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JB commentary

An impact of CCN2–BMP-2 complex upon chondrocyte biology: evoking a signalling pathway bypasses ERK and Smads?

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CCN family 2/connective tissue growth factor (CCN2/CTGF) is a secreted protein that regulates diverse cellular functions. In addition to being a growth factor to transmit mitogen-activated protein kinase (MAPK) signalling into cells, through largely unknown mechanism, CCN2 antagonizes bone morphogenetic proteins (BMPs) by direct interaction. CCN2 and BMPs co-localize in cartilage, and both of them promote proliferation and differentiation of chondrocytes *in vivo* and *in vitro*. However, it was unclear whether these growth factors act cooperatively, or mutually inhibitory in chondrocyte biology. In addition, an information whether the hetero-oligomer of CCN2 and BMPs has any physiological roles in skeletogenesis was completely missing. Takigawa and his colleagues have recently reported that CCN2 and BMP-2 interacted directly to mutually interfere with their downstream ERK and Smad signalling, whereas the CCN2–BMP-2 complex promoted chondrocyte differentiation [Maeda *et al.* (2009) *J. Biochem.* 145, 207–216]. This contradictory finding shed light on a possibility that the complex of CCN2 and BMP-2 is capable of activating an additional non-canonical signalling pathway to promote chondrocyte differentiation.

Keywords: BMP-2/CCN2/chondrocytes/ERK/Smad.

Abbreviations: BMP, bone morphogenetic protein; TGF-β, transforming growth factor-β; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase.

Members of the CCN family of cysteine-rich (CR) secreted protein include **c**ysteine-rich protein 61 (Cyr61)/CCN1, **c**onnective tissue growth factor (CTGF)/CCN2, nephroblastoma over-expressed gene (Nov)/CCN3, Wnt-inducible secreted protein 1 (WISP1)/CCN4, WISP2/CCN5 and WISP3/CCN6. They regulate diverse cell biology, including proliferation, migration, adhesion and extracellular matrix production, in events of angiogenesis, wound healing, fibrosis and skeletogenesis (*1*). During endochondral ossification in developing skeleton, mesenchymal cells condense and undergo chondrogenesis, proliferate and differentiate to perform cartilaginous skeletal rudiments to be replaced by bone tissue. This complicated event is tightly controlled by many factors such as parathyroid hormone-related peptide, fibroblast growth factors, hedgehog proteins, insulin-like growth factors and bone morphogenetic proteins (BMPs) (*2*). *CCN2*, a gene cloned from a chondrocytic cell line HCS-2/8 (*3*), promotes proliferation and differentiation of chondrocytes *in vitro* (*4*). *In vivo*, CCN2 is highly expressed in both pre-hypertrophic chondrocytes and terminally differentiated hypertrophic chondrocytes in developing bone, that loss of *CCN2* gene in mice resulted in chondrodysplasia, characterized by deficiency in proliferation and extracellular matrix production of chondrocytes (*5*). However, precise molecular mechanisms by which CCN2 up-regulates proliferation and differentiation of chondrocytes remain elusive.

CCN proteins are highly conserved and contain four distinct structural modules: an amino-terminal insulin-like growth factor-binding domain (IGFB), followed by a CR/von Willebrand type C domain-containing domain, a thrombospondin type 1 repeat (TSP1) and a carboxyl-terminal cystine knot (CT) domain (Fig. 1A). All of these modules are highly interactive with many other molecules such as aggrecan (*6*), fibronectins (*7*), transforming growth factor-β1 (TGF-β1) (*8*), BMP-4 (*8*) and BMP-7 (*9*). CR module is similar to those found in a BMP antagonist chordin, and is responsible for binding to BMPs (*10*). BMPs are member of TGF-β family, which bind to two distinct Type II and Type I serine/threonine kinase receptors, which in turn phosphorylate receptor-regulated Smads (Smad1, 5 and 8 for BMP signalling) to transduce canonical BMP signalling (Fig. 1B) (*11*). BMPs promote proliferation and differentiation of chondrocytes through Smad1/5/8 (*12*). Binding of CCN2 to BMP-4 inhibited interaction of BMP-4 with BMP receptors, thereby inhibited BMP signalling (*8*). Other CCN family proteins also bind to BMPs to inhibit BMP signalling. For instance, Nov/CCN3 binds to BMP-2 (*13*), WISP1/CCN4 binds to BMP-2 (*14*) and WISP3/CCN6 interacts with BMP-4 (*15*). Among them, interestingly, only WISP-1/CCN4 was not inhibitory to BMP signalling, rather promoted BMP-2 activity by inducing α_5-integrin expression to enhance the accessibility of BMP-2 for bone marrow stroma cells, thereby accelerate bone formation to increase bone volume of the transgenic mice skeleton (*14*). These findings indicate that CCN family proteins can serve as BMP antagonists or agonists in context-dependent manner.

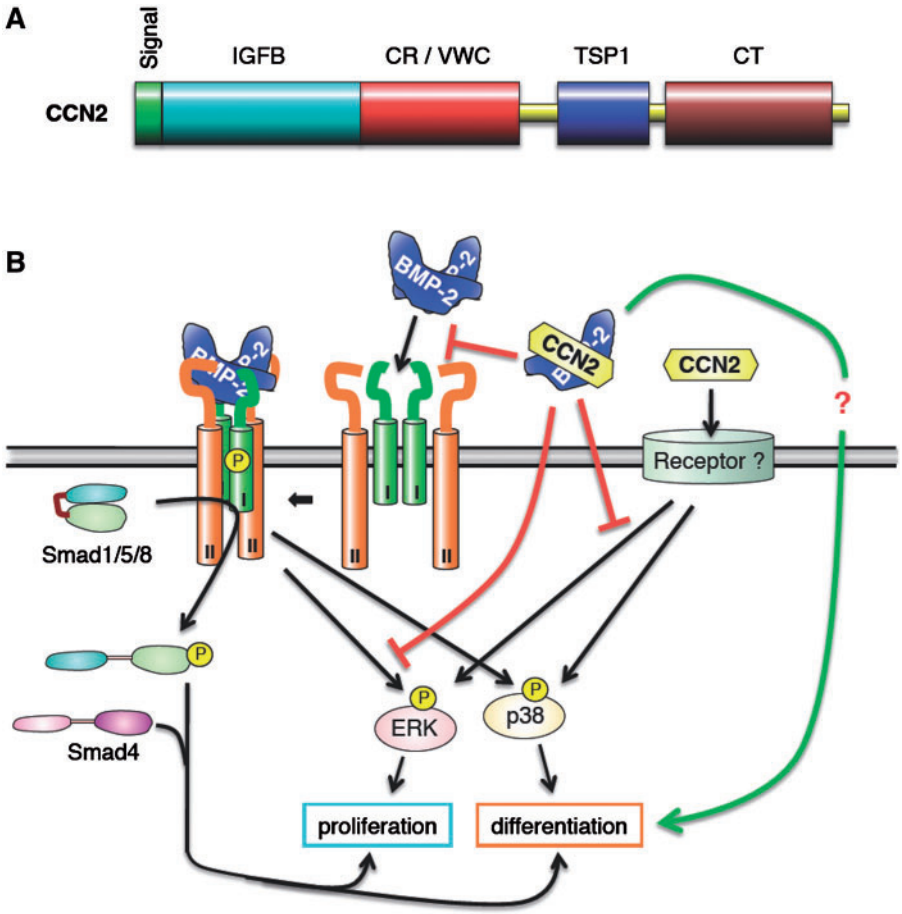

Fig. 1 Signal cross-talk between CCN2 and BMP-2 in chondrocyte proliferation and differentiation. (A) Structural domains of CCN2 protein. IGFB, CR/ VWC, TSP1 repeat and CT domains are shown. (B) Combined application of CCN2 and BMP-2 inhibits BMP-Smad pathway, BMP-ERK pathway, CCN2-ERK pathway and chondrocyte proliferation, whereas promotes chondrocyte differentiation.

In case of Noggin, a representative BMP antagonist, the knock-out mice showed accelerated BMP signalling, which resulted in overgrowth of skeletal elements (*16*). On the other hand, CCN2-deficient mice showed an opposite skeletal phenotype of chondrodysplasia (*5*). These evidences from mouse genetics suggest that a contribution of CCN2 as a BMP antagonist in chondrogenesis is dispensable, or it is possible that CCN2–BMPs complex have an unidentified positive function in cartilage development.

Recently, Takigawa and his colleague (*17*) studied a possible interaction between CCN2 and BMP-2, and the functional impacts of the resulting heterooligomer on chondrocytes. CCN2 interacted with BMP-2 with a dissociation constant (K_D) of 0.77 nM, while CCN2 had been reported to bind to BMP-4 at a K_D of 5 nM (*8*), and to BMP-7 with a K_D of 14 nM (*9*). Not only CR module of CCN2, but also modules of IGFB and CT interacted to BMP-2, among which CT module showed the highest affinity (*17*). Together with the fact that cysteine residues in CR module of CCN2 are 80% homologous to those found in chordin (*10*), BMP-2 is likely to have the strongest affinity to CCN2 among these osteogenic BMPs. As expected, combined application of CCN2 and BMP-2 inhibited both BMP-2-induced phosphorylation of Smad1/5/8 and proliferation of HCS-2/8 (*17*). This was probably achieved though preventing BMP-2 from interacting with BMP receptors, as a case for BMP-4 (Fig. 1B) (*8*). In addition to Smad pathway, BMPs transmit signals through non-Smad pathways, including extracellular signal-regulated kinase (ERK), JNK and p38 mitogen-activated protein kinase (MAPK) pathways (Fig. 1B) (*18*). Co-treatment of CCN2 abolished BMP-2-induced phosphorylation of ERK1/2, whereas did not affect that of p38 (*17*). CCN2 activates ERK pathway and JNK pathway to up-regulate proliferation of chondrocytes, and p38 pathway to promote differentiation of HCS-2/8 cells (Fig. 1B) (*19*, *20*). Interestingly, addition of BMP-2 dramatically suppressed CCN2-induced phosphorylation of ERK1/2, but not that of p38, which in turn completely cancelled the accelerated proliferation of HCS-2/8 cells by CCN2 (*17*). Surprisingly, however, combined treatment of BMP-2 and CCN2 not only showed no inhibition against the promoted chondrocyte differentiation by either factor alone, but also further enhanced the production of cartilage extracellular matrix proteins (*17*). Because the phosphorylation level of p38, responsible for chondrocyte differentiation, was unchanged by BMP-2–CCN2 co-application as mentioned above, this collaborative effect by these two growth

factors on chondrocyte differentiation seemed independent of neither Smad pathway nor p38 MAPK cascade. It should be noted that the effect of combined treatment of BMP-2 and CCN2 on chondrocyte differentiation was 'additive' rather than 'synergistic', therefore, it is not clear whether the complex formation by BMP-2 and CCN2 was indispensable in this context, or not. To date, possible physiological receptors for CCN2 responsible for promoting cell proliferation and differentiation are largely unknown. It is possible that the heterooligomers of BMPs and CCN family proteins bind to some receptors belonging to other signalling systems to evoke an alternative non-canonical signal cascade into certain cells (Fig. 1B).

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Conflict of interest
None declared.

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