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Epicardium-derived fibroblasts in heart development and disease

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Abstract

The majority of cardiac fibroblasts in a mature mammalian heart are derived from the epicardium during prenatal development and reactivate developmental programs during the progression of fibrotic disease. In addition, epicardial activation, proliferation, and fibrosis occur with ischemic, but not hypertensive injury. Here we review cellular and molecular mechanisms that control epicardium-derived cell lineages during development and disease with a focus on cardiac fibroblasts. This article is part of a special issue entitled “Fibrosis and Myocardial Remodeling”.

Keywords

Cardiac fibrosis; epicardium; fibroblast lineage; myocardial infarction

1. Introduction

Cardiac fibrosis occurs in the context of multiple cardiac stresses including myocardial infarction (MI), hypertensive heart disease (HHD), diabetes, and aging [1, 2]. Fibrosis is characterized by increased deposition of extracellular matrix (ECM) proteins, notably fibrillar collagen, which can lead to progressive stiffening, loss of systolic function, and ultimately heart failure [3]. There is emerging evidence that the localization and cell types involved in cardiac fibrosis are distinct, depending on the type of stress exerted on the heart [4]. Activation of the epicardial layer of the heart occurs with cardiac injury, such as MI, but is less apparent in hypertensive heart disease [4, 5]. Recent studies demonstrate that the embryonic epicardium is the predominant source of cardiac fibroblasts (CFs), including those activated during pathologic fibrogenesis [6, 7]. Likewise, regulatory programs that promote the cardiac fibroblast lineage development are reactivated in the adult cardiac fibrotic response, not only in the epicardium, but also in interstitial and perivascular adventitial cardiac fibroblasts [4, 5]. Thus the epicardium and epicardial developmental

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regulatory mechanisms are integral to the initiation and progression of cardiac fibrotic disease.

2. Epicardial development and origins of cardiac fibroblasts

In the developing vertebrate heart, the epicardium is formed with the growth of mesothelial proepicardial progenitors arising at venous and arterial poles of the looped heart [8]. Formation of the epicardial cell layer on the surface of the ventricles is apparent beginning at Embryonic day (E)10.5 in mice, E3 in chick, and during the fourth week of gestation in human embryos [9, 10] [11]. A subset of epicardial epithelial cells undergoes an epithelial-to-mesenchymal transition (EMT) and invades the adjacent myocardium, while others remain on the surface of the heart (Figure 1) [12–14]. Fate mapping and cell lineage studies from the 1990s demonstrated that epicardium-derived cells (EPDCs) differentiate into cardiac fibroblasts and vascular smooth muscle cells of the mature heart [9, 15, 16]. More recent Cre-recombinase-based lineage and cell sorting studies in mice demonstrate that adult cardiac fibroblasts activated with disease are primarily derived from the developing epicardium [6, 7].

Multiple cell signaling pathways regulate epicardial EMT, migration, lineage determination, and differentiation. Transforming growth factor β (TGF β) signaling promotes epicardial EMT, and initiation of epicardial EMT involves Bone Morphogenetic Protein 2 (BMP2) signaling through Type III Transforming Growth Factor β receptor 3 (TGF β R3) [17]. Epicardial EMT also is dependent on Wnt/ β -catenin signaling, and β -catenin is required for oriented cell division and delamination of epithelial cells from the epicardium [12, 13]. Platelet derived growth factor (PDGF) signaling through either PDGFR α or β is required for epicardial EMT, while cardiac fibroblast cell lineage determination and differentiation are dependent specifically on PDGFR α [18, 19]. Calcineurin signaling and NFATc1 activity promote invasion of EPDCs into the myocardium [14]. Likewise TGF β signaling and Myocardin-related transcription factors (MRTFs) also are required for migration of EPDCs and subsequent development of coronary vessels and fibroblast lineages [20, 21]. Retinoic acid (RA) signaling is active in EPDCs on the surface of the heart, and RA treatment inhibits smooth muscle differentiation of EPDCs [22]. Together, these signaling pathways coordinate the initial generation of EPDCs, as well as control the differentiation and maturation of multiple cell types in the developing heart, including cardiac fibroblasts.

The transcription factors Wt1, Tbx18, and Tcf21 are expressed in the epicardium and EPDCs [23]. Differential regulation of these factors is apparent in specific activation of Wt1 and Tcf21, but not Tbx18, by RA signaling in subepicardial mesenchyme [22]. Wt1 has a critical role in epicardial EMT through regulation of Wnt/ β -catenin and Snail transcription factors [13, 24, 25]. Thus Wt1 is necessary for the initial formation of EPDCs, but its expression is downregulated after migration into the myocardium [13]. Tcf21 expression in EPDCs is maintained after they invade the myocardium, and it represses smooth muscle differentiation while activating cardiac fibroblast lineage development [22, 26]. Cre-based lineage studies demonstrate that derivatives of EPDCs expressing Wt1, Tbx18, or Tcf21 differentiate into fibroblasts and vascular smooth muscle cells [27–29]. While contribution of EPDCs to endothelial cells and cardiomyocytes has been a matter of contention, there is

agreement that Wt1, Tbx18, and Tcf21 reliably mark cardiac fibroblast and smooth muscle progenitors that arise from the embryonic epicardium.

3. Location, cellular contributions, and progression of cardiac fibrosis

Cardiac fibrosis results from increased cell proliferation, myofibroblast activation, and synthesis of collagen and matrix remodeling enzymes in cardiac fibroblasts [3]. Investigation of the sources and activation mechanisms of cardiac fibroblasts has been an active area of recent research. The prenatal epicardium is the main source of cardiac fibroblasts in the developing heart with additional contributions from endothelial-derived endocardial cushions [9, 30]. Cell lineage and sorting studies in mice demonstrate that resident fibroblasts generated from epicardial-lineages are the predominant cells that promote adult fibrosis after pressure overload [6, 7]. With cardiac ischemic injury, bone marrow-derived cells infiltrate the area of injury and contribute to scarring, but EPDCs generated during embryogenesis (Wt1Cre lineage) are the main source of fibroblasts that contribute to pathologic ventricular remodeling and fibrosis [31]. Generation of new fibroblasts in adults via EMT from the adult epicardium [32] and vascular endothelium [33] also has been reported. These cell lineage studies are limited by narrow identification of fibroblasts as defined by a collagen1GFP transgene or combinations of cell surface markers, and the full complement of activated myofibroblasts after cardiac injury may include additional cellular origins [34]. However, there is increasing evidence that cardiac fibroblasts originally derived from the embryonic epicardium are primary mediators of cardiac fibrotic disease.

The localization of cardiac fibrosis is distinct depending on the initial cause of disease (Figure 1). Activation of the epicardium and deposition of increased ECM on the epicardial surface of the heart are prevalent with MI and ischemic injury in mice, dogs, and humans [4, 5, 31]. Interestingly epicardial activation occurs both in the area of injury and also in regions remote from injury, supporting a widespread epicardial injury response with certain cardiac stresses. In contrast, perivascular cardiac fibrosis is prevalent under hypertensive conditions, such as pressure overload, while widespread epicardial activation is not observed [4]. Fibroblast activation and increased collagen deposition in the myocardial interstitium are observed with multiple cardiac insults and likely are significant factors in decreased myocardial function and heart failure with fibrosis resulting from a variety of causes. The underlying mechanisms for epicardial activation with ischemic injury and perivascular fibrosis with pressure overload are not known, but could have implications for treatment of fibrotic heart disease of different origins.

4. Epicardial activation with myocardial infarction

In a normal adult heart, the epicardium and subepicardium are a few cell layers thick. After MI or ischemia/reperfusion injury, the number of cells in the epicardium and subepicardial space on the surface of the injured heart is dramatically increased. In mice the epicardial thickness is increased by >6-fold, and collagen deposition surrounding the heart is greatly increased 7d after ischemia/reperfusion injury [4]. In human and dog hearts after MI, increased numbers of subepicardial cells are apparent, in addition to increased epicardial

adipose deposition that is not observed in rodents [4, 5]. Within a week after MI in mice, new EPDCs are generated by epicardial EMT and increased cell proliferation of epicardial cells [5]. This epicardial activation is organ-wide, even in areas remote from injury. Interestingly a similar epicardial activation response occurs in adult zebrafish after cardiac injury and precedes regeneration of the myocardium [35]. While adult mammals have lost the ability to robustly regenerate the myocardium after injury, epicardial activation after injury has been retained. The study of reparative or regenerative functions of activated EPDCs after cardiac injury in mammals is currently an active area of research [36, 37].

Epicardial activation after cardiac injury recapitulates EPDC development during embryogenesis [23]. EMT of the epicardial epithelium is induced, as indicated by expression of Twist1 and Snail1/2, activation of BMP and TGF β signaling, as well as assumption of mesenchymal morphology in culture [4, 5]. The majority of cells in the thickened subepicardial space are derived from the epicardium, as indicated by lineage tracing with tamoxifen-inducible Wt1CreER or infection with an epicardial-specific mesothelin (Msln)-Cre adenovirus [5]. In addition, RA, Notch, and Wnt1/ β -catenin signaling are upregulated in the activated epicardium and newly generated EPDCs [5, 32, 38]. Expression of EPDC transcription factors Wt1, Tbx18, and Tcf21 also is induced in subepicardial cells within a week after injury [4, 5]. The newly generated EPDCs, together with bone marrow-derived cells, contribute to scar formation in the infarcted area [31]. Unlike embryonic EPDCs, adult EPDCs generated after injury largely remain on the surface of the heart, as indicated by fate mapping and cell lineage tracing [5]. However, newly generated EPDCs in adult injured hearts differentiate predominantly into smooth muscle and cardiac fibroblast lineages similar to embryonic EPDCs [4, 5]. Adult EPDCs also secrete multiple angiogenic factors, including VEGFA and FGF2, that can improve cardiac function and reduce scar formation during the cardiac injury response [5].

5. Epicardial developmental pathways activated in cardiac fibroblasts and potential therapeutics

Activation of resident cardiac fibroblasts is apparent in the epicardium, myocardial interstitium, and/or perivascular adventitia during cardiac fibrosis resulting from various pathologic stimuli. The differential localization of cardiac fibrosis with ischemic injury or pressure overload is likely due to distinct populations of infiltrating cells and acute injury response after MI in contrast to biomechanical stimulation of perivascular fibroblasts with hypertensive disease [39, 40]. Human and mouse interstitial CFs in fibrotic hearts express EPDC transcription factors Wt1, Tcf21, and Tbx18, which are differentially regulated in development and also in cardiac fibrotic disease resulting from ischemia or hypertension [4, 23, 31]. Wt1 and Tbx18, expressed early in epicardial lineage development, are preferentially expressed in epicardial and interstitial, but not perivascular fibrosis [4, 13]. In contrast, Tcf21 has a more specific role in fibroblast lineage development and is induced in epicardial, interstitial, and perivascular fibrosis in response to ischemic injury or pressure overload [4, 22, 26]. Lineage tracing with tamoxifen-inducible Cre lines supports widespread activation of Tcf21-derived fibroblasts after pressure overload or MI, whereas Wt1-derived fibroblasts are preferentially activated with ischemic injury but not angiotensin

II-induced pressure overload [29, 31](Xiang, Fang, Yutzey unpublished data). Thus, transcriptional regulatory mechanisms that control EPDC lineages during development also are active in the adult fibrotic response, but exhibit differential induction depending on the nature of cardiac injury.

Multiple signaling mechanisms that control the initial formation and differentiation of EPDCs in the developing embryo are reactivated with fibrotic disease and have been targeted therapeutically. TGF β signaling is activated with cardiac fibrosis and has a critical role in myofibroblast activation and cell migration, as has been demonstrated in epicardial EMT [21]. Likewise, inhibition of TGF β or BMP signaling, required for EPDC development, ameliorates cardiac fibrosis in animal models [41–44]. Cardiomyocyte-specific expression of human stem cell factor also induces epicardial EMT, with increased TGF β signaling and Wt1 expression, leading to increased myocardial arteriogenesis after MI [45]. During acute cardiac ischemic injury, activation of Wnt/ β -catenin signaling is apparent in epicardium, but its role in fibrosis, especially with pressure overload, has not been fully characterized [32]. Interestingly, therapeutic inhibition of Wnt/ β -catenin signaling can suppress fibrosis induced by MI in mice [43, 46]. Additional studies are necessary to define the inductive mechanisms and crosstalk among different pathways in interstitial CFs that lead to activation and progression of fibrosis. These developmental mechanisms represent attractive therapeutic targets for cardiac fibrosis and adverse remodeling that have not yet been fully explored.

6. Perspectives and conclusions

The specific functions and contributions of epicardial fibroblasts activated during adulthood by ischemic injury are not fully known, but could be beneficial. Activated epicardial cells secrete angiogenic factors and can promote arteriogenesis after MI [5, 45]. Epicardial activation can be enhanced by treatment with Thymosin β 4, which enhances neoangiogenesis and generation of new cardiomyocytes after MI [47, 48]. EPDCs that arise in adulthood after MI are heterogeneous and distinct from progenitor populations that arise during development [49]. Multiple epicardium-derived stem cell populations have been described that could have beneficial effects on vascularization or remodeling of the injured myocardium [49, 50]. Follistatin-like1 (FSTL1) has been identified as a factor secreted from epicardial cells that, when delivered via an epicardial patch, promotes resident cardiomyocyte proliferation after MI in swine [51]. Therefore, fibroblasts, as indicated by increased collagen expression, that are derived from injury-induced epicardial expansion might be inherently different from those amplified in the interstitium or perivascular adventitia after injury. It is possible that these activated epicardial cells have reparative functions after cardiac injury as has been observed during zebrafish heart regeneration.

Recent studies have demonstrated that resident cardiac fibroblasts that arose from the epicardium during development are the major source collagen-producing activated fibroblasts in hypertensive injury in mice [6, 7]. The fibrotic response induced by hypertensive injury causes adverse myocardial remodeling and can eventually lead to heart failure. Therapeutic interventions in animal models of heart failure and fibrosis have targeted signaling pathways implicated in cardiac fibroblast development [41, 43, 46]. An

interesting observation from these therapeutic studies is that reduced cardiac fibrosis is accompanied by improved cardiac function supporting paracrine interactions between pathogenic fibroblasts and cardiomyocytes [41, 43, 46]. However, whether these pathways work together or in parallel in the progression of fibrosis is not known. It is also unclear whether these beneficial interventions are dependent on the type of injury, cell types involved in fibrosis, or stage of treatment. Therefore, it is important to consider potential differences among epicardial, perivascular, and interstitial fibrotic responses when developing antifibrotic therapeutic strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

(MI)	Myocardial Infarction
(HHD)	Hypertensive heart disease
(HI)	Hypertensive Injury
(ECM)	Extracellular matrix
(CF)	Cardiac fibroblast
(EMT)	epithelial-to-mesenchymal transition
(EndMT)	endothelial-to-mesenchymal transition
(EPDCs)	epicardium-derived cells

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Highlights

Fibroblasts derived from embryonic epicardium contribute to cardiac fibrosis

Epicardial developmental pathways are active in adult fibrotic heart disease

Epicardial activation and fibrosis occurs with myocardial infarction.

Cardiac fibrosis is differentially localized with ischemic and hypertensive injury

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Figure 1. Generation of cardiac fibroblasts in development and disease

During embryonic development, epicardium-derived cells (EPDCs) invade the myocardium and differentiate into cardiac fibroblasts (blue cells) and vascular smooth muscle cells (pink cells; Left panel). After myocardial infarction (MI), the epicardium is activated and undergoes epithelial-to-mesenchymal transition (EMT), producing increased numbers of subepicardial cells expressing developmental transcription factors Tcf21, Wt1, and Tbx18 with increased collagen deposition (blue background) on the surface of the heart. With hypertensive injury (HI), perivascular adventitial fibrosis (blue) occurs surrounding coronary arteries. Interstitial fibrosis (blue) is apparent with both MI and HI.