Published in final edited form as:
Semin Cell Dev Biol. 2007 February ; 18(1): 84–89. doi:10.1016/j.semcdb.2006.12.013.

Signals from both sides: Control of cardiac development by the endocardium and epicardium

Travis K. Smith and **David M. Bader***
The Stahlman Cardiovascular Research Laboratories, Program for Developmental Biology, Department of Medicine, Vanderbilt University Medical Center, 2200 Pierce Ave, 348 Preston Research Building, Nashville, TN 37232-6300, USA

Abstract

It is readily apparent that the process of heart development is an intricate one, in which cells derived from many embryonic sources coalesce and coordinate their behaviors and development, resulting in the mature heart. The behaviors and mechanisms of this process are complex, and still incompletely understood. However, it is readily apparent that communication between diverse cell types must be involved in this process. The signaling that emanates from epicardial and endocardial sources is the focus of this review.

Keywords

Endocardium; Epicardium; FGF; Retinoic acid; Neuregulin-1

1. Epicardial and endocardial signaling during cardiac development

Exquisite control and regulation is required for development of a properly functioning heart. As the heart progresses from mesoderm to a simple tube to a complex four-chambered structure, each step along this progression requires precise regulation of cellular behavior by a variety of sources. The hypoblast [1,2], epiblast [3], endoderm [4], neural tissue [5-8], endocardium [9-13], and epicardium [9,10,14] all signal to developing/mature muscle or cells that are fated to become myocardium during development. Additionally, the developing/mature myocardium emits signals to several of these structures (as well as to itself) that are necessary for proper development and maintenance of the cardiovascular system [15-19]. The proper transmittal of these signals is dependent on cellular emission of signals, cellular response to these signals, and morphogenetic movements of the organ and embryo that manipulate these communicating cells into the proper position for this communication to occur. Our focus here is to review signaling from the epicardium and endocardium, focusing on events that occur during embryonic development.

2. Embryonic origins of the endocardium, epicardium, and myocardium

While a thorough discussion of the early embryonic development of the components of the mature heart is not within the scope of this review, a brief review of the embryonic origins and mechanisms that give rise to these tissues is useful for this discussion. Numerous excellent and more exhaustive reviews of endocardial [20-24], epicardial [25-29], and myocardial [30-32] development and origins are available. To summarize, endocardial and myocardial precursors

*Corresponding author. Tel.: +1 615 936 1974; fax: +1 615 936 3527. david.bader@vanderbilt.edu (D.M. Bader).

originate in lateral splanchnic mesoderm [33-35]. These cells coalesce to form the cardiac crescent, which then fuses to become the primitive heart tube [36]. The rhythmically contracting primitive heart tube at this point consists of a tube of epithelial endocardium surrounded by an epithelial tube of myocardium, and a layer of cardiac jelly separates these two tubes. This tube displays polarity along the anterior–posterior axis, as gene products expressed at this stage demonstrate that some early cell fate decisions have been made [36-38]. The linear heart tube then begins the process of looping, which establishes the position of the future chambers of the heart. Through this process, the presumptive atria are positioned anterior to the portion of the heart tube that gives rise to the ventricles. During this process of looping, the myocardium induces cells of the endocardium to delaminate and migrate into the cardiac jelly that separates the epithelial myocardium and the epithelial endocardium [39,40]. These cells migrate into the cardiac cushions and undergo differentiation into components of the valves. Endocardial cushions develop in multiple locations during heart development and give rise to several septal/valvular structures [22], along with migratory neural crest that invade the heart and contribute to the aorticopulmonary septum [41]. During the process of looping, the epithelial proepicardium migrates to the surface of the myocardium, proliferates rapidly, and covers the surface of the myocardium [42-44]. This epithelium persists through development and adulthood, comprising the epicardium and pericardium. A subpopulation of cells of the epicardium undergo an epithelial–mesenchymal transition, delaminate from the epithelial epicardium, and infiltrate the developing myocardium [42,45]. These mesenchymal cells give rise to the smooth muscle, vascular endothelium, and cardiac fibroblasts of the coronary vasculature [42].

3. Signals from the endocardium

The endocardium secretes signals that act upon other components of the developing heart in a paracrine manner, as well as factors that act upon the endocardium itself in an autocrine fashion. The endocardium also receives and responds to cues from other tissue sources during heart development, such as VEGF signaling from the myocardium that represses delamination of cells of the endocardial epithelium prior to endocardial cushion formation [17]. These interactions lie outside the scope of this review. In this section, signals from the endocardium to the developing myocardium, cardiac mesenchyme, and endocardium will be discussed (Table 1).

3.1. Neuregulin-1

During heart development, one of the first myocardial activities demonstrated to be affected by signaling from the endocardium is trabeculation of the myocardium. The EGF family member neuregulin-1 (NRG-1), which is expressed at a high level in the endocardium during development, has been demonstrated to act on both myocardium [13,46-50] and endocardial mesenchyme [51]. Neuregulin-1 secreted from the endocardium stimulates heterodimerization of $ErbB_2$ and Erb_4, activating downstream signaling pathways that have been demonstrated to be critical for early cardiac trabeculation via both inactivation of neuregulin-1 [48] and the $ErbB_2$ receptor [47]. NRG-1 also has recently been shown to be sufficient to induce differentiation of cardiomyocytes to components of the cardiac conduction system [13,49, 50]. Through interaction with IGF-1, which is also secreted by the endocardium, NRG-1 controls cardiac chamber morphogenesis through activation of the phosphatidylinositiol 3-kinase [46]. The synergistic effects of IGF-1 and neuregulin-1 also show themselves later in cardiac development, as myocardium treated with these two factors simultaneously displayed an increase in DNA synthesis and expansion of the compact zone and atrioventricular cushions later in development [46]. However, NRG-1 not only acts on cells of the developing myocardium, it also triggers proliferation of endocardially derived mesenchyme during cardiac cushion development through stimulation of the $ErbB_2/ErbB_3$ receptor homodimer [51,52].

To summarize, neuregulin-1 signals from the endocardium control cellular behaviors of myocardium and endocardial mesenchyme, and affect the processes of differentiation and proliferation during cardiac development.

3.2. FGFs

Work from the Markwald laboratory demonstrated that members of the FGF signaling pathway are expressed in the epicardium, endocardium, and myocardium during heart development [53]. Mesenchymal derivatives of the endocardium express FGF-4 and FGFR1-FGFR3. This work indicated that endocardium and endocardially derived mesenchyme likely signaled in an autocrine fashion through the FGF-4 pathway, leading to downstream signal transduction through the Ras and PLC-γ pathways. *In vivo* experiments demonstrated that FGF-4 signaling induced proliferative expansion of the cushion mesenchyme during the formation of cardiac valve leaflets. The Ornitz group later showed that FGF-9, FGF-16, and FGF-20 are all expressed in the endocardium during mouse embryogenesis [9]. These FGFs signal to the myocardium through FGFR1 and FGFR2, stimulating myocyte differentiation and proliferation.

3.3. Endothelins

Endothelins, a family of small signaling peptides, signal through their G-protein coupled receptors, ET_A and ET_B [54-57]. Two enzymes, endothelin-converting enzyme-1 and -2 (ECE-1 and ECE-2), are necessary for conversion of the biologically inactive precursor endothelins into biologically active products [58,59]. Endothelins are highly expressed in various endocardial domains, and receptors for ET family members are expressed in migratory and post-migratory neural crest derivatives of the heart and the myocardium itself [60]. ECEs are expressed in the mesenchyme of endocardial cushions, mesenchyme of the brachial arch arteries, and in endocardial epithelia throughout the heart. Disruptions of endocardial endothelin signaling by inactivation of components of this pathway cause cardiac defects including defects in aortic arches, major arteries, and septum [57,60-62]. Interestingly, these septation defects involve the septation of the outflow tract and the septation of the common ventricle. Thus, endothelin signaling mediates septation by modulating behavior of neural crest derived cells of the OFT and endocardial/myocardial derived cells of the IVS.

3.4. Neurofibromin

Global inactivation of the neurofibromin (Nf1) gene in mice leads to gross cardiac anomalies that cause gestational lethality, including OFT defects, enlarged endocardial cushions, ventricular septal defects, and thinned myocardium [63-65]. The initial interpretation of this phenotype was that these defects were caused by disruption of Nf1 gene activity in neural crest cells and derivatives. However, work by the Epstein laboratory later demonstrated that endocardial specific inactivation of Nf1 gene activity was sufficient to generate a similar phenotype, indicating that endocardial Nf1 activity was more important than previously thought [66]. Additionally, neural crest or myocardium specific inactivation of Nf1 did not yield cardiac defects. Although Nf1 is not secreted from the endocardium, Nf1 activity in the endocardium serves to modulate endocardial Ras GTPase and NFATc activity during heart development [66].

3.5. SDF-1 (PBSF)

Stromal cell-derived factor 1, also called pre-B-cell growth-stimulating factor, is a member of the CXC family of chemokines [67]. Along with a role in hematopoeisis, SDF-1 has a signaling role during development of the heart. Genetic inactivation of either the SDF-1 chemokine or the specific receptor for SDF-1, CXCR4, yield mice that die embryonically and display atrioventricular septum defects [68,69]. *In situ* analysis shows that SDF-1 is expressed in the

endocardium of the mouse during cardiogenesis [68], while the CXCR4 receptor is expressed in the aorticopulmonary septum and the ventricular wall of the heart at this time [68,70]. This expression pattern indicates that endocardially derived SDF-1 signals are critical for septation of the heart.

As described here, it is clear that these endocardium-derived signals are critical for proper morphogenesis of the heart. There are undoubtedly other signals that originate from the endocardium that control developmental processes, both described and yet to be discovered. Additionally, myocardial development is also controlled by the epithelial lining on the outside of the heart muscle—the epicardium.

4. Signals from the epicardium

The proepicardium, epicardium, and epicardially derived cells are critical for normal heart development. These structures not only provide a major portion of the cells that comprise the coronary vasculature, the epicardium also plays an important role in modulating the development of the myocardium. Naturally, as diseases related to the coronary vasculature are a major cause of death, interest in understanding the normal development of the transient and definitive structures of this tissue has in recent years increased greatly. Interestingly, the mechanism by which the coronary vasculature is generated was long thought to be unique. However, recent work by Wilm and colleagues has demonstrated that a very similar mechanism generates components of the vasculature of the gut [71]. Therefore, understanding of signaling mechanisms that play a role in cardiac vasculogenesis may well have implications for development and maintenance of tissues in other organs.

Early indications that the proper development of the myocardium was dependent upon close interaction and signaling from the myocardium were noted in studies involving inactivation of transcription factors and cell adhesion molecules. Knockouts of FOG-2 [72], WT-1 [73], VCAM-1 [74], and α_4 integrin [75] all displayed cardiac defects, most notably abnormally thin myocardium. Some of these genes have expression patterns that are largely restricted to the epicardium, illustrating that disruption of normal epicardial function had deleterious effects on cardiac compaction and proliferation. Here, some of the specific factors that are involved in myocardial regulation by epicardium are discussed.

4.1. Retinoic acid signaling

Cardiovascular developmental defects in experimental settings have long been associated with improper retinoic acid (RA) levels during gestation [76,77]. However, the molecular mechanisms underlying these observations are only recently becoming more apparent. Ingested vitamin A is converted to active retinoids by a series of oxidative reactions *in vivo*. Therefore, presence of components of this biosynthetic pathway are indicators of sources of RA signaling. One of these components, RALDH-2, is highly expressed in the developing epicardium of the mouse [78] and the chicken [79], but not in the myocardium. Global inactivation of one of the receptors for RA, RXR-α, caused embryonic death resulting from cardiovascular defects including myocardial thinning [80-82]. This seemingly indicates that RA–RXR-α signals from the epicardium to the myocardium may be critical for proper cardiogenesis. However, myocardium specific inactivation of RXR-α displayed no cardiac phenotype [83]. Subsequent investigation utilizing an epicardial-specific inactivation of RXR-α yielded a similar phenotype to that of the global inactivation, pointing to the conclusion that an epicardial autocrine RA/RXR-α signaling mechanism may regulate myocardial proliferation by inducing release of another trophic factor(s) from the epicardium [84], an idea which had previously been postulated [14,85]. Early investigations indicate that two of these factors may be FGF-2, which is discussed in greater detail below.

4.2. FGFs

Mikawa and colleagues demonstrated that FGF-2 signaling from the epicardium was necessary for proliferation of myocytes in the outer compact myocardial wall [86], and further elucidation of the mechanism of the regulation of myocyte proliferation by epicardium has followed. However, previous studies had demonstrated only a mild phenotype when FGF-2 was inactivated [87,88]. Later studies demonstrated that FGF-9, -16, and -20 are all expressed in the epicardium, and that these growth factors signal to the myocardium through FGF receptors 1 and 2 [9]. Knockout of these receptors in the myocardium demonstrate that this FGF signaling is critical for proper proliferation and differentiation of the cardiac muscle. These results correspond with those stated previously, namely that RXR-α inactivation in the epicardium led to a similar phenotype, as it has been shown that RA signaling to the epicardium induces expression of FGFs [14,83,85]. Subsequently, Ornitz and colleagues have revealed a role for epicardial FGF signaling for modulation of coronary vasculogenesis as well. This series of experiments shows that epicardial FGF signals induce myocardial activation of Hedgehog activation, that in turn upregulate VEGF-A, -B, -C, and angiopoietin-2 expression [89].

To summarize, evidence indicates that autocrine and paracrine RA signaling to the epicardium induces expression of FGF family members. The epicardium uses these growth factors to signal to the myocardium, thereby controlling myocyte proliferation, differentiation, and coronary vasculogenesis.

Thus, multiple signaling pathways originating from epithelia on either side of the myocardium regulate cardiac developmental processes. Migration, differentiation, and proliferation have all been demonstrated to be regulated by these extrinsic signals. The field's increasing understanding of the developmental regulation exerted by these signaling pathways will lead to a greater understanding of human cardiovascular birth defects, which are one of the most commonly occurring birth defects. Further investigation into these signaling networks may also lead to a better understanding of how extra-myocardial signals support and control myocardial function in the adult organ.

Acknowledgments

The authors would like to thank members of our laboratory for helpful comments and discussion. DMB is supported by NIH HL37675 and NIH HL079050. TKS is supported by AHA 0415252B and NIH HL079050.

Abbreviations

CXCR	CXC chemokine receptor
ECE	endothelin converting enzyme
ErbB	epidermal growth factor receptor
ET	endothelin receptor
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FOG-2	friend of gata 2
IGF-1	insulin-like growth factor 1
IVS	interventricular septum
Nf1	neurofibromin 1
$NFAT_c$	nuclear factor of activated T cells

NRG-1	neuregulin-1
OFT	outflow tract
PBSF	pre-B-cell stimulating factor 1
PLC-γ	phospholipase C γ
RA	retinoic acid
RALDH	retinaldehyde dehydrogenase
RXR-α	retinoic acid receptor α
SDF-1	stromal cell derived factor 1
VCAM-1	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor
WT-1	Wilm's tumor 1

References

[1]. Ladd AN, Yatskievych TA, Antin PB. Regulation of avian cardiac myogenesis by activin/TGFbeta and bone morphogenetic proteins. Dev Biol 1998;204:407–19. [PubMed: 9882479]

[2]. Yatskievych TA, Ladd AN, Antin PB. Induction of cardiac myogenesis in avian pregastrula epiblast: the role of the hypoblast and activin. Development 1997;124:2561–70. [PubMed: 9216998]

[3]. Inagaki T, Garcia-Martinez V, Schoenwolf GC. Regulative ability of the prospective cardiogenic and vasculogenic areas of the primitive streak during avian gastrulation. Dev Dyn 1993;197:57–68. [PubMed: 8400411]

[4]. Schultheiss TM, Xydas S, Lassar AB. Induction of avian cardiac myogenesis by anterior endoderm. Development 1995;121:4203–14. [PubMed: 8575320]

[5]. Climent S, Sarasa M, Villar JM, Murillo-Ferrol NL. Neurogenic cells inhibit the differentiation of cardiogenic cells. Dev Biol 1995;171:130–48. [PubMed: 7556890]

[6]. Jacobson AG. Influences of ectoderm and endoderm on heart differentiation in the newt. Dev Biol 1960;2:138–54. [PubMed: 14406400]

[7]. Jacobson AG. Heart determination in the newt. J Exp Zool 1961;146:139–51. [PubMed: 14450715]

[8]. Jacobson AG, Duncan JT. Heart induction in salamanders. J Exp Zool 1968;167:79–103. [PubMed: 4870429]

[9]. Lavine KJ, Yu K, White AC, Zhang X, Smith C, Partanen J, et al. Endocardial and epicardial derived FGF signals regulate myocardial proliferation and differentiation in vivo. Dev Cell 2005;8:85–95. [PubMed: 15621532]

[10]. Kang JO, Sucov HM. Convergent proliferative response and divergent morphogenic pathways induced by epicardial and endocardial signaling in fetal heart development. Mech Dev 2005;122:57–65. [PubMed: 15582777]

[11]. Mably JD, Mohideen MA, Burns CG, Chen JN, Fishman MC. Heart of glass regulates the concentric growth of the heart in zebrafish. Curr Biol 2003;13:2138–47. [PubMed: 14680629]

[12]. Kuruvilla L, Kartha CC. Molecular mechanisms in endothelial regulation of cardiac function. Mol Cell Biochem 2003;253:113–23. [PubMed: 14619961]

[13]. Milan DJ, Giokas AC, Serluca FC, Peterson RT, MacRae CA. Notch1b and neuregulin are required for specification of central cardiac conduction tissue. Development 2006;133:1125–32. [PubMed: 16481353]

[14]. Chen TH, Chang TC, Kang JO, Choudhary B, Makita T, Tran CM, et al. Epicardial induction of fetal cardiomyocyte proliferation via a retinoic acid-inducible trophic factor. Dev Biol 2002;250:198–207. [PubMed: 12297106]

[15]. Sugi Y, Yamamura H, Okagawa H, Markwald RR. Bone morphogenetic protein-2 can mediate myocardial regulation of atrioventricular cushion mesenchymal cell formation in mice. Dev Biol 2004;269:505–18. [PubMed: 15110716]

[16]. Ma L, Lu MF, Schwartz RJ, Martin JF. Bmp2 is essential for cardiac cushion epithelial–mesenchymal transition and myocardial patterning. Development 2005;132:5601–11. [PubMed: 16314491]

[17]. Chang CP, Neilson JR, Bayle JH, Gestwicki JE, Kuo A, Stankunas K, et al. A field of myocardial–endocardial NFAT signaling underlies heart valve morphogenesis. Cell 2004;118:649–63. [PubMed: 15339668]

[18]. Pennisi DJ, Mikawa T. Normal patterning of the coronary capillary plexus is dependent on the correct transmural gradient of FGF expression in the myocardium. Dev Biol 2005;279:378–90. [PubMed: 15733666]

[19]. Wang J, Sridurongrit S, Dudas M, Thomas P, Nagy A, Schneider MD, et al. Atrioventricular cushion transformation is mediated by ALK2 in the developing mouse heart. Dev Biol 2005;286:299–310. [PubMed: 16140292]

[20]. Brutsaert DL, De Keulenaer GW, Fransen P, Mohan P, Kaluza GL, Andries LJ, et al. The cardiac endothelium: functional morphology, development, and physiology. Prog Cardiovasc Dis 1996;39:239–62. [PubMed: 8970576]

[21]. Brutsaert DL, Fransen P, Andries LJ, De Keulenaer GW, Sys SU. Cardiac endothelium and myocardial function. Cardiovasc Res 1998;38:281–90. [PubMed: 9709389]

[22]. Person AD, Klewer SE, Runyan RB. Cell biology of cardiac cushion development. Int Rev Cytol 2005;243:287–335. [PubMed: 15797462]

[23]. Farrell MJ, Kirby ML. Cell biology of cardiac development. Int Rev Cytol 2001;202:99–158. [PubMed: 11061564]

[24]. Lough J, Sugi Y. Endoderm and heart developmenta. Dev Dyn 2000;217:327–42. [PubMed: 10767078]

[25]. Manner J, Perez-Pomares JM, Macias D, Munoz-Chapuli R. The origin, formation and developmental significance of the epicardium: a review. Cells Tissues Organs 2001;169:89–103. [PubMed: 11399849]

[26]. Reese DE, Mikawa T, Bader DM. Development of the coronary vessel system. Circ Res 2002;91:761–8. [PubMed: 12411389]

[27]. Munoz-Chapuli R, Gonzalez-Iriarte M, Carmona R, Atencia G, Macias D, Perez-Pomares JM. Cellular precursors of the coronary arteries. Tex Heart Inst J 2002;29:243–9. [PubMed: 12484607]

[28]. Mu H, Ohashi R, Lin P, Yao Q, Chen C. Cellular and molecular mechanisms of coronary vessel development. Vasc Med 2005;10:37–44. [PubMed: 15920999]

[29]. 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;55:1070–82. [PubMed: 12383393]

[30]. Eisenberg LM, Markwald RR. Cellular recruitment and the development of the myocardium. Dev Biol 2004;274:225–32. [PubMed: 15385154]

[31]. Gittenberger-de Groot AC, Bartelings MM, Deruiter MC, Poelmann RE. Basics of cardiac development for the understanding of congenital heart malformations. Pediatr Res 2005;57:169–76. [PubMed: 15611355]

[32]. Sedmera D, Pexieder T, Vuillemin M, Thompson RP, Anderson RH. Developmental patterning of the myocardium. Anat Rec 2000;258:319–37. [PubMed: 10737851]

[33]. Stalsberg H. Regional mitotic activity in the precardiac mesoderm and differentiating heart tube in the chick embryo. Dev Biol 1969;20:18–45. [PubMed: 5795847]

[34]. Rawles M. The heart-forming region of the early chick blastoderm. Physiol Zool 1943:22–42.

[35]. Gonzalez-Sanchez A, Bader D. In vitro analysis of cardiac progenitor cell differentiation. Dev Biol 1990;139:197–209. [PubMed: 2328836]

[36]. Yutzey KE, Bader D. Diversification of cardiomyogenic cell lineages during early heart development. Circ Res 1995;77:216–9. [PubMed: 7614708]

[37]. Antin PB, Taylor RG, Yatskievych T. Precardiac mesoderm is specified during gastrulation in quail. Dev Dyn 1994;200:144–54. [PubMed: 7919500]

[38]. Redkar A, Montgomery M, Litvin J. Fate map of early avian cardiac progenitor cells. Development 2001;128:2269–79. [PubMed: 11493546]

[39]. Markwald RR, Fitzharris TP, Manasek FJ. Structural development of endocardial cushions. Am J Anat 1977;148:85–119. [PubMed: 842477]

[40]. Potts JD, Dagle JM, Walder JA, Weeks DL, Runyan RB. Epithelial–mesenchymal transformation of embryonic cardiac endothelial cells is inhibited by a modified antisense oligodeoxynucleotide to transforming growth factor beta 3. Proc Natl Acad Sci USA 1991;88:1516–20. [PubMed: 1996351]

[41]. Kirby ML, Gale TF, Stewart DE. Neural crest cells contribute to normal aorticopulmonary septation. Science 1983;220:1059–61. [PubMed: 6844926]

[42]. Mikawa T, Gourdie RG. Pericardial mesoderm generates a population of coronary smooth muscle cells migrating into the heart along with ingrowth of the epicardial organ. Dev Biol 1996;174:221–32. [PubMed: 8631495]

[43]. Viragh S, Challice CE. The origin of the epicardium and the embryonic myocardial circulation in the mouse. Anat Rec 1981;201:157–68. [PubMed: 7305017]

[44]. Viragh S, Gittenberger-de Groot AC, Poelmann RE, Kalman F. Early development of quail heart epicardium and associated vascular and glandular structures. Anat Embryol (Berl) 1993;188:381–93. [PubMed: 7506502]

[45]. Reese DE, Zavaljevski M, Streiff NL, Bader D. bves: A novel gene expressed during coronary blood vessel development. Dev Biol 1999;209:159–71. [PubMed: 10208750]

[46]. Hertig CM, Kubalak SW, Wang Y, Chien KR. Synergistic roles of neuregulin-1 and insulin-like growth factor-I in activation of the phosphatidylinositol 3-kinase pathway and cardiac chamber morphogenesis. J Biol Chem 1999;274:37362–9. [PubMed: 10601306]

[47]. Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C. Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature 1995;378:394–8. [PubMed: 7477377]

[48]. Meyer D, Birchmeier C. Multiple essential functions of neuregulin in development. Nature 1995;378:386–90. [PubMed: 7477375]

[49]. Patel R, Kos L. Endothelin-1 and neuregulin-1 convert embryonic cardiomyocytes into cells of the conduction system in the mouse. Dev Dyn 2005;233:20–8. [PubMed: 15759273]

[50]. Rentschler S, Zander J, Meyers K, France D, Levine R, Porter G, et al. Neuregulin-1 promotes formation of the murine cardiac conduction system. Proc Natl Acad Sci USA 2002;99:10464–9. [PubMed: 12149465]

[51]. Ford BD, Loeb JA, Fischbach GD. Neuregulin stimulates DNA synthesis in embryonic chick heart cells. Dev Biol 1999;214:139–50. [PubMed: 10491263]

[52]. Erickson SL, O'Shea KS, Ghaboosi N, Loverro L, Frantz G, Bauer M, et al. ErbB3 is required for normal cerebellar and cardiac development: a comparison with ErbB2-and heregulin-deficient mice. Development 1997;124:4999–5011. [PubMed: 9362461]

[53]. Sugi Y, Ito N, Szebenyi G, Myers K, Fallon JF, Mikawa T, et al. Fibroblast growth factor (FGF)-4 can induce proliferation of cardiac cushion mesenchymal cells during early valve leaflet formation. Dev Biol 2003;258:252–63. [PubMed: 12798286]

[54]. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. Nature 1990;348:730–2. [PubMed: 2175396]

[55]. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, et al. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci USA 1989;86:2863–7. [PubMed: 2649896]

[56]. Sakurai T, Yanagisawa M, Takuwa Y, Miyazaki H, Kimura S, Goto K, et al. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature 1990;348:732–5. [PubMed: 2175397]

[57]. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–5. [PubMed: 2451132]

[58]. Emoto N, Yanagisawa M. Endothelin-converting enzyme-2 is a membrane-bound, phosphoramidon-sensitive metalloprotease with acidic pH optimum. J Biol Chem 1995;270:15262–8. [PubMed: 7797512]

[59]. Xu D, Emoto N, Giaid A, Slaughter C, Kaw S, deWit D, et al. ECE-1: a membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1. Cell 1994;78:473–85. [PubMed: 8062389]

[60]. Yanagisawa H, Hammer RE, Richardson JA, Williams SC, Clouthier DE, Yanagisawa M. Role of endothelin-1/endothelin-A receptor-mediated signaling pathway in the aortic arch patterning in mice. J Clin Invest 1998;102:22–33. [PubMed: 9649553]

[61]. Yanagisawa H, Hammer RE, Richardson JA, Emoto N, Williams SC, Takeda S, et al. Disruption of ECE-1 and ECE-2 reveals a role for endothelin-converting enzyme-2 in murine cardiac development. J Clin Invest 2000;105:1373–82. [PubMed: 10811845]

[62]. Yanagisawa H, Yanagisawa M, Kapur RP, Richardson JA, Williams SC, Clouthier DE, et al. Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene. Development 1998;125:825–36. [PubMed: 9449665]

[63]. Brannan CI, Perkins AS, Vogel KS, Ratner N, Nordlund ML, Reid SW, et al. Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. Genes Dev 1994;8:1019–29. [PubMed: 7926784]

[64]. Jacks T, Shih TS, Schmitt EM, Bronson RT, Bernards A, Weinberg RA. Tumour predisposition in mice heterozygous for a targeted mutation in Nf1. Nat Genet 1994;7:353–61. [PubMed: 7920653]

[65]. Lakkis MM, Epstein JA. Neurofibromin modulation of Ras activity is required for normal endocardial–mesenchymal transformation in the developing heart. Development 1998;125:4359–67. [PubMed: 9778496]

[66]. Gitler AD, Zhu Y, Ismat FA, Lu MM, Yamauchi Y, Parada LF, et al. Nf1 has an essential role in endothelial cells. Nat Genet 2003;33:75–9. [PubMed: 12469121]

[67]. Nagasawa T, Kikutani H, Kishimoto T. Molecular cloning and structure of a pre-B-cell growth-stimulating factor. Proc Natl Acad Sci USA 1994;91:2305–9. [PubMed: 8134392]

[68]. Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 1996;382:635–8. [PubMed: 8757135]

[69]. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 1998;393:595–9. [PubMed: 9634238]

[70]. Tachibana K, Hirota S, Iizasa H, Yoshida H, Kawabata K, Kataoka Y, et al. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature 1998;393:591–4. [PubMed: 9634237]

[71]. Wilm B, Ipenberg A, Hastie ND, Burch JB, Bader DM. The serosal mesothelium is a major source of smooth muscle cells of the gut vasculature. Development 2005;132:5317–28. [PubMed: 16284122]

[72]. Tevosian SG, Deconinck AE, Tanaka M, Schinke M, Litovsky SH, Izumo S, et al. FOG-2, a cofactor for GATA transcription factors, is essential for heart morphogenesis and development of coronary vessels from epicardium. Cell 2000;101:729–39. [PubMed: 10892744]

[73]. Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, et al. WT-1 is required for early kidney development. Cell 1993;74:679–91. [PubMed: 8395349]

[74]. Kwee L, Baldwin HS, Shen HM, Stewart CL, Buck C, Buck CA, et al. Defective development of the embryonic and extraembryonic circulatory systems in vascular cell adhesion molecule (VCAM-1) deficient mice. Development 1995;121:489–503. [PubMed: 7539357]

[75]. Yang JT, Rayburn H, Hynes RO. Cell adhesion events mediated by alpha 4 integrins are essential in placental and cardiac development. Development 1995;121:549–60. [PubMed: 7539359]

[76]. Wilson JG, Warkany J. Congenital anomalies of heart and great vessels in offspring of vitamin A-deficient rats. Am J Dis Child 1950;79:963. [PubMed: 15410706]

[77]. Wilson JG, Warkany J. Cardiac and aortic arch anomalies in the offspring of vitamin A deficient rats correlated with similar human anomalies. Pediatrics 1950;5:708–25. [PubMed: 15417271]

[78]. Moss JB, Xavier-Neto J, Shapiro MD, Nayeem SM, McCaffery P, Drager UC, et al. Dynamic patterns of retinoic acid synthesis and response in the developing mammalian heart. Dev Biol 1998;199:55–71. [PubMed: 9676192]

[79]. Xavier-Neto J, Shapiro MD, Houghton L, Rosenthal N. Sequential programs of retinoic acid synthesis in the myocardial and epicardial layers of the developing avian heart. Dev Biol 2000;219:129–41. [PubMed: 10677260]

[80]. Dyson E, Sucov HM, Kubalak SW, Schmid-Schonbein GW, DeLano FA, Evans RM, et al. Atrial-like phenotype is associated with embryonic ventricular failure in retinoid X receptor alpha −/− mice. Proc Natl Acad Sci USA 1995;92:7386–90. [PubMed: 7638202]

[81]. Gruber PJ, Kubalak SW, Pexieder T, Sucov HM, Evans RM, Chien KR. RXR alpha deficiency confers genetic susceptibility for aortic sac, conotruncal, atrioventricular cushion, and ventricular muscle defects in mice. J Clin Invest 1996;98:1332–43. [PubMed: 8823298]

[82]. Sucov HM, Dyson E, Gumeringer CL, Price J, Chien KR, Evans RM. RXR alpha mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis. Genes Dev 1994;8:1007–18. [PubMed: 7926783]

[83]. Chen J, Kubalak SW, Chien KR. Ventricular muscle-restricted targeting of the RXRalpha gene reveals a non-cell-autonomous requirement in cardiac chamber morphogenesis. Development 1998;125:1943–9. [PubMed: 9550726]

[84]. Merki E, Zamora M, Raya A, Kawakami Y, Wang J, Zhang X, et al. Epicardial retinoid X receptor alpha is required for myocardial growth and coronary artery formation. Proc Natl Acad Sci USA 2005;102:18455–60. [PubMed: 16352730]

[85]. Stuckmann I, Evans S, Lassar AB. Erythropoietin and retinoic acid, secreted from the epicardium, are required for cardiac myocyte proliferation. Dev Biol 2003;255:334–49. [PubMed: 12648494]

[86]. Pennisi DJ, Ballard VL, Mikawa T. Epicardium is required for the full rate of myocyte proliferation and levels of expression of myocyte mitogenic factors FGF2 and its receptor, FGFR-1, but not for transmural myocardial patterning in the embryonic chick heart. Dev Dyn 2003;228:161–72. [PubMed: 14517988]

[87]. Schultz JE, Witt SA, Nieman ML, Reiser PJ, Engle SJ, Zhou M, et al. Fibroblast growth factor-2 mediates pressure-induced hypertrophic response. J Clin Invest 1999;104:709–19. [PubMed: 10491406]

[88]. Zhou M, Sutliff RL, Paul RJ, Lorenz JN, Hoying JB, Haudenschild CC, et al. Fibroblast growth factor 2 control of vascular tone. Nat Med 1998;4:201–7. [PubMed: 9461194]

[89]. Lavine KJ, White AC, Park C, Smith CS, Choi K, Long F, et al. Fibroblast growth factor signals regulate a wave of Hedgehog activation that is essential for coronary vascular development. Genes Dev 2006;20:1651–66. [PubMed: 16778080]

Table 1

Signals from the endocardium and epicardium

	Target	Receptor(s)	Response
Endocardial signals			
Neuregulin	Myocardium	$ErbB_2$ + $ErbB_4$	• Cardiac trabeculation • Differentiation to components of conduction system • (w/IGF) increase in DNA synthesis and expansion of compact zone
	Endocardial mesenchyme	$ErbB_1$ + $ErbB_2$	Proliferation of endocardial mesenchyme
FGF-4	Endocardium, endocardial mesenchyme	FGFR1, 2, -3	Activation of Ras and PLC-γ pathways, proliferative expansion of cushion mesenchyme
FGF-9, -16, -29	Myocardium	FGFR1	Differentiation and proliferation of myocytes
Endothelins	Neural crest cells, myocardium, endocardium	ET_A and ET_B	Mediates septation of common ventricle and OFT by signaling to endocardium, myocardium, and NC derivatives
Neurofibromin	Neural crest cells, endocardium, myocardium		Upstream modulator of Ras GTPase and $NFAT_c$ activity. Regulates septation of common ventricle, OFT, and development of endocardial cushions
SDF-1 (PBSF)	Aorticopulmonary wall, myocardium	CXCR4	Mediates septation
Epicardial signals			
Retinoic acid signaling	Epicardium	RXR-α	Autocrine signaling stimulates the release of other trophic factors. Including FGF-2
FGF-2, -9, -16, -20	Myocardium	FGFR1, FGFR2	• Proliferation of myocytes • Differentiation of myocytes • Modulation of vasculogenesis via VEGF-A, -B, -C, and angiopoietin-2 pathways