Published in final edited form as:
Pediatr Cardiol. 2010 April ; 31(3): . doi:10.1007/s00246-009-9614-z.

The Role of Serum Response Factor in Early Coronary Vasculogenesis

Ravi P. Misra
Department of Biochemistry, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA, rmisra@mcw.edu

Defects in vascular development are a major cause of fetal demise and congenital cardiovascular disease. Knowledge is scarce concerning both embryonic vascular cell progenitors and the underlying regulatory mechanisms that determine their commitment to various vessel cell lineages.

During early cardiovascular development, the proepicardium (PE) is the major source of vascular progenitors. The PE is a transient embryonic structure found at stages 16 and 17 in the chick, at 9.5 days postcoitum (dpc) in the mouse, and at approximately 5 weeks in the human that consists of mesothelial and mesenchymal cells overlying the septum transversum (ST). Cells within the PE undergo a complex process of development and differentiation that involves at least two directed waves of migration and epithelial-to-mesenchymal transitions (EMT). The PE cells make contact with the inferior surface of the developing myocardium, migrate over the surface of the heart forming the epicardium, and penetrate into the myocardium and give rise to subepicardial mesenchymal cells (SEMCs). The SEMCs subsequently migrate throughout the developing heart giving rise to the entire coronary vasculature consisting of fibroblasts as well as endothelial and smooth muscle cells [32].

Serum response factor (SRF) is essential for mesoderm formation during embryonic development [2]. A necessary role for SRF in the development of vascular smooth muscle from yet to be characterized PE-derived coronary vascular precursor cells has been demonstrated in avian explant models [15]. The role of SRF in vivo during some of the earliest steps in mammalian PE-mediated vascular development, however, is less well studied, and our recent observations suggest that it likely is more complex. This brief review aims to discuss the role of SRF during early coronary vasculogenesis as it relates to development of the PE.

The Role of the PE in Coronary Vasculogenesis

Generation of the coronary vasculature is a complex process that involves regulation of cell fate determination, cell migration, EMT, and three-dimensional patterning. Development of the vasculature in the heart occurs by two broad yet developmentally distinct processes: (1) angiogenesis, characterized by outgrowth or branching of preformed vessels, and (2) vasculogenesis, characterized by fusion of more delocalized locally formed endothelial vessels. The great vessels of the heart, the thoracic aorta, and the aortic arches are derived from the neural crest and grow by an angiogenic process [5, 32].

In the heart itself, however, vessels are formed by a vasculogenic mechanism involving in situ fusion of angioblasts. Numerous studies have identified mesodermal cells in the dorsal

Correspondence to: Ravi P. Misra.

mesocardium (the PE) as the major source for coronary vascular precursors. These studies led to a model, diagrammed in Fig. 1, in which cells from the PE differentiate and migrate into the heart and give rise to the distinct cell types (endothelial, smooth muscle, and perivascular connective tissue) that make up the coronary vascular system [19, 20].

Various studies have shown the importance of cells derived from the PE in the formation of coronary vessels [22, 23, 32]. The PE is a transient “grapelike” embryonic structure found at stages 16 and 17 in the chick, at approximately 9.25 dpc in the mouse, and at approximately 5 weeks in the human that consists of mesothelial and mesenchymal cells overlying the ST. A transient bridging structure reliably seen in the chick, and recently in the murine systems, has sometimes been termed the proepicardial organ. As depicted in Fig. 2, cells from the PE make contact with the inferior surface of the developing myocardium and migrate over the surface of the heart to form the epicardium. Epicardial cells then penetrate into the myocardium at the junction between the atrium and the ventricle (AV junction) and give rise to SEMCs.

The SEMCs are important for multiple aspects of heart development, giving rise to the entire coronary vasculature and contributing to cardiac fibroblast, endothelial, and smooth muscle cell lineages. Using retroviral tagging and dye-labeling studies, Mikawa and Fischman [19] have provided evidence that these three distinct lineages of the coronary vasculature are already segregated in the PE before the time of migration to the heart, although it appears that the PE cells have not yet differentiated into distinct vascular cell types [20]. To understand the mechanisms that regulate early coronary vasculogenesis, it is necessary to appreciate the central role of the PE in the events underlying three important aspects of vascular development: (1) formation of the PE, (2) formation of the epicardium, and (3) formation of the SEMCs that serve as the direct progenitors of the various vascular cells.

Formation of the PE

Cells that generate the PE begin as an outgrowth of the epithelium associated with the ST. The ST is an outgrowth of the dorsal body wall that divides the embryonic coelom and extends from the dorsal body wall, closing off the pericardial and peritoneal cavities. At early stages, approximately HH17 in avian systems (~9.25 dpc in mice), the PE protrudes from the ST as multiple villous-like projections that in avian systems have a cauliflower-like appearance. These protrusions are covered by the mesothelium and contain numerous mesenchymal cells in an extracellular matrix.

Whereas the organization of the PE has been extensively studied mainly in avian embryos, the factors that control development of the PE are not well known. Watt et al. [38] have shed some light on underlying genetic events involved in formation of the PE in mice. They used a loss-of-function analysis to show a central role for GATA4 in PE formation. Because GATA4-null mice are lethal, Watt et al. [38] used a tetraploid embryo complementation approach for rescue, generating clonal 9.5-dpc $Gata4^{-/-}$ embryos directly from embryonic stem cells. The GATA4-null embryos displayed heart defects characterized by disrupted looping morphogenesis, septation, and a hypoplastic ventricular myocardium.

Surprisingly, the PE was absent in the rescued GATA4-null embryos, and formation of the epicardium was blocked. Therefore, it was proposed that myocardial defects in GATA4-null animals are a secondary consequence of loss of the PE. These findings definitively demonstrate that GATA4 is an essential transcriptional regulatory factor for generation of the PE. To date, GATA4 is the only identified transcription factor known to be essential for genesis of the PE. This likely is largely due to the difficulty carrying out loss of function experiments that specifically address PE development.

Significant effort has been made by numerous labs to identify signaling molecules that induce cardiogenesis within the epiblast. Importantly, explant tissue culture and genetic studies have demonstrated that the endoderm is a source of signals acting to both permit and instruct the mesoderm to adopt a cardiogenic fate [6, 11, 16]. Many signals appear to be involved in the process of specifying cardiac cell fate, although bone morphogenetic proteins (BMPs) acting in concert with fibroblast growth factors (FGFs) have received a great deal of attention as key players in controlling cardiac specification [1, 4].

In contrast to cardiac specification, little is known about how the PE is induced and specified in the mesoderm, although a number of studies suggest that vascular endothelial growth factor (VEGF), BMPs, tumor growth factor-β (TGFβ), and FGFs have important roles in PE development and differentiation [14, 26, 33]. The PE develops from the mesothelium that overlies the liver bud, suggesting that liver primordia may play an important role in PE induction.

Experiments from Ishii et al. [12] have shown that the expression of proepicardial marker genes Wt1, capsulin (epicardin, pod1, Tcf21), and Tbx18 are induced in naïve mesothelial cells by the liver bud, both in vitro and in vivo. Their results suggest that after a specific developmental stage, a large area of the mesothelium becomes competent to express proepicardial marker genes in response to localized liver-derived signals but not signals from other endoderm-derived tissue. The nature of these specific signals still remains to be elucidated.

Formation of the Epicardium

After PE formation, a key event in coronary vasculogenesis is formation of the epicardium. As depicted in Fig. 2, at approximately 9.25 dpc in mouse development, cells from the PE begin to migrate and make contact with the developing heart tube. Groups of epithelial cells move over the heart and eventually form a continuous epithelial sheet to form the epicardium. Although little is known about the underlying mechanisms that control this migration, it clearly is tightly regulated. The PE cells move in a directed manner over the heart and therefore must maintain motility and adhesive characteristics, suggesting that regulation of cell adhesion molecules is critical for this process. Consistent with this, findings have shown that vascular cell adhesion molecule-1 (VCAM-1) and $\alpha_4\beta_1$-integrin knockout mice die in early embryogenesis and lack an epicardium, suggesting that EMT- or EMT-like transitions occur early as PE cells differentiate to form epicardium [34, 39].

Formation of SEMCs

Formation of SEMCs from epicardium requires an EMT that involves $\alpha_4\beta_1$-integrin, WT-1, GATA, VEGF, and FGF: By approximately 12.5 dpc in mice, the epicardium completely surrounds the heart, and some cells lose contact with the epicardial epithelium to become freely migrating mesenchyme undergoing an EMT. These mesenchymal cells move into the subepicardial space and give rise to a population of SEMCs. For this epicardial EMT, findings have shown that VCAM-1, $\alpha_4\beta_1$-integrin, and two zinc finger proteins are required, namely, Wilm's tumor-1 (WT-1) and Friend of GATA 2 (FOG-2), a GATA4 cofactor [32]. The FOG-2-null mice form a complete epicardium but lack mesenchyme and fail to form coronary vessels because of an inability to undergo EMT [8, 35]. Consistent with this, mice homozygous for GATA4 mutant alleles that inactivate the FOG-2-binding domain phenocopy FOG-2-null animals, indicating that the FOG-2/GATA4 complex is required for EMT.

Explant studies also have identified a number of factors presumed to be myocardially derived that can regulate EMT. These include VEGF and FGF, both of which have been

shown to stimulate EMT of avian epicardial cells in vitro [21]. Subepicardial mesenchymal cells migrate throughout the myocardium, where they coalesce to form channels within the connective space to give rise first to endothelial tubes within the myocardium, which subsequently recruit additional SEMCs to differentiate into smooth muscle cells around the endothelial tubes, ultimately linking through a vasculogenic process to form the coronary vessels.

Proepicardium-Derived Nonepicardial Origins of SEMCs

The epicardium appears to originate many of the SEMCs that populate the subepicardial mesenchymal space [27]. However, cell-labeling studies show that epicardially derived mesenchymal cells become smooth muscle cells and adventitial fibroblasts of coronary arteries but not endothelial cells [9]. This suggests that another, separate population of cells move into the subepicardial space and give rise to endothelial cells. The origin of these precursor cells is not known, but it is suggested that they may migrate from the mesothelium of the PE, the dorsal body wall of the intraembryonic coelom, or the dorsal mesocardium near the liver [27, 29, 30, 36].

The fate-mapping studies of Mikawa and Fischman [19] demonstrate that the three distinct lineages of the coronary vasculature are already segregated in the PE before the time of migration into the heart. Together, these observations raise the possibility that in addition to an epicardial origin for SEMCs, there may exist a PE-derived but non-epicardial origin for some SEMCs. The ultimate origin of these cells, however, remains controversial, with some studies suggesting a ST or liver origin [30]. As discussed later, our recent results are consistent with the idea that SRF may be an important regulator of a nonepicardially derived population of SEMCs, suggesting that SRF also may be important for differentiation of a PE-derived endothelial cell population.

The Role of SRF in Early Vascular Development

Serum response factor, a member of the MADS (MCM1, Agamous and Deficiens, SRF) box family of transcription factors, is essential for regulation of early embryonic development [2]. Various lines of evidence discussed later indicate that SRF has a key role in controlling myogenic and nonmyogenic genes that are important and likely to be important for PE-mediated vasculogenesis.

The Definitive Role of SRF During PE-Mediated Vasculogenic Differentiation and Expression of Vascular Smooth Muscle Cell (VSMC) Markers—Using explanted quail PE cells, Landerholm et al. [15] showed that SRF is induced before, and required for, the differentiation of PE cells to smooth muscle cells and that overexpression of SRF is sufficient to induce the expression of smooth muscle markers. As their findings show, before differentiation, explants do not express SRF or smooth muscle markers, but after 24 h in culture, SRF RNA and protein are expressed, as are smooth muscle markers. Their work also suggests that SRF expression contributes to the specification and differentiation of mesenchymal cells in vivo. Whereas SEMCs strongly express SRF as they form the coronary vasculature, the PE-derived epicardium does not express SRF.

Landerholm et al. [15] have interpreted their observations in avian systems to indicate that induction of SRF is important because epicardial cells differentiate to coronary VSMCs. These results are consistent with a variety of studies showing that SRF is a critical regulator of genes associated with the myogenic phenotype. We also have shown that SRF is required for expression of both structural and nonstructural genes important for myofibril assembly and function [3]. However, because Landerholm et al. [15] did not observe SRF expression

in the PE, they did not consider other roles for SRF. As discussed later, other evidence raises the possibility of a broader role for SRF in PE cell differentiation in mammalian systems.

In addition, SRF is important for mediating gene expression in endothelial cells, and findings have shown that SRF is critical for VEGF-induced endothelial cell migration and proliferation [7].

SRF as a Regulator of the Vasculogenesis of PE-Derived Nonepicardial SEMCs—In mouse, we found that endogenous SRF is expressed in a subset of PE cells before migration over the heart [24]. This subset of cells may contribute directly to the coronary vasculature without forming epicardium, raising the possibility that SRF may be an important regulator of a population of PE non-epicardially derived SEMCs.

We also have identified a DNA sequence, termed the proepicardial organ enhancer, in the SRF promoter that controls PE-specific expression of the SRF gene. This enhancer is a 270-bp, E-box/Ets-dependent module that confers PE-specific expression to the SRF promoter, which tentatively has been termed the proepicardial organ enhancer. It is important to note that the expression of intact SRF gene also throughout the myocardium implies that the proepicardial organ enhancer directs expression only to a subset of the cells that express the intact gene. Significantly, this proepicardial organ enhancer directs LacZ reporter expression in a subpopulation of cells within the PE that appear to be marked for vasculogenesis because expression is seen only PE and then in the SEMCs and subsequent nascent coronary vessels but not in the myocardium (Fig. 3). Furthermore, proepicardial organ enhancer–driven LacZ reporter expression is not apparent in the epicardium, similar to expression of the endogenous gene.

The most direct interpretation of these results is that SRF expression is required to differentiate a population of PE cells that directly populate the subepicardial space, contributing to the SEMC population without first differentiating to the epicardium. Although it cannot be ruled out that as SRF-expressing PE cells migrate to form the epicardium, SRF expression is repressed and then reinduced as epicardial cells differentiate to SEMCs. This seems less likely due to the long half-life of the β-galactosidase marker used. This interpretation also is consistent with our observation that in mouse, endogenous SRF, which also has a relatively long half-life of approximately 12–16 h, also is not reliably seen early throughout the epicardium (Kolander and Misra, unpublished observations). These observations are consistent with the model proposed by Mikawa and Fischman [19], in which the PE consists of a chimeric population of cells destined to become distinct cell types of the coronary vasculature [20].

The aforementioned results indicate multiple important roles for SRF in PE development. One role for SRF is to regulate myogenic differentiation. However, the documented ability of SRF to regulate numerous nonmyogenic processes, such as neuronal cell plasticity [13], EMT in carcinoma cells [31], and control of various genes associated with cell growth, suggests that it likely functions in other aspects of PE differentiation in addition to myogenesis. Later, we briefly discuss a potential role for SRF in EMT and migration during PE-mediated vasculogenesis.

SRF and EMT: Does SRF Play a Role in EMT in the PE or in the Epicardial-to-SEMC Transition?—As a critical developmental process, EMT is important for distributing cells from epithelia. Based on the observation that many SRF target genes are important for processes underlying EMT, such as cell movement and motility, it is intriguing to speculate that SRF is involved in controlling the EMTs associated with early coronary vasculogenesis.

A role for SRF as a regulator of EMT is consistent with a well-documented and important role for SRF in regulating Rho-dependent actin and cytoskeletal rearrangements [17]. More direct evidence of a role for SRF in controlling EMT comes from studies investigating multistage models of skin carcinogenesis [31]. In these studies, high SRF levels were correlated with stages of mesenchymal transition, with both SRF-binding activity and protein upregulated during mouse skin tumor progression. Furthermore, in mesenchymal tumor cells, the SRF target genes c-*fos*, *actin*, and *vinculin* were upregulated. Significantly, a dominant inhibitory version of SRF downregulated changes in stress fibers associated with mesenchymal transformation.

SRF and the Epicardial-to-SEMC Transition: During PE-mediated vasculogenesis, at least two significant EMT or EMT-like transitions occur. First, after formation of the epicardium, some epicardial cells become migratory mesenchyme and move into the subepicardial space to form SEMCs. Cells involved in this migration undergo EMT [9, 10, 15, 28, 37].

Majesky and co-authors [15] showed that avian PE explant cultures initially form an epithelial colony that is positive for the epicardial marker cytokeratin and that transcripts for flk-1, Nkx-2.5, GATA4, or smooth muscle markers are undetectable, indicating an absence of endothelial, myocardial, or preformed smooth muscle cells. In freshly isolated PE cells, SRF is undetectable. By 24 h, cytokeratin-positive cells become smooth muscle α-actin-positive. By 72 h, a subset of epicardial cells shows rearrangement of cytoskeletal actin, focal adhesion formation, acquisition of a motile phenotype consistent with EMT, and strong expression of SRF. The EMT of these cells coordinates with expression of the smooth muscle (SM) cell markers calponin, SM22α, and SM γactin.

Expression of these smooth muscle markers requires transcriptionally competent SRF because inhibitory versions of SRF block smooth muscle differentiation. Although inhibitory versions of SRF were able to block expression of smooth muscle markers in these studies, they did not appear to have any effect on mesenchymal transformation as assessed by cytokeratin marker expression. This suggests that at least in avian explant systems, SRF might not be required for the EMT associated with epicardial-to-SEMC differentiation. Whether a similar situation occurs in vivo remains to be determined.

In both quail and mouse systems, SRF is expressed in SEMCs. This is consistent with a model in which SRF may not be required for epicardial-to-SEMC EMT. Rather, SRF is expressed later as SEMCs differentiate to vascular smooth muscle cells and other cell types, possibly under the influence of myocardium-derived factors.

SRF and Migration of Non-Epicardially Derived SEMCs: As discussed earlier, some evidence suggests that at least a subset of the SEMCs populating the subepicardial space have a nonepicardial origin. Although the nature of the transition undergone by these cells is not well documented, it is reasonable to assume that these PE-derived cells must have a directed migration to the SEMC, which appears to occur in an EMT-like manner [23]. Consistent with this idea, molecules associated with EMT are expressed in the PE, including WT-1, α4-integrin, and blood vessel/epicardial substance (BVES).

A potential role for SRF in mediating this process also is supported by our observations in SRF knockout studies [3]. We have found that in SRF-null cardiomyocyte cultures, WT-1 and BVES gene expression is downregulated, and that the WT-1 promoter contains SRF-binding sites, suggesting that SRF may play a role in regulating these genes, which are known to be important for the PE-epicardium transition.

Significantly, our data indicate that SRF regulates numerous other molecules important for cell adhesion that likely are involved in cell PE-EMT- or EMT-like transitions. These include integrin family members (including notably β_1), cadherins, the tight junction protein-1 (ZO-1), VEGF, FGF-2, moesin, thrombospondin, syndecan-2, and junctophilin-2, among others. These observations, together with the observations that SRF appears to be expressed in a subset of PE cells and in SEMCs but not in the epicardium, suggest the hypothesis that SRF expression may be required for the EMT-like transition and migration of PE-derived nonepicardial SEMC precursors as they move from the PE to populate the subepicardial space.

Summary and Model for SRF Action During Early Coronary Vasculogenesis

In summary, the following lines of evidence support an important role for SRF as a regulator of PE-mediated vascular development: (1) SRF, expressed in a vasculogenic pattern, is present before vascular differentiation in the PE; (2) SRF is required for expression of myogenic genes necessary for VSMC differentiation; and (3) SRF controls expression of at least an important subset of nonmyogenic genes shown to be important for vasculogenic EMT.

A Model for the Role of SRF in Early Coronary Vascular Development

As summarized in Fig. 4, we hypothesize that in mammals, SRF is important for at least two distinct yet critical aspects of PE development. First, we posit that SRF is absolutely necessary to control expression of myogenic genes important for differentiation of vascular smooth muscle from PE-derived progenitors, including the epicardium.

Second, we propose that SRF regulates genes important for EMT and EMT-like transitions that occur both during early PE development before expression of vascular markers and migration from the PE and later during EP and SEMC differentiation. Chromatin immunoprecipitation analyses and gene array studies in SRF knockout cells (R. Misra and R. Balza, unpublished data) [3] indicate direct SRF binding to the GATA4 promoter. Therefore, we also hypothesize that SRF is required for expression of GATA4 in the splanchnic mesoderm, and thus that GATA control of PE formation from ST also may be an SRF-regulated process. Because SRF is known to be important for differentiation of SEMCs to VSMCs, we also postulate that SRF is critical for SEMC differentiation to coronary vascular endothelial cells.

Features of this model await further experimental investigation, as do numerous details of the molecular mechanisms and pathways by which SRF exerts regulatory control over vasculogenesis. It also will be interesting to see whether SRF plays a more general role in these aspects of vasculogenesis in other tissues.

Acknowledgments

We thank Drs. John Lough, Stephen Duncan, and Mary Holtz for helpful discussions. Because this review was necessarily limited in scope and size, the author apologizes in advance to those whose work may not have been cited. This study was supported by NIH: R01 HL084636, and Advancing Healthier Wisconsin Grant, and the Sophia Wolffe Quadracci Memorial Fund for Stem Cell Research.

References

1. Alsan BH, Schultheiss TM. Regulation of avian cardio-genesis by FGF8 signaling. Development. 2002; 129:1935–1943. [PubMed: 11934859]

2. Arsenian S, Weinhold B, Oelgeschlager M, Ruther U, Nordheim A. Serum response factor is essential for mesoderm formation during mouse embryogenesis. EMBO J. 1998; 17:6289–6299. [PubMed: 9799237]
3. Balza RO Jr, Misra RP. Role of the serum response factor in regulating contractile apparatus gene expression and sarcomeric integrity in cardiomyocytes. J Biol Chem. 2006; 281:6498–6510. [PubMed: 16368687]
4. Barron M, Gao M, Lough J. Requirement for BMP- and FGF-signaling during cardiogenic induction in nonprecardiac mesoderm is specific, transient, and cooperative. Dev Dyn. 2000; 218:383–393. [PubMed: 10842364]
5. Bernanke DH, Velkey JM. Development of the coronary blood supply: changing concepts and current ideas. Anat Rec. 2002; 269:198–208. [PubMed: 12209558]
6. Brand T. Heart development: molecular insights into cardiac specification and early morphogenesis. Dev Biol. 2003; 258:1–19. [PubMed: 12781678]
7. Chai J, Jones MK, Tarnawski AS. Serum response factor is a critical requirement for VEGF signaling in endothelial cells and VEGF-induced angiogenesis. FASEB J. 2004; 18:1264–1266. [PubMed: 15180964]
8. Crispino JD, Lodish MB, Thurberg BL, Litovsky SH, Collins T, Molkentin JD, Orkin SH. Proper coronary vascular development and heart morphogenesis depend on interaction of GATA-4 with FOG cofactors. Genes Dev. 2001; 15:839–844. [PubMed: 11297508]
9. Dettman RW, Denetclaw W Jr, Ordahl CP, Bristow J. Common epicardial origin of coronary vascular smooth muscle, perivascular fibroblasts, and intermyocardial fibroblasts in the avian heart. Dev Biol. 1998; 193:169–181. [PubMed: 9473322]
10. Gittenberger-de Groot A, Peeters MV, Mentink M. Epicardium-derived cells contribute a novel population to the myocardial wall and the atrioventricular cushions. Circ Res. 1998; 82:1043–1052. [PubMed: 9622157]
11. Harvey RP. Patterning the vertebrate heart. Nat Rev Genet. 2002; 3:544–556. [PubMed: 12094232]
12. Ishii Y, Langberg JD, Hurtado R, Lee S, Mikawa T. Induction of proepicardial marker gene expression by the liver bud. Development. 2007; 134:3627–3637. [PubMed: 17855432]
13. Knoll B, Kretz O, Fiedler C, Alberti S, Schutz G, Frotscher M, Nordheim A. Serum response factor controls neuronal circuit assembly in the hippocampus. Nat Neurosci. 2006; 9:195–204. [PubMed: 16415869]
14. Kruithof BP, van Wijk B, Somi S, Kruithof-de Julio M, Perez Pomares JM, Weesie F, Wessels A, Moorman AF, van den Hoff MJ. BMP and FGF regulate the differentiation of multipotential pericardial mesoderm into the myocardial or epicardial lineage. Dev Biol. 2006; 295:507–522. [PubMed: 16753139]
15. Landerholm TE, Dong XR, Lu J, Belaguli NS, Schwartz RJ, Majesky MW. A role for serum response factor in coronary smooth muscle differentiation from proepicardial cells. Development. 1999; 126:2053–2062. [PubMed: 10207131]
16. Lough J, Sugi Y. Endoderm and heart development. Dev Dyn. 2000; 217:327–342. [PubMed: 10767078]
17. Lu J, Landerholm T, Wei J, Dong X-R, Wu S-P, Liu X, Nagata K, Inagaki M, Majesky M. Coronary smooth muscle differentiation from proepicardial cells requires RhoA-mediated actin reorganization and p160 Rho-kinase activity. Dev Biol. 2001; 240:404–418. [PubMed: 11784072]
18. Mikawa, T. Cardiac lineages. In: Harvey, RP.; Rosenthal, N., editors. Heart development. San Diego: Academic Press; 1999. p. 19-33.
19. Mikawa T, Fischman DA. Retroviral analysis of cardiac morphogenesis: discontinuous formation of coronary vessels. Proc Natl Acad Sci USA. 1992; 89:9504–9508. [PubMed: 1409660]
20. Mikawa T, Gourdie R. Pericardial mesoderm generates a population of coronary smooth muscle cells migrating into the heart along with ingrowth of the epicardial organ. Dev Biol. 1996; 174:221–232. [PubMed: 8631495]
21. Morabito CJ, Dettman RW, Kattan J, Collier JM, Bristow J. Positive and negative regulation of epicardial-mesenchymal transformation during avian heart development. Dev Biol. 2001; 234:204–215. [PubMed: 11356030]

22. Morabito C, Kattan J, Bristow J. Mechanisms of embryonid coronary artery development. Curr Opin Cardiol. 2002; 17:235–241. [PubMed: 12015472]
23. Munoz-Chapuli R, Macias D, Gonzalez-Iriarte M, Carmona R, Atencia G, Perez-Pomares JM. The epicardium and epicardial-derived cells: multiple functions in cardiac development. Rev Esp Cardiol. 2002; 55:1070–1082. [PubMed: 12383393]
24. Nelson TJ, Duncan SA, Misra RP. Conserved enhancer in the serum response factor promoter controls expression during early coronary vasculogenesis. Circ Res. 2004; 94:1059–1066. [PubMed: 15001533]
25. Olivey HE, Compton LA, Barnett JV. Coronary vessel development: the epicardium delivers. Trends Cardiovasc Med. 2004; 14:247–251. [PubMed: 15451517]
26. Olivey HE, Mundell NA, Austin AF, Barnett JV. Transforming growth factor-beta stimulates epithelial-mesenchymal transformation in the proepicardium. Dev Dyn. 2006; 235:50–59. [PubMed: 16245329]
27. Perez-Pomares JM, Macias D, Garcia-Garrido L, Munoz-Chapuli R. Contribution of the primitive epicardium to the subepicardial mesenchyme in hamster and chick embryos. Dev Dyn. 1997; 210:96–105. [PubMed: 9337131]
28. Perez-Pomares JM, Macias D, Garcia-Garrido L, Munoz-Chapuli R. The origin of the subepicardial mesenchyme in the avian embryo: an immunohistochemical and quail-chick chimera study. Dev Biol. 1998; 200:57–68. [PubMed: 9698456]
29. Perez-Pomares JM, Carmona R, Gonzalez-Iriarte M, Atencia G, Wessels A, Munoz-Chapuli R. Origin of coronary endothelial cells from epicardial mesothelium in avian embryos. Int J Dev Biol. 2002; 46:1005–1013. [PubMed: 12533024]
30. Poelmann RE, Gittenberger-de Groot AC, Mentink MM, Bokenkamp R, Hogers B. Development of the cardiac coronary vascular endothelium, studied with antiendothelial antibodies, in chicken-quail chimeras. Circ Res. 1993; 73:559–568. [PubMed: 8348697]
31. Psichari E, Balmain A, Plows D, Zoumpourlis V, Pintzas A. High activity of serum response factor in the mesenchymal transition of epithelial tumor cells is regulated by RhoA signaling. J Biol Chem. 2002; 277:29490–29495. [PubMed: 12039949]
32. Reese DE, Mikawa T, Bader DM. Development of the coronary vessel system. Circ Res. 2002; 91:761–768. [PubMed: 12411389]
33. Schlueter J, Manner J, Brand T. BMP is an important regulator of proepicardial identity in the chick embryo. Dev Biol. 2006; 295:546–558. [PubMed: 16677627]
34. Sengbusch JK, He W, Pinco KA, Yang JT. Dual functions of [alpha]4[beta]1 integrin in epicardial development: initial migration and long-term attachment. J Cell Biol. 2002; 157:873–882. [PubMed: 12021259]
35. Tevosian SG, Deconinck AE, Tanaka M, Schinke M, Litovsky SH, Izumo S, Fujiwara Y, Orkin SH. FOG-2, a cofactor for GATA transcription factors, is essential for heart morphogenesis and development of coronary vessels from epicardium. Cell. 2000; 101:729–739. [PubMed: 10892744]
36. Van den Eijnde SM, Wenink AC, Vermeij-Keers C. Origin of subepicardial cells in rat embryos. Anat Rec. 1995; 242:96–102. [PubMed: 7604987]
37. Vrancken Peeters MP, Gittenberger-de Groot AC, Mentink MM, Poelmann RE. Smooth muscle cells and fibroblasts of the coronary arteries derive from epithelial-mesenchymal transformation of the epicardium. Anat Embryol. 1999; 199:367–378. [PubMed: 10195310]
38. Watt AJ, Battle MA, Li J, Duncan SA. GATA4 is essential for formation of the proepicardium and regulates cardiogenesis. Proc Natl Acad Sci USA. 2004; 101:12573–12578. [PubMed: 15310850]
39. Yang JT, Rayburn H, Hynes RO. Embryonic mesodermal defects in alpha 5 integrin-deficient mice. Development. 1993; 119:1093–1105. [PubMed: 7508365]

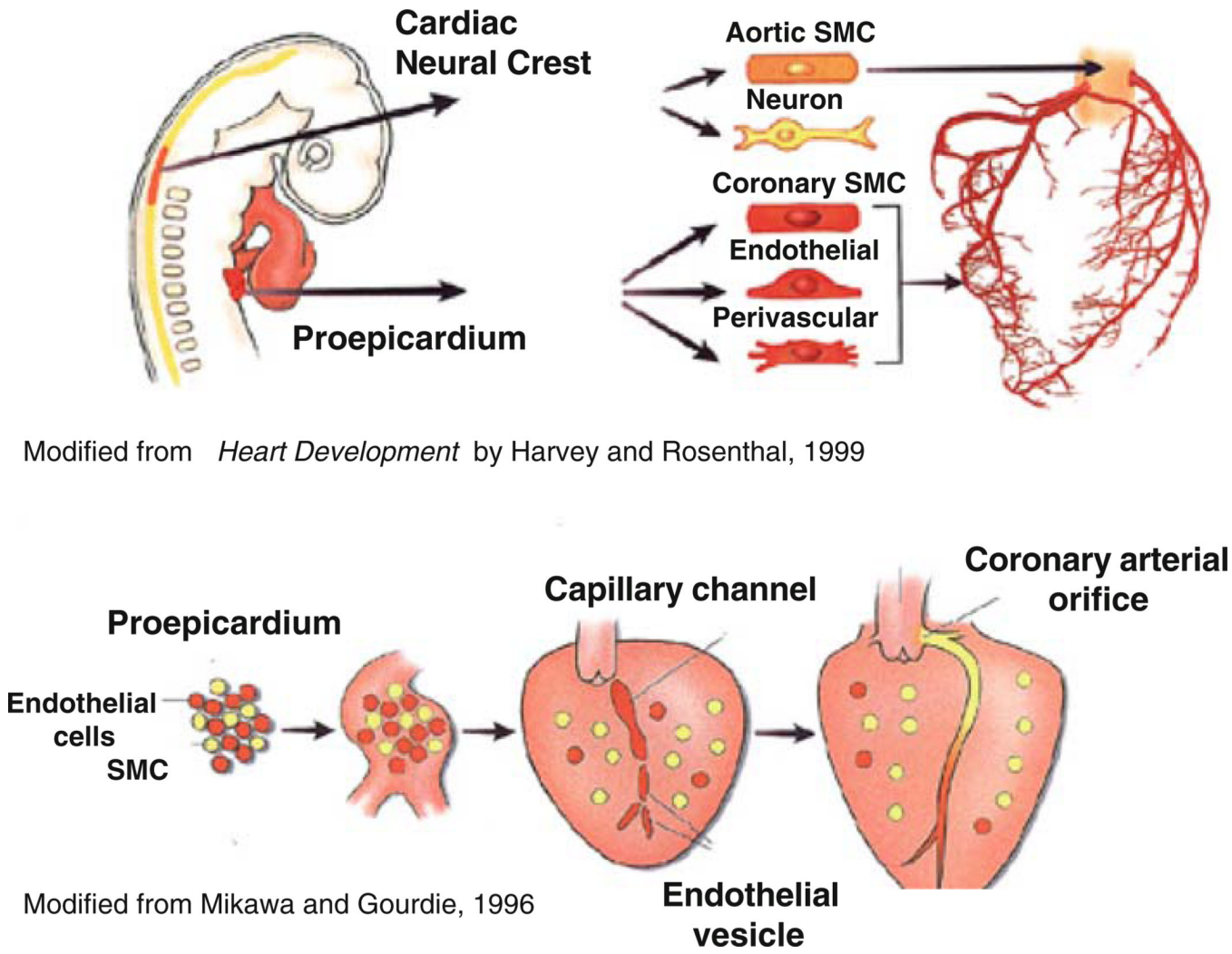

Fig. 1.

Model for the proepicardium (PE)-derived generation of coronary vasculature. The *top* shows lineage of cell types, and the *bottom* shows independent PE-derived smooth muscle cell (SMC) (*yellow/light circles*) and endothelial cell (*red darker/circles*) precursors migrating to the tubular heart. Adapted from [18]

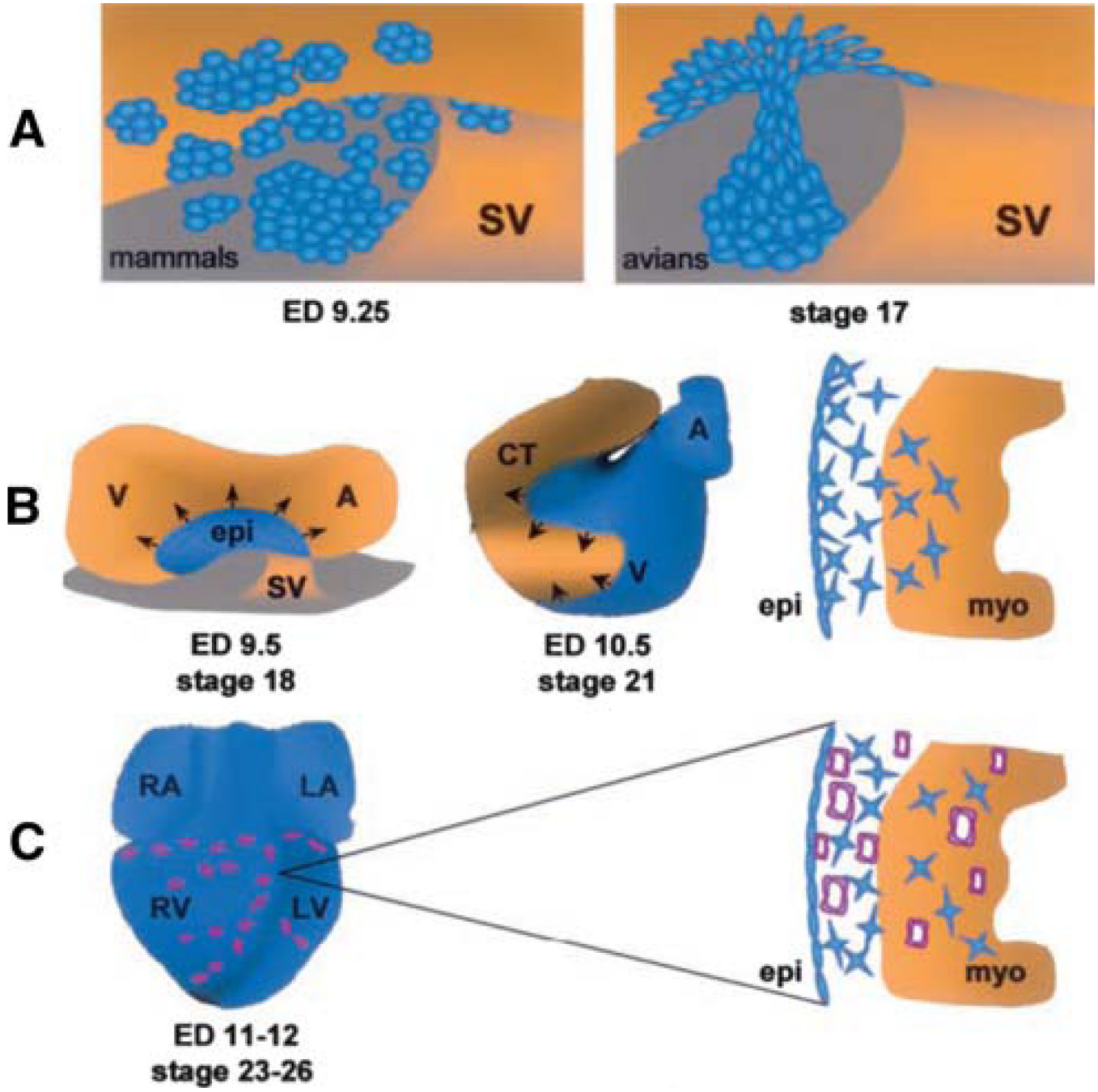

Fig. 2.

Proepicardium (PE)-derived early vasculogenesis. **a** PE transfer and attachment to the myocardium in mammals and avians. At approximately 9.25 days postcoitum (dpc) in the mouse, clusters of cells detach from the PE and travel across the pericardial space to the atrium and ventricle. At attachment, clusters flatten into a monolayer and coalesce to form initial epicardium. In avians, PE cells migrate across an extracellular matrix bridge (PEO) between the PE and the myocardium, then migrate radially from the point of attachment. **b** Epicardial migration and epithelial-to-mesenchymal transition (EMT). The PE-derived cells migrate and proliferate across the surface of the myocardium to form the epicardium (epi). The migration is similar in mammals and avians. Epicardial EMT begins soon after contact

with the myocardium. Epicardially derived mesenchymal cells (SEMCs) are depicted entering the subepicardial space and the myocardium. **c** Vasculogenic assembly. SEMCs coalesce to form endothelial vesicles (*purple/rectangles*). Both subepicardial and intramyocardial vesicles are shown surrounded by epicardially derived mesenchyme (*blue/stars*), which then coalesce to form nascent coronary vessels. Sinus venosus (SV) = septum transversum (ST). Adapted from [25]

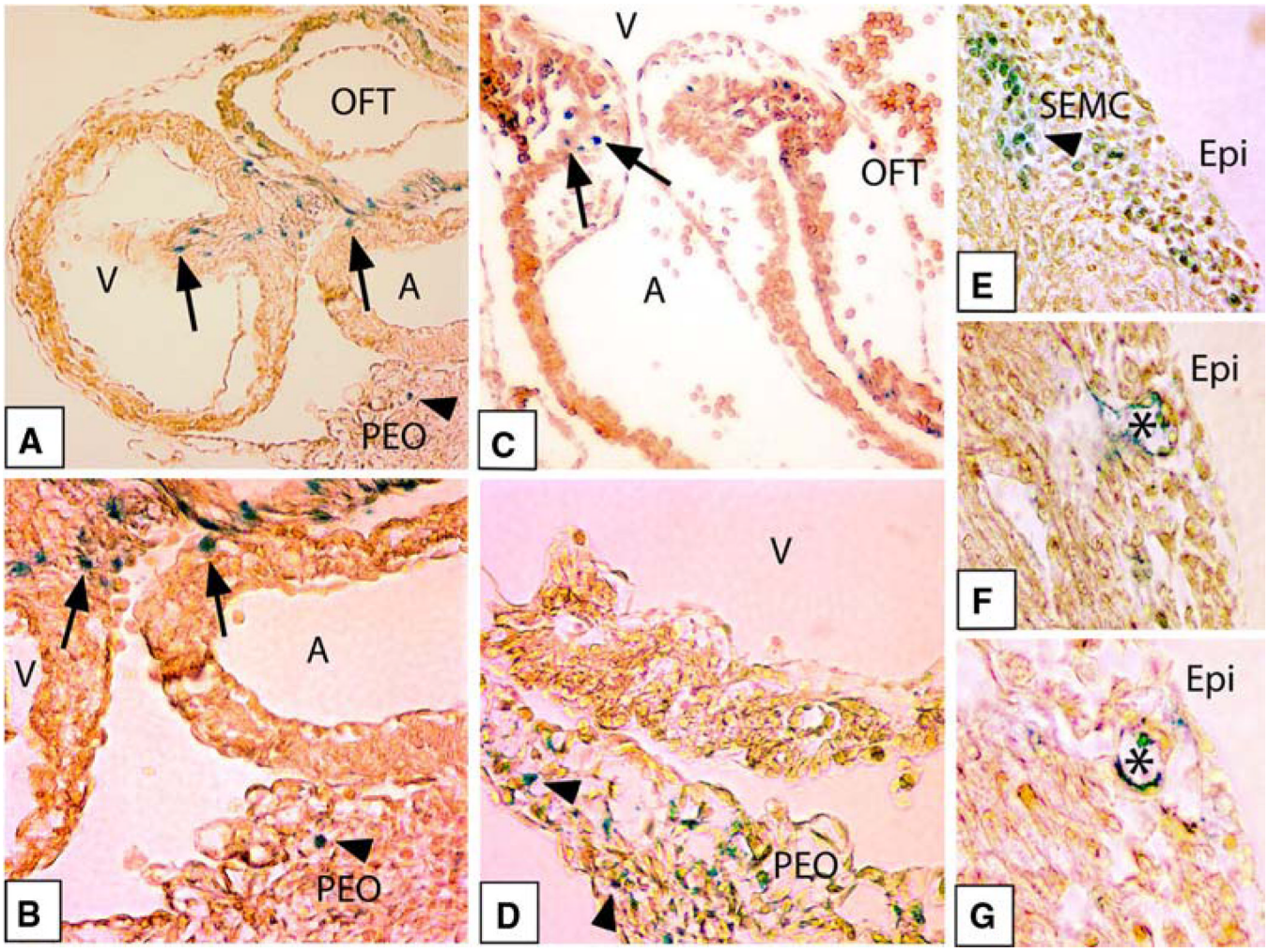

Fig. 3.

The proepicardial organ (PEO)-enhancer-LacZ construct expresses in the proepicardium (PE) and nascent coronary vessels. **a, b** E9.5 embryos containing a PE-LacZ reporter express in cells within the atrium and ventricle (AV) (*arrow*) and the PE (PEO, *arrowhead*). **c, d** E10.5 embryos containing the PE-LacZ reporter express in cells within the Cushing tissue between the AV junction (*arrow*) and the expanding PE (ePE) (*arrowhead*). **e–g** E14.5 embryos containing the ePE-LacZ reporter express in cells within the subepicardial mesenchymal cells (SEMCs) (*arrowhead*) and nascent coronary vessels (*asterisk*) without expressing in the epicardium (epi). Adapted from [24]

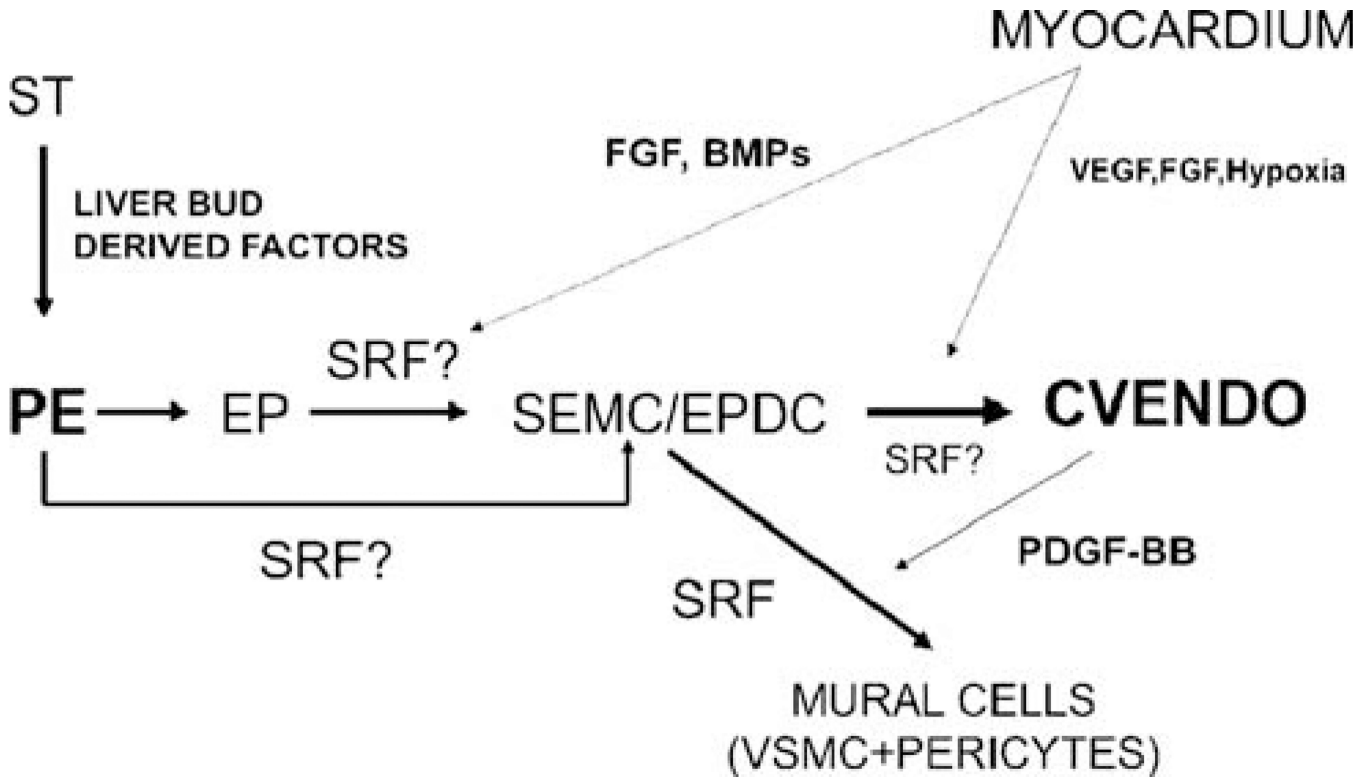

Fig. 4.

Model for proepicardium (PE)-mediated vascular development. In this model, PE arises from the septum transversum (ST) under the influence of liver bud-derived signals, and PE and epicardium (EP) can differentiate to subepicardial mesenchymal cells (SEMCs) under the influence of myocardially released factors. The SEMCs differentiate to endothelial cells under the influence of local cardiac conditions including secretion of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and tissue hypoxia. Endothelial cell-secreted factors, including platelet-derived growth factor (PDGF)-BB, induce SEMCs to differentiate to mural cells