Cellular switching from an epithelial-to-mesenchymal phenotype, and conversely from a mesenchymal-to-epithelial phenotype, are important biological programs that are operative from conception to death in mammalian organisms. Indeed, the capacity of cells to switch between these states has been fundamental to the generation of complex body patterns throughout evolution. Phenotypic switching from an epithelial to mesenchymal cell, termed epithelial-to-mesenchymal transition (EMT), was a paradigm that evolved from numerous observations on early embryonic development, the foundations of which date back to the 1920s and the pioneering work of Johannes Holtfreter on embryo formation and differentiation.1,2 By the late 1960s, seminal chick embryo studies by Elizabeth Hay3 led to the first formal description that epithelial cells can undergo a dramatic phenotypic transformation and give rise to embryonic mesoderm.4 Subsequent studies have revealed that this process is reversible (mesenchymal-to-epithelial transition [MET]), and gradually the term “transition” has come to replace “transformation.”

Given that EMT/MET was initially identified and described by developmental biologists, it is perhaps not surprising that these processes are best understood during embryonic implantation and development. As explored in this review, it is now known that successive waves of cellular transition, from an epithelial to mesenchymal and then back to an epithelial state, are required for normal embryonic patterning and organ formation. In addition, numerous studies that span a broad spectrum of physiological and pathological conditions have expanded our knowledge of EMT/MET and now provide evidence for the important role played by these processes in various adult conditions including fibrosis, wound repair, inflammation, and malignancy. Indeed, our conceptual framework now also encompasses several variations and subcategories of cellular phenotypic switching, including endothelial-to-mesenchymal transition (EndMT).

In this review, epithelial, endothelial, and mesenchymal phenotypic cellular switching will be explored in the cardiovascular system, spanning cardiovascular development through to adult end organ disease. Key areas of recent scientific progress will be examined, including recent developmental and pathological insights, which potentially may lead to novel therapeutic opportunities.

EMT: A Key Role in Early Development

Within days of conception and during very early embryonic implantation, the process of EMT is already operative. Typically at the blastocyst stage, after initial adherence to the uterine lining (decidua), the outer trophoblast sends forward columns of epithelial cells to penetrate the uterine wall.5 At the leading edge of these embryonic cellular columns, epithelial trophoblast cells undergo EMT and invade the underlying maternal decidual interstitum and vessels. These invading embryonic cells ultimately go on to become mesenchymal placental giant cells, participating in the remodeling of the maternal uterine vasculature and securing a functional placental blood supply.5 This embryonic execution of the EMT program establishes cellular phenotypic switching as a key biological paradigm and, interestingly, sets an early precedent for the vascular involvement of this process.

Soon after these events, EMT plays a pivotal role in germ layer specification (ectoderm, mesoderm, endoderm). Epithelial cells from the primitive epiblast layer migrate to the midline and undergo EMT to give rise to mesoderm and endoderm.6 This process is highly ordered in time and space and generates pools of primitive stem/progenitor cells at precise anatomic locations within the embryo, which constitute the primordia of the developing organs. For example, lateral plate mesoderm gives rise to the heart and hematopoietic cells, the paraxial mesoderm to the musculo-skeletal system, and the intermediate mesoderm to the urogenital tract. These events, occurring in tissues that have not previously undergone cellular phenotypic switching, are termed primary EMT (Figure 1).

Having established these primitive mesodermal populations, successive waves of EMT/MET then typically occur before final
organ formation. In the case of the heart, this can involve recurrent waves of EMT/MET before the heart begins to assume a recognizable 4-chambered form (see section “Early Cardiac Formation”). For other organs, such as the kidney, successive waves of EMT/MET are required to ultimately give rise to epithelial structures such as nephrons and nephric ducts.7

**Genetic, Molecular, and Cellular Basis of EMT/MET**

At the core of EMT/MET, fundamental molecular and architectural rearrangements occur to bring about the dramatic cellular changes necessary to switch phenotypes. Underpinning this is a complex network of gene activation and repression programs that are required for the initiation, execution, and maintenance of EMT or MET (Figure 2).

At the cellular level, gross changes in polarity, morphology, functionality, and cell–cell interactions are requisite steps. Epithelial cells are arranged on a basement membrane, exhibiting apico-basal polarity and abundant expression of intercellular adhesion complexes such as E-Cadherin and integrins. In order to adopt a mesenchymal phenotype, these cells must lose cell adhesion by E-Cadherin downregulation and degradation.8,9 Other epithelial proteins such as zonula occludens, cytokeratin and desmoplakin must also be repressed.8–11 Transitioning cells then progressively lose polarity while eroding the basement membrane by matrix metalloproteinase production (matrix metalloproteinases 2, 3, and 9).12,13 Cytoskeletal changes mediated by Rho GTPases induce apical constriction and further structural rearrangements to permit passage through the degraded basement membrane, culminating in delamination from the epithelial layer.14 Completing the transition, cells activate the expression of additional mesenchymal genes and proteins, such as α-smooth muscle actin (αSMA), smooth muscle protein 22α, collagen I and III, vimentin, fibronectin, or fibroblast-specific protein 1 (FSP1; also known as S100A4).8,15–17

Orchestrating these processes, the most widely described regulator of EMT/MET is the transforming growth factor β (TGFβ) superfamily of signaling molecules (TGFβ, Nodal, bone morphogenic proteins [BMPs], and growth and differentiation factors).18–21 Downstream of the receptors for the various TGFβ superfamily members, the Smad family of signal transducers is also of key importance, and in particular Smad2 and Smad3 appear to control EMT program activa-
Classification and Types of EMT

Epithelial-to-mesenchymal transition is classified into 3 types depending on its biological (or pathological) role and the time window in which it occurs. The embryonic and developmental EMT programs described above are classified as type-1 EMT, being distinct in that they do not generally cause fibrosis or give rise to mesenchymal cells with an invasive phenotype. As shown in Figure 3, the remaining types (2 and 3) are operative after birth and are concerned with fibrosis and malignant cellular transformation respectively. Type-2 EMT is extensively described in the literature, and it appears that chronic inflammation may be the sovereign inciting injury that triggers this form of EMT and sets the stage for end organ disease. Although the hypothesis is not without controversy, evidence exists to support type-2 EMT in numerous adult conditions, including those affecting the kidney, liver, skin, intestines, lungs, eyes, and heart (the latter will be considered separately in the section “EndMT Contributes to Cardiac Fibrosis in the Adult Heart”). Type-3 EMT is an important step in malignant cell transformation. Of particular relevance with respect to carcinoma (epithelial cell tumors), EMT is proposed as a critical mechanism for the acquisition of malignant characteristics, with loss of E-Cadherin expression facilitating the delamination and metastasis of transformed epithelial cells.

A further aspect of this classification system requiring clarification is the place of EndMT. The endothelium is a specialized form of squamous epithelial tissue, and as such, EndMT is a subclass of EMT. Accordingly, EndMT may be observed in each of the 3 categories of EMT. For example, the endothelium gives rise to hematopoietic cells during embryonic development (type-1 EMT), and to both fibrosis and malignancy in the adult (types-2 and -3 EMT, respectively).

EMT During Cardiac Development: Valves, Cushions, Neural Crest, and Epicardium-Derived Cells

Early Cardiac Formation

The heart forms via a remarkable series of sequential waves of EMT/MET. As the definitive germ layers emerge in the
developing embryo, cardiac progenitors are among the first epiblast cells to undergo EMT and to migrate out from the primitive streak.46–47 This population of mesenchymal cardiac precursors migrates bilaterally within the lateral plate toward the anterior pole of the embryo47–49 to coalesce in mammals into an anterior cardiac crescent.47 The formation of the celomic cavity divides the lateral plate to give rise to the somatic and splanchnic mesodermal layers. Within the splanchnic layer, primitive cardiac progenitor cells organize into a bilayered epithelium via MET.50 Next, either via another round of EMT/MET involving these mesodermal cardiac progenitor cells or from a separate cell population, the endocardial cells that will line the cardiac structures are formed.51–53 The primitive heart tube soon emerges by folding and remodeling of the cardiac crescent cells. Genetic and direct labeling of cardiac precursor cells at various stages of heart tube development has shown that the primary heart tube only contains the precursors of the left ventricle and that the heart tube development has shown that the primary heart tube directly labeling of cardiac precursor cells at various stages of ing and remodeling of the cardiac crescent cells. Genetic and direct labeling of cardiac precursor cells at various stages of heart tube development has shown that the primary heart tube only contains the precursors of the left ventricle and that the remaining chambers are formed by the progressive infiltration and incorporation of new cardiac precursors into the outflow and inflow poles of the heart tube.54–56 The area of the cardiac crescent that gives rise to the initial heart tube is named the primary heart field whereas the area that remains behind in the pharyngeal region that is added later is called the secondary heart field. Primary and secondary heart field precursors occupy adjacent areas in the cardiac crescent but differ in the mechanism by which they are added to the heart tube (folding versus migration) and in the timing of addition. At the current time, the role of EMT in the migration and incorporation of second heart field precursors remains under investigation.

EndMT Contributes to Valve Formation and Heart Septation

Soon after the primitive heart tube appears, endothelial cells from the region of the forming atrioventricular (A-V) canal and of the outflow tract (OFT) region undergo another round of EMT, or more specifically EndMT because the cells undergoing phenotypic switching are endothelial. At this time, the endocardium and the myocardium are separated by a thick acellular matrix termed the cardiac jelly. As the cardiac chambers start to form, the cardiac jelly gets thicker in the A-V canal and OFT regions, where endothelium-derived mesenchymal cells invade the adjacent cardiac jelly to form endocardial cushion tissue.57 The OFT cushions are the precursors of the semilunar valves whereas the A-V cushions give rise to the A-V septum, the membranous part of the ventricular septum and the mitral and tricuspid valves.58 The extent to which EndMT contributes to these 2 cushion areas differs significantly, and although most of the A-V cushion mesenchyme derives from EndMT,57 most of the OFT cushion derives from pharyngeal mesodermal cells. Subsequently, both cushions receive further specific mesenchymal cellular contributions involving EMT. The OFT region receives an important third mesenchymal population arising from the neural crest, which delaminates by EMT from the neural tube.59 This neural crest–derived population is essential for OFT septation into the aortic and pulmonary trunks; however, it represents a transient population that does not contribute significantly to the definitive heart structures. In the region of the A-V canal, a third mesenchymal population derives from EMT of the epicardium.60,61

EMT and the Epicardium

In parallel with endocardial EndMT and cushion formation, the outermost epicardial layer of the heart is also coming into existence.62 Like the primitive early cardiac progenitor cells, epicardial progenitor cells also arise from the splanchnic mesoderm (likely also via MET). Initially, the cells destined to form the epicardium assemble to create a transitory body of cells termed the proepicardial organ, consisting of an accumulation of pericardial progenitor cells lying adjacent to the sinus venosus (the venous pole of the heart; Figure 4A). These proepicardial cells migrate, or in some species float freely within the pericardial cavity, and attach to the myocardial surface.63–67 There, they proliferate and flatten to cover the embryonic heart as the epicardial sheet. Concomitantly, some epicardial cells undergo EMT and generate a mesenchymal population of epicardium-derived cells (EPDCs). Although a population of EPDCs remain to occupy the extracellular matrix–rich region between the epicardium and myocardium named the subepicardial space, some migrate further to invade the myocardium.

The developmental cellular contributions of EPDCs are controversial and potentially species-dependent. An important contribution of EPDCs is made to the A-V canal cushion mesenchyme where EPDCs merge with endocardium-derived cells to form the mitral and tricuspid valves and cardiac septa.60,61,68 Interestingly, EPDCs do not colonize the OFT cushions, perhaps implying a specific role for these cells in the formation of the tricuspid and mitral but not the pulmonary and aortic valves. Epicardium-derived cells also play a role in coronary vascular formation. Studies in avian species, which included labeling the proepicardium with replication-deficient virus62–69 or the generation of quail-chick chimeras,60–62,70 indicated that EPDCs are the primary source of coronary endothelial cells, coronary vascular smooth muscle cells (cVSMCs) and cardiac fibroblasts. More recently, genetic fate mapping experiments have investigated the fate of EPDCs in the mouse. T-box transcription factor 18 (Tbx18) and Wilms tumor suppressor 1 (Wt1) are broadly expressed in the proepicardium and epicardium during development and represent appropriate markers to trace EPDCs.71–74 Using Cre-based technology, the fate of Tbx18+/Wt1− cells was analyzed,65,75 confirming that EPDCs differentiate into cVSMCs and cardiac fibroblasts. However, in mice, in contrast to the chick, no contribution to coronary endothelial cells was identified for Tbx18+-derived cells and only a minor contribution was found from Wt1− cells. Very recently, Kikuchi et al have also demonstrated that in zebrafish, whereas EPDCs contribute to perivascular cells, they do not give rise to endothelial cell populations.76 Potentially, this discrepancy in the contribution of EPDCs to the endothelium in avian versus other species may be explained by species-specific differences or may reflect differing experimental approaches used to study EPDC fate. It is also possible that the initial observations performed in the chick were not correctly interpreted or that contamination by non-EPDC
endothelial precursor cells migrating together with the proepicardium during grafting/labeling may have occurred.77,78 Consistent with this, genetic evidence from the mouse supports the classic notion that the coronary endothelium derives from the sinus venosus.79 Using inducible vascular endothelial-cadherin-Cre mice to trace endothelial cell clones, it was determined that most coronary veins and arteries derive from sprouts arising at the sinus venosus, with a lesser proportion potentially arising from the endocardium. Importantly, no endothelial clones were linked to the proepicardium, suggesting that this structure does not contribute to endothelial progenitors, although it remains possible that epicardial cells may commit to the endothelial lineage at a later developmental stage, after colonization of the myocardial surface.

Interestingly, analysis of Tbx18+ and Wt1+ epicardium-derived lineages also revealed an unexpected myocardial contribution. Thus, \( \approx 4\% \) of total cardiomyocytes were found to have arisen from Wt1+ cells, contributing mostly to the intraventricular septum (10%), atria (18%), and to a lesser extent the ventricular walls (7%).75 Similarly, the Tbx18+ lineage was found to contribute to cardiomyocytes in the ventricular septum and in scattered areas within the ventricular walls and atria.68 However, these findings remain under scrutiny because Tbx18 expression has been detected in maturing cardiomyocytes, potentially suggesting epicardium-independent Tbx18 activation in these cells.80 Furthermore, studies in zebrafish have also refuted the ability of EPDCs to give rise to cardiomyocytes.76

Most recently, yet another contribution of EPDCs was revealed by Harvey and coworkers, who described a population of mesenchymal stem cell–like cells that occupy a perivascular adventitial niche in the adult mouse.81 Although the precise extent of their normal and pathological cellular contributions remains to be defined, these proepicardium/epicardium-derived stem cells exhibit transgerm layer potency in vitro and in vivo and appear distinct from previously described resident cardiac stem cell populations.

In summary, it is clear that EMT-derived EPDCs support coronary artery development by supplying vascular pericytes and cVSMCs and that they make a major contribution to fibrous cardiac tissues/populations, including resident perivascular fibroblasts and the fibrous cardiac skeleton.60–62,68,69,75,77,78,82 Although the ability of EPDCs, other epicardial cells, or both to give rise to cardiomyocytes and endothelium during development remains controversial,
it is clear that the EMT and MET programs are of key importance during cardiac formation and for epicardial provisioning of specific cell populations.

**Signaling Pathways Governing EndMT/EMT During Cardiovascular Development**

Signaling via the TGFβ superfamily, as previously described in this review, is the major regulator of EMT during cardiac formation, including TGFβ2, TGFβ3, and BMP-2 and the downstream transcription factors Snai1 and Snai2.83 The precise role of these TGFβ isoforms differs between species, and at least in the chick TGFβ2 mediates EndMT via endothelial cell activation and separation whereas TGFβ3 mediates cell invasion into the extracellular matrix.84

In addition to TGFβ, numerous other pathways modulate EMT during cardiac formation and development. Notch signaling is of particular significance during murine cardiac development, functioning to promote endocardial EndMT and with Notch deficient embryos displaying atrophic valve formation.85 Expanding our understanding of these pathways, Luna-Zurita et al86 recently showed that Notch1 is sufficient to activate a cell-autonomous promesenchymal gene-expression program in endocardial cells. Bone morphogenic protein 2 was found to drive endocardial EndMT and mesenchymal cell invasion into the cardiac jelly. Further, myocardial BMP-2 inactivation impaired Notch1 activity,86 suggesting a model in which the interplay between myocardial BMP-2 and endocardial Notch signaling restricts EndMT to prospective valve territory.86,87

Endocardial EndMT is also dependent on receptor tyrosine kinase signaling via the phosphoinositide-3 kinase–phosphoinositide–dependent protein kinase 1–Akt/protein kinase B cascade, which is upstream of Snail. Genetic ablation of dependent protein kinase 1 in endothelial cells leads to embryonic lethality with abnormal vascular remodeling and a failure of endocardial cushion development because of defective EndMT.88 Gata4, an upstream regulator of an Erbb3-Erk pathway, is expressed in the endothelium and mesenchyme of the embryonic A-V valves and is also required for endocardial EndMT. Selective Gata4 inactivation in endothelium-derived cells results in a failure of EndMT and hypocellular endocardial cushions in animal models.89 This phenotype corresponds with that seen in humans, where heterozygous Gata4 mutation is associated with defects in the interatrial or interventricular septum.90,91 Endocardial expression of the protein tyrosine phosphatase SHP2, encoded by the gene PTPN11, also regulates EndMT and endocardial cushion formation. Gain of function PTPN11 mutations are responsible for a significant proportion of cases of Noonan syndrome and cause cardiac valve and septal defects by increasing Erk–mitogen-activated protein kinase activation, probably downstream of Erbb family–receptor tyrosine kinases, thus extending the time window during which endocardial EndMT is operative.92 Endocardial EndMT is also restrained by inhibitory influences such as endocardial cell expression of the gene encoding Nfatc1 (nuclear factor of activated T cells, cytoplasmic 1), which functions to inhibit EndMT and promote semilunar valve elongation in a cell-autonomous manner.93 In addition, cushion mesenchymal cells are especially sensitive to reactive oxygen species–mediated injury, suggesting that oxidative stress may contribute to the formation of congenital cardiac defects.94

Additional specific factors are operative during epicardial EMT and EPDC migration. For example, Wt1 mutant mice display disrupted epicardial formation and reduced numbers of EPDCs and their derivatives.73,95 Consistent with this, conditional ablation of Wt1 in Gata5-Cre lineage cells (marking proepicardium- and epicardium-derived cells) has been reported to reduce epicardial Snai1 and increase E-Cadherin levels, thus inhibiting the EMT program.96 However, the influence of Wt1 on epicardial E-Cadherin is disputed.97 Interestingly, during kidney development Wt1 regulates MET rather than EMT, thereby highlighting the importance of Wt1 in controlling the switch between a mesenchymal and epithelial state.95,98

Potentially upstream of Wt1, epicardial EMT is known to be modulated by thymosin β4 (Tβ4), an actin-binding peptide. Although not required for proper cardiac formation,99 during development Tβ4 is expressed by the myocardium, providing a paracrine stimulus for EPDCs to migrate and differentiate into cVSMCs and facilitating vascular development.100 This observation supports the concept that cross-talk between different regions of the heart during development via non–cell-autonomous signaling may exert a major influence on EMT and EPDCs. As a further example Van Gogh–like 2, a component of the planar cell polarity signaling pathway, is expressed in myocardial cells of the developing heart. However, Van Gogh–like 2 mutant mice with disruption of the planar cell polarity pathway exhibit cardiac defects that involve the epicardium and EPDCs. In these mice, among other defects, reduced fibronectin deposition in the subepicardial space is associated with reduced EPDC migration into the ventricular myocardium where the coronary vessels fail to develop an intact cVSMC layer.101

β-Catenin also regulates epicardial EMT. Conditional epicardial β-catenin deletion using the Gata5-Cre driver line leads to incomplete invasion of EPDCs into the myocardium and disruption of cVSMC formation.102 Wu et al showed that β-catenin affects epicardial EMT by regulating cell spindle orientation and stabilization of adherens junctions.103 These investigators also demonstrated that proliferation is a prerequisite for epicardial cells to become mesenchymal and that the mitotic division plane should be orthogonal to the myocardial surface in order to enable EMT.104 A direct interaction between Wt1 and β-catenin has also recently been suggested on the basis of the observation that β-catenin is downregulated in Wt1 mutant epicardium.97

Platelet-derived growth factor (PDGF) signaling is yet another factor that is able to induce epicardial EMT.15 Whereas platelet-derived growth factor receptor (PDGFR)β is required for EMT and the subsequent differentiation of cVSMCs,104 PDGFRe is required for the subpopulation of EPDCs giving rise to intracardiac fibroblasts.105 Additional factors that regulate EMT/MET during cardiac development are shown in the Table.

Periostin, a secreted protein, is associated with the endocardial EndMT and epicardial EMT programs. Although its role as a true mediator of EndMT/EMT seems less likely, it...
appears to mark EndMT/EMT-derived mesenchymal cells in the developing heart. Periostin knockouts mice exhibit minor leaflet abnormalities in the viable majority. Assessment of collagen production, 3-dimensional lattice formation ability, and TGFβ responsiveness indicate that peristin is required for myocardialization. Likely also involved with endocardial EndMT. 106, 107

**Table. Genes and Proteins Known to be of Importance for EMT/MET and EPDCs During Cardiac Development and in Cardiovascular Disease**

<table>
<thead>
<tr>
<th>Gene/Protein</th>
<th>Expression</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP family (see also TGFβ)</td>
<td>Myocardial</td>
<td>BMP-2 promotes endocardial EndMT and mesenchymal cell invasion into the cardiac jelly and also interacts with endocardial Notch1 to spatially govern endocardial EndMT. 106 BMP-7 attenuates EMT-related cardiac fibrosis. 19</td>
</tr>
<tr>
<td>Connexin 43</td>
<td>Ubiquitous</td>
<td>Governs cell polarity and cytoskeletal integrity. Required for epicardial EMT, EPDC migration and OFT myocardialization.</td>
</tr>
<tr>
<td>FGF family</td>
<td>Differs among family members</td>
<td>Broadly promotes epicardial EMT and regulates other developmental events such as myocardial invasion and endothelial differentiation of EPDC. 108, 109, 111 Epicardial EMT during heart regeneration is dependent on FGF signaling. 110, 111</td>
</tr>
<tr>
<td>Gata4</td>
<td>Proepicardium, endothelium, and mesenchyme of A-V valves</td>
<td>Upstream regulator of ErbB3-Erk pathway regulating endocardial EndMT. 95 Also required for formation of the proepicardium112 and endocardial EndMT. 89 Human Gata4 mutations are associated with atrial and ventricular septal defects. 90, 91</td>
</tr>
<tr>
<td>Nfatc1</td>
<td>Proepicardium, epicardium, valve endocardium</td>
<td>Inhibits EndMT by inhibiting Snail1 and Snail2; promotes cell proliferation and semilunar valve elongation. 93 Promotes invasion of EPDCs into the myocardium by induction of extracellular matrix-degrading enzyme gene expression. 113</td>
</tr>
<tr>
<td>Notch pathway</td>
<td>Epicardium and endocardium</td>
<td>Promotes endocardial EndMT. 83, 86, 87 Notch-deficient embryos have atriopeptic valve formation. 85 Notch pathway includes upstream protein kinase D2, Histone deacetylase 5, Krüppel-like factors, and downstream Hey1, Hey2, and HeyL. 114 Also involved in epicardial/proepicardial development and EPDC differentiation. 92</td>
</tr>
<tr>
<td>PDGF-PDGFR signaling</td>
<td>PDGFs by EPDCs. PDGF by endothelial and other cardiac cells</td>
<td>PDGFRβ required for epicardial EMT and cvSMC differentiation. PDGFRα required for EPDC contributions to cardiac fibroblasts. 15, 104, 105 Inhibition of PDGF signaling disrupts epicardial EMT during cardiac injury repair. 117</td>
</tr>
<tr>
<td>PDK1</td>
<td>Ubiquitous</td>
<td>Endothelial deletion leads to abnormal vascular remodeling and defective endocardial EndMT with decreased Snail expression and embryonic lethality. 96</td>
</tr>
<tr>
<td>Periostin</td>
<td>Fibrous cardiac skeleton and endocardial cushions</td>
<td>Required for maturation and extracellular matrix stabilization of noncardiomyocyte cardiac lineages, with knockout mice exhibiting minor leaflet abnormalities. Marks EndMT/EMT-derived mesenchymal cells in the developing heart. 82, 118</td>
</tr>
<tr>
<td>PTPN11 (encodes SHP2)</td>
<td>Ubiquitous</td>
<td>Gain of function mutations cause human valve and septal defects ( Noonan syndrome) by increasing Erk-MAPK activation and increasing the extent of endocardial EndMT. 92</td>
</tr>
<tr>
<td>Reactive oxygen species</td>
<td>Ubiquitous presence</td>
<td>Affect cushion mesenchymal cells, which may lead to congenital cardiac defects via aberrant EndMT/EMT. 94</td>
</tr>
<tr>
<td>Retinoid X receptor alpha</td>
<td>Ubiquitous</td>
<td>Promotes epicardial EMT via a Wnt signaling pathway. 119</td>
</tr>
<tr>
<td>Tβ4</td>
<td>Myocardium</td>
<td>Dispensable for cardiac development 99 but regulates EPDC migration and differentiation into cvSMCs and facilitates cardiac vascular formation. 100 Stimulates epicardial EMT after injury and enhances cardiac repair. 116, 117</td>
</tr>
<tr>
<td>TGFβ superfamily</td>
<td>Ubiquitous</td>
<td>Critical role in cardiac EndMT/EMT. Includes multiple isoforms, other TGFβ family members (see BMP), and downstream factors. The exact role of each isoform and family member differs between species. 94 TGF signaling is implicated in EndMT-related cardiac fibrosis. 18, 123 ALK2, a TGFβ/BMP receptor, is implicated in EndMT-related vascular calcification. 123 In vitro, while TGFβ induces EMT in proepicardial explants, 114 it may have inhibitory effects in avian epicardial explants. 108</td>
</tr>
<tr>
<td>Vangl2</td>
<td>Myocardial</td>
<td>Vangl2 mutants have reduced subepicardial fibronectin, reduced EPDC migration, enlarged ectopic coronary vessels with deficient cvSMC coverage, and disrupted cardiomyocyte organization. 101</td>
</tr>
<tr>
<td>VEGF</td>
<td>Dynamic temporal expression during development</td>
<td>Promotes endocardial EndMT 125 and possibly epicardial EMT. 110, 126, 127</td>
</tr>
<tr>
<td>Wnt/β-catenin</td>
<td>Epicardium</td>
<td>Regulates EPDC migration and cvSMC formation via effects on cell-spindle orientation and stabilization of adherens junctions. 97, 102, 103, 133</td>
</tr>
<tr>
<td>Wi1</td>
<td>Epicardium</td>
<td>Required for epicardial EMT and EPDC formation. 73, 96, 97 Wi1 is upstream of canonical Wnt/β-catenin signaling. 97</td>
</tr>
</tbody>
</table>

EMT indicates epithelial-to-mesenchymal transition; MET, mesenchymal-to-epithelial transition; EPDCs, epicardium-derived cells; BMP, bone morphogenetic protein; TGFβ, transforming growth factor β; EndMT, endothelial-to-mesenchymal transition; OFT, outflow tract; FGF, fibroblast growth factor; A-V, atrioventricular; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; cvSMC, coronary vascular smooth muscle cells; PDK1, phosphoinositide-dependent protein kinase 1; MAPK, mitogen-activated protein kinase; Tβ4, thymosin β4; VEGF, vascular endothelial growth factor; and Wi1, Wilms tumor suppressor 1.
maturation and extracellular matrix stabilization of noncardiomyocyte lineages of the heart. The utility of manipulating periostin and several of the abovementioned pathways to attenuate cardiac fibrosis, regeneration, and remodeling is currently under investigation.

EMT and EndMT in the Adult Cardiovascular System

Epicardial EMT in the Adult Heart

In the adult heart, the epicardium constitutes a unicellular layer of epithelial cells. Several animal models of myocardial infarction or cardiac damage have shown that an early response to injury consists of the reactivation of epicardium-specific genes usually expressed only during development such as Wt1. Although this epicardial genetic reactivation is initially generalized in nature, it subsequently becomes restricted to the site of damage, suggesting a role for the epicardium during cardiac wound healing. Concomitantly, epicardial cells undergo EMT and proliferate and accumulate in the subepicardial space to form a thickened epicardial cap composed of EPDCs (Figure 4B). In zebrafish, inhibition of fibroblast growth factor signaling by genetic manipulation results in a lack of epicardial marker gene expression within wounds induced by partial apical cardiac amputation, suggesting that during heart regeneration epicardial EMT is dependent on fibroblast growth factor signaling. Similarly, pharmacological inhibition of PDGF signaling was also shown to disrupt epicardial EMT during zebrafish cardiac injury repair. In the mouse, both myocardial infarction and ventricular pressure-overload induce Wnt1- and Notch-regulated activation of epicardial cells, which then undergo EMT and contribute to fibrosis repair. Together these studies indicate that, at least in modified form, the EMT programs in cardiac development may become reactivated in the adult.

Consistent with their fate during cardiac development, lineage tracing of epicardial cells after myocardial infarction in mice has shown that EPDCs give rise to fibroblasts, myofibroblasts, and smooth muscle cells but, at least in the absence of additional stimulation, not to endothelial cells nor cardiomyocytes. Interestingly, after myocardial infarction, epicardial cells were also found to produce trophic factors that promote capillogenesis such as vascular endothelial growth factor A. Confirming this paracrine effect, supplementalation of the epicardium with transplanted EPDCs or EPDC-conditioned media after myocardial infarction improved subsequent cardiac function.

Riley and coworkers have provided significant evidence to demonstrate that Tβ4 can stimulate epicardial EMT after injury and enhance myocardial repair. Using explanted tissues, these investigators provided proof of concept that with Tβ4 stimulation adult murine epicardial cells undergo EMT in vitro and differentiate into smooth muscle cells, fibroblasts, cardiac progenitors, and endothelial cells. In vivo studies of Tβ4 stimulation have shown that this peptide is able to induce a dramatic EPDC contribution to neovascularization after myocardial infarction. Most recently, Riley and coworkers reported that in Tβ4-pretreated mice a small subset of ≈1% of Wt1+ cells can give rise to cardiomyocytes after myocardial infarction. However, although other salutary effects are present, when initiated soon after myocardial infarction Tβ4 treatment does not reprogram epicardial cells into cardiomyocytes. Nevertheless, on the basis of other data suggesting that the epicardium may harbor adult cardiac progenitor cells, intense research efforts are currently underway investigating the possibility that augmenting the normal postinjury response by activating epicardial EMT may be of therapeutic utility after cardiac injury or infarction in humans.

EndMT Contributes to Cardiac Fibrosis in the Adult Heart

Evidence implicating EndMT in cardiac fibrosis has been mounting for several years. In addition to the above studies and as important corroborating data, it is well described that TGFβ plays a major causal role in myocardial fibrosis and diastolic dysfunction through fibroblast activation in pressure-overload rodent models, with systemic administration of TGFβ-neutralizing antibody preventing this process. In a landmark publication in 2007, Kalluri and coworkers demonstrated that EndMT makes a significant contribution to myocardial fibrosis in the adult heart. These investigators used murine models of cardiac fibrosis/heart failure in association with a Cre-lox genetic marking system to identify cells that have ever expressed Tie1 (an endothelial marker), revealing that 27% to 33% of all cardiac fibroblasts are of endothelial origin in these models (Figure 5A). Further, TGFβ1 induced cultured endothelial cells to undergo EndMT in vitro whereas BMP-7 preserved the endothelial phenotype. The use of mice genetically deficient in Smad3 or the systemic administration of recombinant human BMP-7 inhibited EndMT and the progression of cardiac fibrosis in vivo. Treatment with BMP-7 was associated with a decrease in the number of fibroblasts of endothelial origin, providing strong evidence for the importance of EndMT in cardiac fibrosis. These provocative findings suggest that the inhibition of EndMT/EMT with BMP-7 or other means may be a promising target for clinical therapeutic translation in settings such as cardiac fibrosis where EndMT/EMT is injury-causing.

Although the results have yet to be replicated with robust endothelial lineage-tracing systems, supporting evidence for EndMT/EMT has since arisen from other models of cardiac fibrosis including hypertrophic cardiomyopathy, diabetes-induced cardiac fibrosis, and perhaps more controversially with genetic deficiency of plasminogen activator inhibitor-1 in aged but not young mice. For example, in mice with streptozotocin-induced diabetes mellitus, evidence for EndMT was provided by the identification of cells copositive for endothelial (CD31) and mesenchymal (αSMA and fibroblast-specific protein 1) markers (Figure 5B). These investigators also showed that EndMT could be induced in vitro by endothelial cell culture under high-glucose conditions, and that this process was mediated by the endothelial expression of endothelin-1 and TGFβ. Angiotensin II, which is known to have profibrotic effects in the heart and elsewhere, may also promote cardiac EndMT. Although
Evidence is limited, Tang et al. suggested the administration of irbesartan (an angiotensin-II–receptor antagonist) reduces interstitial cardiac fibrosis and left ventricular dysfunction by inhibiting EndMT and adopting a fibroblast-like phenotype. These effects on cardiac EndMT and fibrosis are consistent with the clinical response to angiotensin-II–receptor blockade and offer novel insights into potential mechanisms of action of this class of drug. 

**Other Contributions of EndMT and EMT to the Adult Cardiovascular System**

The origins of vascular calcification, and in particular the origins of the cells that cause heterotopic vascular ossification, is a matter of controversy. Importantly, osteoblasts and chondrocytes, the cells responsible for ossification, are mesenchymal cells. Therefore, the question arises whether osteoblasts and chondrocytes that are operative in adult pathological conditions may be derived from endothelial cells via EndMT. This possibility was investigated using samples from humans suffering fibrodsyplasia ossificans progressiva, a condition in which heterotopic ossification occurs because of a gain of function mutation in the gene encoding ALK2, a member of the TGFβ/BMP family of receptors. In this study, using a combination of in vivo murine-lineage tracing and in vitro cell characterization experiments, Medici et al. provided extensive evidence of an endothelial origin of osteoblasts and chondrocytes via EndMT. Provocatively, in addition to showing that the expression of constitutively active ALK2 in endothelial cells causes EndMT, these investigators showed that it leads to the acquisition of a mesenchymal stem cell–like phenotype. Similar results were obtained by treatment of untransfected endothelial cells with TGFβ2 or BMP-4 in an ALK2-dependent manner. These mesenchymal stem-like cells could be triggered to differentiate into osteoblasts, chondrocytes, or adipocytes. Although it remains to be shown if EndMT contributes to vascular calcification in persons without this genetic mutation, this study lays the foundations for a wider exploration of the role of EndMT in the adult vasculature. Moreover, the possibility that EndMT generates mesenchymal stem-like cells is consistent with other emerging data indicating that MET is involved with the reprogramming of fibroblasts to become pluripotent stem cells. Although the ability to undergo cellular phenotypic switching via EMT/MET is not a core feature of the stem cell repertoire, these data and other material presented in this review suggest that it is involved with aspects of the generation and functionality of certain stem or progenitor cell populations.

Although definitive lineage-tracing studies are yet to be performed, EndMT/EMT is potentially implicated in several other adult pathological cardiovascular conditions. Foremost among these, pulmonary hypertension, regardless of its cause, has been thought to involve EndMT/EMT for several years. This speculation is based on the fact that increased numbers of αSMA-expressing cells is a near-universal finding in remodeled pulmonary vessels and that the major signaling systems associated with EndMT/EMT are also operative in pulmonary hypertension, including the TGFβ, BMP, Smad pathways. In addition, it has been demonstrated that adult pulmonary artery endothelial cells undergo EndMT in vitro. Another chronic inflammatory condition affecting the vasculature is systemic sclerosis. It is believed that EndMT is involved in the microvascular changes seen in this condition. Of relevance, systemic sclerosis bears many of the hallmarks of conditions involving EndMT/EMT, including mesenchymal cell proliferation and TGFβ signaling. EndMT/EMT may also be involved in mitral valve pathology and transplant vasculopathy, although again we reiterate that at the current time the definitive involvement of EndMT/EMT in the above-mentioned conditions remains to be demonstrated using rigorous lineage-tracing systems.

**Conclusions**

Throughout life, EndMT, EMT, and MET are conserved and integral cellular processes that play a diverse role in biological organism formation and pathological end organ disease. Until recently, research in this field was hampered by a lack of specific endothelial and epithelial lineage-tracing systems to follow the fate of these cells as they transition to a mesenchymal phenotype. However, this problem is being progressively overcome and accurate animal models for permanent
genetic cell labeling are now increasingly being used for this purpose. These models differentiate themselves from all prior systems in their specificity, fidelity, and accuracy and enhance our capacity to address the scientific questions proposed. In addition, the possibility of tracking EMT/MET in vivo in humans may soon be a reality with techniques such as fluorodeoxyglucose-positron emission tomography (commonly referred to as FDG-PET) or PET magnetic resonance imaging (PET-MRI) continuing to evolve and now approaching the resolution required for cell-tracing studies.

As discussed, EndMT/EMT appears likely to play a role in numerous chronic cardiovascular disease states such as heart failure and pulmonary hypertension and various forms of chronic vasculopathy. Furthermore, as reviewed elsewhere, chronic inflammatory and malignant diseases are a particularly common finding in older persons.\textsuperscript{158,159} Given that types-2 (inflammatory/fibrosing) and -3 (malignancy-associated) EMT appear to play an important role in the pathology of these conditions, EMT may prove to be a key aspect of age-related morbidity and mortality. The prospect that the therapeutic manipulation of EndMT/EMT may be used in the treatment of these conditions is particularly attractive. As a result, interest and research in this field is expanding rapidly. An increased understanding of the function and contribution of EndMT/EMT during development and in the adult should provide the foundation for the subsequent clinical exploitation of these pathways.

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Disclosures

None.

References


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