Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Cell Lineages and Tissue Boundaries in Cardiac Arterial and Venous Poles : Developmental Patterns, Animal Models, and Implications for Congenital Vascular Diseases

Simonetta Ausoni and Saverio Sartore

Arterioscler Thromb Vasc Biol. 2001;21:312-320
doi: 10.1161/01.ATV.21.3.312
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636

Cell Lineages and Tissue Boundaries in Cardiac Arterial and Venous Poles

Developmental Patterns, Animal Models, and Implications for Congenital Vascular Diseases

Simonetta Ausoni, Saverio Sartore

Abstract—Multiple cell populations with different embryological histories are involved in the morphogenesis of the cardiac arterial and venous poles as well as in the correct alignment and connection of the developing vessels with the cardiac chambers. Formation of the aorta and the pulmonary trunk is a complicated process orchestrated via a specific sequence of highly integrated spatiotemporal events of cell proliferation, migration, differentiation, and apoptosis. The peculiar susceptibility of this intricate cell network to be altered explains the frequency of congenital cardiovascular diseases of the arterial and venous poles. We review this topic from the "vascular point of view," putting major emphasis on (1) the existence of different cell lineages from which smooth muscle cells of the aorticopulmonary trunk can be derived, (2) the establishment of cell/tissue boundaries in the cardiovascular connecting regions, and (3) the animal models that can mimic human congenital defects of the arterial and venous poles of the heart. **(*Arterioscler Thromb Vasc Biol*. 2001;21:312-320.)**

Key Words: congenital cardiovascular diseases ■ tissue boundaries ■ outflow tract ■ inflow tract ■ animal models

The more we treat the theories of our predecessors as myths, the more inclined we shall be to treat our own theories as dogmas.

J.B. Thornton

A high percentage of cardiovascular congenital malformations arise from an abnormal development of the great vessels and an improper alignment with the heart.[1–3] Many of the cardiovascular defects that reach clinical observation are due to an abnormal development of the arterial pole, in particular, aortic arches, aorta, and pulmonary trunk. Abnormalities induced in the venous pole, on the other hand, can mostly be embryonically lethal, as supported by experimental observations in animal models,[4] and are likely to be largely underestimated.

Previous reports in the field have dealt mostly with chamber specification, general heart morphogenesis, and cardiac looping. Instead, this report will discuss cell lineages and cell-signaling pathways in normal and abnormal development of the arterial and venous great vessels, inasmuch as this approach can provide more detailed information on the cell fate within the morphogenetic plan.

In the developing cardiovascular system, cell movements and the establishment of boundaries between the heart and the vessels are responsible for casting the outflow tract (OFT) and the inflow tract (IFT) of the heart, and this is why they represent the main topic of the present review. Development of the coronary vessels, derived from the proepicardium by a unique vasculogenetic process,[5–7] will not be discussed because it has no primary impact on the arterial pole formation.

Pursuant to the aims mentioned above, we will highlight the following aspects: (1) Which cell lineages contribute to the formation of the arterial and venous poles of the heart? (2) How do vascular cells achieve their final identity and position with respect to cardiac cells? (3) What is the role of different cell lineages in the establishment of connections and boundaries? (4) Which "signals" control cell organization temporally and spatially? (5) Which experimental cardiovascular malformations arise from perturbations of these processes?

In the present review, we will present an updated list of animal models carrying defects of either the arterial or venous pole or both. The rapid generation of these models, thanks to the advances in gene-targeting techniques, are now allowing us to probe deeply into the molecular bases of congenital cardiovascular defects in humans and to underscore unexpected similarities and overlaps in the molecular pathways that control cardiac and vascular development.

A Snapshot of Cardiovascular Development

In the developing embryo, the arterial pole consists of an OFT connected to the aortic arch arteries,[8] and the venous pole consists of an IFT connected to the vitelline veins. The OFT has an aortic sac (proximal to the aorta) and a conotruncus (proximal to the ventricle). The IFT has a sinus venosus

Received October 25, 2000; revision accepted November 29, 2000.

From the Department of Biomedical Sciences (S.A., S.S.) and the National Research Council Center of Muscle Biology (S.A., S.S.), Padua, Italy.

Correspondence to Dr Simonetta Ausoni, Department of Biomedical Sciences, University of Padua, Viale G. Colombo, 3, I-35121 Padua, Italy. E-mail ausoni@civ.bio.unipd.it

***Arterioscler Thromb Vasc Biol.* is available at http://www.atvbaha.org**

(proximal to the cardinal veins) and a sinuatrial region (proximal to the atria). The OFT and the IFT are transient embryological regions that undergo profound remodeling during development and result in the formation of cardiac as well as vascular distinct structures. This is why, whenever appropriate, we prefer to use the terms arterial pole and venous pole to indicate the outlet and inlet of the heart instead of OFT and IFT. Formation of the arterial and venous poles in the embryo is a complex morphogenetic event whose detailed analysis is beyond the scope of the present review. Nonetheless, we have summarized the main embryological stages in Figure 1 to help the reader visualize the complicated processes. Figure 1 illustrates the initial differentiation of vascular and cardiac lineages in the cardiogenic area (Figure 1A), the progressive septation of the OFT into the aorta and the pulmonary trunk due to migration of neural crest cells (Figure 1B and 1C), the alignment of the great vessels and the cardiac chambers (Figure 1D), and the remodeling of the vitelline veins, umbilical veins, and cardinal veins so that finally all venous blood enters the right atrium via the superior and inferior caval veins (Figure 1C and 1D). For a detailed embryological analysis, we refer to previous articles.[3,9–11]

Cell Lineages in the Cardiac Arterial and Venous Pole

Endothelial and Endocardial Lineages

The arterial pole contains endocardial cells of the OFT and endothelial cells of the aortic sac and aortic arches. These distinct cell lineages are, at least in the adult, structurally and functionally distinct in terms of tissue permeability, cell-cell contact, and cell communication with adjacent compartments.[12,13] Endothelial precursors of mesodermal origin initiate vasculogenesis and promote the recruitment of surrounding mesenchymal cells to form the definitive smooth muscle cells (SMCs) and fibroblasts of the vascular wall.[14,15] Vascular endothelial growth factors (VEGFs) and their cognate receptors (VEGFR-1, VEGFR-2, VEGFR-3, and neuropilins), angiopoietins and their *Tie* receptors, platelet-derived growth factor, transforming growth factor-β (TGF-β), and the ephrin-Eph receptor system are essential for vasculogenesis and remodeling (see reviews 16,17) and act as carefully orchestrated players in terms of time, space, and dose effect. Unlike the endothelial cells of the aortic sac, endocardial cells of the OFT form 2 endocardial cushions through an epithelial-to-mesenchymal transition.[18] These cushions are essential in forming the aorticopulmonary septum, as demonstrated by the absence of an aorticopulmonary septum in null mice lacking proper endocardial ridges, such as the *Sox-4* mutants (see below).[19]

SMC Lineage From Different Compartments

The great vessels connected to the heart contain SMCs with largely diverse embryological origin. Their mesenchymal progenitor cells may be recruited from local and distant sources (Figure 2), among which there are the neural crest cells. Neural crest cells migrate from the neural folds to the pharyngeal arches. Here, they separate each arch artery and aortic sac from the pharyngeal ectoderm and condense against the lumen to generate its smooth muscle wall.

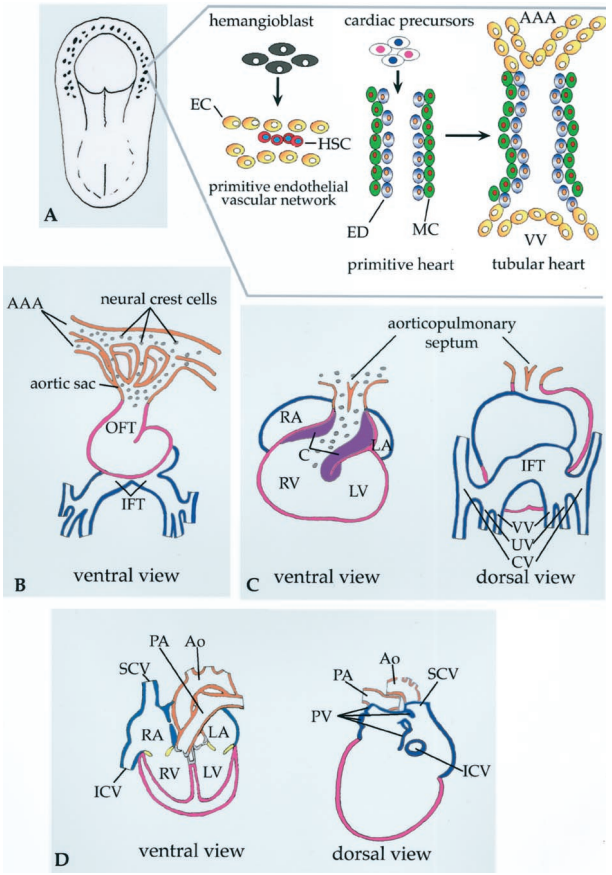

Figure 1. Morphogenetic events in cardiovascular development. A, The primitive endothelial vascular network and the primitive tubular heart arise from precursor cells (hemangioblast and cardiac precursors) in the cardiogenic area of the embryo. EC indicates endothelial cells; MC, mesenchymal cells; HSC, hemopoietic stem cells; ED, endocardium. B, The heart loops to the right-hand side and connects to the aortic arch arteries (AAA) in the arterial pole and to the vitelline veins (VV) in the venous pole. Neural crest cells migrate from the neural folds to the aortic arches and the aortic sac. C, Neural crest cells migrate into the OFT and contribute to the aorticopulmonary septum (ventral view). Also, the endocardial cushions (C), arising from the endocardium, contribute to this process. In the venous pole, the IFT initially receives blood from the vitelline (VV), the umbilical (UV), and the cardinal veins (CV, dorsal view). RA indicates right atrium; RV, right ventricle; and LV, left ventricle. D, OFT septation and remodeling ends with the separation of the aorta and the pulmonary artery, the formation of the interventricular septum, and the formation of aortic and pulmonary valves (ventral view). IFT remodeling occurs through mechanisms of regression, and all venous blood finally enters the right atrium via the superior (SCV) and inferior (ICV) caval veins. PA indicates pulmonary artery; PV, pulmonary vein; LA, left atrium; and Ao, aorta. Embryonic stages in panels A, B, C, and D are day 7–8, day 9.5, day 12.5, and adult, respectively, and refer to mouse development.

subpopulation of neural crest cells invades the OFT and the base of the heart, thus forming the aorticopulmonary septum, pulmonary infundibulum, aortic vestibule, and separation of the great vessels from the right and left ventricles.[23] This explains why ablation of cardiac neural crest cells in the chick embryos leads to a wide variety of malformations, including common trunk and ventricular septal defects (VSDs).[24,25] There is no significant neural crest cell contribution to the formation of the venous pole, even though neural crest–derived SMCs are present in the tunica media of the anterior

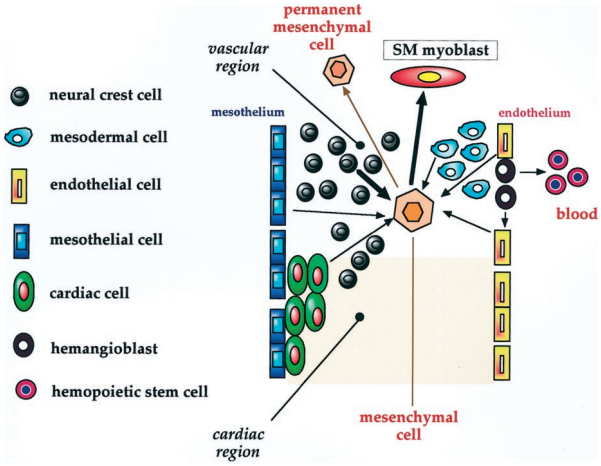

Figure 2. SMC origin in the arterial pole. The diagram summarizes the current knowledge of the cell populations that give rise to the smooth muscle (SM) lineage in the arterial pole. The region between the mesothelium and the endothelium corresponds to the aortic sac in the upper part (vascular region) and to the OFT in the lower part (cardiac region). Thick arrows indicate cell transitions that have been proved to exist; thin arrows indicate possible transitions, whose existence is still contentious and is a matter of ongoing studies. Cell types are indicated on the left side of the figure.

cardinal veins[26] and defects of the venous pole after ablation of cardiac neural crest cells have been described.[27] The properties of the cardiac neural crest cells are quite unique, allowing them to differentiate into SMCs, initiate elastogenesis in the aorta and pulmonary trunk,[28] and control tissue remodeling of the forming vessels. It is likely, therefore, that they represent a specific subpopulation with partially restricted developmental options, as suggested by grafting experiments in which replacement of cardiac neural crest cells by cranial neural crest cells failed to support cardiovascular development.[29] Cardiac neural crest cells are functionally linked by gap junctions, and gap junction communications are associated with SMC differentiation.[30] Furthermore, neural crest cells are responsive to TGF-β in vitro,[31] and TGF-β and BMP-2 and -3 instructively promote SMC differentiation.[32]

There is at least one more major source of mesenchymal cells that differentiate into SMCs. This is the local mesoderm that contributes to the muscular wall of the ascending aorta and to the pulmonary trunk but not to the aortic arch arteries.[33] Distribution of SMCs of mesodermal origin in the aorticopulmonary septum is not clear, because differences in OFT septation have been found in mice and in chicks.[34]

Recent reports indicate the endocardium,[9] the mesothelium,[35] and the myocardium[36] as other possible sources of vascular SMCs, but these contributions, if any, remain speculative and will require further investigation. The fourth possible SMC origin is the endothelium. Endothelial cells transdifferentiate into SMCs and migrate into the media and adventitia in the chick dorsal aorta.[37] Whether endothelial cell transdifferentiation contributes to form the tunica media of other vessels has yet to be investigated. SMC origin from endothelial cells can also be explained differently. Endothelial precursors share a common progenitor, the hemangioblast, with the hemopoietic stem cells (see Figure 1A). The intimate relationship between hemopoietic and vascular cells is exemplified by the common expression of the CD34 cell surface glycoproteins,[38] by the presence of $CD34^+$ cells in the mouse para-aortic mesenchyme,[39] and by the absence of hemopoietic and endothelial cells in the zebra fish mutant *cloche*.[40] Intra-aortic hemopoietic cells can be derived from endothelial cells[41] and play a role in postnatal angiogenesis.[42–44] Whether hemopoietic stem cells also participate in prenatal vasculogenesis is a tempting speculation, but this is still under debate. In conclusion, the mesenchymal SMC progenitor in the arterial pole originates from multiple differentiation pathways. After the mesenchymal cells become associated with the endothelium, a coordinated differentiation program is activated, and smooth muscle–specific contractile and cytoskeletal proteins are synthesized.[45] This process is likely to involve the majority of cells, but it is also formally possible that a minor population of mesenchymal cells persists as a reservoir, with intermediate characteristics of SMC precursors.[46] Ubiquitous or widely expressed transcription factors, such as serum response factor[47–49] and Sp-1/Sp-3,[50] play a role in the smooth muscle–specific gene transcription. A Kruppel-like factor and the BTEB2 protein are required to activate the smooth muscle lineage marker, the SM22 gene, through a TGF-β control element.[51] However, the molecular details of a functional smooth muscle–specific transcription complex remain to be elucidated.

Cardiac Lineage

Septation of the OFT ends with the formation of an outlet septum that separates the 2 great arteries and allows the aorta and the pulmonary artery to drain into the left and right ventricle, respectively. Initially, this septum is a mesenchymal structure originating from the OFT endocardial ridges, but later in development, it becomes muscular through an ingrowth of a newly formed myocardium into the mesenchymal endocardial cushions.[52,53] Impaired myocardialization results in the persistence of an embryonic outlet septum and can lead to a variety of congenital heart diseases, ranging from VSD to double-outlet right ventricle (DORV). There is much evidence to indicate that myocardialization is under multiple control signals from the aortic sac mesenchyme[52] and from the neural crest cells.[30,52,53]

Myocardialization proceeds in the venous pole, too. Here, it is not limited to the heart, but extends up to the forming caval veins and pulmonary veins. In fact, myocardial cells largely contribute to the tunica media of these vessels in rodents[54] and, to a lesser extent, in humans. In transgenic mice expressing the LacZ reporter gene under the control of the cardiac troponin I promoter,[55] we observed that cardiac cells in the pulmonary veins never go beyond well-defined boundaries that correspond to the third bifurcation and never spread to colonize the pulmonary arteries.[56] It is likely, therefore, that endothelial cells and/or SMCs of the veins release “signals” to recruit and set the position of the myocardial cells.

Morphological, Cellular, and Molecular Boundaries in the Arterial and Venous Pole

Formation of the great vessels at the arterial and venous pole involves multiple cell types that rearrange themselves at the correct space in the body to realize a precise morphogenetic plan. The existence of a prepattern on which the heart and the

Animal Mutants With Defects in Cardiac Arterial and Venous Poles

Gene	Reference	Protein	Mutation	Survival	Tissue Expression	Principal Vascular Defects
Defects of arterial pole						
dHAND	74, 75	Tran factor	KO	9.5	NCC derivatives	Aortic arch abnormalities; mesenchyme fails to differentiate into SMCs
MEF2C	76, 77	Tran factor	KO	9.5	Myocardium, SMCs, endothelium	Abnormal vessels, atresia of the aorta
Hox 1.5	78	Tran factor	KO	Perinatal	NCC derivatives	Vascular abnormalities similar to the DGS
Pax-3 (Splotch)	79–81	Tran factor	Spontaneous	13.5–14.5	NCC derivatives	Aortic arch abnormalities, common trunk, abnormal migration of NCCs
Sox-4	19	Tran factor	KO	14	Endocardial cushions	Common trunk, no functional semilunar valves
MFH-1	82	Tran factor	KO	...	NCC derivatives	Aortic arch abnormalities
NF-ATc	83, 84	Tran factor	KO	13.5–17.5	Endocardium, endothelium	No semilunar valve formation, VSD
RAR α/γ	85	RA receptor	KO double mutation	Viable*	ND	Aortic arch abnormalities, common trunk, VSD
RXR α	86	RA receptor	KO	13.5–14.5	ND	Common trunk or incomplete aorticopulmonary septum, DORV, pulmonary artery stenosis, VSD
ET-1	61	Cytokine	KO	Perinatal	Endothelium, endocardium	VSD, common trunk, DORV, pulmonary stenosis, aortic arch abnormalities
ET_A receptor	62	G-protein receptor	KO	Perinatal	NCC derivatives, myocardium	Aortic arch abnormalities, interrupted aortic arch
ECE-1	63	Metalloprotease	KO	Perinatal	Endothelium, endocardium, mesenchyme	Aortic arch abnormalities, VSD, DORV, common trunk
NT-3	87	Growth factor	KO	Perinatal	Endocardial cushions	VSD, common trunk, valve abnormalities, Fallot
Cx43	88, 89	Gap junction protein	KO	Perinatal	NCC derivatives, myocardium	Obstruction of subpulmonary outlet
PDGF-α receptor	90	Tyrosine kinase receptor	KO	15	Endocardial cushions	VSD, DORV, common trunk
TGF-β2	91	Transforming growth factor	KO	Perinatal, postnatal	Endocardial cushions	VSD, DORV, DILV
Df1	92		Deletion	...		Vascular abnormalities similar to DGS
Neurofibromin-1	93	GAP	KO	14.5	Endocardial cushions, myocardium	Common trunk, abnormal endocardial ridges, no functional semilunar valves
Versican (*hdf*)	72	Matrix protein	Insertional mutation	10.5	Endocardial cushions, endothelium, myocardium	No cardiac jelly, no cushions
Hyaluronan synthase-2	73	Matrix protein	KO	9.5–10.0	Endocardial cushions, endothelium, myocardium	Reduction of OFT, no cardiac jelly, no cushions
ActRIIB receptor	94	Serine/threonine receptor	KO	Perinatal	Endocardium, endothelium	Malposition of great arteries
NCC ablation (chick)	25, 27	...				Common trunk, TGA, DORV, VSD
Syrian hamster	95	...	Spontaneous	Viable	...	Bicuspid aortic valve, abnormal origin of the coronary arteries
iv/iv	96	...	Spontaneous	Viable	...	DORV, Fallot
Defects of venosus pole						
COUP-TFII	97	Tran factor	KO	10	Myocardium, vascular mesoderm	Sinus venosus, atrial malformation, cardinal vein malformations
NT-3	87	Growth factor	KO	Perinatal	Endocardial cushions	Sinus venosus malformation, reduction of SMCs in the pulmonary veins
iv/iv	96	...	Spontaneous	...		Common sinus venosus

List shows animal mutants with cardiovascular defects either in the arterial or in the venous pole or in both. Defects of the arterial pole include abnormalities of the aortic arches, aortic sac, and OFT. Defects of the venous pole include abnormalities of the IFT and caval veins. Animal mutants were obtained by targeted disruption of single genes (knockout [KO]). Exceptions are the ablation of neural crest cells in the chick, spontaneous mutants *iv/iv*, Syrian hamster, Splotch mice and the Df1 mouse, derived by deletion of the chromosomal region homologue to human 22q11, and the *hdf* mouse, derived by insertional mutation. RAR α/γ is a double mutant obtained by crossing heterozygous mice for the single mutations. Survival (time) indicates time of death in the embryos, unless differently specified. Tissue expression, restricted to the cardiovascular system, may indicate either protein or mRNA distribution or both. MFH-1 indicates mesenchyme forkhead-1 gene; RAR and RXR, retinoic acid receptors; ET-1, endothelin-1; ET_A, endothelin receptor A; ECE-1, endothelin-converting enzyme-1; NT-3, neurotrophin-3; Cx43, connexin43; PDGF, platelet-derived growth factor; NCC, neural crest cell; *iv/iv*, inversus viscerum; tran, transcription; GAP, GTPase-activating protein; ND, not precisely determined at the cell lineage level; DGS, DiGeorge syndrome; Fallot, tetralogy of Fallot; DILV, double-inlet left ventricle; and TGA, transposition of great arteries.

great vessels are built is suggested by much evidence, most of which pertains to the heart more than to vessel formation. Strict boundaries for Hox gene expression exist in the pharyngeal arches,[57,58] but nothing similar has ever been demonstrated in other vessels. So far, the Hairy-related family members HRT1, HRT2, and HRT3 are the only genes that exhibit distinct expression patterns in the vascular system, with strict boundaries along the anterior-posterior axis.[59] In zebra fish, the *grid-lock* mutation for the *grl* gene, which encodes a Hairy-type basic helix-loop-helix protein, selectively perturbs assembly of the aorta, suggesting that identity of this vessel is determined before the onset of circulation.[60] This is in agreement with the idea that the embryonic circulatory plan is genetically established.

To create strict boundaries between the heart and specific vascular segments, integrated cellular and molecular events are required; these include the following: specific cell-cell and cell-matrix adhesion and cell migration, proliferation, differentiation, and apoptosis. In this respect, neural crest cells may be fundamental because their migration follows a precise colonization territory. In the chick, there is a strict boundary between the aortic arches, ascending aorta, and pulmonary trunk invested by neural crest cells on one side and descending aorta and pulmonary arteries that are completely devoid of neural crest cells on the other side.[9,20,34]

Our hypothesis is that neural crest cells perform 2 functions: (1) they colonize and demarcate the territory where the vasculature will develop (instructive role), and (2) they provide an abundant population of SMC precursors for vascular remodeling (structural role). Interestingly, ablation of neural crest cells indicates that a threshold level of cells is critically important for proper septation and tissue remodeling.[26] Another essential event to guarantee OFT septation is the interplay between endocardial cushions and neural crest cells. In this respect, endothelin-mediated signaling seems to be of great importance. Targeted inactivation of endothelin-1,[61] endothelin receptor A,[62] and endothelin-converting enzyme-1[63] in mice causes abnormal aortic arches, poor development of the endocardial cushions, DORV, and VSD (see Table) similar to the DiGeorge syndrome in humans. Endothelin-1–mediated signals from endocardial and endothelial cells may be relayed to neural crest cells by classic binding to endothelin type A and B receptors. It is interesting that the transcription factors goosecoid,[61] d-HAND, and msx1,[64] are downregulated in mice lacking the endothelin-mediated pathway and that dHAND and msx1 are required for the development of the aortic arches and OFT septation (see below).

What is the significance of boundaries in the embryo, and how are they formed? Tissue boundaries and, hence, morphogenetic patterning are likely to be the result of a combined effect of endogenous and exogenous "driving cues" and "positional cues" (Figure 3). The former impose the correct spatiotemporal migration to cells that may or may not be intrinsically determined for movement; the latter establish and maintain the correct topographic patterns by restricting cell and tissue intermingling. This process can result from a series of molecular events involving cell-cell and/or cell-matrix contact-mediated guidance in a short range. The nervous system, in which guidance signals direct axon formation along defined patterns to establish the neuronal

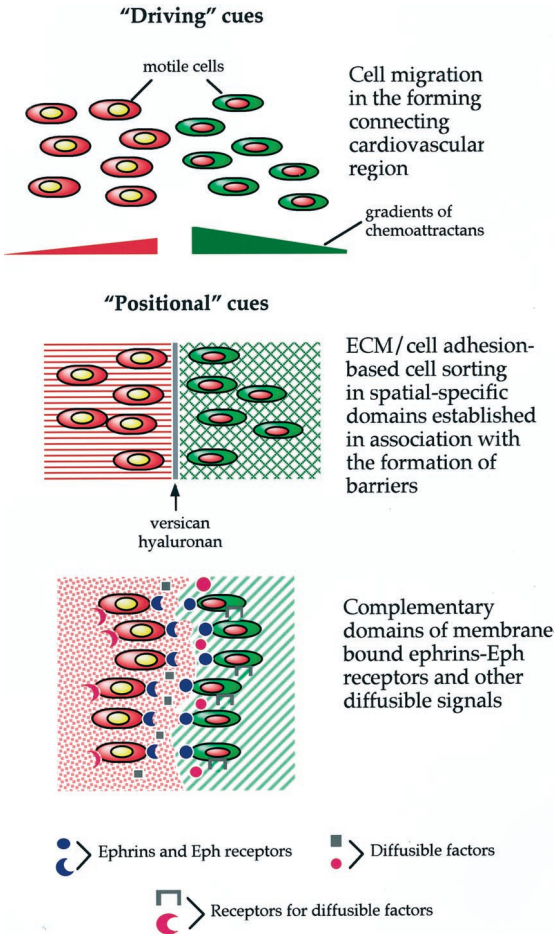

Figure 3. Schematic representation of putative driving cues and positional cues acting on the establishment of boundaries between the heart and the great vessels. ECM indicates extracellular matrix.

connection network, is paradigmatic. It is noteworthy that some of the molecular cues that guide neural crest cell migration and stabilization of neural patterns play a role in vasculogenesis and angiogenesis, too. Three major groups of molecules can be involved in these processes: (1) diffusible molecules, such as semaphorins[65] and netrins,[66] (2) membrane-bound proteins, such as the ephrins-Eph receptor system,[16,17,67,68] and (3) extracellular matrix proteins. The semaphorin Sema3A is able to inhibit endothelial cell motility, capillary formation, and sprouting by competing with VEGF for the neuropilin-1 coreceptor.[65] Other molecules, such as netrins, act as either a chemoattractant or chemorepellent in the nervous system, and netrin-1 transcripts are also present in the developing cardiovascular system.[66] Ephrins and Eph receptors, initially discovered in the nervous system, may mediate cell-cell adhesion or deadhesion, restricting the cellular intermingling and thus establishing and maintaining the appropriate tissue boundary.[16,17,67,68] Recent data indicate that the ephrin-Eph receptors can trigger a local depolymerization of the cytoskeleton, leading to the collapse of filopodia and, hence, producing a direct control over cell migration.[69] In addition, ephrin B2, initially restricted to endothelial cells in the embryo, is also later expressed in the surrounding SMCs.[16] Finally, in the nervous system, the ephrin-Eph receptor system displays complementary gradients that over-

lap those of the fate mapping. This may occur in the establishment of cardiovascular boundaries, too. Ephrins also regulate surface density of integrins $\alpha_{V\beta3}$ and $\alpha_{5\beta1}$, which are known to have a role[70] in early vasculogenesis.

Cell adhesion and extracellular matrix are likely to be involved in setting boundaries between 2 neighboring tissues. For example, in the OFT extracellular matrix components, such as fibronectin, elastin, and laminin, collagens I and VI have a specific temporal and spatial distribution,[71] and versican, a cell adhesion molecule, seems to play a nonpermissive role in cell movements.[72] Lack of versican in the *hdf* mouse mutants[72] and lack of hyaluronan synthase-2[73] in the embryo results in severe cardiovascular defects for impaired endocardial cushion formation. Possible consequences in septation of the OFT cannot be observed in these mutants because of early death in the littermate.

Cell Lineage and Tissue Boundary Defects in Animal Models

Many mouse mutants that reproduce aspects of human congenital cardiovascular defects are currently being generated. These animal models provide an exciting instrument for identifying the factors involved in cardiac morphogenesis and are extremely powerful in establishing the relationship between a genetic defect and its functional consequences in vivo. Animal models with defects in the cardiac arterial and venous pole are listed in the Table, where associated references can be found.[19,25,27,61–63,72–97]

In this section, we will mainly focus on animal models with abnormal development of the arterial pole. Abnormalities of the venous pole will not be reviewed extensively because there are just a few examples of animal mutants with defects in this region. In addition, the origin of these defects is still a matter of discussion for clinicians and embryologists. The only animal model with an exclusive defect in the venous pole is the knockout mouse for the steroid receptor chicken ovalbumin upstream promoter transcription factor II (COUP-TFII). This mutant shows sinoatrial malformations and either poorly formed or collapsed cardinal veins.[97] Early lethality in the littermate occurs because of congestive heart failure, which is presumably due to deficient blood circulation in the rapidly growing embryo.

Analysis of the Table suggests some general comments. The first is related to survival times. Animal mutants with abnormalities in the arterial pole die at around 3 specific periods: embryonic day 10, embryonic days 14 to 15, and perinatally. It may be that the vascular system maturation proceeds according to a spatiotemporal sequence of events, whose completion ensures the correct progression along the developmental pathway. The establishment of a morphogenetic abnormality during this process could not be tolerated if it did not guarantee a successful outcome of embryogenesis. The first decision to be made by the forming organism is related to OFT colonization by neural crest cells. The second concerns the completion of OFT septation and the muscularization of the septum. The third is the activation of pulmonary circulation. Nothing is known about the ways in which such decisions are made, and the use of the term heart failure to indicate the cause of embryonic death may be not always adequate. Some data highlight the role played by altered hemodynamic factors on inducing cardiovascular malformations. The physiological increases in blood volume, pressure, and flow with their inherent increases of shear stress and wall stretching may have a profound impact on the onset of cardiovascular abnormalities. The establishment of an abnormal pulmonary circulation is also a crucial event causing sudden death. For instance, mice lacking connexin43 die neonatally as a consequence of an obstruction of the subpulmonary OFT.[88,89]

A second observation in the interpretation of complex knockout phenotypes is that we need to distinguish, whenever possible, between primary defects and secondary defects. Primary developmental defects originating in the heart can have dramatic consequences for the vessels and vice versa. For example, DORV, characterized by the persistence of an embryonic configuration in which septated aorta and pulmonary trunk drain into the pulmonary ventricle, is associated with VSD. In other cases, malformations of the great vessels can be the consequence of cardiac defects. For example, an abnormal cardiac looping, such as in the *iv*/*iv* mice, can lead to transposition of the great arteries, DORV, and VSD. The interdependence between cardiac and vascular development is well illustrated in the MEF2C knockout mouse, which exhibits myocardial and endocardial defects as well as abnormal vessels and atresia of the great vessels connected to the heart.[76,77] In this model, the lack of the right ventricle and the development of an hypoplastic left ventricle reduces and finally blocks blood flow in the embryo. This results in vascular atresia of the aortic arch arteries that require the blood flow to maintain their normal lumen. In addition, hypoxia produces multiple effects, including the loss of mesenchymal cell and an increased VEGF expression, resulting in vessel enlargement for fusion of adjacent capillaries and altered vascular remodeling.

A third observation is that the same defect can be generated by mutations in different genes. For example, common trunk can be due to genes that act either on the neural crest cells or on the endocardial cushions, suggesting a functional cooperation among different cells in OFT septation. Conversely, one gene mutation can lead to a broad spectrum of abnormalities either because a gene controls the same function in multiple tissues or, more frequently, because a cellular compartment is involved in multiple functions. For example, mutations in genes of the endothelin-mediated pathway lead to aortic arch abnormalities, common trunk, and DORV,[61–63] presumably because endothelin-1 released by the endothelium and the endocardium controls neural crest differentiation, endocardial cushion formation, and myocardialization, 3 closely related events.

Among the human pathologies that best reflect the spectrum of abnormalities observed in these animal mutants are the DiGeorge syndrome and velocardiofacial syndrome. The common features of these syndromes are interrupted aortic arch, OFT malformations, hypoplastic thymus, and parathyroids.[98] Both diseases are due to haploinsufficiency of ≥1 gene in the q11.2 region of chromosome 22. The minimal critical region that is deleted in most DiGeorge patients has been mapped, but the identification of the genes involved is complicated by the fact that some patients show deletions in distinct nonoverlapping regions. Using the Cre-LoxP system, Lindsay et al[74] generated a mouse that carries a deletion (Df1)

homologous to the human deleted region in DiGeorge and velocardiofacial syndrome. The mouse mutant lacks 14 of the almost 30 genes of the DiGeorge critical region and recapitulates most of the human cardiovascular defects but lacks thymic, parathyroid, and craniofacial abnormalities. Thus, it is likely that the DiGeorge syndrome requires the deletion of a whole group of genes and/or regulatory elements that control multiple genes in a cluster.

Future Directions

Our present knowledge about the biology and embryology of OFT and IFT does not allow for the assembling of data in a definitive picture. However, the discovery of new genes and the generation of models for gene function studies in vivo are making progress in this field faster. Identification of genes that regulate commitment and differentiation of SMCs will be essential in understanding how the vascular network arises and is organized. Unfortunately, this search is still in its infancy compared with analogous studies in cardiac and skeletal muscle, but it will certainly profit from the fact that genes involved in cardiac development can also have a profound impact on vascular development. In addition, it will be important to identify (1) the genes that control arterial and venous SMC diversification and (2) the genes involved in induction and maintenance of tissue boundaries in the connecting cardiovascular regions. The lesson derived from the discovery of the ephrin-Eph receptor system for vascular endothelium clearly indicates the direction.

On the other hand, a great effort has to be devoted to the generation of new animal models. The new inducible and conditional knockouts will be extremely useful in this respect. They will help to overcome problems of genes that control some morphogenetic events but cause a premature death. In addition, they will contribute in the dissection of a function whenever a single gene controls multiple morphogenetic events. Although some caution must be used when a direct correlation between mouse mutants (eg, the DiGeorge mouse models) and human diseases is made,[99–101] the availability of more models for studies in vivo is certainly the strategy of choice for disclosing the mechanisms of cardiovascular abnormalities.

Acknowledgments

Preparation of this review was supported by a grant from the Regione Veneto (1998–1999) to S.A and MURST (Role of Smooth Muscle Cells in Atherogenesis: Pharmacological Implications) to S.S. We wish to thank Adriana Gittenberger-de Groot, Department of Anatomy and Embryology, Leiden University Medical Center; Joseph Miano, Center for Cardiovascular Research, University of Rochester Medical Center; and Stefano Schiaffino and Marina Campione, Department of Biomedical Sciences, University of Padova, for critical reading of this review. We would also like to thank Sarah Hansen for her excellent editorial assistance.

References

1. Hoffman J. Incidence of congenital heart disease, I: post-natal incidence. *Pediatr Cardiol*. 1995;16:103–113.
2. Hoffman J. Incidence of congenital heart disease, II: prenatal incidence. *Pediatr Cardiol*. 1995;16:155–165.
3. Srivastava D, Olson EN, A genetic blueprint for cardiac development *Nature*. 2000;407:221–226.
4. Hogers B, DeRuiter MC, Gittenberger-de Groot AC, Poelmann RE. Extraembryonic venous obstructions lead to cardiovascular malformations and can be embryonic lethal. *Cardiovasc Res*. 1997;41:87–99.
5. Mikawa T, Gourdie RG. Pericardial mesoderm generates a population of coronary smooth muscle cells migrating into the heart along with ingrowth of epicardial organ. *Dev Biol*. 1996;173:221–232.
6. Vrancken Peeters MP, Gittenberger-de Groot A, Mentink MM, Poelmann RE. Smooth muscle cells and fibroblasts of the coronary arteries derive from epithelial-mesenchymal transformation of the epicardium. *Anat Embryol (Berl)*. 1999;199:367–378.
7. Gittenberger-de Groot AC, DeRuiter MC, Bergwerff M, Poelmann RE. Smooth muscle cell origin and its relation to heterogeneity in development and disease. *Arterioscler Thromb Vasc Biol*. 1999;19:1589–1594.
8. Pexieder T. Conotruncus and its septation at the advent of the molecular biology era. In: Clark EB, Markwald RR, Takao A, eds. *Developmental Mechanisms of Heart Disease*. New York, NY: Futura Publishing Co Inc; 1995:227–247.
9. Noden DM, Poelmann RE, Gittenberger-de Groot AC. Cell origins and tissue boundaries during outflow tract development. *Trends Cardiovasc Med*. 1995;5:69–75.
10. Ya J, van den Hoff MJB, de Boar PAJ, Tesink-Taekena S, Franco D, Moorman AFM, Lamers WH. Normal development of the outflow tract in the rat. *Circ Res*. 1998;82:464–472.
11. Wessels A, Anderson RH, Markwald RR, Webb S, Brown NA, Viragh S, Moorman AF, Lamers WH. Atrial development in the human heart: an immunohistochemical study with emphasis on the role of mesenchymal tissues. *Anat Rec*. 2000;259:288–300.
12. Brutsaert DL, Fransen P, Andries LJ, De Keulenaer GW, Sys SU. Cardiac endothelium and myocardial function. *Cardiovasc Res*. 1998;38:281–290.
13. Bianchi C, Sellke FW, Del Vecchio RL, Tonks NK, Neel BG. Receptor-type protein-tyrosine phosphatase μ is expressed in specific vascular endothelial beds in vivo. *Exp Cell Res*. 1999;248:329–338.
14. Folkman J, D'Amore PA. Blood vessel formation: what is its molecular basis? *Cell*. 1996;87:1153–115.
15. Risau W. Mechanisms of angiogenesis. *Nature*. 1997;386:671–674.
16. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature*. 2000;407:242–248.
17. Gale NW, Yancopoulos GD. Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development. *Genes Dev*. 1999;13:1055–1066.
18. Mjaatvedt CH, Yamamura H, Wessels A, Ramsdell A, Turner D, Markwald R. Mechanisms of segmentation, septation, and remodeling of the tubular heart: endocardial cushion fate and cardiac looping. In: Harvy RP, Rosenthal N, eds. *Heart Development*. London, UK: Academic Press; 1999:159–177.
19. Ya J, Schilham M, DeBoer P, Moorman A, Clevers H, Lamers W. Sox4-deficiency syndrome in mice is an animal model for common trunk. *Circ Res*. 1998;83:986–994.
20. Le Douarin NM. *The Neural Crest.* Cambridge, UK: Cambridge University Press; 1982.
21. Kirby ML, Gale TF, Stewart DE. Neural crest cells contribute to aorticopulmonary septation. *Science*. 1983;220:1059–1061.
22. Kirby ML, Waldo KL. Neural crest and cardiovascular patterning. *Circ Res*. 1995;77:211–215.
23. Waldo KL, Miyagawa-Tomita S, Kumiski D, Kirby ML. Cardiac neural crest cells provide new insight into septation of the cardiac outflow tract: aortic sac to ventricular septal close. *Dev Biol*. 1998;196:129–144.
24. Kirby ML, Waldo KL. Role of neural crest in congenital heart disease. *Circulation*. 1990;82:332–340.
25. Kirby ML. Alteration of cardiogenesis after neural crest ablation. *Ann N Y Acad Sci*. 1990;588:289–295.
26. Bergwerff M, Verberne ME, DeRuiter MC, Poelmann RE, Gittenberger-de Groot AC. Neural crest cell contribution to the developing circulatory system: implications for vascular morphology? *Circ Res*. 1998;82:221–231.
27. Nishibatake M, Kirby ML, van Mierop LH. Pathogenesis of persistent truncus arteriosus and dextroposed aorta in the chick embryo after neural crest ablation. *Circulation*. 1987;75:255–264.
28. Rosenquist TH, Beall AC. Elastogenic cells in the developing cardiovascular system: smooth muscle, nonmuscle, and cardiac neural crest. *Ann N Y Acad Sci*. 1990;558:106–119.
29. Kirby ML, Turnage KL, Hays BM. Characterization of conotruncal malformations following ablation of "cardiac" neural crest. *Anat Rec*. 1985;213:87–93.
30. Lo CW, Waldo KL, Kirby ML. Gap junction communication and the modulation of cardiac neural crest cells. *Trends Cardiovasc Med*. 1999;9:83–89.

31. Gadson PF Jr, Dalton ML, Patterson E, Svoboda DD, Hutchinson L, Schram D, Rosenquist TH. Differential response of mesoderm- and neural crest-derived smooth muscle to TGF-beta1: regulation of c-myb and alpha1 (I) procollagen genes. *Exp Cell Res*. 1997;230:169–180.
32. Shah NM, Groves AK, Anderson DJ. Alternative neural crest cell fates are instructively promoted by TGFbeta superfamily members. *Cell*. 1996;85:331–343.
33. Le Lièvre CS, Le Douarin NM. Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. *J Embryol Exp Morphol*. 1975;34:125–154.
34. Waldo KL, Lo CW, Kirby ML. Connexin 43 expression reflects neural crest patterns during cardiovascular development. *Dev Biol*. 1999;208: 307–323.
35. Perez-Pomares JM, Macias-Lopez D, Garcia-Garrido L, Munoz-Chapuli R. Immunohistochemical evidence for a mesothelial contribution to the ventral wall of the avian aorta. *Histochem J*. 1999;31:771–779.
36. Ya J, van den Hoff MJB, de Boar PAJ, Tesink-Taekena S, Franco D, Moorman AFM, Lamers WH. Normal development of the outflow tract in the rat. *Circ Res*. 1998;82:464–472.
37. DeRuiter MC, Poelmann RE, VanMunsteren JC, Mironov V, Markwald RR, Gittenberger-de Groot AC. Embryonic endothelial cells transdifferentiate into mesenchymal cells expressing smooth muscle actins in vivo and in vitro. *Circ Res*. 1997;80:444–451.
38. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275:965–967.
39. Wood HB, May G, Healy L, Enver T, Morriss-Kay GM. CD34 expression patterns during early mouse development are related to modes of blood vessel formation and reveal additional sites of hematopoiesis. *Blood*. 1997;90:2300–2311.
40. Stainier DY, Weinstein BM, Detrich MW III, Zon LI, Fishman MC. Cloche, an early acting zebrafish gene is required by both endothelial and hematopoietic lineages. *Development*. 1995;121:3141–3150.
41. Jaffredo T, Gautier R, Eichmann A, Dieterlen-Lièvre F. Intraaortic hemopoietic cells are derived from endothelial cells during ontogeny. *Development*. 1998;125:4575–4583.
42. Mills KR, Kruep D, Saha MS. Elucidating the origins of the vascular system: a fate map of the vascular endothelial and red blood cell lineages in *Xenopus laevis*. *Dev Biol*. 199;209:352–368.
43. Takakura N, Watanabe T, Suenobu S, Yamada Y, Noda T, Ito Y, Satake M, Suda T. A role for hemopoietic stem cells in promoting angiogenesis. *Cell*. 2000;102:199–209.
44. de Bruijn MFTR, Speck NA, Peeters M, Dzierzak E. Definitive hemapoietic stem cells first develop within the major arterial regions of the mouse embryo. *EMBO J*. 2000;19:2465–2474.
45. Sartore S, Franch R, Roelofs M, Chiavegato A. Molecular and cellular phenotypes and their regulation in smooth muscle. *Rev Physiol Biochem Pharmacol*. 1999;134:235–320.
46. Holifield B, Helgason T, Jemelka S, Taylor A, Navran S, Allen J, Seidel C. Differentiated vascular myocytes: are they involved in neointimal formation? *J Clin Invest*. 1996;97:814–825.
47. Kim S, Ip HS, Lu MM, Clendenin C, Parmacek MS. A serum response factor-dependent transcriptional regulatory program identifies distinct smooth muscle cell sublineages. *Mol Cell Biol*. 1997;17:2266–2278.
48. Mack CP, Thompson MM, Lawrenz-Smith S, Owens GK. Smooth muscle alpha-actin CArG elements coordinate formation of a smooth muscle cell-selective, serum response factor-containing activation complex. *Circ Res*. 2000;86:221–232.
49. Mericskay M, Parlakian A, Porteu A, Dandre F, Bonnet J, Paulin D, Li Z. An overlapping CArG/octamer element is required for regulation of desmin gene transcription in arterial smooth muscle cells. *Dev Biol*. 2000;226:192–208.
50. Bierhuizen MF, van Amersfoorth SC, Groenewegen WA, Vliex S, Jongsma HJ. Characterization of the rat connexin40 promoter: two Sp1/Sp3 binding sites contribute to transcriptional activation. *Cardiovasc Res*. 2000;46:511–522.
51. Adam PJ, Regan CP, Hautmann MB, Owens GK. Positive and negative-acting Kruppel-like transcription factors bind a transforming growth factor beta control element required for expression of the smooth muscle cell differentiation marker SM22alpha in vivo. *J Biol Chem*. 2000;275: 37798–37806.
52. van den Hoff MJ, Moorman AF, Ruijter JM, Lamers WH, Bennington RW, Markwald RR, Wessels A. Myocardialization of the cardiac outflow tract. *Dev Biol*. 1999;212:477–490.
53. Poelmann RE, Gittenberger-de Groot AC. A subpopulation of apoptosis-prone cardiac neural crest cells targets to the venous pole: multiple functions in heart development? *Dev Biol*. 1999;207:271–286.
54. Kramer AW, Marks LS. The occurrence of cardiac muscle in the pulmonary veins of rodents. *J Morphol*. 1965;117:135–150.
55. Di Lisi R, Millino C, Calabria E, Altruda F, Schiaffino S, Ausoni S. Combinatorial cis-acting elements control tissue-specific activation of the cardiac troponin I gene in vitro and in vivo. *J Biol Chem*. 1998;273: 25371–25380.
56. Millino C, Sarinella F, Tiveron C, Villa A, Sartore S, Ausoni S. Cardiac and smooth muscle cell contribution to the formation of the murine pulmonary veins. *Dev Dyn*. 2000;218:414–425.
57. Krumlauf R. Hox genes in vertebrate development. *Cell*. 1994;78: 191–201.
58. Bergwerff M, DeRuiter MC, Hall S, Poelmann RE, Gittenberger-de Groot AC. Unique vascular morphology of the fourth aortic arches: possible implications for pathogenesis of type-B aortic arch interruption and anomalous right subclavian artery. *Cardiovasc Res*. 1999;44: 185–196.
59. Nakagawa O, Nakagawa M, Richardson JA, Olson EN, Srivastava D. HRT1, HRT2, and HRT3: a new subclass of bHLH transcription factors marking specific cardiac, somitic, and pharyngeal arch segments. *Dev Biol*. 1999;216:72–84.
60. Zhong TP, Rosenberg M, Mohideen M-APK, Weinstein B, Fishman MC. *Gridlock*, an HLH gene required for assembly of the aorta in zebrafish. *Science*. 2000;287:1820–1824.
61. Kurihara Y, Kurihara H, Oda H, Maemura K, Nagai R, Ishikawa T, Yazaki Y. Aortic arch malformations and ventricular septal defect in mice deficient in endothelin-1. *J Clin Invest*. 1995;96:293–300.
62. Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, Kuwaki T, Kumada M, Hammer RE, Yanagisawa M. Cranial and cardiac neural crest defects in endothelin-A receptor-deficient mice. *Development*. 1998;125:813–824.
63. Yanagisawa H, Yanagisawa M, Yanagisawa H, Yanagisawa M, Kapur RP, Richardson JA, Williams SC, Clouthier DE, de Wit D, Emoto N, et al. Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene. *Development*. 1998;125:825–836.
64. Thomas T, Kurihara H, Yamagishi H, Kurihara Y, Yazaki Y, Olson EN, Srivastava D. A signaling cascade involving endothelin-1, dHAND and msx1 regulates development of neural-crest-derived branchial arch mesenchyme. *Development*. 1998;125:3005–3014.
65. Miao H-Q, Soker S, Feiner L, Alonso JL, Raper JA, Klagsbrun M. Neuropilin-1 mediates collapsin-1/semaphorin III inhibition of endothelial cell motility: functional competition of collapsin-1 and vascular endothelial growth factor. *J Cell Biol*. 1999;146:233–241.
66. Püschel AW. Divergent properties of mouse netrins. *Mech Dev*. 1999; 83:65–75.
67. Wang HU, Chen ZF, Anderson DJ. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell*. 1998;93:741–753.
68. Adams RH, Wilkinson GA, Weiss C, Diella F, Gale NW, Deutsch U, Risau W, Klein R. Roles of ephrinB ligands and EphB receptors in cardiovascular development: demarcation of arterial/venous domains, vascular morphogenesis, and sprouting angiogenesis. *Genes Dev*. 1999; 13:295–306.
69. Mellitzer G, Xu Q, Wilkinson DG. Control of cell behaviour by signaling through Eph receptors and ephrins. *Curr Opin Neurobiol*. 2000; 10:400–408.
70. Huynh-Do U, Stein E, Lane AA, Liu H, Cerretti DP, Daniel TO. Surface densities of ephrin-B1 determine EphB1-coupled activation of cell attachment through $\alpha_{5\beta3}$ and $\alpha_{5\beta1}$ integrins. *EMBO J*. 1999;8:2165–2173.
71. Burke RD, Wang D, Jones VM. Ontogeny of vessel wall components in the outflow tract of the chick. *Anat Embryol (Berl)*. 1994;189:447–456.
72. Yamamura H, Zhang M, Markwald RR, Mjaatveldt CH. A heart segmental defect in the anterior-posterior axis of a transgenic mutant mouse. *Dev Biol*. 1997;186:58–72.
73. Camenisch TD, Spicer AP, Brehm-Gibson T, Biesterfeldt J, Augustine ML, Calabro A Jr, Kubalak S, Klewer SE, McDonald JA. Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. *J Clin Invest*. 2000;106:349–360.
74. Yamagishi H, Garg V, Matsuoka R, Thomas T, Srivastava D. A molecular pathway revealing a genetic basis for human cardiac and craniofacial defects. *Science*. 1999;283:1158–1160.
75. Yamagishi H, Olson EN, Srivastava D. The basic helix-loop-helix transcription factor, dHAND, is required for vascular development. *J Clin Invest*. 2000;105:261–270.

76. Lin Q, Schwarz J, Bucana C, Olson EN. Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. *Science*. 1997;276:1404–1407.
77. Bi W, Drake CJ, Schwarz JJ. The transcription factor MEF2C-null mouse exhibits complex vascular malformations and reduced cardiac expression of angiopoietin 1 and VEGF. *Dev Biol*. 1999;211:255–267.
78. Chisaka O, Capecchi MR. Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene *hox-1.5*. *Nature*. 1991;350:473–479.
79. Franz T. Persistent truncus arteriosus in the Splotch mutant mouse. *Anat Embryol*. 1989;180:457–464.
80. Conway SJ, Henderson DJ, Kirby ML, Anderson RH, Copp AJ. Development of a lethal congenital heart defect in the splotch (Pax3) mutant mouse. *Cardiovasc Res*. 1997;36:163–173.
81. Conway SJ, Henderson DJ, Copp AJ. Pax3 is required for cardiac neural crest migration in the mouse: evidence from the splotch (Sp2H) mutant. *Development*. 1997;124:505–514.
82. Iida K, Koseki H, Kakinuma H, Kato N, Mizutani-Koseki Y, Ohuchi H, Yoshioka H, Noji S, Kawamura K, Kataoka Y, et al. Essential roles of the winged helix transcription factor MFH-1 in aortic arch patterning and skeletogenesis. *Development*. 1997;124:4627–4638.
83. De la Pompa JL, Timmerman LA, Takimoto H, Yoshida H, Elia AJ, Samper E, Potter J, Wakeham A, Marengere L, Lowell Langille B, et al. Role of the NF-Atc transcription factor in morphogenesis of cardiac valves and septum. *Nature*. 1998;392:182–185.
84. Ranger AM, Grusby MJ, Hodge MR, Gravallese EM, de la Brousse FC, Hoey T, Mickanin C, Scott Baldwin H, Glimcher LH. The transcription factor NF-ATc is essential for cardiac valve formation. *Nature*. 1998; 392:186–190.
85. Mendelsohn C, Lohnes D, Decimo D, Lufkin T, LeMeur M, Chambon P, Mark M. Function of the retinoic acid receptors (RARs) during development (II): multiple abnormalities at various stages of organogenesis in RAR double mutants. *Development*. 1994;120:2749–2771.
86. 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;98:1332–1343.
87. Donovan MJ, Hahn R, Tessarollo L, Hempstead BL. Identification of an essential nonneuronal function of neurotrophin 3 in mammalian cardiac development. *Nat Genet*. 1996;14:210–213.
88. Reaume AG, de Sousa PA, Kulkarni S, Langille BL, Zhu D, Davies TC, Juneja SC, Kidder GM, Rossant J. Cardiac malformation in neonatal mice lacking connexin43. *Science*. 1995;267:1831–1834.
89. Ya J, Erdtsieck-Ernste EB, de Boer PA, van Kempen MJ, Jongsma H, Gros D, Moorman AF, Lamers WH. Heart defects in connexin43-deficient mice. *Circ Res*. 1998;82:360–366.
90. Morrison-Graham K, Schatteman GC, Bork T, Bowen-Pope DF, Weston JA. A PDGF receptor mutation in the mouse (Patch) perturbs the development of a non-neuronal subset of neural crest-derived cells. *Development*. 1992;115:133–142.
91. Sanford LP, Ormsby I, Gittenberger-de Groot AC, Sariola H, Friedman R, Boivin GP, Cardell EL, Doetschman T. TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. *Development*. 1997;124:2659–2670.
92. Lindsay EA, Botta A, Jurecic V, Carattini-Rivera S, Cheah YC, Rosenblatt HM, Bradley A, Baldini A. Congenital heart disease in mice deficient for the DiGeorge syndrome region. *Nature*. 1999;401: 379–383.
93. Brannan CI, Perkins AS, Vogel KS, Ratner N, Nordlund ML, Reid SW, Buchberg AM, Jenkins NA, Parada LF, Copeland NG. Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. *Genes Dev*. 1994;8:1019–1029.
94. Oh SP, Li E. The signaling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. *Genes Dev*. 1997;11:1812–1826.
95. Sans-Coma V, Arque JM, Duran AC, Cardo M, Fernandez B. Coronary artery anomalies and bicuspid aortic valves in the Syrian hamster. *Basic Res Cardiol*. 1991;86:148–153.
96. Icardo JM, Sanchez de Vega MJ. Spectrum of heart malformations in mice with situs solitus, situs inversus, and associated visceral heterotaxy. *Circulation*. 1991;84:2547–2558.
97. Pereira FA, Qiu Y, Zhou G, Tsai MJ, Tsai SY. The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. *Genes Dev*. 1999;13:1037–1049.
98. Wilson DI, Burn J, Scambler P, Goodship J. DiGeorge syndrome: part of CATCH 22. *J Med Genet*. 1993;30:852–856.
99. Novelli G, Amati F, Dallapiccola B. Individual haploinsufficient loci and the complex phenotype of DiGeorge syndrome. *Mol Med Today*. 2000;1:10–11.
100. Baldini A. DiGeorge syndrome: complex pathogenesis?: maybe, maybe not. *Mol Med Today*. 2000;6:12.
101. Srivastava D. DiGeorge syndrome: an enigma in mice and men. *Mol Med Today*. 2000;6:13–14.