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Cardiac repair by epicardial EMT: Current targets and a potential role for the primary cilium

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ABSTRACT

Despite therapeutic advances that have prolonged life, myocardial infarction (MI) remains a leading cause of death worldwide and imparts a significant economic burden. The advancement of treatments to improve cardiac repair post-MI requires the discovery of new targeted treatment strategies. Recent studies have highlighted the importance of the epicardial covering of the heart in both cardiac development and lower vertebrate cardiac regeneration. The epicardium serves as a source of cardiac cells including smooth muscle cells, endothelial cells and cardiac fibroblasts. Mammalian adult epicardial cells are typically quiescent. However, the fetal genetic program is reactivated post-MI, and epicardial epithelial-to-mesenchymal transition (EMT) occurs as an inherent mechanism to support neovascularization and cardiac healing. Unfortunately, endogenous EMT is not enough to encourage sufficient repair. Recent developments in our understanding of the mechanisms supporting the EMT process has led to a number of studies directed at augmenting epicardial EMT post-MI. With a focus on the role of the primary cilium, this review outlines the newly demonstrated mechanisms supporting EMT, the role of epicardial EMT in cardiac development, and promising advances in augmenting epicardial EMT as potential therapeutics to support cardiac repair post-MI.

Contents

Abbreviations: BBS8, Bardet-Biedl syndrome 8; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; BMSCs, bone marrow-derived hematopoietic stem cells; ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial-to-mesenchymal transition; EndMT, endothelial-to-mesenchymal transition; EPDCs, epicardium-derived cells; Fstl1, follistatin-like 1; GSK3β, glycogen synthase kinase 3β; HA, hyaluronic acid; HGF, hepatocyte growth factor; Hh, hedgehog; HIF-1α, hypoxia-inducible factor 1α; IFT, intraflagellar transport; IGF, insulin-like growth factor; Jbn, Jouberin; lncRNAs, long non-coding RNAs; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; MEG3, maternally expressed gene 3; MCP-1, monocyte chemoattractant protein 1; MI, myocardial infarction; miRNAs, microRNAs; MMPs, matrix metalloproteinases; ncRNA, non-coding RNA; PCP, planar cell polarity; PI3K, phosphatidylinositol 3-kinase; PDGF, platelet derived growth factor; Ptch, patched; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase; RARs, retinoic acid receptors; RTKs, receptor tyrosine kinases; RXRs, retinoid X receptors; SCF, stem cell factor; Shh, sonic hedgehog; Smo, smoothened; Tβ4, thymosin beta-4; TGF-β, transforming growth factor beta; Tbx18, T-box transcription factor 18; VEGF, vascular endothelial growth factor; Wnt, wingless-type integration site; Wt1, Wilms' tumor 1.

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1. Introduction

Cardiovascular diseases are the second leading cause of death in Canada and the leading cause of death worldwide (Statistics Canada, 2011; World Health Organization, 2015). Specifically, myocardial infarction (MI), often caused by coronary artery disease, is the most common form of sudden cardiac death (Hayashi, Shimizu, & Albert, 2015). There has been significant improvement in therapeutic options for patients to treat MI and the subsequent disease associated with post-MI cardiac compensation. As a result, the in-hospital mortality of patients with acute MI has decreased from 29% to 3% in the last 50 years (de Vreede et al., 1991; Wang, Goodman, et al., 2015). Despite therapeutic advancement, MI and post-MI morbidity pose a significant financial burden for the health care system of most nations. In Canada, MI is the third leading cause of hospitalization and a major driver of prescription drug use (Public Health Agency of Canada, 2009). The annual estimated direct and indirect cost of cardiovascular disease has recently amounted to over $21.2 billion in Canada alone (Tarride et al., 2009). The advent of percutaneous intervention as well as the use of vasodilators and thrombolytics has markedly improved survival post-MI, however, approximately 26% of patients still progress to heart failure (Gerber et al., 2016). Furthermore, the number of patients for whom conventional revascularization techniques fail is increasing. As such, the approach to the treatment of heart failure is continuously evolving, and clinical trials continue to investigate emerging treatment options from stem cells to gene therapy. Recently, the discovery that processes which occur during the development of the cardiovascular system are recapitulated in the disease setting has directed research efforts towards modulating these processes to enhance tissue repair. Epithelial-to-mesenchymal transition (EMT) is one such process that is up-regulated in the setting of MI which supports neovascularization and cardiac healing.

2. Myocardial infarction

The ventricular response to MI consists of a dynamic remodeling process which is often deleterious. Acutely, MI results in tissue damage and cardiomyocyte loss by both apoptosis and necrosis. Myocyte cell death causes cytotoxic spilling and the recruitment of inflammatory cells occurs in the first three or four days post-infarction. In the same time period, both resident and recruited fibroblasts and myofibroblasts produce collagen (types I and III) to form a reparative fibrotic scar (van den Borne et al., 2010). This initial scarring is paramount to the healing process as it prevents rupture of the ventricular wall. Myofibroblasts produce inflammatory cytokines, and exhibit properties of both fibroblasts and smooth muscle cells, thereby contributing to both scar formation and contraction of the granulation tissue. As the scar begins to mature in the early phase of remodeling, more cells are gradually lost by apoptosis and necrosis, and characteristic mural thinning occurs. Over weeks to months, thinning and expansion cause ventricular dilation, which is initially beneficial, compensating for a reduced stroke volume. Ejection fraction, however is persistently depressed and end diastolic pressure continues to rise. Consistent with the Frank-Starling mechanism, reactive compensatory hypertrophy of remaining viable cardiomyocytes occurs. This hypertrophic response allows individual myocytes to work harder to conserve ventricular function, but at a cost of requiring additional blood supply to sustain cell metabolism. An essential component of the remodeling process is neoangiogenesis within the infarct region. Upregulation of pro-angiogenic agents like vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF/FGF-2) within the ischemic area promotes endothelial cell migration and pericyte communication (Ware & Simons, 1997). However, under normal conditions the growing capillary network cannot keep pace with the growing demands of hypertrophied myocardium; this evokes further myocyte necrosis. Along with wall thickening and necrotic cell death, the ventricle muscle stiffens further; the initial reparative fibrotic response becomes exaggerated and the addition of reactive fibrosis causes excessive collagen production, contributing to progressive ventricular dysfunction (Talman & Ruskoaho, 2016).

3. Approaches to improve healing post-MI

Over the last 50 years, major efforts in cardiac research have focused on minimizing infarct expansion, fibroblast collagen deposition, and cardiac hypertrophy following MI. Methods such as inhibiting inflammation, reprogramming cardiac fibroblasts, activating cell cycle reentry, transferring skeletal myoblasts to the infarct region, and transplanting progenitor cells have been tested (Christia & Frangogiannis, 2013; Ptaszek, Mansour, Ruskin, & Chien, 2012). These methods have been met with conflicting results in in vivo models and in the clinic. Early studies in murine models suggested that bone marrow-derived hematopoietic stem cells (BMSCs) introduced into the myocardium following MI could differentiate into cardiomyocytes and aid in repair; however, these cells did not differentiate or incorporate into the myocardium in subsequent studies (Murry et al., 2004). A number of clinical trials using autologous stem or progenitor type cells were initiated in the early 2000's, however meta-analyses of these trials show modest improvements in cardiac function or no significant benefit on ventricular remodeling (Delewi et al., 2014; Jeong et al., 2013; Liu, Duan, et al., 2014; Zhang et al., 2009). Many traditional therapies were also directed at increasing infarct zone microvascularity and promoting angiogenesis. It is thought that if blood flow can be restored to the infarcted or "hibernating" myocardium, fewer cardiomyocytes will be lost. For example, augmented infarct zone angiogenesis has been demonstrated in animal models using VEGF and bFGF therapy (Banai et al., 1994; Yanagisawa-Miwa et al., 1992), however this was not associated with benefit in human trials (Mitsos et al., 2012). To augment neovascularization, research has turned to exploring processes which support coronary artery formation during development, namely EMT.

4. EMT

EMT is a process by which epithelial cells undergo transcriptional reprogramming to transdifferentiate into motile mesenchymal cells (Lamouille, Xu, & Derynck, 2014). Epithelial cells are characterized by defined polarity which is maintained through intercellular junctions including tight junctions, adherens junctions, desmosomes, and gap junctions. EMT involves cell junction disassembly, loss of epithelial cell polarity, and cytoskeletal reorganization via suppression of epithelial genes, and activation of a mesenchymal gene signature. Mediators of EMT are upregulated locally, and their expression and receptor binding initiates an intracellular molecular cascade which supports the cell phenotype switch. For example, the upregulated local expression of transforming growth factor beta (TGF-β) and bFGF activated kinases and small GTPases facilitates degradation of the basement membrane and promotes actin rearrangement and cell movement (von Gise & Pu, 2012). TGF-β stimulates the expression of Snail and Slug, transcription factors which directly repress transcription of epithelial genes. Epithelial E-cadherin is a transmembrane protein that forms epithelial cellular junctions; specifically, E-cadherin is critical to the adhesion belt of adherens junctions (Hartsock & Nelson, 2008). Upon EMT activation, E-cadherin is both cleaved at the cell membrane and endocytosed, and its production is suppressed in favor of N-cadherin, a mesenchymal protein (David & Rajasekaran, 2012; Lamouille et al., 2014). This "cadherin switch" is a hallmark of EMT, and supports the gain of affinity for mesenchymal cells rather than endothelial cells.

Cadherin complexes are connected to the actin cytoskeleton by members of the catenin family. P120-catenin, α-catenin, and β-catenin stabilize E-cadherin at the plasma membrane (Hartsock & Nelson, 2008). However, the dual role of β-catenin implicates it in both the maintenance of an epithelial phenotype and induction of mesenchymal transition. Sequestration of β-catenin at the adherens junction supports maintenance of junction integrity, however the degradation of E-cadherin liberates

β-catenin, allowing its cytoplasmic localization. Wingless-type integration site (Wnt) pathway activation (discussed later) causes β-catenin nuclear translocation and transcriptional activation of Wnt/β-catenin target genes to support EMT (Valenta, Hausmann, & Basler, 2012).

In addition to the cadherin switch, cellular motile capacity is enhanced by actin reorganization, which enables extension of the cytoskeleton. Lamellipodia and filopodia extend from the plasma membrane, and proteolytically degrade the extracellular matrix (Ridley, 2011). Meanwhile, actin is polymerized in the direction of migration (Olson & Sahai, 2009). Further facilitating migration, the intermediate filament composition switches from cytokeratin-rich to vimentin-rich (Mendez, Kojima, & Goldman, 2010). The cell assumes a mesenchymal phenotype and migrates away from the basement membrane. After EMT, cells exhibit a mesenchymal gene signature, and are able to re-differentiate into other cell types.

5. Factors driving EMT

There are numerous molecules implicated in a wide range of overlapping signaling pathways that support EMT. Among others, these include TGF-β, bFGF, hepatocyte growth factor (HGF), insulin-like growth factor (IGF), platelet derived growth factor (PDGF), Notch, Wnt, retinoic acid (RA), hypoxia inducible factor (HIF-1α), and Sonic hedgehog (Shh). The mechanisms of by which these molecules support EMT is outside the scope of this review, but has previously been discussed in the following eloquent reviews (Lamouille et al., 2014; von Gise & Pu, 2012). Another important factor modulating EMT is thymosin beta-4 (Tβ4), an actin-monomer-binding protein known to regulate cell motility (Mannherz & Hannappel, 2009). Most of the pathways through which these molecules exert their functions converge on the transcriptional control of epithelial-specific and mesenchymal-specific proteins, including E-cadherin. Regardless of the predominant EMT pathway upregulated to provoke the initiation of signaling, common downstream effectors of these pathways are transcriptional repressors like Snail, Slug, ZEB1/2 and Twist, which block the E-cadherin promoter region (at E-box elements), resulting in a mesenchymal switch (Germani, Foglio, Capogrossi, Russo, & Limana, 2015; Lamouille et al., 2014). Signaling pathways supporting EMT are summarized in Fig. 1. The focus of this review is on non-coding RNA and the primary cilium.

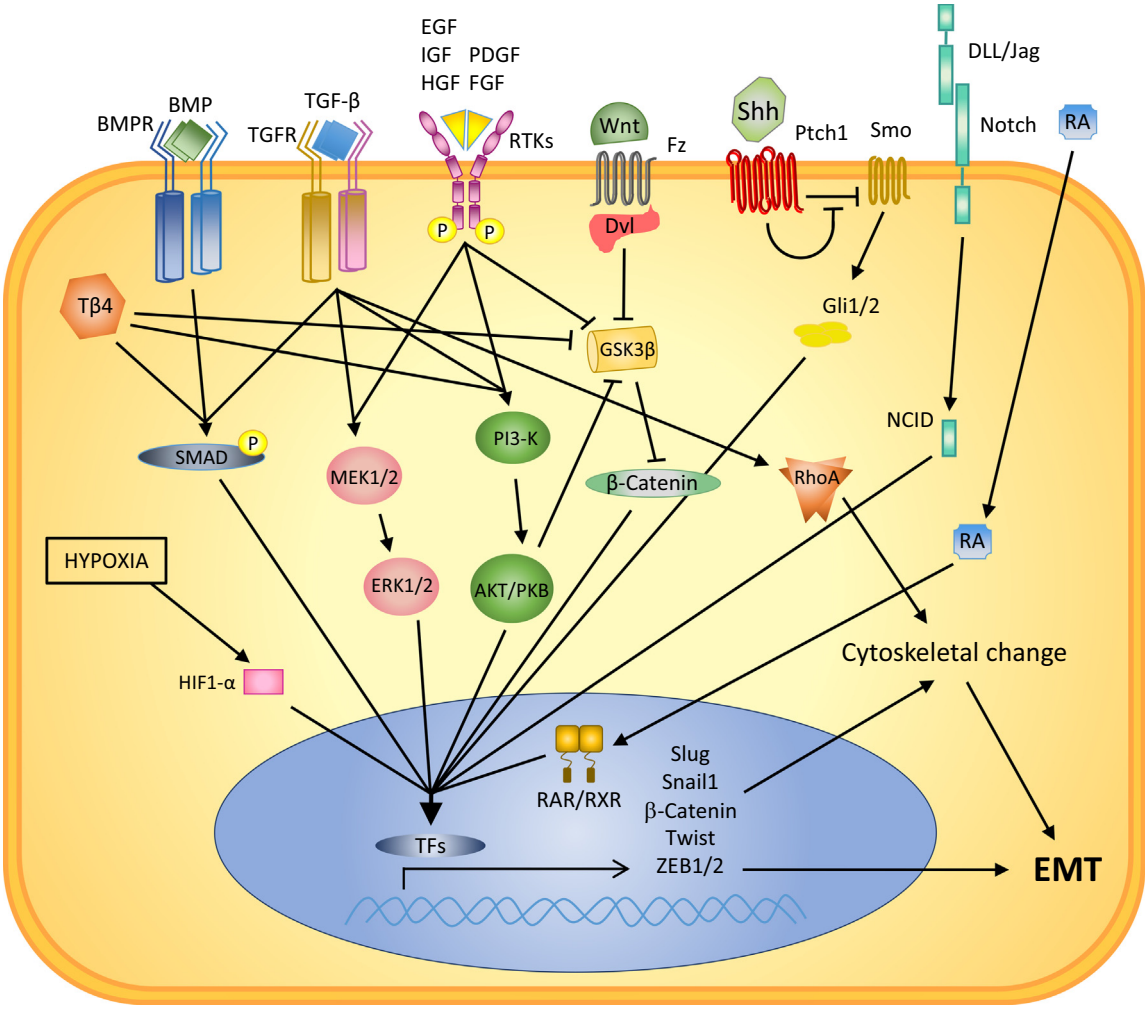

Fig. 1. Signaling pathways mediating EMT. TGF-β and BMP both phosphorylate SMAD proteins, leading to a SMAD phosphorylation cascade. TGF-β also activates the PI3K/AKT and ERK/MAPK pathways which are known for being regulated by ligands of RTKs, including PDGF, FGF, EGF, IGF, and HGF. RTKs inhibit the activity of the destruction complex containing GSK3β, which is inhibited by canonical Wnt signaling. Shh promotes the nuclear translocation of EMT transcription factors by binding its receptor Ptch. The Notch receptor is activated by its membrane bound ligand DLL or Jagged from a neighboring cell to cause nuclear transport of its intracellular domain. Furthermore, RA diffuses through the cell to bind its nuclear receptors (RAR and RXR), which themselves act as transcription factors. In addition to ligand mediated EMT, other mediators, such as the actin sequestering protein Tβ4 are upregulated in EMT to support mesenchymal like cytoskeletal changes. The GTPase RhoA is also activated by Tβ4 and TGF-β, and regulates changes in cell shape. Finally, hypoxia supports the entire process by upregulating transcription of HIF-1α, which in turn binds hypoxia response elements of other genes to cause EMT.

5.1. Non-coding RNA

EMT is a highly regulated event, which proceeds through a wide spectrum of intermediates. Genome wide analysis studies have determined that although the majority of the human (and mouse) genome is transcribed (80%), the bulk (62–75%) of these transcripts do not code for proteins (Djebali et al., 2012; Encode Project Consortium, 2012). Questioning the doctrine of the "central dogma" theory, non-coding RNA (ncRNA) genes produce RNA molecules that are functional in and of themselves and are not merely intermediary molecules that encode proteins. MicroRNAs (miRNAs; 18–24 nucleotides) and long non-coding RNAs (lncRNAs; >200 nucleotides) are two subsets of ncRNA which modulate EMT by transcriptional repression, epigenetic modification, and translational regulation.

miRNAs repress translation by binding the 3′-untranslated region of target mRNAs. Ibrahim et al. (2017) recently demonstrated the importance of miRNAs in EMT by altering the activity of Dicer, the RNase enzyme responsible for cleaving pre-miRNA into miRNA. Forced expression of Dicer prevented hypoxia-induced increase in HIF-1α expression, and reduced cell migration and EMT in hepatocellular carcinoma cells. Furthermore, several miRNAs have been reported to modulate EMT transcription factors and protein components of the cell architecture. One of the most well studied miRNA families involved in EMT is the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429), reviewed by Korpal and Kang (2008). Several studies have demonstrated that members of the miR-200 family target transcriptional repressors of *E-cadherin* and moderate EMT driven by Wnt/β-catenin. (Cong et al., 2013; Korpal, Lee, Hu, & Kang, 2008; Su et al., 2012). Intriguingly, PDGF mediated EMT is augmented in the presence of anti-miR-200 and diminished upon anti-miR-221 treatment (Kong et al., 2009; Su, He, Tian, Hu, & Zhang, 2013). The critical EMT transcription factor *Snail* is also mediated by miRNAs, as demonstrated via 3'UTR binding and translational inhibition by the miR-30 family (Kumarswamy et al., 2012). miRNAs also target components of the cell architecture, thereby affecting epithelial integrity. For example, miR-9 directly targets *E-cadherin* and supports release of β-catenin and mesenchymal transformation in mammary epithelial cells (Ma et al., 2010). The molecular mechanisms by which miRNAs act as critical regulators in determining the fate of transitioning cells are described in recent reviews (Abba, Patil, Leupold, & Allgayer, 2016; Lamouille, Subramanyam, Blelloch, & Derynck, 2013).

More recently, studies have begun to illuminate the role of lncRNAs in EMT. The lncRNAs comprise the largest portion of the non-coding transcriptome, with 96,308 genes and 172,216 transcripts discovered in the human to date (NONCODE database, http://www.noncode.org/analysis.php). A number of lncRNAs have been described to regulate EMT, with some possessing supportive functions, and others repressive functions. For example, the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) has been described to control EMT, apoptosis, cell cycle progression, and angiogenesis (Michalik et al., 2014; Xiang et al., 2016; Xiang, Zhang, Tang, & Li, 2017). *MALAT1* localizes to nuclear bodies (nuclear speckles) within the nucleus, where it is suggested to regulate alternative splicing of several pre-mRNAs (Tripathi et al., 2010). Indeed, *MALAT1* was originally identified as a marker for non-small cell lung cancers with metastatic propensity (Ji et al., 2003). *MALAT1* transcripts have also been found to be upregulated in numerous cancer types and it is thought to contribute to the maintenance of EMT for cell migration and invasion (Gutschner, Hammerle, & Diederichs, 2013; Zhou et al., 2015). Inhibition of *MALAT1* in both in vivo and in vitro models of squamous cell carcinoma reduced cell migration and invasion, expression of EMT markers Slug and β-catenin, and the appearance of mesenchymal markers N-cadherin and Vimentin (Zhou et al., 2015). Furthermore, Yang, Yao, Li, Li, and Wang (2016) demonstrated a direct link between *MALAT1* and TGF-β-induced EMT in human retinal pigment epithelial cells in which knockdown of *MALAT1* using siRNA suppressed TGF-β-mediated morphological changes, α-SMA expression, as well as the down-regulation of E-cadherin. The role of *MALAT1* in epicardial EMT has yet to be elucidated.

Contrastingly, the lncRNA maternally expressed gene 3 (*MEG3*), recently identified through genome-wide mapping, suppresses both TGF-β and SMAD as well as TGF-β receptor expression by binding GA rich sequences (Mondal et al., 2015). Furthermore, *MEG3* was shown to specifically repress EMT in breast cancer cells in a transwell invasion assay; *MEG3* was demonstrated to sponge miRNA-421 to maintain E-cadherin expression (Zhang et al., 2017). As a result, decreased expression of *MEG3* is associated with poor prognosis in cancer patients (Sun et al., 2014). Interestingly, two groups have recently demonstrated a role for *MEG3* in cardiac tissue. In experiments by Piccoli et al. (2017), GapmeR-mediated silencing of *MEG3* in cardiac fibroblasts reduced *MMP-2* transcription. Inhibition of *MEG3 in vivo* prevented transverse aortic constriction induced cardiac fibrosis and cardiomyocyte hypertrophy. Similarly, Gong et al. (2018) recently identified a deleterious response to *MEG3* expression in H9c2 cells, demonstrating that *MEG3* shRNA mediated knockdown alleviates hypoxia-induced cell injury. A careful investigation of mechanisms of *MEG3* action in different cell types and for different cardiac pathologies are warranted in future research. Beyond those mentioned here, a number of lncRNAs are involved in regulating EMT, as is eloquently reviewed by Heery, Finn, Cuffe, and Gray (2017).

5.2. The primary cilium

Outside of signaling mediators, cellular structural changes may alter receptor responses and thus downstream effects of EMT signals. Receptors for cytokines that govern multiple signaling pathways integral to the initiation of EMT are found on small microtubule based sensory organelles called primary cilia (Koefoed, Veland, Pedersen, Larsen, & Christensen, 2014). Primary cilia are non-motile hair-like protrusions that emanate from the cell surface in an antenna-like fashion. Primary cilia are located on almost all polarized, and most non-polarized cell types (Davenport & Yoder, 2005). These organelles possess unique antennal like properties which allow them to detect mechanical and sensory cues and transmit these signals from the cell surrounding to its interior.

The ciliary axoneme is elongated, and continuously turned over via the process of intraflagellar transport (IFT). IFT is a bi-directional transport system which allows the trafficking of large protein complexes (IFT particles) along polarized microtubules in the axoneme (Kozminski, Johnson, Forscher, & Rosenbaum, 1993). The IFT complexes serve as rafts for ciliary cargo such as tubulin, membrane proteins, ion channels, and protein kinases (Lechtreck, 2015). Dysregulation of IFT polypeptides leads to a plethora of human diseases, demonstrating the importance of these molecules for proper ciliary function. These "ciliopathies" are often pleiotropic disorders and include defects in numerous organ systems including the liver, kidney, airway, brain, pancreas, retina, adipose, and heart (Davenport & Yoder, 2005). For example, intraflagellar transport protein 88 (IFT88) (*Tg737*; Polaris protein) is a complex B protein required for primary cilia formation. This requirement was discovered in *Chlamydomonas*, and subsequently determined to contribute to the failure of ciliary function in polycystic kidney disease (Moyer et al., 1994; Pazour et al., 2000). *Tg737* functionality was also shown to be required for various ciliary related processes including proper ependymal cell function in brain ventricles and node cilia functioning in left–right patterning for embryonic development (Taulman, Haycraft, Balkovetz, & Yoder, 2001). *Tg737* knock-out mice die at embryonic day 11.5 due to a variety of developmental abnormalities, including cardiac insufficiency with pericardial sac ballooning (Murcia et al., 2000).

5.2.1. Wnt at the primary cilium

IFT allows the transport of signaling molecules and receptors, turning the cilium into a repository that can harbor proteins and coordinate signaling pathways. Various lines of evidence indicate that the primary cilium plays an inhibitory role in Wnt signaling. Knockout mice for proteins required for primary cilia assembly exhibit hyperactive β-catenin-dependent signaling as compared to wild-type controls (Corbit et al., 2008). It is thought that the β-catenin-positive regulator Jouberin (Jbn) is spatially sequestered in the cilium, constraining movement of β-catenin to the nucleus. To this effect, Lancaster, Schroth, and Gleeson (2011) demonstrated ciliary targeting motifs on the Jbn protein, and increased nuclear co-localization of Jbn and β-catenin upon Kif3a siRNA mediated cilia retraction (Fig. 2). β-catenin is a known transcriptional activator of several EMT related genes, including Snail1 and Zeb1 (Yang et al., 2015). Unlike the canonical pathway, however, the primary cilium is thought to be required for planar cell polarity (PCP, non-canonical Wnt signaling), and core PCP components are depleted in IFT mutants (Cao, Park, & Sun, 2010). Similarly, Cre-mediated conditional mutation of Ift88 in the inner ear, results in disorganization of the planar-polarized orientation of stereocilia (Jones et al., 2008). Furthermore, several core PCP proteins, such as Vangl2, have been shown to localize to the primary cilium axoneme (Borovina, Superina, Voskas, & Ciruna, 2010). May-Simera et al. (2010) demonstrated the requirement for the interaction between Vangl2 with the ciliary/ basal body protein Bardet-Biedl syndrome 8 (BBS8) for the establishment of left–right asymmetry. In this study, a direct interaction between Vangl2 and Ift20 was demonstrated by co-immunoprecipitation, and deletion of Ift20 interrupted PCP-

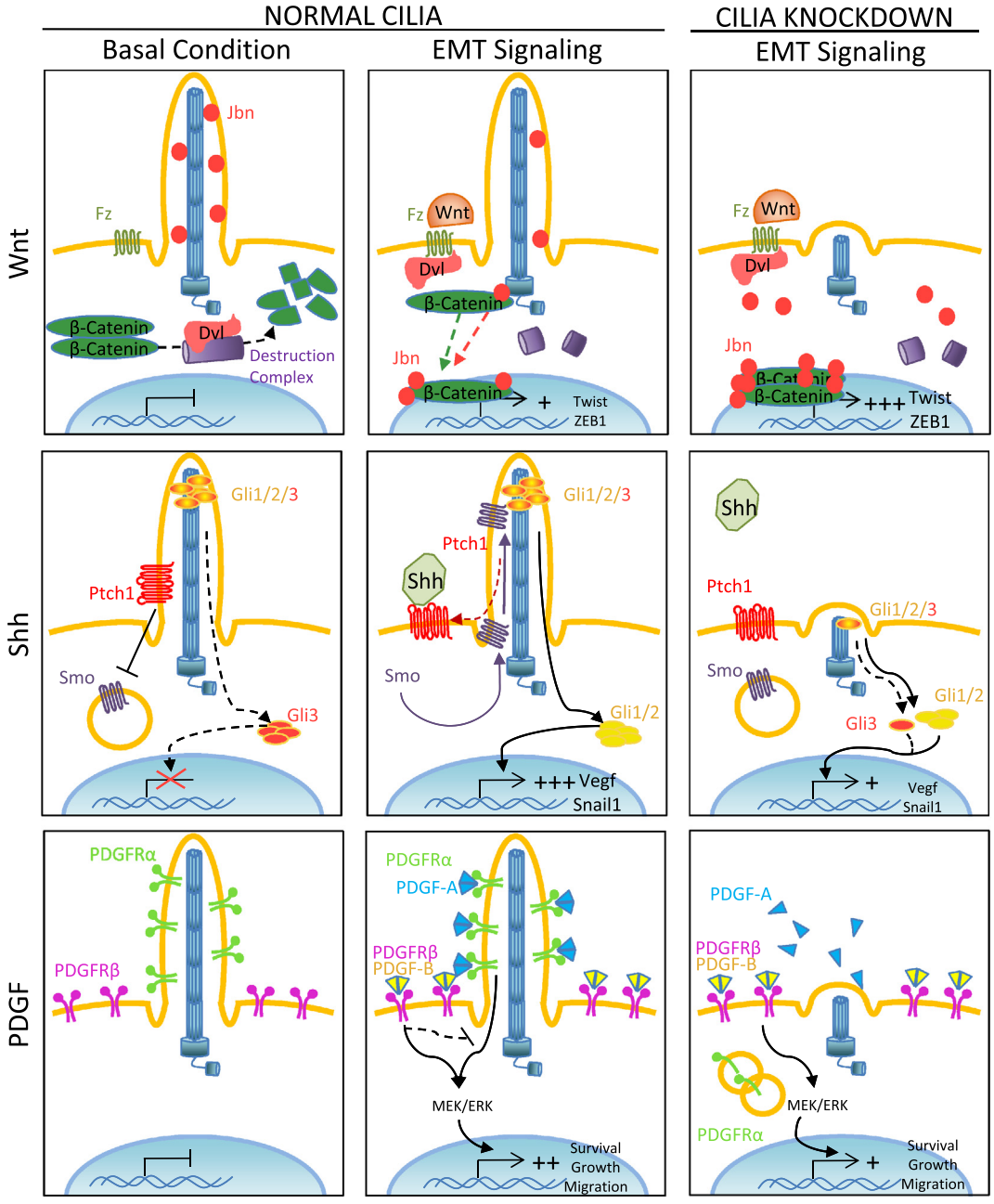

Fig. 2. Signaling associated with the primary cilium. Wnt receptors are associated with the base of the primary cilium. In the absence of the cilium, Jouberin (Jbn) binds β-catenin in the cytoplasm and its nuclear transport is uninhibited, causing hyperactive signaling. Primary cilium also harbors sonic hedgehog (Shh) signaling factors, and its degradation decreases Shh signaling. PDGFα receptors are also associated on the cilium while PDGFβ receptors are only on the plasma membrane. Loss of the cilium attenuates PDGFα mediated responses, but has no effect on PDGFβ signaling.

dependent asymmetric protein accumulation. On the other hand, using *Frizzled-2* knockdown, it has been demonstrated that mediators of non-canonical Wnt/PCP signaling are required for primary cilia formation (Oishi, Kawakami, Raya, Callol-Massot, & Izpisua Belmonte, 2006). Regardless of whether the primary cilium is required for noncanonical Wnt signaling, or vice versa, the two appear to be closely connected, perhaps via various molecules. In either case, mutations in ciliary proteins attenuate Wnt/PCP signaling, resulting in loss of cell polarity – a feature of EMT (Lamouille et al., 2014).

5.2.2. Shh at the primary cilium

In addition to Wnt mediators, Ptch, Smo, and Gli transcription factors are all present within the primary cilium. This localization of Ptch and Smo within the primary cilium is mutually exclusive (Haycraft et al., 2005; Rohatgi, Milenkovic, & Scott, 2007). In a basal state, Ptch is localized to the primary cilium. In the absence of Shh, Ptch tonically inhibits Smo from its entrance into the cilium, thereby repressing Hh mediated transcription. In this instance, Smo cannot act on the Gli factors, and Gli2 is degraded while Gli3 is processed to its repressive form. Alternatively, in the presence of Shh, Ptch is internalized, allowing Smo to enter the primary cilium (Fig. 2). Within the cilium, Smo sends Gli3 to the proteasome for degradation, and activates the Gli2 transcription factor, which enters the nucleus to promote transcription. The involvement of the primary cilium in this process is demonstrated by the requirement of IFT for both Gli2 activator and Gli3 repressor processing. Loss of IFT results in reduced Gli2 activators and Gli3 repressors, causing aberrantly active low level Hh signaling (Caspary, Larkins, & Anderson, 2007; Haycraft et al., 2005). During development, at which time Hh levels are high, disrupting IFT proteins results in neural tube and brain defects (Huangfu et al., 2003). However, in postnatal tissues, the primary cilium inhibits Hh signaling. A loss of primary cilia may activate Hh signaling, which regulates plasticity of the pancreatic epithelium and may contribute to tumorigenesis (Cervantes, Lau, Cano, Borromeo-Austin, & Hebrok, 2010; Hassounah, Bunch, & McDermott, 2012).

5.2.3. PDGF at the primary cilium

The primary cilium is essential for PDGF signaling in growth arrested cells through its receptor PDGFRαα, the homodimer of PDGFRα (Schneider et al., 2005). PDGFRαα is localized to the primary cilium, and in *Tg737*^{orpk} (or Ift88) knockout mutants, embryonic fibroblast cell cycle entrance was blocked. Reduced PDGFRα expression in these cells led to decreased MEK1/2-ERK1/2 activation, delineating a role for the primary cilium and PDGF signaling in growth control. The same group also used micropipette administration to demonstrate the importance of this signaling pathway for directional wound healing, indicating that the primary cilium is required for chemotaxis towards PDGF-AA (Schneider et al., 2010). Defects in PDGF signaling are implicated in a range of diseases including epithelial cancers and vascular disorders (Andrae, Gallini, & Betsholtz, 2008). For example, aberrant PDGF causes EMT and supports cancer metastasis, as demonstrated by reduced experimental metastasis with overexpression of a dominant-negative PDGFR (Jechlinger et al., 2006). PDGF signaling also plays a role in EMT of mesenchymal cells during cardiac development and the growth of the coronary vasculature (van den Akker et al., 2005). However, using immunohistochemistry, these authors demonstrated the presence of PDGF-B and PDGFRββ, rather than PDGFRαα in EPDCs undergoing EMT for coronary artery development. Although Schneider et al. (2005) demonstrated that PDGF-AA signaling through PDGFRαα is associated with the cilium, signaling through PDGFRββ was unaffected by loss of the cilium (Fig. 2). Interestingly, activation of PDGFRββ has been shown to promote deciliation, a phenotype associated with ciliopathies. In experiments with NIH3T3 serum deprived cells, potent deciliation was observed under immunofluorescence microscopy with the addition of PDGF-DD (a PDGFββ agonist), but not PDGF-AA (Nielsen et al., 2015). In these experiments, serum-induced deciliation was markedly impaired with depletion of PDGFRβ using siRNA. Furthermore, isolated deletion of PDGFRβ (PDGFRβ$^{-/-}$) results in an impairment of coronary vascular smooth muscle generation in development, while knockout of PDGFRα alone inhibits the generation of cardiac fibroblasts, differentiating the two PDGF receptors (Mellgren et al., 2008; Smith, Baek, Sung, & Tallquist, 2011). Thus, the type of PDGF may be an important consideration when determining the role of the cilium in PDGF signaling. Specifically, it has been suggested that signaling through PDGFRαα requires the presence of the cilium, while PDGFRββ leads to activation of the downstream MEK/ERK mediators independent of the cilium. In this regard, addition of PDGF-BB to *Tg737*^{orpk} mouse embryonic fibroblasts caused ERK1/2 and AKT phosphorylation while PDGF-AA had no effect (Clement et al., 2013). Notably, PDGF-AA/αα signaling can be restored in ciliary transport mutants by inhibiting mTORC1 with rapamycin, suggesting that while the cilium is not absolutely required for PDGFαα signaling, it does play a key role in augmenting efficiency of PDGFαα mediated responses (Umberger & Caspary, 2015).

5.2.4. Other signaling associated with the primary cilium

In a recent review, Christensen, Morthorst, Mogensen, and Pedersen (2017) eloquently described the role of the primary cilium in coordinating both RTK and TGF-β signaling. In addition to their role in regulating PDGF, primary cilia have been demonstrated to mediate the activity of other RTKs, which autophosphorylate to activate MAPK and PI3K-AKT signaling. The epidermal growth factor receptor (EGFR) is also localized on the primary cilium (Ma, Li, et al., 2005), and loss of the primary cilium in *Kif3a* mutant mice results in apical mislocalization of the EGFR on the tubular epithelial cells (Lin et al., 2003). Although the role of the primary cilium in signaling has not been elucidated, Seeley and Nachury (2009) has suggested that loss of the IFT motor complex causes EGFR accumulation inside the cell and resulting persistent MAPK activity. In other cases, signaling through some RTKs appears to facilitate changes in ciliary structure. For example, IGF-1 binds to IGF-1R on the cilia to support cilia resorption in retinal pigment epithelial cells (Yeh et al., 2013). Interestingly, in this study, IGF-1R was found to be located both on the cilium and on the plasma membrane. IGF-1 receptor neutralizing antibodies blocked ciliary resorption, and addition of IGF-1 alone stimulated resorption and cell cycle reentry. The addition of IGF-1 to *Ift88* mutant cells which lack functional cilia did not support similar mitogenic responses, suggesting that the cilium is required to mediate IGF-1 signaling. Conflictingly, the combination of both IGF-1, EGF or FGF and PDGF-AA was required to mediate ciliary destabilization in mouse embryonic fibroblasts (Jacoby et al., 2009). In this study, addition of IGF-1, EGF or FGF alone did not alter cellular architecture, however when one of these factors was added in combination with PDGF-A the cilium was disassembled, indicating a requirement for PDGF-A and permissive roles for the other RTKs in cilia disassembly. Notch receptors are also located on the primary cilium, however the involvement of the cilium in the regulation of the Notch pathway is a subject of debate. While some groups demonstrate a requirement for the primary cilium in Notch signaling for early development (Ezratty et al., 2011), others show over activation of the Notch pathway upon disruption of ciliary localization (Leitch, Lodh, Prieto-Echague, Badano, & Zaghloul, 2014).

Like RTKs and Notch, TGF-β receptors are located on the primary cilium, and primary cilia appear to be required for TGF-β dependent SMAD signaling (Clement et al., 2013). In this study, immunofluorescence microscopy was used to demonstrate the localization of TGF-β receptors to the tip of the cilium in fibroblasts, and the activated TGF-β receptors migrated to the ciliary base to phosphorylate SMAD2/3 and activate ERK1/2 (Clement et al., 2013). These authors also demonstrated the requirement for the cilium in this process as TGF-β mediated SMAD phosphorylation was significantly attenuated in fibroblasts from *Tg737*^{orpk} mutant cells. In another study however, primary cilia were not required for responses to TGF-β/BMP type ligands. In fact, non-ciliated

endothelial cells from Tg737^{orpk} mutant embryos exhibited a fibroblast phenotype after exposure to fluid flow and enhanced phospho-Smad2 and *Snail1* expression as compared to ciliated cells (Egorova et al., 2011). Upon exposure to increased shear stress (2.5 Pa), ciliated endothelial cells became non-ciliated and EMT marker expression reflected similar propensity for change as Tg737^{orpk} mutant cells. In fact, rescue of the cilium in Tg737^{orpk} mutant cells by Ift88 transfection inhibited EMT. Similarly, Sanchez-Duffhues et al. (2015) demonstrated that endothelial cells lacking primary cilia are prone to undergo morphogenic change in response to BMP; interestingly, BMP rather than TGF-β was required for SMAD phosphorylation in cilium-defective aortic endothelial cells, and this supported β-*catenin* and *Slug* expression. Importantly, Rozycki et al. (2014) demonstrated that the resorption of the primary cilium is involved in regulating TGF-β mediated myofibroblast transition. In this study, wounding to de-couple epithelial intercellular contacts provoked initial cilium growth, and subsequent TGF-β stimulation evoked cilium loss and EMT like transition. Provoking cilium loss with Kif3a siRNA prior to EMT made the cells insensitive to TGF-β, however if the Kif3a siRNA was applied with optimal timing for peak deciliation after TGF-β stimulation, the ensuing cilium loss accelerated the EMT process. Thus, it is likely that ciliary signaling is both ligand, cell type, and condition specific. It is possible that mediators of EMT bind receptors on the primary cilium to induce cilia resorption and downstream signaling that supports EMT, and this process is further potentiated by the addition of other ligands post resorption like BMP.

6. EMT and cardiovascular development

EMT plays a critical role in early development. Beyond its role in the invasion of the uterine lining and forming the three germ layers, EMT is also responsible for cardiac formation. Originating in the proepicardial organ, cells that express the transcription factors Wilms' tumor 1 (Wt1) and T-box transcription factor 18 (Tbx18) in development migrate to the surface of the myocardium to become the epicardial layer at around E9.0–10.5 in mice (Cai et al., 2008; Vicente-Steijn et al., 2015). Many of these cells then undergo EMT to delaminate from the epicardium and form epicardium-derived cells (EDPCs) which migrate into the myocardium as the precursors of the cardiac structures (about E11.5–12.5) (Vicente-Steijn et al., 2015). Specifically, EPDCs contribute to the subendocardial and atrioventricular cushion, cardiac interstitial fibroblasts, and the coronary vasculature (perivascular fibroblasts, coronary smooth muscle cells and endothelial cells) (von Gise & Pu, 2012).

Disruption of numerous mediators of the EMT process have been demonstrated to cause congenital heart defects. For example, the epicardial transcription factor Wt1 is enriched in the embryonic heart, and is required for embryonic coronary artery formation and essential for epicardial EMT (Moore, McInnes, Kreidberg, Hastie, & Schedl, 1999). Wt1 contains a DNA binding domain with a proline-glutamine rich region capable of regulating transcription. Correspondingly, two of the major mediators of EMT, Snail (*Snail*) and E-cadherin (*Cdh1*), are controlled through direct transcriptional regulation by Wt1, thereby affecting cell differentiation (Martinez-Estrada et al., 2010). Furthermore, Guadix et al. (2011) demonstrated Wt1 binding regions in the Raldh2 promoter and the direct transcriptional control of Raldh2 expression by Wt1 in epicardial cells. The developmental deficiencies of mice with a homozygous deletion for Wt1 were reported by Kreidberg et al. (1993). Even epicardial specific Wt1 mutants die from cardiovascular defects between E16.5 and E18.5 (Martinez-Estrada et al., 2010). Similarly, Tbx18 deficient mouse embryos exhibit structural and functional defects of the epicardium and coronary vessels (Wu, Dong, Regan, Su, & Majesky, 2013).

Many of the signaling pathways associated with EMT have been demonstrated to be required for proper embryonic heart development. Both TGF-β1 and TGF-β2 deficient mice develop severe cardiac defects (Bartram et al., 2001; Letterio et al., 1994). Similarly, BMP2 deficiency is embryonic lethal, and BMP2-deficient embryos exhibit malformed cardiac cushions and abnormal cardiac development in the exocoelomic cavity (Ma, Lu, Schwartz, & Martin, 2005; Zhang & Bradley, 1996). Interrupted Notch signaling disturbs smooth muscle differentiation of EPDCs in coronary vascular development, resulting in EPDCs which surround developing arteries but do not provide sufficient support for normal vascular development (Grieskamp, Rudat, Ludtke, Norden, & Kispert, 2011). Likewise, deficiency of either FGF or PDGF attenuates EMT, EPDC invasion into the myocardium, and vessel cell differentiation during coronary vascular formation (Pennisi & Mikawa, 2009; Smith et al., 2011). Using a conditional β-catenin-deletion mutant, Zamora, Manner, and Ruiz-Lozano (2007) demonstrated similar impairment in coronary artery formation due to failed epicardial expansion, blunted epicardial invasion into the myocardium, and impaired differentiation into smooth muscle cells. Jing et al. (2016) demonstrated the importance of HIF-1α mediated Snail expression in epicardial Tbx18^{+} cells for smooth muscle cell differentiation; blocking nuclear accumulation of HIF-1α with 2-methoxyestradiol attenuated hypoxia induced Snail and smooth muscle marker expression in these cells. Similarly, proper RA signaling is required for epicardial EMT for coronary arteriogenesis, as demonstrated by reduced β-catenin and FGF2 expression and abnormal arterial branching in tissue specific retinoic acid receptor mutant (Merki et al., 2005). In this study, arteriogenesis was found to be impaired due to a deficiency in retinoid-dependent Wnt signaling. Furthermore, conditional epicardial deletion of retinoid X receptors (RXR) in development using the Gata5-Cre caused thinning of the subepicardial compartment resulting in a hypoplastic myocardium. The activation of epicardial cells during development is also suggested to cause trophic factor production which contributes to the proliferation of cardiomyocytes. Conditioned media from epicardial cell cultures causes proliferation of primary fetal cardiomyocytes. Interestingly, trophic factor production is augmented by RA treatment, and inhibited by a retinoid receptor antagonist (Chen et al., 2002). Reduced trophic factor production may have also been responsible for the hypoplastic myocardium observed by Merki et al. (2005) in their retinoic acid receptor (RAR) mutant mice.

Outside of growth factors and cytokines, miRNAs and primary cilia which regulate EMT also play a role in cardiac development. To this end, Singh, Lu, Massera, and Epstein (2011) demonstrated that regulation of miRNAs by Dicer is critical for epicardial EMT and coronary smooth muscle cell differentiation during development. In this study, epicardial-specific deletion of Dicer impaired EMT, epicardial cell proliferation and coronary vessel development, resulting in 100% postnatal fatality. Likewise, multiple human ciliopathies have cardiac abnormalities such as dextrocardia, aortic stenosis, and septum defects (Koefoed et al., 2014). Mutations in cilia proteins in animal models results in congenital heart defects (Clement et al., 2009; Li et al., 2015). Specifically, Ift88-null mice develop hearts with ventricular dilation, reduced myocardial trabeculation, and outflow tract abnormalities (Clement et al., 2009). In this study, it was demonstrated that Hh signaling is associated with the cilium in cardiomyocytes, suggesting its loss could be responsible for the observed cardiac phenotype of these Ift88-null mice. Furthermore, both endocardial and epicardial cells in the heart are ciliated. However, under the influence of high shear stress (as in cardiac development), cells become devoid of cilia as the pressure signals the microtubules in these organelles to dissociate (Van der Heiden et al., 2006). Interestingly, FGF receptor knockdown or FGF inhibition downregulates expression of core ciliary proteins and reduces the ciliary length during development (Neugebauer, Amack, Peterson, Bisgrove, & Yost, 2009). Importantly, the absence of ciliary function in the embryonic mouse heart coincides with endocardial–to–mesenchymal transition (EndMT) (Egorova et al., 2011).

7. Cardiac repair

7.1. Insight from regenerative models

Until recently, the dogma dictating an incapacity for complete mammalian cardiac healing was in sharp contrast with a complete regeneration in non-mammalian vertebrate models, such as that demonstrated by zebrafish from cardiac apex resection (Jopling et al., 2010; Poss, Wilson, & Keating, 2002). Notably, two surgical models of mammalian cardiac regeneration in the neonatal mouse have been developed (Blom, Lu, Arnold, & Feng, 2016; Haubner et al., 2012; Porrello et al., 2011). However, the regenerative capacity in neonatal mice was retained only in the early neonatal period, and was lost shortly after birth by P7. Evidence from coronary corrosion casts indicates that a vascular response restores perfusion during neonatal heart regeneration (Porrello et al., 2013). Furthermore, EMT genes are upregulated post-cardiac apex resection in the neonate, implicating epicardial EMT in neovascularization (Porrello et al., 2011). Indeed, data from zebrafish models designates the epicardium as the cellular source of the regenerated vascular compartment during cardiac regeneration, corroborating this hypothesis (Kikuchi et al., 2011); in this study, Cre-mediated lineage tracing of Tcf21^{+} (epicardin) epicardial cells demonstrated differentiation into mesenchymal perivascular cells, but not cardiomyocytes. In other experiments, the zebrafish epicardium re-expressed Wt1, Tbx18 and aldh1a2 after cardiac injury, and Wt1^{+} cells were shown to differentiate into perivascular cells (Gonzalez-Rosa, Peralta, & Mercader, 2012; Lepilina et al., 2006). Disruption of mediators of EMT including PDGF and FGF disturb neovascularization during zebrafish heart regeneration, demonstrating the importance of EMT mediated repair (Kim et al., 2010; Lepilina et al., 2006). Similarly, treatment with the Hh antagonist cyclopamine blunts the epicardial regenerative capacity post-apex resection, and transplantation of Shh-soaked beads to the ventricular base stimulates epicardial regeneration (Wang, Cao, Dickson, & Poss, 2015). Furthermore, hyaluronic acid (HA) is a glycosaminoglycan known for its role in formation of granulation tissue for wound healing. Recently, HA and its receptor Hmmr have been implicated in epicardial EMT for cardiac remodeling. Knockdown of Hmmr in a zebrafish ventricular apex resection model reduced epicardial cell migration and coronary vascular formation, causing permanent fibrosis (Missinato, Tobita, Romano, Carroll, & Tsang, 2015).

7.2. EPDCs and cardiac repair

In adult mammals, this dormant fetal growth program is reactivated after MI by stimuli such as increased wall stress, which activates mechanical stretch receptors, triggering immediate early genes such as c-jun, c-fos, and c-myc (Gidh-Jain, Huang, Jain, Gick, & El-Sherif, 1998; Reiss et al., 1993; Sadoshima, Jahn, Takahashi, Kulik, & Izumo, 1992). Hypoxia from injury induces hypoxia-inducible factor-1 alpha (HIF-1α), which is responsible for the upregulation of VEGF and other pro-angiogenic factors that are implicated in epicardial EMT (Forsythe et al., 1996; Tao, Doughman, Yang, Ramirez-Bergeron, & Watanabe, 2013). Indeed, fetal epicardial markers are also re-expressed after MI in adult mice. This is particularly important in subsets of EPDCs that express Wt1 and Tbx18, markers of embryonic cardiovascular progenitors (van Wijk, Gunst, Moorman, & van den Hoff, 2012). These cells are thought to contribute to angiogenesis and stem cell recruitment post-MI through paracrine effects by up-regulating molecules such as FGF, PDGF, and monocyte chemoattractant protein 1 (MCP-1) (Zhou et al., 2011). Within the infarcted myocardium, some endothelial cells also express Wt1. Since Wt1 promotes endothelial cell proliferation, the Wt1^{+} endothelial cells may contribute to the angiogenic response (Duim, Kurakula, Goumans, & Kruithof, 2015). Recently, post-MI EPDCs were also shown to express the ectoenzyme CD73 on their cell surface, which can convert extracellular ATP and NAD to adenosine (Hesse et al., 2017). Excess extracellular adenosine stimulated the release of cytokines like IL-6, IL-11, and VEGF. These authors also demonstrated the release of the fetal protein tenascin-C from EPDCs, which stimulated EPDC migration. While this milieu may support angiogenesis, it may also contribute by polarizing the inflammatory response to support cardiac repair.

The immune response has been demonstrated to be important in both the acute and long-term responses to MI (Frangogiannis, 2014). In general, the innate system is activated in the acute setting and plays a role in wound clearance and scar formation. Overactive adaptive immune responses however, are thought to accentuate deleterious remodeling and contribute to heart failure. For example, the importance of macrophages for cardiac repair was demonstrated by failure of the neonatal cardiac regenerative capacity in mice depleted of macrophages by administration of clodronate liposomes pre and post-MI (Aurora et al., 2014). In a recent study, the epicardium was shown to modulate the adaptive immune response via epicardial Hippo signaling (Ramjee et al., 2017). Indeed, YAP/TAZ signaling, which activates Wnt and IGF pathways, was shown to be required for neonatal cardiac regeneration (Xin et al., 2013). Cardiac specific Yap knockout mice retained an extensive fibrotic scar 26 days after infarction induced on postnatal day 2. Ramjee et al. (2017) further demonstrated the critical importance of epicardial YAP/TAZ in suppressing post-infarct inflammatory responses by recruiting T-regulatory cells. In this study, loss of epicardial Hippo signaling by tamoxifen mediated *Yap* and *Taz* deletion ($Wt1^{CreERT2/+}Yap^{f/f}Taz^{f/f}$) did not affect expansion of EPDCs, but did result in reduced interferon expression and a profound inflammatory response leading to increased fibrosis and mortality post-MI.

Beyond paracrine signaling, Wt1^{+} epicardial cells are able to recapitulate their embryonic profile and undergo EMT. This embryonic reprogramming is responsible for detachment, inward movement and mitosis of transitioning epicardial cells post-MI. Once transitioned, differentiated EPDCs lose Wt1 expression (Moore et al., 1999). Post-MI, epicardial cells express mesenchymal markers rather than epicardial ones, implying their mesenchymal transition (Zhou et al., 2011). The ability of epicardial cells to undergo EMT in humans was confirmed in isolated primary adult epicardial cells grown in culture, which demonstrated the potential to undergo EMT and re-differentiate (van Tuyn et al., 2007). Most authors agree that EPDCs possess the capacity to re-differentiate into vascular smooth muscle cells and myofibroblasts, but less likely endothelial cells. Interestingly, however, some authors have suggested that transitioning epicardial cells may re-differentiate into cardiomyocytes. For example, van Wijk et al. (2012) used Wt1^{Cre};R26R mice to show that EPDCs form a limited number of cardiomyocytes 1 and 3 months post-MI. However, these cardiomyocytes did not display the typical cardiomyocyte architecture, but rather co-expressed the cardiomyocyte markers, cardiac troponin I and SERCA2a. Whether these cells indeed differentiate into cardiomyocytes during cardiac development or post-MI is the subject of debate (Cai et al., 2008; Manner, 1999; Smart et al., 2011; Zhou et al., 2012). Regardless of the differentiation potential of EPDCs into cardiomyocytes, the endogenous capacity of adult EPDCs is clearly not sufficient to replace the lost cardiomyocytes post-MI.

7.3. EPDC and cardiac fibrosis

As reviewed by Fang, Xiang, Braitsch, and Yutzey (2016), numerous sources have also demonstrated the fibrotic differentiation potential of EPDCs in both development and disease. It is possible that different mediators of EMT support the differential linage determination of EPDCs. For example, as mentioned previously, PDGFRβ activation supports coronary vascular smooth muscle cell differentiation, while PDGFα activity encourages fibroblast fate specification (Smith et al., 2011). Interestingly, EPDC derived, and not bone marrow cell derived, cardiac fibroblasts are thought to contribute the majority of cardiac fibroblasts to the post-ischemic myocardium (Ruiz-Villalba et al., 2015). To discern this, irradiated *Wt1Cre*-YFP^{+} mice were transplanted

with mRFP bone marrow and subject to MI. Although both YFP^+ and RFP^+ cells infiltrated the myocardium in response to MI, YFP^+ EPDCs represented the majority of retained fibroblasts post-MI. However, these fibroblasts were suggested to be derived mainly from embryonic EPDC precursors that differentiated during development which subsequently proliferate post-MI in response to signals such as SDF-1, rather than by adult epicardial EMT. Regardless of the time of differentiation, this fibrotic response post-MI is critical to preserving cardiac function. In a recent study, the importance of EPDCs in fibrosis post-MI was demonstrated. In this study, disruption of Wnt/β-catenin signaling in epicardial cells using Wt1-Cre-mediated excision of β-catenin impaired epicardial EMT mediated fibroblast differentiation, and worsened cardiac dysfunction post-MI (Duan et al., 2012). As mentioned previously, the initial fibrotic response is important for scar formation to mitigate further damage and prevent ventricular rupture. However, long-term fibrotic responses are associated with progression to heart failure. Since the activation of epicardial cells post-MI is transient (van Wijk et al., 2012), perhaps the epicardial fibrotic response is beneficial for the acute phase of healing. Indeed, as exemplified by Zhou et al. (2011), most studies indicate that epicardial activation reduces infarct size and LV stiffness post-MI.

8. EMT for post-MI repair

8.1. Gain of function approaches

Enhancing the EMT process may augment EPDC signaling, as well as infarct and peri-infarct angiogenesis in the adult, thereby providing a target for therapeutic development (Fig. 3). In pre-clinical animal models, overexpression approaches for growth factors and cytokines have demonstrated the potential of enhancing EMT for cardiac repair. Our laboratory recently demonstrated that the contribution of EPDC progenitors can be augmented in the adult by other factors related to embryogenesis, including stem cell factor (SCF) (Xiang, Liu, Lu, Jones, & Feng, 2014). Overexpression of membrane-associated human SCF in the murine myocardium augmented cardiac repair. The breakdown of the epicardial basement membrane post-MI allowed SCF expressed by the myocyte to interact with the c-Kit receptor on the epicardium. This induced the expression of the key EMT regulator, TGF-β, enhancing epicardial activation and the production of EPDCs, contributing to arteriogenesis (Xiang et al., 2014). Furthermore, using a lineage tracing approach to fate map $Wt1^+$ cells ($ROSA^{mTmG}$;$Wt1^{CreER}$ mice), we demonstrated the transdifferentiation of EPDCs into SMA^+ cells (myofibroblasts or vascular smooth muscle cells), but not cardiomyocytes or endothelial cells. Importantly, cardiac specific SCF overexpression decreases infarct size, inhibits cardiac remodeling, and improves myocardial function and animal survival post-MI (Xiang et al., 2009). Interestingly, Zakharova, Nural-Guvener, and Gaballa (2012) found that most $c\text{-}Kit^+$ cells derived from atrial explants express both Wt1 and Notch in culture, and these cells undergo EMT upon Notch stimulation, supporting the role of Notch mediated epicardial activation in cardiac repair. Not surprisingly, using a transgenic Notch reporter mouse, others demonstrated that MI amplifies Notch-activated EPDCs, which support a reparative response (Russell et al., 2011). Interestingly, a novel population of $c\text{-}Kit^+$ cells termed cardiac telocytes have been discovered to be present in large numbers in the epicardial compartment (Popescu et al., 2010). These cells are also positive for PDGFRβ and the progenitor cell marker CD34. Cardiac telocytes are thought to

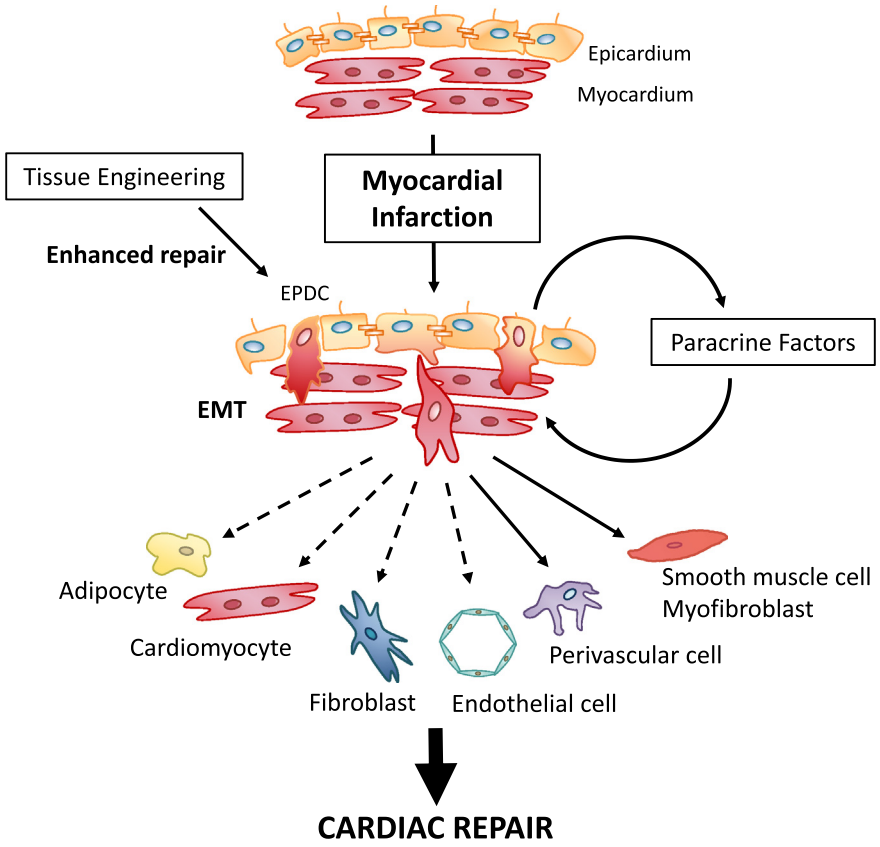

Fig. 3. Schematic diagram underlying epicardial EMT post-MI. Epicardial cells are activated by post-MI stimuli to undergo EMT and become EPDCs. EPDCs release paracrine factors which further support epicardial activity. These paracrine factors also support stem cell recruitment while enhancing angiogenesis, arteriogenesis, cardiomyocyte proliferation, and modulating the immune response. Post-MI, EPDCs re-differentiate into smooth muscle cells, myofibroblasts, pericytes, and potentially contribute endothelial cells, fibroblasts, cardiomyocytes, and adipocytes. This process can be enhanced by administration of exogenous materials which may contain growth factors and cytokines using epicardial patches, extracellular matrix scaffolds, and hydrogels. Exogenous material may also be delivered through ultrasound mediated microbubble delivery. All of these processes support cardiac repair post-MI.

contribute to intercellular communications via atypical cellular junctions and shedding of numerous microvesicles and multivesicular bodies which may contain RNAs and proteins (Gherghiceanu & Popescu, 2012). Work by Zhao and colleagues has revealed that the network of cardiac telocytes in the infarct area are destroyed post-MI, but transplantation of telocytes significantly improves vessel density in the infarct and peri-infarct zone, reduces infarct size, and improves myocardial function (Zhao et al., 2013).

8.2. Roles of growth factors and cytokines

Hypoxia causes epicardial and myocardial secretion of many of the factors which support EMT post-MI. While these factors diffuse across the ventricular wall and affect adjacent myocytes, some factors are released into the pericardial fluid. Evidence suggests that trophic factors within pericardial fluid from MI patients supports epicardial cell proliferation and Wt1 expression when injected into the pericardial cavity of non-infarcted mouse hearts (Limana et al., 2010). Subsequently, the same group identified clusterin, a secreted glycoprotein, in the pericardial fluid from patients with acute MI (Foglio et al., 2015). Clusterin treatment alone after acute MI *in vivo* supported epicardial EMT which enhanced arteriogenesis and reduced cardiomyocyte apoptosis. Similarly, exogenous administration of several other factors which support epicardial EMT have been demonstrated to promote repair. For example, intramyocardial injection of HGF complexed to an IgG carrier enhanced vascular integrity post-MI in a rat model (Rao et al., 2015). Similarly, intramyocardial injection of a constitutively active plasmid hybrid encoding HIF-1α and herpes simplex virus VP16 enhanced angiogenesis and improved regional blood flow (Shyu et al., 2002). Although HIF-1α administration was determined to enhance VEGF expression, this group did not discern the cellular source of improved angiogenesis; whether new vessels came from an epicardial source or pre-existing vessels was not investigated. The role of VEGF in direct epicardial activation post-MI has been confirmed using synthetic modified RNA (modRNA). In this study, intramyocardial injection of VEGF-A modRNA resulted in epicardial progenitor cell mobilization into the myocardium (Zangi et al., 2013). Cultured EPDCs treated with VEGF-A modRNA demonstrated endothelial expression characteristics, and treated hearts had improved $Wt1^+$ cell proliferation and reduced scar area. Lineage tracing with $Wt1^{CreERT2}$ mice demonstrated EPDC expression of both cardiomyocyte and endothelial markers after VEGF-A modRNA treatment.

Contrastingly, while most of these factors have been demonstrated to improve cardiac repair, a recent study elucidated a deleterious role of growth factor mediated epicardial activation (Zangi et al., 2017). While many EPDCs contribute to intramyocardial vessels, some EPDCs differentiate into fat cells. Using Wt1-CreER;$Rosa26^{RFP/+}$ mice for lineage tracing, Liu, Huang, et al. (2014) demonstrated co-immunostaining of epicardial cells with perilipin, an adipocyte marker. Furthermore, in the study by Zangi et al., administration of IGF-1 after MI supported the differentiation of EPDCs into epicardial adipocytes. Although the effect of this differentiation process on infarct size and fibrosis was not discussed, the authors suggested that this type of EPDC differentiation may contribute to worsening coronary artery disease (Zangi et al., 2017). While others have demonstrated the ability of IGF-1 to support cardiac repair, preserving both ejection fraction and capillary density (Koudstaal et al., 2014), this recent data should be considered when evaluating therapies using IGF-1.

A body of research surrounding Tβ4 has also demonstrated its potential as a biological stimulus for adult epicardial activation. Cardiac specific, $Nkx2.5^{Cre}$-driven shRNA knockdown of Tβ4 in mice resulted in embryonic lethality at E14.5–15.5 with abnormal heart and coronary artery development, suggesting an essential role of Tβ4 in coronary formation during embryogenesis (Smart et al., 2007). Tβ4 was also shown to stimulate the outgrowth of adult epicardial cells in explant culture, which is typically lost after the neonatal period. Subsequently, the same group demonstrated that pretreatment of Tβ4 in adult mice by intraperitoneal injection augments neovascularization and stabilizes vascular plexus formation post-MI (Smart et al., 2011). In this study, EPDCs were found to migrate to peri-vascular areas and express PECAM and α-smooth muscle actin. Furthermore, pretreatment of Tβ4 has been shown to increase the time window of neonatal heart regeneration to postnatal day 7, by supporting $Wt1^+$ EPDC migration into the myocardial region (Rui et al., 2014). Unfortunately, however the report by Zhou et al. (2012) questioned the ability of Tβ4 to promote EPDC mobilization; the report suggested that Tβ4 supports EPDC activation and differentiation into smooth muscle cells, but these cells do not migrate substantially into the myocardium. These authors also stipulated that $Wt1^+$ EPDCs activated by Tβ4 treatment post-MI do not differentiate into cardiomyocytes or endothelial cells, but may provide beneficial paracrine factors for repair (Zhou et al., 2012). This discrepancy may, in part, be explained by the epigenetic regulation and chromatin accessibility of key epicardial, EMT and cardiomyocyte genes, which were found to be induced by Tβ4 pre-treatment.

Recent evidence implicates Tβ4 in epigenetic regulation and chromatin accessibility. Brahma-related gene 1 (BRG1) is an ATPase subunit essential for SWI/SNF mediated chromatin-remodeling. Using chromatin immunoprecipitation-sequencing, Vieira et al. (2017) demonstrated BRG1-SWI/SNF activity at evolutionary conserved regions of the Wt1 locus, causing chromatin remodeling. Not only did this study show that BRG1 is required for Wt1 activation in EPDCs, authors also provided evidence for Tβ4 as a co-activator to enhance Wt1 transcription. Tβ4 co-immunoprecipitated with BRG1, and its expression pattern was spatiotemporally equivalent to BRG1. Priming of Tβ4 knockout mice with Tβ4 augmented Wt1 reactivation post-MI, confirming the requirement for Tβ4 in epicardial response to stress (Vieira et al., 2017).

However, in sharp contrast to the study by Smart et al. (2007), neither global nor cardiac specific Tβ4 deficient mice show any abnormalities in embryonic heart development, coronary artery development or adult cardiac function (Banerjee et al., 2012). These authors suggest that the difference between these two studies is most likely due to the off-target effects of the shRNA approach used by Smart et al. (2007). Additionally, Tβ4 treatment in pigs before ischemia or after reperfusion does not appear to be cardioprotective in the short term i.e., within 30 h of ischemia/reperfusion injury (Stark et al., 2016). Furthermore, a phase II clinical trial on the safety and efficacy of Tβ4 treatment in patients with acute MI was suspended in 2015, citing that the drug product manufacturer was not GMP compliant (NCT01311518). Thus, the potential of Tβ4 as a treatment of MI remains to be determined.

Epicardial expression of trophic factors appears to be critical not only for mediating angiogenesis, but also cardiomyocyte survival and division to support cardiac repair. In 2011, the importance of EPDCs as a contributor to fibroblasts and smooth muscle cells, and paracrine mediators for adult cardiac repair was demonstrated (Zhou et al., 2011). MI induced EPDC mesenchymal change and EPDC conditioned medium stimulated endothelial cell proliferation and vessel assembly. Epicardial cells secreted proangiogenic factors such as VEGFa, FGF, PDGF and MCP1. Subsequently, epicardial cells were found to produce follistatin-like 1 (Fstl1), a cardiogenic mitogen (Wei et al., 2015). In this study, collagen patches treated with conditioned media from epicardial cells significantly improved cardiac function as compared to control patches. Similarly, collagen patches conditioned with Fstl1 significantly improved cardiac function, but this effect was not observed with Fstl1-transgenic mice that overexpressed Fstl1 in the myocardium. These results indicate that epicardial specific Fstl1 supports myocyte proliferation and cardiac repair. Improved function was also associated with increased vascularization (Wei et al., 2015).

8.3. Other mediators of epicardial activation

Beyond growth factors and cytokines, the role of non-coding RNA in EMT has prompted investigation into modulating these factors to support cardiac repair. As reviewed elsewhere, many miRNAs and lncRNAs

have been investigated for their role in supporting angiogenesis and cardiomyocyte survival and proliferation (Guo, Luo, Liu, & Xu, 2017). More recently, however, a role for miRNAs in specifically regulating epicardial EMT to improve post-MI recovery has been demonstrated (Seeger et al., 2016). The *let-7* family of miRNAs is composed of 13 members and regulates numerous functions including cell proliferation and differentiation (Roush & Slack, 2008). The expression of *let-7* family members was increased post-MI, and this increase could be blocked by administration of a let-7 anti-miR. Anti-miR administration supported epicardial cell recruitment, EMT, and cardiac functional improvement (Seeger et al., 2016). Contrastingly, in another investigation, miR-21 supported *Timp-1* expression and *E-cadherin* loss in TGF-β induced EMT of EPDCs while anti-miR for miR-21 blocked epicardial EMT (Bronnum et al., 2013). As the understanding of ncRNA is still in its infancy, it is expected that modulation of other ncRNA targets will demonstrate efficacy in supporting epicardial EMT for cardiac repair in the future.

Like the emerging investigations in ncRNA and cardiovascular disease, the role of the primary cilium in cardiac EMT has not been well investigated post-MI. As mentioned above, the primary cilium has recently been established as a mediator of both EMT and cardiac development. In a recent investigation from our laboratory, we uncovered a role for the primary cilium in cardiac repair (Blom, Lu, Kim, & Feng, 2016). Using a model of MI in postnatal day 7 mice, we demonstrated the efficacy of promoting ciliary disassembly in prolonging the age-dependent limitation on cardiac regeneration. In this study, shRNA for Ift88, a protein vital for ciliary function, supported epicardial EMT and cardiac regeneration in mice beyond the neonatal regenerative window. Our recent work also confirms the importance of the primary cilium in mediating epicardial EMT in the adult mice post-MI, and suggests the primary cilium as a target for supporting post-MI repair (Blom et al., unpublished observations).

8.4. Cells and tissue engineering

As discussed above, transplantation of progenitor cells to the myocardium has shown benefit in pre-clinical models, but has met challenges in clinical application. In an early study by Winter et al. (2007), transplantation of human adult EPDCs into infarcted mouse hearts preserved ventricular function at 2 weeks, and up to 6 weeks-post MI. Engrafted EPDCs expressed α-smooth muscle actin and von Willebrand factor, but not markers of cardiomyocytes. Also, engrafted EPDCs did not necessarily integrate into vessel walls, and few were detected at the long-term time point of 6 weeks; thus, authors suggested that the contribution of the EPDCs to vasculogenesis was mostly mediated via a paracrine mechanism. In a subsequent study, co-transplantation of human EPDCs and cardiomyocyte progenitor cells further improved cardiac function beyond the transplantation of one cell type alone (Winter et al., 2009). These authors speculated that this effect was the result of communication between co-transplanted cells causing complementary secretion of paracrine factors.

Interestingly, others have demonstrated that the transplantation of progenitor cells to the ischemic myocardium provides superior reparative effects when placed epicardially as compared to injection intramyocardially. The epicardial application of a cardiogenic scaffold harboring human cardiac-derived cardiomyocyte progenitor cells in a porous 3D printed hyaluronic acid/gelatin based biomaterial supports cell engraftment and vascular differentiation, as well as long-term survival in a mouse model of MI (Gaetani et al., 2015). Engrafted cells expressed troponin I and CD31 at 1 month post-engraftment. Tano et al. (2014) also demonstrated that epicardial placement of mesenchymal stromal cell-sheets produces enhanced production of the paracrine factors *IGF-1*, *MMP-2* and *HIF-1*α, molecules expressed during epicardial EMT. Mesenchymal sheet cells expressed PECAM1 on day 3 post-placement, however these cells did not appear to migrate into the heart. Furthermore, these cells did not differentiate into cardiac cells expressing troponin T, implicating the paracrine mediators in the observed improvement in cardiac function. This type of cell transplantation strategy has been employed in a phase I clinical trial to treat cardiomyopathy patients using autologous cells. Although not all patients in this trial experienced MI as their primary injury, the cell-sheet implants appear to possess a promising safety profile and support some functional recovery (Miyagawa et al., 2017). Larger studies are warranted to further explore both the use of cell-sheet therapy for cardiac repair in the clinical setting, particularly post-acute MI, and the mechanism by which cell-sheets produce their effects.

Although the implementation of cells and genetic material has been controversial in large animal models and in the clinical setting, recent development in biomaterials such as patches and hydrogels offers promise for continued development of the field. For example, while Tβ4 has demonstrated conflicting long-term results in larger animals and through one time administration via IP injection or intramyocardial delivery, the use of biomaterials seems to remarkably improve its effectiveness. Using ultrasound-targeted microbubble delivery of human Tβ4 under a transposon plasmid system, Chen, Shimoda, Chen, and Grayburn (2013) demonstrated significant Tβ4 overexpression in rat hearts for three months. Using this delivery method, $Wt1^+$ progenitor cells increased in number, and began to express cardiac troponin T in the myocardium. Microbubble Tβ4 delivery also supported prolonged angiogenesis and arteriogenesis, measured by alpha-smooth muscle actin, VEGF, Tie-2 and PECAM mRNA, as well as coronary artery and capillary density. Finally, Tβ4 induced proliferation of cardiomyocytes in the long-term (3 months), indicating self-renewing potential. Similarly, Chiu, Reis, Momen, and Radisic (2012) enhanced Tβ4 delivery using a biomaterial approach by controlled release from collagen-chitosan hydrogels. Hydrogel type materials had been previously demonstrated to impart structural reinforcement, but were unable to prevent deleterious remodeling post-MI (Rane et al., 2011). Controlled release of Tβ4 with a collagen-chitosan hydrogel improved vascularization and reduced tissue loss (Chiu et al., 2012). Similarly, Boopathy et al. (2015) used hydrogels to administer the *Notch* ligands, which resulted in improved cardiac function and angiogenesis post-MI. Hydrogel mediated-*Notch* ligand administration significantly augmented the number of Ki67-positive, lectin-positive endothelial cells. Another group demonstrated that an epicardially placed hydrogel containing bFGF reduced infarct size and increased microvascularity in a rabbit model of MI (Fujita et al., 2005).

Furthermore, epicardial based biomaterial patches have gained recognition for their supportive capabilities post-MI. In addition to the study on Fstl1 mentioned above (Wei et al., 2015), others have demonstrated the utility of epicardial patching to aid in the delivery of substances to support epicardial mediated repair. Beyond the direct mechanical benefit of patching the epicardium, which provides structural support and restricts ventricular wall dilation (Serpooshan et al., 2013), patches containing cells or cytokines and growth factors are able to further enhance epicardial mediated repair. For example, bFGF-enriched extracellular matrix (ECM) biomaterial patches in a rat MI model improved long term ejection fraction beyond the ECM patch alone (Mewhort, Turnbull, Meijndert, Ngu, & Fedak, 2014).

A recent study in rats demonstrated the ability of a mesenchymal stem cell-loaded cardiac patch to secrete HIF-1α, Tβ4, VEGF and SDF-1 to support activation of $Wt1^+$ EPDCs, which migrated deep into the myocardium and differentiated into smooth muscle cells, and potentially a small number of cardiomyocytes (Wang et al., 2017). Unfortunately, as has been argued previously, the sparse availability of autologous cells for transplantation may limit the feasibility of this type of therapeutic approach. Other cell types for cardiac patches can be primed for directed differentiation such that they support repair. For example, Zhao et al. (2017) demonstrated that stimulation of the Wnt and RA pathways can direct cardiac progenitor cells to an epicardial cell fate, and these cells are supportive of smooth muscle cell

differentiation rather than a fibrotic response. In this study, human pluripotent stem cells (hPSCs) were directed to differentiate into $Tbx18^{+}/Wt1^{+}$ cobblestone shaped epicardial like cells by RA and Wnt respectively; these cells were then induced to transform to a mesenchymal phenotype using bFGF and TGF-β1 administration. While the use of primed cells in cardiac patches is gaining popularity, the ability to direct hPSCs into desired cell types is becoming increasingly feasible. Recently, Witty et al. (2014) and Iyer et al. (2015) were also able to differentiate hPSCs into epicardial-like cells via stimulation with BMP, Wnt and RA pathways. Combining this type of approach with hydrogels or patches could provide a sufficient source of accessible cells which would possess the characteristics of EPDCs to support cardiac repair.

8.5. Small molecules

It has become increasingly apparent that small molecular compounds may be efficacious in aiding with treatment modalities for various types of disease. Recent studies have explored the use of small molecules to enhance epicardial differentiation and EMT in rodent models of MI. Sasaki, Hwang, Nguyen, Kloner, and Kahn (2013) showed that a small molecule Wnt signaling modulator ICG-001 decreases β-catenin/CREB binding protein (CBP) interaction, while concomitantly increasing the β-catenin/p300 interaction. They further demonstrated that ICG-001 promotes epicardial EMT and improves cardiac function in rats with MI. In the study by Zhao et al. (2017) the Wnt pathway was stimulated with the use of a small molecule GSK-3 inhibitor, CHIR99021, to induce Wnt signaling and epicardial differentiation from hESCs. Similarly, Bao et al. (2016) recently demonstrated 95% $Wt1^{+}$ epicardial cell differentiation from hPSCs using three GSK-3 small molecule inhibitors: CHIR99021, CHIR98014 and BIO-acetoxime. This group additionally demonstrated efficacy of using TGF-β receptor inhibitors A83-01, RepSox, SB505124, and SB431542 in expanding the epicardial cell population. Importantly, these epicardial cells can be seeded on cardiac-fibroblast-derived extracellular matrix patches. In a mouse-MI model, application of these epicardial seeded patches onto the heart surface resulted in significant EMT and smooth-muscle-like and fibroblast-like cell differentiation within the mid-myocardium (Bao et al., 2016). However, longer-term efficacy of this patch on myocardial function post-MI remains to be determined. Additionally, primary human EPDCs have been used to screen 7400 structurally diverse compounds that are known to modulate a broad range of biological targets (Paunovic et al., 2017). The phenotypic screens have identified small molecules that promote human EPDC proliferation. It is expected that some of these small molecules can be further modified to optimize their metabolic and pharmacokinetic properties for *in vivo* studies.

9. Conclusions

Over the last two decades, the understanding of the contribution of the epicardium and its role in cardiac development and repair has increased dramatically. Seminal work using zebrafish demonstrated the importance of EPDCs in the regenerating myocardium. More recently, molecular tools and fate-mapping have demonstrated the contribution of EPDCs to the developing mammalian heart. This led to the discovery of EPDC activation in adult mammals upon cardiac injury. Efficacies of augmenting epicardial EMT post-MI have been demonstrated using growth factors and cytokine mediators, as well as innovative biomaterials in animal models (Fig. 3). Similarly, the novel understanding of different levels of gene regulation has revealed the involvement of ncRNA in this process. miRNAs and lncRNAs play a pivotal role in cardiogenesis and understanding their roles in adult epicardial EMT may provide additional targets for therapeutic intervention. Likewise, the primary cilium, which regulates signaling by EMT mediators, may serve as a promising target to augment epicardial responses post-MI. Although these promising advances have been made in the search for therapeutics to support cardiac repair, a suitable treatment has yet to be successful in large clinical trials. Continued investigation into the power of the epicardium to both mediate angiogenesis and support trophic factors for cardiac repair and/or regeneration may provide the key to unveiling a cure for heart failure.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Abba, M. L., Patil, N., Leupold, J. H., & Allgayer, H. (2016). MicroRNA regulation of epithelial to mesenchymal transition. *Journal of Clinical Medicine 5*, 8.

Andrae, J., Gallini, R., & Betsholtz, C. (2008). Role of platelet-derived growth factors in physiology and medicine. *Genes & Development 22*, 1276–1312.

Aurora, A. B., Porrello, E. R., Tan, W., Mahmoud, A. I., Hill, J. A., Bassel-Duby, R., ... Olson, E. N. (2014). Macrophages are required for neonatal heart regeneration. *The Journal of Clinical Investigation 124*, 1382–1392.

Banai, S., Jaklitsch, M. T., Shou, M., Lazarous, D. F., Scheinowitz, M., Biro, S., ... Unger, E. F. (1994). Angiogenic-induced enhancement of collateral blood flow to ischemic myocardium by vascular endothelial growth factor in dogs. *Circulation 89*, 2183–2189.

Banerjee, I., Zhang, J., Moore-Morris, T., Lange, S., Shen, T., Dalton, N. D., ... Chen, J. (2012). Thymosin beta 4 is dispensable for murine cardiac development and function. *Circulation Research 110*, 456–464.

Bao, X., Lian, X., Hacker, T. A., Schmuck, E. G., Qian, T., Bhute, V. J., ... Palecek, S. P. (2016). Long-term self-renewing human epicardial cells generated from pluripotent stem cells under defined xeno-free conditions. *Nature Biomedical Engineering 1*, 0003.

Bartram, U., Molin, D. G., Wisse, L. J., Mohamad, A., Sanford, L. P., Doetschman, T., ... Gittenberger-de Groot, A. C. (2001). Double-outlet right ventricle and overriding tricuspid valve reflect disturbances of looping, myocardialization, endocardial cushion differentiation, and apoptosis in TGF-β2-knockout mice. *Circulation 103*, 2745–2752.

Blom, J. N., Lu, X., Arnold, P., & Feng, Q. (2016). Myocardial infarction in neonatal mice, a model of cardiac regeneration. *Journal of Visualized Experiments 111*, e54100.

Blom, J. N., Lu, X., Kim, M. Y., & Feng, Q. (2016). Primary cilia disassembly promotes mammalian cardiac regeneration. *The FASEB Journal 30*(Suppl. 1), 1207.7.

Boopathy, A. V., Martinez, M. D., Smith, A. W., Brown, M. E., Garcia, A. J., & Davis, M. E. (2015). Intramyocardial delivery of notch ligand-containing hydrogels improves cardiac function and angiogenesis following infarction. *Tissue Engineering. Part A 21*, 2315–2322.

Borovina, A., Superina, S., Voskas, D., & Ciruna, B. (2010). Vangl2 directs the posterior tilting and asymmetric localization of motile primary cilia. *Nature Cell Biology 12*, 407–412.

Bronnum, H., Andersen, D. C., Schneider, M., Sandberg, M. B., Eskildsen, T., Nielsen, S. B., ... Sheikh, S. P. (2013). miR-21 promotes fibrogenic epithelial-to-mesenchymal transition of epicardial mesothelial cells involving programmed cell death 4 and Sprouty-1. *PLoS One 8*, e56280.

Cai, C. L., Martin, J. C., Sun, Y., Cui, L., Wang, L., Ouyang, K., ... Evans, S. M. (2008). A myocardial lineage derives from Tbx18 epicardial cells. *Nature 454*, 104–108.

Cao, Y., Park, A., & Sun, Z. (2010). Intraflagellar transport proteins are essential for cilia formation and for planar cell polarity. *Journal of the American Society of Nephrology: JASN 21*, 1326–1333.

Caspary, T., Larkins, C. E., & Anderson, K. V. (2007). The graded response to sonic hedgehog depends on cilia architecture. *Developmental Cell 12*, 767–778.

Cervantes, S., Lau, J., Cano, D. A., Borromeo-Austin, C., & Hebrok, M. (2010). Primary cilia regulate Gli/hedgehog activation in pancreas. *Proceedings of the National Academy of Sciences of the United States of America 107*, 10109–10114.

Chen, T., Chang, T. C., Kang, J. O., Choudhary, B., Makita, T., Tran, C. M., ... Sucov, H. M. (2002). Epicardial induction of fetal cardiomyocyte proliferation via a retinoic acid-inducible trophic factor. *Developmental Biology 250*, 198–207.

Chen, S., Shimoda, M., Chen, J., & Grayburn, P. A. (2013). Stimulation of adult resident cardiac progenitor cells by durable myocardial expression of thymosin beta 4 with ultrasound-targeted microbubble delivery. *Gene Therapy 20*, 225–233.

Chiu, L. L., Reis, L. A., Momen, A., & Radisic, M. (2012). Controlled release of thymosin β4 from injected collagen-chitosan hydrogels promotes angiogenesis and prevents tissue loss after myocardial infarction. *Regenerative Medicine 7*, 523–533.

Christensen, S. T., Morthorst, S. K., Mogensen, J. B., & Pedersen, L. B. (2017). Primary cilia and coordination of receptor tyrosine kinase (RTK) and transforming growth factor β (TGF-β) signaling. *Cold Spring Harbor Perspectives in Biology 9*, a02816.

Christia, P., & Frangogiannis, N. G. (2013). Targeting inflammatory pathways in myocardial infarction. *European Journal of Clinical Investigation 43*, 986–995.

Clement, C. A., Ajbro, K. D., Koefoed, K., Vestergaard, M. L., Veland, I. R., Henriques de Jesus, M. P., ... Christensen, S. T. (2013). TGF-β signaling is associated with endocytosis at the pocket region of the primary cilium. *Cell Reports 3*, 1806–1814.

Clement, C. A., Kristensen, S. G., Mollgard, K., Pazour, G. J., Yoder, B. K., Larsen, L. A., & Christensen, S. T. (2009). The primary cilium coordinates early cardiogenesis and hedgehog signaling in cardiomyocyte differentiation. *Journal of Cell Science 122*, 3070–3082.

Cong, N., Du, P., Zhang, A., Shen, F., Su, J., Pu, P., ... Zhang, Q. (2013). Downregulated microRNA-200a promotes EMT and tumor growth through the Wnt/β-catenin pathway by targeting the E-cadherin repressors ZEB1/ZEB2 in gastric adenocarcinoma. *Oncology Reports 29*, 1579–1587.

Corbit, K. C., Shyer, A. E., Dowdle, W. E., Gaulden, J., Singla, V., Chen, M. H., ... Reiter, J. F. (2008). Kif3a constrains β-catenin-dependent Wnt signalling through dual ciliary and non-ciliary mechanisms. *Nature Cell Biology 10*, 70–76.

Davenport, J. R., & Yoder, B. K. (2005). An incredible decade for the primary cilium: A look at a once-forgotten organelle. *American Journal of Physiology. Renal Physiology 289*, F1159–1169.

David, J. M., & Rajasekaran, A. K. (2012). Dishonorable discharge: The oncogenic roles of cleaved E-cadherin fragments. *Cancer Research 72*, 2917–2923.

de Vreede, J. J., Gorgels, A. P., Verstraaten, G. M., Vermeer, F., Dassen, W. R., & Wellens, H. J. (1991). Did prognosis after acute myocardial infarction change during the past 30 years? A meta-analysis. *Journal of the American College of Cardiology 18*, 698–706.

Delewi, R., Hirsch, A., Tijssen, J. G., Schachinger, V., Wojakowski, W., Roncalli, J., ... Piek, J. J. (2014). Impact of intracoronary bone marrow cell therapy on left ventricular function in the setting of ST-segment elevation myocardial infarction: A collaborative meta-analysis. *European Heart Journal 35*, 989–998.

Djebali, S., Davis, C. A., Merkel, A., Dobin, A., Lassmann, T., Mortazavi, A., ... Gingeras, T. R. (2012). Landscape of transcription in human cells. *Nature 489*, 101–108.

Duan, J., Gherghe, C., Liu, D., Hamlett, E., Srikantha, L., Rodgers, L., ... Deb, A. (2012). Wnt1/β-catenin injury response activates the epicardium and cardiac fibroblasts to promote cardiac repair. *The EMBO Journal 31*, 429–442.

Duim, S. N., Kurakula, K., Goumans, M. J., & Kruithof, B. P. (2015). Cardiac endothelial cells express Wilms' tumor-1: Wt1 expression in the developing, adult and infarcted heart. *Journal of Molecular and Cellular Cardiology 81*, 127–135.

Egorova, A. D., Khedoe, P. P., Goumans, M. J., Yoder, B. K., Nauli, S. M., ten Dijke, P., ... Hierck, B. P. (2011). Lack of primary cilia primes shear-induced endothelial-to-mesenchymal transition. *Circulation Research 108*, 1093–1101.

Encode Project Consortium (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature 489*, 57–74.

Ezratty, E. J., Stokes, N., Chai, S., Shah, A. S., Williams, S. E., & Fuchs, E. (2011). A role for the primary cilium in notch signaling and epidermal differentiation during skin development. *Cell 145*, 1129–1141.

Fang, M., Xiang, F. L., Braitsch, C. M., & Yutzey, K. E. (2016). Epicardium-derived fibroblasts in heart development and disease. *Journal of Molecular and Cellular Cardiology 91*, 23–27.

Foglio, E., Puddighinu, G., Fasanaro, P., D'Arcangelo, D., Perrone, G. A., Mocini, D., ... Limana, F. (2015). Exosomal clusterin, identified in the pericardial fluid, improves myocardial performance following MI through epicardial activation, enhanced arteriogenesis and reduced apoptosis. *International Journal of Cardiology 197*, 333–347.

Forsythe, J. A., Jiang, B. H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D., & Semenza, G. L. (1996). Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Molecular and Cellular Biology 16*, 4604–4613.

Frangogiannis, N. G. (2014). The immune system and the remodeling infarcted heart: Cell biological insights and therapeutic opportunities. *Journal of Cardiovascular Pharmacology 63*, 185–195.

Fujita, M., Ishihara, M., Morimoto, Y., Simizu, M., Saito, Y., Yura, H., ... Maehara, T. (2005). Efficacy of photocrosslinkable chitosan hydrogel containing fibroblast growth factor-2 in a rabbit model of chronic myocardial infarction. *The Journal of Surgical Research 126*, 27–33.

Gaetani, R., Feyen, D. A., Verhage, V., Slaats, R., Messina, E., Christman, K. L., ... Sluijter, J. P. (2015). Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction. *Biomaterials 61*, 339–348.

Gerber, Y., Weston, S. A., Enriquez-Sarano, M., Berardi, C., Chamberlain, A. M., Manemann, S. M., ... Roger, V. L. (2016). Mortality associated with heart failure after myocardial infarction: A contemporary community perspective. *Circulation. Heart Failure 9*, e002460.

Germani, A., Foglio, E., Capogrossi, M. C., Russo, M. A., & Limana, F. (2015). Generation of cardiac progenitor cells through epicardial to mesenchymal transition. *Journal of Molecular Medicine (Berlin, Germany) 93*, 735–748.

Gherghiceanu, M., & Popescu, L. M. (2012). Cardiac telocytes - Their junctions and functional implications. *Cell and Tissue Research 348*, 265–279.

Gidh-Jain, M., Huang, B., Jain, P., Gick, G., & El-Sherif, N. (1998). Alterations in cardiac gene expression during ventricular remodeling following experimental myocardial infarction. *Journal of Molecular and Cellular Cardiology 30*, 627–637.

Gong, L., Xu, H., Chang, H., Tong, Y., Zhang, T., & Guo, G. (2018). Knockdown of long non-coding RNA MEG3 protects H9c2 cells from hypoxia-induced injury by targeting microRNA-183. *Journal of Cellular Biochemistry 119*, 1429–1440.

Gonzalez-Rosa, J. M., Peralta, M., & Mercader, N. (2012). Pan-epicardial lineage tracing reveals that epicardium derived cells give rise to myofibroblasts and perivascular cells during zebrafish heart regeneration. *Developmental Biology 370*, 173–186.

Grieskamp, T., Rudat, C., Ludtke, T. H., Norden, J., & Kispert, A. (2011). Notch signaling regulates smooth muscle differentiation of epicardium-derived cells. *Circulation Research 108*, 813–823.

Guadix, J. A., Ruiz-Villalba, A., Lettice, L., Velecela, V., Munoz-Chapuli, R., Hastie, N. D., ... Martinez-Estrada, O. M. (2011). Wt1 controls retinoic acid signalling in embryonic epicardium through transcriptional activation of Raldh2. *Development 138*, 1093–1097.

Guo, Y., Luo, F., Liu, Q., & Xu, D. (2017). Regulatory non-coding RNAs in acute myocardial infarction. *Journal of Cellular and Molecular Medicine 21*, 1013–1023.

Gutschner, T., Hammerle, M., & Diederichs, S. (2013). MALAT1 — A paradigm for long noncoding RNA function in cancer. *Journal of Molecular Medicine (Berlin, Germany) 91*, 791–801.

Hartsock, A., & Nelson, W. J. (2008). Adherens and tight junctions: Structure, function and connections to the actin cytoskeleton. *Biochimica et Biophysica Acta 1778*, 660–669.

Hassounah, N. B., Bunch, T. A., & McDermott, K. M. (2012). Molecular pathways: The role of primary cilia in cancer progression and therapeutics with a focus on hedgehog signaling. *Clinical Cancer Research 18*, 2429–2435.

Haubner, B. J., Adamowicz-Brice, M., Khadayate, S., Tiefenthaler, V., Metzler, B., Aitman, T., & Penninger, J. M. (2012). Complete cardiac regeneration in a mouse model of myocardial infarction. *Aging (Albany NY) 4*, 966–977.

Hayashi, M., Shimizu, W., & Albert, C. M. (2015). The spectrum of epidemiology underlying sudden cardiac death. *Circulation Research 116*, 1887–1906.

Haycraft, C. J., Banizs, B., Aydin-Son, Y., Zhang, Q., Michaud, E. J., & Yoder, B. K. (2005). Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS Genetics 1*, e53.

Heery, R., Finn, S. P., Cuffe, S., & Gray, S. G. (2017). Long non-coding RNAs: Key regulators of epithelial-mesenchymal transition, tumour drug resistance and cancer stem cells. *Cancer 9*, 38.

Hesse, J., Leberling, S., Boden, E., Friebe, D., Schmidt, T., Ding, Z., ... Schrader, J. (2017). CD73-derived adenosine and tenascin-C control cytokine production by epicardium-derived cells formed after myocardial infarction. *FASEB Journal 31*, 3040–3053.

Huangfu, D., Liu, A., Rakeman, A. S., Murcia, N. S., Niswander, L., & Anderson, K. V. (2003). Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature 426*, 83–87.

Ibrahim, A. A., Schmithals, C., Kowarz, E., Koberle, V., Kakoschky, B., Pleli, T., ... Piiper, A. (2017). Hypoxia causes downregulation of dicer in hepatocellular carcinoma, which is required for upregulation of hypoxia-inducible factor 1α and epithelial-mesenchymal transition. *Clinical Cancer Research*, 1–10.

Iyer, D., Gambardella, L., Bernard, W. G., Serrano, F., Mascetti, V. L., Pedersen, R. A., ... Sinha, S. (2015). Robust derivation of epicardium and its differentiated smooth muscle cell progeny from human pluripotent stem cells. *Development 142*, 1528–1541.

Jacoby, M., Cox, J. J., Gayral, S., Hampshire, D. J., Ayub, M., Blockmans, M., ... Schurmans, S. (2009). INPP5E mutations cause primary cilium signaling defects, ciliary instability and ciliopathies in human and mouse. *Nature Genetics 41*, 1027–1031.

Jechlinger, M., Sommer, A., Moriggl, R., Seither, P., Kraut, N., Capodiecci, P., ... Grunert, S. (2006). Autocrine PDGFR signaling promotes mammary cancer metastasis. *The Journal of Clinical Investigation 116*, 1561–1570.

Jeong, H., Yim, H. W., Cho, Y., Park, H. J., Jeong, S., Kim, H. B., ... Kim, H. (2013). The effect of rigorous study design in the research of autologous bone marrow-derived mononuclear cell transfer in patients with acute myocardial infarction. *Stem Cell Research & Therapy 4*, 82.

Ji, P., Diederichs, S., Wang, W., Boing, S., Metzger, R., Schneider, P. M., ... Muller-Tidow, C. (2003). MALAT-1, a novel noncoding RNA, and thymosin β4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene 22*, 8031–8041.

Jing, X., Gao, Y., Xiao, S., Qin, Q., Wei, X., Yan, Y., ... She, Q. (2016). Hypoxia induced the differentiation of Tbx18-positive epicardial cells to CoSMCs. *Scientific Reports 6*(30468).

Jones, C., Roper, V. C., Foucher, I., Qian, D., Banizs, B., Petit, C., ... Chen, P. (2008). Ciliary proteins link basal body polarization to planar cell polarity regulation. *Nature Genetics 40*, 69–77.

Jopling, C., Sleep, E., Raya, M., Marti, M., Raya, A., & Izpisua Belmonte, J. C. (2010). Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature 464*, 606–609.

Kikuchi, K., Gupta, V., Wang, J., Holdway, J. E., Wills, A. A., Fang, Y., & Poss, K. D. (2011). Tcf21^{+} epicardial cells adopt non-myocardial fates during zebrafish heart development and regeneration. *Development 138*, 2895–2902.

Kim, J., Wu, Q., Zhang, Y., Wiens, K. M., Huang, Y., Rubin, N., ... Lien, C. L. (2010). PDGF signaling is required for epicardial function and blood vessel formation in regenerating zebrafish hearts. *Proceedings of the National Academy of Sciences of the United States of America 107*, 17206–17210.

Koefoed, K., Veland, I. R., Pedersen, L. B., Larsen, L. A., & Christensen, S. T. (2014). Cilia and coordination of signaling networks during heart development. *Organogenesis 10*, 108–125.

Kong, D., Li, Y., Wang, Z., Banerjee, S., Ahmad, A., Kim, H. R., & Sarkar, F. H. (2009). miR-200 regulates PDGF-D-mediated epithelial-mesenchymal transition, adhesion, and invasion of prostate cancer cells. *Stem Cells 27*, 1712–1721.

Korpal, M., & Kang, Y. (2008). The emerging role of miR-200 family of microRNAs in epithelial-mesenchymal transition and cancer metastasis. *RNA Biology 5*, 115–119.

Korpal, M., Lee, E. S., Hu, G., & Kang, Y. (2008). The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *The Journal of Biological Chemistry 283*, 14910–14914.

Koudstaal, S., Bastings, M. M., Feyen, D. A., Waring, C. D., van Slochteren, F. J., Dankers, P. Y. , ... Chamuleau, S. A. (2014). Sustained delivery of insulin-like growth factor-1/hepatocyte growth factor stimulates endogenous cardiac repair in the chronic infarcted pig heart. *Journal of Cardiovascular Translational Research 7*, 232–241.

Kozminski, K. G., Johnson, K. A., Forscher, P., & Rosenbaum, J. L. (1993). A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proceedings of the National Academy of Sciences of the United States of America 90*, 5519–5523.

Kreidberg, J. A., Sariola, H., Loring, J. M., Maeda, M., Pelletier, J., Housman, D., & Jaenisch, R. (1993). WT-1 is required for early kidney development. *Cell 74*, 679–691.

Kumarswamy, R., Mudduluru, G., Ceppi, P., Muppala, S., Kozlowski, M., Niklinski, J., ... Allgayer, H. (2012). MicroRNA-30a inhibits epithelial-to-mesenchymal transition by targeting Snai1 and is downregulated in non-small cell lung cancer. *International Journal of Cancer 130*, 2044–2053.

Lamouille, S., Subramanyam, D., Blelloch, R., & Derynck, R. (2013). Regulation of epithelial-mesenchymal and mesenchymal-epithelial transitions by microRNAs. *Current Opinion in Cell Biology 25*, 200–207.

Lamouille, S., Xu, J., & Derynck, R. (2014). Molecular mechanisms of epithelial-mesenchymal transition. *Nature Reviews. Molecular Cell Biology 15*, 178–196.

Lancaster, M. A., Schroth, J., & Gleeson, J. G. (2011). Subcellular spatial regulation of canonical Wnt signalling at the primary cilium. *Nature Cell Biology 13*, 700–707.

Lechtreck, K. F. (2015). IFT-cargo interactions and protein transport in cilia. *Trends in Biochemical Sciences 40*, 765–778.

Leitch, C. C., Lodh, S., Prieto-Echague, V., Badano, J. L., & Zaghloul, N. A. (2014). Basal body proteins regulate notch signaling through endosomal trafficking. *Journal of Cell Science 127*, 2407–2419.

Lepilina, A., Coon, A. N., Kikuchi, K., Holdway, J. E., Roberts, R. W., Burns, C. G., & Poss, K. D. (2006). A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell 127*, 607–619.

Letterio, J. J., Geiser, A. G., Kulkarni, A. B., Roche, N. S., Sporn, M. B., & Roberts, A. B. (1994). Maternal rescue of transforming growth factor-beta 1 null mice. *Science 264*, 1936–1938.

Li, Y., Klena, N. T., Gabriel, G. C., Liu, X., Kim, A. J., Lemke, K., ... Lo, C. W. (2015). Global genetic analysis in mice unveils central role for cilia in congenital heart disease. *Nature 521*, 520–524.

Limana, F., Bertolami, C., Mangoni, A., Di Carlo, A., Avitabile, D., Mocini, D., ... Capogrossi, M. C. (2010). Myocardial infarction induces embryonic reprogramming of epicardial c-kit^{+} cells: Role of the pericardial fluid. *Journal of Molecular and Cellular Cardiology 48*, 609–618.

Lin, F., Hiesberger, T., Cordes, K., Sinclair, A. M., Goldstein, L. S., Somlo, S., & Igarashi, P. (2003). Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. *Proceedings of the National Academy of Sciences of the United States of America 100*, 5286–5291.

Liu, B., Duan, C. Y., Luo, C. F., Ou, C. W., Sun, K., Wu, Z. Y., ... Chen, M. S. (2014). Effectiveness and safety of selected bone marrow stem cells on left ventricular function in patients with acute myocardial infarction: A meta-analysis of randomized controlled trials. *International Journal of Cardiology 177*, 764–770.

Liu, Q., Huang, X., Oh, J. H., Lin, R. Z., Duan, S., Yu, Y., ... Zhou, B. (2014). Epicardium-to-fat transition in injured heart. *Cell Research 24*, 1367–1369.

Ma, R., Li, W. P., Rundle, D., Kong, J., Akbarali, H. I., & Tsiokas, L. (2005). PKD2 functions as an epidermal growth factor-activated plasma membrane channel. *Molecular and Cellular Biology 25*, 8285–8298.

Ma, L., Lu, M. F., Schwartz, R. J., & Martin, J. F. (2005). Bmp2 is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. *Development 132*, 5601–5611.

Ma, L., Young, J., Prabhala, H., Pan, E., Mestdagh, P., Muth, D., ... Weinberg, R. A. (2010). miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. *Nature Cell Biology 12*, 247–256.

Manner, J. (1999). Does the subepicardial mesenchyme contribute myocardioblasts to the myocardium of the chick embryo heart? A quail-chick chimera study tracing the fate of the epicardial primordium. *The Anatomical Record 255*, 212–226.

Mannherz, H. G., & Hannappel, E. (2009). The beta-thymosins: Intracellular and extracellular activities of a versatile actin binding protein family. *Cell Motility and the Cytoskeleton 66*, 839–851.

Martinez-Estrada, O. M., Lettice, L. A., Essafi, A., Guadix, J. A., Slight, J., Velecela, V., ... Hastie, N. D. (2010). Wt1 is required for cardiovascular progenitor cell formation through transcriptional control of snail and E-cadherin. *Nature Genetics 42*, 89–93.

May-Simera, H. L., Kai, M., Hernandez, V., Osborn, D. P., Tada, M., & Beales, P. L. (2010). Bbs8, together with the planar cell polarity protein Vangl2, is required to establish left-right asymmetry in zebrafish. *Developmental Biology 345*, 215–225.

Mellgren, A. M., Smith, C. L., Olsen, G. S., Eskiocak, B., Zhou, B., Kazi, M. N., ... Tallquist, M. D. (2008). Platelet-derived growth factor receptor beta signaling is required for efficient epicardial cell migration and development of two distinct coronary vascular smooth muscle cell populations. *Circulation Research 103*, 1393–1401.

Mendez, M. G., Kojima, S., & Goldman, R. D. (2010). Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition. *The FASEB Journal 24*, 1838–1851.

Merki, E., Zamora, M., Raya, A., Kawakami, Y., Wang, J., Zhang, X., ... Ruiz-Lozano, P. (2005). Epicardial retinoid X receptor α is required for myocardial growth and coronary artery formation. *Proceedings of the National Academy of Sciences of the United States of America 102*, 18455–18460.

Mewhort, H. E., Turnbull, J. D., Meijndert, H. C., Ngu, J. M., & Fedak, P. W. (2014). Epicardial infarct repair with basic fibroblast growth factor-enhanced CorMatrix-ECM biomaterial attenuates postischemic cardiac remodeling. *The Journal of Thoracic and Cardiovascular Surgery 147*, 1650–1659.

Michalik, K. M., You, X., Manavski, Y., Doddaballapur, A., Zornig, M., Braun, T., ... Dimmeler, S. (2014). Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. *Circulation Research 114*, 1389–1397.

Missinato, M. A., Tobita, K., Romano, N., Carroll, J. A., & Tsang, M. (2015). Extracellular component hyaluronic acid and its receptor Hmmr are required for epicardial EMT during heart regeneration. *Cardiovascular Research 107*, 487–498.

Mitsos, S., Katsanos, K., Koletsis, E., Kagadis, G. C., Anastasiou, N., Diamantopoulos, A., ... Dougenis, D. (2012). Therapeutic angiogenesis for myocardial ischemia revisited: Basic biological concepts and focus on latest clinical trials. *Angiogenesis 15*, 1–22.

Miyagawa, S., Domae, K., Yoshikawa, Y., Fukushima, S., Nakamura, T., Saito, A., ... Sawa, Y. (2017). Phase I clinical trial of autologous stem cell-sheet transplantation therapy for treating cardiomyopathy. *Journal of the American Heart Association 6*, e003918.

Mondal, T., Subhash, S., Vaid, R., Enroth, S., Uday, S., Reinius, B., ... Kanduri, C. (2015). MEG3 long noncoding RNA regulates the TGF-β pathway genes through formation of RNA-DNA triplex structures. *Nature Communications 6*, 7743.

Moore, A. W., McInnes, L., Kreidberg, J., Hastie, N. D., & Schedl, A. (1999). YAC complementation shows a requirement for Wt1 in the development of epicardium, adrenal gland and throughout nephrogenesis. *Development 126*, 1845–1857.

Moyer, J. H., Lee-Tischler, M. J., Kwon, H. Y., Schrick, J. J., Avner, E. D., Sweeney, W. E., ... Woychik, R. P. (1994). Candidate gene associated with a mutation causing recessive polycystic kidney disease in mice. *Science 264*, 1329–1333.

Murcia, N. S., Richards, W. G., Yoder, B. K., Mucenski, M. L., Dunlap, J. R., & Woychik, R. P. (2000). The oak ridge polycystic kidney (Orpk) disease gene is required for left-right axis determination. *Development 127*, 2347–2355.

Murry, C. E., Soonpaa, M. H., Reinecke, H., Nakajima, H., Nakajima, H. O., Rubart, M., ... Field, L. J. (2004). Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature 428*, 664–668.

Neugebauer, J. M., Amack, J. D., Peterson, A. G., Bisgrove, B. W., & Yost, H. J. (2009). FGF signalling during embryo development regulates cilia length in diverse epithelia. *Nature 458*, 651–654.

Nielsen, B. S., Malinda, R. R., Schmid, F. M., Pedersen, S. F., Christensen, S. T., & Pedersen, L. B. (2015). PDGFRβ and oncogenic mutant PDGFRα D842V promote disassembly of primary cilia through a PLCγ- and AURKA-dependent mechanism. *Journal of Cell Science 128*, 3543–3549.

Oishi, I., Kawakami, Y., Raya, A., Callol-Massot, C., & Izpisua Belmonte, J. C. (2006). Regulation of primary cilia formation and left-right patterning in zebrafish by a noncanonical Wnt signaling mediator, duboraya. *Nature Genetics 38*, 1316–1322.

Olson, M. F., & Sahai, E. (2009). The actin cytoskeleton in cancer cell motility. *Clinical & Experimental Metastasis 26*, 273–287.

Paunovic, A. I., Drowley, L., Nordqvist, A., Ericson, E., Mouchet, E., Jonebring, A., ... Plowright, A. T. (2017). Phenotypic screen for cardiac regeneration identifies molecules with differential activity in human epicardium-derived cells versus cardiac fibroblasts. *ACS Chemical Biology 12*, 132–141.

Pazour, G. J., Dickert, B. L., Vucica, Y., Seeley, E. S., Rosenbaum, J. L., Witman, G. B., & Cole, D. G. (2000). Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *The Journal of Cell Biology 151*, 709–718.

Pennisi, D. J., & Mikawa, T. (2009). FGFR-1 is required by epicardium-derived cells for myocardial invasion and correct coronary vascular lineage differentiation. *Developmental Biology 328*, 148–159.

Piccoli, M. T., Gupta, S. K., Viereck, J., Foinquinos, A., Samolovac, S., Kramer, F. L., ... Thum, T. (2017). Inhibition of the cardiac fibroblast-enriched lncRNA Meg3 prevents cardiac fibrosis and diastolic dysfunction. *Circulation Research 121*, 575–583.

Popescu, L. M., Manole, C. G., Gherghiceanu, M., Ardelean, A., Nicolescu, M. I., Hinescu, M. E., & Kostin, S. (2010). Telocytes in human epicardium. *Journal of Cellular and Molecular Medicine 14*, 2085–2093.

Porrello, E. R., Mahmoud, A. I., Simpson, E., Hill, J. A., Richardson, J. A., Olson, E. N., & Sadek, H. A. (2011). Transient regenerative potential of the neonatal mouse heart. *Science 331*, 1078–1080.

Porrello, E. R., Mahmoud, A. I., Simpson, E., Johnson, B. A., Grinsfelder, D., Canseco, D., ... Sadek, H. A. (2013). Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. *Proceedings of the National Academy of Sciences of the United States of America 110*, 187–192.

Poss, K. D., Wilson, L. G., & Keating, M. T. (2002). Heart regeneration in zebrafish. *Science 298*, 2188–2190.

Ptaszek, L. M., Mansour, M., Ruskin, J. N., & Chien, K. R. (2012). Towards regenerative therapy for cardiac disease. *Lancet 379*, 933–942.

Public Health Agency of Canada (2009). Tracking heart and stroke in Canada 2009. *Chronic diseases in Canada*.

Ramjee, V., Li, D., Manderfield, L. J., Liu, F., Engleka, K. A., Aghajanian, H., ... Epstein, J. A. (2017). Epicardial YAP/TAZ orchestrate an immunosuppressive response following myocardial infarction. *The Journal of Clinical Investigation 127*, 899–911.

Rane, A. A., Chuang, J. S., Shah, A., Hu, D. P., Dalton, N. D., Gu, Y., ... Christman, K. L. (2011). Increased infarct wall thickness by a bio-inert material is insufficient to prevent negative left ventricular remodeling after myocardial infarction. *PLoS One 6*, e21571.

Rao, K. S., Aronshtam, A., McElory-Yaggy, K. L., Bakondi, B., VanBuren, P., Sobel, B. E., & Spees, J. L. (2015). Human epicardial cell-conditioned medium contains HGF/IgG complexes that phosphorylate RYK and protect against vascular injury. *Cardiovascular Research 107*, 277–286.

Reiss, K., Capasso, J. M., Huang, H. E., Meggs, L. G., Li, P., & Anversa, P. (1993). ANG II receptors, c-myc, and c-jun in myocytes after myocardial infarction and ventricular failure. *The American Journal of Physiology 264*, H760–769.

Ridley, A. J. (2011). Life at the leading edge. *Cell 145*, 1012–1022.

Rohatgi, R., Milenkovic, L., & Scott, M. P. (2007). Patched1 regulates hedgehog signaling at the primary cilium. *Science 317*, 372–376.

Roush, S., & Slack, F. J. (2008). The let-7 family of microRNAs. *Trends in Cell Biology 18*, 505–516.

Rozycki, M., Lodyga, M., Lam, J., Miranda, M. Z., Fatyol, K., Speight, P., & Kapus, A. (2014). The fate of the primary cilium during myofibroblast transition. *Molecular Biology of the Cell 25*, 643–657.

Rui, L., Yu, N., Hong, L., Feng, H., Chunyong, H., Jian, M., ... Shengshou, H. (2014). Extending the time window of mammalian heart regeneration by thymosin β4. *Journal of Cellular and Molecular Medicine 18*, 2417–2424.

Ruiz-Villalba, A., Simon, A. M., Pogontke, C., Castillo, M. I., Abizanda, G., Pelacho, B., ... Perez-Pomares, J. M. (2015). Interacting resident epicardium-derived fibroblasts

and recruited bone marrow cells form myocardial infarction scar. *Journal of the American College of Cardiology 65*, 2057–2066.
Russell, J. L., Goetsch, S. C., Gaiano, N. R., Hill, J. A., Olson, E. N., & Schneider, J. W. (2011). A dynamic notch injury response activates epicardium and contributes to fibrosis repair. *Circulation Research 108*, 51–59.
Sadoshima, J., Jahn, L., Takahashi, T., Kulik, T. J., & Izumo, S. (1992). Molecular characterization of the stretch-induced adaptation of cultured cardiac cells. An in vitro model of load-induced cardiac hypertrophy. *The Journal of Biological Chemistry 267*, 10551–10560.
Sanchez-Duffhues, G., de Vinuesa, A. G., Lindeman, J. H., Mulder-Stapel, A., DeRuiter, M. C., Van Munsteren, C., ... Ten Dijke, P. (2015). SLUG is expressed in endothelial cells lacking primary cilia to promote cellular calcification. *Arteriosclerosis, Thrombosis, and Vascular Biology 35*, 616–627.
Sasaki, T., Hwang, H., Nguyen, C., Kloner, R. A., & Kahn, M. (2013). The small molecule Wnt signaling modulator ICG-001 improves contractile function in chronically infarcted rat myocardium. *PLoS One 8*, e75010.
Schneider, L., Cammer, M., Lehman, J., Nielsen, S. K., Guerra, C. F., Veland, I. R., ... Christensen, S. T. (2010). Directional cell migration and chemotaxis in wound healing response to PDGF-AA are coordinated by the primary cilium in fibroblasts. *Cellular Physiology and Biochemistry 25*, 279–292.
Schneider, L., Clement, C. A., Teilmann, S. C., Pazour, G. J., Hoffmann, E. K., Satir, P., & Christensen, S. T. (2005). PDGFRαα signaling is regulated through the primary cilium in fibroblasts. *Current Biology 15*, 1861–1866.
Seeger, T., Xu, Q. F., Muhly-Reinholz, M., Fischer, A., Kremp, E. M., Zeiher, A. M., & Dimmeler, S. (2016). Inhibition of let-7 augments the recruitment of epicardial cells and improves cardiac function after myocardial infarction. *Journal of Molecular and Cellular Cardiology 94*, 145–152.
Seeley, E. S., & Nachury, M. V. (2009). Constructing and deconstructing roles for the primary cilium in tissue architecture and cancer. *Methods in Cell Biology 94*, 299–313.
Serpooshan, V., Zhao, M., Metzler, S. A., Wei, K., Shah, P. B., Wang, A., ... Ruiz-Lozano, P. (2013). The effect of bioengineered acellular collagen patch on cardiac remodeling and ventricular function post myocardial infarction. *Biomaterials 34*, 9048–9055.
Shyu, K. G., Wang, M. T., Wang, B. W., Chang, C. C., Leu, J. G., Kuan, P., & Chang, H. (2002). Intramyocardial injection of naked DNA encoding HIF-1α/VP16 hybrid to enhance angiogenesis in an acute myocardial infarction model in the rat. *Cardiovascular Research 54*, 576–583.
Singh, M. K., Lu, M. M., Massera, D., & Epstein, J. A. (2011). MicroRNA-processing enzyme dicer is required in epicardium for coronary vasculature development. *The Journal of Biological Chemistry 286*, 41036–41045.
Smart, N., Bollini, S., Dube, K. N., Vieira, J. M., Zhou, B., Davidson, S., ... Riley, P. R. (2011). De novo cardiomyocytes from within the activated adult heart after injury. *Nature 474*, 640–644.
Smart, N., Risebro, C. A., Melville, A. A., Moses, K., Schwartz, R. J., Chien, K. R., & Riley, P. R. (2007). Thymosin β4 induces adult epicardial progenitor mobilization and neovascularization. *Nature 445*, 177–182.
Smith, C. L., Baek, S. T., Sung, C. Y., & Tallquist, M. D. (2011). Epicardial-derived cell epithelial-to-mesenchymal transition and fate specification require PDGF receptor signaling. *Circulation Research 108*, e15–26.
Stark, C. K., Tarkia, M., Kentala, R., Malmberg, M., Vahasilta, T., Savo, M., ... Koskenvuo, J. (2016). Systemic dosing of thymosin beta 4 before and after ischemia does not attenuate global myocardial Iischemia-reperfusion injury in pigs. *Frontiers in Pharmacology 7*, 115.
Statistics Canada (2011). *The 10 leading causes of death, 2011. Health Fact Sheets.*
Su, A., He, S., Tian, B., Hu, W., & Zhang, Z. (2013). MicroRNA-221 mediates the effects of PDGF-BB on migration, proliferation, and the epithelial-mesenchymal transition in pancreatic cancer cells. *PLoS One 8*, e71309.
Su, J., Zhang, A., Shi, Z., Ma, F., Pu, P., Wang, T., ... Zhang, Q. (2012). MicroRNA-200a suppresses the Wnt/β-catenin signaling pathway by interacting with β-catenin. *International Journal of Oncology 40*, 1162–1170.
Sun, M., Xia, R., Jin, F., Xu, T., Liu, Z., De, W., & Liu, X. (2014). Downregulated long noncoding RNA MEG3 is associated with poor prognosis and promotes cell proliferation in gastric cancer. *Tumour Biology 35*, 1065–1073.
Talman, V., & Ruskoaho, H. (2016). Cardiac fibrosis in myocardial infarction-from repair and remodeling to regeneration. *Cell and Tissue Research 365*, 563–581.
Tano, N., Narita, T., Kaneko, M., Ikebe, C., Coppen, S. R., Campbell, N. G., ... Suzuki, K. (2014). Epicardial placement of mesenchymal stromal cell-sheets for the treatment of ischemic cardiomyopathy; in vivo proof-of-concept study. *Molecular Therapy 22*, 1864–1871.
Tao, J., Doughman, Y., Yang, K., Ramirez-Bergeron, D., & Watanabe, M. (2013). Epicardial HIF signaling regulates vascular precursor cell invasion into the myocardium. *Developmental Biology 376*, 136–149.
Tarride, J. E., Lim, M., DesMeules, M., Luo, W., Burke, N., O'Reilly, D., ... Goeree, R. (2009). A review of the cost of cardiovascular disease. *The Canadian Journal of Cardiology 25*, e195–202.
Taulman, P. D., Haycraft, C. J., Balkovetz, D. F., & Yoder, B. K. (2001). Polaris, a protein involved in left-right axis patterning, localizes to basal bodies and cilia. *Molecular Biology of the Cell 12*, 589–599.
Tripathi, V., Ellis, J. D., Shen, Z., Song, D. Y., Pan, Q., Watt, A. T., ... Prasanth, K. V. (2010). The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Molecular Cell 39*, 925–938.
Umberger, N. L., & Caspary, T. (2015). Ciliary transport regulates PDGF-AA/αα signaling via elevated mammalian target of rapamycin signaling and diminished PP2A activity. *Molecular Biology of the Cell 26*, 350–358.
Valenta, T., Hausmann, G., & Basler, K. (2012). The many faces and functions of β-catenin. *The EMBO Journal 31*, 2714–2736.
van den Akker, N. M., Lie-Venema, H., Maas, S., Eralp, I., DeRuiter, M. C., Poelmann, R. E., & Gittenberger-De Groot, A. C. (2005). Platelet-derived growth factors in the developing avian heart and maturating coronary vasculature. *Developmental Dynamics 233*, 1579–1588.
van den Borne, S. W., Diez, J., Blankesteijn, W. M., Verjans, J., Hofstra, L., & Narula, J. (2010). Myocardial remodeling after infarction: The role of myofibroblasts. *Nature Reviews. Cardiology 7*, 30–37.
Van der Heiden, K., Groenendijk, B. C., Hierck, B. P., Hogers, B., Koerten, H. K., Mommaas, A. M., ... Poelmann, R. E. (2006). Monocilia on chicken embryonic endocardium in low shear stress areas. *Developmental Dynamics 235*, 19–28.
van Tuyn, J., Atsma, D. E., Winter, E. M., van der Velde-van Dijke, I., Pijnappels, D. A., Bax, N. A., ... de Vries, A. A. (2007). Epicardial cells of human adults can undergo an epithelial-to-mesenchymal transition and obtain characteristics of smooth muscle cells in vitro. *Stem Cells 25*, 271–278.
van Wijk, B., Gunst, Q. D., Moorman, A. F., & van den Hoff, M. J. (2012). Cardiac regeneration from activated epicardium. *PLoS One 7*, e44692.
Vicente-Steijn, R., Scherptong, R. W., Kruithof, B. P., Duim, S. N., Goumans, M. J., Wisse, L. J., ... Jongbloed, M. R. (2015). Regional differences in WT-1 and Tcf21 expression during ventricular development: Implications for myocardial compaction. *PLoS One 10*, e0136025.
Vieira, J. M., Howard, S., Villa Del Campo, C., Bollini, S., Dube, K. N., Masters, M., ... Riley, P. R. (2017). BRG1-SWI/SNF-dependent regulation of the Wt1 transcriptional landscape mediates epicardial activity during heart development and disease. *Nature Communications 8*, 16034.
von Gise, A., & Pu, W. T. (2012). Endocardial and epicardial epithelial to mesenchymal transitions in heart development and disease. *Circulation Research 110*, 1628–1645.
Wang, J., Cao, J., Dickson, A. L., & Poss, K. D. (2015). Epicardial regeneration is guided by cardiac outflow tract and hedgehog signalling. *Nature 522*, 226–230.
Wang, J. Y., Goodman, S. G., Saltzman, I., Wong, G. C., Huynh, T., Dery, J. P., ... Canadian Registry of Acute Coronary Events, I (2015). Cardiovascular risk factors and in-hospital mortality in acute coronary syndromes: Insights from the Canadian global registry of acute coronary events. *The Canadian Journal of Cardiology 31*, 1455–1461.
Wang, Q. L., Wang, H. J., Li, Z. H., Wang, Y. L., Wu, X. P., & Tan, Y. Z. (2017). Mesenchymal stem cell-loaded cardiac patch promotes epicardial activation and repair of the infarcted myocardium. *Journal of Cellular and Molecular Medicine 21*, 1751–1766.
Ware, J. A., & Simons, M. (1997). Angiogenesis in ischemic heart disease. *Nature Medicine 3*, 158–164.
Wei, K., Serpooshan, V., Hurtado, C., Diez-Cunado, M., Zhao, M., Maruyama, S., ... Ruiz-Lozano, P. (2015). Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. *Nature 525*, 479–485.
Winter, E. M., Grauss, R. W., Hogers, B., van Tuyn, J., van der Geest, R., Lie-Venema, H., ... Gittenberger-de Groot, A. C. (2007). Preservation of left ventricular function and attenuation of remodeling after transplantation of human epicardium-derived cells into the infarcted mouse heart. *Circulation 116*, 917–927.
Winter, E. M., van Oorschot, A. A., Hogers, B., van der Graaf, L. M., Doevendans, P. A., Poelmann, R. E., ... Goumans, M. J. (2009). A new direction for cardiac regeneration therapy: Application of synergistically acting epicardium-derived cells and cardiomyocyte progenitor cells. *Circulation. Heart Failure 2*, 643–653.
Witty, A. D., Mihic, A., Tam, R. Y., Fisher, S. A., Mikryukov, A., Shoichet, M. S., ... Keller, G. (2014). Generation of the epicardial lineage from human pluripotent stem cells. *Nature Biotechnology 32*, 1026–1035.
World Health Organization (2015). *The top 10 causes of death. Fact Sheet 310, 937–952.*
Wu, S. P., Dong, X. R., Regan, J. N., Su, C., & Majesky, M. W. (2013). Tbx18 regulates development of the epicardium and coronary vessels. *Developmental Biology 383*, 307–320.
Xiang, J., Guo, S., Jiang, S., Xu, Y., Li, J., Li, L., & Xiang, J. (2016). Silencing of long non-coding RNA MALAT1 promotes apoptosis of glioma cells. *Journal of Korean Medical Science 31*, 688–694.
Xiang, F. L., Liu, Y., Lu, X., Jones, D. L., & Feng, Q. (2014). Cardiac-specific overexpression of human stem cell factor promotes epicardial activation and arteriogenesis after myocardial infarction. *Circulation. Heart Failure 7*, 831–842.
Xiang, F. L., Lu, X., Hammoud, L., Zhu, P., Chidiac, P., Robbins, J., & Feng, Q. (2009). Cardiomyocyte-specific overexpression of human stem cell factor improves cardiac function and survival after myocardial infarction in mice. *Circulation 120*, 1065–1074.
Xiang, Y., Zhang, Y., Tang, Y., & Li, Q. (2017). MALAT1 modulates TGF-beta1-induced endothelial-to-mesenchymal transition through downregulation of miR-145. *Cellular Physiology and Biochemistry 42*, 357–372.
Xin, M., Kim, Y., Sutherland, L. B., Murakami, M., Qi, X., McAnally, J., ... Olson, E. N. (2013). Hippo pathway effector Yap promotes cardiac regeneration. *Proceedings of the National Academy of Sciences of the United States of America 110*, 13839–13844.
Yanagisawa-Miwa, A., Uchida, Y., Nakamura, F., Tomaru, T., Kido, H., Kamijo, T., et al. (1992). Salvage of infarcted myocardium by angiogenic action of basic fibroblast growth factor. *Science 257*, 1401–1403.
Yang, X., Li, L., Huang, Q., Xu, W., Cai, X., Zhang, J., ... Xiao, J. (2015). Wnt signaling through Snail1 and Zeb1 regulates bone metastasis in lung cancer. *American Journal of Cancer Research 5*, 748–755.
Yang, S., Yao, H., Li, M., Li, H., & Wang, F. (2016). Long non-coding RNA MALAT1 mediates transforming growth factor β1-induced epithelial-mesenchymal transition of retinal pigment epithelial cells. *PLoS One 11*, e0152687.
Yeh, C., Li, A., Chuang, J. Z., Saito, M., Caceres, A., & Sung, C. H. (2013). IGF-1 activates a cilium-localized noncanonical Gbetagamma signaling pathway that regulates cell-cycle progression. *Developmental Cell 26*, 358–368.
Zakharova, L., Nural-Guvener, H., & Gaballa, M. A. (2012). Cardiac explant-derived cells are regulated by Notch-modulated mesenchymal transition. *PLoS One 7*, e37800.
Zamora, M., Manner, J., & Ruiz-Lozano, P. (2007). Epicardium-derived progenitor cells require beta-catenin for coronary artery formation. *Proceedings of the National Academy of Sciences of the United States of America 104*, 18109–18114.

Zangi, L., Lui, K. O., von Gise, A., Ma, Q., Ebina, W., Ptaszek, L. M., ... Chien, K. R. (2013). Modified mRNA directs the fate of heart progenitor cells and induces vascular regeneration after myocardial infarction. *Nature Biotechnology 31*, 898–907.

Zangi, L., Oliveira, M. S., Ye, L. Y., Ma, Q., Sultana, N., Hadas, Y., ... Chien, K. R. (2017). Insulin-like growth factor 1 receptor-dependent pathway drives epicardial adipose tissue formation after myocardial injury. *Circulation 135*, 59–72.

Zhang, H., & Bradley, A. (1996). Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development 122*, 2977–2986.

Zhang, W., Shi, S., Jiang, J., Li, X., Lu, H., & Ren, F. (2017). LncRNA MEG3 inhibits cell epithelial-mesenchymal transition by sponging miR-421 targeting E-cadherin in breast cancer. *Biomedicine & Pharmacotherapy 91*, 312–319.

Zhang, S. N., Sun, A. J., Ge, J. B., Yao, K., Huang, Z. Y., Wang, K. Q., & Zou, Y. Z. (2009). Intracoronary autologous bone marrow stem cells transfer for patients with acute myocardial infarction: A meta-analysis of randomised controlled trials. *International Journal of Cardiology 136*, 178–185.

Zhao, J., Cao, H., Tian, L., Huo, W., Zhai, K., Wang, P., ... Ma, Y. (2017). Efficient differentiation of TBX18^{+}/WT1^{+} epicardial-like cells from human pluripotent stem cells using small molecular compounds. *Stem Cells and Development 26*, 528–540.

Zhao, B., Chen, S., Liu, J., Yuan, Z., Qi, X., Qin, J., ... Cai, D. (2013). Cardiac telocytes were decreased during myocardial infarction and their therapeutic effects for ischaemic heart in rat. *Journal of Cellular and Molecular Medicine 17*, 123–133.

Zhou, B., Honor, L. B., He, H., Ma, Q., Oh, J. H., Butterfield, C., ... Pu, W. T. (2011). Adult mouse epicardium modulates myocardial injury by secreting paracrine factors. *The Journal of Clinical Investigation 121*, 1894–1904.

Zhou, B., Honor, L. B., Ma, Q., Oh, J. H., Lin, R. Z., Melero-Martin, J. M., ... Pu, W. T. (2012). Thymosin beta 4 treatment after myocardial infarction does not reprogram epicardial cells into cardiomyocytes. *Journal of Molecular and Cellular Cardiology 52*, 43–47.

Zhou, X., Liu, S., Cai, G., Kong, L., Zhang, T., Ren, Y., ... Wang, X. (2015). Long non coding RNA MALAT1 promotes tumor growth and metastasis by inducing epithelial-mesenchymal transition in oral squamous cell carcinoma. *Scientific Reports 5*(15972).