The Arterial Epicardium: A Developmental Approach to Cardiac Disease and Repair

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Abstract

The significance of the epicardium that covers the heart and the roots of the great arteries should not be underestimated as it is a major component with impact on development, disease, and repair. The epicardium differentiates from the proepicardial organ located at the venous pole (vPEO). The differentiation capacities of the vPEO into epicardium-derived cells (EPDCs) have been extensively described. A hitherto escaped part of the epicardium derives from a second proepicardial organ located at the arterial pole (aPEO) and covers the intrapericardial part of the aorta and pulmonary trunk. In avian and mouse embryos, disturbance of epicardium differentiation causes a spectrum of cardiac anomalies including coronary artery abnormalities, deficient annulus fibrosis with rhythm disturbances, valve malformations, and non-compaction cardiomyopathies. Late in prenatal life the epicardium becomes dormant, losing the activity of many genes.

In human cardiac diseases, both arterial and venous epicardium can be activated again into EPDCs. The epicardial reactivation observed after experimental

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myocardial infarction and during aneurysm formation of the ascending aorta provides clinical relevance. EPDCs applied for cell therapy demonstrate repair processes synergistic with the resident cardiac progenitor stem cells that probably share an embryonic origin with EPDCs. Future therapeutic strategies might be possible addressing cell autonomous-based and signaling capacities of the adult epicardium.

Keywords
Epicardium • Venous pole • Arterial epicardium • Cardiac disease • Congenital anomlies

2.1 Origin of the Epicardium

The cardiogenic mesoderm is referred to as first heart field, flanked medially by second heart field (SHF) mesoderm. The addition of SHF-derived cardiac mesoderm enables the formation of all cardiac components. A secondary layer will cover the complete myocardial tube (Fig. 2.1) and the developing roots of the great arteries. At the venous pole, the vPEO develops from which the epicardium (cEP) spreads over the cardiac tube up to the ventriculo-arterial junction [1]. Here, the cEP meets the PEO located at the arterial pole (Fig. 2.2) [2] forming arterial epicardium (aEP) that is continuous with the pericardium. Both PEO structures and the spreading cEP and aEP express Wilms' tumor-1 suppressor gene (WT-1) among others and harbor endothelial and mesenchymal cells.

2.2 Epicardium-Derived Cells (EPDCs)

Epicardial cells lose epithelial contacts by epithelial–mesenchymal transition (EMT) and EPDCs relocate subepicardially [3, 4]. Early EPDCs invade the thin myocardial wall. Proliferation of the myocardium depends on both endocardial- and epicardial-derived signals, including Raldh2 [5] with a spatiotemporal difference between right and left ventricle (Fig. 2.1). The aEP starts EMT slightly later than cEP, and ensuing EPDCs can be detected in the outer layers of the developing great arteries and at the myocardial–endocardial cushion interface (Fig. 2.2). Epicardial heterogeneity with a subset of cells taking part in the initial EMT wave provides the myocardium with the main number of the future interstitial fibroblasts. The next wave of EMT correlates with the formation of the fibrous atrioventricular annulus [6] and contributes to part of the atrioventricular cushion cells. At the ventriculo-arterial junction aEP migrates into the outflow tract forming the future arterial annuli and partake in the semilunar valves [7].

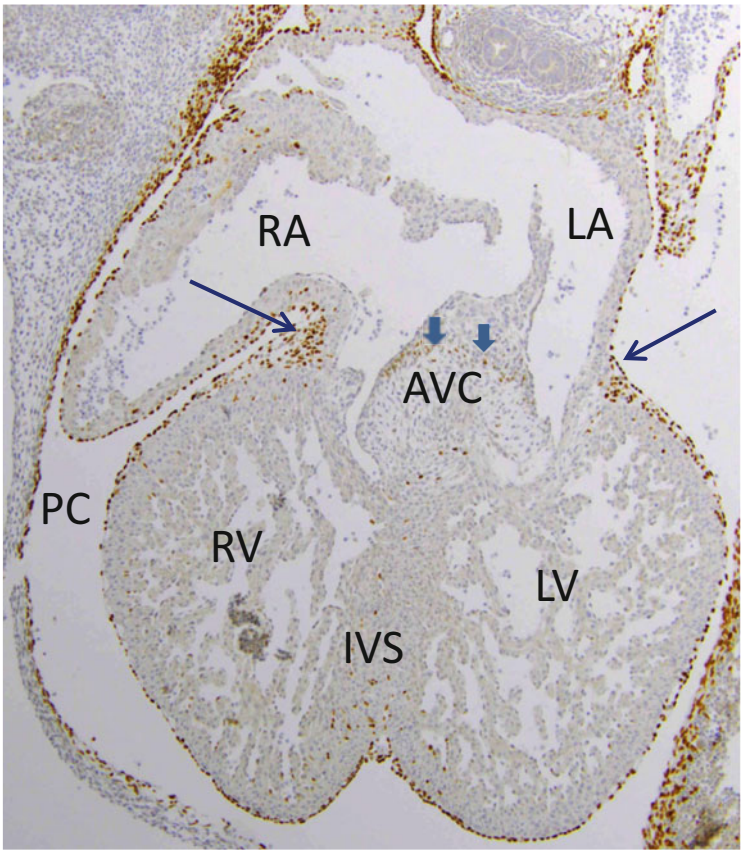

Fig. 2.1 Four-chamber view of a mouse heart (ED12.5) immunostained for WT1. Note brown cells lining the pericardial cavity (PC), including the epicardium. The AV groove is indicated (*arrows*). The left ventricular (LV) wall contains hardly EPDCs, whereas some are present in the RV and in the interventricular septum (IVS). Note the presence of EPDCs at the border of the AV cushion and the interatrial septum (*short arrows*)

2.3 Heterogeneity of Epicardial Cells

2.3.1 The Cardiac Fibroblast

The epicardium is the main source of the interstitial, the fibrous annulus, and the coronary adventitial fibroblasts [7, 8]. The endocardial cells lining the cushions are the other source of the valve fibroblasts [3].

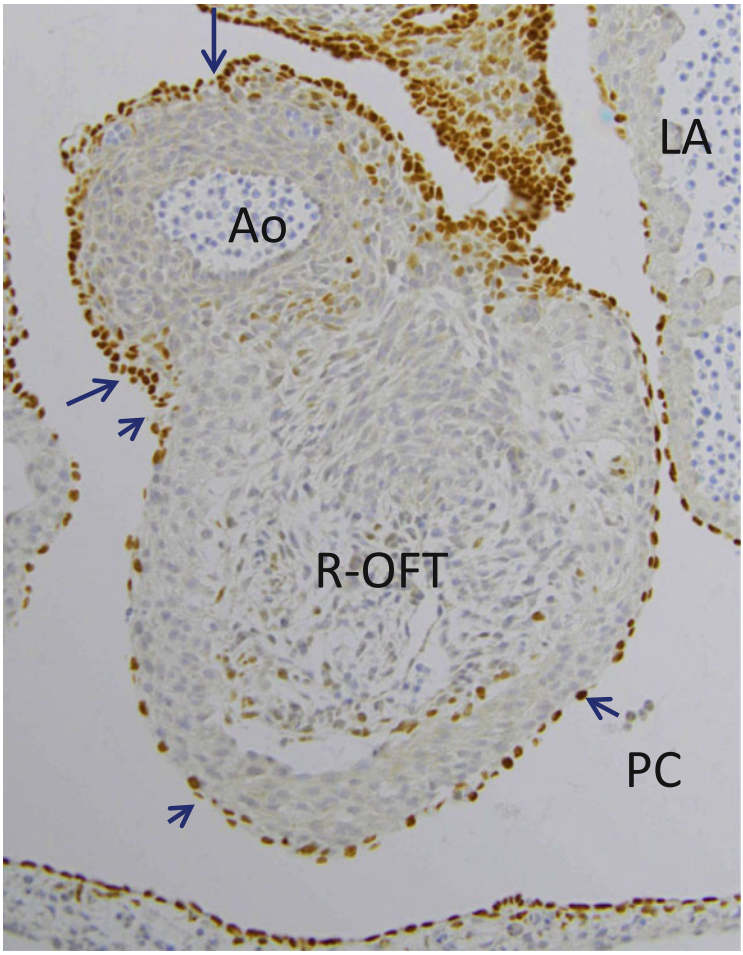

Fig. 2.2 Cross section of aorta (Ao) and right ventricular outflow tract (R-OFT). Aortic epicardium is densely packed and cuboid (*arrows*), whereas cEP is squamous (*short arrows*). Note EPDCs at the border of R-OFT cushions and myocardium and between Ao and R-OFT

2.3.2 Arterial Smooth Muscle Cell

After ingrowth of the peritruncal coronary capillary plexus into the aorta, EPDCs surround the main coronary vessels and differentiate into smooth muscle cells (SMC). Differentiation into SMCs is regulated by many genes including FGF, VEGF, Notch, SRF, and PDGFRb and their ligands [8]. Quail–chicken chimera studies demonstrated the timing of EMT and required cell interactions [4].

2.3.3 Endothelial Cells

The origin of the coronary endothelium is still under debate. Using a quail vPEO transplanted into the isochronous chick as reviewed in [10] pointed out that coronary ECs do not derive from the coelomic lining but from endothelial cells from the sinus venosus sprouting into the stalk of the vPEO. This is also supported by studies using transgenic mice [11]. We have shown that the sinus venosus-derived endothelial cells express the "arterial" Notch1, underlining the plasticity of these cells [9]. The discussion on the origin of the coronary endothelium is kept alive as specific compartments produce restricted numbers of ECs [12, 13].

2.3.4 Cardiomyocytes

Conditional reporter mice studies, using WT-1 and Tbx18 as epicardial marker, suggested that a population of EPDCs might differentiate into a myocardial phenotype. However, based on interaction between BMP and FGF, some of the progenitors of cardiomyocytes in the SHF share the same markers with cells in the underlying mesoderm of the SHF and the epicardial population [14]. Other data, including quail–chicken chimera, also do not support EPDC–cardiomyocyte transition [3].

2.3.5 The Purkinje Fiber

The Purkinje fiber is a specialized cardiomyocyte induced by endothelin produced by endocardium and endothelium. We have postulated an essential interaction between EPDCs and endocardial-/endothelial-derived factors after vPEO tracing and inhibition experiments [15].

2.4 Congenital and Adult Cardiac Disease

In a large-screen microarray [16], no epicardium-specific gene has been encountered. Therefore, it is challenging to attribute specific cardiac malformations and diseases to epicardial malfunctioning. However, in animal models it is possible to link the epicardium to certain cardiac defects and diseases. We are dealing with complex tissues in which epicardial cells are essential.

2.4.1 Non-compaction

The most relevant cardiomyopathy resulting from abnormal EPDC function is the primary left ventricular non-compaction cardiomyopathy [4] demonstrating a spongious myocardium usually including the ventricular septum. Differences in

amount and timing of LV and RV invasion by EPDCs might account for predilection of the LV. With respect to congenital heart disease, a spongious ventricular septum can be connected with muscular VSDs.

2.4.2 Conduction System Anomalies

The main components of the conduction system are myocardial in origin. Clinically, it has been hypothesized that the genetically determined long QT syndrome is linked to abnormal Purkinje fiber function. Indirectly, the abnormal formation of the fibrous annulus with persisting accessory pathways can result in reentry tachycardias. PEO inhibition in chick embryos showed defective atrioventricular isolation, delaying the shift from a base-to-apex to an apex-to-base conduction [17].

2.4.3 Valvulopathies

PEO inhibition can lead to deficient AV valve formation [18, 19]. Furthermore, abnormal differentiation including defective undermining of the valve leaflet is similar to Ebstein's anomaly of the tricuspid valve as observed in combination with accessory pathways [4]. EPDCs of aEP origin are found in the outflow tract cushions (Fig. 2.2) probably acting via Notch signaling and hence influencing bicuspid aortic valve formation.

2.4.4 Coronary Vascular Anomalies

Experimental studies disturbing normal coronary development result in a number of malformations that associate human congenital pattern variations with abnormal ventriculo-coronary-arterial communications (fistulae). Fistulae found in avian models with absent coronary arterial orifices in the aorta [20] resembling coronary malformations found in pulmonary atresia without VSD are hypothesized to be a primary coronary vascular disease [21].

2.5 Cardiovascular Repair

Myocardial infarction. Different approaches have focused on the potential of the adult epicardial cell after myocardial infarction (MI). A c-kit-positive subepicardial population indicates renewed epicardial activity with acquisition of stem cell characteristics [22]. Using a retrovirally induced fluorescent Katushka labeling of dormant epicardium showed EPDCs that migrated into the myocardium and differentiated into a myofibroblast phenotype. The activated epicardium and EPDCs reexpressed WT1 not only in the MI border zone but also in remote areas. Another approach is represented by epicardial cells cocultured with

cardiomyocytes in which EPDCs change myocardial alignment and contraction [23]. A direct approach was provided by grafting human adult atrial epicardial cells [24] cultured in vitro from a cobblestone epithelium into spindle shape, thereby acquiring characteristics of mesenchymal stem cells. Injection of these adult human EPDCs into immune-incompetent mice resulted in a marked improvement of cardiac function [24] indicative of repair. Combined injection with adult human cardiomyocyte progenitors (CMPCs) aimed at induction of cardiomyocyte regeneration [25] which showed an additive effect on remodeling, although no new cardiac cell types (endothelial cells, fibroblasts, SMCs, or cardiomyocytes) could be traced to human origin. The capacities, expressed within the normal embryonic state, seem to be preserved in adult life and in disease states.

2.6 Future Directions and Clinical Applications

Many positive effects of EPDCs either after injection or by stimulation of the dormant native epicardial covering of the heart are due to a paracrine mechanism [22, 24, 25]. These findings bear important potential for drug and cell-based therapeutic approaches to stimulate the native epicardium in repair of the ischemic cardiac wall. An underdeveloped area is the priming of grafts taken from the pericardium for use in cardiac or arterial repair.

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