REVIEW ARTICLE

Biological characteristics of CCN proteins in tumor development

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Summary

The extracellular matrix (ECM) is no longer regarded as inert, rather it has multiple versatile physiologic functions. Its diverse composition is implicated in each step of cancer progression including inflammation, angiogenesis, tumor invasion and metastasis. In addition to structural proteins, the ECM also contains a family of non-structural proteins called matricellular proteins. The six secreted CCN proteins, which belong to the matricellular protein family, include the following: Cyr61 (CCN1), CTGF (CCN2), Nov (CCN3), WISP-1 (CCN4), WISP-2 (CCN5) and WISP-3(CCN6). These pro-

teins are capable of modulating a variety of biological processes in health as well as in disease conditions. In tumor development and in tumor microenvironment, CCN proteins can influence multiple facets of pathophysiological processes including cellular proliferation, invasion and metastasis. This review has attempted a cohesive look at the CCN family protein functions in a tumor-specific manner.

Key words: cancer, CCN proteins, cell signaling, extracellular matrix, matricellular proteins

Introduction

The extracellular matrix (ECM) consists of diverse groups of proteins including matricellular proteins, which are a family of non-structural matrix proteins capable of modulating a variety of biological processes. Members of this family include a large number of structurally unrelated proteins such as thrombospondins, osteopontin, and the CCN proteins. Overall, they play a highly complex role in health and disease conditions [1]. It is expected that these proteins are critical for the development of an appropriate tumor microenvironment by establishing complex interactions/crosstalk among cancer cells and various components of the surrounding stroma such as different ECM constituents, inflammatory cells, and numerous molecules of signaling pathways.

The CCN (Cyr61-CTGF-Nov) family consists of six members: cysteine-rich protein 61 (Cyr61

or CCN1), connective tissue growth factor (CTGF or CCN2), nephroblastoma overexpressed protein (Nov or CCN3), Wnt-inducible secreted protein-1 (WISP-1 or CCN4), WISP-2 (CCN5) and WISP-3 (CCN6). A growing body of evidence suggests that the CCN proteins are involved in different aspects of cancer development [2-5]. It has been shown that CCN proteins can modulate the signals of several proteins including integrins, Wnt, and transforming growth factor-β (TGF-β); many of the functions of these proteins are also related to tumor growth [2,3].

Studies have revealed a link between CCN proteins and various cancer risk factors. One such important risk factor is chronic inflammation. Recent reports documented that CCN proteins have been strongly implicated in the inflammatory process [6,7]. Expression of CCN has been shown to

be regulated by diverse inflammatory mediators including TGF-β, prostaglandins, and extracellular matrix enzymes [8]. It has been hypothesized that the CCN family proteins may play a central role in signaling the tumor microenvironment of hepatocellular carcinoma (HCC) [9]. In general, HCC takes place in inflammatory conditions that could be associated, for example, with infection by hepatitis B virus (HBV) or hepatitis C virus (HCV). Other agents that have associated etiological roles in HCC are alcohol intake, obesity and cigarette smoking - all of which can generate a chronic inflammatory environment [10]. Interestingly, CCN1 expression level was reported to be correlated with smoking in non-small cell lung cancer (NSCLC) [11].

Obesity-related inflammation has been linked with a number of health problems such as cardiovascular disorders, insulin resistance/type 2 diabetes, and certain cancers. In different obesity-related diseases, many components of matricellular proteins including CCN have been shown to be altered and influence the pathological processes. For instance, CCN1 can cause endothelial cell dysfunction in atherosclerosis [12]; CCN2 may play an important role in diabetic pathology [13,14]; and all CCN proteins probably affect breast cancer development [2,15]. Intriguingly, reports show a close connection between different CCN proteins and tumor-associated biochemical risk factors, e.g., estrogenic influence [16], pro-inflammatory adipokines–such as tumor necrosis factor alpha (TNFα) and leptin [12,17], as well as enhanced activity of growth factors, for instance insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) [5,16,18]. Obviously, higher VEGF action promotes increased angiogenesis and/or lymphangiogenesis, which is supportive for cell proliferation and tumor growth. In this review article, an attempt has been made to focus on CCN proteins' functions in the tumor microenvironment.

CCN1: Cyr61

In certain cancers Cyr61 can induce tumorigenesis, cancer progression and/or invasion, such as in gliomas, ovarian carcinoma, and prostate cancer [19-21]. In other cancers, including NSCLC, Cyr61 promoted apoptosis and impeded tumor growth [22]. Astonishingly, elevated expressions of Cyr61 have been found in colorectal cancer, gastric cancer, and HCC; however its levels declined when these cancers became more advanced

[23-25]. The available data on melanoma suggests that Cyr61 can act as a tumor-suppressor gene; Dobroff et al. [26] found that Cyr61 reduced tumor growth and metastases, decreased angiogenesis and matrix metalloproteinase-2 (MMP-2) expression, as well as stimulated apoptosis in vivo. Cyr61 may also decrease the metastatic ability of melanoma cells by altering vascular cell adhesion molecule 1 (VCAM-1) activity [27].

Overexpressed Cyr61 has been associated with more invasive breast cancer phenotypes, due to increased cell growth stimulated invasion and metastases, partly via up-regulated VEGF and increased tumor vascularization in a VEGF-independent manner [28-30]. Interestingly, Sarkissyan et al. [31] showed that IGF-1 can induce Cyr61 expression, resulting in reduced E-cadherin expression in breast cancer. Zhang et al. [32] provided evidence that 17β-estradiol exposure induced Cyr61 synthesis in MCF-7 breast cancer cells. Epidermal growth factor (EGF) can also up-regulate Cyr61 in MCF-7 cells, suggesting a synergistic regulation of Cyr61 in carcinogenesis [33]. In addition, Cyr61 can induce estrogen-independence and anti-estrogen resistance to bring about an aggressive breast cancer phenotype [28]. In concordance, Lin et al. [34] observed that over-expressed Cyr61 in MCF-7 cells had a remarkable resistance to apoptosis against chemotherapeutic agents.

Abnormal expression of Cyr61 has been demonstrated to be associated with osteosarcoma progression; Cyr61 can promote osteogenesis by increasing osteoblast differentiation and impeding osteoclast formation [35]. Enticingly, Cyr61 levels correlated with a poor prognosis regardless of metastasis [36]. In addition, Hou et al. [35] showed that in osteosarcoma the abnormal expression of Cyr61 induced mesenchymal transition and promoted lung metastases, via αvβ5 integrin, Raf-1, mitogen-activated protein kinase kinase (MEK), extracellular signal-regulated kinase (ERK), and Elk-1 signal transduction pathways. They also demonstrated that the knockdown of Cyr61 inhibited in vitro tumor cell invasion and migration, along with in vivo lung metastases.

CCN2: CTGF

Increased levels of CTGF have been linked with an advanced aggressive state of breast cancer, esophageal cancer, gastric cancer, osteosarcoma, hepatocellular carcinoma and glioblastoma [37-42]. In a breast cancer cell line, Chien et al. [43] demonstrated that forced overexpression
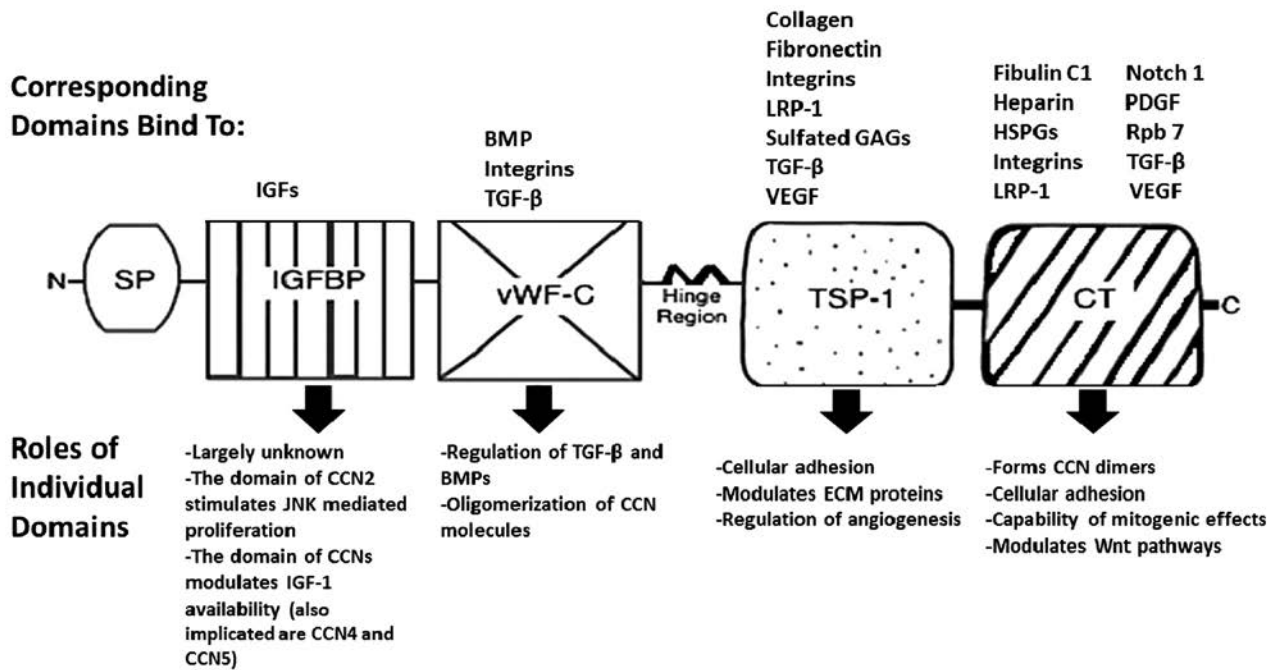
Figure 1. General structure of CCN proteins. The general structure of the CCN proteins consists of the signal peptide (SP), insulin-like growth factor binding domain (IGFBP), von Willebrand factor type C domain (vWF-C), thrombospondin type-1 repeat (TSP-1), and a carboxy-terminal domain (CT) which contains a cysteine knot (not depicted). CCN5 (WISP-2) lacks CT domain. Listed above each domain, are some of the corresponding known binding partners. Underneath each domain are the corresponding known or suspected roles. Thought-provokingly, more than one domain can bind to the same protein, thus the interactions of the full-length protein may be needed to bring about certain functions. A hinge region exists, which is susceptible to cleavage by matrix metalloproteases -1, -2, -3, -7, -9, -13, as well as by elastase and plasmin. IGFs: Insulin like growth factors, BMP: bone morphogenic protein, TGF: transforming growth factor, LRP-1: LDL receptor protein-1, GAGs: glycosaminoglycans, VEGF: vascular endothelial growth factor, HSPGs: heparin sulphated proteoglycans, PDGF: platelet-derived growth factor, Rbp 7: Retinol Binding Protein 7, JNK: c-Jun Kinase.

of CTGF led to stimulation of angiogenesis and migration, and suggested that the CT domain of CTGF (Figure 1) was critically important for the migratory ability.

The expression of CTGF has also been seen in murine melanoma cells; Sha and Leask [44] demonstrated that CTGF was expressed in B16 (F10) melanoma cells and was downstream of the oncogenic MEK/ERK pathway. Finger et al. [45] reported that hypoxia was a key factor in up-regulating CTGF expression, promoting melanoma progression and metastases. In addition, they revealed the effectiveness of an antibody against CTGF to impede metastatic melanoma tumor progression *in vivo*.

CCN3: NOV

CCN3 has tumor suppressive and oncogenic properties, dependent on tumor type [46-48]. Bohlig et al. [47] reported that the CCN3 gene could be transcriptionally up-regulated by the tumor suppressor p53. Nevertheless, elevated levels of CCN3 have been demonstrated to be associated with prostate cancer with bone metastasis and osteosarcoma with poor prognosis [46,49]. CCN3 can promote cell motility and invasion in certain cancers, and has been implicated in the progression of chondrosarcoma and Ewing's sarcoma [50].

While increased levels of CCN3 have been linked to higher metastatic rates in melanoma, decreased CCN3 expression has also been observed in melanomas to promote cancer progression, revealing the complexity in the role of CCN3 [51,52]. In melanocytes, CCN3 can regulate cell adhesion and maintain correct spatial localization to the basement membrane. Therefore, in early tumorigenesis down-regulation of CCN3 may be needed for the tumor cells to locally invade. Subsequently with progression and visceral metastases of melanoma, CCN3 levels are then overexpressed [51-53]. Of note, two different isoforms have been identified in melanoma cells, which can further account for the diverse functions [53].

Chen et al. [54] showed that increased CCN3 expression stimulated cell migration, by up-regulating the intracellular adhesion molecule-1 (ICAM-1) levels in a prostate cancer cell line. They showed that the knockdown of CCN3 resulted in a decrease in both cell migration in vitro and me-

tastases to bone in vivo. However, divergent data appear to exist in regards to breast cancer. Elevated CCN3 levels were demonstrated to be associated with breast cancer cells that metastasized and caused osteolysis of bone [55]. Recently, Dobson et al. [48] provided evidence that CCN3 prevented invasion by metastatic breast cancer cells and that silencing CCN3 in MDA-MB-231 cells resulted in less migratory and more invasive cells.

CCN4: WISP-1

Conflicting findings have been reported in WISP-1 (CCN4), which possesses oncogenic and tumor suppressive roles in different cancers. In breast cancer, it was documented that WISP-1 levels were decreased in tumor cells compared to normal breast tissue and that the expression was less in the more aggressive tumor types [56]. On the contrary, Klinke [57] focused on a larger sample size and on invasive breast cancer to reveal that an increased WISP-1 gene expression was associated with oncogenic transformation.

In murine models, up-regulated WISP-1 levels were demonstrated in highly metastatic D122 Lewis lung carcinoma pulmonary metastasis and B16-F10.9 melanoma cell lines, as compared to the primary tumors [58]. However, Soon et al. [59] found that WISP-1 overexpression in H460 lung cancer cells prevented lung metastases as well as *in vitro* cell invasion and mortality. In melanoma cells, Kulkarni et al. [60] showed that up-regulated tumor-derived WISP-1 inhibited the cells' response to anti-tumor immunity enhancer interleukin-12. Shao et al. [61] provided evidence that the Notch1 pathway increased WISP-1 secretion, inhibiting melanoma growth and tumor angiogenesis. Taken altogether, perhaps WISP-1 is not needed for tumor growth in melanoma, but is crucial for progression and cancer metastases.

Ono et al. [62] found elevated WISP-1 levels in early stage prostate cancer in mice and humans. Antibodies against WISP-1 led to a decrease in both growth of the tumor xenograft cell line and metastases to bone. Additionally, the study observed that Cyr61 expression in prostate cancer was consistent with a lower recurrence risk and an inverse relationship with p53 levels. WISP-1 has been shown to activate the anti-apoptotic Akt/PKB signaling pathway, rendering cells resistant to apoptosis following DNA damage by preventing the mitochondrial release of cytochrome C and the up-regulation of anti-apoptotic Bcl-x$_L$, which protects cells from p53-dependent cell death [63].

CCN5: WISP-2

WISP-2 (CCN5) can suppress proliferation and attenuate invasive phenotypes in certain cancers [64,65]; it can also act as a tumor suppressor [66]. Differing data exist in regards to WISP-2 in breast cancer, which must be correlated to the phenotype of the cancer. Studies have shown WISP-2 expression to be predominately in pre-neoplastic disorders, i.e., atypical ductal hyperplasia and non-invasive ductal carcinoma in situ (DCIS) [65,67]. The mRNA transcript of WISP-2 was undetectable in normal mammary cells as well as in poorly differentiated highly aggressive breast cancer cell lines, while elevated levels were observed in non-invasive and more differentiated breast cancer cell phenotypes [68]. Fritah et al. showed that the knockdown of WISP-2 in the MCF-7 breast cancer cell line induced an estradiol-independent growth, which was found to be associated with loss of estrogen receptor-α (ERα) expression and promoted epithelial-to-mesenchymal transition (EMT) [68]. Importantly, the loss of WISP-2 expression has also been seen to result in a triple negative (ER⁻/PR⁻/HER2⁻) breast cancer cell line [67]. Ferrand et al. successfully demonstrated a glucocorticoid-induced up-regulation of WISP-2 in estrogen receptor-negative breast cancer, which led to a reduction in cellular proliferation and invasive phenotype [67].

CCN6: WISP-3

WISP-3 (CCN6) can function in a tumor-suppressor manner in certain cancers. WISP-3 has been shown to be expressed in normal breast epithelium but down-regulated or lost in many invasive carcinomas [69]. A study revealed that its levels were dramatically reduced in aggressive breast cancer, particularly breast cancer with axillary lymph node metastases and in inflammatory breast cancer [70]. WISP-3 may be crucial in maintaining normal epithelial morphology and regulating anoikis in breast cancer cells. Huang et al. [71] showed that in human mammary epithelial cells, WISP-3 knockdown led to a resistance to anoikis and an increased anchorage-independent growth. Pal et al. [72] demonstrated that WISP-3 overexpression reduced invasion and distant metastases of breast cancer cells, while decreased levels led to disruption of acinar morphogenesis and promoted mammary epithelial cell invasion. Moreover, the loss of WISP-3 has been seen as a trigger for EMT [73].

Table 1. Association of different CCN proteins with three important groups of the intracellular signaling system

Family members	Integrins	Transforming growth factor-β	Wnt signaling
CCN1	Via $\alpha_v\beta_5$, CCN1 promotes tumor growth; stimulates proliferation, EMT and migration in pancreatic cancer cells, and induces VEGF secretion in breast cancer cells. Interaction with $\alpha_6\beta_1$ inhibits tumor growth and induces apoptosis. As a ligand to $\alpha_4\beta_1$, reduces metastases in melanoma cells.	CCN1 sequesters TGF-β leading to decreased TGF-β signaling and fibrogenesis in primary portal myofibroblasts. In macrophages, CCN1 downregulates the TGF-β1 gene. However, TGF-β increases CCN1 expression in osteoblasts.	Wnt3a stimulation induces CCN1 expression in mesenchymal stem cells; β-catenin activation increases CCN1 expression in HCC cells. Elevated CCN1 levels activate β-catenin signaling in gastric epithelial cells promoting wound healing; in glioma cells enhancing tumorigenicity; in NSCLC cells suppressing growth; in ESCC promoting oncogenesis.
CCN2	Participates in cytoskeleton rearrangement and migration of breast cancer cells through $\alpha_v\beta_3$. Interactions with integrin $\alpha_5\beta_1$, promote keratinocyte adhesion to fibronectin and migration. Other known ligands of CCN2 include: $\alpha_M\beta_2$ associated with cell adhesion to monocytes and macrophages; $\alpha_{IIb}\beta_3$ contributes to the adhesion of activated platelets.	A cofactor of TGF-β, to undergo active cell adhesion; TGF-β induces CCN2 synthesis and CCN2 could be a downstream mediator of TGF-β1 actions. In mesangial cells, CCN2 enhances TGF-β/Smad signaling pathway and represses inhibitors.	Canonical Wnt signaling activates the CCN2 promoter in NIH3T3 fibroblasts; in mesenchymal cells Wnt3a induces CCN2 expression in early stages of osteogenic differentiation. CCN2 activates the canonical Wnt pathway, stimulates β-catenin nuclear translocation in mesangial cells. In chondrocytes, β-catenin induces CCN2 expression.
CCN3	CCN3 stimulated COX-2 expression enhances migration in osteosarcoma via $\alpha_v\beta_5$. Interaction with $\alpha_v\beta_3$ induces cell migration and has been associated with ICAM-1 expression in prostate cancer cells. Increases MMP-13 to stimulate migration via $\alpha_v\beta_3/\alpha_v\beta_5$ receptor in chondrosarcoma cells and can also interact with $\alpha_5\beta_1$ and $\alpha_6\beta_1$ on endothelial cells to stimulate cell adhesion.	TGF-β suppresses CCN3 expression in adrenocortical, glial, mesangial cells, osteoblasts and nucleus pulposus cells; TGF-β signaling induces the CCN3 promoter via Smad3 and enhances its repression through MAPK signaling.	Overexpressed CCN3 antagonizes Wnt 3 activity in cells of the osteoblastic lineage. In NIH3T3 fibroblasts, CCN3 mRNA is only modestly induced by Wnt3a. Fibroblasts treated with Wnt3a and TGF-β, show a synergistic effect to elevate CCN3 expression.
CCN4	Acts as a ligand for $\alpha_v\beta_5$ and $\alpha_6\beta_1$, and increases VCAM-1 expression. Interaction with $\alpha_v\beta_3$ induces migration in oral squamous cell carcinoma and promotes VEGF-A expression to regulate angiogenesis. Mediates VSMC adhesion, migration and proliferation through $\alpha_5\beta_1$.	TGF-β increases CCN4 expression in osteoblasts and in a hepatic stellate cell line. In lung fibroblasts, TGF-β1 induces CCN4 expression, which can be reversed by miR-30a and miR-92a.	Both Wnt-1 and β-catenin signaling induce WISP-1 gene expression. Wnt-11 induces WISP-1 expression in fibroblasts. In Wnt-1-expressing tumor cells, CCN4 is predominately in the stromal fibroblasts; CCN4 expression is induced by Wnt-1-expressing mammary glands.
CCN5	It has been shown to bind to integrin $\alpha_v\beta_3$ in VSMCs and in podosomes to regulate matrix degradation.	TGF-β induces CCN5 mRNA. CCN5 inhibits TGF-β signaling preventing EMT in cancer cells.	CCN5 is downstream of Wnt-1 signaling. Secreted CCN5 activates canonical WNT in mesenchymal precursor cells.
CCN6	Interaction with $\alpha_v\beta_3$ and $\alpha_v\beta_5$ is associated with increased ICAM-1 expression enhancing migration in chondrosarcoma cells.	CCN6 antagonizes BMP4, a TGF-β superfamily ligand and attenuates invasion in breast cancer cells.	CCN6 may be downstream of Wnt-1 signaling. Silenced CCN6 in gastric cancer cells attenuates Wnt/β-catenin signaling.

EMT:epithelial-to-mesenchymal transition, VEGF:vascular endothelial growth factor, HCC:hepatocellular carcinoma, NSCLC:non-small cell lung cancer, ESCC:esophageal squamous cell carcinoma, COX:cyclooxygenase, ICAM:intercellular adhesion molecule, MMP:matrix metalloproteinase, VCAM:vascular cell adhesion molecule, VSMC:vascular smooth muscle cell, miR-30a and miR-92a:Small non-coding RNA/microRNA precursor

CCN Proteins Intertwined

In general, the promotion of cell proliferation and tumor growth have been associated with CCN1, CCN2, and CCN4, while the suppression of cell proliferation and tumor growth have been linked to CCN3, CCN5 and CCN6; however, exceptions exist [74]. Perhaps, these perplexing features of the different CCN proteins indicate their connection with a number of diverse signaling components such as the various members of integrins and TGF-β family (Table 1), and their involvement in cellular processes where malignant cells may exploit like tissue invasion and the organization of tumor microenvironment. Nevertheless, it is of great value to examine the CCN family members as a whole in cancers. Ohgawara et al. [75] evaluated all six of the CCN proteins in a breast cancer cell line and recorded that the CCN2/CCN3 ratio was a parameter associated with a highly metastatic phenotype to bone, while a higher ratio of CCN1/CCN3 was noted in highly invasive MDA-MB-231 cells. ER status has been shown to be a valuable predictor of Cyr61 expression, whereas the HER-2/neu status was an important factor for WISP-1 expression [76]. In addition, Cyr61 along with CTGF, have exhibited a drug resistance role in breast cancer. Lai et al. [77] demonstrated that resistance to paclitaxel in breast cancer treatment was completely reversed when both Cyr61 and CTGF were blocked.

The expressions of Cyr61, CTGF and WISP-1 have been observed in esophageal squamous cell carcinoma (ESCC) in association with high tumor grade, metastases and/or poor prognosis [38,78,79]. The silencing of the Cyr61 gene in an ESCC cell line led to an inhibition of cell growth, adhesion and invasion [80]. Bartel et al. [81] reported an inverse expression between Cyr61 and CTGF in both borderline and invasive ovarian cancer (IOC). The highest levels of Cyr61 were seen in IOC, and the study demonstrated that the independent loss of CTGF was a poor prognostic marker in IOC [81]. In gastrointestinal tract cancers WISP-3 has been reported to be up-regulated and acting in an oncogenic fashion, whereas WISP-2 behaved as a tumor suppressor [82,83]. WISP-1 may promote colorectal cancer (CRC) progression, with elevated levels associated with poorly differentiated, high grade tumors and a worse prognosis [84]. While CTGF can inhibit CRC metastasis, the overexpression of Cyr61 has

been shown to increase invasion *in vitro* and promote lung metastases *in vivo* [83].

Conclusions

The ECM is composed of diverse components including glycosaminoglycans, hyaluronic acid, collagen, laminin, fibronectin etc. and thus accomplishes a myriad of functions. The interaction between the CCN proteins themselves, as well as other environmental components, is an intricately complex process that, if precisely understood, could provide invaluable insight into various pathological conditions. The interactions among ECM proteins and their pathways are tissue-specific and phenotype-specific. Therefore, no single member of the CCN family can be generally considered as oncogenic or tumor suppressive. Even within the same tissues, the same CCN protein may carry out different functions during the formation, maintenance and/or progression of cancers.

Precisely examining the entire CCN protein family in various stages of different cancers could lead to a clearer understanding of the proteins' functions and to decreased discrepancies. Potentially, this understanding would provide a basis for the creation of new biomarkers to improve earlier detection of disease, improve prediction of disease severity, and assist in therapeutic assessments and interventions. On the other hand, there are other matricellular proteins that significantly influence various pathologic processes. Understanding the crucial role of the ECM and its proteins may lead to the development of interventions to cease and hopefully reverse inflammation, in order to prevent the progression to carcinogenesis. The microenvironment mediators continue to provide us abundant opportunities for study. As our understanding of the ECM in disease becomes clearer, it will continue to open doors towards important clinical implications to better combat human diseases.

Conflict of interests

The authors declare no conflict of interests.

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