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Original contribution

Bone-marrow–derived CXCR4-positive tissue-committed stem cell recruitment in human right ventricular remodeling

Chiara Castellani PhD, Bsc[a], Massimo Padalino MD, PhD[b], Paolo China MD[c], Marny Fedrigo MD[a], Carla Frescura MD[a], Ornella Milanesi MD[d], Giovanni Stellin MD[b], Gaetano Thiene MD, FRCP[a], Annalisa Angelini MD, FESC[a,*]

[a]*Department of Medical-Diagnostic Sciences and Special Therapies, University of Padua, Medical School, 35121 Padua, Italy*
[b]*Pediatric and Congenital Cardiovascular Surgery Unit, University of Padua, Medical School, 35121 Padua, Italy*
[c]*Department of Cardiothoracic and Vascular Sciences, University of Padua, Medical School, 35121 Padua, Italy*
[d]*Clinical Cardiology, Pediatric Cardiology, University of Padua, Medical School, 35121 Padua, Italy*

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Summary The epicardium contributes to cardiac formation, particularly during embryogenesis. It remains to be seen if it is also involved in postnatal myocardial homeostasis. This study evaluates the topographic distribution of stem cells (c-Kit) and extracardiac progenitor cells (CXCR4+) and their contribution to ventricular remodeling in a model of pressure volume overload leading to right ventricle hypertrophy. Eleven specimens with hypoplastic left heart syndrome were evaluated and compared with 6 normal hearts from subjects matched for age and weight. All underwent Norwood procedure with the right ventricle becoming a systemic one, with pressure and volume overload leading to right ventricle remodeling. Transmural cardiac tissue samples from the right ventricle were analyzed by immunohistochemistry and morphometry. This is the first study to demonstrate that c-Kit–positive progenitor cells and tissue-committed stem cells (CXCR4+/CD45−) are higher in children with systemic right ventricle remodeling. We also show that the localization of cardiac progenitor and recruited CXCR4+ stem cells in the myocardium is site specific in hearts with right ventricle hypertrophy. These cells are mainly scattered in the interstitium of the epicardial layer. In contrast, myocyte proliferation is not a key process in right ventricular hypertrophy. Induced by the overexpression of SDF-1α by the myocardium, CXCR4 cell mobilization resembles SDF-1 homing factor distribution, showing transmural enhanced expression from the endocardium toward the epicardium. The study provides evidences of the site-specific epicardial localization of stem cells in a model of pressure/volume overload and suggests that the epicardium acts as a permissive niche in normal and pathologic conditions.

1. Introduction

Myocardial regeneration is thought to be mediated by multipotent cardiac stem cells (CSCs) residing in the heart and giving rise to new myocytes and vascular structures.

* Corresponding author. Department of Medico-Diagnostic Sciences and Special Therapies, Pathological Anatomy-Cardiovascular Pathology, University of Padua Medical School, 35121 Padova, Italy.
E-mail address: annalisa.angelini@unipd.it (A. Angelini).
URL: http://anpat.unipd.it/ (A. Angelini).

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A variety of studies have reported CSCs in the mouse, dog, rat, and human adult myocardium [1-3]. It has been seen that cardiomyocyte regeneration takes place in humans with ischemic cardiomyopathy and following sustained pressure overload mediated by systemic hypertension and aortic stenosis [4]. Cardiac chimerism offers a unique opportunity to study myocyte and vascular structure formation from primitive cells resident in or homed to the heart from circulation or from the recipient atrial cuffs in heart transplantation. Minami et al [5] and Angelini et al [6], who studied chimerism in males receiving hearts from females, demonstrated that migratory cells participate in the formation of almost all cardiac structures.

Stem cells are of paramount importance in embryogenesis, but they also retain some functions in adults in whom they can replace senescent cells and regenerate damaged organs [7]. Although it is well established that the epicardium contributes to cardiac fibroblasts during embryogenesis, it is unclear if it is involved in postnatal recruitment in normal or diseased hearts.

The adult myocardium is enveloped in a layer of epithelial cells coming from a population of pluripotent stem cells—called *epicardially derived cells* (EPDCs)—that delaminate from the epicardium and migrate into the subepicardium, acquiring a mesenchymal phenotype. This process is called *epithelial-to-mesenchymal transition*. EPDCs are pluripotent stem cells that are extremely important in cardiac development, contributing to several cell lineages and secreting factors that modulate myocardial growth [8,9].

Myocardial regeneration, regarded as heart repair, is also thought to originate from bone-marrow–derived CXCR4-positive stem cells made up of 2 populations: CXCR4+/CD45+ hematopoietic stem cells and CXCR4+/CD45− nonhematopoietic, tissue-committed stem cells (TCSCs). Limited numbers of TCSCs circulate in the peripheral blood and can be mobilized by cytokine stimulation under specific pathophysiologic conditions [10,11].

Myocardial hypertrophy is the mechanism by which the adult heart seems to compensate for workload increase. In fetal hearts, instead, a hyperplastic event is the most important means of myocardial growth. Shortly after birth, when heart loading conditions suddenly change, there is a shift from a hyperplastic to hypertrophic growth [12], considered the predominant myocardial response to heavier loading conditions [13].

It has recently been demonstrated that a stronger pressure and volume workload can increase the right ventricle's (RV's) weight and free wall thickness in rats at all ages. These changes are mostly the outcome of cardiomyocyte hypertrophy causing no apparent increase in cell number [14].

The increment in ventricular mass is predominantly the result of protein synthesis and an increase in cell size. The existence of proliferating cardiac progenitor cells and the influx of bone-marrow–derived cells developing into cardiomyocytes have recently been demonstrated, but their contribution to RV overload adaptation is not entirely clear [15]. Although the effect of pressure overload has been amply investigated in the adult heart and during the development of left ventricle hypertrophy, little is known about potential changes occurring in patients with hypoplastic left heart syndrome (HLHS) following the 3 stages of the Norwood procedure attempting to correct this defect.

The aim of this study was to investigate (*a*) the presence and topographic distribution of stem and cardiac progenitor cells to identify stem cell populations potentially involved in cardiac regeneration during ventricular remodeling and (*b*) the contribution of resident and extracardiac progenitor cells to the stem cell intracardiac pool.

2. Materials and methods

2.1. Patients

Eleven consecutive specimens of HLHS were obtained from the anatomical collection of congenital heart disease samples conserved at the Institute of Pathological Anatomy at the University of Padua. All of these had undergone the Norwood procedure to correct the congenital defect (see below). To correct the hypoplasia in the left ventricle, surgeons must transform the right one into a systemic ventricle that supports both systemic and pulmonary circulation. That is why, following surgery, these hearts are considered a model of right pressure and volume overload and ventricle hypertrophy. These specimens were compared with 6 normal hearts showing no signs of structural anomalies from patients matched for age and weight who died of non–cardiac-related causes (Table 1).

2.2. Norwood procedure

2.2.1. Definition

HLHS occurs when there is failure in the development of the systemic or left ventricle. Varying degrees of hypoplasia or atresia of the aorta, mitral valve, and left ventricle may occur. Coarctation of the aorta is a frequent association. The newborn with HLHS is dependent upon a patent ductus arteriosus for systemic perfusion. These patients exhibit a

Table 1 Group characteristics

	Controls	Pathologic samples	*P*
n	6	11	
Age (d)	153.33 ± 166.7	113.54 ± 125.02	.623
Heart weight (g)	76.33 ± 49.84	94.96 ± 89.13	.63
RV free wall thickness(mm)	1.88 ± 0.27	3.86 ± 1.37	.0006

NOTE. Ventricular wall thickness was measured postmortem. Data are expressed as mean ± SD. The *P* value indicates the statistical significance for the differences between both groups (unpaired Student *t* test).