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Review

Epicardial Lineages and Cardiac Repair

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Abstract: The death of cardiac myocytes resulting from myocardial infarction is a major cause of heart failure worldwide. Effective therapies for regenerating lost cardiac myocytes are lacking. Recently, the epicardium has been implicated as a source of inflammatory cytokines, growth factors and progenitor cells that modulate the response to myocardial injury. During embryonic development, epicardially-derived cells have the potential to differentiate into multiple cardiac lineages, including fibroblasts, vascular smooth muscle and potentially other cell types. In the healthy adult heart, epicardial cells are thought to be generally quiescent. However, injury of the adult heart results in reactivation of a developmental gene program in the epicardium, which leads to increased epicardial cell proliferation and differentiation of epicardium-derived cells (EPDCs) into various cardiac lineages. Recent work suggests that epicardial reactivation after injury is accompanied by, and contributes to, a robust inflammatory response. In this review, we describe the current status of research related to epicardial biology in cardiac development and regeneration, highlighting important recent discoveries and ongoing controversies.

Keywords: epicardium; proepicardium; epithelial-to-mesenchymal transition (EMT); cardiac regeneration

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1. Introduction

The development of the epicardium is conserved through the evolution of vertebrates. The epicardial layer of the heart derives from an embryonic anlage, known as the proepicardial organ (PEO), which is a transient structure that arises from the mesothelium of the septum transversum. The outer lining of the PEO is covered with epithelial cells, while mesenchymal cells compose the core [1–4]. Early heart development begins with the formation of the linear heart tube derived from lateral plate mesoderm. Prior to heart looping, the heart tube has only two layers: endocardium and myocardium. After looping has occurred, at ~E9.5 in the mouse, cells from the PEO migrate onto the surface of the heart to form a third heart layer: embryonic epicardium [1–4]. Proepicardial cells migrate either as individual cells (in mammals) or as a sheet of cells (in birds) and cover the developing myocardium [5]. Soon after the epicardium is formed, a subset of these epithelial cells undergoes mesenchymal transformation and invades the subepicardial space and underlying myocardium and differentiates into multiple cardiac cell types, while other cells remain on the surface of the heart and form the definitive epicardium [1,4,6–8] (Figure 1). Many secreted factors, extracellular matrix molecules, enzymes, transcription factors and signaling molecules, have been implicated in PEO formation, epicardium formation, epicardial epithelial-to-mesenchymal transformation (EMT) and epicardially-derived cell (EPDC) proliferation, survival and migration [6,9,10]. For example, α4-integrin, a cell adhesion receptor expressed on epicardial cells, binds to fibronectin and vascular cell adhesion molecule-1 (VCAM-1) and regulates PEO migration. Genetic deletion of *α4-integrin* or *VCAM-1* results in a heart without an epicardium, due to impaired proepicardial cell migration [11–13]. *Podoplanin* deletion results in reduction of PEO size and decreased attachment to myocardium [14]. Loss of *Dicer*, a microRNA-processing enzyme, in the epicardium results in coronary vasculature defects, due to reduced epicardial cell proliferation, differentiation and EMT [9]. *Wt1* expression in the epicardium is necessary for coronary vasculature development [15,16]. Wt1 modulates epicardial EMT by regulating Wnt and retinoic acid signaling during development [15,16]. Tbx18, a member of the T-box family of transcription factors, is expressed in the proepicardium, the epicardium, the ventricular septum, the left ventricular myocardium and the sinus horns of the mouse heart [17,18]. Although loss of *Tbx18* in the epicardium does not affect epicardial development and function (perhaps due to functional redundancy by other T-box factors), epicardial expression of a transcriptional activator version of Tbx18 (Tbx18VP16) results in premature smooth muscle differentiation of epicardial cells, due to activated transforming growth factor-beta receptor (TGFßR) and Notch signaling in the embryonic epicardium [19]. Nfatc1, a transcription factor regulated by calcium and calcineurin, is expressed by a subset of cells in the PEO, epicardium and coronary vessels [20]. Genetic studies suggest that Nfatc1 is required for promoting EPDCs invasion into myocardium [20]. Tallquist and colleagues identified neurofibromin (encoded by the *Nf1* gene) as a key mediator of epicardial EMT [21]. Loss of *Nf1* in epicardial cells results in increased EMT, EPDC proliferation and subsequent differentiation into fibroblasts and smooth muscle cells [21].

For many decades, epicardium was considered to be a passive fibrous lining surrounding the myocardium. However, studies in the last 10 years have suggested an active role for epicardium in development, disease and regeneration. For example, epicardium is a source of multipotent progenitor cells, which give rise to important components of the developing heart (Figures 1 and 2) [22–26].

Moreover, reciprocal paracrine signaling between the epicardium and myocardium regulates coronary vasculature development, as well as cardiomyocyte proliferation and differentiation [8,10,27–29] (Figure 3). Myocardial injury of the adult heart leads to reactivation of an embryonic gene program in epicardial cells. Factors released from activated epicardium modulate cardiac regeneration and function, at least in part by promoting angiogenesis (Figures 3 and 4) [10,27,30,31]. Despite the critical role of the epicardium during development, physiological functions in the adult heart remain poorly understood.

Figure 1. Epicardial derivatives contribute to cardiac lineages. Epicardial cells undergo epithelial-to-mesenchymal transition (EMT), delaminate from the epicardium, invade the underlying myocardium and differentiate into various cardiac lineages, including fibroblasts, smooth muscle cells, endothelial cells and, perhaps, cardiomyocytes.

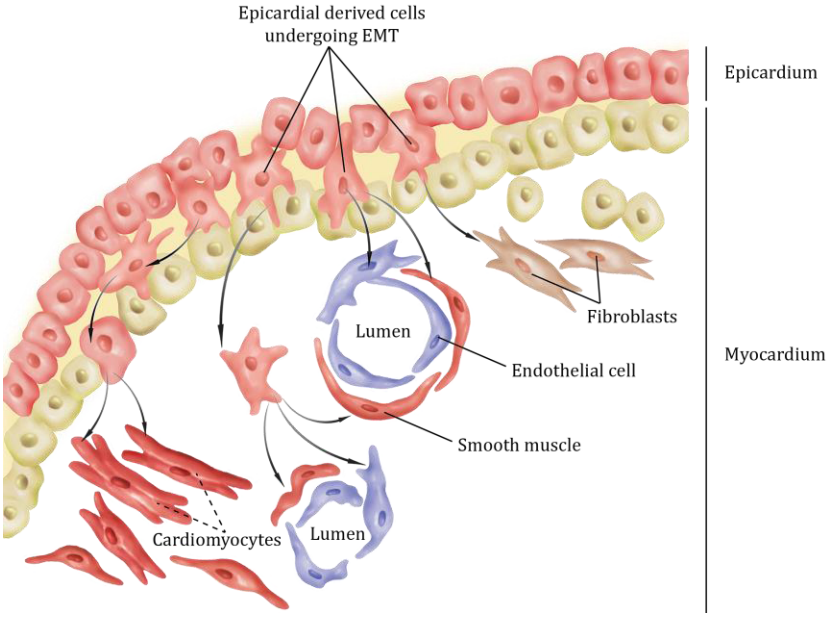


Figure 2. Heterogeneous proepicardial precursors and their fates. Distinct proepicardial sub-compartments variably give rise to coronary endothelial cells, vascular smooth muscle cells, fibroblasts and perhaps cardiomyocytes. Arrows represent differentiation potential. Dotted arrows indicate unresolved potential.

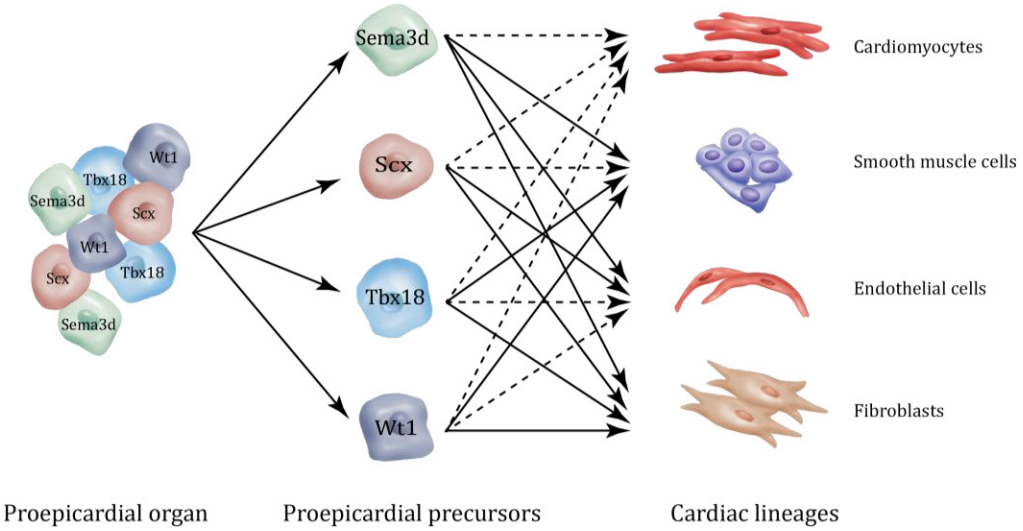


Figure 3. Reciprocal interactions between epicardium and myocardium during cardiac development. Retinoic acid (RA) modulates epicardial EMT and coronary vascular development by regulating Fgf2 and Wnt/β-catenin signaling. RA also activates Fgf9 expression in the epicardium, which signals via receptors Fgfr1/2 to regulate myocardial proliferation and differentiation. Signals from the myocardium, such as Thymosin β4, platelet-derived growth factors (PDGFs) and fibroblast growth factors (FGFs), regulate epicardial cell differentiation.

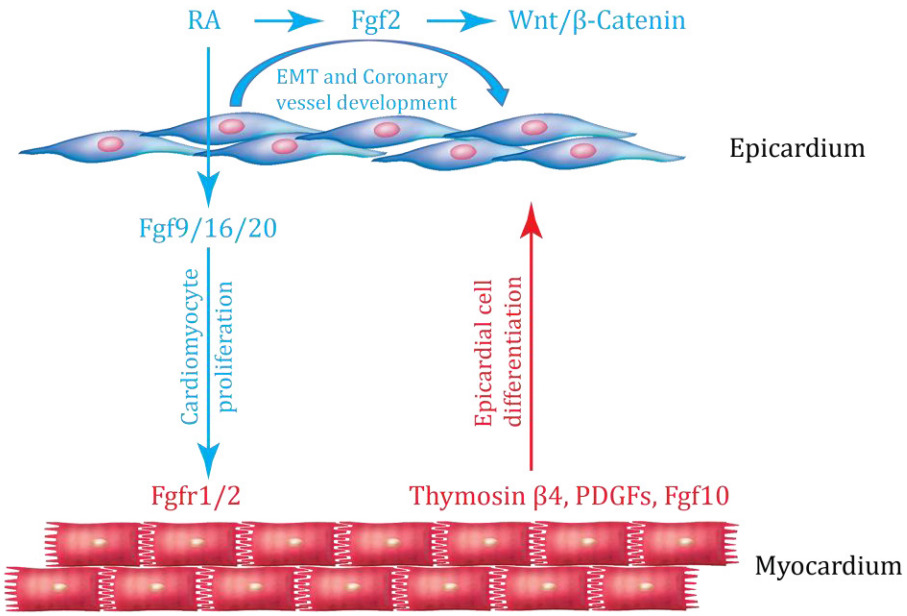
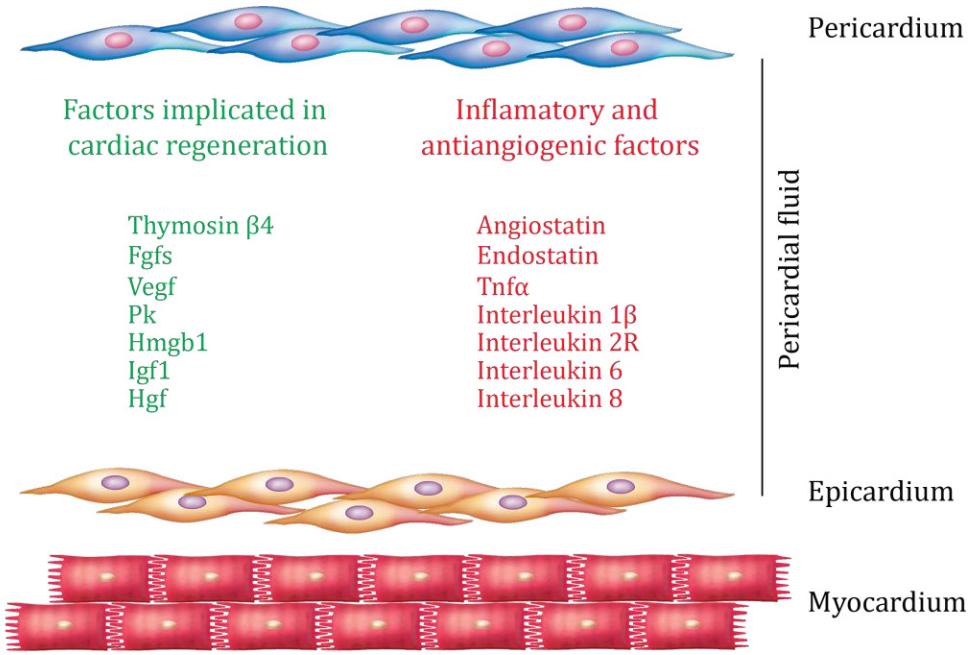


Figure 4. Regenerative and inflammatory response after myocardial injury. Factors accumulate in the pericardial fluid after myocardial infarction that may affect both epicardial and myocardial function and which are implicated in cardiac regeneration and inflammation. Recent studies suggest that the balance between regenerative and inflammatory factors can affect repair and subsequent cardiac function.



2. Epicardial Lineages

Fate mapping analysis of early cardiac multipotent precursors that express either *Nkx2.5* or *Isl1* suggest that these cells contribute to cardiomyocyte, smooth muscle and endothelial lineages of the mature heart [32,33]. However, the relationship between early cardiac progenitors (marked by *Nkx2.5* or *Isl1*) and the PEO was not fully elucidated in these initial studies. Recent work by Zhou *et al*. demonstrates that Wilm's Tumor 1 (Wt1) is expressed in the PEO, epicardium and epicardium-derived cells (EPDCs) [24]. *Wt1*-expressing proepicardial progenitors are derived from *Nkx2.5* and *Isl1*-expressing secondary heart field precursors [34]. *Nkx2.5* and *Wt1* are not actively co-expressed in the PEO, but are either sequentially expressed or only transiently co-expressed in a subset of PEO precursors [34]. Unlike *Isl1*, expression of *Nkx2.5* is necessary for the formation of the PEO, since loss of *Nkx2.5* resulted in abnormal PEO formation and reduced *Wt1* expression [34].

The proepicardial contribution to various cardiac lineages has been disputed, due to conflicting findings in chick and mouse. The epicardial cells begin to cover the myocardium at the same time that coronary precursor cells first appear in the heart tube [1]. Retroviral labeling experiments demonstrate that single vasculogenic cells of the proepicardium differentiate into cardiac endothelial, smooth muscle cells and perivascular connective tissue cells [1]. Further studies using diI labeling and quail-chick chimeras have established the PEO as a source of both cardiovascular smooth muscle and endothelial cells in chick [35–38]. However initial fate mapping studies in mice did not identify a significant proepicardial contribution to the cardiac endothelium [19,23,24,26].

The PEO was thought to be a genetically homogeneous tissue. However, recent studies in both chick and mouse suggest that the PEO is a highly heterogeneous collection of genetically distinct sub-populations of cells with distinct functions and downstream fates (Figure 2). In a recent study, we, with our collaborators, demonstrated that *semaphorin 3D (Sema3D), scleraxis (Scx), Wt1* and *Tbx18* could mark distinct sub-populations of progenitor cells within the PEO. *Wt1*- and *Tbx18*-expressing proepicardial cells differentiate into smooth muscle cells and fibroblasts, but rarely, if ever, into coronary endothelium [23,24,26]. In contrast, *Scx*- and *Sema3D*-expressing progenitors within the PEO differentiate into smooth muscle cells, fibroblasts and coronary endothelium [25]. In addition, they also contribute to two other vascular endothelial tissues: the sinus venosus and the endocardium. *Scx* and *Sema3D* expression partially overlaps in the proepicardium, but is distinct from cells expressing the canonical epicardial markers, *Tbx18* and *Wt1* [25]. This might explain why fate mapping using mouse *Wt1* and *Tbx18* cre lines does not detect the proepicardial contribution to coronary endothelium.

Further, two independent studies have recently demonstrated that during cardiac development, a subpopulation of proepicardial cells marked by either *Tbx18* or *Wt1* expression have the potential to differentiate into cardiomyocytes [23,24]. This could have significant clinical implications as a potential source for regenerative cardiomyocytes in adults [39]. However, the conclusions that *Wt1* and *Tbx18* expressing epicardial cells can give rise to cardiomyocytes have been challenged based on the suggestion that *Wt1* and *Tbx18* are normally expressed by some cardiomyocytes, thus confounding the interpretation of the cre-based fate-mapping results. This controversy remains unresolved [18,23,24,26].

Cardiac fibroblasts compose up to two-thirds of the cells in the heart and regulate structural, mechanical, biochemical and electrical properties during development and disease [40,41]. During

embryonic development, the epicardium is considered the primary source of cardiac fibroblasts, although more work is needed to fully define the embryonic origins of all cardiac fibroblasts [42]. Despite their significant role, surprisingly little is known about the factors that determine the cardiac fibroblast lineage. *PDGF* receptors (*PDGFRα* and *PDGFRβ*) are expressed in the embryonic epicardium [43–46] and contribute to fibroblast lineage specification. *PDGFRα* deletion in the epicardium results in a deficit in cardiac fibroblast formation, whereas cardiac vascular smooth muscle cell (cVSMC) development is unperturbed [46]. Inactivation of *PDGFRβ* perturbs cVSMC development without affecting cardiac fibroblasts [45]. A recent study by Tallquist and colleagues identified *Tcf21* (*epicardin/Pod1/capsulin*) as an essential regulator of cardiac fibroblast cell fate determination [47]. Epicardial fate mapping in a *Tcf21* null background demonstrates that epicardial cell differentiation into cardiac fibroblasts is significantly compromised [47].

Epicardial activation and re-expression of a fetal gene program following myocardial injury contributes significantly to cardiac fibrosis and scar formation [10,22,41,48]. Recent work by Huang *et al*. suggests that epicardium-mediated injury induces an inflammatory and regenerative response [49]. Considering their significant role during cardiac regeneration, it is important to better understand factors that lead to fibrosis and to design strategies manipulating epicardial activation after cardiac injury to decrease fibrosis and enhance regeneration.

3. Interaction between Epicardium and Myocardium

The embryonic epicardium is a source of growth factors required for coronary vasculature development and for proliferation and differentiation of underlying myocardial cells [10,27–29]. Early evidence suggesting that epicardium plays a significant role in modulating myocardial development arose from chick experiments in which blocking or photoablation of proepicardial cells resulted in a thin myocardial compact layer within the ventricular wall, poor cardiac function and embryonic lethality [50–52]. This suggests that soluble factors are released from the epicardium and act in a paracrine manner to regulate myocardial proliferation and differentiation. In recent years, several of these paracrine signaling pathways have been elucidated, including retinoic acid (RA), fibroblast growth factors (FGFs) and Wnt/β-catenin (Figure 3).

A number of studies have suggested that RA is important for early development of mammalian embryos [53]. RA exerts its functions by signaling to nuclear hormone receptors, including retinoid A and retinoid X receptors (RARs and RXRs, respectively). The phenotype of mouse embryos carrying a null mutation of the gene encoding *retinaldehyde dehydrogenase 2* (*Raldh2*), which participates in RA synthesis, suggests that RA signaling is required for cardiac looping and chamber morphogenesis [53]. Epicardial RA is required for normal myocardial growth [28,54]. Blockade of RA signaling in intact chick heart slices impairs myocardial cell proliferation [55]. However, administration of RA to heart slices cultured in the absence of the epicardium failed to rescue cardiac myocyte proliferation in these experiments, suggesting that RA does not have a direct mitogenic effect on myocardium [55]. These results suggest that epicardium may modulate myocardial growth by producing a mitogen whose expression is regulated by RA. This finding was further supported by a genetic experiment in which deletion of *RXRα* in ventricular cardiomyocytes did not affect the thickness of the ventricular myocardium [56]. In agreement with this model, conditional deletion of *RXRα* in the epicardium using

Gata5-Cre resulted in abnormal cardiac development, characterized by epicardial detachment and thinning of the myocardial compartment [54].

One function of RA in the epicardium may be to induce expression of fibroblast growth factors, including Fgf2 and Fgf9, to regulate cardiomyocyte proliferation and differentiation [28]. Fgf16 and Fgf20 are also produced by epicardium, but RA does not regulate their expression [28]. Loss of *Fgf9* results in ventricular hypoplasia and a significant reduction in the myocardial proliferation rate [28]. Epicardial-specific *RXRα* mutants show decreased *Fgf2* gene expression in the epicardium [54], and Fgf2 can activate epicardial Wnt signaling through activation of *Wnt9b* [54]. Loss of *RXRα* in epicardium produces thinned myocardium and, also, persistent ventricular expression of *atrial myosin light chain* (*mlc2a*), which is normally down-regulated [54]; thus, both proliferation and differentiation appears to be affected. However, one caveat with these studies is that *Gata5*-Cre, used to delete *RXRα* in epicardium, is not entirely restricted to epicardial tissue and can display "leakiness" in myocardium, as well. Thus, further studies using alternative epicardial cre lines, such as *Sema3d*-Cre or *Wt1*-Cre, should be performed to validate this model. Recent work by Brade *et al*. demonstrates that hepatic RA signaling is required for induction of EPO expression in the liver [57]. Secreted EPO then travels to the epicardium and induces IGF2 expression, which is secreted by the epicardium to stimulate myocardial growth [57,58]. In adult hearts, these developmental signaling pathways are reactivated in the epicardium and modulate epicardial EMT. For example, cardiac injury activates Wnt1 expression in the epicardium and epicardial-derived fibroblasts [59].

FGFs regulate cardiac development by binding and signaling to their receptors (FGFRs). Among FGFRs, only *Fgfr1* and *Fgfr2* are expressed on cardiomyocytes [52,60]. Loss of *Fgfr1* and *Fgfr2* in the myocardium results in hypoplastic heart [28]; similar to *Fgf9* nulls. Additional studies suggest that Fgf9-Fgfr1/2 signaling is also required for coronary vasculature development. Myocardial Fgf9-Fgfr1/2 signaling activates Hedgehog signaling, which is essential for *vascular endothelial growth factor* (*VEGF*)-*A*, *VEGF-B*, *VEGF-C* and *angiopoietin-2* (*Ang2*) expression and, thereby, regulation of coronary vasculature development [54].

In addition to mitogenesis, FGF signaling has been implicated in regulating cardiac morphogenesis and lineage specification during embryonic development. In quail embryos, *Fgfr1* is expressed in the proepicardium and epicardium-derived cells. Fgfr1 is required for epicardial EMT and coronary vasculature development [60]. Variation of Fgfr1 levels in the proepicardium affects epicardial EMT and myocardial invasion and differentiation of epicardium-derived cells [60]. Loss of *Fgfr1* leads to a reduction in the ability of EPDCs to invade the underlying myocardium [60], while overexpression promotes epicardial EMT and biases the differentiation of EPDCs into endothelial cells over smooth muscle cells [60].

Signals from the myocardium can reciprocally modulate epicardial EMT (Figure 3). Early evidence to support this idea came from *in vitro* experiments showing that conditioned media derived from cultured cardiomyocytes can increase epicardial EMT [61,62]. Indeed, Fgf10 produced by myocardium can modulate epicardial EMT and EPDC migration [63]. Multiple other factors expressed by myocardium have been shown to regulate epicardial development and function. Thymosin β4 is an actin-binding protein expressed in the developing myocardium, but not in the epicardium [64,65]. Cardiomyocyte-specific deletion of *Thymosin β4* produces impaired formation of coronary vasculature, suggesting that Thymosin β4 acts on epicardium in a paracrine manner [65]. The addition

of Thymosin β4 to epicardial explants significantly increases the number of epicardial-derived endothelial and smooth muscle cells [65]. In adult hearts, systemic administration of Thymosin β4 leads to induction of coronary vessel growth, myocardial progenitor mobilization and activation of embryonic developmental programs in adult epicardium [66]. Notably, however, recent work by Banerjee *et al*. demonstrates that *Thymosin β4* is dispensable for embryonic viability and vascular development in mice [67,68].

Platelet-derived growth factors (PDGFs) may also participate in myocardial-epicardial communication. During development, *PdgfA* is expressed by the myocardium, while PDGF receptors, *Pdgfrα* and *Pdgfrβ*, are expressed by epicardial cells [45,46]. Genetic deletion of both PDGF receptors in the epicardium results in abnormal epicardial EMT and EPDC formation [45,46]. Taken together, these studies suggest that a PDGF signal from the myocardium is important for epicardial development and function.

4. Epicardium and Cardiac Regeneration

The adult mammalian heart does not undergo significant regeneration following injury and, as a consequence, ischemic heart disease results in permanent myocardial damage, impaired pump function and heart failure. In lower vertebrates, however, efficient myocardial regeneration occurs after damage [69–76]. For example, the zebrafish heart undergoes minimal scarring and exhibits a robust regenerative response upon injury [71,74]. Zebrafish hearts can fully regenerate within a period of 30 to 60 days after surgical removal of as much as 20% of the ventricle [71], a cryoinjury or a genetic ablation of over 60% of the cardiomyocytes [77–80]. Cardiac regeneration in adult zebrafish is not achieved by proliferation and differentiation of cardiac stem cells, as initially suggested, but by activation, de-differentiation and proliferation of mature cardiomyocytes [75,76,81]. After de-differentiation, cardiomyocytes undergo a subsequent maturation step to completely restore cardiac function. Ultimately, newly generated cardiac myocytes are functionally integrated with the preexisting myocardium with little or no evidence of the injury, suggesting complete myocardial regeneration [75,76,81]. These studies have encouraged scientists to determine whether the adult mammalian heart is incapable of cardiac myocyte replacement or whether it retains a low-level capacity for regeneration, which could be therapeutically amplified.

The traditional view that the adult mammalian heart is incapable of cardiomyocyte renewal has been recently challenged. Studies have shown that humans and other mammals have a limited capacity for myocardial regeneration that is insufficient to restore cardiac function after injury [7,39,82–85]. Porrello *et al*. demonstrated that the neonatal mouse heart has a robust regeneration potential similar to the zebrafish heart [86]. A one-day-old mouse has the capacity to fully regenerate after partial surgical resection of the ventricles (~15% of the ventricle) within a period of 21 days [86]. Resected hearts show complete restoration of cardiac function after 60 days [86]. This regenerative response is characterized by cardiomyocyte proliferation with minimal hypertrophy or fibrosis. Genetic lineage tracing studies using an inducible cardiomyocyte specific cre line (αMHC-MerCreMer mice) demonstrate that the majority of newly formed cardiomyocytes within the regenerated ventricle are derived from preexisting cardiomyocytes [86]. Myocardial infarction of the neonatal heart also results in a robust regenerative response and recovery within 21 days. During this regenerative process, a

possible contribution from stem or epicardial progenitor cells was not ruled out. Importantly, the regenerative capability of the neonatal heart is lost by seven days of age.

The role of the epicardium during cardiac regeneration has been recently explored [69–76]. During zebrafish heart regeneration, the epicardium is activated and re-expresses developmental genes [73,74]. Upon myocardial resection, epicardial cells rapidly expand and create a new epithelial cover for the exposed myocardium [73]. A subset of activated epicardial cells undergoes EMT and contributes to new vasculature for the regenerating cardiac muscle [73,74]. In zebrafish, cardiac injury induces expression of Fgf17b by myocardium and its receptors (*Fgfr2* and *Fgfr4*) on EPDCs [73]. Blocking FGF signaling results in reduction of epicardial EMT and impaired neovascularization, leading to poor cardiac regeneration [73]. Although different organisms have different regenerative capacities, components of the injury response are conserved, including the activation of the epicardium and the contribution of epicardium-derived cells (EPDCs) to the repair process [27,48,49,73,74].

In mammals, the epicardium is reactivated after myocardial infarction [10,27,31,48,49,59]. Activated epicardial cells then undergo EMT and differentiate into fibroblasts and smooth muscle cells. However, little or no epicardial contribution towards cardiomyocytes or endothelial cells is observed in the adult after injury [27,48]. Activated epicardium secretes angiogenic factors, and treatment with epicardium-derived conditioned media significantly reduces infarct size and improves cardiac function [27].

Although available data suggest that differentiation of EPDCs into cardiomyocytes after myocardial damage is rare or non-existent, Riley and co-workers demonstrated that thymosin β4 priming before myocardial infarction results in a significant increase in the number of epicardium-derived cardiomyocytes [87]. However, work by Pu and coworkers demonstrated that thymosin β4 treatment after myocardial infarction did not alter the fate of epicardially-derived cells [88,89]. Differentiation of EPDCs into either cardiomyocyte or endothelial lineages was not observed [88,89]. Thus, while intriguing, the potential clinical utility and mechanism of action of thymosin ß4 remains to be fully elucidated.

Additional lines of evidence have implicated epicardium not just as a signaling center, but also as a source of cardiovascular progenitors. Harvey and colleagues identified a population of cardiac resident progenitor cells, called cardiac colony-forming units-fibroblast (cCFU-Fs) [90,91]. Genetic lineage tracing experiments showed that cCFU-Fs are derived from epicardium and have the potential to differentiate into smooth muscle cells, cardiac muscle and adipose tissue *in vitro* [90]. The role of cCFU-Fs during cardiac regeneration will require further examination.

Recent work by Olson and colleagues highlights the critical role of inflammation and inflammatory mediators produced by epicardium in the cardiac response to injury [49]. By analyzing conserved enhancer regions in genes expressed during epicardial activation, the authors implicated the family of transcription factor C/EBPs (CCAAT-enhancer binding proteins) as critical mediators of epicardial gene expression after injury [49]. C/EBP inhibition resulted in significantly improved contractile function and decreased myocardial fibrosis after injury [49]. Interestingly, the authors found that the inflammatory response after injury, marked by neutrophil influx to the infarcted area, was significantly reduced after C/EBP inhibition [49]. This study implicates epicardial-derived cytokines in the inflammatory response and the neutrophil infiltration that occurs after cardiac injury. Modulation of

this response may have both beneficial and damaging effects and may offer an opportunity for modulation of the postulated balance between fibrosis and regeneration during the injury response.

Consistent with this finding, molecules released from adult epicardium and other cardiac tissues can be found in pericardial fluid, and these factors may affect cardiac function under normal or pathological conditions [10]. Several molecules have been identified in the pericardial fluid of normal and diseased heart, and their levels correlate with regenerative [92–98] and inflammatory responses [99–102] (Figure 4). Examples include FGFs [92,93], VEGF [92,94,95], hepatocyte growth factor [96], high-mobility group box 1 protein-96 and insulin-like growth factor-1 [96,98]. Growth factors present in pericardial fluid can affect proliferation and differentiation of cardiac progenitors towards myocardial and vascular lineages [103–105]. The pericardial fluid of ischemic heart patients also shows higher levels of inflammatory factors [99–102]. For example, high levels of angiostatin [99], endostatin [100], tumor necrosis factor-alpha [102] and interleukins [101,102] were detected in ischemic heart disease patients compared to controls. An elevated level of inflammatory factors may adversely affect the regenerative process, causing more fibrosis and less regeneration.

5. Conclusions and Future Perspectives

In summary, the epicardium has emerged as a fascinating and dynamic tissue that can contribute to a variety of cardiac lineages in the embryo and in the adult. Reciprocal signaling between epicardium and myocardium regulates cardiac growth and cell fate decisions, and paracrine factors emerging from activated epicardium after injury can modulate the repair process. A fine balance between scar formation and myocardial regeneration may provide a therapeutic window to enhance the degree to which new myocytes are produced after damage to the heart, and epicardial regulation of the inflammatory response may significantly impact this process. Rather than a quiescent and uninteresting covering for the heart, the epicardium is an active participant in homeostasis, development and disease and will be the focus of exciting investigation for years to come.

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Conflict of Interest

The authors declare no conflict of interest.

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