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Acetaldehyde dehydrogenase 2 (ALDH2) deficiency exacerbates pressure overload-induced cardiac dysfunction by inhibiting Beclin-1 dependent autophagy pathway ☆

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ABSTRACT

Mitochondrial aldehyde dehydrogenase 2 (ALDH2) was demonstrated to play cardioprotective roles in cardiovascular diseases. Nonetheless, little is known about the roles and mechanisms of ALDH2 in pressure overload-induced cardiac damages. In this study, we revealed that ALDH2 deficiency overtly exacerbated transverse aortic constriction (TAC)-induced cardiac dysfunction. Cardiomyocyte enlargement was observed in both WT and ALDH2 −/− mice in HE-stained myocardial tissue samples at 8 weeks post TAC surgery. Mitochondrial morphology and structure were also significantly damaged post TAC surgery and the changes were aggravated in ALDH2 −/− TAC hearts. ALDH2 deficiency also depressed myocardial autophagy in hearts at 8 weeks post TAC surgery with a potential mechanism of repressing the expression of Beclin-1 and promoting the interaction between Bcl-2 and Beclin-1. These data indicate that ALDH2 deficiency exacerbates the pressure overload induced cardiac dysfunction partly by inhibiting Beclin-1 dependent autophagy pathway.
This article is part of a Special Issue entitled: Autophagy and protein quality control in cardiometabolic diseases.

1. Introduction

Heart failure is a leading cause of mobility and mortality worldwide and it is the final result of many different insults to the myocardium. Clinical and experimental studies have demonstrated that chronic excessive pressure overload is a significant risk factor for heart failure and sudden death [1,2]. It is known that the initial growth response to pressure overload is an important compensatory process and thought to be beneficial for maintaining the normal heart function. Nonetheless, continued pressure overload, such as in the case of resistant hypertension could initiate a series of pathological cardiac remodeling, and eventually lead to heart failure [3]. The pathological progress is characterized by the enlargement of cardiomyocyte size, activation of "fetal gene program", cytoskeletal reorganization and irreversible decompensation of contractile dysfunction [4,5].

Pathological cardiac hypertrophy is often accompanied with activation of anabolism and catabolism [6] and the imbalance between anabolism and catabolism often results in the dysfunction of intracellular organelles and structural remodeling. Autophagy, an evolutionary conserved process, plays a crucial role in regulating the degradation and recycling of damaged protein and intracellular organelles [7]. It has been shown in the past decades that autophagy dysfunction was linked with a variety of heart disease models, including ischemia/reperfusion or ischemic injury, alcoholic cardiomyopathy and diabetic cardiomyopathy, and pressure overload-induced heart failure [8–11]. However, the precise role of autophagy in the pathological process of cardiac geometry and function post pressure overload stimulation still remains controversial [1,8]. Some studies have suggested that augmented autophagy aggravated pathological cardiac hypertrophy and heart failure, [12,13], while on the contrary other works found that upregulation of autophagy is an adaptive response to pressure overload stress [14]. Several autophagy related signaling molecules, which actively participated in the pathological process post pressure overload, have been identified now, i.e., the classical AMP-activated protein kinase–mammalian target of rapamycin pathway (AMPK–mTOR-autophagy), Beclin-1 dependent pathway and JNK-Foxo3a signaling pathways [15–17].

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Mitochondrial aldehyde dehydrogenase 2 (ALDH2), one of 19 members of the ALDH gene family in humans, has been shown to play a critical role in the metabolism of acetaldehyde and other toxic aldehydes such as 4-hydroxy 2-nonenol (4-HNE) [9,18,19]. Evidences of our researches and others have demonstrated a cardioprotective role of ALDH2 activation in attenuating ischemic injury, toxic damage and alcoholic cardiomyopathy [18,20,21]. Interestingly, autophagy has proved to be indispensable in the above-mentioned injuries [18,20,21]. Furthermore, the prevalence of hypertension and related pressure overload injuries has been steadily increasing worldwide now. It is known that approximately half the Asian population is carrying one copy of the mutant ALDH2 gene [22]. The interaction between hypertension and ALDH2 genotype remains unknown now. We speculate that ALDH2 might also play an important role in the process of pressure overload-induced heart failure via regulating autophagy.

Thus, this study was designed to elucidate the role of ALDH2 on pressure overload injury and related mechanisms focusing on autophagy. Changes of cardiac function and autophagy signaling were examined in wild-type (WT) and ALDH2 knockout (ALDH2 −/−) mice with or without the transverse aortic constriction (TAC) surgery.

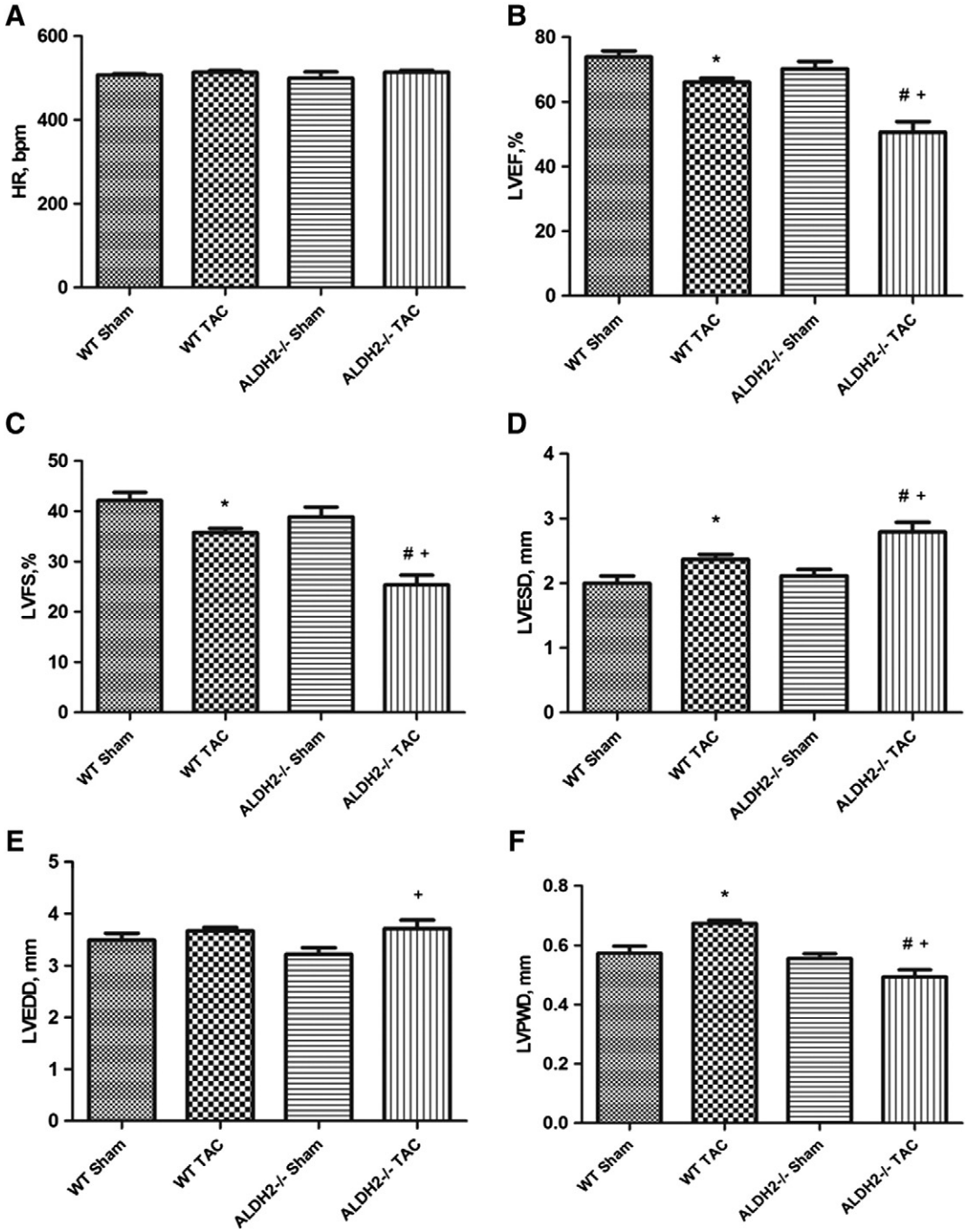

Fig. 1. Effect of ALDH2 deficiency on pressure overload-induced LV dysfunction. Quantitative analysis of heart rate (HR; A), left ventricular ejection fraction (LVEF; B), left ventricular fractional shortening (LVFS; C), left ventricular end-systolic diameter (LVESD; D), left ventricular end-diastolic diameter (LVEDD; E), left ventricular diastole posterior wall dimension (LVPWD; F). Mean ± SEM, $^{*}P < 0.05$ vs. WT Sham group, $^{\#}P < 0.05$ vs. WT TAC group, $^{+}P < 0.05$ vs. ALDH2 −/− sham group.

2. Materials and methods

2.1. Experimental animals and TAC surgery

All animal procedures described in this study were approved by the Animal Care and Use Committee of Fudan University and the surgery procedure was in compliance with the guidelines of "the Guide for the Care and Use of Laboratory Animals" Academy Press (NIH Publication No. 85–23, revised 1996). Adult male WT mice (C57BL/6, 8 weeks old, weighing 22–25 g) were bought from the Shanghai Animal Administration Center (Shanghai, China). ALDH2 −/− mice were produced by method described previously [22]. The TAC model was used to generate pressure overload-induced hypertrophy and heart failure. Animals were randomly assigned to sham or TAC group and observed at 8 weeks post surgery. In brief, mice were anesthetized with isoflurane and were placed in a supine position. The chest was opened and the transverse aortic was dissected free at the aortic arch level of surrounding tissues and muscles. A 5-0 nylon suture was tied around the aorta and a blunted 27-gauge needle which was removed after the ligation. The sham group underwent all operation procedures apart from the ligation. Mice were housed at room temperature under a 12-hour light/dark cycle with free access to water and standard laboratory mouse chow.

2.2. Echocardiographic assessment

Transthoracic echocardiography was performed using a 30-MHz high-frequency scan head as previously described (VisualSonics Vevo770; VisualSonics Inc. Toronto, Canada) to evaluate cardiac geometry and function. Left ventricular (LV) parasternal long-axis view was recorded. All the data were averaged measurements of at least 5 cardiac cycles of every mice in M-mode, including left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS), left ventricular end-systolic diameter (LVESD), left ventricular end-diasolic diameter (LVEDD) and left ventricular diastole posterior wall dimension (LVPWD).

2.3. Histological analysis

The heart was arrested after anesthesia by ketamine (intraperitoneal injection, i.p. 50 mg/kg). Heart weight and lung wet weight were measured. For histological analysis, heart tissues were fixed in 4% neutral formaldehyde at room temperature for more than 24 h. The specimen was embedded in paraffin and serial sections were cut at 4 μm in the short axis at the papillary muscle lever, and stained with hematoxylin and eosin (HE) to analyze the cardiomyocyte morphology and cross-sectional areas. Cardiomyocytes with clear myofiber outlines and integral nucleus were measured to analyze the cross-sectional area (CSA) on a high-resolution digital microscope (×400) by the image analysis system (QwinV3, Leica, Wetzlar, Germany). The cross-sectional area of cardiomyocytes was quantified from 3 slices of each heart, 5 high power random fields from each section, results were averaged.

2.4. Transmission electron microscopy (TEM)

Left ventricular tissues were dissected into small cubic pieces ≦1 mm3 and fixed with 2.5% glutaraldehyde (pH 7.4) for more than 2 h. After being washed in 0.1 M phosphate buffer (PB) for 3 times, the tissues were fixed in 1% osmium tetroxide. Then samples were dehydrated by graded ethanol with the last dehydrated procedure in 90% acetone.

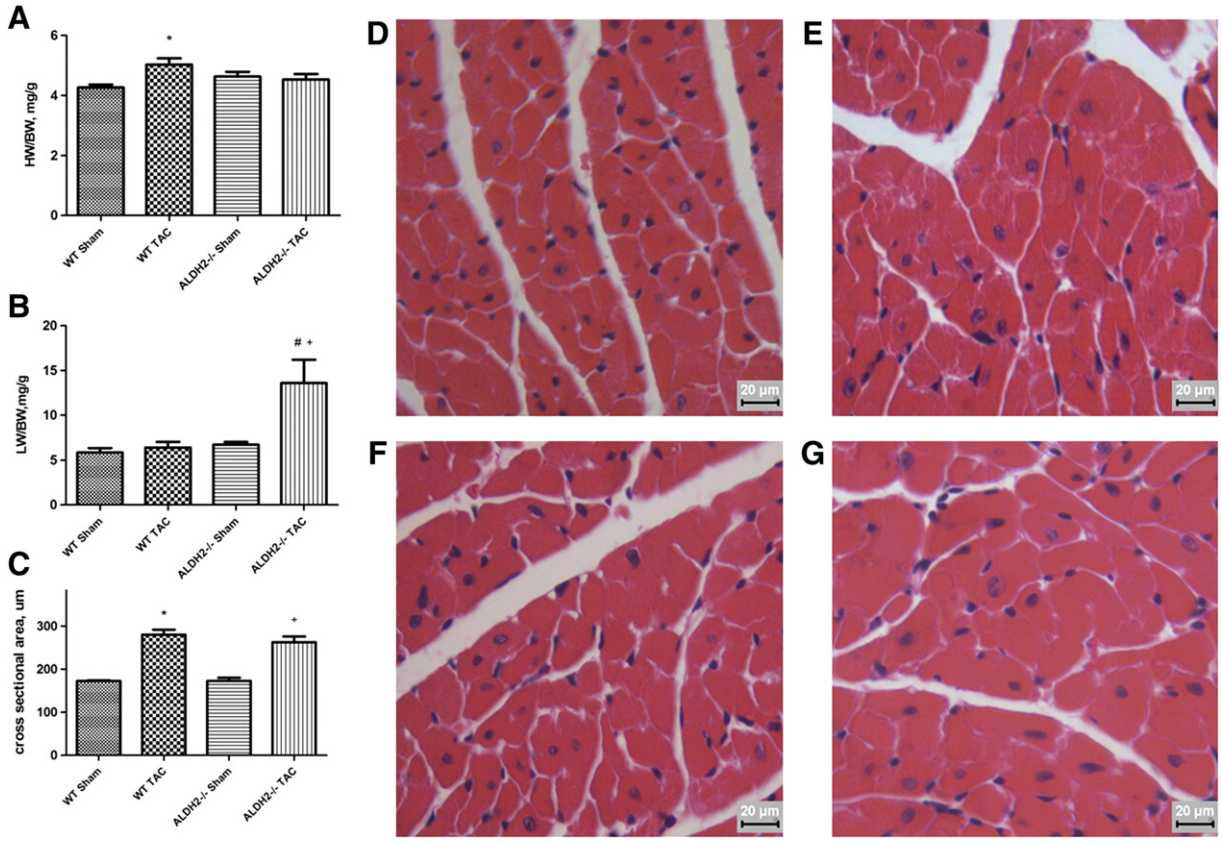

Fig. 2. Hypertrophic and histological characteristics in WT and ALDH2 −/− mice with or without TAC surgery. Quantitative analysis of heart-to-body weight ratio (HW/BW; A) and lung-to-body weight ratio (LW/BW; B). Representative images of hematoxylin and eosin (HE)-staining displaying transverse myocardial sections of left ventricular myocardium (×400) from WT sham group (D), WT TAC group (E), ALDH2 −/− sham group (F), ALDH2 −/− TAC group (G). C, Quantitative analysis of cardiomyocyte cross section areas (CSA) in each group. Scale bar, 20 μm. Mean ± SEM, $^{*}P < 0.05$ vs. WT sham group, $^{\#}P < 0.05$ vs. WT TAC group, $^{+}P < 0.05$ vs. ALDH2 −/− sham group.

All above procedures were made at 4 °C. After being embedded in Epon Araldite and fixed, ultrathin sections (50–60 nm) were cut using an LKB-I ultramicrotome (Leika, Germany) and stained with 3% uranyl acetate and lead citrate. Images were captured with the CM-120 transmission electron microscope (Philip, Holland).

2.5. Western blot analysis

Hearts were quickly-frozen in liquid nitrogen and stored at −80 °C. Procedures of protein extraction and Western blot were carried out as described previously [11]. Polyclonal rabbit antibodies against phosphorylated AMPKα (pAMPKα) at Thr172 (2535S), total AMPKα (2532S), Beclin-1 (3738S), p62 (5114S), LC3B (3868S), phosphorylated mTOR at Ser2448 (5536S), total mTOR (2983S; 1:1000; Rabbit; Cell Signaling Technology Inc. Boston, USA); ALDH2 (1:1000; Rabbit; Santa Cruz Biotechnology Inc. Dallas, USA) and horseradish peroxidase-conjugated secondary antibody (KANGCHEN Biotechnology, Nanjing, China) were examined by standard Western blotting. Respective proteins were analyzed with GAPDH serving as the loading control.

2.6. Co-immunoprecipitation

The co-immunoprecipitation (Co-IP) assay was performed as previously reported [23]. Briefly, protein was firstly incubated with Protein A/G PLUS-Agorase Immunoprecipitation Reagant (Santa Cruz, sc-2003) and certain amount of supernatant (500 μg protein) was transferred to a tube. Beclin-1 primary antibody (1:50) and 20 μl resuspended agarose were added to the tube and the mixture was incubated at 4 °C on a rocker platform overnight. After being washed with lysate, the agarose was eluted with SDS/PAGE loading buffer and then subjected to SDS/PAGE and Western blotting.

2.7. Statistical analysis

Data were presented as mean ± SEM. Multi-group comparison was performed by one-way ANOVA followed by a Tukey's test for post hoc analysis. Analysis was performed with GraphPad Prism 5.0 software (GraphPad, San Diego, CA). A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. ALDH2 deficiency exacerbates pressure overload-induced cardiac dysfunction in mice

Echocardiographic examination was performed at 8 weeks post TAC or sham surgery. Increased LVESD, decreased LVEF and LVFS were found while LVEDD remained unchanged in WT mice post TAC surgery. Although ALDH2 deficiency failed to affect any of the echocardiographic parameters measured in the absence of pressure overload exposure, it exacerbated these effects and increased LVEDD after TAC surgery (Fig. 1B–E). Besides, LVPWD in WT TAC mice was much thicker than in the WT sham group, while it was reversed to be much thinner in ALDH2 −/− TAC mice (Fig. 1F). In addition, the heart-to-body weight ratio (HW/BW) was significantly increased in WT mice at 8 weeks post TAC surgery, however, this effect disappeared in ALDH2 −/− TAC mice (Fig. 2A), which was more or less consistent with the echocardiographic result of LVPWD. The lung-to-body weight ratio (LW/BW) was

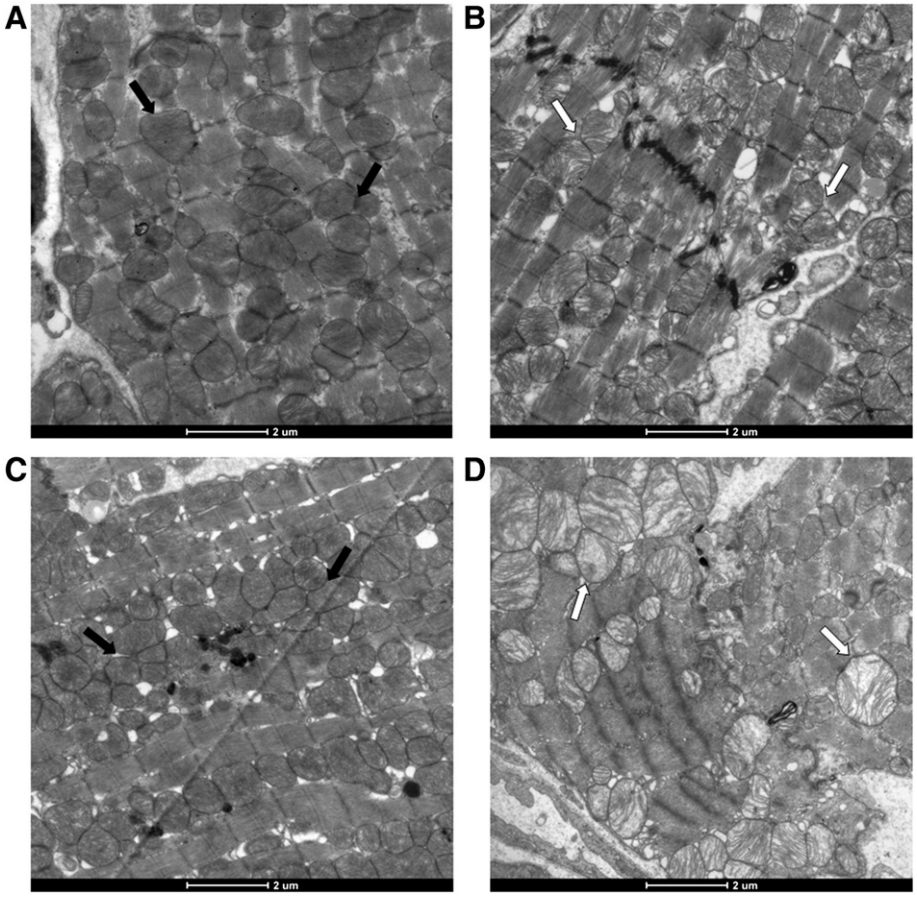

Fig. 3. Ultrastructural changes in left ventricular myocardium from WT and ALDH2 −/− mice with or without TAC surgery. Representative transmission electron microscopy (TEM) images of left ventricular from WT sham group (A), WT TAC group (B), ALDH2 −/− sham group (C), ALDH2 −/− TAC group (D). Normal mitochondria (black arrows) and damaged mitochondria (white arrows) are presented in the figure. Scale bar, 2 μm.

remarkably increased in ALDH2 −/− (but not WT) mice after TAC challenge (Fig. 2B). These results suggest that ALDH2 depletion accentuates pressure overload-induced contractile dysfunction and cardiac remodeling.

3.2. ALDH2 Deficiency augments histological changes induced by pressure overload in mice

To evaluate the pressure overload-induced structural changes in WT and ALDH2 −/− mice, myocardial histology was evaluated. HE staining showed that TAC surgery did not affect the arrangement and structure of cardiomyocytes (Fig. 2D–G), however, similarly increased CSA was obtained (Fig. 2C) both in WT and ALDH2 −/− mice at 8 weeks post TAC surgery.

As pressure overload-induced heart injury is often accompanied by mitochondrial damage [24], TEM was conducted to analyze the changes of mitochondrial morphology. In the absence of TAC challenge, no difference was found in myocardial ultrastructure between WT and ALDH2 −/− mice, and the mitochondria were arranged normally in the organized sarcomeres (black arrows in Fig. 3A and C). However, swelling changes and disorganized cristae were evidenced in mitochondria of the WT-TAC mice (white arrows in Fig. 3B). More aggravated changes were observed in ALDH2 −/− TAC mice, characterized by much larger mitochondria, vacuolar changes and disintegrate cristae (white arrows in Fig. 3D). These observations indicate that ALDH2 deficiency exacerbates pressure overload-induced mitochondrial injury.

3.3. ALDH2 deficiency exacerbates pressure overload- induced inhibition of autophagy in mice

Our results showed that ALDH2 protein expression was remarkably reduced in response to pressure overload (Fig.4A and B). Recent studies suggested a role of autophagy in pressure overload induced cardiac injury [15,25]. Our data depicted that pressure overload significantly suppressed myocardial autophagy (LC3II-to-LC3I ratio) without affecting autophagy flux (evidenced by invariant autophagy adaptor protein p62). ALDH2 itself did not affect myocardial autophagy, however, it accentuated pressure overload-induced inhibition of autophagy (Fig. 4A and C–F). These data suggest that autophagy inhibition is linked with aggravated pressure overload-induced cardiac dysfunction and myocardial injury in ALDH2 −/− mice.

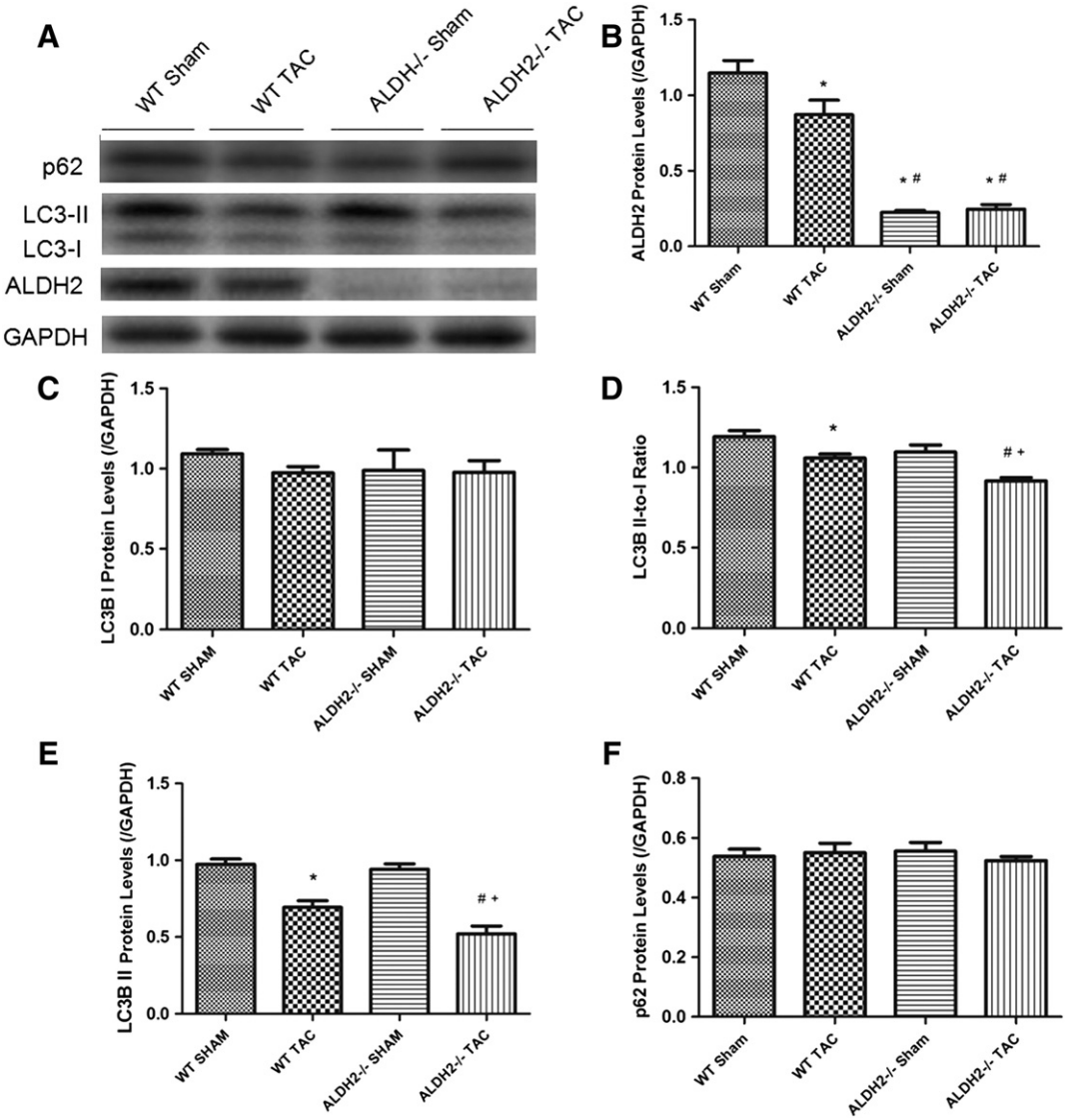

Fig. 4. Effect of ALDH2 deficiency on pressure overload-induced changes in myocardial autophagy. A, Representative gel blots of p62, LC3BI/II, ALDH2 and GAPDH (as loading control) using specific antibodies. B, ALDH2 expression. C, LC3B I expression. D, LC3B II expression. E, LC3B II-to-I tatio. F, p62 expression. Mean ± SEM, $^{*}P < 0.05$ vs WT sham group, $^{\#}P < 0.05$ vs WT TAC group, $^{+}P < 0.05$ vs ALDH2 −/− sham group.

3.4. ALDH2 deficiency further suppresses pressure overload-induced Beclin-1 expression and promotes the interaction between Bcl-2 and Beclin-1

To further analyze a potential signaling pathway involved in pressure overload and/or ALDH2 deficiency-elicited cardiac autophagic response, Beclin-1 dependent autophagy pathway was examined in our study. As shown in Fig. 5A and C, dampened expression of Beclin-1 was found in WT-TAC mice while it was much lower in ALDH2 −/− TAC mice. Beclin-1 (a BH3-only protein) binds to the BH3 domain of Bcl-2 and enhanced interaction between them would restrain autophagy [26,27]. To examine the interaction of the complex, immunoprecipitation was performed in WT and ALDH2 −/− mice hearts with or without pressure overload challenge. As shown in Fig. 5B and D, ALDH2 deficiency itself did not affect the Bcl-2-to-Beclin-1 ratio, but it promoted the interaction between Bcl-2 and Beclin-1 after TAC surgery. These data favor the notion that ALDH2 deficiency accentuates pressure overload-inhibited autophagy via suppressing Beclin-1 expression and promoting the interaction of Bcl-2 and Beclin-1.

3.5. ALDH2 deficiency accentuates pressure overload-activated AMPK–mTOR pathway

To explore the potential role of mTOR, a key regulator of autophagy, in pressure overload-induced cardiac remodeling, phosphorylated mTOR and its upstream signaling regulator AMPK were examined. Our results indicated that pressure overload markedly activated the phosphorylation of AMPK at Thr172 and depressed the phosphorylation of mTOR at Ser2448 without affecting AMPK and mTOR expression. Although ALDH2 deficiency itself did not affect the phosphorylation of these two molecules, it significantly accentuated pressure overload-induced changes in the phosphorylation of AMPK and mTOR (Fig. 6). Besides, AMPK could be activated following declined supplements of energy and metabolic disorder, which was an important pathological mechanism in pressure overload-induced heart failure [28–30]. These findings suggest that ALDH2 deficiency could inhibit pressure overload induced AMPK–mTOR pathway activation and this mechanism might associate with inadequate energy supplement in case of pressure overload.

4. Discussion

Our results suggested that pressure overload-induced cardiac anomalies were exacerbated by ALDH2 deficiency and pressure overload also dampened Beclin-1 dependent autophagy signaling, these effects were amplified by ALDH2 deficiency. Furthermore, we found that ALDH2 deficiency promoted the interaction of Beclin-1 and Bcl-2 post TAC surgery. Although evidence is accumulating that ALDH2 depicted a beneficial role against alcohol, acetaldehyde and toxic aldehyde-induced cardiac injury [2,9], data from present study represented another possible protective effect of ALDH2 which was capable of counteracting pressure overload induced myocardial injury.

Cardiac remodeling is characterized by increased cardiomyocyte size at the early stage of pressure overload, while contractile dysfunction and dilated cardiomyopathy often represent end-stage heart failure after sustained pathological stress [31,32]. In our hands, both WT mice and ALDH2 −/− mice developed overt contractile dysfunction as evidenced by reduced LVEF, and LVFS and increased LVESD at 8 weeks post pressure overload challenge. Combined with hypertrophic findings on LVPWD, HW/BW and CSA, WT-TAC mice presented in a maladaptive functional stage accompanied with adaptive geometric responses. These results are consistent with former report showing dampened cardiomyocyte contractile function in hypertrophic mice [33]. However, ALDH2 −/− TAC mice were already in functional and geometric maladaptive stages, as evidenced by increased LW/BW and much thinner wall thickness (Fig. 1F). Our results showed that pressure overload stimulation similarly increased CSA both in WT and ALDH2 −/− mice. The discrepancy between HW/BW and CSA could be explained by the possibility that functional changes might occur prior to the morphological responses during the progression from hypertrophy to heart failure. In addition, the extracellular matrix (ECM) remodeling may partially account for it during progressive cardiac dysfunction [34], as characterized by imbalance between ECM degradation and accumulation. Since more serious cardiac dysfunction was found in ALDH2 −/− mice subjected to pressure overload, we speculate that this result might partly be related to the more drastically ECM degradation in ALDH2 −/− TAC mice compared to that in WT TAC mice. Moreover, pressure overload challenge significantly reduced the myocardial expression of ALDH2. Taken together, ALDH2 deficiency definitely accelerates the pathological

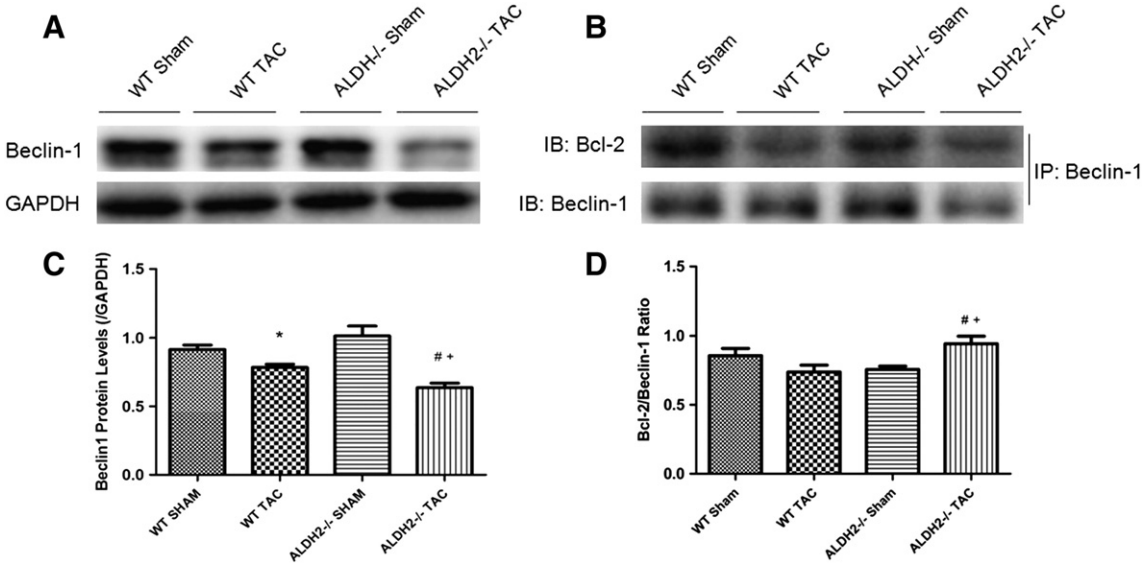

Fig. 5. Effect of ALDH2 deficiency on pressure overload-induced changes in Beclin-1 and interaction of Bcl-2 and Beclin-1. A, Representative gel blots of Beclin-1 and GAPDH (as loading control). C, Beclin-1 expression. B, Representative gel blots of Bcl-2 and Beclin-1 using specific antibodies. IP, immunoprecipitation; IB, immunoblot. D, Bcl-2-to-Beclin-1 Ratio. Mean ± SEM, $^{*}P < 0.05$ vs WT sham group, $^{\#}P < 0.05$ vs WT TAC group, $^{+}P < 0.05$ vs ALDH2 −/− sham group.

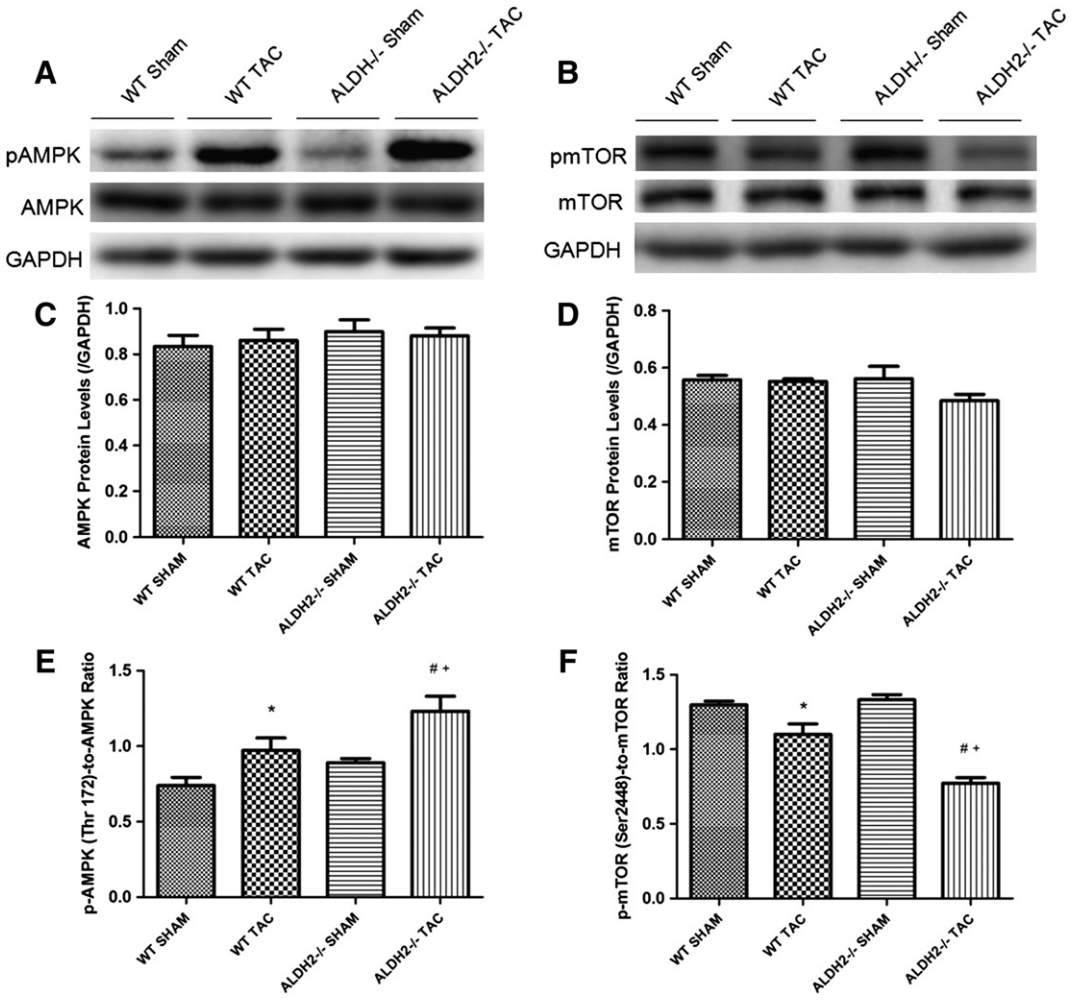

Fig. 6. Western blot analysis exhibiting phosphorylation of AMPK and mTOR in myocardium from WT and ALDH2 −/− mice with or without TAC surgery. A, Representative gel blots depicting levels of total and phosphorylated AMP-activated protein kinase (AMPK, A) and mammalian target of rapamycin (mTOR, B) and GAPDH (as loading control). C, Total AMPK expression. D, Total mTOR expression. E, AMPK phosphorylation expression (Thr172, pAMPK-to-AMPK ratio). F, mTOR phosphorylation expression (Ser2448, pmTOR-to-mTOR ratio). Mean ± SEM, $^{*}P < 0.05$ vs WT sham group, $^{\#}P < 0.05$ vs WT TAC group, $^{+}P < 0.05$ vs ALDH2 −/− sham group.

progression under pressure overload stress, suggesting that ALDH2 is indispensable in pressure overload-induced heart failure.

Our data showed that autophagy was inhibited in the remodeling hearts post pressure overload, in other words, autophagy was detrimental to cardiac function under pressure overload. Increasing evidence has depicted certain range of autophagy was essential to cardiac geometry and function under stress conditions, including hypertrophy, ischemia/reperfusion, and pressure overload-induced heart failure [1,8]. Since autophagy activation is needed to promote cell survival by degradation and turnover of damaged organelles in case of various disease conditions [1], it is reasonable to speculate the negative effects of autophagy inhibition in TAC mice. Although autophagy is well accepted to be a critical mediator under hemodynamic stress, it remains controversy with regard to its precise effect in this situation. Some studies have reported that autophagic activity was overt induced post pressure overload stress and the amplified autophagic response was associated with severe pathological remodeling [17,35,36]. Nonetheless, other researchers have indicated that autophagic suppression accentuated hemodynamic stress-induced cardiac hypertrophy and dysfunction [1,14]. Possible explanations for the observed discrepancies could be the inhomogeneity of different experimental settings and intervention periods. Intriguingly, the dual characteristics of autophagy have also been shown in other organ system and diseases [37]. Data from our group revealed that ALDH2 deficiency exacerbated the decline of autophagy after TAC surgery, which was in parallel with worse cardiac function in ALDH2 −/− TAC mice. Besides, p62 expression, an autophagy adaptor (as evidenced by autophagic flux), remained unchanged among various groups, suggesting that neither pressure overload nor ALDH2 deficiency affected autolysosome fusion.

Previous studies demonstrate that Beclin-1, a platform protein that interacts with several different proteins, including a complex with class III PI3K and Bcl-2, plays an important role in the autophagosome formation [26,38,39]. Alterations of Bcl-2 expression or phosphorylation could reflect changes on the interaction of Bcl-2/Beclin-1 complex, thus, affect the autophagy regulation process. Our study revealed that ALDH2 deficiency exacerbated pressure overload-induced suppression of autophagy as shown by inhibited Beclin-1. Although pressure overload did not inhibit the amount of Bcl-2 that immunoprecipitated with Beclin-1 in WT mice, the ratio of Bcl-2-to-Beclin-1 was significantly increased in ALDH2-/- TAC mice. In other words, ALDH2 deficiency promoted the interaction of Bcl-2 and Beclin-1 under pressure overload challenge, which was in parallel with the further depressed myocardial autophagy. Taken together, our data suggested that dampened Beclin-1-autophagy cascade in response to pressure overload under ALDH2 deficiency, leading to exacerbated cardiac geometric and functional anomalies.

Moreover, upregulated AMPK-mTOR pathway was shown in mice at 8 weeks post TAC surgery, theoretically, these will increase the expression of LC3B II according to the mTOR-dependent autophagy pathway [15]. However, it was disaccord with our current results on autophagic

marker, LC3B II and Beclin-1. We considered that AMPK, as an integral global coordinator of the metabolic stress response, can be activated following declined supplement of ATP, which serves as another pathological mechanism of pressure overload injury [30]. Accordingly, metabolism abnormalities might be a promoter of AMPK-mTOR pathway without regulating autophagy or its effect was covered by dampened Beclin-1 dependent autophagy pathway. In parallel, more severe damages of the mitochondria in ALDH2 −/− TAC mice (as shown in Fig. 3) could be induced by the excessive activation of AMPK–mTOR pathway. Further work is needed to define the accurate influence or interaction between the two pathways in pathological cardiac remodeling.

Former studies have demonstrated ALDH2 was capable of mitigating cardiac remodeling and contractile dysfunction in alcoholic cardiomyopathy [40]. Clinical researches have also certified that ALDH2 gene polymorphism contribute to the early onset alcoholism [41]. Our data showed that ALDH2 deficiency exacerbated pressure overload-induced hypertrophy and cardiac dysfunction in mice. This finding is in line with previous clinical studies in that ALDH2 polymorphism was closely associated with hypertension [42,43]. However, convincing clinical and experimental studies on interaction between ALDH2 gene polymorphisms and heart function following chronic pressure overload are still lacking. Further clinical studies are warranted to explore the association between ALDH2 gene and pressure overload-induced cardiomyopathy. Since half the of Asian populations carrying one copy of the mutant ALDH2 gene [22], based on our finding, they might suffer more severe disease consequences in case of hypertension, future studies are warranted to verify the impact of ALDH2 genotype between Asian and western hypertensive patients.

LW/BW ratio was significantly increased in ALDH2 −/− mice at 8 weeks post TAC. Two factors might be responsible for this finding. First of all, ALDH2 −/− mice were compelling in more serious stage of cardiac dysfunction as evidenced by significantly reduced LVEF, LVFS and increased LVESD. On the other hand, the limited absorptivity of gauze which we used to absorb the lung surface liquid might also contribute to the observed the high LW/BW ratio in ALDH2 −/− mice at 8 weeks post TAC.

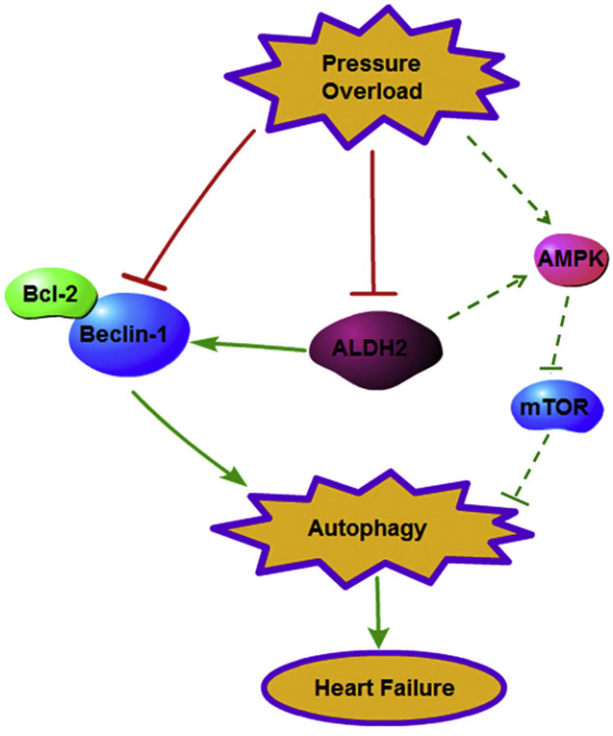

Fig. 7. Schematic diagram depicting the role of ALDH2 in pressure overload-induced changes in autophagy and cardiac function. Pressure overload suppresses expression of myocardium ALDH2, which participates in pressure overload-induced inhibition of Beclin-1 and in promoting the interaction of Bcl-2 and Beclin-1. Besides, ALDH2 also take part in the activation of AMPK–mTOR pathway possibly induced by energy deficiency. The Beclin-1 dependent pathway plays a key role in pressure overload-induced inhibition of autophagy, leading to heart failure. Arrows denote stimulation whereas the lines with a "T" ending represent inhibition.

5. Conclusion

In summary, our results suggest that ALDH2 deficiency exacerbated pressure overload-induced cardiac dysfunction, possibly through suppression of Beclin-1 dependent autophagy pathway (Fig. 7). Restoration of autophagy function may thus be an approach to attenuate cardiac geometric and functional abnormalities in ALDH2 −/− mice after pressure overload. Our findings further suggest that individuals carrying low levels or mutated ALDH2 alleles are prone to cardiac dysfunction under chronic hemodynamic stress such as hypertension and activating autophagy might serve as a potential therapeutic strategy in the population.

Conflict of interests

All authors declare no conflict of interest.

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