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EDITORIAL COMMENT

Understanding the Role of CCN Matricellular Proteins in Myocardial Fibrosis*

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Heart failure (HF) is a leading cause of morbidity and mortality and generates high health care-related costs, putting a great burden on both patients and society. HF therapy given on the basis of counteracting neurohormonal activation appears to have reached a plateau, thus highlighting the need to treat the underlying metabolic, functional, and structural alterations of the failing heart not adequately targeted by current pharmacological agents. In this conceptual framework, reactive diffuse interstitial myocardial fibrosis (MF), regardless of its origin, is a structural hallmark of the failing heart that has attracted the attention of the scientific community during the last decades.

Although the cellular sources involved in alterations of fibrillar collagen (namely, type I collagen) turnover leading to MF have been relatively well characterized, only a minority of factors that regulate the fibrotic response of the myocardium in cardiac diseases has already been identified. Therefore, investigating new mechanisms not targeted by currently available therapies is essential. In this regard, during the past few years, a number of studies have focused on the role of matricellular proteins. These macromolecules are nonstructural extracellular matrix proteins that are expressed at low levels in the heart during normal postnatal life but are dramatically up-regulated during cardiac injury (1). Matricellular proteins affect the function of cardiac fibroblasts, inflammatory cells, and cardiomyocytes by coordinating and integrating cell-cell and cell-matrix interactions, thus playing an important role in the regulation of inflammatory, hypertrophic, fibrotic, and angiogenic pathways in the myocardium.

The CCN family of matricellular proteins is a complex family of multifunctional molecules designated CCN1 to CCN6. The CCN acronym was introduced from the names of the first 3 members of the family to be discovered: cysteine-rich protein 61 (Cyr61), connective tissue growth factor (CTGF), and nephroblastoma overexpressed gene (NOV) (2). Available evidence suggests that CCN proteins participate in the pathogenesis of organ and tissue fibrosis, but play opposing roles (1). For instance, Yoon et al. (3) reported that, compared with wild-type mice, transgenic (Tg) mice overexpressing CCN2 exhibited increased MF, overactivation of the transforming growth factor-beta (TGF-β) signaling pathway, and deterioration of cardiac function in response to transverse aortic constriction (TAC)-induced pressure overload. On the contrary, MF and cardiac dysfunction associated with TAC were prevented in Tg mice overexpressing CCN5 (also known as WNT1-inducible signaling pathway protein-2 [WISP2]) (3). In addition, myocardial TGF-β signaling pathway was inhibited in CCN5 Tg mice subjected to TAC (3). These data raise the hypothesis that the imbalance between CCN2 and CCN5 may contribute to regulations of the fibrotic response of the myocardium to injury.

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In this issue of the *Journal*, the same investigators provide new clinical and experimental indirect evidence supporting this hypothesis (4). In fact, they report that CCN5 expression was reduced and CCN2 expression was increased in explanted hearts obtained from patients with MF and HF at the time of cardiac transplantation and in hearts from mice with

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MF and HF induced by TAC. In addition, the investigators confirm the antifibrotic effect of CCN5, demonstrating that, in mice with established MF and cardiac dysfunction due to TAC, adeno-associated virus-mediated cardiac overexpression of CCN5 was associated with regression of established MF and preservation of cardiac function, as well as inhibition of the TGF-β signaling pathway. Furthermore, they provide interesting data showing that, in hearts from CCN5 Tg mice, endothelial-mesenchymal transition and fibroblast-to-myofibroblast transdifferentiation were inhibited but that myofibroblast apoptosis was stimulated. In vitro experiments performed in human coronary endothelial cells, and rat cardiac fibroblasts and myofibroblasts exposed to CCN5-containing conditioned medium corroborated the previously mentioned in vivo effects of CCN5 on mouse cardiac cells. From these findings, the investigators suggest that the cardiac antifibrotic effect of CCN5 may be due, in part, to reduction in the number of cardiac myofibroblasts. These cells participate in continuous collagen tissue formation through their ongoing production of signaling molecules that promote fibrogenesis in an autocrine manner (5).

The consideration of structure-activity relationships of CCNs may shed some light on the molecular mechanisms involved in the effects of CCN2 and CCN5 on MF. A prototypical CCN protein contains an N-terminal secretory signal peptide and 4 functional domains: 1) an insulin-like growth factor-binding protein-like module; 2) a von Willebrand factor type C repeat module (vWC); 3) a thrombospondin type 1 repeat module; and 4) a cysteine knot-containing module (CT) (3). It has been shown that CCN2 binds directly to TGF-β through the vWC domain and facilitates the binding of TGF-β to its receptors via the CT domain (6). It is therefore possible that CCN5 can bind to TGF-β through its intact vWC domain, but, as it lacks the CT domain (7), it prevents the binding of TGF-β to its receptors and the subsequent activation of its signaling pathway, thus avoiding the fibrogenic cell responses to this cytokine.

How does CCN5 differ from other candidate molecules to regulate the fibrotic response of the myocardium to injury? On the basis of the considerations made in the previous paragraph, it is conceivable that CCN5 may represent a naturally occurring dominant negative molecule that modulates the profibrotic effects of CCN2 (and likely other profibrotic factors) by blocking the TGF-β signaling pathway in cardiac fibroblasts. The weakness of this view is its limited generalizability, as the information currently available focuses on MF developed in conditions of hemodynamic injury, and no data are yet available in the setting of cardiac injury due to ischemia and metabolic derangements, among others. Additionally, the relationship of CCN5 to other members of the CCN family with apparent roles in fibrosis (e.g., in vitro data suggest that CCN1 and CCN3 may exert antifibrotic actions) has not yet been investigated. Interestingly, in myocardial tissue from CCN5 Tg mice analyzed by Jeong et al. (4), α-SMA is detected in vascular smooth muscle cells. Thus, whether beyond its effect on fibroblast differentiation CCN5 also regulates vascular smooth muscle cell behavior in coronary vessels as it does in other blood vessels (8) deserves further study. Finally, MF is not just a matter of quantitative excess of collagen tissue, but also of changes in the quality of collagen fibers (e.g., immunochemical phenotype, degree of cross-linking, stiffness, among others). Whether CCN5 and other CCNs also modify the quality of the myocardial collagen network requires to be investigated.

One additional question remains regarding how the role of CCN5 in MF translates to the clinical arena. Because of its decreased expression in the failing human heart (4), the level of CCN5 determined from plasma or serum could serve as a biomarker for HF patients. Even more, the assessment of both CCN5 and CCN2 could provide an idea of the balance between these 2 matricellular proteins with opposite effects on MF. However, much of the clinical potential of targeting individual CCNs in therapy is still largely unexplored, as translational studies are still in early stages and none are in the field of MF. Future studies are required to clarify how combinatorial therapies that simultaneously down-regulate CCN2 and up-regulate CCN5 impact on MF.

In summary, the paper by Jeong et al. (4) provides novel information that advances our knowledge of how CCN5 functions mechanistically to reverse established MF and protect cardiac function. In addition, their findings reinforce the notion that the impact of CCNs on myocardial collagen network must be viewed in terms of the balance between family members with opposing effects. Therefore, the investigators deserve praise for their continued effort to clarify the contributing role of CCN proteins to the structural alterations of the failing heart.

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