Published in final edited form as:

Cell Mol Life Sci. 2011 October ; 68(19): 3209–3217. doi:10.1007/s00018-011-0782-7.

Roles for CCN2 in normal physiological processes

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Abstract

CCN2, also known as connective tissue growth factor, is a member of the CCN (CCN1-6) family of modular matricellular proteins. Analysis of CCN2 function *in vivo* has focused primarily on its key role as a mediator of excess ECM synthesis in multiple fibrotic diseases. However, CCN2 and related family members are widely expressed during development. Recent studies using new genetic models are revealing that CCN2 has essential roles in the development of many tissues. This review focuses on current and emerging data on CCN2 and its functions in chondrogenesis and angiogenesis, and on new studies showing that CCN2 has essential functions during embryonic and postnatal development in a number of epithelial tissues.

Keywords

CCN2; CTGF; connective tissue growth factor

Introduction

CCN2, also known as connective tissue growth factor (CTGF) is a member of the CCN (CCN1-6) family of modular matricellular proteins [1]. Analysis of CCN2 function *in vivo* has focused primarily on its key role as a mediator of excess extracellular matrix (ECM) synthesis in multiple fibrotic diseases. However, CCN2 and related family members are widely expressed during development, and modulate cell adhesion, proliferation, survival, migration, and extracellular matrix production in diverse cell types. A role for CCN2 as an essential regulator of chondrogenesis during development has been established, but recent studies have provided new insights into the mechanisms of CCN2 action in cartilage. Other studies are revealing that CCN2 has essential roles in the development of many other tissues. This review focuses on current and emerging data on CCN2 and its functions throughout embryonic and postnatal development.

CCN2 Structural Domains and Functions

CCN2 and the five other members of the CCN family (CCN1/Cyr61, CCN3/Nov, CCN4/WISP-1/ELM1, CCN5/WISP-2/CTGF-L and CCN6/WISP-3) are cysteine-rich matricellular proteins that contain an N-terminal secretory peptide, followed by four multi-functional domains with a diverse array of binding partners that potentially impact multiple signaling mechanisms [2]. Proteins that interact with CCN2 through recognition of these domains include integrins, low-density lipoprotein receptor-related proteins (LRPs), growth factors, and ECM components (Fig. 1). The first domain shares homology to insulin-like growth

factor binding proteins (IGFBPs), but has very low affinity for IGF [3]. The second domain encodes a von Willebrand type C (VWC) repeat. This motif mediates CCN2 interactions with integrins $\alpha v\beta 3$, $\alpha v\beta 5$ and with growth factors such as bone morphogenetic proteins (BMPs) and transforming growth factor β (TGF β) [1,4,5,6]. Recently, the IGFBP and VWC domains, which comprise the N-terminal half of CCN2, have been shown to bind to aggrecan, the major proteoglycan produced by chondrocytes [7]. The third domain is a type-1 thrombospondin (TSP) repeat, known to mediate the ability of TSP to bind to ECM proteins, matrix metalloproteinases (MMPs), and integrins. This domain modulates CCN2 interactions with VEGF and LRP1 [6,8]. The final C-terminal (CT) motif contains a cysteine knot similar to those present in many growth factors, including members of the TGF β super family, platelet derived growth factor (PDGF), and nerve growth factor (NGF). This domain is also found in other secreted proteins, including WISE, slit, and mucins [9,10,11]. It mediates CCN2 interactions with LRP6, fibronectin, perlecan, and fibulin-1 [12,13,14].

Interactions between CCN2 and these binding partners mediate the effects of CCN2 on cell proliferation, survival, differentiation, adhesion, migration, and ECM production [15]. However, to date, none of the *in vivo* activities of CCN2 have been unequivocally attributed to any of these specific interactions. This is due in part to the fact that the *in vivo* roles of CCN2 in development and homeostasis have not been completely defined, and because CCN2 most likely mediates its effects through multiple mechanisms that are tissue specific.

CCN2 Expression during embryonic and postnatal development

CCN2 is conserved in chordates but is not found in invertebrates. Where studied, its pattern of expression appears to be largely conserved. For example, in zebrafish, *Xenopus*, and mouse, CCN2 expression is first detected in somites, floorplate, and notochord [16,17,18]. The role of CCN2 in these tissues is essentially unknown. In zebrafish, the injection of morpholinos against CCN2 led to developmental delays and distortion of the notochord. However, the nature of these defects has not been clarified, and they are not seen in *Ccn2* null mice or in *Xenopus* embryos treated with morpholinos [1,12,18,19]. CCN2 is co-expressed with the related family member CCN1 in the notochord (unpublished data), raising the possibility of overlapping functions for these CCN proteins in early stages of notochord formation.

CCN2 is expressed in a wide variety of structures at later stages of development. In the mouse, CCN2 is expressed at high levels in the developing cartilage and vasculature [18,20,21]. Other prominent sites of expression include various epithelial structures, such as bronchial and alveolar epithelium of the lung, pancreatic duct and endocrine cells, and salivary glands [20,22]. As discussed below, recent studies indicate that CCN2 plays essential roles in the development of many of these structures. CCN2 is also expressed in maturing neurons, and in sensory organs such as the otic placode [6,16,17,20,23]. To date, the functions of CCN2 in neural and sensory structures have not been investigated.

CCN2 expression is maintained in adult tissues, and is increased in pathological conditions such as fibrosis, atherosclerosis, osteoarthritis and certain cancers [24]. In the adult mouse, CCN2 is expressed in the reproductive system, where it has been implicated in control of uterine cellular growth, adhesion, migration, and ECM production during the estrous cycle and pregnancy [25].

CCN2 and fibrosis

In vivo studies of CCN2 function have focused almost entirely on its role in fibrosis. Fibrosis is caused by excessive extracellular matrix (ECM) deposition mediated by activated

contractile fibroblasts termed myofibroblasts [26]. CCN2 over-expression is a hallmark of fibrosis in multiple tissues, including skin, liver, heart, lung, and kidney, and is widely thought to be required to mediate the profibrotic effects of TGF β . However, the extent to which CCN2 causes fibrosis, (or is merely a marker of fibrosis in different organs), is unknown. A number of excellent reviews on this topic have been published recently [15,27,28]. Hence, we focus here on the development of several new transgenic models of CCN2 over-expression, which have revealed unexpected actions of CCN2 in the onset and progression of fibrosis *in vivo*.

The expression of CCN2 during normal wound healing and in various fibrotic diseases [26,29] has prompted many studies of CCN2 function in wound healing and in chemically induced fibrosis [26]. However, *in vivo* studies have been limited, in part because suitable transgenic models have not been available. Mice over-expressing CCN2 in podocytes [29] or liver [30] are normal. Increased fibrosis was observed in these transgenic models only upon tissue injury. These findings may indicate that CCN2 alone is not sufficient to induce fibrosis in these organs. An alternative explanation is that levels of CCN2 expression were not high enough to induce fibrosis. A new transgenic model, described below, suggests the latter possibility may be true with respect to the kidney.

Mice over-expressing CCN2 under the control of the fibroblast-specific enhancer of type I collagen (Col1-CCN2) develop systemic multi-organ fibrosis. Excess ECM production was noted in dermis, kidney and lung. Immunostaining revealed a large increase in the number of myofibroblasts, and consistent with previous *in vitro* studies, fibroblasts from transgenic mice exhibited increased migratory capability and excessive ECM production [31]. Unlike the podocyte-specific transgenic mice discussed above [29], basement membrane thickening and increased ECM deposition around blood vessels were noted in the kidneys of Col1-CCN2 transgenics. Consistent with the notion that the difference in susceptibility to kidney fibrosis between these two transgenic models relates to CCN2 levels, these effects were dose-dependent, as mice carrying two copies of the Col1-CCN2 transgene insertion had a shorter lifespan and earlier onset of fibrosis than did mice heterozygous for the transgene insertion [31].

Recently, Liu et al. generated a conditional mouse model in which CCN2 was deleted specifically in fibroblasts and smooth muscle cells [32]. Consistent with the findings that CCN2 over-expression in fibroblasts induced systemic fibrosis [31], loss of CCN2 resulted in a marked decrease in bleomycin induced-skin fibrosis [32]. Remarkably, these effects were not TGF β dependent, nor did the loss of CCN2 impact the ability of TGF β to induce α -smooth muscle actin (α -SMA) or collagen type I expression [32]. The attenuated fibrosis in *Ccn2* conditional mutants was attributed to defective myofibroblast migration and recruitment to the site of fibrosis [32]. Thus, these gain- and loss-of-function studies suggest that CCN2 is sufficient to induce fibrosis, and challenge the dogma that CCN2 is required to mediate the pro-fibrotic effects of TGF β .

The Col1-CCN2 transgenic phenotype described above contrasts with that seen in transgenic mice in which CCN2 expression from the endogenous locus can be increased or decreased [31,33]. This latter mouse model was developed by introduction of a 3'UTR cassette into the *Ccn2* locus that alters *Ccn2* mRNA stability relative to the native *Ccn2* 3'UTR. Cre-mediated recombination excises this 3'UTR and replaces it with one that yields increased *Ccn2* mRNA stability compared to the native *Ccn2* 3'UTR. This novel strategy, when used in conjunction with *Ccn2* null mice, permits analysis of the effects of altering *Ccn2* expression from 30% of normal to up to nine-fold higher than normal [33]. Mice with 30% of normal *Ccn2* expression were indistinguishable from wild type littermates, whereas nine-fold over-expression led to developmental delay, craniofacial defects, and early embryonic

lethality [33]. The cause of lethality was not investigated in this study, but both the time of lethality and the presence of hemorrhage and altered microvasculature in transgenic over-expressers suggests a cardiovascular defect. The few over-expressers that survived to adulthood exhibited moderate cranial and axial skeletal defects, but no fibrosis. However, these surviving mice were found to exhibit incomplete Cre-mediated recombination [33]. While the present study demonstrates that CCN2 has potent effects during development, it was not informative with respect to adult tissues. However, when used in conjunction with tissue-specific inducible Cre alleles, this transgenic model is likely to be highly informative with respect to CCN2 function in adult tissues that normally express this protein.

Transgenic models have led to unexpected insights into CCN2 function in the heart. Although CCN2 is highly expressed in the developing heart, it is unknown whether CCN2 is required for cardiac development. CCN2 is also markedly elevated during cardiac fibrosis, and can stimulate cardiac smooth muscle cell proliferation [34]. Two recent papers reported the generation of transgenic mice over-expressing CCN2 in the heart [35,36]. Surprisingly, neither study found any evidence of fibrosis, either basally, or in response to cardiac ischemia/reperfusion injury or acute pressure-overload. In the first model, there was no fibrosis, but induction of an adaptive hypertrophic response that may have contributed to the finding that transgenics were no more susceptible than wild type littermates to ischemia/reperfusion injury [35]. In the second transgenic model, although CCN2 transgenic hearts exhibited increased myocardial pro-collagen and fibronectin expression, this was counteracted by increased MMP3 levels [36]. When challenged with cardiac ischemia/reperfusion injury, CCN2 over-expression was actually cardio-protective [36].

The discrepancies between these models may be a consequence of the different promoters used. Both alpha-myosin heavy chain (α -MHC) and myosin light chain-2 (MLC-2) are expressed early in developing cardiomyocytes, but as development proceeds, α -MHC expression is confined mainly to the atria, whereas MLC-2 is expressed predominantly in the ventricle [37]. These promoters may also be differentially regulated during cardiac hypertrophy. Nonetheless, these results clearly indicate that CCN2 is not always profibrotic, and can have protective functions in adult tissues. Studies to elucidate the mechanisms underlying these cardioprotective functions are clearly warranted.

Overall, the effects of CCN2 over-expression appear to be both dose-dependent and context specific. While the above models have confirmed the expected contribution of CCN2 to the onset and/or progression of fibrosis in several tissues in adults, they have led to the discovery of protective functions in others. Given the vast number of growth factors, ECM components, integrins, and other receptors that can interact with CCN2 *in vitro*, it is likely that CCN2 can exert very different functions at different doses within a given tissue.

CCN2 function in development

The most prominent sites of CCN2 expression in developing embryos are the skeletal and cardiovascular systems [20,22,38], and expression in these tissues persists in adults [39]. Essential functions for CCN2 have been demonstrated in multiple aspects of skeletogenesis and angiogenesis. Other recent studies are revealing roles in epithelial tissues. We review some of the major findings from *in vivo* studies below.

Lung

Both gain- and loss-of-function studies support an essential role for CCN2 in lung formation. Similar to the effects of CCN2 over-expression in hepatocytes, CCN2 adenoviral over-expression in the adult mouse lung is unable on its own to induce chronic fibrosis, but does increase susceptibility to fibrosis following chemical treatment [41,42]. In contrast,

CCN2 over-expression in airway epithelium [43] or alveolar type II epithelial cells [44] during the first two weeks of postnatal life in transgenic mice led to thicker alveolar septa, characterized by increased cell proliferation, collagen and fibronectin deposition, myofibroblast differentiation, and fibrosis [43]. Increased levels of integrin-linked kinase (ILK) activation were noted, raising the possibility that the phenotype reflects the ability of CCN2 to engage integrins directly, and/or to increase the expression of other ECM components that bind to these integrins. Moreover, increased stabilization and nuclear translocation of β -catenin, a well-known target of ILK [45] that mediates fibrosis in multiple organs (eg, [46,47,48]) was seen in CCN2 transgenic lungs. These findings, along with the TGF β -independent ability of CCN2 to induce fibrosis found in the Col1-CCN2 transgenics discussed previously [32], suggest that CCN2 may mediate its pro-fibrotic effects to a significant degree through Wnt pathways. A number of studies have shown that CCN2 expression is induced by canonical Wnt signaling [49,50]. The demonstration that CCN2 over-expression in turn induces stabilization of β -catenin raises the possibility that CCN2 is an essential component of a positive feedback loop regulating levels of canonical Wnt signaling.

A loss-of-function study also provides strong support for an essential role for CCN2 in alveolarization during lung maturation [51]. Examination of *Ccn2* null mice revealed hypoplastic lungs, with reduced cell proliferation, a phenotype opposite to that seen in the above transgenic over-expression models. This study did not examine the consequences of loss of CCN2 on canonical Wnt pathways. However, the authors reported decreased expression of PDGF receptor beta (PDGFR β), which is primarily expressed by pericytes [51,52]. Whether the decreased expression of PDGFR β in *Ccn2*^{-/-} lungs translates to altered levels of PDGF-mediated signal transduction is unknown. However, an investigation of the role of CCN2 in PDGF signaling is warranted, especially in light of the potent pro-fibrotic properties of PDGF [53].

Skeletal tissues

CCN2 appears to have essential functions in multiple skeletal tissues. CCN2 function has been studied most extensively in cartilage, but recent studies have demonstrated roles in osteoblasts, osteoclasts, intervertebral discs, and cranial sutures.

Cartilage—Endochondral bone formation is a dynamic process in which mesenchymal cells condense and differentiate, resulting in the formation of a growth plate. Chondrocytes in the growth plate are arranged in distinct zones, which include a slowly proliferating reserve population, the rapidly proliferating columnar population, followed by a post-mitotic prehypertrophic zone and finally a terminally differentiated hypertrophic zone. Hypertrophic chondrocytes produce a matrix that subsequently promotes vascular invasion and osteoblast migration, leading to replacement of the cartilage template with bone [54,55].

CCN2 is not expressed in mesenchymal condensations, but can be detected at the earliest stages of growth plate formation [18]. CCN2 is expressed in proliferating chondrocytes at low levels, and at higher levels in hypertrophic cells [38]. The expression of *Ccn2* in growth plate chondrocytes was shown recently to be positively regulated by canonical Wnt pathways via a TCF/LEF motif in the *Ccn2* promoter [50]. This finding accounts for the high levels of expression of CCN2 in hypertrophic chondrocytes, which contain the highest levels of stabilized β -catenin in the growth plate [50].

Ccn2 null mice exhibit lethality at birth, accompanied by severe chondrodysplasia [18]. *Ccn2*^{-/-} chondrocytes exhibited decreased rates of proliferation and survival. These defects were attributed to defective production of multiple ECM components, including type II collagen and aggrecan, the major collagen and proteoglycan, respectively, in cartilage

[18,56]. While the precise mechanisms by which CCN2 mediates its essential role in maintenance of ECM production in the growth plate are unknown, integrins are likely to play key roles. CCN2 is a ligand for integrin $\alpha 5 \beta 1$ in chondrocytes, and this integrin is important for chondrocyte adhesion and survival [56]. Moreover, the loss of CCN2 results in a significant decrease in $\alpha 5$ integrin levels and in levels of activation of the downstream effectors of integrins, including focal adhesion kinase (FAK) and extracellular regulated protein kinase [57] in chondrocytes [56,58].

One of the most unexpected findings in *Ccn2* null mice is the expansion of the hypertrophic zone. This was accompanied by decreased expression of VEGF in these cells [18]. VEGF is essential for growth plate angiogenesis [59], and *Ccn2*^{-/-} growth plates exhibit defective vascularization of the growth plate. Thus, a key function for CCN2 in the hypertrophic zone is to maintain VEGF levels. We investigated the mechanisms by which CCN2 induces VEGF expression in chondrocytes. Hypoxia inducible factor-1 α (HIF-1 α) is expressed by chondrocytes and required for chondrocyte survival [58]. It is also a potent inducer of VEGF in many tissues. We found that HIF-1 α levels are decreased in the growth plates of *Ccn2* mutants; similar results are seen in isolated chondrocytes where recombinant CCN2 induces HIF-1 α , demonstrating that the effects of CCN2 on HIF-1 α are direct in chondrocytes [60]. The mechanisms underlying CCN2-mediated regulation of HIF-1 α are unknown.

While the above results demonstrate that CCN2 is essential for chondrogenesis during development, whether it is required for maintenance of cartilage in adults is an important unanswered question. We have analyzed CCN2 expression in postnatal cartilage using a BAC transgenic line in which green fluorescent protein (GFP) is expressed under the control of the *Ccn2* locus [61] (Fig. 2). In 1-2 month old mice, CCN2 is expressed in the intervertebral disc (Fig 2A), a derivative of the notochord (which also expresses CCN2) in the mouse. It is also expressed in all appendicular and axial cartilaginous elements, with the highest levels of expression in hypertrophic zone of the growth plate in both axial (rib) and appendicular elements (Fig. 2B,C). CCN2 expression persists in articular cartilage, suggesting an essential role in cartilage maintenance in adults [62].

On the one hand, CCN2 is expressed in articular chondrocytes and can stimulate articular cartilage repair and regeneration *in vivo* [62]. On the other hand, CCN2 levels are elevated in osteoarthritic (OA) cartilage [62,63], and adenoviral over-expression of CCN2 in knee joints leads to transient fibrosis and cartilage damage [63]. These disparate outcomes most likely reflect different levels of CCN2 expression, which in turn, may lead to different levels of HIF activity. For example, low levels of HIF-1 α are anabolic for cartilage [64]. However, HIF-2 α promotes aspects of hypertrophy in the growth plate, and OA in articular cartilage [60]. It is thus conceivable that low levels of CCN2 are required to maintain levels of HIF activity that promote anabolic functions in growth plate and articular cartilage, but that the higher levels of CCN2 found in the hypertrophic zone and in OA cartilage promote HIF-2 α expression, leading to cartilage damage.

Bone—CCN2 also appears to have effects on bone formation *in vivo*. This was initially demonstrated by Kawaki et al. using *Ccn2* null mice [65]. Owing to neonatal lethality, this analysis did not address the role of CCN2 in adult bone remodeling. However, *Ccn2* null mice were shown to exhibit a reduction in bone matrix synthesis, osteoblast proliferation, maturation and mineralization *in vivo*. At least some of these effects are likely to be direct, as opposed to a secondary consequence of defective endochondral ossification, as the osteogenic response in calvarial osteoblasts isolated from *Ccn2* mutants was significantly reduced *in vitro* [65]. These studies were extended recently by Canalis et al. to examine consequences of postnatal loss of CCN2 in osteoblasts [66]. In this study, the authors observed osteopenia, but only in male adult mice [66]. As in the study by Kawaki et al.,

isolated calvarial osteoblasts were found to exhibit diminished osteoblast function. Of note, the conditional allele of CCN2 used in the study by Canalis et al. led to reductions in *Ccn2* mRNA levels ranging from 20-80%, and inactivation throughout the limb bud reportedly led to a phenotype similar to that seen in *Ccn2*^{+/-} mice [66]. This is in contrast to the bent bones seen in *Ccn2* null mice [18]. Thus while the full extent of CCN2 action in bone is as yet uncertain, it is clear that CCN2 is an important regulator of osteoblast function. Its mechanism of action is completely unknown in this tissue.

CCN2 is abundantly expressed in cranial sutures (Fig. 2D). Premature mineralization of cranial sutures leads to craniosynostosis, which restricts skull growth and causes disfigurement. A recent study showed that CCN2 expression can prevent craniosynostosis in a rat model [67]. While sutures appear to be unaffected in *Ccn2*^{-/-} neonates (unpublished data), it is conceivable that CCN2 plays a role postnatally in regulating suture patency.

Roles in angiogenesis

CCN2 was first isolated from human umbilical vascular endothelial cells (HUVECs) [21], and is highly expressed in all major and micro- vasculature including coronary vessels of the heart, vasculature of the kidneys, lung and liver (Fig. 2E-G) [20,22,67]. The physiological roles of CCN2 in angiogenesis are unclear, however, as CCN2 has both pro- and anti-angiogenic activities *in vitro*. For example, CCN2 induces angiogenesis in the cornea [68], and induces neovascularization *in vitro* through engagement of integrins. CCN2 is a ligand for $\alpha 6\beta 1$ and $\alpha v\beta 3$ in endothelial cells [40,68,69]. However, anti-angiogenic activities have also been reported. CCN2 binds to and sequesters VEGF in an inactive form [8] and combined administration of CCN2 and VEGF in a mouse model of hindlimb ischemia inhibits VEGF induced angiogenesis [70]. Taken together, these studies implicate CCN2 as an essential regulator of angiogenesis, but do not address its role *in vivo*.

CCN2 global knock out mice exhibit defective growth plate angiogenesis as a result of decreased levels of vascular endothelial growth factor (VEGF) in hypertrophic cartilage [18]. However whether CCN2 also plays a direct role in the vasculature has not been demonstrated. One study compared basal and VEGF-induced outgrowth of vessels from metatarsals of wild type and *Ccn2* mutant neonatal mice to conclude that CCN2 is not required for angiogenesis [71]. However, the large amount of variation in outgrowth that was observed may obscure real differences, and the images shown in the paper reveal considerably less outgrowth from *Ccn2*^{-/-} metatarsals treated with VEGF [71].

On the other hand, a second study by the same group showed that the thickening of the basal lamina of retinal capillaries that occurs during streptozotocin (STZ)-induced diabetes in mice was completely prevented in *Ccn2*^{+/-} mice [72]. Similarly, glomerular basement membrane thickening was prevented in diabetic *Ccn2*^{+/-} mice compared to WT littermates [73]. *Ccn2*^{-/-} mice could not be analyzed in these studies owing to their neonatal lethality. Nonetheless, these results raise the possibility of a role for CCN2 in the formation of endothelial basement membranes. Other evidence for a role for CCN2 in endothelial basement membrane formation comes from the findings that one of the most prominent features of Col1-CCN2 [31] transgenic mice was a thickening of endothelial basement membranes.

Each of these studies addresses a role for CCN2 in vascular fibrosis; over-expression promotes basement membrane thickening, whereas decreased CCN2 expression prevents it. However, whether CCN2 plays an essential role in normal angiogenesis has not been established. The survival of *Ccn2*^{-/-} mice to birth indicates that vasculogenesis, the initial formation of blood vessels during development, is not impaired. However, we have analyzed vasculature from *Ccn2*^{-/-} mice, and have uncovered evidence that CCN2 is required for

vascular remodeling, a process that takes place at midgestation stages and postnatally *in vivo*, is impaired in *Ccn2* mutants (manuscript in preparation).

Insights and Future Directions

Genetic models are revealing that CCN2 has essential functions for tissue formation and maintenance that extend beyond its ability to induce tissue fibrosis. It is likely that developmental functions for CCN2 beyond those discussed here will be discovered. For example, a role has recently been discovered for CCN2 in the formation of the endocrine pancreas [74]. CCN2 is widely expressed in other secretory epithelial tissues, raising the possibility that CCN2 has a much broader role during development than currently appreciated [25]. A major unknown is the extent to which CCN2 and other members of the family have overlapping functions. Members of the CCN family can engage similar sets of integrins and bind to similar ECM components to mediate similar effects *in vitro* [24], and their patterns of expression overlap in many tissues [75]. Given the many integrins, growth factors and their receptors, and ECM components that have been shown to interact with CCNs, whether individual CCN proteins have similar vs. distinct functions will likely prove to be cell-type specific. Finally, the mechanisms by which CCNs mediate their effects *in vivo* are essentially unknown. While we uncovered a role for integrins as mediators of the effects of CCN2 in chondrogenesis, it is highly likely that CCN2 uses additional mechanisms in this and other tissues.

Acknowledgments

This work was supported by NIH grants R01 AR052686 to KML and the UCLA Vascular Biology Training Grant (Ruth L. Kirschstein National Research Service Award T32HL69766) to FHG.

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Figure 1. Functional Domains of CCN Family Members

The CCN family is comprised of six members that contain four conserved functional domains. Each domain is likely to contribute both independently and cooperatively to the overall function of the CCN family members. The insulin-like growth factor binding protein (IGFBP) domain and the von Willebrand factor C (VWC) domain both bind to aggrecan. The VWC domain also binds to BMPs and TGFβ, integrins and matrix metalloproteinases (MMPs). The third motif is the thrombospondin (TSP) domain, which interacts with integrins, growth factors, vascular endothelial growth factor (VEGF) and low-density lipoprotein receptor-related proteins (LRPs). The last structural module is the C-terminal domain, which contains the cysteine knot motif. This motif binds to integrins, heparin sulfate proteoglycans (HSPGs), fibronectin and LRPs. CCN5 lacks this structural motif, but can still function similarly to other CCN family members. Downstream signaling via integrins activates multiple cell signaling pathways that mediate cell survival, migration and ECM production.

Figure 2. CCN2 eGFP Expression in the adult mouse

CCN2 expression was examined in 1-month old male mice. (A) Strong CCN2 expression was observed in vertebral bodies and intervertebral discs, (B) CCN2 is expressed in ribs within the cartilaginous ventral segments, as compared to undetectable expression in the ossified dorsal rib segments. (C, C') hypertrophic chondrocytes in the femoral growth plate and in articular cartilage. C' is a magnification of the growth plate in C. (D) CCN2 is highly expressed in cranial sutures. Expression was also observed in both major and minor vasculature including coronary arteries (E), kidneys (F) and lungs (G). Intervertebral disc (IVD), sternebra (St), rib ventral segment (Rv), rib dorsal segments (Rd), femoral condyle (Fc), vessel (v), hypertrophic chondrocytes (Hc), cranial sutures (Cs), atria (At), ventricle (Vt), renal cortex (Rc), renal medulla (Rm), bronchus (Br). Images were taken on a Leica MZ 16F bright field/GFP dissecting scope.