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Review

Evidence of epigenetic tags in cardiac fibrosis

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ABSTRACT

In cardiac fibrosis, following an injury or a stress, non-functional fibrotic tissue substitutes normal myocardium, thus leading to progressive heart failure. Activated fibroblasts are principal determinants of cardiac fibrosis by producing excessive fibrotic extracellular matrix and causing hypertrophy of cardiomyocytes. Epigenetic changes, such as DNA methylation, histone modifications, and miRNAs have been involved in these mechanisms. Therefore, there is a strong interest in reverting such epigenetic transformations in order to arrest myocardial fibrotic degeneration. Demethylating agents, such as 5-aza-2′-deoxycytidine, 5-azacytidine, some selective histone deacetylase inhibitors, including mocetinostat, trichostatin A, and MPT0E014, have a direct action on important inducers of cardiac fibrosis. Also dietary compounds, such as resveratrol, can suppress the differentiation of fibroblasts to myofibroblasts. Although *in vivo* and *in vitro* studies suggest specific epigenetic therapies to treat cardiac fibrosis, the related clinical trials are still lacking. A better understanding of the epigenetic effects of dietary compounds (*e.g.* curcumin and green tea catechins) on the onset and progression of cardiac fibrosis, will allow the identification of protective dietary patterns and/or the generation of novel potential epidrugs.

Contents

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Introduction

One-third of naturally occurring deaths worldwide is associated with organ fibrosis and failure. Among fibrotic diseases, cardiac fibrosis is of particular relevance and understanding its etiopathogenesis may be beneficial in reducing cardiac morbidity and mortality [1,2].

Cardiac fibrosis, similarly to other cardiovascular diseases, results from the complex interaction of genetic, epigenetic, and environmental factors, such as lifestyle and diet. This knowledge led to the identification of biomarkers and therapeutic targets for the clinical management of patients [3–8].

Cardiac fibrosis is an injury- or stress-induced remodeling process of the heart, which causes the substitution of myocardium with non-functional fibrotic tissue, thus leading to impaired ventricular systolic and diastolic function, as well as atrial and ventricular arrhythmias and heart failure [9,10]. Cardiac myocytes occupy approximately 75% of normal myocardial tissue volume, but they account for only ≈30% of cell numbers. The majority of the remaining cells in the myocardium are non-myocytes: ≈60% endothelial cells, ≈13% fibroblasts, ≈6% vascular smooth muscle cells/pericytes. Other cell types, such as hematopoietic-derived cells and vascular smooth muscle cells, represent comparatively small populations [11].

Cardiac fibroblasts play numerous roles in cardiac development and remodeling and in defining cardiac structure and function. Following an injury, resident cardiac fibroblasts, but, possibly, also bone marrow-derived circulating fibrocytes, get activated, migrate to the site of injury, transdifferentiate into myofibroblasts, and secrete extracellular matrix (ECM) components [1,12]. This remodeling activity involves pathological changes that include chamber dilation, cardiomyocyte hypertrophy, and apoptosis, ultimately leading to heart failure. In particular, increased deposition of collagen and other matrix proteins promotes scar formation, which is critical for reparative healing of the myocardium. Conversely, sustained cardiac injury causes chronic myofibroblast activation and proliferation, leading to imbalanced collagen/matrix metalloproteinase secretion and interstitial fibrosis, myocyte ischemia, and arrhythmogenicity [13].

Many biochemical mediators participate to the development of cardiac fibrosis: inflammatory cytokines, chemokines, reactive oxygen species (ROS), mast cell-derived proteases, endothelin-1, the renin/angiotensin/aldosterone system, matricellular proteins, and growth factors [*e.g.* transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF)]. TGF-β can stimulate myofibroblasts to produce collagen and fibronectin, which, through a positive feedback, improve the TGF-β-induced ECM deposition [12,14].

In addition to resident cardiac fibroblasts and, possibly, bone marrow-derived fibroblasts, fibroblast-like cells arise from endothelial cells, through an endothelial-to-mesenchymal transition (EndMT) mediated by TGF-β1 [15]. Whereas EndMT is physiological occurring during embryonic heart development, abnormal activation of EndMT in adult mice contributes to the onset and progression of fibrosis [16]. Therefore, the process leading to the formation of new fibroblasts from endothelial cells appears to be a promising therapeutic target for cardiac fibrosis. However, the molecular bases of this TGF-β-induced EndMT have not been clarified to date, thus greatly limiting the potential of clinical intervention [17].

Within this context, it has been reported that aberrant deposition of ECM is associated with epigenetic changes in ECM-producing genes, such as those encoding for collagen, laminin, and fibronectin [1]. The occurrence of epigenetic changes in activated fibroblasts during cardiac fibrosis not only add novel mechanistic comprehension of the disease, but also represents a crucial issue for the identification of new cellular targets that could be manipulated for clinical purposes [18].

In this review, we examine the influence of epigenetics on the onset and progression of cardiac fibrosis and discuss the recent insight into the epigenetic signatures that might be useful for the prevention, diagnosis, and follow up of cardiac fibrosis. Furthermore, we debate the emerging anti-fibrotic epigenetic therapies and the potential beneficial effects on fibrotic heart remodeling of dietary compounds with epigenetic activity.

Epigenetics in cardiac fibrosis

Here the major epigenetic modifications that are better characterized and associated with cardiac fibrosis are reported (Fig. 1).

DNA methylation

Recent evidence suggests a role of DNA methylation in cardiac fibrosis. As previously mentioned, two key pro-fibrotic stimuli are TGF-β and hypoxia. In human coronary endothelial cells, TGF-β and hypoxia silence RASAL1 (a Ras-GTPase-activating protein) gene by inducing aberrant methylation of its promoter, thus increasing Ras-GTP activity and enhancing EndMT. Moreover, also in fibrotic heart samples from patients with end-stage heart failure and from mice, after 4 weeks since ascending aortic constriction, RASAL1 promoter hypermethylation, increase of Ras activity, and of EndMT markers were observed [19,20]. Interestingly, in both the *in vivo* and *in vitro* models, BMP7 could reverse the induced RASAL1 promoter methylation through hydroxymethylation by TET3, an enzyme of the ten-eleven translocation [19].

In vitro, hypoxia can stimulate human cardiac fibroblasts to express HIF-1α which, in turn, upregulates DNA-methylating genes DNMT1 and DNMT3a/3b. As a result, changes in DNA methylation occur and pro-fibrotic genes are activated [21]. In activated rat cardiac fibroblasts, increased DNMT3a expression is also associated with the up-regulation of ERK1/2, and with down-regulation of RASSF1a, a tumor suppressor gene involved in fibroblast activation and cardiac fibrosis [22]. In the same cellular model, knockdown of DNMT3a induced an increase of the expression of RASSF1a and the DNMT3a inhibitor 5-aza-2′-deoxycytidine (5-AzadC) prevented the loss of RASSF1a expression, thus blocking PDGF-BB-induced cardiac fibroblast proliferation [22]. Moreover, 5-AzadC has been reported to revert also the pro-fibrotic effects of TGF-β on human ventricular cardiac fibroblast cell line ventricular cardiac fibroblasts. In particular, this compound reduced the expression of collagen I, collagen III, and α-smooth muscle actin genes [23].

Overall, these findings support the importance of DNA methylation in the onset and progression of cardiac fibrosis and underline the potential beneficial effects of DNA methylation inhibitors in both prevention and treatment of this degenerative heart disease.

It is not surprising that epigenome-wide association studies (EWAS) addressing the relevance of DNA methylation in cardiac fibrosis are lacking. Indeed, EWAS analyze the epigenetic state at several loci in a group of people and evaluate possible associations of these epigenetic characteristics and traits of interest, such as a disease or an environmental stimulus [24]. In particular, it is impractical to obtain biological samples for most diseases including cardiac fibrosis and, consequently, DNA methylation has been often measured in blood DNA. However, the use of blood in replacement of the tissue of interest is questionable and should be first validated by providing evidence that both the tissue of interest and the surrogate tissue respond with similar epigenetic changes to a specific stimulus. Presently, this is not demonstrated

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