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Invited Mini Review

Role of the CCN protein family in cancer

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The CCN protein family is composed of six matricellular proteins, which serve regulatory roles rather than structural roles in the extracellular matrix. First identified as secreted proteins which are induced by oncogenes, the acronym CCN came from the names of the first three members: CYR61, CTGF, and NOV. All six members of the CCN family consist of four cysteine-rich modular domains. CCN proteins are known to regulate cell adhesion, proliferation, differentiation, and apoptosis. In addition, CCN proteins are associated with cardiovascular and skeletal development, injury repair, inflammation, and cancer. They function either through binding to integrin receptors or by regulating the expression and activity of growth factors and cytokines. Given their diverse roles related to the pathology of certain diseases such as fibrosis, arthritis, atherosclerosis, diabetic nephropathy, retinopathy, and cancer, there are many emerging studies targeting CCN protein signaling pathways in attempts to elucidate their potentials as therapeutic targets. [BMB Reports 2018; 51(10): 486-492]

INTRODUCTION

The extracellular matrix (ECM), which is known to occupy the extracellular space between cells, serve not only as structural supports, but also as modulators of diverse cell functions (1). Of the ECM protein families, matricellular proteins are secreted proteins which do not function primarily as structural proteins but rather by regulating cellular functions through interacting with bioeffector molecules such as cell-surface receptors, growth factors, cytokines, and hormones (2). Matricellular proteins include thrombospondin 1 and 2, osteopontin, tenascin C and X, SPARC, SC1/hevin, and the CCN protein family (2). The CCN protein family plays significant roles in biological processes such as embryonic development, inflammation, and cancer, suggesting their

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potential as therapeutic targets (3).

The CCN protein family consists of six members: cysteine-rich angiogenic inducer 61 (Cyr 61; CCN1), connective tissue growth factor (CTGF; CCN2), nephroblastoma overexpressed (NOV; CCN3), and WNT-inducible signaling pathway protein 1, 2, and 3 (WISP 1, 2 and 3; CCN4, 5 and 6). The first three members, Cyr 61, CTGF, and NOV, which gave a name and acronym to the family, were first identified as immediate early gene products of growth factors or tumor transformation related genes (4-6). The six members of the CCN protein family, except for CCN5 which lacks the cysteine-knot (CT) motif, share four conserved protein motifs: the insulin-like growth factor binding protein (IGFBP) motif, von Willebrand factor C-like (VWC) motif, thrombospondin type 1 repeat (TSR) motif, and carboxy-terminal CT motif (Fig. 1). An amino-terminal signal peptide is followed by these four motifs. The VWC and TSR motif is known to be associated in cell-cell interactions and the CT motif is known to be involved in CCN protein dimerization and receptor binding (7). The multimodular structure of the CCN proteins indicate that they may interact with other proteins to exert biological functions. They are known to interact with cell surface integrins, growth factors, cytokines, matrix metalloproteinases (MMPs), and other ECM proteins, such as fibronectin and vitronectin (8).

Since the members of the CCN protein family act as signaling components of the ECM, they are known to be involved in biological processes such as cell adhesion, skeletal development, chondrogenesis, angiogenesis, wound repair, proliferation, and tumorigenesis (8).

NORMAL BIOLOGICAL FUNCTIONS OF THE CCN PROTEINS

Cell adhesion and migration

As CCN proteins are part of the ECM, one of their primary functions is the regulation of cell adhesion and migration. CCN1 and CCN2 are known to be related to cellular adhesion in diverse cell types. Additionally, CCN2 is required for ECM contraction (9). CCN3 can increase the adhesion of melanocytes to type IV collagen through discoidin domain receptor 1, a receptor tyrosine kinase (10). CCN proteins can also induce adhesion through heparan sulfate proteoglycans (HSPGs) and integrins. In human skin fibroblasts, adhesion to CCN1 and CCN2 through $\alpha_6\beta_1$-HSPGs induces focal adhesion, actin cytoskeleton rearrangement, and the development of filopodia
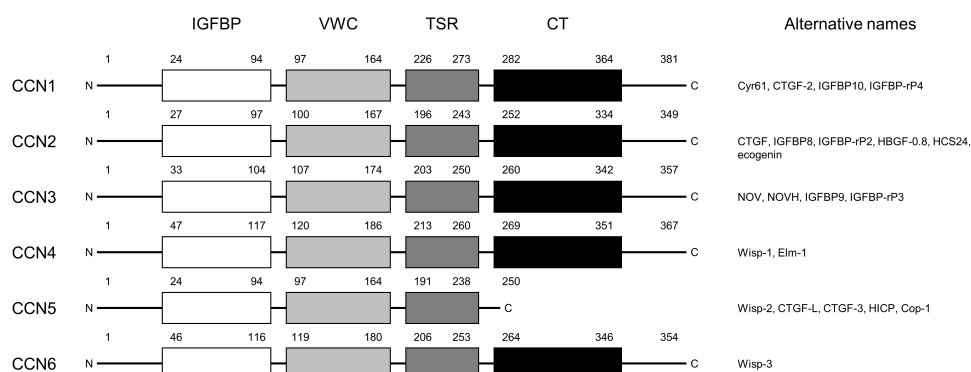
Fig. 1. Structure and nomenclature of CCN protein family members. (Left) The amino acid location of the four conserved motifs (IGFBP, VWC, TSR, and CT) are represented as Arabic numerals. (Right) Alternative names for the CCN proteins are indicated. Abbreviations: Cyr61, cysteine rich 61; CTGF-2, connective tissue growth factor 2; IGFBP10, insulin-like growth factor-binding protein 10; IGFBP-rP4, IGFBP-related protein 4; HBGF-0.8, heparin-binding growth factor 0.8; HCS24, hypertrophic chondrocyte specific 24; NOV, nephroblastoma overexpressed gene; NOVH, human nov gene; Wisp, Wnt-inducible secreted protein; Elm-1, expressed in low metastatic cells; HICP, heparin-induced CCN-like protein; and Cop-1, card-only protein 1.

and lamellipodia (11). CCN3 is known to induce adhesion of endothelial cells, vascular smooth muscle cells, and fibroblasts through HSPGs and integrins (8). CCN1, CCN2, and CCN3 all promote cell migration in mesenchymal cells (12-14). CCN4 and CCN5 inhibit cell migration in lung cancer cells and smooth muscle cells, respectively (15, 16).

Cell proliferation

The effects of CCN proteins related to cell proliferation are CCN protein-specific. CCN1 and CCN2 were originally identified as early-response genes related to cell growth (17). In contrast, CCN3 is considered to be an antiproliferative gene (18). Further studies indicated that CCN proteins promote the proliferation of osteoblasts and chondrocytes. CCN1 and CCN2 increases cell proliferation in vascular smooth muscle cells (19, 20). In contrast, CCN3 and CCN5 inhibit cell proliferation in those cells (16, 21). CCN2 is also known to be involved in the mitogen-activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK) signaling pathway, which is related to cell cycle progression (22).

Osteogenesis and chondrogenesis

All CCN proteins are known to be involved in osteogenesis and chondrogenesis. CCN1 and CCN2 can each promote osteocyte and chondrocyte differentiation (23). Transforming growth factor-β (TGF-β) increases CCN1, CCN2, and CCN5, but decreases CCN4 mRNA and protein expression in osteoblasts (24). CCN2 and CCN3 can interact with bone morphogenetic protein-2 (BMP-2) and inhibit chondrocyte and osteocyte differentiation, respectively (24, 25). CCN4, on the other hand, increases osteogenesis by enhancing BMP-2 activity (26).

Wound repair and angiogenesis

CCN proteins are well known to have angiogenic activities since they interact with diverse growth factors and integrins (27). CCN1 was first discovered to be related to angiogenesis by using human microvascular endothelial cells (28). Further studies indicated that CCN1, CCN2, and CCN3 can induce angiogenesis *in vivo* through integrin $\alpha v \beta 3$ dependent pathways (14, 29-31). In addition, CCN proteins can inhibit angiogenesis. CCN2 is known to suppress angiogenesis by binding to vascular endothelial growth factor (VEGF), then negatively regulating the angiogenic activity of VEGF (32). The processes of wound healing which include angiogenesis, adhesion, vascularization, and proliferation, are known to be regulated by CCN proteins. CCN1 expression is upregulated in liver regeneration (17, 33). CCN1, CCN2, and CCN3 expression are increased during cutaneous wound repair (17, 34, 35).

CCN PROTEIN FUNCTIONS IN TUMORIGENESIS

In many type of cancers, aberrant CCN protein expression is known to be related to tumorigenesis (36-39). However, although they have similar protein structures, each member of the CCN protein family may play different roles within the same or across different types of cancer.

CCN1

CCN1 expression is known to be upregulated in prostate, ovarian, endometrial, and pancreatic cancer cells (40-43). CCN1 is known to enhance cell migration in prostate cancer (44). In addition, CCN1 expression is elevated in breast cancer, leading to increased invasiveness (43). Tsai *et al.* (2000) showed that CCN1 acts as a ligand for integrin $\alpha v \beta 3$ and is related to breast cancer progression (45). They also

demonstrated that CCN1 acts as a downstream of heregulin (HRG) and that CCN1-neutralizing antibodies decreased migration of HRG-positive breast cancer cells (45). One study demonstrated that CCN1 expression is associated with the status of the tumor suppressor gene, p53. They showed that CCN1 was highly expressed in cell lines with mutant and null p53, while low expression of CCN1 was found in cell lines with wild-type p53 (41). In addition, CCN1 is overexpressed in highly tumorigenic glioma cell lines, and forced expression of CCN1 in U343 cells resulted in the activation of the phosphatidylinositol-3-kinase/Akt signaling pathway, leading to the inhibition of the pro-apoptotic protein, Bad (46). On the other hand, CCN1 has been shown to be downregulated in lung and gastric cancer (47, 48). Chien *et al.* (2004), in contrary to Watari *et al.* (2009), demonstrated that endometrial cancer cells have lower expressions of CCN1 compared to their normal counterparts, and overexpression of CCN1 decreased endometrial cancer cell growth and induced apoptosis (49).

CCN2

CCN2 overexpression is known to be related to poor prognosis in chondrosarcomas, enchondromas, rhabdomyosarcomas, pancreatic cancer, esophageal cancer, and breast cancer (50-54). Chien *et al.* (2011) demonstrated that CCN2 overexpression in a breast cancer cell line resulted in increased migration and angiogenesis, and that the increased migration was dependent of the CT domain of CCN2 protein (55). In contrast, Jiang *et al.* (2004) analyzed the mRNA and protein expression level of CCN2 in 122 human breast tumors and concluded that CCN2 may act as a tumor suppressor in breast cancer given the results of CCN2 being downregulated in tumor tissues compared to the normal tissues and that CCN2 overexpressing patients have better prognoses than patients with low CCN2 (52). Additionally, knockdown of CCN2 resulted in decreased pancreatic tumor growth in mouse, indicating that CCN2 may be a good therapeutic target in pancreatic cancer (53). CCN2 overexpression leads to increased breast cancer metastasis to the bone and results in poor-prognosis (56). Shimo *et al.* (2009) showed that CCN2 is associated with the osteolytic metastasis of breast cancer through the PKA- and PKC-dependent activation of ERK, and that the neutralization of CCN2 using CCN2-specfic antibodies decreased bone metastasis *in vivo* (57).

CCN3

CCN3 has been shown to have antiproliferative effects in glioma cells (58, 59). Bleau *et al.* (2007) demonstrated that secreted CCN3 leads to decreased cell proliferation in glioma, and these antiproliferative effects could be neutralized by antibodies that specifically recognize the C-terminal domain of CCN3 (58). They also showed that the CT domain of CCN3 is responsible for the role of CCN3 in cell growth inhibition (58). In addition, CCN3 is able to negatively regulate cell proliferation in choriocarcinoma cells through interacting with a gap junction protein, connexin 43 (60). Benini *et al.* (2005) showed that CCN3-overexpressing Ewing's sarcoma cells had reduced cell proliferation but increased migration and invasion (61). In melanoma, CCN3 protein expression is downregulated in invasive cell lines and the forced expression of CCN3 inhibited the proliferation and invasion of melanoma cells (62). In contrast to reports describing the antiproliferative roles of CCN3 in cancer, there have been studies showing the role of CCN3 as an oncogene. CCN3 is overexpressed in rhabdomyosarcoma, cartilage tumors, and prostate cancer (63, 64). Glukhova *et al.* (2001) demonstrated that CCN3 expression and secretion is increased in nephroblastoma and related to poor prognosis (65). In addition, a study done with

Table 1. Role of CCN proteins in cancer

CCN proteins	Type of Cancer	Role	Ref.
CCN1	Prostate cancer	Enhance cell migration	37
	Breast cancer	Increase invasiveness	36
		Related to cancer progression	38
	Glioma	Inhibits apoptosis	39
	Gastric cancer	Inversely related to MMP-7 expression	41
CCN2	Breast cancer	Increase migration and angiogenesis	48
		Increase bone metastasis	49, 50
	Pancreatic cancer	Increase tumor growth	46
CCN3	Glioma	Decrease cell proliferation	51
	Choriocarcinoma	Negatively regulate cell proliferation	53
	Ewing's sarcoma	Decrease cell proliferation and increase migration	54
	Melanoma	Decrease proliferation and invasion	55
CCN4	Oral cancer	Increase cell migration	63
	Melanoma	Attenuates growth and metastasis	64, 65
	Lung cancer	Decrease migration and invasion	8
CCN5	Breast cancer	Decrease proliferation and invasion	74
CCN6	Breast cancer	Decrease proliferation and invasion	76

56 paired tissue samples of human cervical cancer and their normal counterparts showed that CCN3 mRNA and protein is overexpressed in cervical cancer (66).

CCN4

CCN4 is known to be overexpressed in colon, colorectal, breast, and lung cancer (47, 67-69). Chuang *et al*. (2013) demonstrated that CCN4 increased cell migration in oral squamous cell carcinoma through integrin αvβ3 activation and intercellular adhesion molecule-1 expression (70). In contrast, in melanoma, CCN4 expression is inversely correlated to that metastatic potential of K-1735 mouse melanoma cells (71, 72). Additionally, overexpression of CCN4 in highly metastatic K-1735 cells attenuated growth rates and metastasis *in vivo* (72). Soon *et al*. (2003) showed that the forced expression of CCN4 in H460 lung cancer cells resulted in decreased cell migration and invasion *in vitro* and metastasis *in vivo* through the downregulation of Rac (15). A cohort study done conducted on 122 human breast cancer tissues and 32 normal breast tissues indicated that CCN4 mRNA and protein was relatively downregulated in patients with worse prognosis (73). CCN4 expression has been evaluated in chondrosarcomas and enchondromas with various grades and the results found showed that high grade tumors had lower expressions of CCN4 (74).

CCN5

CCN5 is downregulated in human leiomyomas, pancreatic adenocarcinoma, salivary gland tumors, colon tumors, gallbladder cancer, and colorectal cancer (67, 68, 75-78). In hepatocellular carcinoma and adrenocorticotropic hormone-secreting pituitary tumors, CCN5 is upregulated compared to in their normal counterpart tissues (79, 80). In breast cancer, CCN5 expression is low in aggressive breast cancer cell lines (81). The forced expression of CCN5 into MDA-MB-231, an invasive breast cancer cell line, resulted in decreased cell proliferation and invasion (81). Banerjee *et al*. (2008) demonstrated that the expression profile of CCN5 changes during the course of breast cancer progression (82). In normal breast tissues, CCN5 is undetectable, while its expression is increased in noninvasive breast cancer lesions (82). When noninvasive breast cancer progresses into an invasive type, CCN5 mRNA and protein expression is downregulated by genes such as Snail, MMP-2, and MMP-9 (82).

CCN6

Forced CCN6 expression into an inflammatory breast cancer cell line that resulted in decreased invasion and cell proliferation *in vitro* and cell growth *in vivo* (83). Lorenzatti *et al*. (2011) demonstrated that CCN6 expression level is low in aggressive breast cancer cells, and that recombinant human CCN6 protein attenuates the insulin-like growth factor-1 (IGF-1) signaling pathway and downregulates ZEB1, a transcription factor which is known to be an epithelial-to-

mesenchymal transition activator (84). In addition, chromatin immunoprecipitation assays revealed that the inhibition of CCN6 upregulates Snail and ZEB1 binding to the E-cadherin promoter, which act as transcriptional repressors of E-cadherin in breast cancer (85). In contrast, CCN6 is overexpressed in 63% of human colon tumors and seems to be associated with tumorigenesis in colon cancer (67). In addition, CCN6 was identified as being a novel gene related to colorectal cancers with microsatellite instability (86).

CONCLUSION

CCN family proteins play roles in diverse cellular functions and have different expression profiles among different tissues and organs. Although all six members of the CCN protein family share similar protein structures, their roles are tightly regulated in a spatiotemporal matter rather than playing the redundant roles of other proteins in the same family (7, 18). CCN proteins are known to interact with receptors such as integrins, HSPGs, IGFs, and lipoprotein receptor-related proteins (87, 88). In addition, CCN proteins can bind to other growth factors and cytokines including TGF-β, VEGF, fibroblast growth factor 2, and BMPs, altering their biological functions (32, 89, 90). In cancer, the dysregulated expression of CCN proteins is often associated with tumorigenesis and cancer progression (91). Although it differs among various types of cancer, in general, CCN1, CCN2, and CCN4 are known to be related to tumor progression and play roles as oncogenes while CCN3, CCN5, and CCN6 are associated with inhibiting tumor progression and play tumor suppressor roles (Table 1). Since the current literature has certain limitations in clarifying the exact role of CCN proteins in controversial areas, continued studies could help reveal the therapeutic potential of CCN proteins in cancer.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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