Review of: "The CCN Family of Proteins: A Critical Approach to the Multi-Modular Structure of the CCN Domains"

Håvard Attramadal[1]

1 University of Oslo, Norway

Potential competing interests: No potential competing interests to declare.

In this review, the author aims to discuss the potential roles assigned to the four constitutive modules of CCN proteins and to provide a critical view of the structural basis for their interactions and functions.

CCN proteins constitute a family of 6 secreted proteins sharing a tetramodular organization consisting of an insulin-like growth factor binding protein (IGFBP) homology domain, a von Willebrand factor type C repeat (vWC) homology domain, a thrombospondin type 1 repeat (TSP1) homology domain, and a carboxyl-terminal domain with a cystine knot motif (CK). Although the primary structures of these domains are all highly conserved among CCN proteins, their homology to similar structural domains in other proteins may be less well conserved. For example, the crystal structure of the TSP1 homology domain of CCN3 revealed that the cysteine connectivity differed from those of TSP1 domains found in many other proteins. Although there is substantial data on the biological functions of CCN proteins, in particular CCN1 and CCN2, the molecular mechanisms by which CCN proteins exert their actions are still poorly understood.

Although this review presents some interesting structure-function aspects of CCN proteins, the limited and skewed presentation of the scientific literature on CCN proteins detracts from the overall quality of the review.

The major criticism is that the author promotes a reductionist view of the multi-modular organization of CCN proteins, implying that each structural domain has its separate functions and that the function of CCN proteins is simply an add-up of the functions of the individual domains. The author uses the example from the evolution of the biosynthetic pathway of pyrimidine from bacteria to eukaryotes, where the first three steps of the biosynthetic pathways, originally encoded by three separate genes in bacteria, are catalyzed by a single polypeptide containing all three enzymatic activities. The author presents his view in a rather dogmatic way and without critical considerations.

The overall tertiary structure of a CCN protein has still not been solved. Yet, data from Alphafold predictions indicate that the structure of the various domains may interact to affect overall functions (**Monsen VT and Attramadal H.*J Cell Commun Signal* 2023, 17 (2): 371-390**). Thus, the author should also acknowledge the possibility that CCN proteins are more than the sum of the functions of 4 individual domains and that the tertiary structure of 2 more domains may constitute the principal functions of a CCN protein.

As correctly pointed out by the reviewer, the various domains of CCN proteins have been reported to interact with a number of other proteins in the extracellular matrix. However, the review fails to acknowledge that the scientific evidence for these interactions is varying; some have a relatively solid scientific foundation, whereas others may be regarded as

incidental reports. The author's view that CCN proteins may be regarded as multi-sensing stations in the extracellular matrix coordinating complex functions is just one possible scenario. Thermodynamically, it is difficult to understand how such a view could allow CCN proteins to perform dynamic independent functions. In this respect, the author should discuss the literature demonstrating that CCN proteins may instigate rapid signaling responses and activate intracellular pathways highly similar to growth factors. The author also ought to acknowledge the evidence demonstrating that CCN proteins are secreted as preproproteins requiring proteolytic activation by cleavage of the non-structured hinge domain between the 2nd and the 3rd domain, thereby releasing a bioactive carboxyl-terminal fragment consisting of domains III and IV. The CK domain is well established to be a structural entity involved in the homo-dimerization of proteins and is found in many autocrine/paracrine factors (e.g., TGF-beta; **Zhou, Y-F and Springer, TA. *Blood*. 123;1785-1793, 2014**) or hormones (e.g., growth hormone and other glycoprotein hormones; **Hearn MT and Gomme PT. J Mol Recognit. 13(5);223-278, 2000**), working as homo-dimeric entities. In this respect, the author also needs to acknowledge the recently reported evidence of CCN3 as a maternal hormone from the central nervous system (Arcuate-KISS1 neurons) to act on bone mineralization as an osteoanabolic hormone (**Babey ME et al. *Nature* 632; 357-365, 2024**).

Additional points of criticism:

1. The title of the chapter "the general picture" is not in agreement with the literature and should be modified.
2. The author refers to a recent study from the Park group (Song et al., 2022) reporting that the TSP1 domain from CCN5 is required, but not sufficient by itself, to exert the anti-fibrotic effects of CCN5 (e.g., reduction of expression of the fibrotic markers fibronectin and alpha smooth muscle actin (αSMA) in fibroblasts). The Park group reported that the TSP1 domain, when fused with either the IGFBP or the vWC domain, was sufficient to exert the anti-fibrotic actions of CCN5. Combining motifs in this manner, however, will most likely generate highly different tertiary structures. Assigning functional roles to domains based on such a strategy is not meaningful. Given the low solubility of the isolated TSP1 domain, it is plausible that the IGFBP and vWC domains could act as chaperones, promoting expression, correct folding, and increased solubility of the TSP1 domain in the Song study, similarly to the roles of other fusion partners of the TSP1 domain as reported by **Zolfaghari, et al. J Biol Chem 299 (1); 102803, 2023**. The author ought to discuss these issues.