Multipotent Islet-1 Cardiovascular Progenitors in Development and Disease

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During the past several years, advances at the intersection of cardiovascular development and heart stem cell biology have begun to reshape our view of the fundamental logic that drives the formation of discrete tissue components in the mammalian heart. Although many of the critical genes that control cardiac myogenesis have been identified, our understanding of how a highly diverse and specialized subset of heart cell lineages arises from mesodermal precursors and is subsequently assembled into distinct muscle chambers, coronary arterial tree and large vessels, valvular tissue, and conduction system/pacemaker cells remains at a relatively primitive stage. Recent studies have uncovered a diverse group of closely related heart progenitors that are central in controlling and coordinating these complex steps of cardiogenesis. Understanding the pathways that control their formation, renewal, and subsequent conversion to specific differentiated progeny forms the underpinning for unraveling the pathways for congenital heart disease and has direct relevance to cardiovascular regenerative medicine. This current brief review highlights the discovery and delineation of the role of Islet-1 cardiovascular progenitors in the generation of diverse heart cell lineages and how the implications of these findings are revising our classification and thinking about congenital heart disease in general.

The mammalian heart is a complex multichambered organ consisting of two ventricles, two atria, outflow tract and inflow tract, conduction system, valves, endocardium, and epicardium. The proper function of the whole heart requires a highly coordinated process of differentiation and integration of all of the cellular components (Olson 2006; Srivastava 2006). One of the central questions in cardiovascular biology relates to how these various components are assembled from early mesodermal precursors located in the heart field in the early mammalian embryo.

The cardiac precursor cells are specified in the anterior part of the primitive streak shortly after gastrulation. These precursor cells migrate toward the anterior lateral plate mesoderm and form a pair of cardiac crescents, the first visible and morphologically distinguishable structure of the cardiac primordia. Endocardial tubes are already formed in each of cardiac crescents at this stage. As the neuroectoderm develops rapidly at this stage, the heart-forming region is pushed ventrally and fuses to form a single linear heart tube. It has been long thought that the identity of each heart chamber is already specified at an early stage within each segment of the linear heart tube—outflow tract, right ventricle, left ventricle, atria, and sinus venosus, from the cranial to caudal direction.

The discovery of the second progenitor source has begun to challenge this classical segmental prespecification view of cardiogenesis and is reshaping our view of congenital heart abnormalities (Buckingham et al. 2005). This second source of cardiac progenitors is formed in the dorsomedial part of the anterior lateral plate mesoderm adjacent to the

cardiac crescent (Fig. 1). Recently, this extra-crescent heart field (second heart field; SHF) was revisited and characterized by several different techniques: dye labeling, detection of specific molecular markers, and retrospective single-cell-fate tracing experiments (Kelly et al. 2001; Mjaatvedt et al. 2001; Waldo et al. 2001; Cai et al. 2003; Dodou et al. 2004; Meilhac et al. 2004). Different methods and different markers have determined slightly different populations of the cardiac progenitors, which has led to the initial confusion of the definition of SHF and anterior heart field (AHF). The SHF is well delineated by the expression of *Isl1*, a member of the LIM homeodomain transcription factor family (Cai et al. 2003), whereas several other molecular markers, such as *FGF8/10*, *Wnt11*, and a specific *Mef2c* enhancer, label the anterior subset of SHF. In this chapter, “SHF” is used in a broader sense, including both the anterior heart field subset and the posterior subset that mainly contributes to the atria and inflow tract.

The dorsal mesocardium, the mesentery that suspends the linear heart tube, is initially continuous to the splanchnic mesoderm at any level at the linear heart tube stage. Cardiac progenitor cells in the splanchnic mesoderm continue to migrate through the dorsal mesocardium. As the dorsal mesocardium breaks down, the heart tube becomes connected to the body wall only at the arterial pole and the venous pole. From this point, the SHF progenitors migrate to the heart tube at either pole.

The rightward looping of the heart tube is then triggered by the addition of second heart field progenitors onto the scaffold of the primary heart tube and subsequent segmental ballooning of outer curvature of each chamber. The rapid growth of each segment places the forming atria and inflow portion above the ventricular chambers. The rightward looping is the first appearance of left-right asymmetry in the heart. Obviously, the rapid growth of the polar compart-

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Figure 1. Morphogenesis of the mouse embryonic heart. The primordia of the heart are first morphologically recognizable as the cardiac crescent. Whereas the cells in the first heart field (FHF, pink) mainly contribute to the formation of the cardiac crescent, the cells in the second heart field (SHF, blue) appear in extra-crescent tissue. At around E8, bilateral crescents fuse in the midline to form a single linear heart tube. The primary heart tube is mainly composed of FHF cells, but SHF cells start to migrate toward the primary heart tube through the dorsal mesocardium, which is still continuous to the heart tube at any level at this stage. As the dorsal mesocardium dissolves, the heart tube become suspended by two poles, creating two subpopulations of SHF cells: anterior/arterial pole/outflow tract population and posterior/venous pole/inflow tract population. The anterior subpopulation gives rise to the myocardium of the outflow tract and right ventricle and acquires the ventricular phenotype. The posterior population contributes mainly to atrial myocytes and acquires the atrial phenotype. Concomitant with this, the heart tube starts to undergo rightward looping (d loop).

ments by itself is not the primary initiator of the asymmetry but an enhancer of preexisting laterality information.

After the cardiac chambers are positioned as seen in the postnatal heart, the heart undergoes a series of septation events. The formation of the conotruncal part of the heart requires the contribution of two distinct cell types: cardiac neural crest and second heart field progenitors. These two populations have distinct developmental origins, but they migrate through the pharyngeal mesoderm in a closely related pathway (Kelly and Buckingham 2002). Cardiac neural crest cells arise from the dorsal neural tube at the level of rhombomeres 6–8 and migrate into the arterial pole of the looping heart tube (Stoller and Epstein 2005; Hutson and Kirby 2007; Snider et al. 2007). This specific population of the neural crest contributes to the septum of the outflow tract and the smooth muscle wall of the ascending aorta, aortic arch, and orifice of the left and right coronary arteries. Simultaneously to this septation event, the outflow myocardium of the second heart field origin undergoes shortening. Ablation of either cell population results in the hypoplasia and rotation defects of the cardiac outflow tract (Yelbuz et al. 2002; Baldini 2005). Therefore, the interaction of these two populations has an important role in the morphogenesis of the cardiac outflow tract (Waldo et al. 2005a).

Endocardial cushions also contribute to the formation of septa as well as the valves. After the completion of the

looping, endocardial cells at both ends of the heart tube—outflow tract and atrioventricular (AV) canal—transform into mesenchymal cell types and migrate into the cardiac jelly between endocardial and myocardial layers to form endocardial cushions. Cushions in the outflow portion are the primordia of the outflow septum and semilunar valves, and the cushion in the AV canal gives rise to atrial and ventricular septa and the AV valves.

CELL-FATE MAP

The earliest process of mammalian cardiogenesis has largely been a black box, partly because of the technical inaccessibility to the embryo at this stage. Instead, this step has been intensively studied in lower vertebrates (chick, amphibians, zebra fish) and nonvertebrate (fly) models. These nonmammalian models suggested that at least part of the endothelial/endocardial lineage shares a common origin with cardiac precursors. Recent advances in mouse genetics and embryonic stem (ES) cell technology have enabled us to study this process at the cellular level in mammals (Fig. 2). Mesodermal precursor cells expressing *Flk1/Brachyury (T)* and early cardiac progenitors labeled by *Isl1/Nkx2.5/Flk1* are shown to be capable of differentiating into cardiac, smooth muscle, and endothelial lineages from single cells (Garry and Olson 2006; Kattman et

Figure 2. A model for cell-fate map of cardiac progenitors. Putative common precursor cells are specified from mesodermal precursors shortly after gastrulation. This common precursors give rise to first (*Isl1*[−]) and second (*Isl1*⁺) heart field progenitors (FHF and SHF). The SHF population can be further subdivided into anterior and posterior secondary heart field subpopulations. The FHF population gives rise to the left ventricle (red); the anterior SHF population gives rise to the outflow tract, right ventricle, and AV nodal cells; and the posterior SHF population contributes mainly to atrial myocytes and SA nodal cells. Proepicardial progenitor cells may share a common origin with SHF progenitors (they also give rise to cardiomyocytes, express early cardiac markers *Isl1* and *Nkx2.5*, and arise in close vicinity to the posterior SHF). (LV) Left ventricle; (OFT) outflow tract; (RV) right ventricle; (SA) sinoatrial; (AV) atrioventricular; (SAN) sinoatrial node.

al. 2006, 2007; Moretti et al. 2006; Martin-Puig et al. 2008; Yang et al. 2008). *Mesp1* acts as a master regulator during the commitment of mesodermal cells into cardiac lineages (Bondue et al. 2008; Lindsley et al. 2008; Wu 2008). Whereas endothelial lineages separate from cardiac and smooth muscle lineages at an early stage, cardiac and smooth muscle lineages are closely related until later stages of cardiogenesis (Wu et al. 2006). Smooth muscle cells appear to be recruited locally from a wide range of mesodermal tissues and differentiate in response to local inductive signals (Waldo and Kirby 1993; Topouzis and Majesky 1996; Waldo et al. 2005b; Majesky 2007).

Using *in vivo* retrospective clonal assays, Meilhac et al. (2004) showed that cardiac progenitors come from two major origins corresponding to the FHF and SHF. Both FHF and SHF progenitors contribute to most of the cardiac regions except for the left ventricle (mainly FHF) and outflow tract (mainly SHF), challenging the classical segmental view of cardiogenesis. Furthermore, they also speculate that these two lineages likely originate from putative common precursors. It remains to be answered whether there is a fundamental functional difference other than the marker expression between these two populations and whether these two are mutually exchangeable. Indeed, there are no morphological differences between the progenitors in FHF and SHF at a cellular level, nor are there

clear morphological boundaries at the cardiac crescent stage. Even in the postnatal heart, there are no obvious morphological differences among cardiomyocytes in OFT/RV and LV at the cellular level. Recent analysis suggests the possibility that *Isl1* is transiently expressed in the FHF (Prall et al. 2007), although lineage-tracing studies support the concept of a distinct SHF subset of progenitors. Thus, the argument still remains as to whether it is relevant to define so-called SHF as another “organ field” of the heart (Abu-Issa et al. 2004; Moorman et al. 2007). Current studies designed to purify and tag specific subsets of FHF and SHF heart progenitors from ES cells and early-stage embryos should help to resolve this issue.

The breakdown of dorsal mesocardium further subdivides SHF into two major cardiac progenitor populations: anterior/arterial pole/outflow populations and posterior/venous pole/inflow populations (Fig. 3). The anterior population is labeled by several molecular markers, including fibroblast growth factors (FGFs), bone morphogenetic protein (BMPs), *Wnt11*, and a specific enhancer of *Mef2c* (Kelly et al. 2001; Cai et al. 2003; Dodou et al. 2004; Lin et al. 2007), and eventually acquires the ventricular phenotype contributing to the outflow tract and right ventricle. The posterior population eventually gives rise to atrial myocardium (Galli et al. 2008). The posterior population may also contribute to multiple lineages, such as the

Figure 3. Migration of anterior and posterior population of SHF progenitors. Shortly after the formation of the linear heart tube, the anterior SHF progenitors migrate to the arterial pole of the primitive heart tube. This population is marked by FGF8/10 and the specific enhancer of *Mef2c*. The anterior population eventually acquires the ventricular phenotype and gives rise to the cardiomyocytes in the outflow tract and right ventricle, as well as smooth muscle cells in the base of the ascending aorta. The posterior subpopulation of the SHF migrates toward the venous pole and contributes to atrial myocytes and myocardial sleeves, as well as to smooth muscle cells in the cardiac inflow. Note that the proepicardium arises from the boundary between the embryonic liver and sinus venosus and thus spatially and temporally continuous to the posterior SHF.

Tbx18⁺ population (Christoffels et al. 2006) and mediastinal myocardial population (Anderson et al. 2006; Snarr et al. 2007a,b). Identification of additional specific molecular markers will further help to establish the cellular hierarchy of cardiac lineages (Laugwitz et al. 2008).

Recent findings suggest that the proepicardium may be another source of cardiomyocytes (Cai et al. 2008; Zhou et al. 2008). The proepicardium is a pair of grape-like clusters of epithelial cells that arise between the sinus venosus and liver primordium. Using retroviral tagging methods, Mikawa and Gourdie (1996) proved the speculation by Goor and Lillehei (1975) that the proepicardium is the source of the coronary vasculature. A combinatorial approach using mouse genetic models and the ES system showed that proepicardial cells also contribute to some of the cardiomyocytes (Cai et al. 2008; Zhou et al. 2008). These proepicardial progenitor cells are positive for both *Isl1* and *Nkx2.5*. Given that the proepicardium is spatially and temporally continuous to the posterior population of SHF, the proepicardial progenitors may share a common origin with cardiac progenitors in the SHF.

Apart from the argument regarding the expression of *Isl1* in the FHF, *Isl1* is expressed in most of the other cardiac lineages, as well as the proepicardial lineages. Hence, *Isl1* labels a pool of various cardiac progenitor subpopulations. Although *Isl1* is down-regulated as the cardiomyocytes mature, it stays on until a later stage in some subsets and turns off early in other subsets. Notably, these diverse progenitor populations arise from the spatiotemporal continuity of the *Isl1*-positive field of the primordial heart. The common function of the *Isl1* transcription factor in these diverse populations is of interest. Obviously, *Isl1* is expressed in proliferative cardiac progenitors. Our recent data show that *Isl1* expression strongly correlates with cell cycle activity (A. Nakano et al., unpubl.). Therefore, *Isl1* may well be regarded as a marker for proliferative progenitors during their transition to cardiomyocytes as well as for the cells early in the SHF lineage.

CONGENITAL HEART DISEASE AND *ISL1* PROGENITOR

Many monogenic heart diseases result in closely related phenotypes, suggesting that abnormalities of cardiac progenitors resulting from these genetic deficiencies may be fundamental to the pathogenesis of congenital heart disease. Identification of the cellular level of the defects in the cardiac progenitor lineages in these diseases should link molecular function of genes to the pathogenesis of congenital heart defects. Figure 4 displays several of the human congenital heart defects associated with gene mutations. Although studies in model systems have shown that *Isl1* is expressed in a variety of cardiac lineages and has a pivotal role in the transcription network during cardiogenesis (Black 2007), it is currently of major interest to determine if *Isl1* genetic variation might relate to human congenital heart abnormalities.

CARDIAC OUTFLOW TRACT AND WNT/ β -CATENIN SIGNALING

Many of the key major signaling molecules such as BMP, FGF, Shh, Wnt, and Notch are also implicated in multiple processes of cardiogenesis. Among these signals, the Wnt/ β -catenin pathway is unique in that it has a highly context-dependent role during cardiogenesis. Early in avian cardiogenesis, canonical Wnt signals from the neuroectoderm are known to inhibit the specification of cardiac progenitors, resulting in a crescent-shaped heart-forming region (Marvin et al. 2001; Schneider and Mercola 2001; Tzahor and Lassar 2001). Mouse ES-based data mostly suggest a positive effect of Wnt/ β -catenin signaling on the appearance of beating cardiomyocytes (49–52). Genetic loss- and gain-of-function experiments using various Cre mice mostly resulted in the conclusion that Wnt/ β -catenin is involved in expansion of the cardiac progenitor population (Naito et al. 2006; Cohen et al. 2007; Klaus et al. 2007; Kwon et al.

Figure 4. Distribution of Isl1-positive progenitor-derived cells and congenital heart disease. (AS) Aortic stenosis; (ASD) atrial septal defect; (AV) atrioventricular; (MS) mitral stenosis; (PDA) patent ductus arteriosus; (PS) pulmonary stenosis; (PV) pulmonary vein; (TAPVR/PAPVR) total/partial anomalous pulmonary venous return; (TGA) transposition of great arteries; (TOF) tetralogy of Fallot.

	Isl1-derived cells in the lesion	Congenital anomalies
Outflow tract defects		
Aortic stenosis		
Fibromuscular subvalvar	◦	NKX2.5 (A)
Bicuspid aortic valve	◦	Notch2 (Alagille) (B)
Supravalvar (SVAS)	◦	Elastin (Williams-Beuren) (C)
Pulmonary stenosis	◦	NF1 (D), Jag1 (Alagille) (E), Notch2 (Alagille) (B), PTPN11/Shp2 (Noonan) (F), MEK1/2 (G), B-Raf/H/K-Ras (H), GATA4 (I)
Tetralogy of Fallot		
RV/OFT obstruction, overriding aorta	◦	Jag1 (Alagille) (E), Notch2 (Alagille) (B), NKX2.5 (A)
Double-outlet right ventricles	◦	CFC1 (J), NKX2.5 (A), GATA4 (I)
Truncus arteriosus	◦	TBX1 (DiGeorge) (K), VEGF promoter (DiGeorge) (L)
Transposition of great arteries	◦	ZIC3 (M), CFC1 (J), THRAP2 (N)
Inflow tract defects		
Cor triatriatum	?	
TAPVR and PAPVR	?	
Anomalies of systemic veins	◦	NKX2.5 (A)
Septation defects		
VSD	◦	TBX1 (DiGeorge) (O), TBX5 (P), CHD7 (CHARGE) (Q), Sema3E (CHARGE) (R), NKX2.5 (A), GATA4 (I)
ASD	◦	TBX5 (P), CHD7 (CHARGE) (Q), Sema3E (CHARGE) (R), NKX2.5 (A), GATA4 (I), TBX20 (S), MYH6 (T)
AVSD (endocardial cushion defect)	◦	GJA1 (U), CRELD1 (V), FOG2 (W), GATA4 (I)
Valvular defects		
Congenital MS	?	
Tricuspid atresia	?	NKX2.5 (Epstein) (A)
Defects in aortic remodeling		
Patent ductus arteriosus (usually secondary)	◦	TFAP2 β (X)
Coarctation of the aorta	◦	TBX1 (DiGeorge) (O)
Aortopulmonary septal defect (AP window)	◦	
Coronary defects		
Anomalous origin of coronary arteries	◦	
Congenital distal coronary defects (fistula, aneurysm, stenosis, hypoplasia)	◦	
Congenital cardiac conduction system defects		
Congenital sinus nodal dysfunction	◦	SCN5A (Y)
WPW syndrome	?	PRKAG2 (Z)
Congenital AV block	◦	TBX5 (P), NKX2.5 (A)
Long QT syndrome	x	KCNQ1, KCNH2, KCNE1, KCNE2, CACNA1c, CAV3, SCN5A, SCN4B, HCN4
RBBB	?	TBX5 (P)
Left ventricular defects		
Hypoplastic left heart syndrome	x	GJA1 (U)
Left ventricular noncompaction syndrome	x	DTNA (ZZ), NKX2.5 (Epstein) (A)

References: (A) Schott et al. 1998; Benson et al. 1999; Kasahara et al. 2000; Goldmuntz et al. 2001; Gutierrez-Roelens et al. 2002; Ikeda et al. 2002; Watanabe et al. 2002; McElhinney et al. 2003; Kasahara and Benson 2004; Pashmforoush et al. 2004; (B) McDaniell et al. 2006; (C) Ewart et al. 1993; (D) Li et al. 1992; Bahuau et al. 1996, 1998; Lin et al. 2000; (E) Li et al. 1997a; Oda et al. 1997; (F) Tartaglia et al. 2001; (G) Rodriguez-Viciana et al. 2006; (H) Aoki et al. 2005; Niihori et al. 2006; Schubbert et al. 2006; (I) Pehlivan et al. 1999; Garg et al. 2003; Hirayama-Yamada et al. 2005; (J) Bamford et al. 2000; Goldmuntz et al. 2002; (K) Jerome and Papaioannou 2001; Lindsay et al. 2001; Merscher et al. 2001; Yagi et al. 2003; (L) Stalmans et al. 2003; (M) Gebbia et al. 1997; (N) Muncke et al. 2003; (O) Jerome and Papaioannou 2001; Lindsay et al. 2001; Merscher et al. 2001; (P) Basson et al. 1997; Li et al. 1997b; (Q) Vissers et al. 2004; (R) Lalani et al. 2004; (S) Kirk et al. 2007; (T) Ching et al. 2005; (U) Dasgupta et al. 2001; (V) Robinson et al. 2003; Zatyka et al. 2005; Maslen et al. 2006; (W) Pizzuti et al. 2003; (X) Satoda et al. 2000; Zhao et al. 2001; (Y) Benson et al. 2003; (Z) Gollob et al. 2001a,b; (ZZ) Ichida et al. 2001.

2007; Qyang et al. 2007; Ueno et al. 2007). These results reflect the difference in each experimental system. First, Wnt/ β -catenin has various roles at different stages during cardiogenesis (stage dependency) (Tzahor 2007). Second, it is likely that the Wnt/ β -catenin pathway has a different role in various subsets of the progenitors (sublineage dependency). Third, many of the key signaling molecules and transcription factors are known to have different roles at different dosages and different gradients (dosage and gradient dependency). Fourth, in some cases, it is difficult to segregate specification, migration, and proliferation of the Isl1 progenitors in some models. Thus, the Wnt/ β -catenin pathway has a highly context-dependent role. However, despite all of the limitations of each experimental system, it is clear that the Wnt/ β -catenin pathway is one of the most promising signaling molecules from the viewpoint of future therapeutic application.

ANCHORING ATRIAL CHAMBERS AND GREAT VEINS IN THE CARDIAC INFLOW TRACT

As discussed above, the SHF delineated by Isl1 can be subdivided into two major cardiac populations: the anterior subpopulation in the arterial pole and the posterior subpopulation in the venous pole of the heart tube (Fig. 3) (Galli et al. 2008). Isl1-positive cardiac progenitors that populate in the anterior SHF/arterial pole of the primitive heart tube give rise to both cardiomyocytes and smooth muscle cells in the cardiac outflow tract (Waldo et al. 2005b). Analogous to this observation, we have recently found that Isl1-positive cardiac progenitors in the posterior SHF/venous pole give rise to atrial cardiomyocytes as well as smooth muscle cells in the cardiac inflow region (A. Nakano et al., unpubl.). Single-cell analysis using a cardiac mesenchymal feeder system revealed that this lineage diversification is controlled by the bipotency of

atrial progenitors that express Isl1 and sarcolipin (SLN), a marker for committed atrial cells.

An important biological question is why these Isl1-positive cardiac progenitors show bipotency until a relatively late stage and how this bipotency serves to coordinate the morphogenesis of atrial chambers and the great veins. One possibility is that bipotency is required to connect the boundary between the atrial chambers and great veins and to maintain the cardiac syncytium. In fact, when the differentiation of atrial progenitors is inhibited, murine embryos overexpressing β -catenin in the atrial lineage (SLN^{cre/+}; β cat^{ex3/+}) showed significantly smaller atria and inflow tracts compared with control littermates (Fig. 5). Some of the mutants examined showed a massive hemorrhage, possibly leaking from the boundary between atrial chamber and inflow veins. These data suggest that bipotency of atrial progenitors is required for anchoring the atrial chamber to the great veins and forming functional syncytia that facilitate the dynamism of the central circulation system. From a developmental perspective, it implies that the cells in the boundary of two different tissues have to be phenotypically plastic in order to glue these tissues and maintain anatomical and functional continuity. It would be of interest to determine whether the anterior/arterial pole population of the cardiac progenitors displays similar bipotency during cardiogenesis.

CONGENITAL HEART DISEASE AFFECTING THE DEVELOPMENT OF CARDIAC INFLOW TRACT

The defect in the connection of the atrial chamber and great veins possibly has an important role in the pathogenesis of several particular congenital heart malformations (Fig. 4). Anomalous pulmonary venous return denotes a spectrum of malformations in which at least one pulmonary vein connects to the right atrium directly or

Figure 5. Inhibition of the differentiation of atrial progenitors results in the malformation of the boundary between the atrial chamber and great veins. The differentiation of atrial progenitors is inhibited by the overexpressing active form of β -catenin. The SLN^{cre/+}; β cat^{ex3/+} embryo showed significantly smaller atria (black arrows) and a narrower vena cava (white arrows). One of three mutants showed a massive hemorrhage, possibly due to the malconnection of the atrial chamber and great veins (arrowheads). These data suggest that the proper differentiation of atrial progenitors is required for anchoring the vascular smooth muscle wall to the myocardium of atria and for generating functional syncytium. (LV) Left ventricle; (LA) left atrium.

through a systemic vein(s). This anomaly can be asymptomatic depending on the severity of the left-to-right shunt, and the incidence may be more common than we find from clinical cases. Cor triatriatum is another congenital heart disease in which the connection of pulmonary veins and left atrium is affected. The inadequate integration of the common pulmonary vein results in the formation of two chambers: the accessory chamber (enlarged common pulmonary vein) and the true left atrium. Mice have a common pulmonary vein that collects blood from four lobar pulmonary veins. Pulmonary vein stenosis is a congenital heart disease characterized by recurrent obstruction of pulmonary veins by proliferation of spindle-shaped myofibroblasts of the junction between the pulmonary vein and the left atrial chamber (Sadr et al. 2000). Compared with the anomalies on the left side, the connection problem of the systemic veins to the right side of the heart is rare. Precise analysis of these human congenital diseases in the inflow tract will answer the questions of how the myocardial sleeves develop, how the boundary of atrial myocardium and vascular smooth muscle cells is formed during cardiogenesis, and the biological significance of the plasticity of atrial cells.

RETRODIFFERENTIATION

The Isl1/SLN atrial progenitors from the posterior/venous part of the SHF maintain their migratory and proliferative potential even until midgestation. Consistent with this, Isl1 expression continues at E13.5 in mouse atria (Sun et al. 2007). The late development of atrial progenitors is likely linked to the fact that atrial progenitors remain bipotent until later stages of heart development. Atrial cells can acquire a smooth muscle and ventricular phenotype even after birth. Interestingly, this phenotypic plasticity is at least partially acquired by a retrodifferentiation process. Upon culture on cardiac feeder or upon in vivo injury, atrial cells reenter the cell cycle and re-express the Isl1 that is not normally expressed in postnatal atrial myocytes. The cellular plasticity of the atrial cells in the inflow may be related to the pathogenesis of atrial fibrillation.

A number of stem/progenitor cell populations have been identified in the postnatal heart including c-kit-positive and Scf-positive stem/progenitor cells and Isl1-positive progenitors that are embedded in cardiac mesenchyme and considered to be developmental remnants of the secondary heart field (Orlic et al. 2001; Beltrami et al. 2003; Oh et al. 2003; Laugwitz et al. 2005). Researchers speculate that regenerating zebra fish myocardium also arises from undifferentiated cardiac progenitors (Lepilina et al. 2006). The discovery of these preexisting stem/progenitor cells offers a great opportunity to regenerate the lost cardiomyocytes (Murry et al. 2005; Srivastava and Ivey 2006; Rubart and Field 2008). However, besides the activation of these residential undifferentiated stem/progenitor cells (dedifferentiation-independent regeneration), a trace of another mechanism of tissue regeneration may also take place in the heart: mature cells dedifferentiate, reenter the cell cycle, and redifferentiate another mature cell type (dedifferentia-

tion-dependent mechanism). Further analysis using defined markers will lead to a better understanding of the pathogenesis of the above-mentioned congenital heart diseases, the normal developmental process of the atrial lineage, the cellular mechanism of their plasticity, and eventually the clinical usage of Isl1-positive progenitor cells derived from atria.

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