Abstract

Abnormal Accumulation of Reactive Oxygen Species from Pitx2 Mutation Could Cause HLHS

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Hypoplastic Left Heart Syndrome (HLHS) is a highly rare congenital heart defect in which the left ventricle is critically hypoplastic or atretic. At the cellular level, changes in ventricular structure include myocardial growth and myofibroblast apoptosis. Ultimately, this progressive remodeling can result in heart failure and, in HLHS, prematurity death. Recent progress in understanding these mechanisms of myocardial remodeling has led to the evidence that reactive oxygen species (ROS) and oxidative stress play a central role in regulating the phenotype of cardiac myocytes.

We hypothesize that ROS generates developmentally characteristic remodeling due to pitx2 dysfunction in the left ventricle, coupled with the low and high stress placed on the interventricular septum (IVS) and the left ventricular (LV) wall during cardiac contraction, respectively, causes HLHS. Genetic tests of mouse models and human HLHS cases support this proposed further investigation. Further investigation could open the door to curative gene therapy and the many years of suffering and complications with surgical procedures for affected infants.

Hypoplastic Left Heart Syndrome (HLHS) is a complex heart disease with a sharply shrunken ejection fraction and increased left ventricular mass, which develops with several severe dysmorphologies of left heart structures, particularly the left ventricle and the mitral and aortic valves, which are either completely aortic or abnormally small. The prevalence of HLHS is known to be around 0.02% of the national population, and since it is one of the most common congenital heart diseases to manage, 30% of affected newborns do not survive through adulthood.

In utero, prenatal diagnosis is often superior to post-birth electrocardiography, echocardiograms, MRI, and cardiac catheterization due to the potential ability to attempt fetal cardiac intervention and plan in advance for effective delivery and immediate stabilization.

Since an underdeveloped left ventricle results in the breakdown of systemic blood circulation, newborns affected with HLHS require immediate surgical intervention for survival (1). The current standard of care involves the Norwood Procedure where surgical repair aims to increase cardiac output and bypass the poorly functioning left heart by connecting the right ventricle with the ascending aorta, thus providing systemic blood circulation.

Although genetics seem to play a large role, because the underlying cause remains unclear, HLHS is a disease that is not curable but rather only reduce the harmful impacts of the disease (14). Better understanding of the molecular mechanisms that contribute to HLHS would allow for interventions with more promising objectives.

Hypothesis

The differential mechanical strain placed on tissues in the interventricular septum and in the left ventricular wall is potentially an important component necessary for ROS-mediated developmental destabilization to cause HLHS.

In the presence of ROS, similar cells in low strain undergo apoptosis while cells in high strain experience hypertrophy, we hypothesize that Pitx2 mutation-mediated ROS buildup in the left ventricle causes the simultaneous thinning of the IV septum and thinning of the LV wall observed in HLHS.

Hypothesis

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We postulate for potential Pitx2 interactions and transcriptional activities significant to Pitx2’s role in the development of HLHS, immunoprecipitation assays would be performed to evaluate interactions between ROS and Pitx2. After testing for potential binding candidates in Pitx2 pulldowns, we would utilize immunohistochemistry to investigate the effect of ROS accumulation on the loci formation of Pitx2 and its potential ROS-dependent binding partners. Genetic knockouts of these partners would then be investigated to determine the importance of these two components in investigating the effect of their interactions on Pitx2 on cellular growth and development in left ventricular development.

The degree of Pitx2 mutations in cell lines could be varied such that a partial loss of function could be tested. ROS stress in radioactive oxygen could also be artificially added to the mutated cells to measure the binding of the ROS to the regions of the genome dependent on the structural stress conditions.

The mimicking of birth complications of the offspring, examination of the heart of the offspring, and genomic analysis would verify the findings. This would further give us an insight into how HLHS is linked to Pitx2 mutation. If the concentration of ROS are also found to be elevated in the heart, along with the selective hypertrophy and apoptosis via immunostaining, we would support our hypothesis.

References


Significance & Innovation

A pitx2 mutant cardiomyocyte cell line was expected to accumulate ROS. Then, modulation like expansion microscopy and atomic force microscopy (AFM) was performed to evaluate cell morphology.

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Low Strain Conditions Afford Apoptosis

A. Apoptotic cells are present within an accumulation of ROS and appear to act via the mitochondrial pathway of cytotoxicity, resulting in apoptotic cell death (Sawyer et al. 2002). (b) Cardiomyocytes were exposed to increasing levels of H2O2 and mitochondrial RNA was monitored via qPCR and Western blotted the copy number of MDH (Suzumura et al. 2002). (c) Effect of the H2O2 ROS on cardiac cells. Exposure leads to cell death through apoptosis as determined by cell death ELISA (van Harmsdonk et al. 1999).

Low Strain Conditions Afford Hypertrophy

A. Hypertrophied cardiomyocytes

B. Hypertrophied cardiomyocytes

C. Hypertrophied cardiomyocytes

D. Hypertrophied cardiomyocytes

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