transcript levels of IGF-I and IGF-II, resulting in increased bone growth (28). Conversely, CCN6 decreases the IGF-1-induced activation of the IGF-IR, and two of its main downstream signalling molecules, insulin receptor substrate 1 (IRS1) and extracellular signal-regulated kinase (ERK)-1/2 in inflammatory breast cancer cells (29). Downregulation of CCN6 enhances the effects of IGF-I and increases the growth, motility and invasiveness of human mammary epithelial cells (30).

Other receptors. CCNs have been reported to bind receptors, such as LRPs (21). CCN2 is known to regulate the cell adhesion and modulation of Wnt signalling in certain cell types by binding to LRP-1 and LRP-6 (22,23). CCN2 binds bone morphogenetic protein-4 (BMP-4) and TGF-β1 through its VWC domain leading to the inhibition of BMP and TGF-β signalling (31). CCN2 binds VEGF through its TSP and CT domains and inhibits VEGF-induced angiogenesis (32). CCN3 binds to the epidermal growth factor (EGF)-like repeat region of Notch1 via its CT domain. The CCN3-Notch association exerts a positive effect on the Notch signalling pathway and suppresses the differentiation of certain myogenic cells (33). Other receptors include ECM protein (fibulin 1C), a calcium-binding protein (S100A4), ion channels (calcium voltage-independent and Cx43 gap junction) and a subunit of

Table IV. Expression profiles of CCN family members: Breakdown by developmental stage.

Breakdown by developmental stage	CCN1 (TPM)	CCN2 (TPM)	CCN3 (TPM)	CCN4 (TPM)	CCN5 (TPM)	CCN6 (TPM)
Embryoid body	214	457	0	57	0	0
Blastocyst	32	48	0	0	0	16
Fetus	53	132	52	25	39	7
Neonate	386	514	32	0	0	0
Infant	0	85	0	0	0	0
Juvenile	377	197	17	0	0	0
Adult	201	289	26	14	40	2

TPM, transcripts per million.

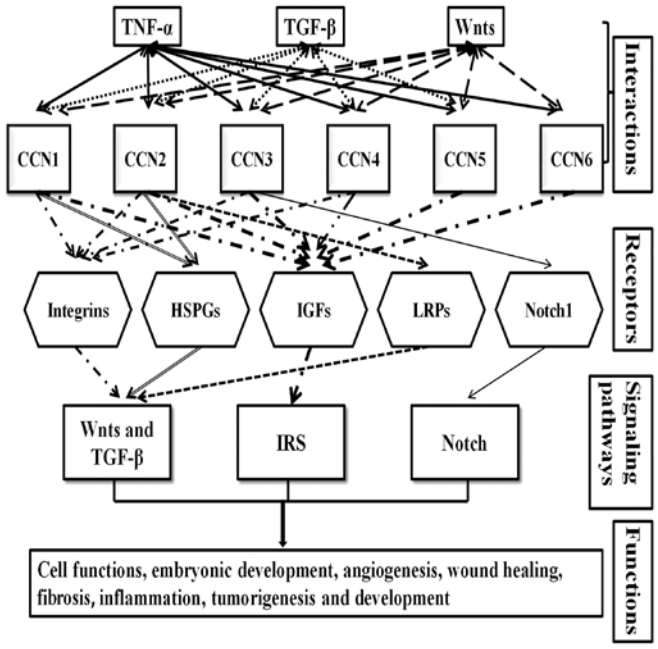

Figure 2. CCN protein interactions with receptors and other main cytokines. Top panels: TNF-α, TGF-β and Wnts and their downstream molecules interact with CCNs; Wnts: Wnt signalling pathway molecules; middle panel: CCNs and their well-known receptors; bottom panel: CCNs regulate cell functions, embryonic development, angiogenesis, wound healing, fibrosis, inflammation, tumorigenesis and development via different receptors and signaling pathways.

RNA polymerase II, which have also been reported to interact with CCNs (34-36).

4. Interactions with other cytokines

TNF-α. TNF-α regulates CCN1 and CCN2 in a cell-type-specific manner. TNF-α represses CCN1 and CCN2 expression in chondrocytes but induces CCN1 expression in osteoblasts and CCN2 expression in synovial cells (37-39). Kular *et al* identified that TNF-α stimulated CCN3, CCN4 and CCN6 expression in melanocytes, cardiac myocytes and fibroblasts and fibroblast-like synoviocytes, respectively. By contrast, TNF-α stimulated CCN3 expression but exerted an inhibitory effect on CCN4 expression in cultured astrocytes (40).

TGF-β. TGF-β has been reported to promote the expression of CCN1, CCN2, CCN4 and CCN5 but represses the expression of CCN3 in chondrosarcoma-derived HCS-2/8 and murine osteoblastic cells (37,41). By contrast, the expression levels of CCN2, CCN3 and CCN4 were inversely correlated with TGF-β in leiomyomas (42). Thus, CCN2 is closely associated with TGF-β as this interaction represses the expression of TGF-β signalling inhibitors (such as Smad7) through the VWC domain (43).

5. Other signalling pathways

CCNs have been shown to be associated with the Wnt signalling pathway (4,41-49). Knockdown of CCN1 expression reduced the Wnt3A-induced oestrogenic differentiation demonstrating that CCN1 expression may be involved in the Wnt3A-induced osteoblast differentiation of mesenchymal stem cells (44). On the other hand, overexpression of CCN1 has also been shown to induce the expression of Wnt/β-catenin transcriptional targets and the formation of secondary body axes (45). Overexpression of CCN2 has been shown to induce the expression of Wnt/β-catenin transcriptional target genes of c-myc and cyclin D1 (46), whereas the overexpression of CCN2 decreased the effects of Wnt3 (47). Notably, CCN3 has been shown to inhibit Wnt/β-catenin signalling pathway through the suppression of BMP-2 activity (48). WISPs (CCN4, CCN5 and CCN6) have been associated with Wnt-1-induced transformation (4,49).

CCN2 has been shown to induce chondrocyte differentiation, through a p38 mitogen-activated protein kinase (p38/MAPK), and proliferation, through the p44/42 MAPK/ERK (49).

6. CCNs in pathophysiological disorders

CCNs and pathophysiological cell functions. The functions of CCNs have been revealed in a wide range of cell types, regulating their cell functions through a variety of mechanisms. CCN1 increased cell adhesion and migration through the integrin α6β1-HSPG co-receptors in fibroblasts, endothelial cells and vascular smooth muscle cells (50,51). In endothelial cells, CCN1 has also been shown to promote cell adhesion, migration, survival, growth factor-induced

Table V. Integrins are associated with the functions of CCN proteins.

Integrins	Involved CCN members	Cell functions affected	Other ligands
α2β1	CCN1	Migration, invasion, motility, lymphangiogenesis	Laminin, collagen, thrombospondin, E-cadherin, tenascin
α5β1	CCN2, CCN3	Adhesion, growth, survival, angiogenesis	Fibronectin, osteopontin, fibrillin, thrombospondin, ADAM, COMP, L1
α6β1	CCN1, CCN2, CCN3	Adhesion, growth	Laminin, thrombospondin, ADAM
αDβ2	CCN1	Adhesion	ICAM, VCAM-1, fibrinogen, fibronectin, vitronectin, plasminogen
αMβ2	CCN1, CCN2	Adhesion	ICAM, iC3b, factor X, fibrinogen, ICAM-4, heparin
αvβ3	CCN1, CCN2, CCN3	Angiogenesis, adhesion, migration, survival, growth	Fibrinogen, vitronectin, thrombospondin, fibrillin, tenascin, PECAM-1, fibronectin, osteopontin, BSP, MFG-E8, ADAM-15, COMP, ICAM-4, MMP, FGF-2, uPA, uPAR, L1, angiostatin, plasmin, cardiotoxin, LAP-TGF-β, Del-1
αvβ5	CCN1, CCN2, CCN3, CCN4	Growth, survival, angiogenesis	Osteopontin, BSP, vitronectin, LAP-TGF-β
αIIbβ3	CCN1, CCN2	Hemostasis, thrombosis	Fibrinogen, thrombospondin, fibronectin, vitronectin, ICAM-4, L1, CD40 ligand

ADAM, a disintegrin and metalloprotease; BSP, bone sialic protein; COMP, cartilage oligomeric matrix protein; L1, CD171; LAP-TGF-β, TGF-β latencyassociated peptide; iC3b, inactivated complement component 3; PECAM-1, platelet and endothelial cell adhesion molecule 1; uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor; VEGF, vascular endothelial growth factor.

mitogenesis and endothelial tubule formation via integrin α6β1 (52). CCN2 promoted the adhesion and migration of microvascular endothelial cells through an integrin-αvβ3-dependent mechanism (53). CCN3 increased the adhesion of normal melanocytes to collagen type IV (54). However, CCN3 expression was also decreased immediately after wounding or re-epithelialization (55), indicating the ability of CCN3 to negatively regulate fibroblast proliferation. CCN4 stimulated the migration and proliferation through integrin α5β1 in vascular smooth muscle cells (56). CCN4 has also been verified to promote the proliferation of hepatic stellate cells *in vitro* (57). CCN5 increased cell proliferation and survival against Streptozotocin in pancreatic cells (58). However, in vascular smooth muscle cells, CCN5 negatively regulated smooth muscle cell proliferation and motility (59). An inhibitory effect on *in vitro* growth of the human mammary epithelial cells function was also assigned to CCN6 (60).

CCNs in embryonic development and angiogenesis. CCN expression profiles appear to be integral to the development of several key organ systems. CCN1 expression has been closely associated with the development of skeletal, cardiovascular, and neuronal systems during mice embryogenesis, best demonstrated by a CCN1 knockout mice model which exhibited aberrations in vascular development (61,62). CCN2 knockout mice died at birth, due to respiratory failure resulting from hypoplastic lungs and poor thoracic development (63). A CCN2 knockdown zebrafish model showed bone defects and disruption in notochord development (64). CCN3 mutant mice exhibited skeletal and cardiac abnormalities, such as cardiomyopathy, muscle atrophy, and cataract formation (65). Evidence suggests that CCN4 has an an important regulatory function in skeletal growth and bone repair (66). The role of CCN5 remains unclear; however, it may serve a multifunctional purpose in developing mice and human embryos (67). CCN6 mutations in humans cause autosomal recessive skeletal disease progressive pseudorheumatoid dysplasia, a juvenile-onset joint degenerative disease (68). However, CCN6-null or CCN6-overexpression mice exhibited no observable phenotype (69). These findings from CCN knockout mice models together with their known expression profiles in the developmental stages (Table IV) suggest that CCN1 and CCN2 play an essential role, while the other four members may play a regulatory role, in human embryonic development.

Wound healing. CCN1 and CCN2 are involved in tissue repair, as the increased expression of the two CCNs has been observed during cutaneous wound healing, liver regeneration, in the heart after myocardial infarction and after bone fracture (70-74). Xu *et al* showed that CCN2 acted as a downstream effector of TGF-β enhancing the production of scar tissue indicating that the suppression of CCN2 may prevent a progressive fibrotic response to TGF-β stimulation (75). Of note, CCN3 transcripts were decreased during the first three days after wound formation or re-epithelialization (55).

Fibrosis. CCN2 mRNA expression has been observed in fibrotic lesions (76-80). However, this pattern has not been observed in early non-fibrotic or atrophic lesions. The serum level of CCN2 protein was significantly increased and correlated with skin sclerosis and lung fibrosis in patients. These results indicate that CCN2 co-operates with TGF-β to maintain and possibly even exacerbate fibrosis (76). Evidence has shown that either CCN2 mRNA or the application of

Table VI. Regulations of CCN members in various types of cancer: Clinical specimens and/or cancer cells *in vitro*.

Tumor type (arranged A-Z)	CCN1	CCN2	CCN3	CCN4	CCN5	CCN6
Breast cancer	↑	↑		↓	↑/↓	↓
Cervical cancer			↑			
Chondrosarcomas	↓	↑	↑	↓		
Chronic myeloid leukaemia			↓			
CRC	↑	↑/↓		↑	↓	↑
Enchondromas		↑				
Endometrial cancer	↑/↓					
Esophageal cancer		↑				
Gallbladder cancer					↓	
Gastric cancers	↓					↑
Glioma	↑		↓			
Liver cancer		↓			↑	↑
Lung cancer	↓	↓		↓		
Malignant adrenocortical tumors			↓			
Melanoma			↓	↓		
Oral carcinoma				↑		
Ovarian cancer	↑	↓				
Pancreatic cancer		↑			↓	
Pituitary tumors					↑	
Prostate cancer	↑		↑			
Salivary gland tumors					↓	
Rhabdomyosarcoma		↑				
Wilms' tumor		↓	↑			

↑, positive correlation or upregulation; ↓, negative correlation or downregulation; ↑/↓, controversial regulations; CRC, colorectal cancer.

exogenous CCN2 protein was required for the development of persistent fibrosis in a mouse fibrosis model (77,78). Lipson *et al* reported that the inhibition of CCN2 was capable of preventing and reversing the process of fibrosis in liver and diabetic nephropathy models (79). CCN5 overexpression inhibited profibrotic phenotypes via the PI3K/Akt signalling pathway in lung fibroblasts and in mice (80).

Inflammation. Bacteria, such as *Yersinia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Staphylococcus aureus*, have been shown to induce CCN1 and CCN2 expression in epithelial cells, indicating that CCN1 and CCN2 overexpression may be useful in the adaptation of epithelial cells in stressful situations (81). HeLa cells infected by *Coxsackievirus B3* induced CCN1 activation via JNK to mediate cell death (82). Bacteria-derived lipid factors have also been shown to induce CCN1 and CCN2 during infections (83,84).

7. CCNs in cancers

CCNs, except CCN5, have four highly conservative functional domains, but play different roles in the same cancer type. Each CCN member may also play different roles in varying cancer types through different signalling pathways (Fig. 2 and Table VI). Some CCN members have already been associated with cancer staging and prognosis as well as contributing to tumorigenesis or metastasis formation (85-93). Other CCN members have been considered as diagnostic or prognostic markers and therapeutic target genes in certain cancer types (46,103,104,119,120).

CCN1. CCN1 mRNA and protein levels are increased in ovarian cancer cells and may play an important role in ovarian carcinogenesis (85). CCN1 is upregulated in prostate cancer cell lines and tumor tissues and is associated with the status of the tumor-suppressor gene p53 (86). CCN1 has also been shown to enhance prostate cancer cell migration via alterations of function to integrins (87). An immunohistochemical analysis of 112 human glioma and normal brain specimens showed that the levels of tumor-associated CCN1 protein were increased with tumor grade ($P<0.001$), and this trend was verified with similar results identified in glioma cells (88). These results have identified a CCN1-dependent pathway that mediates cell growth, cell migration, and long-lasting signalling events in glioma cell lines and possibly astroglial malignancies. CCN1 is overexpressed in U343 glioma cells and has been linked with the integrin-linked kinase-mediated Akt and β-catenin-TCF/Lef signalling pathways (89). CCN1 is a transcriptional target of Hh-GLI signalling leading to increased vascularity and spontaneous metastasis of breast cancer cells (90). Zuo *et al* demonstrated that the overexpression

of CCN1 in breast cancer is associated with the tumorigenesis, migration and invasion of cancer cells (6). CCN1 was expressed in ~30% of invasive breast cancer biopsies and played a role in breast cancer progression, possibly through its interactions with the avb3 receptor (91). CCN1 was found to be overexpressed in patients with endometrial carcinoma and indicative of a poor prognosis (92). CCN1 has also been shown to be overexpressed and correlate with invasion and metastasis in CRC (93).

Other studies, however, have shown different results. For instance, CCN1 expression was found to be reduced in endometrial cancer and lung cancer tissues compared to their paired normal tissues (94,95). Notably, the expression levels of CCN1 were reduced in high-grade chondrosarcomas and advanced gastric cancers (96,97).

CCN2. CCN2 mRNA and protein levels are increased in murine and human rhabdomyosarcoma cells (98). Overexpression of CCN2 increases breast cancer cell migration in Boyden chamber assays and promotes angiogenesis in chorioallantoic membrane assays compared to control cells *in vitro* (99). By contrast, a reduced expression of CCN2 in clinical breast cancer samples based on a qPCR study is associated with poor prognosis (P=0.021), metastasis (P=0.012), local recurrence (P=0.0024) and mortality (P=0.0072) (100). Similarly, findings in CRC are controversial. CCN2 may play an oncogenic role in the progression of well-differentiated CRC (101). However, Lin *et al* showed that lower CCN2 expression levels in CRC patients were associated with a higher peritoneal recurrence rate. Additionally, CCN2 overexpression decreased the incidence of peritoneal carcinomatosis and increased the rate of mice survival, but significantly decreased CRC cell adhesion ability *in vitro* (102). CCN2 overexpression was also found to be associated with poor prognosis in oesophageal squamous cell carcinoma, pancreatic cancer, high-grade chondrosarcomas and enchondromas (46,100,103,104).

However, evidence suggests opposing roles for CCN2 (102,105-109). In these studies, CCN2 acted as an inhibitor, tumor suppressor or a positive prognostic indicator. CCN2 overexpression plays an important inhibitory role on cell proliferation in non-small cell lung cancer cell lines (105). By contrast, in ovarian tumorigenesis, inactivation of the *CCN2* gene may play a role in disease progression (106). CCN2 expression is decreased in Wilms' tumors and a high CCN2 expression exhibits improved prognostic features in intrahepatic cholangiocarcinoma and CRC patients (102,107-109).

CCN3. In human prostate cancer, Maillard *et al* revealed that CCN3 overexpression in cancer cell lines compared with their epithelial localizations was consistent with a role for CCN3 in prostatic tumorigenesis (110). Manara *et al* found the primary musculoskeletal tumors that developed lung and/or bone metachronous metastases also exhibited CCN3 overexpression (111). A similar effect was observed for CCN3 in bone malignancies and cervical cancer, suggesting it acts as a promoter of tumor growth and thus a poor prognostic indicator (112,113). The involvement of CCN3 in cervical cancer has been confirmed by a subsequent study (114). CCN3 transcripts and protein levels were increased in cervical cancer tissues when compared with the corresponding normal tissues. Overexpression of CCN3 was significantly associated with the stage of the disease (P=0.017) and with lymph node involvement (P=0.006). These results suggest that the overexpression of CCN3 is associated with a poorer prognosis in cervical cancer (114).

Other cancer types have resulted in inconsistent results compared to those mentioned above. CCN3-transfected glioma cells induced tumors to a lesser degree than their parental counterparts, which did not express detectable amounts of CCN3 (115). *In vitro*, CCN3 exerted an antiproliferative effect and interfered with the S/G2 transition of the cell cycle, thereby inducing an artificial accumulation of glioblastoma cells (G59) at the S phase (116). CCN3 restored cell growth regulatory properties that were absent in chronic myeloid leukaemia and sensitized chronic myeloid leukaemia cells to imatinib-induced apoptosis (117). CCN3 protein levels were significantly modified in malignant adrenocortical tumors, but not in benign adrenocortical tumors (118). CCN3 suppressed the cell proliferation via interaction with the gap junction protein Connexin43 in glioma cells, and high levels of CCN3 reduced tumorigenicity, resulting in a lower rate of metastasis (119,120). CCN3 *in vitro* has been reported to decrease the transcription and activation of matrix metalloproteinases and suppress the invasion of melanoma cells, indicating that the downregulation of CCN3 expression is a potential mechanism for melanoma progression (121).

CCN4. CCN4 is downstream of Wnt-1 signalling and CCN4 overexpression in colon cancer and may play a role in colon tumorigenesis (4). It has been revealed that CCN4 transcripts are expressed at higher levels in tumor samples compared to normal tissue, and are higher in patients with Dukes' stage B and C compared to Dukes' A. Thus, CCN4 appears to act as a factor for stimulating aggressiveness in colon cancer (15). A similar behavior pattern was observed in oral squamous cell carcinoma cells as CCN4 enhanced their expression by increasing ICAM-1 expression through the αvβ3 integrin receptor and the ASK1, JNK/p38 and AP-1 signal transduction pathways (122).

By contrast, CCN4 inhibited the growth and metastasis of melanoma cells and its expression is increased in low metastatic cells compared to high metastatic cells (123,124). CCN4 overexpression inhibits the motility and invasion of lung cancer cells through the inhibition of Rac activation *in vitro* (125). Similar results have been identified in clinical specimens in which CCN4 has been shown to be reduced in chondrosarcoma and breast cancer with poor prognosis, suggesting it is a putative tumor suppressor (126,127).

CCN5. CCN5 has been shown to be increased in hepatocellular carcinoma compared to paired normal tissues (128), as well as in adrenocorticotropic hormone-secreting pituitary tumors compared to normal pituitaries (129). However, previous findings focusing on the role of CCN5 in breast cancer remain controversial. Ji *et al* reported that CCN5 mRNA and protein levels were increased in some breast cancer cells and in breast tumors from patients with poor prognosis (130). However, CCN5 mRNA and protein levels were significantly reduced as the cancer progressed from a non-invasive to invasive type in breast cancer, and CCN5

mRNA and protein levels were almost undetectable in poorly differentiated cancers compared to the moderately or well-differentiated samples (131). *In vitro* studies have shown that CCN5 was a negative regulator of growth, migration and invasion of breast cancer cells (132,133).

CCN5 exhibits differing effects in other cancer types. For example, Yang *et al* examined CCN5 protein expression in 46 squamous cell/adenosquamous carcinoma samples and 80 adenocarcinoma samples using immunohistochemistry. The results of that study showed that the loss of CCN5 expression was associated with the metastasis, invasion and poor prognosis of gallbladder cancer (134). CCN5 mRNA and protein expression levels have been shown to be reduced in pancreatic adenocarcinoma, salivary gland tumors and CRC compared with the respective paired normal tissues (4,15,135,136).

CCN6. CCN6 was overexpressed in 63% of the colon tumors analyzed and may be downstream of Wnt-1 signalling, thus playing a role in colon tumorigenesis (4). A similar result was obtained in a microsatellite instability subtype of CRC (4,137). However, previous findings revealed that there is no significant difference in CCN6 mRNA levels expressed in the majority of CRC in comparison with paired normal tissues (15). CCN6 transcripts may also play a positive role in the development of hepatocellular carcinoma (138). Knockdown of CCN6 expression suppressed gastric cancer cell proliferation and migration via the Wnt/β-catenin signalling pathway *in vitro*, while a high expression of CCN6 indicated poor prognosis in a gastric cancer clinical cohort (139).

CCN6 mRNA was reduced in 80% of poor outcome cases of breast cancer, and was found to be essential to induce the process of epithelial-mesenchymal transition (EMT) in breast cancer (60). CCN6 overexpression inhibited cell growth and invasiveness in breast cancer cell lines (140) and CCN6 expression was reduced in breast cancer samples compared to paired normal tissues (141). Taken together, the evidence suggests CCN6 is a putative tumor suppressor in breast cancer.

8. Conclusion and perspectives

The perturbed expression of CCNs has been observed in a variety of malignancies. The aberrant expression of certain CCNs is associated with disease progression and poor prognosis. Different CCNs may play contrasting roles in the same cancer, while the same CCN may play different roles in various types of cancer. Further investigations may highlight their clinical relevance and application for predicting prognosis. CCNs comprise four functional domains and exhibit differential expression and functions in different cells and tissues albeit CCN5 lacks a CT module. CCNs can regulate cell functions by acting as ligands for integrins, heparin, and HSPGs, which are regulated by certain growth factors and cytokines, including IGFs, TGF-α and TGF-β, to fulfil their role in the consequent physiological and pathological events. Additionally, CCNs interact with a variety of receptors and cytokines by modulating downstream signal transduction. Insight into the detailed mechanisms involved in CCN-mediated regulation may be useful in understanding their roles and functions in tumorigenesis and cancer metastasis. This may provide new avenues for target therapy in certain malignancies.

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