

WHAT'S IN AN INTRON? CCN1 MRNA SPLICING IN CANCER

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Abstract

The CCN family of matricellular signaling regulators shares a common domain structure. Variants of individual CCN proteins exist, which contain different combinations of these domains. Although mRNA splicing is likely to play a key role on CCN biology, this hypothesis has not been thoroughly tested. In a recent report, Hirschfeld and colleagues (Cancer Res 69:2082-90, 2009), show that CCN1 (cyr61) mRNA is normally present in a form in which intron 3 is retained. In cancers, or upon hypoxia, intron 3 is removed resulting in the appearance of CCN1 protein. The significance of this paper is discussed.

The CCN family of matricellular signaling modifiers are intimately involved with crucial biological processes, such as proliferation, differentiation, survival, adhesion and migration (Leask and Abraham, 2006; Holbourn et al, 2008; Mason, 2009). As such, CCN proteins are key contributors to process such as development, tissue remodeling, fibrosis and cancers. The CCN family of genes comprises six members which possess specific, non redundant functions despite their high degree of structural identity (Leask and Abraham, 2006; Kennedy et al., 2007). The CCN proteins possess four distinct structural modules (IGFBP, VWC, TSP1, and CT) which resemble, respectively, Insulin-

like Growth Factor Binding Proteins, the Von Willebrand factor, Thrombospondin 1, and growth factors that contain a cysteine knot motif [including platelet-derived (PDGF), nerve growth factor (NGF) and transforming growth factor- β] (Bork, 1993).

It has long been appreciated that individual CCN proteins are present not only as full-length proteins, but also as fragments containing various combinations of entire modules. For example, a fragment containing the heparin-binding CT domain of CCN2 was purified based on bioactivity from pig uterine flushings (Brigstock et al., 1997). Moreover, it has been shown that the amino-terminal fragment of CCN2 (containing only the IGFBP and VWC domains) is an effective surrogate biomarker for fibrosis (Leask et al., 2009). Furthermore, in the case of avian nephroblastoma, a CCN3 protein lacking the amino-proximal (IGFBP) domain is likely to exist (Joliot et al., 1992) and in cultured human tumor cells truncated CCN3 proteins lacking the two first domains have been identified (Kyurkchiev et al., 2004). Also, CCN3 proteins lacking the TSP1 domain are developmentally regulated in human kidney (Subramaniam et al., 2008). In addition, a variant CCN4 (wisp1) protein, lacking the VWC module, has been identified in scirrhous gastric carcinomas (Tanaka et al., 2001).

The different versions of CCN proteins are likely to arise due to differential mRNA splicing. Although the group of B. Perbal (1997) detected two different mRNA phenotypes of human CCN1 (Cyr61) in a Northern blot analysis of tumor cells from the nervous system, and concluded that alternative splicing might play a key role in cancer, this hypothesis was never further investigated.

CCN1 (Cyr61) is overexpressed in tumors, and promotes angiogenesis via integrin signaling (Lau and Lam, 1999). Hypoxia is known to induce the transcriptional activation of pathways involved in angiogenesis, growth factor signaling, and tissue invasion and is therefore a potential key regulator of tumor growth, and hypoxia induces CCN1 mRNA expression, through a HIF response element in the CCN1 promoter (Lin et al., 2008). In a recent report, Hirschfeld and colleagues (2009) carefully analyzed the expression pattern of human CCN1 in breast cancer cell lines and tissue samples. Surprisingly, they found that breast carcinogenesis was accompanied by a shift in a CCN1 mRNA which retained intron 3 toward a CCN1 mRNA which correctly lacked intron 3. Hypoxia also resulted in the correct splicing of CCN1 mRNA. Moreover, analyses of matched pairs of invasive breast cancers and corresponding non-cancerous tissue revealed a very strong induction of the correctly spliced CCN1 transcript in invasive breast cancers. An antibody recognizing the hinge portion of the CCN1 molecule, located between the VWC and TSP1, was used to recognize CCN1, CCN1 was not detected in samples possessing the intron3-containing mRNA; indeed, the loss of intron3 correlated with the appearance of CCN1 protein.

Whether the retention of intron 3 resulted in any CCN1 protein at all was not assessed; it is possible that the incompletely spliced transcript may result in no CCN1 protein, or perhaps a severely truncated protein. Nonetheless, these data suggest that domain-specific CCN antibodies may represent unique tools for cancer prognosis (Lazar et al., 2007) but also imply that different fragments of CCN proteins may have different

functions. Moreover, individual CCN modules interact with members of the TGF β family, fibronectin, integrins and heparan sulfate containing proteoglycans (Leask and Abraham, 2006). Thus the different isoforms of individual CCN proteins may possess different biological functions based on the modules they contain. As an example, mice deleted for entire CCN genes may have different phenotypes than mice deleted for individual modules (Heath et al., 2008; Perbal, 2007; Leask, 2007). Indeed, full length and variant CCN proteins might functionally compete and antagonize in normal and pathological conditions. These considerations are important for considering the overall in vivo effect of CCN proteins at particular junctures of development, homeostasis and pathologies.

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