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Aldehyde dehydrogenase 2 deficiency blunts compensatory cardiac hypertrophy through modulating Akt phosphorylation early after transverse aorta constriction in mice

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ABSTRACT

Aims: This study was designed to examine the impact of mitochondrial aldehyde dehydrogenase 2 (ALDH2) on transverse aorta constriction (TAC)-induced cardiac hypertrophy and related molecular mechanisms using an ALDH2 knockout (ALDH2 −/−) murine model.
Methods: Male wild-type and ALDH2 −/− mice were subjected to TAC or sham operation (n = 6–8 for each group). After two weeks, cardiac function was assessed by echocardiography and hemodynamic measurements. Myocardial phosphorylated and total PI3K, the catalytic subunit of PI3Ks (p110α and p110γ), Akt, and total PTEN levels were detected by Western blotting. Cardiomyocytes were stretched for 6 h in vitro in the presence or absence of Alda-1 (a small-molecule activator of ALDH2) prior to assessment of phosphorylated PI3K, Akt and total PTEN expressions by Western blot.
Results: Heart to body weight ratio and left ventricular posterior wall thickness as well as the cross-sectional area of cardiomyocyte were significantly lower in ALDH2 −/− mice than in wild-type mice following TAC. Western blot analysis showed p110γ was upregulated post TAC in both wild-type mice and ALDH2 −/− mice, phosphorylation of Akt was disrupted, PTEN expression was upregulated in ALDH2 −/− mice post TAC while phosphorylated PI3K, p110α and p110γ expression was similar between ALDH2 −/− and wild-type mice post TAC. In vitro, phosphorylation of Akt was significantly accentuated and PTEN expression was reduced while PI3K phosphorylation remained unchanged in stretched cardiomyocytes pretreated by Alda-1 compared to stretched cardiomyocytes treated by saline.
Conclusions: Our results show that ALDH2 deficiency attenuates compensatory cardiac hypertrophy through regulating Akt but not PI3K phosphorylation early after TAC in mice.

1. Introduction

Cardiac hypertrophy is a physiological and pathological responses to chronic pressure or volume overload [1]. Observed changes in cardiomyocytes during the development of cardiac hypertrophy include: rapid induction of proto-oncogenes and heat shock protein genes and increased rate of protein synthesis [2]. Cardiac hypertrophy is initially beneficial in terms of maintaining cardiac function through normalizing left ventricular wall stress, while cardiac function will ultimately be impaired in the course of long-standing cardiac hypertrophy process [3–4].

Multiple signalings are involved in the pathogenesis of cardiac hypertrophy. Among them, the phosphatidylinositol-3 kinase (PI3K)/Akt cascade is considered as a major mediator governing cardiac hypertrophy. It is known that the PI3K system exerts a fundamental role in cell survival and growth [5]. The phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a membrane-bound lipid phosphatase and functions essentially as a negative regulator of PI3K signaling in multiple systems [6]. In the heart, loss of PTEN leads to increase of Akt phosphorylation [7].

Mitochondrial aldehyde dehydrogenase 2 (ALDH2), acting as a key mitochondrial enzyme for maintaining normal mitochondrial function, was shown to be an important endogenous protective factor in a variety of cardiac injuries, including endoplasmic

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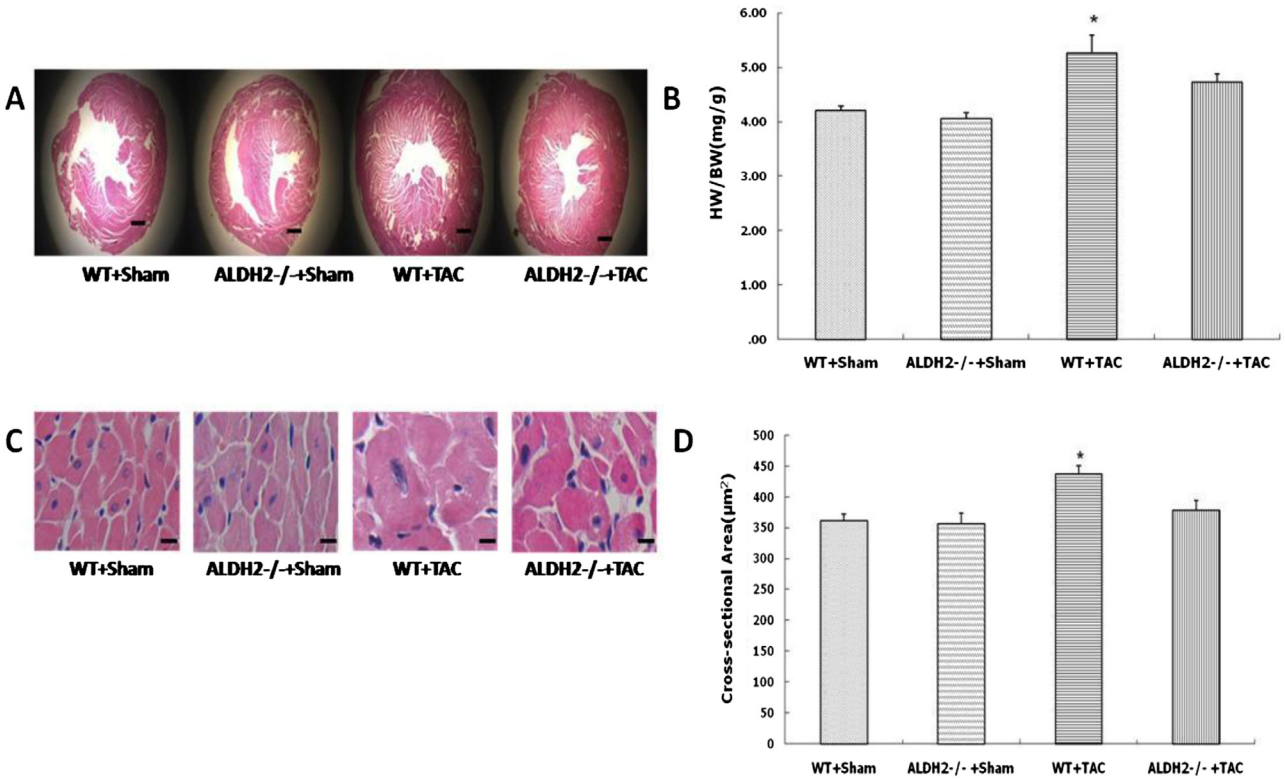

Fig. 1. Heart weight and cardiomyocyte cross-sectional area (CSA) in the wild type and ALDH2 −/− mice at 2 weeks after TAC. A) Transection view of heart (HE staining, scale bar: 40 μm). B) Heart to body weight ratio (HW/BW, mg/g). HW/BW increased significantly in wild-type TAC group, but the increase of this index was not significant in ALDH2 −/− + TAC group at two weeks after surgery. Microscopic view of cardiomyocyte (HE staining, scale bar: 10 μm). D) Bar graph of cardiomyocyte CSA. CSA increased significantly in wild-type TAC group, but not in ALDH2 −/− + TAC group at two weeks after surgery.*$p < 0.05$ versus wild-type sham group.

reticulum (ER) stress, ischemia reperfusion (I/R) injury and so on by our group and other researchers [8–11]. The role of ALDH2 on pressure-overload induced cardiac hypertrophy has not been fully understood. In this study, we explored the role of ALDH2 in transverse aorta constriction (TAC)-induced compensatory cardiac hypertrophy using an ALDH2 knockout (ALDH2 −/−) murine model.

2. Methods

2.1. Animals

Male wild-type C57 BL/6 and ALDH2 −/− mice (10–12 weeks old) were used for this study. Generation and characterization of the ALDH2 −/− mice using gene targeting in embryonic stem cells were described in detail previously [12]. The genotype was confirmed by PCR. Mice were kept on a 12 h/12 h light/dark cycle in temperature-controlled rooms and fed with clean water and standard lab-chow. All experimental procedures were approved by the Animal Care and Use Committee of Zhongshan Hospital, Fudan University.

2.2. Transverse aortic constriction

Mice were anaesthetized with ketamine (5 mg/kg). Anaesthesia was maintained by isoflurane inhalation (1.5–2.5%). After opening the chest, the transverse aorta of ALDH2 −/− or wild-type mice were ligated between the truncus brachiocephalicus and the left common carotid artery by tying a 7–0 silk suture against a 26-gauge needle. Sham-operated ALDH2 −/− and wild-type mice underwent the same procedure without ligation of the aorta [13].

2.3. Echocardiography

At pre-operation, one and two weeks after operation, mice were anaesthetized with isoflurane and body temperature was maintained by placing the mice on a heating pad (37.0 °C). 2-D echocardiography was performed using a 30-MHz high-frequency scan head as previously described (VisualSonics Vevo770; VisualSonics Inc. Toronto, Canada) [13]. Left ventricular end-diastolic posterior wall thickness (LVPWd) and end-systolic posterior wall thickness (LVPWs), fractional shortening (FS), ejection fraction (EF) were measured.

2.4. Hemodynamic measurements

After echocardiographic examination at two weeks after operation, hemodynamic studies were performed. Briefly, mice were anaesthetized and a Millar catheter (Millar 1.4F, SPR 835, Millar Instruments, Inc. Houston, TX) was inserted into the right carotid artery and then, advanced into the LV. The transducer was connected to Power Lab system (AD Instruments, Castle Hill, Australia) and heart rate (HR), LV systolic pressure, LV contractility (dP/dtmax and dP/dtmin) were recorded [14].

2.5. Histological analysis

Post hemodynamic examination, mice were sacrificed under deep anaesthesia, excised hearts were weighed, perfused with PBS followed by 4% polyformaldehyde and fixed in 10% formalin for histological analysis. Paraffin embedded hearts were sectioned at 4-um thickness and stained with hematoxylin and eosin (H–E). Photographs were taken by high-resolution digital image analysis system (QwinV3, Leica, Wetzlar, Germany), at magnification of 400. The cross-sectional area of cardiomyocytes (CSA) was quantified from 5 slices for each heart, 10 high power fields from each section were analyzed [15].