Pediatric End-Stage Failing Hearts Demonstrate Increased Cardiac Stem Cells

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Background. **We sought to determine the location, expression, and characterization of cardiac stem cells (CSCs) in children with end-stage heart failure (ESHF). We hypothesized ESHF myocardium would contain an increased number of CSCs relative to age-matched healthy myocardium, and ESHF-derived CSCs would have diminished functional capacity as evidenced by reduced telomere length.**

Methods. **Tissue samples were obtained from the explanted hearts of children undergoing heart transplantation with ESHF, defined as New York Heart Association class III or IV and ejection fraction less than 0.20, and from age-matched congenital heart disease patients with normal myocardium. The expression profile of cardiac-specific stem cell markers was determined using quantitative real time polymerase chain reaction and immunofluorescence. Cardiac stem cell growth reserve was assessed with telomere length.**

Results. **There were 15 ESHF and 15 age-matched congenital heart disease patients. End-stage heart failure myocardium demonstrated increased expression of c-kit$^+$ and islet-1$^+$ CSCs by 2.0- and 2.5-fold, respectively, compared with myocardium from congenital heart disease patients. There was no difference in expression of c-kit$^+$ CSCs with advancing age from infants to children in ESHF myocardium. The c-kit$^+$ CSCs isolated from ESHF patients demonstrated significantly reduced telomere length, suggesting a diminished functional capability in these cells (8.1 ± 0.6 kbp versus 6.3 ± 0.3 kbp; $p = 0.015$).**

Conclusions. **End-stage heart failure myocardium demonstrated an age-independent increase in CSCs relative to healthy myocardium; however, these CSCs from ESHF patients may have diminished proliferative ability and reduced functionality as an autologous cell therapy candidate. Further investigation is necessary to determine the role of ESHF-derived CSCs within the myocardium.**

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The prevalence of pediatric heart failure has increased dramatically in the last three decades, resulting in frequent hospitalizations, development of significant comorbidities, and protracted heart transplantation waiting-list times [1]. Given the current limitations in medical therapy and the limited number of donors available for pediatric heart transplantation, the potential for an effective cell-based therapy for pediatric heart failure patients is an attractive option. During the last decade, the discovery and characterization of a resident pool of cardiac stem cells (CSCs) has led to a surge of preclinical research and the recent completion of two promising phase I trials in adults with ischemic cardiomyopathy [2, 3]. Specifically, c-kit$^+$ CSCs have been shown to be multipotent, clonogenic, and self-renewing in in vitro and in vivo regenerative assays, and are characterized by a phenotype negative for the hematopoietic and endothelial markers CD45, CD34, and CD31 [2, 4]. The cellular and molecular processes underlying these beneficial effects have yet to be defined, but are thought to be driven by paracrine-mediated signaling, epigenetic modifications, and interaction with endogenous cardiac- or bone marrow–derived progenitor cells [5]. As mechanistic studies evolve, however, a number of important translational questions remain regarding the role of resident CSCs in the developing myocardium of pediatric patients.

We and others have previously shown the enhanced regenerative capacity of CSCs derived from pediatric patients compared with adults, and also the increased number of resident CSCs present in neonatal and infant myocardium when compared with that from older children, adolescents, and adults [6–8]. However, the relative role of endogenous CSCs in the setting of pediatric end-stage heart failure (ESHF) has not been extensively studied, nor has the effect of ESHF on stem cell functionality. Recently, the correlation between a more robust telomere-telomerase axis and population doubling time of the CSCs was found to be highly predictive of negative left ventricular remodeling after coronary artery bypass grafting and improved ventricular function [9]. These

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results imply the importance of maintaining a strong telomere-telomerase axis that will directly influence the functional abilities of the c-kit$^+$ CSCs. Whether a similar association exists between the ESHF myocardium-derived CSCs and their telomere-telomerase axis has yet to be explored, and could provide important insights into the functionality of ESHF-derived c-kit$^+$ CSCs as a potential cell therapy product for clinical use.

We sought to explore the differences in CSC expression between ESHF myocardium and normal myocardium from pediatric patients with congenital heart disease (CHD). Others have shown that the number of resident CSCs increase by 29- and 14-fold in adults with acute and chronic myocardial infarction, respectively [10], and by 3-fold in the right ventricle of children with pressure-overload single ventricles [11]. Accordingly, we hypothesized that in the setting of ESHF, pediatric patients would demonstrate enhanced expression of endogenous CSCs relative to age-matched CHD patients with normal myocardium. Furthermore, we predicted that c-kit$^+$ CSCs isolated from ESHF patients would exhibit differential growth properties when compared with c-kit$^+$ CSCs isolated from CHD patients with normal myocardium, as evidenced by differences in telomere length and population doubling time.

Patients and Methods

Study Patients

This study was approved by the University of Maryland and Lurie Children's Hospital, Chicago Institutional Review Board, and parental consent was provided for use of all cardiac tissue biopsy specimens. Tissue specimens were obtained from the right atrial appendage (RAA) of pediatric patients with normal myocardium or ESHF during congenital cardiac surgery or at the time of heart transplantation. End-stage heart failure was defined as New York Heart Association class III or IV and ejection fraction less than 0.20. Right atrial appendage samples were also obtained from age-matched patients with normal myocardium and no clinical evidence of heart failure undergoing routine congenital cardiac surgery for CHD.

Generation of Cardiac Stem Cells

C-kit$^+$ CSCs were isolated from RAA samples as previously described [3]. Briefly, samples were minced in Ham's F12 medium (cat. no. 12-615F; Lonza Group Ltd, Basel, Switzerland) and digested with 1 to 2 mg/mL of collagenase type II (cat. no. 4177; Worthington Biochemical Corp, Lakewood, NJ). Samples were then incubated on a shaker for 30 to 45 minutes at 37°C. After collagenase treatment, cells were washed twice with growth medium (Ham's F12, 10% fetal bovine serum, 10 ng/mL recombinant human fibroblast growth factor-basic, 0.2 mmol/L L-glutathione, and 5 U/mL human erythropoietin 250) before being plated. At subconfluency, cells were trypsinized and sorted for c-kit$^+$ with microbeads (CD117 MicroBead Kit human cat. no. 130-091-332; Miltenyi Biotec, Bergisch Gladbach, Germany) as per kit instructions. The c-kit$^+$ cells were collected and cultured in c-kit growth media.

Characterization of c-kit$^+$ Cardiac Stem Cells

Cardiac stem cells at P3 to P5 were evaluated by flow cytometry using a Becton-Dickinson FACS caliber (San Jose, CA) with 10,000 events collected. Cells were incubated with fluorochrome-conjugated primary antibodies against c-kit, the hematopoietic lineage surface markers (CD34 and CD45), the mesenchymal stromal lineage

Table 1. Patient Characteristics

Patient	Age	ESHF Diagnosis	CHD Diagnosis
1	3 mo	Congenital cardiomyopathy (HLHS)	VSD
2	5 mo	Congenital cardiomyopathy (DORV, UAVSD)	VSD
3	8 mo	Congenital cardiomyopathy (HLHS)	VSD
4	12.5 mo	Dilated cardiomyopathy	VSD
5	1 y	Congenital cardiomyopathy (HLHS)	VSD
6	13 mo	Dilated cardiomyopathy	VSD
7	2 y	Dilated cardiomyopathy	VSD
8	7 y	Myocarditis[a]	VSD
9	9 y	Dilated cardiomyopathy	VSD
10	10 y	Dilated cardiomyopathy	VSD
11	11 y	Dilated cardiomyopathy	VSD
12	12 y	Idiopathic cardiomyopathy	VSD
13	12 y	Dilated cardiomyopathy	VSD
14	13 y	Dilated cardiomyopathy	VSD
15	14 y	Dilated cardiomyopathy	VSD

[a] Decompensated heart failure ensued after recovery from acute myocarditis.

CHD = congenital heart disease; DORV = double-outlet right ventricle; ESHF = end-stage heart failure; HLHS = hypoplastic left heart syndrome; UAVSD = unbalanced atrioventricular septal defect; VSD = ventricular septal defect.