CCN1 and CCN2: blood brothers in angiogenic action

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Abstract CCN2/connective tissue growth factor (CTGF) is a matricellular protein essential for skeletal development during embryogenesis. In adulthood, aberrant *CCN2* expression is associated with many malignancies and fibrosis of virtually every organ. Despite its prominent expression in endothelial cells in the vasculature, the role of CCN2 in vessel development was unknown. In a recent study, Hall-Glenn et al. (PLoS ONE 7:e30562) have revealed the role of CCN2 in developmental angiogenesis through a detailed analysis of how CCN2 mediates the interaction between vascular endothelial cells and pericytes. In addition, CCN2 also regulates endothelial basement membrane formation during vessel formation. Here I compare the angiogenic activities of CCN2 during embryogenesis to those of its homologous family member CCN1 (CYR61), which is essential for cardiovascular development. Understanding the angiogenic actions of CCN1 and CCN2 may have implication in the development of therapeutic strategies targeting these proteins for the treatment of diseases such as cancer and fibrosis.

Keywords Angiogenesis · Development · Pericytes · Blood vessels · Smooth muscle cells · Cancer · Fibrosis

The CCN family of matricellular proteins are crucial for embryonic development and play important roles in inflammation, wound healing, and injury repair in adulthood (Jun and Lau 2011). Deregulation of CCN proteins is found in myriad diseases associated with chronic inflammation or tissue injury, including fibrosis, cancer, atherosclerosis,

arthritis, and diabetic nephropathy (Jun and Lau 2011). As recent studies have generated enthusiasm for targeting CCN proteins as potential therapeutic approaches for some of these diseases, understanding the full range of CCN activities is of critical importance for further uncovering their roles in disease pathobiology and for identifying potential pitfalls or side effects in therapies that block their function.

Ccn1 and *Ccn2* are expressed in similar organ systems during embryonic development, most prominently in the cardiovascular, skeletal, and nervous systems. Despite these similarities, targeted disruptions in *Ccn1* and *Ccn2* in mice engender very different phenotypes. *Ccn1*-null mice are embryonic lethal due to severe defects in cardiac valvuloseptal morphogenesis, impaired placentation, and loss of vascular integrity (Mo et al. 2002; Mo and Lau 2006). By contrast, *Ccn2*-null mice suffer perinatal lethality due to respiratory distress caused by severe skeletal defects (Ivkovic et al. 2003). Although CCN2 is known to induce angiogenesis in vivo in rat cornea (Babic et al. 1999) and in chick chorioallantoic membranes (Shimo et al. 1999), it can also inhibit vascular endothelial growth factor A (VEGF)-induced angiogenesis by binding to VEGF and sequestering it in an inactive form (Inoki et al. 2002; Hashimoto et al. 2002). The specific role of CCN2 in developmental or pathological angiogenesis, if any, was unknown.

A recently paper by Hall-Glenn et al. has provided compelling evidence that CCN2 is critical for developmental angiogenesis (Hall-Glenn et al. 2012). *Ccn2* is expressed in endothelial cells of large vessels, arterioles and capillaries from E13.5 through term. *Ccn2*^{-/-} embryos show normal early vessel formation, but vascular defects begin to emerge by E14.5. In large blood vessels the endothelium is supported by smooth muscle cells and elastic fibers (tunica media), whereas in microvessels the interaction between endothelial cells and the surrounding pericytes is crucial for regulating vascular stability and capillary permeability. The large vessels in *Ccn2*^{-/-} embryos are enlarged, and

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although there is no deficiency in the coverage of these vessels by smooth muscle cells, the smooth muscle cells in the tunica media appear less spindle-like and more heterogeneous in size than in wild type. By contrast, the microvessels in *Ccn2*^{-/-} mice are incompletely covered by pericytes. There is no difference between microvessels of *Ccn2*^{-/-} and wild type mice in the number of PCNA-positive proliferating cells or TUNEL-positive apoptotic cells, suggesting that the defects may lie in recruitment of pericytes to *Ccn2*^{-/-} microvessels and interaction between pericytes and the endothelium.

Interestingly, *Ccn2*^{-/-} mice express a lower level of angiopoietin 1 (Ang1), a glycoprotein secreted mainly by mural cells and functions to stabilize endothelial-pericyte interactions. At the same time, the level of VEGF is elevated. Recent studies indicate that Ang1 is not required for pericyte recruitment, but plays a crucial role in restraining angiogenesis during wound healing and protecting the glomerular microvasculature in diabetic nephropathy (Jeansson et al. 2011). As CCN2 is intimately involved in the pathobiology of wound healing and diabetic nephropathy (Mason 2009), these findings suggest that CCN2 may potentially act in part through perturbing the expression of *Ang1* in these contexts. Furthermore, Hall-Glenn et al. noticed that the *Ccn2*^{-/-} microvascular phenotypes resemble those of mice lacking platelet-derived growth factor-B (PDGF-B) or its receptor, PDGFR β . The interaction of PDGF-B produced by endothelial cells with PDGFR β expressed on pericytes is critical for the recruitment of pericytes to new blood vessels (Lindahl et al. 1997). CCN2 induces PDGF-B expression and enhances PDGF-B-induced AKT activation in endothelial cells, indicating that CCN2 potentiates PDGF signaling between endothelial cells and pericytes. In addition to impaired pericyte-endothelial interaction and deficient PDGF signaling, *Ccn2*^{-/-} mice also show defects in basement membrane organization. Fibronectin (FN) forms a matrix that provides an organizational framework for the assembly of permanent basement membrane components such as collagen type IV. The expression of FN and its association with blood vessels are both decreased in *Ccn2*^{-/-} mice. Accordingly, the incorporation of type IV collagen in the vascular basement membranes appears diminished and discontinuous, even though CCN2 does not affect *Col4a2* expression.

In contrast to these microvascular phenotypes in *Ccn2*^{-/-} mice, the most notable vascular defect in *Ccn1*^{-/-} mice occur in large vessels (Mo et al. 2002). *Ccn1* is highly expressed in endothelial cells and in smooth muscle cells throughout development. Like *Ccn2* mutant mice, *Ccn1*^{-/-} mice do not show any defects in vasculogenesis and yolk sac development is normal, although they suffer extensive impairment in the vasculature. About 30 % of *Ccn1*^{-/-} embryos die by E10.5 due to failure in chorioallantoic fusion, or attachment

of the umbilical cord to the placenta. Of those that progress pass this step in development, a significant percentage experience placental defects. At the chorioallantoic junction where the umbilical cord and placenta join, the umbilical artery and vein bifurcate to form a network of blood vessels that penetrate and populate the placenta, forming part of the placental labyrinth in which exchange of nutrient and waste between mother and fetus occurs. *Ccn1*^{-/-} mice are impaired in this placental vessel bifurcation, leading to placental deficiency with a severe shortage of vessels that penetrate into the placental labyrinth. This defect is linked to deficient *VEGF-C* expression in the *Ccn1*^{-/-} chorioallantoic junction, although expression of *VEGF-A*, *Ang1*, *Ang2*, and the angiopoietin receptor *Tie2* is unaffected.

Severe defects also occur in large vessels elsewhere in the *Ccn1*^{-/-} embryo, most notably in the dorsal aorta (Mo et al. 2002). The vessels are often enormously dilated with a lumen several-fold larger than normal, resembling a large aneurysm. Coverage of the vessels by smooth muscle cells and pericytes is deficient, and the vascular cells are highly apoptotic. The endothelial basement membrane is also disorganized, with endothelial cells loosely associated with the basement membrane or detached from it. Not surprisingly, these vessels are prone to rupture, leading to hemorrhage and edema.

Together, these results demonstrate that both CCN1 and CCN2 are critically involved in developmental angiogenesis. Furthermore, CCN3, another member of the CCN family, has also been shown to induce angiogenesis in vivo (Lin et al. 2003), although its specific contribution to developmental angiogenesis is currently unknown. Mechanistically, CCN1, CCN2, and CCN3 can induce angiogenesis through their direct binding to integrin $\alpha_v\beta_3$ on endothelial cells (Babic et al. 1998; Babic et al. 1999; Leu et al. 2002; Lin et al. 2003), and/or by regulating the expression or activity of other angiogenic factors such as VEGFs (Chen et al. 2001; Ivkovic et al. 2003; Hashimoto et al. 2002; Inoki et al. 2002; Perbal 2008). The possibility that these CCN family members may serve redundant functions or orchestrate overlapping or complementary aspects of developmental angiogenesis is intriguing, and further analysis using double knockout mice will be informative in this regard. The angiogenic activities of these CCN proteins may also play a role in pathological angiogenesis, for example in enhancing vascular density in tumors (Babic et al. 1998; Yang et al. 2005). Recent studies have generated interest in targeting CCN proteins in therapeutic treatment of various diseases, and clinical trials are underway to test the use of humanized anti-CCN2 antibodies in treating cancer, fibrosis, and diabetes (Jun and Lau 2011). Understanding the specific effects of CCNs in vessel formation and maturation will have implications on how and when these therapies might be safely applied.

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