

CCN3: A Novel function in vivo

A. Leask

Abnormal skeletal and cardiac development, cardiomyopathy, muscle atrophy and cataracts in mice with a targeted disruption of the Nov (Ccn3) gene
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Development, tissue regeneration and disease are regulated through complex interactions among molecules which signal to regulate cellular processes such as proliferation, survival, differentiation, adhesion and migration. The CCN family of matricellular proteins is a central player in regulating several of these signaling molecules (reviewed Leask and Abraham, 2006). Thus CCN family members profoundly affect cellular behavior in development, wound healing, tissue homeostasis and in a range of pathologies, including fibrosis and cancer. There are six members of the CCN family: CCN1 (Cyr61), CCN2 (connective tissue growth factor, CTGF), CCN3 (Nov), CCN4 (WISP1), CCN5 (WISP2/rCOP-1) and CCN6 (WISP3). Their effects are mediated by four cysteine-rich conserved domains which are shared by all members of the family, with the exception of WISP2 which lacks the C-terminal domain (Bork, 1993; Perbal et al., 2004). Through these domains, CCN proteins interact with a variety of extra- cellular signaling molecules, thereby regulating their activities.

Recent evidence has shown that, in spite of their structural similarity, CCN proteins have a diverse range of activities. In particular, results revealed using genetically-deficient mice and cells derived thereof have revealed essential non-redundant functions for CCN family members. Targeted disruption of CCN2/CTGF identified a role in coordinating chondrogenesis and angiogenesis (Ivkovic et al., 2003) while knock out mice lacking CCN1/CYR61 show that it is required for placental development and vascular integrity (Mo et al., 2002). Experiments using CCN2-deficient cells have shown that CCN2 is required for optimal adhesion, contraction, migration and gene transcription both basally and in response to fibronectin, TGF β 1 and micromass culture (Chen et al., 2004; Shi-wen et al., 2006; Kennedy et al., 2007; Nishida et al., 2007; Pala et al., 2008).

CCN3/nov (nephroblastoma overexpressed) was initially identified in a chick nephroblastoma caused by the MAV-1 retrovirus (Joliot et al., 1992). CCN3 is over-expressed in all MAV-induced avian nephroblastomas studied and deregulated in a variety of other tumor types, including Wilm's tumors (Chevalier et al., 1998) and musculoskeletal tumors (Manara et al., 2002). Although these results suggest that CCN3 may contribute to tumorigenesis, whether CCN3 plays an essential role in vivo is unknown.

To investigate the in vivo function of CCN3, Heath and colleagues (2008) generated mice carrying a targeted mutation of CCN3. These mice produce no full length NOV protein, but express at a barely detectable level a mutant NOV protein that lacks the von Willebrand domain. Mutating CCN3 resulted in abnormal skeletal and cardiac development, including joint abnormalities, cardiomyopathy, and premature tissue degeneration culminating in muscle atrophy and cataracts in adult mice.

In development at E16.5, CCN3 expression was found in the mesenchyme surrounding cartilage condensations, and in the tendons and myotendinous junctions. Chondrocytes were enlarged and the region in which chondrocytes were present was enlarged. Ossification was severely impaired in mutant embryos. These results differed significantly from those seen in CCN2 knockout mice which die shortly after birth with severely malformed rib cages (Ivkovic et al., 2003). In these latter animals, delayed ossification was also observed, but these mice had enlarged pre-hypertrophic/hypertrophic zones and thinner bone collars. In contrast, in CCN3 mutant mice, the size of the prehypertrophic/hypertrophic zone was reduced, and the bone collars were increased in thickness.

The basis of the phenotype of the CCN3-deficient mice was not explored mechanistically. It is unclear whether the phenotypes observed are due to the loss of full length CCN3, or to possible novel functions of the very low level of mutant CCN3 expression. Moreover, expression of key players in chondrogenesis and ossification (e.g., the sox family of transcription factors) were not examined. Moreover, it has been recently shown that CCN2, but not CCN3, is induced by Wnt3a, which plays a key role in signaling in chondrogenesis, osteoblastogenesis, and osteoclastogenesis (Si et al., 2006; Chen et al., 2008). An intriguing possibility exists that the differential results between the CCN2 and CCN3 animals may arise due to differential responses in expression to Wnt3a.

Nonetheless, the results presented by Heath and colleagues (2008) are intriguing at they point to clear functional differences in vivo among the different CCN family members. These differences which may reflect divergences not only regarding the proteins with which they interact with, but also regarding the expression patterns in response to various stimuli in vivo.

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