JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
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PUBLISHED BY ELSEVIER

VOL. 67, NO. 17, 2016
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2016.02.048

EDITORIAL COMMENT

Fibroblast Senescence as a Therapeutic Target of Myocardial Fibrosis

Beyond Spironolactone?*

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A major pathophysiological component of cardiac remodeling during heart failure (HF), cardiac fibrosis has become a target for therapeutic intervention. Additionally, compelling evidence indicates a key role of cardiac fibrosis in myocardial malfunctioning during aging. Cardiac fibrosis is a complex phenomenon resulting from aberrant activation of various cell types and signaling pathways as a consequence of injury or damage to tissue. It develops over a time course that also depends upon the type of *noxa* activation of tissue-specific repair programs, resulting in the subsequent activation of proliferation and migration of fibroblasts from different myocardial locations to the injury site, where they synthesize extracellular matrix (ECM) (1,2).

Tissue repair through the synthesis of new ECM by fibroblasts is beneficial, particularly after myocardial infarction. However, prolonged activation of this process results in excess scar tissue formation, increased ECM deposition, and therefore, "bad fibrosis" with consequent alterations of inotropic and lusitropic characteristics of the myocardial tissue. Functionally, myocardial fibrosis within the infarct scar favors the initiation and perpetuation of arrhythmias by uncontrolled overproduction of collagenous septa, which separate bundles of cardiomyocytes, producing structural discontinuities of the impulse propagation (zig-zag conduction) (3). The understanding of myocardial fibrosis at cellular and molecular levels has greatly benefited from the use of genetic tools over the last decade. Several studies have identified the origin of resident cardiac fibroblasts (4) and the complex network of signaling pathways contributing to the control of gene expression of these cell types, ultimately determining the fibrotic phenotype. The definition of these signaling events is a prerequisite for potential targets of pharmacological intervention.

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In this issue of the *Journal*, the study by Meyer et al. (5) adds knowledge to this field by describing a link between cell senescence and myocardial fibrosis and then providing evidence of an essential role of premature senescence in abrogating cardiac fibrosis. Through the use of animal models of cardiac disease and confirmation on human heart biopsies, the authors present data suggesting that cardiac fibroblasts undergo premature senescence during the course of fibrogenesis. Similar to other examples, such as liver fibrosis (6), genetic inactivation of the fibroblast cellular senescence program resulted in aggravated fibrosis and cardiac dysfunction. Conversely, induction of senescence limited fibrosis and promoted cardioprotection.

More specifically, the authors started from the observation that under pressure overload induced by transverse aortic constriction in mice, the senescent markers p21$^{CIP/WAF1}$, senescent-associated β-galactosidase, and p16^{INK4a} were significantly increased in the perivascular fibrotic areas of the myocardium, particularly in cells positive for markers typical of myocardial fibroblasts (vimentin, platelet-derived growth factor receptor-α). Similar results were found

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

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in human biopsies of patients with heart disease. The authors then proved the causality of fibroblast senescence and myocardial fibrosis by performing experiments using knockout mice for both the tumor suppressor gene p53—a gene that regulates cell cycle progression and is inactivated in a large percentage of tumors, genotoxic stress, and senescence, particularly in cycling cells—and the $p16^{INK4a}$ inhibitor of cyclin-dependent kinases, which blocks cell cycle progression (7). In the $Trp53^{-/-}$ $p16^{INK4a-/-}$ double knockout mouse, pressure overload was found to aggravate fibrosis. To further prove their point, Meyer et al. (5) used a cardiotropic vector (adeno-associated vector 9) to overexpress cysteine-rich angiogenic inducer 61 (Cyr61) in vivo. Cyr61, a protein secreted at sites of wound healing, induces fibroblast senescence through the activation of p53 and $p16^{INK4a}$ pathways. Data strongly suggested a role for premature senescence as an essential antifibrotic mechanism, with consequent therapeutic implications for HF.

Naturally, there are aspects that need further clarification. The authors have used constitutive knockout models, and thus it may well be that cell types other than fibroblasts are affected, although not to the same degree, by the lack of p53 or $p16^{INK4a}$. In particular, p53 is a regulator of transcription that is virtually active in all cell types, including growth-arrested ones. Also, a major issue in the field of myocardial fibrosis relates to the source of fibroblasts: from which cells do they originate? Are they derived from resident myocardial cells? Although there is consensus on the epicardial and endocardial origin of myocardial fibroblasts during development (8–12), the origin of adult fibroblasts, particularly in pathological states, is much more debated. Barring resident fibroblasts activated by specific proliferating signals, it has been proposed that they could come from circulating hematopoietic progenitor cells, the transition of endothelium to mesenchymal cells, or the epicardial epithelial-to-mesenchymal transition (13–15). However, a recent report, in which multiple independent murine Cre lines and a collagen1a1-*GFP* fusion reporter were used for specifically labeling fibroblasts, proved that pressure overload promoted comparable proliferation and activation of 2 resident fibroblast lineages, including a previously described epicardial population and a population of endothelial origin (16). Although the contribution of different cell types to the ECM-producing fibroblasts goes beyond the scope of this paper, it would be of interest to understand whether the genetic manipulation of p53 or $p16^{INK4a}$ affects 1 specific fibroblast progenitor or another.

A third aspect relates to the definition of “fibroblasts” and “myofibroblasts.” Fibroblasts have a spindle-shaped formation, reside in the majority of tissues and organs of the body, and produce ECM molecules. They are typically characterized by expression of vimentin and absence of desmin and smooth muscle alpha actin (SMα-actin). Fibroblasts originate from the mesenchyme; they portray a diverse phenotypic variability and can be observed as non-contractile fibroblasts, protomyofibroblasts, or contractile myofibroblasts (17). Indeed, myofibroblasts are associated with synthesis and secretion of ECM molecules, such as collagens, proteoglycans, and fibronectin, and can be distinguished from fibroblasts by their expression of de novo SMα-actin in stress fibers and various ECM proteins. Although myofibroblasts express SMα-actin, they can be distinguished from actual smooth muscle cells by their lack of desmin and smooth muscle myosin expression. The origin of myofibroblasts is debated, and may be precursors as a result of transdifferentiation of resident fibroblasts, circulating bone-marrow-derived progenitor cells, circulating adventitial or interstitial fibroblasts undergoing a phenotype switch, or smooth muscle cells. How and to what extent myofibroblasts contribute to fibrosis is a matter of investigation (18,19).

As of today, aldosterone antagonists are the only consolidated therapeutic approach for limiting the consequences of an uncontrolled fibrosis during HF-induced remodeling. The clarification of the molecular mechanisms of myocardial fibroblast homeostasis will further enhance our knowledge in this field of investigation, possibly leading to the discovery of more powerful and specific modulators of fibrosis, hopefully beyond spironolactone.

ACKNOWLEDGMENT The authors thank Michael V.G. Latronico for editorial support.

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KEY WORDS aging, fibroblast, gene expression, heart failure, tumor suppressor genes