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EDITORIAL COMMENT

Connecting the Dots for Connective Tissue Growth Factor Roles in Cardiac Wound Healing After Myocardial Infarction*

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In response to myocardial infarction (MI), the formation of scar comprised of extracellular matrix (ECM) is essential to maintain structure of the left ventricle (LV); however, too much or different ECM composition can generate an LV that is overly stiff and increases pre-load to the myocardium. Connective tissue growth factor (CTGF) (also known as CCN2) is a matricellular protein that influences fibroblast activation, cell migration, and cardiomyocyte hypertrophy (1). Cardiac fibroblast-mediated production of macrophage-recruiting chemokines are induced by CTGF (2,3). CTGF is low in the healthy adult heart and is markedly up-regulated in response to cardiac injury (4,5). CTGF gene expression is induced as early as 2 days after MI and remains elevated for up to 8 weeks (4,6). Therefore, understanding the mechanisms whereby CTGF regulates LV remodeling will provide insight into cardiac wound healing and help to elucidate additional targets that may be of therapeutic use.

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In the study by Vainio et al. (7) in this issue of *JACC: Basic to Translational Science*, the potential of CTGF monoclonal antibody (mAb) therapy was tested in 3 different study protocols in mice: one inhibiting during the initial inflammation and scar formation period, a second evaluating chronic administration effects in a permanent occlusion MI model, and the third examining acute effects following ischemia and reperfusion (7). CTGF mAb during the early proliferative phase of MI limited infarct expansion, increased survival, and limited the development of LV systolic dysfunction. Starting administration later reduced remote fibrosis and myocyte hypertrophy. The mechanisms of action were to modulate development, inflammation, and ECM genes to promote repair. Jnk signaling in fibroblasts was identified as a major node of action.

This paper is interesting because CTGF is known for its role in activating fibroblast polarization to an ECM synthesizing cell phenotype (8), yet its inhibition enhanced rather than impaired repair. This report also highlights that timing is a crucial factor for consideration in drug administration, as different benefits were seen when the mAb was started at 3 days versus 7 days after MI and was evaluated at 1 week versus 7 weeks.

Protocol 1. The first protocol started mAb administration at 3 days after MI and evaluated at day 7 after MI. Under this administration, they observed less reduction in ejection fraction at 1 week, indicating that CTGF treatment slowed the progression of

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LV dilation. There was increased survival, although the cause was not given; rupture, acute heart failure indicated by lung congestion, and sudden cardiac death due to arrhythmias are the 3 causes typically observed. There was less infarct scar thinning and infarct expansion. From these findings, the authors conclude that enhanced ejection fraction and fractional shortening meant improved systolic physiology. Improved systolic physiology indicates myocyte actions versus diastolic physiology that indicates ECM differences. Because diastolic function also contributes to these equations and neither alone showed differences, the effect was likely due to the combination. The improvement in systolic properties is not likely due to preservation of myocytes in the infarct region, because initiation at 3 days after MI would not limit ischemic injury. The effect, therefore, was on surviving myocytes in the remote and border zones. Because treatment was started 3 days after MI surgery, it would have been good to see the day 3 echocardiography results to show that the 2 groups started out treatment looking the same. Day 7 was an appropriate time to evaluate, as most of inflammation and ECM responses occur by this time (9).

Protocol 2. The second protocol started mAb administration 1 week after MI and evaluated at week 7 MI. They observed reduced ECM accumulation (i.e., collagen) in the remote region. Myocyte size and LV mass were reduced, indicating a tempered hypertrophic response to MI. Infarct size was not different, as would be expected since treatment started 1 week after MI, a time when salvage would not be expected. RNA-seq showed repair (inflammation and ECM genes) and development genes increased with mAb treatment. The 2 most prominent development genes were Nkx2.5 and Gata4. This protocol revealed transforming growth factor (TGF) β-independent signaling stimulated by CTGF, which provides new targets for therapeutic exploration.

Protocol 3. The third protocol started mAb administration 24 h before MI (a prevention rather than inhibition strategy) and evaluated after 30 min ischemia and 3 or 24 h reperfusion. This protocol revealed findings that are in contrast to a previous report using cardiac myocyte-specific overexpression of rat CTGF, which showed protection from acute ischemia/reperfusion injury (10). Using the CTGF mAb strategy, the current study noted protection with inhibition, opposite the overexpression strategy used previously. These results highlight that translational protocols often do not recapitulate genetic models. We also have seen that matrix metalloproteinase-9 null and inhibition strategies show divergent effects on MI remodeling (11,12), highlighting the distinction between modifying gene expression under artificial conditions and using clinically relevant antibody or inhibitor strategies. Although therapeutic efficacy was not determined by measuring Ab concentrations in plasma or LV, it is likely that 100% inhibition was not achieved, providing another difference from gene deletion strategies. This protocol shows that the effects of the antibody are not acute and are not myocyte-centric, consistent with the other 2 study protocols showing that inflammation and ECM were the primary molecular targets.

Combined, the 3 study protocols reveal a lot about CTGF roles in MI wound healing. Standards have been set up for ischemia studies, and for the most part these are met in this study (9). At the same time, there were a few study limitations that should be noted. Because all 3 study protocols were distinct, results cannot be interwoven among them. Protocols 1 and 2 are translational, whereas protocol 3 is preventative.

The heart rate in the sham group (Table S1 in Vainio et al. [7]) was under 400 beats/min, and fractional shortening was an average of 25%, which is low for control mice (13). It is unusual for heart rate to increase with MI in the mouse permanent occlusion model, and a lack of wall thinning at day 7 after MI is not typical (9,13). It is likely there was wall thinning and infarction was achieved, based on the histological section shown in Figure 2C in Vainio et al. (7). The results combined indicate some technical issues with echocardiography acquisition that may be complicating data interpretation.

The 30-min ischemia period was the minimum time needed to induce infarction, and a lack of effect may indicate that minimal damage occurred. This protocol would not mimic the patient scenario, where 30 min to reperfusion is not the usual treatment window. The early increase in Jun kinase 2 and signal transducer and activator of transcription (STAT)3 to then signal fibroblast activation could indicate that CTGF treatment was stimulating a much earlier activation than typically seen.

Knockdown of CTGF in cardiac fibroblasts increases expression of CCN5 (3). Whereas CTGF promotes fibroblast activation, ECM accumulation, and cardiac hypertrophy, CCN5 has opposing effects (5). CCN5 was not measured in this study, and whether the improved cardiac outcomes in response to CTGF mAb are due to suppression of CTGF or up-regulation of CCN5 would be of interest to determine in future studies.

Regardless of the study limitations, the study by the Kerkela team reveals several mechanisms whereby CTGF is regulating negative components of

cardiac wound repair after MI through effects on propagating inflammation and ECM accumulation in the remote region. This study also highlights the benefits of using translational protocols to bridge between genetic mouse models and clinical application.

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