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Review

DNA methylation in cardiac fibrosis: New advances and perspectives

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ABSTRACT

Cardiac fibrosis is characterized by net accumulation of extracellular matrix (ECM) proteins in the cardiac interstitium, and contributes to both systolic and diastolic dysfunction in many cardiac pathophysiologic conditions. More specifically, cardiac fibroblasts are activated by a variety of pathological stimuli, thereby undergoing proliferation, differentiation to myofibroblasts, and production of various cytokines and ECM proteins. Thus, understanding the biological processes of cardiac fibroblasts will provide novel insights into the underlying mechanisms of cardiac fibrosis. DNA methylation is an important epigenetic mechanism, which often occurs in response to environmental stimuli and is crucial in regulating gene expression. The aberrant methylation of CpG island promoters of selected genes is the prominent epigenetic mechanism by which gene transcription can be effectively silenced. Aberrant hypermethylation of a few selected genes such as RASSF1A plays an important role in facilitating fibrotic fibroblast activation and in driving fibrosis. In this review we will discuss the mechanisms of DNA methylation and their implications for cardiac fibroblasts activation and fibrosis. Control of DNA methylation may serve as a new strategy for anti-fibrotic therapy.

Contents

Abbreviations: ECM, extracellular matrix; α-SMA, α-smooth muscle actin; EMT, epithelial to mesenchymal transition; ET-1, endothelin-1; DNMT, DNA methyltransferase; MeCP2, methyl-CpG-binding protein 2; MBD, methylcytosine-binding domains; miRNA, microRNAs; SAM, S-adenylmethionine; TNF-α, tumor necrosis factor-alpha; LPS, lipopolysaccharide; ANGII, angiotensin II; HDAC, Histone deacetylase.

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1. Introduction

Fibrosis is the morphological correlate of chronic organ impairment (Ozkurt et al., 2014). It is estimated that one-third of naturally occurring deaths worldwide are associated with fibrosis (Hansen et al., 2013; Zeisberg and Kalluri, 2013). Cardiac fibrosis is characterized by net accumulation of extracellular matrix proteins in the cardiac interstitium, and contributes to both systolic and diastolic dysfunction in many cardiac pathophysiologic conditions (Qi et al., 2014; Yu et al., 2014). In general, fibrosis is considered a pathological wound-repair process (Luther et al., 2013; Wang et al., 2013c). The rationale for such thinking is obvious when pathological fibrosis and physiological wound repair are being compared: both processes are associated with deposition of extracellular matrix (ECM), although ECM deposition during fibrosis is excessive in comparison and does not spontaneously resolve, which can lead to permanent scarring in heart (Seok et al., 2014; Ulm et al., 2014). Although the fibroblast activation and proliferation early after cardiac injury are critical for maintaining cardiac integrity and function, the persistence of fibroblasts long after injury leads to chronic scarring and adverse ventricular remodeling (Kinoshita et al., 2014; Ning and Jiang, 2013; Sarrazy et al., 2014). To date, little is known about molecular mechanisms that decide between physiological repair and fibrosis responses are only incompletely understood.

Recent studies have implied that DNA methylation play an important role in determining the response of cardiac tissue injury and fibrosis (Duygu et al., 2013; Kim et al., 2014; Tao et al., 2013). DNA methylation is an essential epigenetic modification on chromosomes that plays an important role in the regulation of gene transcription (Baubec and Schubeler, 2014; Lokk et al., 2014). DNA methylation typically involves the addition of a methyl group to the 5′ position of the cytosine pyrimidine ring of a CpG dinucleotide (Koo et al., 2014; Theruvathu et al., 2013). As the cytosine and guanine contents are greater than 50% in the DNA sequence of mammals, the high CG content regions can be hypermethylated causing transcriptional silencing (Nautiyal et al., 2010; Zhao et al., 2013). Here we review new insights into the role of DNA methylation in fibroblast activation and cardiac fibrosis.

2. The role of activated fibroblasts in cardiac fibrosis

Cardiac fibroblasts are the most abundant cell type in the mammalian heart and comprise approximately two-thirds of the total number of cardiac cell types (Shinde and Frangogiannis, 2013; Sinfield et al., 2013). Activated fibroblasts, as the major source of ECM, are principal mediators of fibrosis (Goldsmith et al., 2014; Park et al., 2014). Cardiac fibroblasts are activated by a variety of pathological stimuli, such as myocardial injury, oxidative stress, mechanical stretch, and elevated autocrine–paracrine mediators, thereby undergoing proliferation, differentiation to myofibroblasts, and production of various cytokines and ECM proteins (Goldsmith et al., 2014; Huang et al., 2010; Yue et al., 2013). Fibroblasts in the heart play a critical function in the secretion and modulation of ECM critical for optimal cellular architecture and mechanical stability required for its mechanical function (El Hajj et al., 2014; Vistnes et al., 2014). Cardiac fibroblasts are also intimately involved in both adaptive and nonadaptive responses to cardiac injury (Crawford et al., 2012). Cardiac fibroblasts display unique biological functions, including increased production of fibrillar type I and type III collagens, expression of α-smooth muscle actin (α-SMA), a molecular marker of activated myofibroblasts, and reduction in the expression of genes encoding ECM-degradative enzymes (Aguilar et al., 2014; Tao et al., 2014b). There are several important sources of the myofibroblasts encountered in the cardiac fibrosis tissues (Lu et al., 2013; Mesquita et al., 2014). For example, activation and proliferation of tissue resident fibroblasts or perivascular and vascular adventitial fibroblasts in response to specific signals from infiltrating inflammatory cells resulting in activation of quiescent fibroblasts to myofibroblast phenotype (Wang et al., 2013a,b). Moreover, transdifferentiation of epithelial cells to myofibroblasts, a process known as epithelial to mesenchymal transition (EMT), which is induced by TGF-β1, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), tumor necrosis factor (TNF)-α, connective tissue growth factor (CTGF), and monocyte chemoattractant protein (MCP)-1 to stimulate the proliferation and phenotypic differentiation of fibroblasts into myofibroblasts (Baek and Tallquist, 2012; Correa-Costa et al., 2014; Ishikawa et al., 2013). In EMT, epithelial cells lose their epithelial characteristics, including E-cadherin expression and apical-basal polarity, and reorganize their cytoskeleton to acquire a motile behavior and the phenotype of myofibroblasts including the expression of α-SMA and fibroblast specific proteins such as type I collagen (DeLaughter et al., 2013; Lachaud et al., 2013; Piera-Velazquez et al., 2011). There is a growing realization that the clinical manifestations and the molecular pathogenesis of a given fibrosis disease are highly heterogeneous (Mocumbi and Falase, 2013; Ramos et al., 2012). Despite this heterogeneity, however, the final common result is the excessive production of ECM (Agrinier et al., 2013). Thus, the focus of this review will be on the cellular alterations and molecular pathways that play critical roles in the fibrotic response and to discuss potential strategies that may allow identification of potential therapeutic targets.

3. Overview of DNA methylation

Epigenetic refers to heritable changes in gene expression, which are not a result of changes in the DNA sequence, but rather due to alterations related to the packaging or translation of genetic information (Heard and Martienssen, 2014; Nguyen, 2014). DNA methylation, which is the addition of a methyl group at the C5 position of the cytosine ring, generating 5-methyl cytosine (Espada et al., 2014; Leger et al., 2014). Methylation occurs on cytosine residues that precede a guanosine in the DNA sequence (De Prins et al., 2013; Lin et al., 2013; Yang et al., 2010). While these CpGs are relatively rare in the genome as a whole, they are often clustered in short stretches of DNA of 300–3000 base pairs (CpG islands) (Dyson et al., 2014). The DNA methylation reaction is catalyzed by DNA methyltransferases (DNMTs) (Qian and Xu, 2014). In humans there are three known enzymes that can methylated DNA, named DNA methyltransferases DNMT1, DNMT3a and DNMT3b (Haggarty et al., 2013). They catalyze the transfer of a methyl group from S-adenylmethionine (SAM) to the fifth carbon of cytosine, forming 5-methylcytosine (5mC) (Esse et al., 2013; Iacobazzi et al., 2013).

DNMT1 shows preference for hemimethylated DNA in vitro, which is consistent with its role as a maintenance DNMT, whereas DNMT3a and DNMT3b methylate unmethylated and methylated DNA at an equal rate which is consistent with a de novo DNMT role (Fagan et al., 2013; Leppert and Matarazzo, 2014). Deletions of the major DNA methyltransferases, DNMT1, DNMT3a and DNMT3b, lead to early lethality during embryogenesis (Mortusewicz et al., 2005). In addition, enzymes that are involved in de-methylation, including iterative oxidation of methylated cytosine and subsequent base excision by repair enzymes, are crucial and their targeted deletion in mice also results in embryonic lethality (Page-Lariviere and Sirard, 2014).

DNA methylation is associated with the silencing of gene expression (Ping et al., 2014; Wu et al., 2014). DNA methylation inhibits gene expression by several mechanisms. The methylation of promoter sequences inhibits the binding of some transcription factors (Paonessa et al., 2013). The methyl