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Harnessing Epicardial Progenitor Cells and Their Derivatives for Rescue and Repair of Cardiac Tissue After Myocardial Infarction

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Abstract

Purpose of review—Ischemic heart disease and stroke lead to the greatest number of deaths worldwide. Despite decreased time to intervention and improvements in the standard of care, 1 out of 5 patients that survive a myocardial infarction (MI) still face long-term chronic heart failure and a 5-year mortality rate of about 50%. Based on their multi-potency for differentiation and paracrine activity, epicardial cells and their derivatives have potential to rescue jeopardized tissue and/or promote cardiac regeneration. Here we review the diagnosis and treatment of MI, basic epicardial cell biology, and potential treatment strategies designed to harness the reparative properties of epicardial cells.

Recent Findings—During cardiac development, epicardial cells covering the surface of the heart generate migratory progenitor cells that contribute to the coronary vasculature and the interstitial fibroblasts. Epicardial cells also produce paracrine signals required for myocardial expansion and cardiac growth. In adults with myocardial infarction, epicardial cells and their derivatives provide paracrine factors that affect myocardial remodeling and repair. At present, the intrinsic mechanisms and extrinsic signals that regulate epicardial cell fate and paracrine activity in adults remain poorly understood.

Summary—Human diseases that result in heart failure due to negative remodeling or extensive loss of viable cardiac tissue require new, effective treatments. Improved understanding of epicardial cell function(s) and epicardial-mediated secretion of growth factors, cytokines and hormones during cardiac growth, homeostasis and injury may lead to new ways to treat patients with myocardial infarction.

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Conflict of Interest

Krithika S. Rao declares no potential conflict of interest.

Jeffrey L. Spees holds patents relevant to human epicardial cells and is co-founder of Samba BioLogics, Inc.

Human and Animal rights and Informed Consent

This article contains no studies with human and animal subjects performed by any of the authors.

Keywords

Epicardial; cardiac; progenitor; EPDC; differentiation; paracrine

INTRODUCTION

Ischemic heart disease that includes myocardial infarction (MI) is a leading cause of death worldwide among both men and women. In 2012, there were an estimated 7.4 million deaths due to ischemic heart disease, three quarters of which occurred in low- and middle-income countries (<http://www.healthdata.org/gbd>). Every year in the United States, an estimated 790,000 people have MI, with 114,000 cases resulting in death. Over the last decade, despite about a 36% fall in mortality rates, MI remains a leading cause of morbidity and a major healthcare burden [1].

Myocardial ischemia occurs during interruption of blood flow to the myocardium (heart muscle), creating a mismatch between the myocardial oxygen demand and supply in the tissue. Ischemia is often caused by atherosclerotic plaque formation in the large coronary arteries. Plaque formation is a slow and chronic process responsible for narrowing of the coronary blood vessels and consequent decrease in blood flow through them. An MI occurs after disruption of an atherosclerotic plaque that subsequently lodges in and blocks the coronary artery (thrombotic occlusion). Myocardial infarction (MI) refers to the necrosis (death) of cardiomyocytes that results from ischemia.

An MI creates a central zone of necrotic non-viable myocardium with a surrounding peri-infarct that consists of viable, but jeopardized tissue. The peri-infarct region represents myocardium that can be salvaged by timely restoration of blood supply through the occluded vessels (i.e. reperfusion). A greater time delay between an infarction event and reperfusion results in a corresponding increase in the expansion of the area of necrosis into the bordering peri-infarct regions [2–5].

Notably, a single bout of MI can result in the death of one billion cardiomyocytes [6]. The widespread death of cardiomyocytes after MI causes the left ventricle (LV) wall to thin, thereby increasing the pressure and volume load on the heart. To compensate for this structural change, the LV undergoes dilatation (i.e. volumetric enlargement of the ventricle lumen); this originates from the infarct region and spreads through the chamber. Anatomically, MI can be either transmural or non-transmural. A transmural infarct spreads across the 3 main heart layers in cross section: the epicardium (outer layer), the myocardium (middle layer) and the endocardium (innermost layer). A non-transmural MI is limited to the endocardium and inner myocardium, which are the least vascularized regions in the heart.

Early after MI, the formerly healthy, contractile cardiomyocytes are replaced by noncontractile, akinetic fibrotic scar tissue composed of myofibroblasts; this is critical to prevent ventricular rupture and to maintain the pumping efficiency of the heart and cardiac output [7–9]. However, increasing stress on the surviving cardiomyocytes and extensive scarring eventually result in compensatory changes in LV shape, size, mass, volume and physiology. This is referred to as “negative” LV remodeling because it adversely affects

cardiac function. Although cardiomyocyte necrosis is the major cause of negative remodeling, death of blood vessels, fibroblasts and other cell types also contribute to subsequent changes in heart physiology [10]. Along with resident interstitial cardiac fibroblasts [11] and cells that derive from circulating, bone marrow-derived fibrocytes [12], epicardial-derived cells that originate from the surface of the heart also generate both fibroblasts and myofibroblasts that contribute to ventricular scar formation after MI [13].

Current Diagnosis and Treatment of MI

In the clinic, MI is classified based on the electrical activity of the heart as determined from electrocardiographic (ECG/EKG) measurements (e.g. ST segment elevation). Another commonly used diagnostic tool for examining cardiac function after MI is Echocardiography (ECHO). In ECHO, Doppler ultrasound is used to provide two- or three-dimensional imaging of the heart and its wall motion in real time. Other tools to determine infarct size include Magnetic Resonance Imaging (MRI) and assay of circulating biomarkers of injured myocardium such as cardiac troponin or creatine kinase. The clinical classifications of MI provide guidelines for the course of treatment and patient management.

Treatment of MI is determined on a patient-to-patient basis with the common goals of rescuing as much of the jeopardized myocardial tissue as possible, relief from angina (chest pain), and prevention of further injury or related complications. Reperfusion therapy for the prompt restoration of blood flow and oxygenation to provide relief from symptoms is performed in patients by mechanical and/or chemical interventions. Percutaneous coronary intervention (PCI) is a non-surgical procedure that is often performed for the re-canalization of macroscopic coronary arteries. In certain severe cases, Coronary Artery Bypass Grafts (CABG) are utilized to restore cardiac flow by creating new routes of blood flow through vascular grafting [14–16]. Alone or combined with mechanical interventions, patients may also receive thrombolytic drugs to help chemically dissolve blood clots; these drugs can both release the occlusion and prevent further thrombosis (e.g. aspirin, IV unfractionated heparin, tissue Plasminogen Activator, IV streptokinase). Patients often receive drugs that help to prevent additional events of myocardial infarction (e.g. nitroglycerides, beta-blockers, ACE inhibitors)[14–16]. In the United States, mechanical and chemical interventions combined with improved emergency response and primary care have achieved more than a 95% survival rate for any given individual presenting with MI to a hospital. Still, however, 1 out of every 5 survivors will develop chronic heart failure (CHF) in the long-term and face a 5-year mortality of ~50% [1].

The Epicardium

The epicardium is a single layer of epithelial (mesothelial cells) that covers the entire heart surface. It plays key roles in signaling to the myocardium and provides a progenitor cell source for different cardiac lineages. Physical removal of the epicardial layer in developing avian hearts was shown to inhibit cardiomyocyte growth, leading to a thin-walled ventricle and the arrest of coronary vessel development [17,18]. Likewise, knockout of genes required for epicardial survival and function in mice such as *beta-catenin* [19], *Wt1* [20], *Yap* and *Taz* (Hippo signaling mediators) [21], *Mrtfa* and *Mrtfb* (Myocardin-related transcription factors) [22], *Vcam1* (alpha4beta1 integrin) [23], *Tbx5* [24], *Tbx18* [25], *Gata4* [26], and *Notch*

[27,28] resulted in similar cardiac phenotypes. Epicardial cells are promising candidates for cardiac cell transplantation after MI based on several specialized properties that include their role as progenitor cells during cardiac development, their regulation of cardiac tissue morphogenesis by protein/peptide secretion, and emerging evidence that they modulate inflammation after cardiac injury [29,30].

Epicardial Cell Biology During Development

Lineage-tracing studies in developing chick and quail embryos have provided insights into the origin and fate of the epicardium. It derives from the proepicardial organ (epithelium attached to the embryonic diaphragm). In mice, around embryonic day 9.5 (E9.5) proepicardial cells migrate to completely engulf the myocardial and endocardial layers. Around E10.5 it forms an epithelial covering (i.e. “epicardium”) that surrounds the entire developing cardiac structure. From this stage onwards it is responsible for directing events that help to complete cardiac development.

Between E11.5 and 13.5, epicardial cells undergo epithelial-to-mesenchymal transformation (EMT), providing a population of mesenchymal cells and precursors that migrate into the myocardium. Retinoic acid (RA) and erythropoietin (Epo) signaling to the epicardial cells stimulates them to secrete mitogens that are required for myocyte proliferation and maturation; this induces maximal heart growth. Notably, mice deficient in RA or Epo signaling were reported to have severe myocyte hypoplasia. In addition to PDGF, epicardial cells were shown to secrete FGF9, FGF16 and FGF20, factors that signal to FGFR1 and FGFR2 on myocytes to mediate myocardial growth [31–33]. Insulin-like growth factor 2 (IGF2) expression by embryonic mouse epicardial cells as well as the receptors for Insulin and Insulin-like growth factor-1 were also required for normal cardiac growth [34].

Adult Epicardium

During adulthood, the mammalian epicardium is primarily thought to be quiescent. After injury, however, selected epicardial cells are activated, gain expression of *Wt-1*, *Raldh2*, *Tbx18* and *Gata4*, proliferate, undergo EMT, and migrate into the myocardium. Lineage-tracing studies based on these epicardial-associated transcription factors demonstrated migration of a subset of epicardial derivatives into the myocardium. Of interest, these embryonic epicardial markers are typically only present during development and not expressed in the normal, healthy adult heart.

Differentiation Potential of Epicardial Progenitor Cells

The fate of epicardial-derived cells after migration into the myocardium is the subject of numerous debates that stem from conflicting observations in different experimental models. Multiple lineage-tracing studies have shown that the majority of epicardial cells differentiate into cardiac interstitial fibroblasts and vascular smooth muscle cells that line large cardiac arteries and arterioles [35–42] (Fig. 1). Lineage-tracing studies of cardiac progenitors demonstrated a population of cells with a proepicardial origin that differentiated into vascular endothelial cells [43,44]. In contrast, the epicardium has not been directly shown to contribute endothelial cells to the heart. This raises the possibility that a different progenitor exists within the proepicardium that migrates separately and is distinct from epicardial-

derived progenitor cells, based on its ability generate endothelial cells. Other experiments performed with quail/chick chimeras during embryogenesis suggest that the epicardial mesothelium itself is a source of endothelial progenitor cells. In these studies, retroviral and fluorescent reporter labeling used to compare derivatives from the proepicardial and epicardial compartments suggested that a portion of the population of coronary endothelial cells derive from epicardial cells, after they undergo EMT [43]. Similarly, whereas a subpopulation of proepicardial cells has been shown to contribute to the cardiac myocyte pool, whether or not epicardial-derived progenitor cells or their derivatives differentiate into cardiac myocytes in adults remains controversial [45–48] (Fig. 1). Relevant to metabolic syndrome and cardiovascular disease, adult epicardial cells exposed to signaling through the peroxisome proliferator activated receptor gamma (PPAR γ) pathway during EMT differentiate into adipocytes, contributing to epicardial fat [49]. This finding is important clinically because excessive epicardial fat lying between the myocardium and the visceral pericardium in humans contributes to a pro-inflammatory phenotype, vascular dysfunction, and increased cardiovascular risk [50].

Utilizing Epicardial Cells for Cardiac Cell Transplantation

Based on our current understanding of organ resident adult stem cells and the known roles of the epicardium, this cell population satisfies certain key criteria to be defined as stem/progenitor cells for the heart. Therefore epicardial cells and their derivatives can potentially be a source of cell replacement cells after MI or of mitogens that preserve and/or repair tissue after injury. To date, few studies have explored the potential of epicardial cells and their derivatives as candidates for cell engraftment. This is due, in part to, limitations in knowledge and lack of appropriate tools for epicardial cell-specific isolation and *in vivo* experimentation. In order to reproducibly isolate a pure population of cells from donors, multiple epicardial cell-specific surface epitopes (i.e. markers) need to be identified. Ideally, this combination of epitopes would be unique to the native epicardial cell population; this approach has successfully been used to identify and isolate stem/progenitor cells in several other organs [51–53]. Additionally, we need to investigate key changes in cell signals both received and secreted by epicardial cells during tissue homeostasis, injury and recovery. Our current understanding of intracellular and extracellular epicardial signaling is largely limited to studies of developing embryos. Thus, there is need for a comprehensive examination of signaling events and pathways that control epicardial cell proliferation, EMT and migration in the adult, which can be eventually targeted to improve or modify injury repair. Multiple studies in mice with MI have demonstrated that epicardial cells in areas bordering injured tissue become activated and proliferate, undergo EMT, migrate into the myocardium and secrete factors that promote angiogenesis and fibrosis within and adjacent to areas with infarction [54,55]. To determine the potential benefits of EPDC transplantation after MI, Winter *et al.* (2007) isolated “spindle-like cells” by removing the epicardial layer from human atrial tissue explants and culturing “EPicardial-Derived Cells” (EPDC) [56]. These cells were culture-expanded and transplanted into the hearts of immune-deficient mice with MI (permanent ligation model, intra-muscular cell administration). For up to 6 weeks after MI and EPDC transplantation, they detected differences several parameters of cardiac function. Notably, despite the observed improvements in cardiac function, cell engraftment was not detectable at the 6-week time point [56]. The improved cardiac function Winter *et*

al. (2007) observed in the absence of sustained cellular engraftment suggests alternative mechanisms of benefit such as the paracrine action of factors secreted by the administered EPDCs.

Paracrine Action as a Beneficial Mechanism in Cell Therapy

By secreting the appropriate concentrations of growth factors, cytokines, chemokines, hormones, matricellular proteins and other factors within their local environment(s), and over the correct timeframe(s), progenitor cells can strongly influence complex cellular processes such as tissue morphogenesis during development. In a similar fashion, to orchestrate remodeling and/or repair after disease or tissue injury, the paracrine activity of adult progenitor cells can modify the phenotype and function of multiple somatic cell types, including immune cells and even other stem/progenitor cells [57–59].

To date, the paracrine biology of adult progenitor cells has been most intensively studied in the bone marrow compartment. Bone marrow contains Hematopoietic Stem Cells (HSCs) and their niches and represents the primary center for hematopoiesis and formation of the major blood cell lineages (i.e. lymphoid and myeloid). HSC survival, proliferation, differentiation and migration are all controlled by paracrine signals released within bone marrow by mesenchymal cells (i.e. multipotent stromal cells, MSC) and endothelial cells that reside in close physical proximity to the HSC along the bone endosteum and within vascular sinusoids (i.e. HSC niches) [60–63]. Since we can isolate, expand and manipulate MSCs and endothelial cells *ex vivo*, factors they secrete may be identified from conditioned medium by complementary proteomic methods such as ELISA and immunoblotting, Mass Spectrometry, and phospho-receptor tyrosine kinase (RTK) arrays. The biologic activity of particular species can then be evaluated by depleting their expression with the use of a variety of tools such as neutralizing antisera, siRNA or viral shRNA vectors, CRISPR/Cas9, or transgenic approaches (e.g. Cre/loxP). Purified paracrine factors, themselves, may be screened to identify drug candidates for use as therapeutics (e.g. biologics) or used to determine new drug targets; these factors could be targeted for neutralization with antibodies or pharmacologic inhibitors.

Therapeutic approaches where purified factors such as receptor agonists can be directly administered at optimized concentrations may provide multiple advantages over treatment with cells that include: ease of preparation, storage/handling and administration. In addition to avoid challenges such as immune-mismatch [64], another important advantage of paracrine-based therapy over cell therapy is the ability to rapidly modify treatment dosage when desired relative to a possible sustained effect from engrafted cells post transplantation.

Beginning with the use of bone marrow-derived mononuclear cells, patients in many cell therapy trials to treat MI showed modest improvements in cardiac function including: increased ejection fraction, fractional shortening, stroke volume and cardiac output [65,66]. The initial excitement over these improvements motivated numerous debates concerning the safety of autologous versus heterologous transplantation, benefit of intracoronary versus systemic delivery, and the logistics of isolating and expanding large cell populations to derive optimal benefit from the interventional procedure to deliver the treatment [67–69]. Among these concerns, the question regarding mechanism of action for cardiac cell therapy

or, specifically, the changes at the cellular and molecular levels that influenced function has been an expanding field of investigation [64, 70–75]. Since myocytes are the cellular functional units of the heart, the overt expectation was that cell therapy worked to improve cardiac function by differentiation of stem cells into cardiomyocytes, thereby increasing their numbers. However, most trials failed to detect significant engraftment of transplanted cells. Furthermore, stem cell transplantation did not stimulate the proliferation of myocytes in numbers adequate to improve cardiac function. It should be noted that transplantation of myocytes alone has also been performed by many investigators, but presents a formidable challenge in terms of ensuring *in vivo* survival, proliferation, and integration of grafted cells within the existing cardiomyocyte network. In addition, if the grafted cells differentiate into mature myocytes, they must form GAP junctions with neighboring cardiac myocytes and couple with them electrically to avoid potentially fatal arrhythmia. In the absence of large numbers of transplanted cells to account for the observed functional benefits, the secretion and/or release of paracrine-acting factors (i.e. paracrine hypothesis) became a more plausible and accepted mechanism responsible for much of the benefits observed following cell transplantation [70–75].

Use of epicardial cells-secreted factors to treat MI

Investigation into the paracrine biology of adult epicardial cells has been inspired by a strong body of research focused on cardiac developmental processes where multiple paracrine signals from the epicardium guide myocardial growth. Analysis of genes expressed by epicardial cells indicate that the epicardium is a notable source of trophic, cytoprotective and angiogenic factors [38,76–79]. In a mouse model of MI, Zhou et al. (2011) demonstrated that treatment with murine EPDC-conditioned medium reduced infarct size and improved cardiac function post-MI [38]. With the use of antibody arrays that detect selected angiogenic factors, they identified VEGFA, SDF1, MCP1, IGFBP2, IGFBP3, IGFBP9, and IGFBP10 as secreted proteins present in medium conditioned by murine EPDCs. Furthermore, by antibody-mediated neutralization assays, they identified bFGF (FGF2) and VEGFA as two paracrine factors produced by murine EPDCs that improved angiogenesis [38].

In a rat model of MI with reperfusion, we found that intra-arterial (left ventricle lumen) administration of human EPDC-conditioned medium (EPI CdM) was vasoprotective. By ELISA, we detected numerous, potentially beneficial factors secreted by human EPDCs including HGF, VEGFA, SDF-1, and angiopoietin-1 [80]. Notably, in cell protection assays of primary cardiac artery endothelial cells that were exposed to simulated ischemia (nutrient deprivation and hypoxia; 1% oxygen), only removal of HGF from EPI CdM was found to significantly reduce its cytoprotective effects. In addition, we observed that about 30% of the HGF in human epicardial cell conditioned medium associated with polyclonal IgG into unique complexes that stimulated the RYK (related to tyrosine kinase) receptor in endothelial cells as well as c-Met (i.e. HGF receptor); these complexes conferred greater protection during hypoxia relative to HGF alone [80].

Wei et al. (2015) identified follistatin-like 1 (Fstl1) as an epicardial-secreted factor capable of stimulating cardiomyocytes to enter the cell cycle and divide [81]. Furthermore,

application of recombinant human Fstl1 with the use of an epicardial patch improved cardiac function and increased survival in mouse and swine models of MI [81]. Additional work is needed to define the identities, concentrations, locations, and temporal release profiles for the many proteins/peptides and other molecules produced by epicardial cells that support myocardial rescue/repair and vascular protection, angiogenesis and post-natal vasculogenesis *in vivo*.

Isolation of epicardial cells from human cardiac tissue

With prior IRB approval, we and others have isolated human epicardial cells from atrial appendages commonly removed during cardiac bypass surgery [80,82–84]. In the presence of fetal calf serum, human epicardial progenitor cells with an epithelial (mesothelial) phenotype can be generated from atrial tissue explants and expanded in culture. However, within a few days, the keratin-expressing epithelial-like cells begin to undergo EMT, differentiating into vimentin-expressing mesenchymal cells (i.e. precursors of fibroblasts, myofibroblasts and smooth muscle cells [81,82–84]. Recently, Moerkamp et al. (2016) reported a valuable new model for the study of naïve human fetal and adult epicardial cells and EMT into EPDCs. By culturing isolated epithelial-like epicardial cells with medium containing a pharmacologic inhibitor of Alk5, a TGF-beta receptor, they were able to maintain and expand epicardial cells with epithelial characteristics [84]. Importantly, the system they developed may be useful as a screening platform to better understand epicardial lineage commitment and differentiation into myocytes or endothelial cells.

Generation of epicardial cells from human pluripotent cell sources

Embryonic Stem cells (ES cells) were first isolated in 1998 from the inner cell mass of the blastocyst during embryogenesis [85]. ES cells retain the ability to differentiate into nearly any cell type when exposed to appropriate hormones and growth factors [86]. Early transplantation studies with ES cells into animal models of MI resulted in teratoma formation and immune complications [87]. Further understanding of human ES cell biology helped to establish protocols for deriving cardiomyocytes *ex vivo* and *in vivo*, and coupling them with the existing myocardial structures after transplantation. These advances resulted in reports of 20–40% improvement in cardiac function in animals with MI [88–91]. To date, pluripotent cells and their derivatives have not been approved for cardiac clinical trials in the USA. However, one trial has begun in France using Islet1⁺/CD15⁺ progenitors derived from committed ES cells (www.clinicaltrials.gov).

In 2006, the research group of Shinya Yamanaka, M.D., Ph.D., in Japan made a landmark discovery (awarded the Nobel prize within 7 years) and published methods to reprogram differentiated skin fibroblasts into Pluripotent Stem Cells (induced PSC, iPSC) by expressing 4 key transcription factors: Oct-3/4, Sox2, Klf4, and c-Myc [92]. Similar to ES cells, the iPSCs could be efficiently differentiated into highly pure cardiomyocytes using unique culture conditions such as embryoid body formation, and gene expression or chemical induction. The iPSCs thus provided a valuable new resource for cell replacement that did not carry the ethical/religious stigma associated with the destruction of human embryos during production of ES cell lines [93,94]. Grafting of iPSCs to the hearts of animals with MI demonstrated preservation of existing structures, electrical coupling with

pre-existing myocytes, and improved cardiac function [95,96]. Aside from their potential as a cellular therapeutic to provide cell replacement, iPS cells have also been used in other applications. For example, cardiomyocyte lines have been generated from different donor-derived iPSCs for genetic studies, drug screening and for creating patient-specific cell banks [97,98]. Notably, however, wide-spread clinical use of iPSCs or their derivatives will demand a significantly deeper understanding of cellular properties including clonal capacity, signals that control their proliferation and differentiation and their immune modulatory effects, among others.

Through a combination of staged, activating BMP and/or Wnt or retinoic acid signals, it is now possible to generate epicardial stem/progenitor cells from either ES cells or iPS cells [99–102]. Since iPS cells can readily be produced from any human donor, this important advance will facilitate investigation of particular genetic loci or mutations and how they impact human epicardial cell development and function. Importantly, iPSC-derived epicardial cells retain their ability to graft cardiac tissue and migrate within it [101]. As with ES- and iPSC-derived cardiac myocytes, which differ in sarcomeric structure and function relative to adult myocytes, it will be important to fully characterize epicardial cells produced from pluripotent cell sources (i.e. embryonic or fetal in nature) to understand if and how they differ from their adult counterparts. Nonetheless, the pluripotent cell-derived epicardial cells provide a powerful system to better understand the cell signals, epigenetic events, and transcriptional program responsible for determining epicardial cell fate, paracrine activity, and other functions after cardiac injury.

Creation of epicardial cells by direct reprogramming of other somatic cell types

Yamanaka's paradigm shifting research in cellular reprogramming provided also a conceptual framework for a different form of reprogramming that did not require cells return to the embryonic state. Because the new form of reprogramming took a fully differentiated cell with a stable phenotype and transformed it directly into a completely different cell type (even one from a different tissue or germ layer), this form became known as "direct reprogramming". Several groups have published different combinations of virally-expressed transcription factors capable of reprogramming adult fibroblasts directly into cardiac myocytes with varying degrees of efficiency. The basic cocktail consists of Gata4, Mef2C and Tbx5 (GMT)[103,104], which becomes more efficient with the addition of Hand2 [105]. Other ways to further improve cardiac reprogramming efficiency include chemical inhibition of Notch, TGF beta, and/or Wnt signaling [106,107]. Notably, viral transduction with GMT is not sufficient for direct reprogramming of human non-myocyte cells into cardiac myocytes. However, it is possible by adding expression of other transcription factors such as myocardin and mesp1 or miRNAs [108–110]. In fact, a specialized combination of miRNAs alone (miRNA1, 133, 208, and 499) was sufficient for direct conversion to cardiac myocytes, and this protocol was 10× more efficient with addition of a JAK1 inhibitor [109]. One group reported direct reprogramming to cardiac myocytes using only chemical induction [111]. While highly targeted methods of miRNA and/or chemical delivery will still be important to optimize, it is noteworthy that direct reprogramming with miRNAs or chemicals alone will obviate the need for viral infection.

Based on their multipotency for differentiation and their repertoire(s) of paracrine factors, epicardial cells and their derivatives have clinical promise as cellular therapeutics to rescue and repair cardiac tissue after myocardial infarction. Because they can engraft and migrate within injured cardiac tissue, epicardial cells are also interesting candidates to target for temporally-controlled direct reprogramming into cardiac myocytes. In this manner, it may be possible to graft epicardial cells, allow them to migrate into areas of myocardium with necrosis, and then to differentiate them into cardiac muscle as opposed to scar. In addition, screening of epicardial paracrine activity and responses in the context of tissue homeostasis, growth, and repair offers the opportunity to identify proteins/peptides and hormones that can be produced, purified, and utilized for clinical application. Future collaboration between research scientists and clinicians and standardized methods for isolation of human epicardial cells, grafting strategies, and manipulation of epicardial cell fate will build a path toward delivery of powerful new epicardial-based therapeutics to patients with MI.

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■ Of importance

■ ■ Of major importance

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Fig. 1. Epicardial cell EMT and migration into the myocardium

In response to cardiac growth or injury, epicardial cells on the heart surface proliferate, undergo epithelial-to-mesenchymal transformation (EMT) and generate a population of progenitor cells in the subepicardial mesenchyme (a.k.a. EPicardial-Derived Cells, EPDCs). Within the subepicardial tissue, the EPDCs may proliferate further prior to migrating into the myocardium where they differentiate to generate adventitial fibroblasts, interstitial fibroblasts, myofibroblasts, and vascular smooth muscle cells. Alternatively, in the presence of PPAR γ , epicardial cells EMT into adipocytes that contribute to epicardial/sub-epicardial adipose tissue. Although proepicardial progenitors have been clearly shown to differentiate into both endothelial cells and cardiomyocytes, the ability of EPDCs to differentiate into endothelial cells or cardiomyocytes in adult mammals remains controversial. If such differentiation does occur in adult mammals, it is likely to be highly context-dependent in terms of cardiac growth (e.g. after running exercise), cardiac injury and/or age of subject.