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Original article

Maintenance of adult cardiac function requires the chromatin factor Asxl2

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ABSTRACT

During development and differentiation, cell type-specific chromatin configurations are set up to facilitate cell type-specific gene expression. Defects in the establishment or the maintenance of the correct chromatin configuration have been associated with diseases ranging from leukemia to muscular dystrophy. The heart expresses many chromatin factors, and we are only beginning to understand their roles in heart development and function. We have previously shown that the chromatin regulator Asxl2 is highly expressed in the murine heart both during development and adulthood. In the absence of Asxl2, there is a significant reduction in trimethylation of histone H3 lysine 27 (H3K27), a histone mark associated with lineage-specific silencing of developmental genes. Here we present evidence that Asxl2 is required for the long-term maintenance of ventricular function and for the maintenance of normal cardiac gene expression. *Asxl2*$^{-/-}$ hearts displayed progressive deterioration of ventricular function. By 10 months of age, there was ~37% reduction in fractional shortening in *Asxl2*$^{-/-}$ hearts compared to wild-type. Analysis of the expression of myofibril proteins suggests that *Asxl2* is required for the repression of β-MHC. *Asxl2*$^{-/-}$ hearts did not exhibit hypertrophy, suggesting that the de-repression of β-MHC was not the result of hypertrophic response. Instead, Asxl2 and the histone methyltansferase Ezh2 co-localize to β-MHC promoter, suggesting that Asxl2 directly represses β-MHC. Interrogation of the CardioGenomics database revealed that *ASXL2* is down-regulated in the hearts of patients with ischemic or idiopathic dilated cardiomyopathy. We propose that chromatin factors like Asxl2 function in the adult heart to regulate cell type- and stage-specific patterns of gene expression, and the disruption of such regulation may be involved in the etiology and/or development of certain forms of human heart disease.

1. Introduction

Transcriptional regulation plays critical roles in heart development and function [1–6]. During embryonic development, the process of heart morphogenesis requires precise regulation of gene expression. Aberrations in the temporal or spatial pattern of gene expression lie at the root of multiple forms of congenital heart defects (CHD). Postnatally, gene expression is fine-tuned to meet the contractile need of adult life. There is little cardiomyocyte turnover in the adult heart, and the appropriate gene expression pattern has to be maintained for a life time. Mutations in a number of transcription factors or changes in the dosage of transcription factors have been shown to cause cardiac dysfunction in humans or in animal models, highlighting the importance of transcriptional regulation in the adult heart [7–11].

In recent years, chromatin has emerged as an important layer of transcriptional regulation. Many chromatin-associated proteins have been identified, and studies have shed light on how these chromatin factors can modify chromatin configuration to either facilitate or inhibit transcription. Accumulating evidence suggests that a substantial amount of transcriptional regulation in the heart takes place at the chromatin level [12–21]. However, much remains to be learned about which chromatin factors are involved, which genes they regulate, what functional mechanisms are used and whether/how deregulation contributes to heart diseases.

Polycomb Group (PcG) and Trithorax Group (TrxG) proteins are two highly conserved protein families that regulate transcription by modifying chromatin structure [22–25]. PcG proteins form several complexes to create repressive chromatin structure and maintain long-term silencing of target genes. For example, Polycomb Repressive Complex 2 (PRC2) generates trimethylated histone H3 lysine 27 (H3K27me3), a histone mark of silent chromatin. Polycomb Repressive Complex 1 (PRC1) has chromatin compaction activity. TrxG proteins also form multiple complexes but create active chromatin structure and antagonize PcG-mediated repression. Components of the PcG/TrxG system

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are expressed in the heart both during development and in the adult. Several pieces of evidence have implicated the system in transcriptional regulation in both embryonic and postnatal hearts. For example, studies of mice mutant for *Rae28* suggest that proper heart morphogenesis requires PcG activity. *Rae28* mutant mice display severe defects in an early and important step of cardiac morphogenesis, cardiac looping, which takes place between E8.5 and E9.5 [17,18]. Postnatal overexpression of *Rae28* causes dilated cardiomyopathy, cardiomyocyte apoptosis, abnormal myofibrils, and severe heart failure [19]. The TrxG protein Brg1 promotes cardiomyocyte proliferation and regulates the activity of multiple cardiac transcription factors in a dosage-dependent manner during heart development [20,21]. In adult cardiomyocytes, Brg1 is required for stress-induced hypertrophy and the pathological α-MHC to β-MHC shift [20].

We have previously generated a mutant mouse model for the chromatin factor Asxl2. We showed that Asxl2 is an enhancer of PcG activity and that Asxl2 deficiency has a significant impact on the level of bulk H3K27me3 [26]. In addition, two Asxl2 homologs have been shown to form a complex with the histone deubiquitinase Calypso/BAP1 and promote deubiquitination of histone H2A [27]. *Asxl2* is highly expressed in the heart throughout development and during adult life. To better understand the role of *Asxl2* in the heart, we carried out a longitudinal study of *Asxl2* $^{-/-}$ mice. Our data indicate that Asxl2 is required for the long-term maintenance of ventricular function and for repression of *β-MHC*. Asxl2 is likely a direct regulator of *β-MHC* and this regulation may involve the PcG protein Ezh2. Finally, *ASXL2* is down-regulated in human patients with ischemic or idiopathic dilated cardiomyopathy.

2. Materials and methods

2.1. Animal breeding

All mice used in this study were in C57BL/6 J × 129Sv F1 background because *Asxl2* $^{-/-}$ animals in either C57BL/6 J or 129Sv inbred background die perinatally. *Asxl2* $^{+/-}$ females in 129Sv inbred background were mated to *Asxl2* $^{+/-}$ males in C57BL/6 J inbred background to produce *Asxl2* $^{-/-}$ animals and wild-type littermates. The genetic compositions of the experimental and control animals were identical except at the *Asxl2* locus.

2.2. Echocardiography

Transthoracic echocardiography was performed while under isoflurane anesthesia and positive pressure ventilation. Transthoracic two-dimensional targeted M-mode and pulsed-wave Doppler echocardiography was performed with a 30-MHz mechanical transducer attached to a VisualSonics Vevo 770 system (Visual Sonics, Toronto, ON, Canada).

2.3. Hemodynamic measurements

Hemodynamic measurements were performed on 5-month-old wild-type and *Asxl2* $^{-/-}$ mice. Mice were anesthetized with isoflurane (1.5%) and injected with etomidate (10 mg/kg body weight; I.P.) for intubation. Anesthesia was maintained at 1% isoflurane and mice were ventilated with a Harvard Respirator at a rate of 140 breaths per minute and a 250 μm volume. A medial laparotomy exposed the diaphragm and a Millar Pressure/Volume transducer (SPR-839) was inserted into the left ventricle through an apical puncture. Steady state measurements of pressure/volume loops were recorded and the inferior vena cava was occluded to derive load-independent measurements of the end-systolic pressure/volume relation.

2.4. SDS-PAGE gel electrophoresis

Myofibril proteins were prepared and separated on SDS-PAGE as previously described [28]. The gels were subjected to either Coomassie staining to visualize all proteins or to Pro-Q Diamond staining (Molecular Probes, Eugene, OR) to visualize phosphorylated proteins. Alternatively, separated proteins were transferred to PVDF membrane and subjected to Western blot analysis.

For high-resolution SDS-PAGE, samples were loaded on 6% SDS-PAGE gels, run for 30 h at 4 °C, and subjected to silver staining. Proportions of α- and β-MHC were determined using densitometry.

2.5. Blood pressure measurement

Blood pressures of male mice ranging from 1-month to 10-months of age were measured in unanesthetized mice using an NIBP-8 tail-cuff blood pressure monitor (Columbus Instruments, Columbus, Ohio). Animals were acclimated to the restrainer and the warming compartment for 30 min/day for at least 3 days. On the day of the experiment, animals were acclimated in the apparatus for 20 min before measurements were taken. The sensor cuff pressure was set at 45 mm Hg and the occlusion cuff pressure was 200 mm Hg. Each data point (for one animal at a specific age) represents the average of 10 or more sequential measurements, spaced at a minimum of one minute intervals.

2.6. Real-time RT-PCR

Real-time RT-PCRs were performed on an ABI Prism 7900HT sequence detection system (Applied Biosystems) using the SuperScript™ III Platinum SYBR Green One-Step qRT-PCR kit (Invitrogen). The expression level of each gene analyzed was normalized against that of 18S rRNA or *β-Actin* in the same sample. For each gene, two wild-type and two mutant animals were analyzed. Detailed information on primer sequences and PCR conditions is given in the Supplementary material.

2.7. Chromatin immunoprecipitation

Nuclei were collected from formaldehyde-fixed, homogenized adult heart tissues. Chromatin was sheared by sonication and immunoprecipitated with KC17 anti-Asxl2 antibody, anti-Ezh2 antibody (AC22, Millipore) or rabbit IgG (Invitrogen). ChIP-ed DNA was analyzed by PCR using primers specific for conserved regions in the β-MHC promoter (b1-b5). Detailed conditions and primers sequences are provided in Supplementary material.

2.8. Histology and immunofluorescence

Paraffin embedded heart sections were made using standard protocols. H&E stainings were performed on 8 um sections; wheat-germ agglutinin-fluorescein staining was performed on 5 um sections.

2.9. Adult cardiomyocyte size measurement

Cardiomyocyte size was measured for two pairs of 6-month-old *Asxl2* $^{-/-}$ and wild-type hearts. Isolation of adult cardiomyocytes was performed as previously described [29]. Cardiomyocytes were plated in the presence of butanedione monoxime (BDM), a contraction inhibitor, and allowed to attach for 3 h. Images of live cardiomyocytes were taken. The length, width and area of cells were measured with ImageJ. 124–262 individual cardiomyocytes were measured for each heart.