JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 5, NO. 5, 2020

EDITORIAL COMMENT

TGF-β Inhibitor CILP as a Novel Biomarker for Cardiac Fibrosis*

Sonja Groß, MSc,[a] Thomas Thum, MD, PhD[a,b]

The clinical and socioeconomic relevance of cardiovascular diseases worldwide eventuated in comprehensive research aiming to unravel molecular mechanisms of developing end-stage heart failure. However, investigations toward improved diagnostic options have been prioritized recently as a first step to enable a fine-tuned personalized treatment. Non-invasive clinical tools are mainly focusing on robust circulating biomarkers, which can be easily detected in patient sera. The major challenge, however, is to identify disease- and organ-specific molecules that are dysregulated in different pathological stages.

One significant hallmark of cardiac diseases is the nascent myocardial fibrosis evolving almost independently of the primary disorder. In general, cardiac fibrosis can be classified according to the anatomical region or based on disease progression—acute or chronic—and, in this context, the functional response of the cardiac fibroblasts (1). Beginning with the latter, the initial and physiological response of fibroblasts to mechanical stress or injury is to stabilize the tissue while preserving the cardiac function. This phase is termed compensated, because the pump function remains stationary, but is accompanied by left ventricular hypertrophy and an activation of the fibroblasts to produce a stabilizing extracellular matrix (ECM). Continuous overload or pumping disability results in the following decompensated phase, making it impossible to achieve a sufficient cardiac output (2,3). A transition from one phase to the next is a complicated process; therefore, the classification of different stages of fibrosis and heart failure is very challenging. Nevertheless, two heterogeneous classifications emerged depending on the phenotype: heart failure with: 1) reduced; or 2) preserved ejection fraction (4). Reduced pump efficiency is mainly caused by ischemic heart events triggering an injury-induced immune response and excessive cardiomyocyte loss. Whereas the second disorder is a chronic progression towards hypertrophy and severe fibrosis, related to diastolic dysfunction. This complexity of fibrotic phenotypes can only be observed by visual diagnostic tools and more specifically by invasive operations to take biopsies for analysis.

To overcome the problematic assessment, the group of Park et al. (5) investigated novel circulating biomarkers in the context of early heart failure stages, allowing specific medication at an early time point of the disease. They performed a ribonucleic acid-sequencing analysis of pressure overload-induced hypertrophic mouse hearts 7 days after transverse aortic constriction and compared the gene expression profile with sham-operated animals. Of note, at this stage, interstitial fibrosis can already be observed and an enlargement of the ventricle occurs as part of the compensatory mechanism (6).

The ribonucleic acid-sequencing results were filtered on the basis of novelty in context of heart failure, protein secretion, and reported roles in ECM formation or remodeling. Three different proteins, namely LTBP2 (latent TGF-β-binding protein 2),

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the [a]Institute for Molecular and Translational Therapeutic Strategies, Hannover Medical School, Germany, Hannover, Germany; and the [b]REBIRTH Center for Translational Regenerative Medicine, Hannover Medical School, Hannover, Germany. Both authors received funding from the Deutsche Forschungsgemeinschaft (grant TH 903/20-1). Dr. Thum has filed and licensed patents about noncoding RNAs; and is the founder and a shareholder of Cardior Pharmaceuticals GmbH.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2020.03.013

COMP (cartilage oligomeric matrix protein), and CILP (cartilage intermediate layer protein 1) were highly up-regulated and further characterized in murine and human in vitro systems as well as in cardiac hypertrophy or myocardial infarction mouse models. Specific up-regulation of all Three genes was confirmed by quantitative polymerase chain reaction and immunocytochemistry stainings, where a functional involvement in the fibrotic response seemed to be most likely. As a proof of clinical relevance, protein levels were additionally analyzed in serum of patients with heart failure. LTBP2 and COMP are both associated with ECM turnover by promoting the formation of dense collagen fibers; however, their levels in human plasma were not significantly altered. Park et al. (5) hypothesized, that these proteins are indeed highly induced and secreted, but instead of being released into the blood vessels, they bind to ECM components and reside within the heart, thus making them unavailable as blood-based biomarkers.

However, CILP seemed to be a promising candidate as a biomarker for cardiac fibrosis because it was significantly up-regulated in cardiac fibroblasts (7,8), which was additionally confirmed by immunocytochemistry stainings of transverse aortic constriction or myocardial infarction hearts. The CILP protein is a precursor for two different variants: specific cleavage at a furin cleavage site leads to a larger N-terminal and a shorter C-terminal fragment, whereas full-length CILP has been reported to be functional, although detailed roles have not been elucidated yet (9). In this study (5), a special focus was directed on full-length CILP, its up-regulation during cardiac fibrosis, and the regulated secretion on disease. All Three protein variants interfere with the transforming growth factor (TGF)-β signaling, and especially the N-terminal fragment and full-length CILP are capable of binding TGF-β due to the common thrombospondin-1 domain (7). As TGF-β is the major regulator of the profibrotic response, not only in the heart, these findings are contradictory (10). A possible explanation would be a negative feedback loop, where induction with TGF-β primarily results in the activation of profibrotic programs, but subsequently restricts the signaling (7). Besides this function, specific yet undiscovered roles in promoting fibrosis are conceivable. In terms of significantly reduced serum levels in patients suffering from heart failure, full-length CILP expression is up-regulated, whereas the secretion might be remarkably diminished, preventing the binding-induced inhibition of TGF-β in the interstitial space. Alternatively, the expression of CILP in diseased hearts might be not sufficient to robustly inhibit the strong effect of TGF-β, reinforced by the findings of Zhang et al. (11) that revealed a cardioprotective effect on *Cilp* overexpression in mice. Therefore, transformed myofibroblasts maintain a strong TGFβ signaling and further promote cardiac fibrosis.

In summary, the study by Park et al. (5) identified three potential biomarkers as indicators for cardiovascular fibrosis in several disease contexts. The analyzed proteins can serve as early signs for cardiac remodeling, as these were already up-regulated 7 days after transverse aortic constriction surgery in mice. Functions of LTBP2 and COMP have already been reported in different organs; therefore, the markers are only suitable as general fibrosis-associated genes (12). However, specific roles especially for the full-length CILP protein still remain unclear. The underlying mechanism of enhanced gene expression on TGF-β stimulation beginning in the initial phase, combined with reduced serum levels in patients with heart failure warrants further in-depth analysis. A temporal gene expression profile is of major interest, which might allow specific attribution to different pathological stages. Finally, a role in other fibrotic diseases should be considered to evaluate the potential of full-length CILP as a specific biomarker for cardiovascular fibrosis.

ACKNOWLEDGMENT The authors thank Christian Bär for proofreading.

ADDRESS FOR CORRESPONDENCE: Prof. Thomas Thum, Hannover Medical School, Institute of Molecular and Translational Therapeutic Strategies, Carl-Neuberg-Strasse 1, Hannover, Lower Saxony 30625, Germany. E-mail: thum.thomas@mh-hannover.de.

REFERENCES

1. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. Cell Mol Life Sci 2014;71:549–74.

2. Travers JG, Kamal FA, Robbins J, Yutzey KE, Blaxall BC. Cardiac fibrosis: the fibroblast awakens. Circ Res 2016;118:1021–40.

3. Fan D, Takawale A, Lee J, Kassiri Z. Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. Fibrogenesis Tissue Repair 2012;5:15.

4. Oatmen KE, Cull E, Spinale FG. Heart failure as interstitial cancer: emergence of a malignant fibroblast phenotype. Nat Rev Cardiol 2019 Nov 4 [E-pub ahead of print].

5. Park S, Ranjbarvaziri S, Zhao P, Ardehali R. Cardiac fibrosis is associated with decreased circulating levels of full-length CILP in heart failure. J Am Coll Cardiol Basic Trans Science 2020;5:432–43.

6. Platt MJ, Huber JS, Romanova N, Brunt KR, Simpson JA. Pathophysiological mapping of experimental heart failure: left and right ventricular remodeling in transverse aortic constriction is temporally, kinetically and structurally distinct. Front Physiol 2018;9:472.

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 5, NO. 5, 2020
MAY 2020:444–6

7. Shindo K, Asakura M, Min KD, et al. Cartilage intermediate layer protein 1 suppresses TGF-β signaling in cardiac fibroblasts. Int J Gerontol 2017;11:67–74.

8. van Nieuwenhoven FA, Munts C, Op't Veld RC, et al. Cartilage intermediate layer protein 1 (CILP1): a novel mediator of cardiac extracellular matrix remodelling. Sci Rep 2017;7:16042.

9. Lorenzo P, Neame P, Sommarin Y, Heinegård D. Cloning and deduced amino acid sequence of a novel cartilage protein (CILP) identifies a proform including a nucleotide pyrophosphohydrolase. J Biol Chem 1998;273:23469–75.

10. Khalil H, Kanisicak O, Prasad V, et al. Fibroblast-specific TGF-β-Smad2/3 signaling underlies cardiac fibrosis. J Clin Invest 2017;127:3770–83.

11. Zhang CL, Zhao Q, Liang H, et al. Cartilage intermediate layer protein-1 alleviates pressure overload-induced cardiac fibrosis via interfering TGF-β1 signaling. J Mol Cell Cardiol 2018;116:135–44.

12. Zachou K, Gabeta S, Shums Z, et al. COMP serum levels: a new non-invasive biomarker of liver fibrosis in patients with chronic viral hepatitis. Eur J Intern Med 2017;38:83–8.

KEY WORDS biomarker, cardiac fibrosis, CILP (cartilage intermediate layer protein 1), TGF-β signaling