

Review article

The aspects of CCN protein family on breast cancer

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ABSTRACT:

CCNs are specific type of matricellular proteins, which are essential signaling molecules, and play multiple roles in multicellular eukaryotes. This family of proteins consists of six separate members in mammals. The architecture of CCN proteins is multimodular and comprises four distinct motifs. CCN proteins achieve their specific physiological functions by binding to integrin receptors. The CCN family has been implicated in both cure and disease with impacts on biological interactions, such as cell adhesion, chemotaxis and migration, mitogenesis, cell survival, angiogenesis, differentiation, tumorigenesis, immune functions, chondrogenesis, and wound healing. Breast cancer is the most commonly diagnosed cancer worldwide and the leading cause of cancer mortality among women triggered by atypical expression of CCNs. A favorable or unfavorable association between various CCNs has been reported in patients with breast carcinomas. Aberrant expression of CCN1 intensifies the proliferation of epithelial cells that line the lobes and ducts of the breast. Evidence also shows that the expression of CCN2 can ameliorate tumor growth and metastasis. However, CCN3 (NOV), CCN5 (WISP-2), and CCN6 (WISP-3) are consistent with neoplastic development and metastasis repression. Particular CCN members can develop tumors and cancer progression, whereas others can competitively counter the processes. Several studies have been conducted on CCN proteins and cancer in recent years. In our study, we intend to provide an overview of those research works while keeping breast carcinoma on focus. We believe that the importance of the CCN protein family in breast cancer should be reconsidered.

Keywords: Breast cancer; CCN proteins; Metastasis; Tumorigenesis

INTRODUCTION

Members the CCN family are matricellular proteins that are present in the extracellular matrix (ECM). Unlike most other ECM proteins, they serve a regulatory role instead of structural [1]. The CCN family comprises six homologous proteins (CCN1–CCN6). They share a conserved motif and mosaic structure, yet serve diverse functions, with CCN5 as the only exception [2]. The first three (CCN1-CCN3) of CCN proteins provided the acronym CCN [3]. The other members included WISP-1 to WISP-3 (Figure 2). Breast cancer is the most frequently reported cancer, and the leading cause of cancer-related mortality in women globally [4]. The human breast is composed of lobes that produce milk and ducts that carry the milk to the nipple, and all of these are surrounded by muscle and fat tissues [5]. Ductal carcinoma and lobular carcinoma are the predominant types of breast cancer. However, carcinomas of the connective tissues surrounding the lobe and ducts are not defined as breast cancer, as they are not unique to the breast. Furthermore, invasive ductal carcinoma is more aggressive than invasive lobular carcinoma [6][7][8].

Since CCN proteins have diverse functionality, their involvement in cancer and breast cancer, in particular, lacks uniformity. CCN1 and CCN2 are closely associated with cell proliferation and migration, and thus, can contribute directly to tumorigenesis and tumor development [9][10]. Increased expression of CCN1 and CCN4 is associated with breast cancer metastasis through the lymphatic vessels that surround the lymph nodes connecting the breast in the armpit [11][12]. CCN3, CCN5, and CCN6 play an antiproliferative role, where their expression can inhibit breast cancer growth, and their absence can induce carcinoma [13][14][15][16]. In breast carcinoma, the positive or negative relationship of CCNs (CCN1–CCN6) has been specified in this review. The association of CCN proteins with numerous activities indicates that any alterations or malfunction of CCN protein activity can trigger a cascade of events in human cells. This information would help in designing drugs and therapeutics targeting CCN proteins in breast cancer.

NOMENCLATURE

The six matricellular proteins (CCN1–CCN6) belong to the CCN protein family (Figure 1). Several genes encoding the CCNs are documented to be composed of four motives (cysteine-rich) with a well multimodular structure (conserved). It is estimated that the N-terminal motif bears the consensus sequence (GCGCCXXC), which is highly conserved and has IGF-binding ability. Additionally, this sequence is followed by an N-terminal signal peptide [17][18][19][20]. The multimodular organization implies that CCNs can act reciprocally with other proteins to exercise similar functions. CCNs' biological functions may be dependent on the availability of interacting substrates and proteins. This would also describe the peculiar biochemical consequences and the wide spectrum of pathological and physiological roles of the members of the CCN family. The CCN protein family is known to mediate epithelial-stromal cross-talk. Therefore, they interact with some key signaling molecules, especially Notch1 and cell surface integrins [19][21]. The position of CCN proteins is well documented in the ECM, although a variety of experiments have shown that some of them may function intracellularly or have uncertain properties [22].

CCN GENE FAMILY

The six different members of the CCN gene family include Cyr61 (CCN1), CTGF (CCN2), NOV (CCN3), WISP-1 (CCN4), WISP-2 (CCN5), and WISP-3 (CCN6) [11][19]. Several biological functions, such as cell growth, ECM regulation, cell adhesion, migration, wound healing and angiogenesis, and diseases including fibrosis, cancer and vascular diseases are controlled by expression of the CCN gene family [23][24]. CCN proteins are not growth factors; but, they can modify the signaling of other molecules associated with the ECM [25]. Cyr61 (CCN1) is an angiogenic ECM-associated inducer that functions as an integrin receptor ligand, and its expression is closely related to incidence of breast cancer. CTGF (CCN2, connective tissue growth factor) was initially recognized as a protein secreted by human endothelial cultured cells. The protein encoded by the CCN2/CTGF gene is a mitogen associated with platelet-derived growth factor. NOV expression was found to be an integrative site for various types of tumors. CCN4 (WNT1) is a matricellular protein with a cysteine-rich motif.

The WNT1 gene is involved in tumor development through the activation of β -catenin, and is considered to be a novel target against toxic cell degeneration. WNT1 is activated in the Wnt/ β -catenin signaling pathway, mostly as a controller that is correlated with cellular function [26][27].

CCN5, previously known as WISP-2, rCop-1, COP-1, HICP and CTGF-L, can perform a regulatory role by reducing epithelial-to-mesenchymal transition (EMT) in both breast and pancreatic cancer cells. In noninvasive breast cancer cell lines, several genetic exposures disrupt CCN5, which is responsible for invasive cell phenotypes [14]. It was detected as a heparin-induced gene, and acts as a growth-arresting gene in vascular smooth muscle cells (SMCs) [28]. CCN6/WISP3 is rich (IGF) in the binding protein domain. Additionally, the WISP3 gene plays an important role in mitochondrial electron transport system [29].

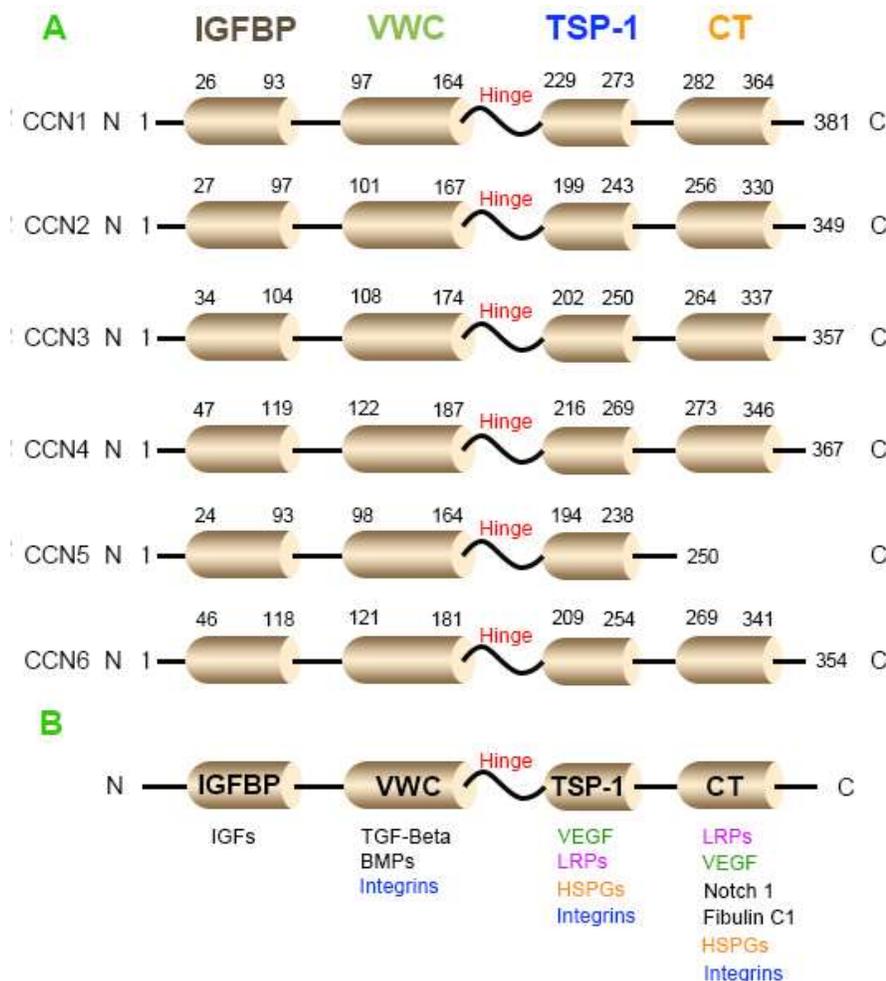


Figure 1: (A) CCNs protein structure. CCNs provides a comparative estimate for the position of four structural areas (CT, TSP-1, VWC, and IGFBP). There is a hinge area separating two N-terminal fields and two C-terminal domains, except for CCN5 (WISP-2). (B) CCNs four domains and their supportive attaching allies. Integrins can attach to the domains CT, VWC, and TSP-1; LRP, HSPGs, and VEGF can adhere to the TSP-1 and the CT domain.

MOLECULAR MAKEUP OF CCN

The molecular makeup of CCNs is conserved among members and various species. CCNs have four exclusive modules with noteworthy structural characteristics and minor exceptions [30]. The IGF-IGFBP (insulin-like growth factor binding protein-like), von Willebrand factor type C (VWC), thrombospondin 1 (TSP1), and cysteine-knot-containing (CT) are named as Modules I, II, III, and IV, respectively (Figure 2). These modules are accompanied by a peptide of an N-terminal that is correlated with signal secretion. The characteristics of the CCNs are highly dependent on the order of the Modules. These Modules contain hydrophobic, polar, and cysteine residues [31]. CCN5 is the only exception to the alignment of these Modules in the CCN family, as it lacks the fourth CT Module. The CCNs' cysteine ratio is comparatively larger, about 10% by mass, containing 38 cysteines separated into 17 retained zones, and extended across four fields. Some dissimilarities have been observed between CCN5 (WISP-2) and CCN6 (WISP-3). CCN5 has no CT domain as it lacks 10 cysteine amino acids [32]. The two N-terminal Modules (I and II) and two C-terminal Modules (III and IV) of the CCN proteins are separated by a linker with a variable amino acid composition [33]. Modules II and III are connected by the “hinge region,” which is responsive to cleavage by proteinases [21]. A conserved exon encodes these structural modules, and the CCN genes are speculated to be products of exon shuffling [34].

The types of binding partners and ligands depend on several molecular structures of the functional domains of the CCN protein. The binding partners are specific to domains: IGFs attach to IGFBP; BMPs, TGF- β , and integrins interact with VWC; VEGF, HSPGs, LDL receptor proteins (LRPs), and integrins interact with TSP-1, and VEGF, integrins, LRP, Notch1, fibulin-1C, HSPGs, and integrins interact with CT (Figure 2) [2] [34] [35]. Thus, their action, half-life, bioavailability, binding to other protein moieties, and

regulation time of the CCN portion is directly associated with the modular configuration [3]. Structural heterogeneity in modular configurations also facilitates their various functions.

CCN RECEPTORS

CCN proteins exert their functions in two different ways: interaction with cell surface receptors and with receptor ligands. Studies of CCN proteins at the molecular level show that extracellular signal-regulated kinases (ERKs), phosphoinositide 3-kinase (PI3K), and small GTPases of the Rho family act at the activation site to trigger the signaling cascades in the cellular context [36][37].

Endeavors have been made to discover particular receptors of CCN proteins. CCNs can interact with unique and specific signaling receptors. They are capable of binding to particular integrin proteins via individual domains, some of which are distinguished based on peptide inhibition studies [38][39]. Kireeva et al. first showed that CCNs can attach to α -v β -3 integrin proteins [40]. The integrins can bind with α - v β - 3, α - 5 β - 1, α - 6 β - 1 [41]; but, it is unclear whether CCN family members exhibit preferential binding to alpha or beta integrin subunits [42]. CCN1 and CCN2 are ligands of integrins alpha-II beta-3 in the platelets and alpha-M beta-3 in monocytes [38].

CCN1 and CCN2 also bind to heparan sulfate-containing proteoglycans (HSPGs) through a heparin-binding domain [43]. CCN2 interaction with tyrosine kinase is also reported [44]. CCN3 may function by direct binding to integrin receptors and other receptors such as NOTCH1 and fibulin-1C (FBLN1).

Apoptosis in fibroblasts is induced by CCN1 (Cyr61); syndecan 4 and integrin are required for this process [45][46][47][48]. CCN2 can also attach to low-density lipoprotein (LDL) receptor-related protein 1 (LRP1) via Module III, and LDL-receptor related protein 6 (LRP6) via the CT domain [49][50][51]. Integrin and HSPGs are functional receptors of CCN4; and, they can also bind to decorin and biglycan [41][52].

POSSIBLE ROLE IN TUMORIGENESIS AND BREAST CANCER

CCN proteins are involved in different aspects of cancer development. Individual CCN proteins may also act differently in many cancer types via various signaling pathways, even though they share similar structures. Some of them have already been found to be

cancer inducers. CCN proteins can modify the signals of several proteins, including integrins, Wnt, and transforming growth factor- β (TGF- β). These protein functions are also related to tumorigenesis or metastasis, and studies indicate their association with several cancer risk factors. Chronic inflammation is a major risk factor [35][53]. Deregulated healing of lesions is also a risk factor in cancer progression. In the early stages of neoplastic transformation, inflammation is responsible for speeding up the development of primary neoplasia into cancer by inducing mutagens, such as reactive oxygen species [36]. Aberrant expression of CCN proteins is responsible for the regulation of diverse inflammatory mediators, including TGF- β , prostaglandins, and ECM enzymes. Moreover, estrogenic influence, pro-inflammatory adipokines such as tumor necrosis factor-alpha (TNF α), and leptin are also reported to be tumor-associated biochemical risk factors [53]. In breast tumors, both the steroid-dependent proteins—CCN1 and CCN2—are overexpressed; these proteins are also estrogen-inducible [54]. Estrogenic GPR30 signaling promotes the proliferation and migration in breast cancer cells via CCN2 [55]. CCN family members play a crucial role as downstream mediators of estrogen- and progesterone-regulated cell proliferation [54]. A recent study has shown that CCN1, CCN2, CCN3, and CCN4 are pro-tumorigenic and may be responsible for human breast carcinoma, while CCN5 and CCN6 have anti-tumorigenic effects (Figure 2). Additionally, abnormal expression of CCN proteins increases proliferative activity and induces tumor formation [11][56].

CCN1: CYR61

Aberrant Cyr61 expression has been observed in various classes and categories of cancer [57]. Studies have indicated overexpression of Cyr61 in approximately 30% of breast carcinomas than in normal breast tissues. This correlation was particularly valid for estrogen receptor (ER) positive–HER-2/neu negative tumors [58]. CCN1 exerts positive effects on tumorigenicity, which are distinguished by decreased proliferation of normal cells, consequently leading to epithelial hyperplasia or sclerosing adenosis. After developing carcinoma, several changes in cellular functions can be recognized, such as an elevated expression of oncogenes (e.g., c-Myc), significant drop in the expression of tumor-suppressor genes (e.g., p53, Rb), modifications in cell structure such as increase

in expression of vimentin, loss of cell adhesion that may implicate E-cadherin and integrins, and overexpression of angiogenic factors (e.g., VEGF, FGF). CCN1 is overexpressed in rapidly dividing breast cancer cells. Increased mRNA expression of Cyr61 in estrogen-induced MCF-7 cells indicates their involvement in estrogen-mediated breast cancer pathogenesis. Aggressive human breast carcinoma cells can bypass estrogen (E2) requirement for growth, which is the most familiar pathological evolution during breast cancer development [57]. Exposure to 17 β -estradiol influences Cyr61 production in MCF-7 breast cancer cells. Epidermal growth factor (EGF) can also upregulate Cyr61 in MCF-7 cells, suggesting a synergistic regulation of Cyr61 in the initiation of cancer formation. In addition, Cyr61 can induce estrogen-independence and anti-estrogen resistance to generate an invasive breast cancer phenotype [53]. In primary breast tumors, progesterone may be a novel facilitator in progesterone receptor (PR)-positive, ER-negative breast cancer [11]. Activity of ErbB-2/3/4 receptor signaling pathway, stimulated by growth factor heregulin (HRG), is correlated more frequently with aggressive breast cancer. Studies have revealed that breast cancer progression can be induced by HRG, along with the reduced function of estrogen receptor (ER) and response to E2, invasion, and metastasis. Cyr61 is also defined as a gene associated with HRG-induced invasive breast carcinoma, as it is expressed in MDA-MB-231 human breast cancer cells. Higher expression of Cyr61 is correlated directly with HRG overexpression and inversely with ER expression [57]. Quantitative PCR analysis identified considerably higher levels of CCN1 in tumor tissues than in normal tissues. CCN1 was significantly expressed in TNM stage-3/4 tumors than in TNM stage-1 tumors (P $\frac{1}{4}$ 0:05 and 0.106, respectively) [56]. CCN1 is a transcriptional target of Hh-GLI signaling that results in extended vascularity and spontaneous metastasis in breast cancer cells. Additionally, elevated levels of CCN1 were associated with lymph node metastases and a worse chance of recovery in breast cancer patients. Overexpression of CCN1 in MCF7 cells resulted in an increase in tumor size and vascularization of xenografts, as documented in nude mice. In vitro, estradiol mediated mRNA and protein from CCN1 in MCF7 cells were induced, and CCN1 was adequate to produce antiestrogen resistance and estradiol independence [56][58]. These studies concluded

that CCN1 was implicated in the progression to more advanced stages of breast cancer [11]. Therefore, CCN1 is currently known as one of the potential targets for chemotherapy against breast cancers [30].

CCN2: CTGF

Increased CCN2 expression leads to increased breast cancer metastasis [59]. Jiang et al. (2004) performed an analytical study of the mRNA and protein levels of CCN2 in 122 human breast tumor samples. The study determined that patients with overexpression of CCN2 have better breast cancer prognoses than those with low CCN2 expression [56]. Further, ectopic aberrant expression of CTGF in a breast cancer cell line stimulated angiogenesis and migration, and the CT domain of CTGF was critical for migratory capability [53][60]. CCN2 is related to osteolytic metastasis of breast cancer via the PKA- and PKC-dependent activation of ERK [59][61]. Induction of GPR30 signaling by estrogen or hydroxytamoxifen (OHT) in ER-negative human breast cancer cells activates the transcription factor network, which corresponds to that stimulated by serum in fibroblasts. Potential CTGF genes appear to be targets for these transcription factors. It has been shown that CTGF stimulates cell proliferation and mediates GPR30-induced cell migration. The stimulation of GPR30 by OHT also induces CTGF in fibroblasts obtained from breast tumor biopsies, which may be related to the development of invasive breast tumors in response to endogenous estrogens or administration of OHT used for endocrine therapy [55]. Moreover, an *in vivo* study documented that neutralization of CCN2 using CCN2-specific antibodies reduced bone metastasis [60].

CCN3: NOV

CCN3 is involved in tumor development, which was amongst the first member to be identified of the CCN family [2]. The various biological features responsible for the Nephroblastoma gene (Nov) represent the regulatory action of this growth that extends beyond the normal response to growth stimulation [19]. The antiproliferative protein CCN3 could be an oncogenic or tumor suppressor depending on the type of tumor. CCN3 gene may be transcriptionally upregulated by the tumor suppressor p53 [53]. A marginal and non-significant reduction in expression of CTGF has been observed in TNM stage-2/3/4 tumors than in TNM stage-1 tumors. The changes in the levels of Nov

transcripts were similar to those of CTGF, except that TNM stage-4 tumors showed significantly lower levels of Nov than TNM stage-1 tumors ($P = 0.0048$) [56]. Further, any change in CCN protein structure or expression is associated with cancer development; CCN3 is associated with tumorigenesis in non-solid cancer types. Gilmour et al. reported that CCN3 is downregulated in human chronic myeloid leukemia (CML) cell lines and primary CML cells than in normal bone marrow cells. CCN3 expression is regulated by BCR/ABL kinase in CML cells. Decreased levels of CCN3 were found in patients with CML, consistent with elevated expression of BCR/ABL kinase. Recovered patients showed normal CCN3 expression after they returned to health. Adding different proteins that combine with CCN3, C. Naus presented results established that CCN3 physically interacts with the C-terminus of Connexin 43 (Cx43). The association between Cx43 expression and reduced tumorigenicity provides a possible explanation for the role of CCN3, in which its full-length expression correlates with a growth-suppressed phenotype in numerous cell systems including glioblastoma, choriocarcinoma, and Ewing's sarcoma. Cx43 and CCN3 in glioblastoma cells were shown to colocalize at the plasma membrane. A study by Koeffler et al. has established that CCN proteins can act as oncogenes or tumor suppressors, depending on the type of cancer considered [62]. Additionally, CCN3 is the most studied CCN protein for tumor typing and prognosis [2].

CCN4: WISP-1

Limited literature related to CCN4 indicates that it has pro-tumorigenic features and functionality in breast cancer. In breast cancer samples, the expression of CCN4 mRNA was increased compared to that in standard breast cancer. High and low CCN4 mRNA levels in breast cancer patients were associated with poor outcomes [12][63]. CCN4 overexpression has facilitated the development of MCF7 tumors in xenograft studies [64]. In breast carcinoma, WISP-1 levels were lower in tumor cells than in normal breast tissue, which were comparatively less than in more invasive tumor types. In contrast, in invasive breast cancer, enhanced expression of WISP-1 is related to oncogenic transformation [53].

CCN5: WISP-2

CCN5 plays an antitumorigenic role in breast cancer. CCN5 expression in breast cancer is weak in the antagonistic lines of breast cancer cells. Inhibition of CCN5 in invasive breast cancer cell lines, such as MDA-MB-231, reduces cell proliferation and invasion [65]. Expression profile of CCN5 changes during proliferation and progression of invasive breast cancer. Expression of CCN5 is less in normal breast tissue, and it increases in breast cancer lesions [66]. When noninvasive breast cancer progresses to an invasive type, the expression of CCN5 mRNA and proteins is reduced by genes such as Snai1, MMP-2, and MMP-9 [22]. WISP-2 can also act as a tumor suppressor. Studies have shown expression of WISP-2 to be predominant in preneoplastic disorders, such as atypical ductal hyperplasia and noninvasive ductal carcinoma in situ (DCIS). Knockdown of WISP-2 in MCF-7 cells induced estrogen-independent growth, which was associated with decreased ER α expression and increased EMT. Moreover, triple-negative (ER-/PR-/HER2-) breast cancer cell lines are also observed to be a result of decreased WISP-2 expression. In ER-negative breast cancer, glucocorticoid-induced upregulation of WISP-2 results in reduction in cellular proliferation and invasive phenotype [53].

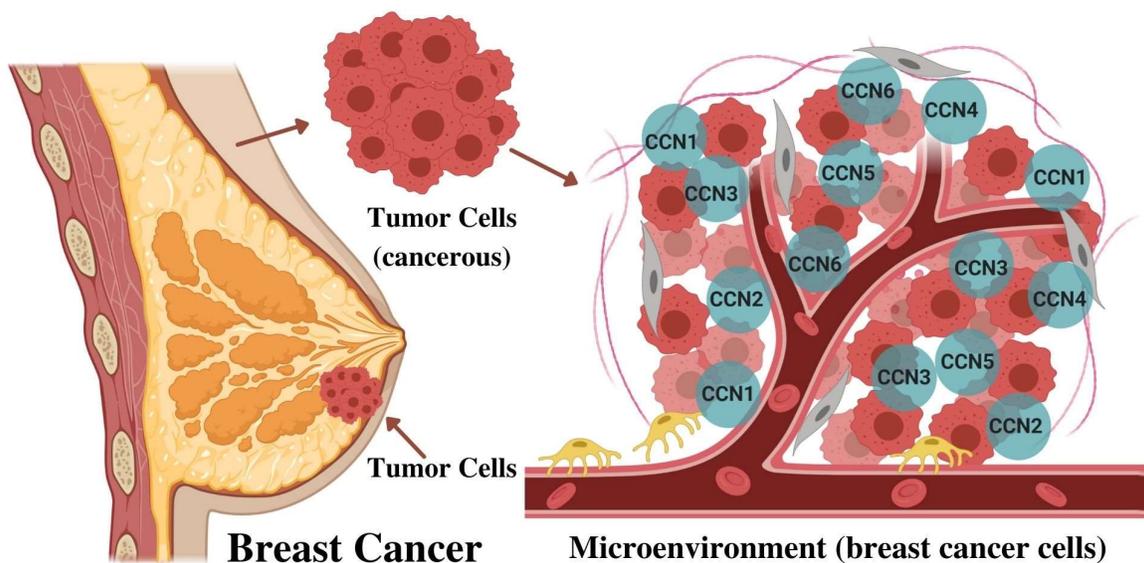


Figure 2: Role of CCN proteins in breast cancer progression or suppression. In microenvironment, the CCN proteins of CCN1/Cyr61, CCN2/CTGF, CCN3/Nov, and CCN4/WISP1 induced tumor, whereas CCN5/WISP2 and CCN6/WISP3 have tumor-suppressing action. Depending on tumor nature, CCN3 and CCN4 may play a dual role in both tumor-promoting or suppressing action.

CCN6: WISP-3

CCN6 plays a crucial role in tumor suppression in certain cancers, including breast cancer. WISP-3 is expressed in the normal mammary epithelium, but is downregulated or absent in some invasive carcinomas. One study found that its levels decreased significantly in invasive breast cancer, especially in breast cancer with axillary lymph node metastases and inflammatory breast cancer. WISP-3 is important for maintaining normal epithelial morphology in breast cancer cells. In human mammalian epithelial cells, knockdown of WISP-3 leads to resistance to anoikis and increases anchorage-specific growth. Overexpression of WISP-3 decreases invasion and distant metastasis in breast cancer cells, while its downregulation disrupts acinar morphogenesis and cell invasion in propagated mammary epithelial cells [53]. In breast cancer, CCN6 mRNA levels were reduced by 80% with poor outcomes, which was necessary to induce the EMT. Ectopic expression of CCN6 in an inflammatory breast cancer cell line led to reduced cell invasion and proliferation *in vitro* and cell growth *in vivo* [16]. Lorenzatti et al. (2011) provided a practical exhibition and explanation of low CCN6 expression in antagonistic breast cancer cells [67]. Moreover, the CCN6 protein is considered a newer regulator of the epithelial phenotype in breast cancer [68]. CCN6 can also act as a tumor suppressor in breast carcinoma [35]. Based on the study by Klier et al., CCN6 protein has recently been shown to play an important role in the development and progression in breast cancer [69].

CONCLUSION

The role of CCN proteins is studied in development, angiogenesis and fibrosis, but there is convincing evidence of their critical and vital role in breast tumorigenesis. The expression profiles of CCN proteins vary in the breast tissue. It is evident that numerous cellular functions are regulated by CCNs. Even though all six members of CCNs share the same protein structure, their role is strictly regulated [59]. Breast cancer often

develops from several sources such as an E2-dependent, non-metastatic, antiestrogen-sensitive phenotype to an E2-independent, antiestrogen-resistant, highly invasive, and metastatic phenotype [70]. Thus, a single member of the CCN family can be oncogenic or tumor-suppressive depending on the nature of the breast tumors [53]. Furthermore, deregulated expression of CCN proteins is frequently involved in tumorigenesis and cancer progression. While the expression of CCN proteins varies in different cancer types, CCN1, CCN2, CCN3 and CCN4 participate in tumor development and act as oncogenes, but CCN5 and CCN6 inhibit tumor growth and play tumor-suppressive roles in breast carcinomas [59]. However, more than a decade of laboratory research has shown that CCN6 plays an inhibitory role in the growth, metastasis, and invasion in breast cancer [16][67][71][72][73]. CCN3 and CCN4 sometimes exhibit a dual nature, either oncogenic or tumor suppressive. The same CCN protein can perform different functions during cancer formation, maintenance, and progression. Accurately examining the expression of the whole CCN protein family at different stages of breast cancer provides a clear idea of their significance. This understanding would provide a basis for the development of new biomarkers to improve early detection and prognosis of the disease, and assist in clinical assessment and intervention. Nevertheless, other extracellular matrix proteins significantly affect various pathogenesis. Understanding the importance of ECM and its proteins may help avoid interventions aimed at preventing carcinogenesis and reverse inflammation. Mediators of the microenvironment continue to provide opportunities to important clinical implications for a better fight against breast cancer [53]. Moreover, their roles in angiogenesis, tumor growth and other diseases are important areas for future research, and a complete understanding of their involvement in these processes may suggest novel therapeutic strategies [9].

Authors contribution

This work was a collaboration among all the authors. ASMS, TAH, HAF, and KAA designed outlines and drafted the manuscript. ASMS, KAA, MS, AM, TAH, and SKP wrote the initial draft of the manuscript. MJU, MAR, and HAF reviewed the scientific contents described in the manuscript. All authors read and approved the final submitted version of the manuscript.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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