Cell Cycle Arrest-Driven Fibrosis
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Heart disease is a major cause of death worldwide. Though the etiology and mechanisms of heart disease are wide-ranging, including arrhythmia, hypertension, cardiomyopathy, and myocardial infarction, most are related to cardiac fibrosis. Fibrosis is the excessive accumulation of extracellular matrix (ECM) and is related to a poor prognosis, because excessive fibrosis can lead to increased left ventricular wall stiffness and decreased mechanoelectric coupling.

There are several well-known mediators that induce cardiac fibrosis, such as transforming growth factor (TGF), Wnt signaling, and the renin-angiotensin-aldosterone system. Though the inhibition of these signaling pathways may be beneficial in anti-fibrotic therapies, few drugs targeting them are used in clinical practice. At the present time, clinical data on anti-fibrotic drugs is limited, despite promising data having been produced by experimental models. Therefore, new targets for anti-fibrotic therapy should be addressed.

Wake, et al. evaluated the collagen production of cultured fibroblasts under serum deprivation. So far, the fibroblast-activity including cell proliferation and profibrotic effects have seemed to be activated in parallel. However, the results of this study clearly showed that collagen production measured by protein level in serum-deprived fibroblasts was significantly increased, compared to those in normal growth media. However, the mRNA level of the type I collagen gene did not increase. On the other hand, serum depleted-fibroblasts had decreased procollagen synthesis and cell proliferation do not necessarily occur at the same time in fibroblasts.

This report has provided a new insight into cardiac fibrosis and anti-fibrotic therapy. Many anti-fibrotic therapies based on the results from animal models target profibrotic factors, such as TGF-β and Wnt signaling. However, the synthesis and degradation of ECM occur continually to maintain a balanced tissue structure; therefore, the secretion or degradation of ECM without synthesis is also a potential target of anti-fibrotic therapy. Thus, it is important to understand the mechanism of ECM secretion and degradation.

Cardiac fibroblasts (CFs) are the most important type of cell in cardiac fibrosis, because their major role is the production of collagen. Under cardiac stresses like myocardial ischemia (MI) and hypertension, CFs can increase the production of collagen both in vitro and in vivo, resulting in the excessive accumulation of fibrillary collagen. The mechanism of exactly how ECM is degraded by CFs still remains largely unknown. The phagocytosis of collagen by gingival fibroblasts and the phagocytosis of dead cells by activated CFs, myofibroblasts, have been reported. However, the phagocytosis of ECM by CFs has not yet been demonstrated.

The heart is composed of various types of cells, including pericytes, white blood cells, smooth muscle cells, cardiomyocytes, and endothelial cells, so the influence of non-cardiomyocytes on interactions between other cells and their roles might also be important. For example, cardiac macrophages regulate cardiac fibrosis. Monocytes and macrophages not only play important roles in the progression of fibrosis but also mediate the resolution of fibrosis in many organs. Monocytes and macrophages are highly heterogeneous, and their diversity enables them to exert a wide range of functions from pro-fibrotic to anti-fibrotic responses. Most studies on the relationship between cardiac macrophages and CFs have been related to pro-fibrotic reactions. In response to cardiac stress, cardiac macrophages secrete pro-inflammatory mediators, including interleukin (IL)-1β, IL-6, and tumor necrosis factor-α and pro-fibrotic growth factors like TGF-β and platelet-derived growth factor. Under these stimulations, CFs differentiate into myofibroblasts, which secrete ECM, leading to its accumulation.

Cardiac macrophages have cell-surface markers, which separate them into several subgroups. One of the most used markers of cardiac macrophages is Ly-6C, an inflammatory marker expressed by macrophages in many organs. In general, Ly-6C macrophages are more closely related to the inflammatory response than Ly-6C macrophages. In the angiotensin II-mediated cardiac fibrosis model, Ly-6C+CD206+ cardiac macrophages may...
have a cardio-protective role in preventing excessive tissue injury.\(^{22}\) In a model of hepatic fibrosis, Ly-6C\(^{+}\) macrophages expressed high levels of matrix metalloproteinases (MMPs), suggesting that this subset has a role in the anti-fibrotic response.\(^{23}\)

Most MMPs have a catalytic domain and a hemopexin domain. Both domains are important in the degradation of collagen, and the role of the latter domain is thought to bind and unwind collagen, allowing the former domain access to the appropriate cleavage site.\(^{24}\) From their name and the roles mentioned above, MMPs seem to have an important role in degrading ECM. However, MMPs are not always related to anti-fibrotic processes and may be related to pro-fibrotic processes.\(^{25,26}\) In a lung fibrosis model, some of the MMPs lead to lung fibrosis, one mechanism of which involves the induction of fibroblast migration followed by the production of collagen.\(^{25}\) Also, in a heart fibrosis model, MMPs may have a pro-fibrotic role, because the deletion of MMP-9 in aged mice resulted in a reduced level of fibrosis in the left ventricle possibly due to the activation of latent TGF-β by MMP-9.\(^{26}\) Therefore, MMPs are not necessarily anti-fibrotic enzymes.

Another anti-fibrotic factor, bone morphogenetic protein-7 (BMP-7), which is a member of the TGF-β family, may have potential as a novel anti-fibrotic therapy.\(^{27}\) It is reported that BMP-7 induces the anti-fibrotic phosphorylation of SMAD1/5/8, which opposes the TGF-β mediated phosphorylation of S SMAD2/3.\(^{28}\) In a model of pre-diabetic cardiomyopathy, BMP-7 activated infiltrated monocytes, transforming them into anti-inflammatory M2 macrophages, leading to the attenuation of cardiac fibrosis.\(^{29}\) In a model of chronic renal injury in mice, the administration of recombinant BMP-7 reduced damage to renal tubular epithelial cells through the negative regulation of TGF-β/SMAD signaling.\(^{30}\) Though the systemic administration of BMP-7 seems to have anti-fibrotic potential, it has a short half-life, necessitating the use of high doses to achieve pharmacological effects.\(^{31}\)

In conclusion, fibrosis is related to a poor prognosis in many organs, so the identification of a new anti-fibrotic therapy is much needed. Though ECM is produced and degraded repeatedly, most research aiming to develop therapeutic approaches for fibrosis has focused on the inhibition of ECM production. Post transcriptional regulation or secretion of ECM coupling with cell cycle arrest is also a potential target for fibrosis (Figure).

**Disclosures**

**Conflicts of interest:** None.

**References**

1. Pagidipati NJ, Gaziano TA. Estimating deaths from cardiovascular disease: a review of global methodologies of mortality measurement. Circulation 2013; 127: 749-56.
2. Berk BC, Fujiwara K, Lehoux S. ECM remodeling in hypertensive heart disease. J Clin Invest 2007; 117: 568-75.
3. Aoki T, Fukumoto Y, Sugimura K, et al. Prognostic impact of myocardial interstitial fibrosis in non-ischemic heart failure. Circ J 2011; 75: 2605-13.
4. Shanbhag SM, Greve AM, Aspelund T, et al. Prevalence and prognosis of ischaemic and non-ischaemic myocardial fibrosis in older adults. Eur Heart J 2019; 40: 529-38.
5. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. Cell Mol Life Sci 2014; 71: 549-74.
6. Fang L, Murphy AJ, Dart AM. A clinical perspective of anti-fibrotic therapies for cardiovascular disease. Front Pharmacol 2017; 8: 186.
7. McVicker BL, Bennett RG. Novel anti-fibrotic therapies. Front Pharmacol 2017; 8: 318.
8. Wake M, Takeda N, Isagawa T, et al. Cell Cycle Perturbation Induces Collagen Production in Fibroblasts. Int Heart J 2019; 60: 958-63.
9. Xiang FL, Fang M, Yutzey KE. Loss of beta-catenin in resident cardiac fibroblasts attenuates fibrosis induced by pressure overload in mice. Nat Commun 2017; 8: 712.
10. Khalil H, Kanisicak O, Prasad V, et al. Fibroblast-specific TGF-beta-Smad2/3 signaling underlies cardiac fibrosis. J Clin Invest 2017; 127: 3770-83.
11. Lombardi R, Betocchi S, Losi MA, et al. Myocardial collagen

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**Figure.** Anti-fibrosis therapy. The amount of extracellular matrix (ECM) is regulated by the synthesis and degradation; therefore, the reduction of ECM will be achieved by the inhibition of synthesis or the activation of degradation. Regarding the former, trials in humans have been performed and failed in spite of encouraging results in animal models. Regarding the latter, little is known, and targeting these pathways will potentially lead to anti-fibrosis therapy.
turnover in hypertrophic cardiomyopathy. Circulation 2003; 108: 1455-60.
12. Doppler SA, Carvalho C, Lahm H, et al. Cardiac fibroblasts: more than mechanical support. J Thorac Dis 2017; 9: S36-51.
13. Bhide VM, Laschinger CA, Arora PD, et al. Collagen phagocytosis by fibroblasts is regulated by decorin. J Biol Chem 2005; 280: 23103-13.
14. Nakaya M, Watarai K, Tajima M, et al. Cardiac myofibroblast engulfment of dead cells facilitates recovery after myocardial infarction. J Clin Invest 2017; 127: 383-401.
15. Anversa P, Olivetti G, Melissari M, Loud AV. Stereological measurement of cellular and subcellular hypertrophy and hyperplasia in the papillary muscle of adult rat. J Mol Cell Cardiol 1980; 12: 781-95.
16. Hulsmans M, Sager HB, Roh JD, et al. Cardiac macrophages promote diastolic dysfunction. J Exp Med 2018; 215: 423-40.
17. Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. Semin Liver Dis 2010; 30: 245-57.
18. Nahrendorf M, Swirski FK. Monocyte and macrophage heterogeneity in the heart. Circ Res 2013; 112: 1624-33.
19. Heidt T, Courties G, Dutta P, et al. Differential contribution of monocytes to heart macrophages in steady-state and after myocardial infarction. Circ Res 2014; 115: 284-95.
20. Epelman S, Lavine KJ, Beaudin AE, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. Immunity 2014; 40: 91-104.
21. Fujiu K, Wang J, Nagai R. Cardioprotective function of cardiac macrophages. Cardiovasc Res 2014; 102: 232-9.
22. Falkenheim A, de Antuero R, Rosin N, et al. Nonclassical resident macrophages are important determinants in the development of myocardial fibrosis. Am J Pathol 2015; 185: 927-42.
23. Ramachandran P, Pellicoro A, Vernon MA, et al. Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. Proc Natl Acad Sci U S A 2012; 109: E3186-95.
24. McKleroy W, Lee TH, Atabai K. Always clean up your mess: targeting collagen degradation to treat tissue fibrosis. Am J Physiol Lung Cell Mol Physiol 2013; 304: L709-21.
25. Yamashita CM, Dolgonos L, Zemans RL, et al. Matrix metalloproteinase 3 is a mediator of pulmonary fibrosis. Am J Pathol 2011; 179: 1733-45.
26. Chiao YA, Ramirez TA, Zamilpa R, et al. Matrix metalloproteinase-9 deletion attenuates myocardial fibrosis and diastolic dysfunction in ageing mice. Cardiovasc Res 2012; 96: 444-55.
27. Ozkaynak E, Rueger DC, Drier EA, et al. Opi1 cDNA encodes an osteogenic protein in the TGF-beta family. EMBO J 1990; 9: 2085-93.
28. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-β family signalling. Nature 2003; 9: 577-84.
29. Urbina PD, Singla K. BMP-7 attenuates adverse cardiac remodeling mediated through M2 macrophages in prediabetic cardiomyopathy. Am J Physiol Heart Circ Physiol 2014; 307: H762-72.
30. Zeisberg M, Hanai J, Sagimoto H, et al. BMP-7 counteracts TGF-β1-induced epithelial-mesenchymal transition and reverses chronic renal injury. Nat Med 2003; 9: 964-8.
31. Vukicevic S, Basic V, Rogic D, et al. Osteogenic protein-1 (bone morphogenetic protein-7) reduces severity of injury after ischemic acute renal failure in rat. J Clin Invest 1998; 102: 202-14.