REVIEW

CCN family proteins and angiogenesis: from embryo to adulthood

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Abstract The CCN family is a novel class of extracellular signal modulators that has been recently established. Typical members are composed of four conserved modules connected tandem, each of which is rich in cysteines and highly interactive with other molecules. The mammalian CCN family consists of six members, most of which have been described as multifunctional factors for the developmental process of mesenchymal tissue including blood vessel formation/induction. Particularly, the angiogenic properties of the three classical members, CCN1, 2 and 3 have so far been characterized, and their physiological and pathological significance has thus been indicated. Recent research has uncovered a unique mechanism regarding these proteins in promoting and/or modulating developmental, physiological and pathological angiogenic events. Namely, CCN proteins exert their ability to drive angiogenesis, not by stimulating a particular behavior of a particular type of cells, but by manipulating the cell communication networks that integrate most of the associated molecules/cells toward angiogenesis. In this article, the role of the CCN proteins in a variety of angiogenic events as an organizer of microenvironmental cell society is comprehensively described, together with a brief summary of the recent findings on each CCN family member relevant to angiogenesis including cardiovascular development and diseases.

Introduction

Angiogenesis is a critical procedure that is involved in a variety of biological events in vertebrates [1]. First and foremost, the formation of the placenta and cardiovascular system are definitely indispensable for mammalian embryonic development, which thus leads to fundamental organogenesis [2]. After development, growth of most of organs and tissues is supported by the expanding blood stream network. In adult animals, even though continuous angiogenesis may no longer occur, physiological angiogenesis is readily initiated as a part of the normal biological response, where additional oxygen, nutrition or cellular infiltration is demanded. Angiogenic events occur under the collaboration of vascular endothelial cells and other types of cells of various origins in the microenvironment. During this process, several molecules that are known as angiogenic factors, such as vascular endothelial growth factor (VEGF), play the role of the key commander [2, 3]. Under the signals emitted by such commanders, a vast number of different extracellular matrix (ECM) components, such as adhesion molecules, and tools for the reconstruction of ECM, including proteinases, are produced by those cells. Then blood vessels are finally manufactured at the requested site based on a properly organized ECM structure. Obviously, in order to establish such an integrated construction, certain molecules have to organize the information from the commander and

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then have to assist the tissue components to be placed correctly. We thus consider that the CCN proteins should be managing these difficult tasks [4–8].

The first member of the CCN family emerged in 1989 under the initial name of Cyr61, because of its cysteine-rich primary structure [9]. Two years later, the second member was identified as a secreted protein with mitogenic and chemotactic activities for fibroblasts from human vascular endothelial cells, and thus it was designated connective tissue growth factor (CTGF) [10]. Then, the next year, a nephroblastoma-overexpressed protein (NOV) with significant homology to Cyr61 and CTGF was identified as a truncated form in a chicken nephroblastoma [11]. Three genes encoding structurally similar proteins are necessary and sufficient to make up a gene family. As such, the initial word "CCN" was born and given to this family of genes in 1993 based on a proposal by Bork, by assembling the initials of the three classical members in order [4]. Thereafter, three additional members were newly reported by 1998 with the proper names totally irrelevant to the family name, CCN [12–15]. However, since most of the initial names given to each member were gradually revealed to be contradictory and misleading, a unified nomenclature was thus proposed in 2003 under the agreement of several major researchers in the CCN research field [16]. Today, all of the CCN family members are usually represented by a unified name, such as CCN1 or CCN2, rather than by classical names.

The CCN family proteins: multi-handed modulator of the extracellular information network

The primary structure of the CCN family proteins is quite unique. The basic organization of these proteins is, in a sense, a relatively simple one that consists of four conserved modules aligned in order. From the N-terminus, the insulin-like growth factor binding protein (IGFBP) module, von Willebrand factor type C repeat (VWC) module, thrombospondin type 1 repeat (TSP1) module and carboxyl-terminal (CT) module, which is absent only in CCN5, are connected in tandem, while no other functional domain exists [4–8]. Interestingly, all of these four modules are highly interactive with other proteins and proteoglycans [17–25]. Furthermore, these modules are encoded by single separate exons on the mRNAs, thus indicating that this gene family has evolved through exon shuffling, and thus each module is structurally independent from the others. This structure is as unusual as a tool

composed of four hands or adaptors without any other devices. Such a tool may appear awkward, however, if duly used, it can be useful in a variety of situations. In fact, the CCN proteins exert multiple functions in different biological situations [26–33], which sometimes yields an apparently contradictory biological outcome induced by the same CCN protein under different conditions. For example, CCN2 was shown to induce apoptosis when it was overexpressed in breast cancer cells [34], while it is also known as a promoter of metastasis in the same type of cells in vivo [35, 36]. Therefore, even though the association with malignancies has been reported in all six members of the CCN family, the role of these proteins in carcinogenesis and malignant phenotypes still remains controversial [11, 12, 15, 37–45]. In terms of the development of mesenchymal tissue, most of the CCN family members have been shown to act as a promoter of cell growth and differentiation [26, 31–33, 46–49], with a few exceptions such as CCN3 with myoblasts [22]. In the case of the best-characterized CCN2, this factor is known to promote the proliferation and differentiation of fibroblasts, chondrocytes, osteoblasts and periodontal ligament cells as well as vascular endothelial cells [18, 26, 31–33, 48, 49]. How can a CCN protein perform such multiple jobs? The answer may be found in the novel modular structure of this family. Using its four (three for CCN5) conserved modules, a CCN protein is able to interact with multiple molecules in the microenvironment, thus resulting in the modulation of the extracellular molecular network therein. The molecular counterparts of the CCN proteins include adhesion molecules [5, 18, 21, 28, 50], cell surface signal transducing receptors [24, 25], proteoglycans [19, 28, 51] and even growth factors [19, 20]. CCN proteins may therefore also be called an integrator/modulator of extracellular information. The angiogenic effects of the CCN proteins are also the outcome of the negotiations with other molecules through the communication via four modules (Fig. 1).

General mechanism of the angiogenic action of the CCN family proteins

According to the phenotypes of conventional KO mice of each CCN family member [56–58], although few members turn out to play a critical role in embryonic vascular development, no CCN family member may be defined as a master molecule of angiogenesis. In fact, even in the CCN1-KO with the most severe defect in angiogenesis, the formation of the blood vessels itself
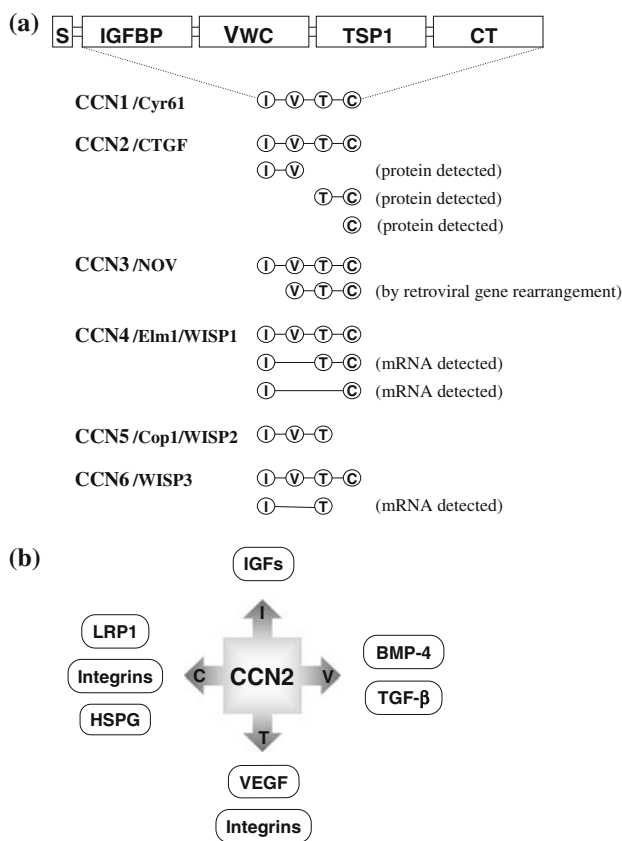
Fig. 1 (A) The primary structure of the CCN family proteins and their reported variants. The general structure is composed of four conserved modules is illustrated as a nascent translation product with a signal peptide for secretion (S) at the top. The module names abbreviated here are fully described in the text. The modules are then further abbreviated into a single letter when describing the structure of each member or its variant. Namely, "I", "V", "T" and "C" represent IGFBP, VWC, TSP1 and CT modules, respectively. In addition to the names under the unified nomenclature, a few classical and well-known names are also presented. As briefly noted in the figure, CCN2 variant proteins were reported by Brigstock et al. [52] Kubota et al. [53], Hinton et al. [54] and Boes et al. [55]. The N-terminal truncated form of CCN3 was identified in nephroblastomas by Joliot et al. [11]. In the cases of CCN4 and CCN6, variants were confirmed at mRNA level, which were generated through the alternative mRNA splicing [40–42]. (B) Multiple molecular interactions by the CCN proteins as exemplified by CCN2. In this panel also, each module is indicated by a single letter abbreviation, as explained above. The abbreviation HSPG stands for heparan sulfate proteoglycans. All of the other abbreviations are explained in the text

was distinctly observed until placental blood vessel fusion [58], thus representing its role rather as a developmental modulator.

The development of the embryonic vascular system is initiated under the signals mediated by bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs), as is generally observed in a number of morphogenetic events. In response to these signals, angioblasts and hematopoietic cells construct blood islands and vessels at proper temporal and spatial locations, which are regulated by a number of growth factors, such as VEGF, transforming growth factor (TGF)-β, platelet-derived growth factors (PDGFs), angiopoietin, sonic hedgehog (Shh), and ephrins, together with their receptors [2]. Of note, several CCN family members are known to interact with these growth factors [17, 20] or regulate the gene expression of some of them, and vice versa [39, 59]. As a result, these CCN family members are anticipated to modulate the effects of these growth factors toward the proper development of the cardiovascular system.

The other critical function of the CCN protein in angiogenesis is probably exerted under the interaction with the extracellular matrix (ECM) molecules and cell-surface adhesion molecule. This functional aspect of these CCN proteins may be metaphorized by a molecular scaffold for ECM construction. By interacting with integrins, fibronectins and other proteins and proteoglycans, several CCN proteins have been shown to promote adhesion and migration of various types of cells, including vascular endothelial cells (Table 1) [18, 28, 46, 47, 51]. In this context, the role of integrins in determining target cells for the CCN proteins is of particular interest, since several distinct subunits are differentially displayed onto the cells that are associated with angiogenic events. In addition to these two major mechanisms, several CCN proteins are capable of inducing the production of proteins that are critical for ECM remodeling which accompanies the physiological and pathological angiogenesis [27, 36] (Fig. 2).

The angiogenic property of each CCN family member

Until today, the angiogenic properties have been analyzed and reported for the three classical members. Concerning the other three members, CCN4, 5 and 6, no clear evidence has yet been presented so far. First, in this section, the current knowledge related to angiogenesis is summarized for CCN1, 2 and 3 individually (Fig. 3).

CCN1/Cyr61

CCN1 is not only the first identified member of the CCN family, but also the first member to have been described as an angiogenic factor. In 1998, Babic et al. reported the positive effect of CCN1 on angiogenesis and tumor growth [60]. Utilizing a baculovirus vector

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

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

[a] vascular endothelial cells, [b] vascular smooth muscle cells, [c] not determined

system to produce recombinant CCN1, they found that CCN1 was capable of enhancing migration and adhesion of microvascular endothelial cells in vitro, and inducing neovascularization on cornea in vivo. These effects were efficiently inhibited by the addition of CCN1-specific antibody, thus confirming the specificity of the observed effects. However, in order to obtain angiogenic effects comparable to basic fibroblast growth factor (bFGF), CCN1 of much higher concentration was required than bFGF. This dose-discrepancy might be due to either lower activity of the recombinant CCN1 than the intact one, or different mechanism of CCN1 in exerting angiogenic effects from that of bFGF.

The mechanism of the CCN1 action to enhance neovascularization by the promotion of migration and adhesion is mainly mediated by integrins (Fig. 1). Integrins are cell surface adhesion receptors that consist of two subunits, α and β. The α subunit is associated with divalent cations, whereas the β subunit contains a talin-binding domain, and thus is thought to transmit extracellular signals into the cells by activating focal adhesion kinase (FAK) [61]. In addition to the CCN family members, a number of different proteins have been identified as ligands for integrins. Interestingly, although most of the other ligands interact with integrins with a conserved peptide motif, arginine–glycine–aspartic acid (RGD), no such peptide sequence has been identified in the integrin binding sites in CCN1 [5, 7]. For CCN1-induced angiogenesis, integrin $\alpha_v\beta_3$ was initially shown to mediate the molecular interaction to realize the biological outcome [61]. A subsequent investigation with umbilical vein endothelial cells indicated that the angiogenic action mediated by integrin $\alpha_v\beta_3$ required its activation, whereas another integrin $\alpha_6\beta_1$ constitutively supported the early proangiogenic events induced by CCN1 [62]. Furthermore, a recent report has described the collaboration of CCN1,

integrin $\alpha_6\beta_1$ and heparan sulfate proteoglycans to be able to promote the adhesion and chemotaxis of vascular smooth muscle cells [63]. Therefore, CCN1 is expected to play a significant role in organizing endothelial cells to construct almost all types of blood vessel structures. In addition to such integrin-mediated effects, CCN1 is known to promote the proliferation of vascular endothelial cells in an ECM-dependent manner in collaboration with other signaling molecules. Namely, owing to the strong affinity of CCN1 to heparan sulfate proteoglycans, it was found to displace another ECM-associated growth factor, such as bFGF, which eventually enhanced the proliferation of those cells [64].

Consistent with the findings above, conventional *ccn1* KO mice that were created in 2002 displayed severe defects in angiogenesis during embryonic development [58]. The KO embryos suffered from defect in chorioallantoic fusion and placental vascular inefficiency. Such phenotypic changes were the most severe among all of the CCN KO mice analyzed, thus indicating angiogenesis to be a major mission of CCN1. In addition, since the observed vascular malformation was characterized by a specific defect in the vessel bifurcation related to the local impairment of VEGF gene expression, the collaboration of VEGF and CCN1 may thus be critical for the proper embryonic vascular development [58].

CCN2/CTGF

It is worthy of note that human CCN2 was originally discovered in, and purified from the conditioned medium of cultured vein endothelial cells [10]. Thereafter, following the characterization of CCN1 as a proangiogenic protein, the angiogenic potential of CCN2 was uncovered by two research groups independently. In 1998, Shimo et al. reported that knocking

down the *ccn2* expression resulted in the suppression of the proliferation and migration of normal vascular endothelial cells [48]. The next year, two articles directly describing the angiogenic activity of CCN2 in vitro and in vivo were published [18, 48]. Indeed, integrins were again revealed to be critical functional counterparts of CCN2 [18]. Furthermore, the CCN2-binding integrins known today are almost the same as those binding to CCN1 [7]. As expected, the enhanced migration and survival of endothelial cells by CCN2

were shown to be mediated by integrin $\alpha_v\beta_3$ [18]. The binding site in CCN2 to this particular integrin was located in the CT module, comprising a peptide sequence of NH_2–IRTPKISKPIKFELSG–COOH, which does not contain any RGD motif of integrin binding. As such, CCN2 is believed to promote angiogenesis via the same mechanism as that of CCN1 [7]. However, their roles in embryonic angiogenesis are not the same, which is probably due to the four-dimensional difference in their gene expression patterns as realized
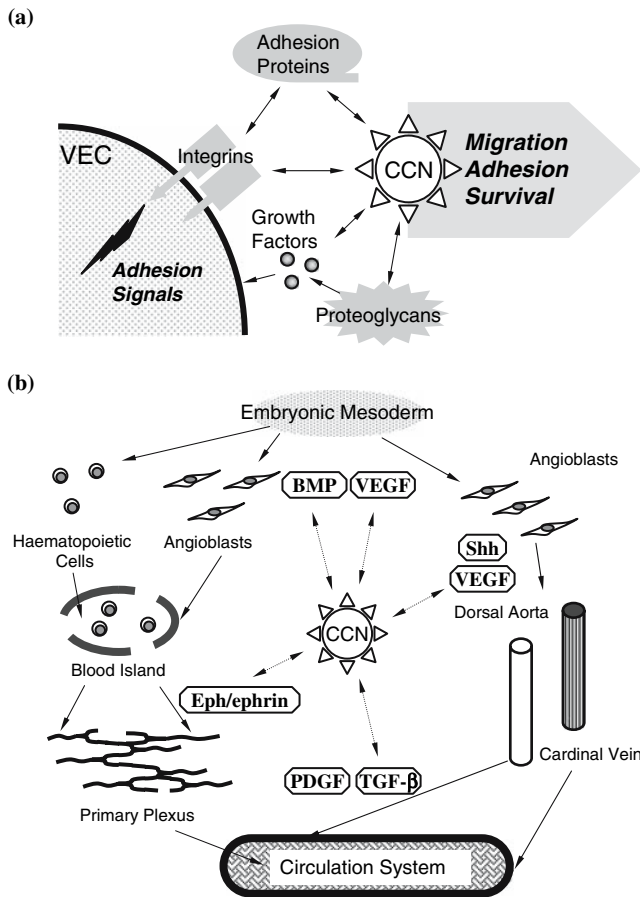
(a)

(b)

Fig. 2 (**A**) The general mechanism of angiogenic action of the CCN proteins. Through the interaction with integrins, angiogenic CCN proteins, such as CCN1, CCN2 and CCN3, promote the migration, adhesion and survival of vascular endothelial cells. CCN proteins are also known to bind to several ECM components including proteoglycans and adhesion proteins, which furnishes microenvironmental basement for the neovascularization by modulating the ECM architecture. The function of other growth factors is occasionally modulated by the direct interaction with these CCN proteins. The abbreviation ''VEC'' stands for ''vascular endothelial cell''. (**B**) The hypothetical roles of CCN proteins in the regulation of the development of embryonic cardiovascular systems at early stages. Based on the findings obtained in vitro, the possible interactions of CCN proteins with the signaling molecules involved are illustrated. All of the full designations of the abbreviated names can be found in the text. Eph is the receptor of ephrin
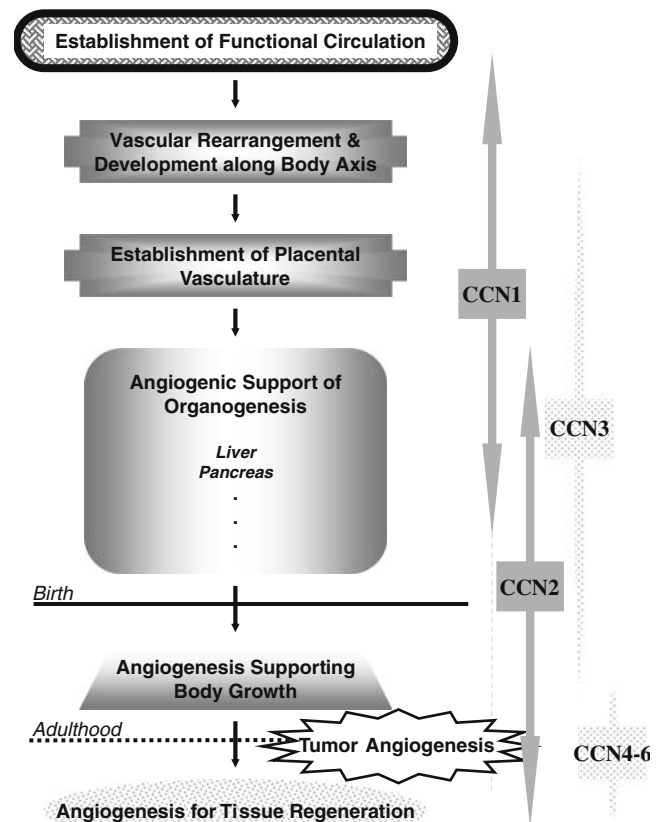

Fig. 3 Assignment of the functioning stages for angiogenic CCN proteins in the angiogenic events throughout the life. According to the accumulating findings, CCN1 appears to play a critical role in the earlier stages of embryonic vascular development, while its function at later stages, such as tissue regeneration, may not be ruled out. In contrast, CCN2 is required for the angiogenic events at later stages of development and it is obviously involved in the regeneration of various tissues. Although no strict requirement of CCN3 in particular angiogenic events has yet been specified, it is plausible that CCN3 supports the CCN1 and CCN2 function in certain aspects of angiogenic events during the development and maintenance of the life. The angiogenic roles of CCN4, 5 and 6 still remain to be investigated further, since an association of these proteins with malignancies has been suggested

by different gene regulatory systems. It should be also noted that CCN2 is processed into subfragments containing the CT module, which retains biological activities at least in part [65]. The functional significance of these subfragments still remains to be addressed, but they may represent a CCN2-specific biological role that is not assigned to CCN1.

The involvement of CCN2 in embryonic vascular development was also represented in the phenotypic changes in conventional CCN2 KO mice, albeit at a less striking level than in the CCN1 KO mice [56]. In CCN2 KO mice, no profound defect was observed in placental vascular development, and thus embryos usually survive until delivery. However, these CCN2 KO mice are also lethal upon delivery, probably due to the functional failure caused by a skeletal defect. A histological examination revealed the endochondral ossification process that determines the growth of most bones to be perturbed, which was characterized by the defect of vascular invasion into terminally differentiated layers of chondrocytes. Such phenotypes represent a critical role of CCN2 not only in cartilage growth, but also in angiogenesis during endochondral ossification. Endochondral ossification continues until the end of body growth. Therefore, CCN2 remains as a critical angiogenic factor for the skeletal growth of humans for longer than 10 years, but the task of CCN2 is not yet over, even after that.

Accumulating evidence has indicated that CCN2 is a major CCN family protein that promotes wound healing and tissue regeneration, which is also supported by the fact that CCN2 gene expression is temporally and spatially regulated during foliculogenesis and luteogenesis [66]. Moreover, although CCN2 is dispensable for embryonic vascular fusion to placenta, it is believed to facilitate the capillary development from maternal vessels in the placenta [30, 67], thus indicating that CCN2 also plays a role in mammalian reproduction.

In addition, in the case of CCN2, a novel role as an angiogenic modulator as well as a proanigiogenic molecule has been suspected, which is based on an investigation of the molecular interaction of CCN2 and VEGF [20]. According to the report by Inoki et al., when CCN2 co-existed, the VEGF-induced angiogenesis was rather repressed, although CCN2 is capable of inducing angiogenesis to some extent per se, which was based upon the direct interaction of CCN2 with VEGF, using the TSP1 and CT modules as interface. Of note, CCN2 and VEGF are capable of inducing gene expression in each other in vascular endothelial cells [7, 39, 59]. Considering the role of VEGF as a critical player in angiogenic events, the role of CCN2 as a modulator of the major information network is thus herein emphasized. Finally, one should not overlook the ability of CCN2 to promote the synthesis of ECM components, such as collagens, which are needed for the construction of a vascular bed that is prerequisite for neovascularization [65].

CCN3/NOV

As expected by the structural similarity with the other two classical CCN family members, the pro-angiogenic effects of CCN3 were investigated and reported in 2003, in which CCN3 was entitled "angiogenic regulator". The mechanism of angiogenic action is also quite similar to CCN1 and 2, which is mediated by integrins $\alpha_v\beta_3$, $\alpha_6\beta_1$ and $\alpha_5\beta_1$, together with heparan sulfate proteoglycans [47]. Since the amino acid sequence of CCN3 does not contain any RGD motif, interaction of CCN3 and these integrins are carried out with proper peptide motif(s), as observed in CCN1 and CCN2. Unexpectedly, conventional KO mice of CCN3 appeared to display no distinct phenotypic abnormalities (Katsube et al., personal communication), thus representing the functional redundancy of the classical three members in embryonic vascular development and body growth. Nevertheless, it is still possible for CCN3 to act as a critical angiogenic modulator at particular biological situations, where both CCN1 and CCN2 are unavailable.

CCN4/Elm1/WISP1, CCN5/COP1/WISP2 and CCN6/WISP3

To date, although the involvement of CCN4 in mesenchymal tissue development has been described, no evidence has yet been reported concerning its angiogenic activity. In contrast, interesting findings in relation to vascular biology have been reported in the case of CCN5. Surprisingly, CCN5 was found to rather inhibit the proliferation of vascular smooth muscle cells (Table 1) [68]. Interestingly, CCN5 is the only member that lacks the CT module. Since the CT module generally acts as an interface for binding to proteoglycans, this inhibitory effect may thus be regarded as a *trans*-dominant effect on the ECM-associated action of CCN proteins. Nevertheless, the last CCN family member, CCN6, was shown to act as an antagonist of tumor angiogenesis, albeit the retention of the CT module [69]. These apparently contradictory findings may thus be due to the different experimental conditions and cellular background employed, or they may represent other mechanisms of action than the ones hypothesized above.

The ccn family members in the development of cardiovascular system

According to the molecular histochemical analysis of developing embryos, a few CCN family member genes are significantly expressed during cardiovascular development. Particularly, ccn1 and ccn2 gene expression appears to be prominent in the mesenchymes of developing cardiovascular tissues and/or the developed vasculature, thus indicating a certain role as a modulator of the ECM therein [58, 66, 67]. However, no CCN family member has yet been shown to be fundamentally essential for the initial development of the cardiovascular system itself. Even in the CCN1 KO mice, the primary vascular plexus was found to form normally. The vascular plexus next develops into a specified vein and artery leading to the establishment of circulatory flow, where Notch signaling plays a critical role. Interestingly, even though CCN1 KO mice showed dorsal aorta formation, the arterial wall was abnormally fragile and thus frequently caused hemorrhaging in the KO embryo. Together with the failure of chorioallantoic fusion and labyrinth formation in placenta observed in these mice, CCN1 is believed to be responsible for the vascular integrity, particularly in the arteries [58]. It is therefore anticipated that CCN1 should be required to organize the ECM network throughout the embryonic vascular development. Unexpectedly, CCN3 appeared to be dispensable for cardiovascular development, although it is known to interact with Notch molecules during embryonic development [22]. After the establishment of the arterial and venous fate, the vascular plexus has to be rearranged, following the morphological program, which is mediated by signaling molecules including Shh and BMP (Fig. 2). CCN2 is known to interact with several of such signaling molecules, but it appeared to be unnecessary for this process, as it is represented by no obvious malfunction in the cardiovascular system of CCN2 KO mice, at least before delivery. Collectively, the differential roles of the three functionally similar proangiogenic CCN family members in the development of cardiovascular system can be summarized: CCN1 is the primary modulator of the specialized ECM structure in cardiovascular development, whereas CCN2 is produced as a supplemental molecule that can partially substitute the role of CCN1. In spite of its angiogenic activities, CCN3 may not be critically involved in this particular biological process, as indicated by no distinct evidence for gene expression therein and no abnormal phenotypes associated with the KO mice.

Role of the CCN proteins in tumor angiogenesis, progression and metastasis

It is a critical issue for tumors to induce neovascularization in establishing the supply of nutrition and oxygen toward further growth, and also in preparing the entrance leading to metastasis. Therefore, the angiogenic potential of a neoplasm is one of the determinants of its malignant phenotypes, and it is represented by the quality and quantity of the angiogenesis-associated factors released from the tumor cells [1]. The CCN family proteins have been attracting the interest of a number of oncologists from two different viewpoints. The first one is based on the above-mentioned angiogenic effects of the three classical members, whereas the second arose from the association of certain malignancies with the overexpression of normal or truncated forms of particular members. The possible involvement of the truncated forms caused by alternative splicing or retroviral gene modification in a few animal and human malignancies has been indicated in CCN3, 4 and 6 [11, 41–43]. In the case of the full-length intact CCN proteins, a number of studies investigated the relationship of their gene expression and malignant phenotypes, and most of those found that CCN proteins themselves rather repress the proliferation and invasiveness of the tumor cells [34, 38, 40, 70]. However, a few studies have also described a significant relationship between the metastatic phenotypes and the CCN family member gene expression in vivo [35, 45], which can be ascribed to their angiogenic effects acted in both paracrine and ECM-associated (matricrine) manners.

Among the angiogenic CCN family members, the role in tumor angiogenesis has been best characterized in CCN2. Along with the substantial growth of solid tumors, the tumor cells deep inside of the tumor suffer from hypoxia. The hypoxic induction of angiogenic factors is thus a critical property that allows the tumor to grow further. It is already known that VEGF is induced by hypoxia via the induction of hypoxia inducible factor 1 (HIF1) which transactivates the vegf gene transcription [3]. Interestingly, it has been recently uncovered that CCN2 is induced by hypoxia through different regulatory systems. The production of the CCN2 molecule is enhanced by hypoxia through multiple steps except for mRNA transcription. In terms of gene expression, hypoxic stress increases the level of ccn2 mRNA without changing the transcription activity of the ccn2 gene [36, 71]. The steady-state mRNA level is determined by the balance of the formation and degradation of the mRNA. The hypoxic signal controls

the latter step to prolong the half-life or *ccn2* mRNA, which results in the net increase of the *ccn2* gene expression [71]. This post-transcriptional regulation is mediated by a *cis*-acting element of structure-anchored repression (CAESAR) in the 3′-untranslated region (UTR) of the *ccn2* mRNA [71–73]. The other mechanism of the hypoxic overproduction of CCN2 is a post-translational one that is based on the ECM-associated behavior of CCN2. After hypoxia, the CCN2 trapped in the ECM is rapidly released, which confers an apparent increase in the secretory production of CCN2 [36, 71]. This event is thought to be mediated by the induction and activation of matrix remodeling enzymes, such as matrix metalloproteinases [36]. Consequently, CCN2 is now regarded as a central mediator of hypoxia-inducible angiogenic factor of certain malignancies. It should be also noted that CCN2 has recently been described as one of the critical mediators that determine the highly metastatic phenotype in breast cancers [35].

Cardiovascular disorder and the CCN proteins

Before the unification of the terminology, CCN2 was widely known as connective tissue growth factor (CTGF). In pathological point of view, the name, CTGF, deserves to represent the effect of CCN2, since CCN2 is associated with fibrotic disorders of a variety of organs and tissues [74–77]. The involvement of CCN2 in cardiac fibrosis has also been evaluated [78], and a significant relationship has been identified between the plasma CCN2 level and the cardiac function parameters of patients with heart failure, such as myocardial infarction (Koitabashi et al., in preparation). These findings suggest the utility of CCN2 as a diagnostic and prognostic marker for heart failure, which is one of the most frequent adult diseases in advanced countries. Arteriosclerosis is another major clinical complexity of adult humans. The prognosis of this disorder highly depends on the fragility of the arterial wall that is deranged by the formation of the atherosclerotic plaque. The initial finding that CCN2 is highly expressed in advanced atherosclerosis lesions was reported in 1997 [79]. An increased expression of CCN1 was also observed in the atherosclerotic vessels but it appeared to be angiotensin II dependent [80]. Notably, the accumulation of CCN2 in the plaque has also been observed as well [81]. The pathogenic role of this accumulation of CCN2 is unclear, but it was shown to stimulate monocyte/macrophage migration in vitro [23]. Since the atherosclerotic plaque is formed by the accumulation of cholesterol in oxidized low-density

lipoprotein (LDL) taken up by macrophages, these findings should therefore not be overlooked. In addition, CCN2 is a possible mediator of aortic fibrosis by a distinct pathway. Further investigations with human aortic endothelial cells showed CCN2 to be produced by an increased blood flow [82] and by stimulation with oxidized LDL though TGF-β [83]. Probably, CCN2 is also engaged in the regeneration of damaged cardiovascular tissue, but it ends up insufficient; hence fibrous tissue replaces the normal functional tissue after all. Therefore, minor therapeutic modifications of such regenerative action of CCN2 toward a better direction may potentially improve the states of those diseases.

Conclusions and prospects

Embryonic angiogenesis is the most fundamental step leading to organogenesis. A classical CCN family member, CCN1, is a critical modulator of the extracellular components in constructing most types of integrated vascular architecture throughout the establishment of the cardiovascular system. A similar role is assigned to another classical member, CCN2, in a different developmental process. Based on the accumulated data in vitro and the phenotypic changes observed in the KO mice, CCN2 is now believed to be such a central modulator of extracellular signaling and molecular architecture in the endochondral ossification process. Endochondral ossification is the most fundamental step for skeletalogenesis in vertebrates, which remains active until the onset of adulthood. After body growth is over, CCN2 continues to play a central role in modulating regenerative events and angiogenesis upon request [84, 85], which sometimes is advantageous for the metastasis of tumors. CCN1 was also indicated to mediate the regeneration of particular tissues [86]. Therefore, both CCN1 and CCN2 are engaged in similar missions in different mesenchymes, following different genetic programs encoding the proper temporal and spatial gene expression (Fig. 3). Since CCN3 is also angiogenic and it is expressed in developing mesenchymes, it is therefore expected to be a central modulator of the extracellular microenvironment of certain tissues. However, specific tissues in which CCN3 plays a critical role still remain to be identified. The same hypothesis can be proposed for the other CCN family members. Research in the future may help to clarify the roles CCN4, CCN5 and CCN6 in a particular cell society as a molecular modulator of the extracellular network.

From a clinical point of view, tumor angiogenesis is one of the major targets of chemotherapy of malig-

nancies [87]. Unlike vascular development, the major modulator of this process has been shown to be the CCN2 molecule, which is thus now regarded as a potential target of anti-angiogenic therapy [88, 89]. In fact, a recent study has shown that an anti-angiogenic agent was capable of inhibiting the ccn2 gene induction by VEGF in human vascular endothelial cells [90]. All of the CCN family members are kept silent in adult animals after development and body growth. Therefore, an anti-CCN strategy may provide a safer choice without adverse effects than those with other agents, such as metabolic antagonists. In particular, CCN2 is involved in tumor angiogenesis, atherosclerosis and cardiac fibrosis, which constitute the pathological background of all of three major diseases in human society of advanced countries. Millions of patients may thus be awaiting the advance of CCN research regarding the fields of cardiology, angiology and oncology.

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