

Review

Mitochondrial aldehyde dehydrogenase in myocardial ischemia-reperfusion injury: from bench to bedside

PANG Jiao-Jiao^{1,2}, Linzi A. Barton¹, CHEN Yu-Guo^{2,*}, REN Jun^{1,3,*}

¹Center for Cardiovascular Research and Alternative Medicine, University of Wyoming College of Health Sciences, Laramie, WY 82071, USA; ²Department of Emergency, Qilu Hospital of Shandong University, Jinan, Shandong 250012, China; ³Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai 200032, China

Abstract: Acute myocardial infarction is one of the major causes of mortality worldwide. Reperfusion in a timely fashion is the most effective way to limit infarct size. However, reperfusion can itself prompt further myocardial injury. This phenomenon is commonly known as myocardial ischemia-reperfusion (IR) injury. Mitochondrial aldehyde dehydrogenase (ALDH2) is an enzyme metabolizing acetaldehyde and toxic aldehydes. Increasing evidence has revealed a cardioprotective role of ALDH2 in myocardial IR injury. Evidence from animal studies has shown that ALDH2 diminishes acute myocardial infarct size, ameliorates cardiac dysfunction and prevents reperfusion arrhythmias. The activity of ALDH2 is severely compromised if it is encoded by the mutant *ALDH2*2* gene, with an incidence of approximately 40% in Asian populations. Epidemiological surveys in the Asian population have depicted that ALDH2 polymorphism is closely associated with higher prevalence of acute myocardial infarction and coronary artery disease. Therefore, targeting ALDH2 may represent a promising avenue to protect against IR injury. This review recapitulates the underlying mechanisms involved in the protective effect of ALDH2 in cardiac IR injury. Translational potential of ALDH2 in the management of coronary heart disease is also discussed.

Key words: ALDH2; myocardial ischemia-reperfusion; 4-HNE; apoptosis; autophagy; mitochondrial injury; ER stress

线粒体乙醛脱氢酶在心肌缺血再灌注中的作用——从实验台到临床

庞佼佼^{1,2}, Linzi A. Barton¹, 陈玉国^{2,*}, 任骏^{1,3,*}

¹怀俄明大学健康科学学院心血管研究与转化医学中心, 拉勒米 WY 82071, 美国; ²山东大学齐鲁医院急诊科, 济南 250012; ³复旦大学中山医院上海心血管病研究所, 上海 200032

摘要: 急性心肌梗死是目前严重威胁人类健康的致死疾病之一, 及时再灌注是限制心肌梗死面积的最有效方法, 然而, 再灌注本身会导致进一步的心肌损伤。线粒体乙醛脱氢酶(mitochondrial aldehyde dehydrogenase, ALDH2)被广泛熟知为代谢乙醛的酶。越来越多的证据表明ALDH2在心肌缺血再灌注中起到心肌保护的作用。动物实验表明ALDH2可减少心肌梗死面积, 减轻心功能紊乱, 防止再灌注性心律失常的发生。突变型*ALDH2*2*基因编码的ALDH2酶活性显著降低, 而该基因的突变率在亚洲人群中约为40%。在亚洲人中的流行病学调查表明, *ALDH2*基因多态性与急性心肌梗死面积和冠状动脉疾病发生率紧密相关。因此, 将ALDH2作为治疗心肌缺血再灌注损伤的靶点将很有前景。本文综述ALDH2对缺血再灌注心肌的保护作用及机制, 并探讨ALDH2的转化应用研究前景。

关键词: ALDH2; 心肌缺血再灌注损伤; 4-HNE; 凋亡; 自噬; 线粒体损伤; 内质网应激

中图分类号: R541.4

Received 2015-05-20 Accepted 2015-09-09

*Co-corresponding authors. REN Jun, Tel: +1-307-766-6131; E-mail: jren@uwyo.edu; CHEN Yu-Guo, Tel: +86-531-82166844; E-mail: chen919085@126.com

1 Ischemia-reperfusion (IR) injury

Coronary artery disease (CAD) is commonly referred to as the interruption of coronary blood supply to myocardium, with acute myocardial infarction (AMI) being the most severe manifestation and consequence of CAD. AMI is the leading cause of morbidity and mortality in the world^[1]. Up-to-date, the most widely employed effective maneuver for AMI is to apply timely reperfusion in order to limit myocardial infarct size^[2]. Paradoxically, such process of reperfusion may itself trigger myocardial injury and cardiomyocyte death. Thus the process of “IR” may not only mitigate the benefits of reperfusion, but also contribute to up to 50% of the ultimate myocardial infarct, resulting in cardiac arrhythmias, heart failure and death^[3]. Thus, protecting cardiomyocytes against IR injury should be a very important therapeutic strategy for the management of AMI. Unfortunately, efforts to protect the heart against IR injury remain dismal.

With the advance of medical technology, the clinical approaches of both preconditioning^[4] and postconditioning^[5, 6] have greatly improved the clinical outcome of IR injury. More recent evidence has revealed a role of mitochondrial aldehyde dehydrogenase (ALDH2) in the onset and progression of myocardial IR injury. ALDH2 is widely known for its role in the detoxification of the alcohol metabolite acetaldehyde. The cardioprotective role of ALDH2 was first reported by Mochly-Rosen's group in 2008^[7]. Through an unbiased proteomic search, they've found that activation of ALDH2 is correlated with reduced ischemic cardiac damage in rodent models. Then, by using Alda-1 [N-(1,3-benzodioxol-5-ylmethyl)-2,6-dichlorobenzamide], a small molecule ALDH2 activator, prior to ischemia, infarct size was found to be significantly reduced. In another word, activation of ALDH2 by Alda-1 prior to ischemic event can mimic preconditioning. The potential role of Alda-1 as a drug to limit ischemic damage has drawn broad attention recently, leading to the notion of ALDH2 as a novel cardioprotective factor and a promising therapeutic target for CAD^[8–11]. The underlying mechanisms for the cardioprotective actions of ALDH2 have been extensively examined. In this review, we will summarize the current understanding of the mechanisms contributing to the effect of ALDH2 in IR injury. Translational potential of Alda-1 in the management of coronary heart disease will also be discussed.

2 Physiology of ALDH2

ALDH2 is the mitochondrial isoform of ALDH superfamily^[12], which is located in mitochondrial matrix to remove the aldehyde substrates. ALDH2 is a main enzyme in ethanol metabolism. Ethanol is mainly eliminated through oxidation, first catalyzed by alcohol dehydrogenase (ADH) to acetaldehyde, and then catalyzed by ALDH2 to acetic acid. In addition to acetaldehyde, ALDH2 also removes toxic aldehydes such as 4-hydroxy-2-nonenal (4-HNE)^[11, 13], a primary detrimental product of lipid peroxidation. Structurally, ALDH2 is a tetrameric allosteric enzyme located in a wide range of organs including the heart. *ALDH2* gene often displays genetic polymorphism. *ALDH2*1* is defined as the wild-type genotype with full enzymatic activity, while *ALDH2*2* represents the mutant genotype. *ALDH2*2* encodes inactive subunit, containing a glutamate to lysine mutation at the position 487 (E487K)^[14]. With such change in any of the four subunits of *ALDH2* gene, ALDH2 enzymatic activity can be severely compromised. Heterozygotes *ALDH2*1/2* only exhibit 30%–40% of normal ALDH2 enzymatic activity, while the mutant homozygotes *ALDH2*2/2* display negligible activity^[7]. The mutation of *ALDH2* occurs commonly in East Asian populations (up to 40%). The incidence of *ALDH2* genetic polymorphism is rather rare in Caucasians, with merely 8% among all ethnicities, as opposed to 40% in the East Asian population^[15].

Although ALDH2 is an ethanol metabolic enzyme, a unique cardioprotective role of ALDH2 has been noted through both epidemiological surveys and experimental studies. Epidemiological surveys in the East Asian populations have revealed that, compared to those with the wild-type *ALDH2*1/1* genotype, the mutant *ALDH2*2* allele carriers endure much higher risks of AMI and the overall incidence for cardiovascular disease^[16], as well as higher risks of independent risk factors for cardiovascular diseases, such as diabetes mellitus^[17, 18], hypertension^[19] and dyslipidemia^[20]. Evidence from animal studies depicted that, activation or overexpression of ALDH2 offers beneficial effect in a wide array of myocardial pathologies including IR injury^[7, 11, 21], alcoholic cardiomyopathy^[22], diabetic cardiomyopathy^[23], septic cardiomyopathy^[24] and heart failure^[25, 26]. For IR injury, activation or overexpression of ALDH2 was shown to protect cardiac injury by diminishing acute myocardial infarction size, ameliorating cardiac dys-

function and preventing reperfusion arrhythmias^[10, 11, 27, 28]. The mechanisms involved in the beneficial effect of ALDH2 in IR injury are quite complicated. Regulation of reactive oxygen species (ROS)^[11, 13, 28], mitochondrial dysfunction^[29], apoptosis^[23, 25, 30], autophagy^[11, 13] and endoplasmic reticulum (ER) stress^[31] were reported to play a role in ALDH2-offered cardiovascular response. In particular, accumulation of ROS is often considered the early event for an array of detrimental outcome. ALDH2 is believed to suppress ROS production through detoxification of aldehydes, a main avenue for the cardiac protective role of ALDH2. Moreover, Akt and AMPK, which play vital roles in many cell signaling pathways, can be regulated by ALDH2 in various pathological conditions. Regulation of Akt and AMPK is considered a main route that underlies the cytoprotective property of ALDH2 including detoxification of aldehydes^[11].

3 ALDH2, reactive aldehydes, ROS and mitochondrial injury

Myocardial IR is accompanied by ROS accumulation in reperfusion phase due to the sudden re-supply of oxygen^[32]. Mitochondria are not only critical organelles for ATP production in cardiomyocytes^[33], but also the main source and the target of ROS^[34, 35]. ROS can oxidize cell membranes and organelle lipids with peroxide ions. This lipid peroxidation further leads to an increase in reactive aldehydes^[36–38]. Reactive aldehydes are capable of modifying key enzymes and genes by forming covalent adducts^[39–42]. Reactive aldehydes also trigger the opening of mitochondrial permeability transition pores (MPTP) and inhibit electron transport chain, resulting in mitochondrial injury^[28, 43]. Reactive aldehydes can, in turn, trigger higher levels of ROS^[39]. ALDH2 can diminish the detrimental effect of reactive aldehydes and stop this positive feedback. Normal expression and activation of ALDH2 is expected to attenuate ROS generation and preserve mitochondrial function.

A plethora of evidence has indicated the effect of ALDH2 on diminishing reactive aldehydes, attenuating ROS generation and reducing mitochondrial injury. The activation of ALDH2 significantly reduced 4-HNE formation and ROS accumulation during IR in rodent models^[7, 11, 28], while inhibition of ALDH2 during IR significantly increased 4-HNE and ROS levels, aggravating cardiomyocyte function^[44]. Though direct evi-

dence is still lacking for a role of ALDH2 in mitochondrial dysfunction in IR injury, a number of reports have been seen demonstrating that ALDH2 activation protects mitochondrial function in several models of cardiac dysfunction^[24, 28, 29]. In post-myocardial infarction cardiomyopathy, ALDH2 activation was reported to prevent inhibition of mitochondrial electron transport chain complexes (I and V) and excessive release of H₂O₂^[28]. Similar findings were noted in heart failure^[29]. In addition, ALDH2 activation also suppressed mitochondrial Ca²⁺-induced MPTP opening and cytochrome C release^[29]. In LPS-induced septic model of cardiac dysfunction, a marked opening of MPTP was observed, which was prevented by ALDH2 activation^[24].

Given the detrimental roles of aldehydes, ROS and mitochondrial injury in cardiac pathology, it is not surprising that cardioprotective effect of ALDH2 may work through regulation of ROS, detoxification of aldehydes and reservation of mitochondrial function, which will be discussed in the following sections.

4 ALDH2 and apoptosis

Apoptosis is a highly regulated, adenosine triphosphate (ATP)-dependent cellular death program that follows well-orchestrated signaling pathways resulting in cell shrinkage, plasma membrane blebbing, nuclear condensation, mitochondrial dysfunction and DNA fragmentation. This is followed by the formation of apoptotic bodies, which quickly undergo phagocytosis by macrophages. It was reported that during cardiac IR injury, prolonged periods of ischemia lead to an increase in necrosis rate, while reperfusion enhances apoptosis levels due to restoration of oxygen and glucose as well as the regeneration of ATP^[45, 46]. Apoptosis contributes to a loss of cardiomyocytes, which are terminally differentiated cells and irreplaceable. Thus, inhibition of apoptosis can reduce the extent of injury and preserve cardiac function. Inhibition of apoptosis during IR has been shown to reduce myocardial infarct size up to 50%–70% and attenuate IR-associated cardiac dysfunction^[47, 48].

Apoptosis during IR is closely associated with ROS accumulation^[49, 50]. ALDH2 has been shown to be an anti-apoptotic enzyme involved in oxidative stress-induced cell apoptosis^[30, 49, 51, 52]. A recent study using induced pluripotent stem cell (iPSC)-derived human cardiomyocytes showed that levels of 4-HNE, ROS, and apoptosis were much higher in the mutant human

ALDH2*1/2 cardiomyocytes compared with those in wild-type cardiomyocytes, especially under ischemic conditions^[49]. When given the activator of ALDH2, Alda-1, levels of 4-HNE, ROS, and apoptosis were significantly decreased. Up-to-date, two independent apoptotic pathways have been identified namely the extrinsic (or receptor-mediated) and the intrinsic (or mitochondrial-mediated) pathways. Extrinsic pathway is activated when pathological stimuli lead to the binding of ligands, such as Fas ligand or tumor necrosis factor- α (TNF- α), to death receptors, following activation of caspases^[53]. Intracellular stress that promotes mitochondrial damage is capable of triggering mitochondria-mediated apoptosis^[53, 54]. The inner membrane of mitochondria contains important functional proteins (e.g. cytochrome C)^[55]. ROS generation as a result of mitochondrial damage leads to the peroxidation of lipids in the inner mitochondrial membrane, resulting in cytochrome C release from the inner mitochondrial membrane to the cytosol, a key step leading to apoptosome formation and caspase-dependent cell death^[54, 56]. ALDH2 may inhibit cytochrome C release in mitochondria-mediated apoptosis by attenuating ROS generation. Nonetheless, further study is warranted to elucidate such scheme.

ROS is known to turn on the JNK/c-Jun signaling pathway, which triggers apoptosis through both extrinsic and intrinsic pathways^[25, 49]. Excessive ROS-activated JNK signaling under ischemic condition was reported in ALDH2-deficient cardiomyocytes, resulting in increased expression of c-Jun, *en route* to exacerbated inhibition of proliferation and induction of apoptosis in ALDH2-deficient cardiomyocytes^[49]. Besides, ALDH2 may inhibit apoptosis through ERK1/2^[52] and p53 pathways^[25].

5 ALDH2 and autophagy

Autophagy, together with apoptosis and necrosis, plays an important role in the “survival and death” procedure to maintain myocardial homeostasis under IR conditions. Under IR conditions, autophagy is upregulated^[57, 58]. Though the purpose of autophagy may be hereby myocardial protection^[59–63], there is evidence suggesting that autophagy can lead to the demise of cardiomyocytes^[13, 64–67]. Thus, autophagy plays a critical and double role in cardiomyocyte survival. Mitochondrial injury can induce autophagy, called mitophagy. Following IR, many autophagosomes were found containing

mitochondria^[68–70].

ALDH2, as an independent factor in cardioprotective signaling pathways, plays an important role in the regulation of IR-induced autophagy. ALDH2 was reported to regulate both classical AMPK/Akt/mTOR pathway and Beclin-1 pathway. By examining the ischemia and reperfusion phases separately, our group found that ALDH2 enhanced the beneficial role of autophagy in ischemia by activating LKB1/AMPK/mTOR pathway, while helped to avoid the detrimental function of excessive autophagy in reperfusion through inducing PTEN/Akt/mTOR pathway^[11], as shown in Fig. 1 and 2. ROS and aldehydes play important roles here as well. Results from our own laboratory showed that 4-HNE

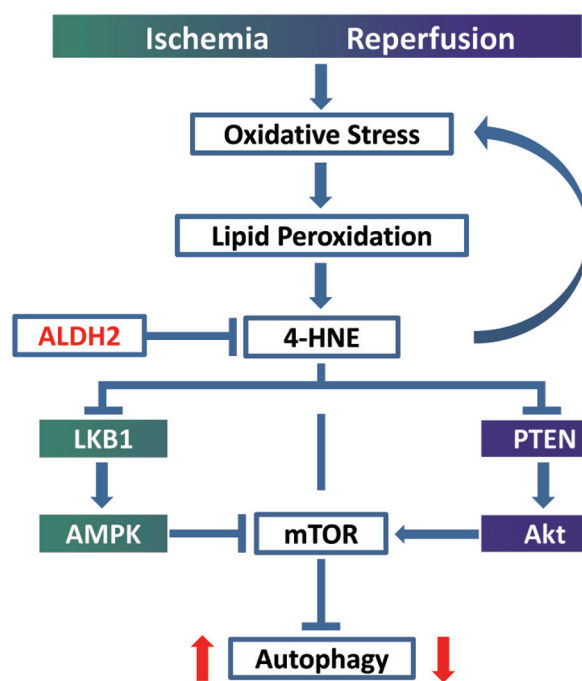


Fig. 1. Autophagy pathway in ischemia-reperfusion and the beneficial role of ALDH2. Ischemia-reperfusion can induce oxidative stress, which leads to lipid peroxidation and production of 4-HNE. 4-HNE can in turn augment oxidative stress. 4-HNE also plays a detrimental role in both ischemia and reperfusion phases. In the ischemic phase, induction of AMPK by LKB1 can inhibit mTOR, an autophagy inhibitory factor, thereby increasing autophagy levels – a beneficial effect during ischemia. However, 4-HNE can inhibit LKB1, the inducer of AMPK. In the reperfusion phase, induction of Akt by PTEN can induce mTOR to avoid excessive autophagy. Yet 4-HNE can inhibit PTEN and decrease the Akt induction. ALDH2 can significantly decrease the level of 4-HNE, thus restoring the AMPK/mTOR/autophagy and Akt/mTOR/autophagy pathways during ischemia and reperfusion respectively.

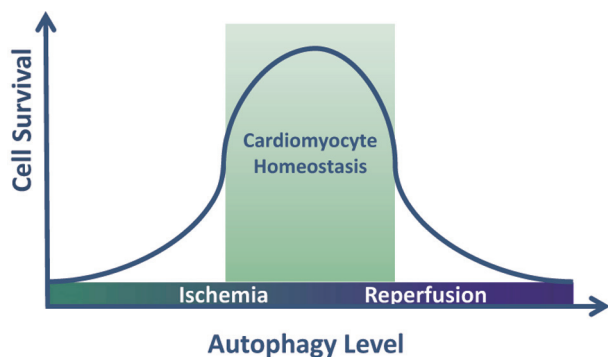


Fig. 2. The relationship between autophagy and cell survival in ischemia-reperfusion. In the ischemic phase, autophagy is useful for the removal and recycling of damaged organelles, so as the autophagy level increases, the cell survival increases. While in the reperfusion phase the autophagy level keeps increasing, which can lead to the demise of cardiomyocytes. Therefore, maintaining the autophagy level in an appropriate range is a way to preserve cardiomyocyte homeostasis.

compromises the LKB1 and PTEN signaling, which are upstream molecules of Akt and AMPK^[11]. The result that ALDH2 may affect Beclin-1 dependent autophagy was found by Junbo Ge's group in a pressure overload-induced cardiac dysfunction mouse model. Beclin-1 can bind Vps34, a class III PI3 kinase, to form the core of initiation complex required for the initiation of the formation of the autophagosome in autophagy. However, the core of initiation complex can be disrupted by the interaction of Beclin-1 with Bcl-2^[71]. Using *ALDH2*^{-/-} mice, Ge's group found that ALDH2 deficiency exacerbated pressure overload-induced inhibition of autophagy by suppressing Beclin-1 expression and promoting the interaction between Bcl-2 and Beclin-1^[26].

Mitophagy is a type of selective autophagy aiming to degrade defective mitochondria and prevent cell death^[72]. However, excessive mitophagy is detrimental for cell survival, the same as autophagy. The PTEN-induced putative kinase 1 (PINK1)/Parkin pathway is one of the important pathways to regulate mitophagy. It is reported that increased oxidative stress inhibits mitophagy by modification and inactivation of Parkin^[73]. However, the precise regulatory mechanism of mitophagy in IR injury is still poorly understood. Given the beneficial role of ALDH2 to control ROS, prevent mitochondrial injury and regulate autophagy, mitophagy may play a role in ALDH2-offered protection although further evidence is needed to consolidate this point.

6 ALDH2 and ER stress

The ER is a membranous intracellular network responsible for folding and processing newly synthesized proteins. Under normal conditions, about 30% of proteins are misfolded and thereby degraded by ER-associated degradation (ERAD)^[74], while the correctly folded proteins are transported to the Golgi apparatus. But this process can be easily disturbed, especially under pathological conditions^[75], causing ER stress. ER stress is initially considered an adaptive response by triggering the unfolded protein response (UPR) to maintain ER homeostasis. Nonetheless, excessive ER stress leads to inflammation, cell apoptosis and autophagy, which play an important role in the development and progression of cardiovascular diseases^[76], including myocardial IR injury. ER chaperones such as GRP78 and three pathways are involved in the ER stress response. These pathways are the inositol-requiring protein-1 (IRE1) pathway, the protein kinase RNA-like ER kinase (PERK) pathway and the activating transcription factor-6 (ATF6) pathway^[77].

The effect of ALDH2 on ER stress was first detected by our lab in the chronic alcohol ingestion-induced myocardial insulin resistance mouse model. Our studies showed that chronic alcohol intake upregulated ER stress markers^[78, 79]. ER stress was already known to interfere with insulin signaling^[80-82], and we showed that ALDH2 ameliorated insulin resistance in this model^[79]. Further scrutiny is warranted to explore the relationship of ALDH2 and ER stress. The result of this study showed that ALDH2 reversed alcohol-induced myocardial ER stress^[79]. Subsequently, the pivotal role of ALDH2 in the regulation of ER stress in cardiac dysfunction was demonstrated using *ALDH2* knockout mice^[83]. Another independent study using *ALDH2* transgenic mice also confirmed the beneficial role of ALDH2 against ER stress-induced cardiac dysfunction involving correction of autophagy^[31]. Regarding myocardial IR injury, studies provided evidence that ER stress was possibly initiated through ROS generation^[76, 84] which then triggered cardiomyocyte apoptosis^[85, 86]. Nonetheless, there is still a lack of direct evidence for the involvement for ALDH2 in this ER stress-ROS generation process. Although these studies have suggested a link between ALDH2 and ER stress, further research is warranted, especially in the field of myocardial IR injury.

7 Translational potentials and approaches

Given the important role of ALDH2 in cardiac protection and the high incidence of *ALDH2* gene mutation in East Asian population, ALDH2 is a potential therapeutic target for cardiac injury. As far as we know, there are several natural and synthetic ALDH2 activators, among which Alda-1^[87] is the most specific and effective one. Alda-1, a small molecule, is unique for its capacity not only to enhance the activity of wild-type ALDH2, but also to restore the function of inactive ALDH2. It can enhance the wild-type ALDH2 activity by two-fold, restore activity of the mutant ALDH2*2/1 to the near wild-type level, and increase the activity of ALDH2*2/2 by ten-fold^[7].

In rodent models, the potential role of Alda-1 to treat cardiac IR injury has received demonstration. It has shown that treatment with Alda-1 decreases 4-HNE protein adducts, reduces cardiac infarction size, and ameliorates damaged cardiac function^[7, 11, 28]. For exam-

ple, using Alda-1 prior to ischemia followed by reperfusion, the small molecule enzymatic activator may mimic preconditioning function and reduce cardiac IR injury^[7]. After permanent left anterior descending artery (LAD) occlusion surgery, Alda-1 treatment prevented aldehyde overload, mitochondrial dysfunction and improved cardiac function^[28]. These studies have shown a rather promising role of Alda-1 in clinical settings of disease intervention.

However, there is still a major gap for clinical use of Alda-1. Much work is needed prior to the clinical application of Alda-1. On pharmacokinetics, the adverse effects or even the effects of Alda-1 on patients suffering from ischemic heart diseases still deserve further research.

8 Summary

ALDH2 is capable of protecting against cardiac IR injury, while the underlying mechanisms are quite

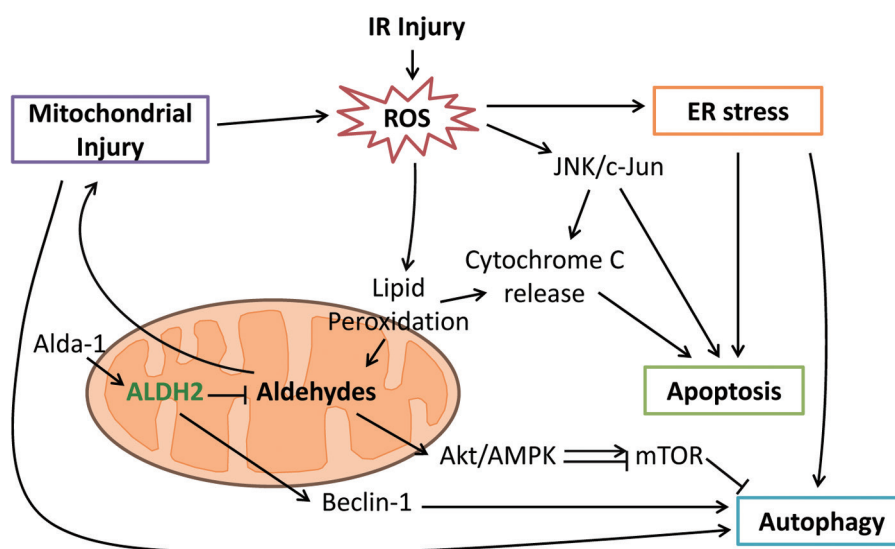


Fig. 3. Schematic diagram depicting the signaling mechanisms behind the protective effect of ALDH2 against myocardial IR injury. During ischemia/reperfusion, especially in reperfusion phase, ROS level is highly elevated by the sudden supply of oxygen, which is followed by lipid peroxidation and aldehyde generation. Aldehydes can induce oxidative stress, and cause mitochondrial injury by inducing opening of mitochondrial permeability transition pores (MPTP) and inhibition of the electron transport chain. Mitochondrial injury can also lead to ROS generation, which forms a positive feedback loop. Lipid peroxidation causes cytochrome C release from the inner membrane of mitochondria to the cytoplasm, which is a key step to inducing apoptosis. ROS can also directly activate the JNK/c-Jun pathway to induce apoptosis. JNK/c-Jun can induce apoptosis with or without cytochrome C. ALDH2, which can be activated by Alda-1, can inhibit apoptosis by stopping the positive feedback loop. Aldehydes can inhibit the activation of Akt and AMPK by compromising the LKB1 and PTEN signaling. Thus, in different conditions of IR injury, ALDH2 plays different roles to induce or inhibit autophagy to protect cardiomyocytes by regulating Akt/AMPK/mTOR pathway as indicated in Fig. 1 and 2. ALDH2 may also regulate autophagy through the Beclin-1 pathway in IR. Mitochondrial injury can directly induce autophagy, called mitophagy, which may be inhibited by ALDH2. ROS can also lead to ER stress, which further cause cardiac dysfunction through apoptosis or autophagy. ALDH2 can suppress ER stress by inhibiting ROS generation.

complicated. In our opinion, detoxification of reactive aldehydes, reduction of ROS generation and protection of mitochondrial function seems to play a central role in many pathways that lead to ER stress, apoptosis and autophagy, as depicted in Fig. 3. ALDH2 may exert a positive feedback control of these signaling machineries. In fact, the role of ROS is rather complicated in IR injury. Under appropriate levels, ROS may serve as signaling molecules to activate the pro-survival factors, including ALDH2^[88]. The novel ALDH2 agonist, Alda-1, has shown potential to treat cardiac IR injury, which means a lot to *ALDH2**2 carriers especially. However, it demands more efforts for better understanding the underlying mechanisms involved in the cardioprotective property of ALDH2 and the promises of applying Alda-1 in the management of acute CAD.

REFERENCES

- Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB, American Heart Association Statistics C, Stroke Statistics S. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation* 2015; 131(4): e29–e322.
- Gerczuk PZ, Kloner RA. An update on cardioprotection: a review of the latest adjunctive therapies to limit myocardial infarction size in clinical trials. *J Am Coll Cardiol* 2012; 59(11): 969–978.
- Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 2007; 357(11): 1121–1135.
- Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; 74(5): 1124–1136.
- Vinten-Johansen J, Zhao ZQ, Zatta AJ, Kin H, Halkos ME, Kerendi F. Postconditioning--A new link in nature's armor against myocardial ischemia-reperfusion injury. *Basic Res Cardiol* 2005; 100(4): 295–310.
- Kloner RA, Rezkalla SH. Preconditioning, postconditioning and their application to clinical cardiology. *Cardiovasc Res* 2006; 70(2): 297–307.
- Chen CH, Budas GR, Churchill EN, Disatnik MH, Hurley TD, Mochly-Rosen D. Activation of aldehyde dehydrogenase-2 reduces ischemic damage to the heart. *Science* 2008; 321(5895): 1493–1495.
- Churchill EN, Disatnik MH, Mochly-Rosen D. Time-dependent and ethanol-induced cardiac protection from ischemia mediated by mitochondrial translocation of varepsilonPKC and activation of aldehyde dehydrogenase 2. *J Mol Cell Cardiol* 2009; 46(2): 278–284.
- Budas GR, Disatnik MH, Chen CH, Mochly-Rosen D. Activation of aldehyde dehydrogenase 2 (ALDH2) confers cardioprotection in protein kinase C epsilon (PKCvarepsilon) knockout mice. *J Mol Cell Cardiol* 2010; 48(4): 757–764.
- Koda K, Salazar-Rodriguez M, Corti F, Chan NY, Estephan R, Silver RB, Mochly-Rosen D, Levi R. Aldehyde dehydrogenase activation prevents reperfusion arrhythmias by inhibiting local renin release from cardiac mast cells. *Circulation* 2010; 122(8): 771–781.
- Ma H, Guo R, Yu L, Zhang Y, Ren J. Aldehyde dehydrogenase 2 (ALDH2) rescues myocardial ischaemia/reperfusion injury: role of autophagy paradox and toxic aldehyde. *Eur Heart J* 2011; 32(8): 1025–1038.
- Jackson B, Brocker C, Thompson DC, Black W, Vasiliou K, Nebert DW, Vasiliou V. Update on the aldehyde dehydrogenase gene (ALDH) superfamily. *Hum Genomics* 2011; 5(4): 283–303.
- Sun A, Cheng Y, Zhang Y, Zhang Q, Wang S, Tian S, Zou Y, Hu K, Ren J, Ge J. Aldehyde dehydrogenase 2 ameliorates doxorubicin-induced myocardial dysfunction through detoxification of 4-HNE and suppression of autophagy. *J Mol Cell Cardiol* 2014; 71: 92–104.
- Ehrig T, Bosron WF, Li TK. Alcohol and aldehyde dehydrogenase. *Alcohol Alcohol* 1990; 25(2–3): 105–116.
- Shibuya A, Yoshida A. Frequency of the atypical aldehyde dehydrogenase-2 gene (ALDH2(2)) in Japanese and Caucasians. *Am J Hum Genet* 1988; 43(5): 741–743.
- Xu F, Sun Y, Shang R, Li M, Cui L, Cui Z, Chen Y. The Glu504Lys polymorphism of aldehyde dehydrogenase 2 contributes to development of coronary artery disease. *Tohoku J Exp Med* 2014; 234(2): 143–150.
- Xu F, Chen Y, Lv R, Zhang H, Tian H, Bian Y, Feng J, Sun Y, Li R, Wang R, Zhang Y. ALDH2 genetic polymorphism and the risk of type II diabetes mellitus in CAD patients. *Hypertens Res* 2010; 33(1): 49–55.
- Yokoyama A, Mizukami T, Matsui T, Yokoyama T, Kimura M, Matsushita S, Higuchi S, Maruyama K. Genetic polymorphisms of alcohol dehydrogenase-1B and aldehyde dehydrogenase-2 and liver cirrhosis, chronic calcific pancreatitis, diabetes mellitus, and hypertension among Japanese alcoholic men. *Alcohol Clin Exp Res* 2013; 37(8): 1391–1401.
- Zhang SY, Chan SW, Zhou X, Chen XL, Mok DK, Lin ZX, Wang YH. Meta-analysis of association between ALDH2 rs671 polymorphism and essential hypertension in Asian

- populations. *Herz* 2015; 40 Suppl2: 203–208.
- 20 Kotani K, Sakane N, Yamada T. Association of an aldehyde dehydrogenase 2 (ALDH2) gene polymorphism with hyper-low-density lipoprotein cholesterolemia in a Japanese population. *Ethn Dis* 2012; 22(3): 324–328.
 - 21 Luo XJ, Liu B, Ma QL, Peng J. Mitochondrial aldehyde dehydrogenase, a potential drug target for protection of heart and brain from ischemia/reperfusion injury. *Curr Drug Targets* 2014; 15(10): 948–955.
 - 22 Zhang Y, Ren J. ALDH2 in alcoholic heart diseases: molecular mechanism and clinical implications. *Pharmacol Ther* 2011; 132(1): 86–95.
 - 23 Guo Y, Yu W, Sun D, Wang J, Li C, Zhang R, Babcock SA, Li Y, Liu M, Ma M, Shen M, Zeng C, Li N, He W, Zou Q, Zhang Y, Wang H. A novel protective mechanism for mitochondrial aldehyde dehydrogenase (ALDH2) in type 1 diabetes-induced cardiac dysfunction: role of AMPK-regulated autophagy. *Biochim Biophys Acta* 2015; 1852(2): 319–331.
 - 24 Hu Y, Yan JB, Zheng MZ, Song XH, Wang LL, Shen YL, Chen YY. Mitochondrial aldehyde dehydrogenase activity protects against lipopolysaccharide-induced cardiac dysfunction in rats. *Mol Med Rep* 2015; 11(2): 1509–1515.
 - 25 Sun A, Zou Y, Wang P, Xu D, Gong H, Wang S, Qin Y, Zhang P, Chen Y, Harada M, Isse T, Kawamoto T, Fan H, Yang P, Akazawa H, Nagai T, Takano H, Ping P, Komuro I, Ge J. Mitochondrial aldehyde dehydrogenase 2 plays protective roles in heart failure after myocardial infarction via suppression of the cytosolic JNK/p53 pathway in mice. *J Am Heart Assoc* 2014; 3(5): e000779.
 - 26 Shen C, Wang C, Fan F, Yang Z, Cao Q, Liu X, Sun X, Zhao X, Wang P, Ma X, Zhu H, Dong Z, Zou Y, Hu K, Sun A, Ge J. Acetaldehyde dehydrogenase 2 (ALDH2) deficiency exacerbates pressure overload-induced cardiac dysfunction by inhibiting Beclin-1 dependent autophagy pathway. *Biochim Biophys Acta* 2015; 1852(2): 310–318.
 - 27 Bian Y, Chen YG, Xu F, Xue L, Ji WQ, Zhang Y. The polymorphism in aldehyde dehydrogenase-2 gene is associated with elevated plasma levels of high-sensitivity C-reactive protein in the early phase of myocardial infarction. *Tohoku J Exp Med* 2010; 221(2): 107–112.
 - 28 Gomes KM, Bechara LR, Lima VM, Ribeiro MA, Campos JC, Dourado PM, Kowaltowski AJ, Mochly-Rosen D, Ferreira JC. Aldehydic load and aldehyde dehydrogenase 2 profile during the progression of post-myocardial infarction cardiomyopathy: benefits of Alda-1. *Int J Cardiol* 2015; 179: 129–138.
 - 29 Gomes KM, Campos JC, Bechara LR, Queliconi B, Lima VM, Disatnik MH, Magno P, Chen CH, Brum PC, Kowaltowski AJ, Mochly-Rosen D, Ferreira JC. Aldehyde dehydrogenase 2 activation in heart failure restores mitochondrial function and improves ventricular function and remodeling. *Cardiovasc Res* 2014; 103(4): 498–508.
 - 30 Wang HJ (王洪巨), Kang PF, Ye HW, Yu Y, Wang XM, Gao Q. Anti-apoptotic role of mitochondrial aldehyde dehydrogenase 2 in myocardial ischemia/reperfusion injury in diabetic rats. *J South Med Univ (南方医科大学学报)* 2012; 32(3): 345–348 (Chinese, English abstract).
 - 31 Zhang B, Zhang Y, La Cour KH, Richmond KL, Wang XM, Ren J. Mitochondrial aldehyde dehydrogenase obliterates endoplasmic reticulum stress-induced cardiac contractile dysfunction via correction of autophagy. *Biochim Biophys Acta* 2013; 1832(4): 574–584.
 - 32 Ferrari R, Ceconi C, Curello S, Cargnoni A, De Giuli F, Visioli O. Occurrence of oxidative stress during myocardial reperfusion. *Mol Cell Biochem* 1992; 111(1–2): 61–69.
 - 33 Harris DA, Das AM. Control of mitochondrial ATP synthesis in the heart. *Biochem J* 1991; 280(Pt 3): 561–573.
 - 34 Figueira TR, Barros MH, Camargo AA, Castilho RF, Ferreira JC, Kowaltowski AJ, Sluse FE, Souza-Pinto NC, Vercesi AE. Mitochondria as a source of reactive oxygen and nitrogen species: from molecular mechanisms to human health. *Antioxid Redox Signal* 2013; 18(16): 2029–2074.
 - 35 Anderson EJ, Katunga LA, Willis MS. Mitochondria as a source and target of lipid peroxidation products in healthy and diseased heart. *Clin Exp Pharmacol Physiol* 2012; 39(2): 179–193.
 - 36 Ceconi C, Cargnoni A, Pasini E, Condorelli E, Currello S, Ferrari R. Lipid peroxidation during myocardial reperfusion. *Mol Cell Biochem* 1992; 111(1–2): 49–54.
 - 37 Ayala A, Munoz MF, Arguelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev* 2014; 2014: 360438.
 - 38 Yadav UC, Ramana KV. Regulation of NF-kappaB-induced inflammatory signaling by lipid peroxidation-derived aldehydes. *Oxid Med Cell Longev* 2013; 2013: 690545.
 - 39 Mali VR, Palaniyandi SS. Regulation and therapeutic strategies of 4-hydroxy-2-nonenal metabolism in heart disease. *Free Radic Res* 2014; 48(3): 251–263.
 - 40 Brooks PJ, Zakhari S. Acetaldehyde and the genome: beyond nuclear DNA adducts and carcinogenesis. *Environ Mol Mutagen* 2014; 55(2): 77–91.
 - 41 Balbo S, Brooks PJ. Implications of acetaldehyde-derived DNA adducts for understanding alcohol-related carcinogenesis. *Adv Exp Med Biol* 2015; 815: 71–88.
 - 42 Maier CS, Chavez J, Wang J, Wu J. Protein adducts of aldehydic lipid peroxidation products identification and characterization of protein adducts using an aldehyde/keto-reactive probe in combination with mass spectrometry. *Methods Enzymol* 2010; 473: 305–330.

- 43 Irwin WA, Gaspers LD, Thomas JA. Inhibition of the mitochondrial permeability transition by aldehydes. *Biochem Biophys Res Commun* 2002; 291(2): 215–219.
- 44 Wang HJ, Kang PF, Wu WJ, Tang Y, Pan QQ, Ye HW, Tang B, Li ZH, Gao Q. Changes in cardiac mitochondrial aldehyde dehydrogenase 2 activity in relation to oxidative stress and inflammatory injury in diabetic rats. *Mol Med Rep* 2013; 8(2): 686–690.
- 45 Buja LM. Myocardial ischemia and reperfusion injury. *Cardiovasc Pathol* 2005; 14(4): 170–175.
- 46 Freude B, Masters TN, Robicsek F, Fokin A, Kostin S, Zimmermann R, Ullmann C, Lorenz-Meyer S, Schaper J. Apoptosis is initiated by myocardial ischemia and executed during reperfusion. *J Mol Cell Cardiol* 2000; 32(2): 197–208.
- 47 Lee P, Sata M, Lefer DJ, Factor SM, Walsh K, Kitsis RN. Fas pathway is a critical mediator of cardiac myocyte death and MI during ischemia-reperfusion *in vivo*. *Am J Physiol Heart Circ Physiol* 2003; 284(2): H456–H463.
- 48 Chen Z, Chua CC, Ho YS, Hamdy RC, Chua BH. Overexpression of Bcl-2 attenuates apoptosis and protects against myocardial I/R injury in transgenic mice. *Am J Physiol Heart Circ Physiol* 2001; 280(5): H2313–H2320.
- 49 Ebert AD, Kodo K, Liang P, Wu H, Huber BC, Riegler J, Churko J, Lee J, de Almeida P, Lan F, Diecke S, Burrigle PW, Gold JD, Mochly-Rosen D, Wu JC. Characterization of the molecular mechanisms underlying increased ischemic damage in the aldehyde dehydrogenase 2 genetic polymorphism using a human induced pluripotent stem cell model system. *Sci Transl Med* 2014; 6(255): 255ra130.
- 50 Zhao ZQ. Oxidative stress-elicited myocardial apoptosis during reperfusion. *Curr Opin Pharmacol* 2004; 4(2): 159–165.
- 51 Fan F, Sun A, Zhao H, Liu X, Zhang W, Jin X, Wang C, Ma X, Shen C, Zou Y, Hu K, Ge J. MicroRNA-34a promotes cardiomyocyte apoptosis post myocardial infarction through down-regulating aldehyde dehydrogenase 2. *Curr Pharm Des* 2013; 19(27): 4865–4873.
- 52 Zhang P, Xu D, Wang S, Fu H, Wang K, Zou Y, Sun A, Ge J. Inhibition of aldehyde dehydrogenase 2 activity enhances antimycin-induced rat cardiomyocytes apoptosis through activation of MAPK signaling pathway. *Biomed Pharmacother* 2011; 65(8): 590–593.
- 53 Favalaro B, Allocati N, Graziano V, Di Ilio C, De Laurenzi V. Role of apoptosis in disease. *Aging (Albany NY)* 2012; 4(5): 330–349.
- 54 Cosentino K, Garcia-Saez AJ. Mitochondrial alterations in apoptosis. *Chem Phys Lipids* 2014; 181: 62–75.
- 55 Vladimirov YA, Proskurnina EV, Alekseev AV. Molecular mechanisms of apoptosis. structure of cytochrome c-cardiolipin complex. *Biochemistry (Mosc)* 2013; 78(10): 1086–1097.
- 56 Kagan VE, Tyurina YY, Bayir H, Chu CT, Kapralov AA, Vlasova, II, Belikova NA, Tyurin VA, Amoscato A, Epperly M, Greenberger J, Dekosky S, Shvedova AA, Jiang J. The “pro-apoptotic genes” get out of mitochondria: oxidative lipidomics and redox activity of cytochrome c/cardiolipin complexes. *Chem Biol Interact* 2006; 163(1–2): 15–28.
- 57 Gustafsson AB, Gottlieb RA. Eat your heart out: Role of autophagy in myocardial ischemia/reperfusion. *Autophagy* 2008; 4(4): 416–421.
- 58 Singh KK, Yanagawa B, Quan A, Wang R, Garg A, Khan R, Pan Y, Wheatcroft MD, Lovren F, Teoh H, Verma S. Autophagy gene fingerprint in human ischemia and reperfusion. *J Thorac Cardiovasc Surg* 2014; 147(3): 1065–1072.
- 59 Yitzhaki S, Huang C, Liu W, Lee Y, Gustafsson AB, Mentzer RM Jr, Gottlieb RA. Autophagy is required for preconditioning by the adenosine A1 receptor-selective agonist CCPA. *Basic Res Cardiol* 2009; 104(2): 157–167.
- 60 Huang C, Yitzhaki S, Perry CN, Liu W, Giricz Z, Mentzer RM Jr, Gottlieb RA. Autophagy induced by ischemic preconditioning is essential for cardioprotection. *J Cardiovasc Transl Res* 2010; 3(4): 365–373.
- 61 Roe ND, Xu X, Kandadi MR, Hu N, Pang J, Weiser-Evans MC, Ren J. Targeted deletion of PTEN in cardiomyocytes renders cardiac contractile dysfunction through interruption of Pink1-AMPK signaling and autophagy. *Biochim Biophys Acta* 2015; 1852(2): 290–298.
- 62 Zhang Y, Han X, Hu N, Huff AF, Gao F, Ren J. Akt2 knockout alleviates prolonged caloric restriction-induced change in cardiac contractile function through regulation of autophagy. *J Mol Cell Cardiol* 2014; 71: 81–91.
- 63 Guo R, Zhang Y, Turdi S, Ren J. Adiponectin knockout accentuates high fat diet-induced obesity and cardiac dysfunction: role of autophagy. *Biochim Biophys Acta* 2013; 1832(8): 1136–1148.
- 64 Kandadi MR, Roe ND, Ren J. Autophagy inhibition rescues against leptin-induced cardiac contractile dysfunction. *Curr Pharm Des* 2014; 20(4): 675–683.
- 65 Wang Q, Yang L, Hua Y, Nair S, Xu X, Ren J. AMP-activated protein kinase deficiency rescues paraquat-induced cardiac contractile dysfunction through an autophagy-dependent mechanism. *Toxicol Sci* 2014; 142(1): 6–20.
- 66 Yang L, Hu N, Jiang S, Zou Y, Yang J, Xiong L, Ren J. Heavy metal scavenger metallothionein attenuates ER stress-induced myocardial contractile anomalies: role of autophagy. *Toxicol Lett* 2014; 225(3): 333–341.
- 67 Kandadi MR, Yu X, Frankel AE, Ren J. Cardiac-specific catalase overexpression rescues anthrax lethal toxin-induced cardiac contractile dysfunction: role of oxidative stress and autophagy. *BMC Med* 2012; 10: 134.

- 68 Sybers HD, Ingwall J, DeLuca M. Autophagy in cardiac myocytes. *Recent Adv Stud Cardiac Struct Metab* 1976; 12: 453–463.
- 69 Decker RS, Wildenthal K. Lysosomal alterations in hypoxic and reoxygenated hearts. I. Ultrastructural and cytochemical changes. *Am J Pathol* 1980; 98(2): 425–444.
- 70 Hamacher-Brady A, Brady NR, Logue SE, Sayen MR, Jinno M, Kirshenbaum LA, Gottlieb RA, Gustafsson AB. Response to myocardial ischemia/reperfusion injury involves Bnip3 and autophagy. *Cell Death Differ* 2007; 14(1): 146–157.
- 71 Dodson M, Darley-Usmar V, Zhang J. Cellular metabolic and autophagic pathways: traffic control by redox signaling. *Free Radic Biol Med* 2013; 63: 207–221.
- 72 Delbridge LM, Mellor KM, Taylor DJ, Gottlieb RA. Myocardial autophagic energy stress responses - macroautophagy, mitophagy and glycopagy. *Am J Physiol Heart Circ Physiol* 2015; 308(10): H1194–H1204.
- 73 Kubli DA, Gustafsson AB. Mitochondria and mitophagy: the yin and yang of cell death control. *Circ Res* 2012; 111(9): 1208–1221.
- 74 Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR. Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* 2000; 404(6779): 770–774.
- 75 Minamino T, Kitakaze M. ER stress in cardiovascular disease. *J Mol Cell Cardiol* 2010; 48(6): 1105–1110.
- 76 Wang Z, Wang Y, Ye J, Lu X, Cheng Y, Xiang L, Chen L, Feng W, Shi H, Yu X, Lin L, Zhang H, Xiao J, Li X. bFGF attenuates endoplasmic reticulum stress and mitochondrial injury on myocardial ischaemia/reperfusion via activation of PI3K/Akt/ERK1/2 pathway. *J Cell Mol Med* 2015; 19(3): 595–607.
- 77 Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 2007; 8(7): 519–529.
- 78 Li SY, Ren J. Cardiac overexpression of alcohol dehydrogenase exacerbates chronic ethanol ingestion-induced myocardial dysfunction and hypertrophy: role of insulin signaling and ER stress. *J Mol Cell Cardiol* 2008; 44(6): 992–1001.
- 79 Li SY, Gilbert SA, Li Q, Ren J. Aldehyde dehydrogenase-2 (ALDH2) ameliorates chronic alcohol ingestion-induced myocardial insulin resistance and endoplasmic reticulum stress. *J Mol Cell Cardiol* 2009; 47(2): 247–255.
- 80 Araki E, Oyadomari S, Mori M. Impact of endoplasmic reticulum stress pathway on pancreatic beta-cells and diabetes mellitus. *Exp Biol Med (Maywood)* 2003; 228(10): 1213–1217.
- 81 Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 2004; 306(5695): 457–461.
- 82 Nakatani Y, Kaneto H, Kawamori D, Yoshiuchi K, Hatazaki M, Matsuoka TA, Ozawa K, Ogawa S, Hori M, Yamasaki Y, Matsuhisa M. Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. *J Biol Chem* 2005; 280(1): 847–851.
- 83 Liao J, Sun A, Xie Y, Isse T, Kawamoto T, Zou Y, Ge J. Aldehyde dehydrogenase-2 deficiency aggravates cardiac dysfunction elicited by endoplasmic reticulum stress induction. *Mol Med* 2012; 18: 785–793.
- 84 Ding W, Yang L, Zhang M, Gu Y. Reactive oxygen species-mediated endoplasmic reticulum stress contributes to aldosterone-induced apoptosis in tubular epithelial cells. *Biochem Biophys Res Commun* 2012; 418(3): 451–456.
- 85 Li Z, Zhang T, Dai H, Liu G, Wang H, Sun Y, Zhang Y, Ge Z. Involvement of endoplasmic reticulum stress in myocardial apoptosis of streptozocin-induced diabetic rats. *J Clin Biochem Nutr* 2007; 41(1): 58–67.
- 86 Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature* 2000; 403(6765): 98–103.
- 87 Dolle L, Gao B. Pharmacological chaperone therapies: Can aldehyde dehydrogenase activator make us healthier? *J Hepatol* 2015; 62(6): 1228–1230.
- 88 Kalogeris T, Bao Y, Korhuis RJ. Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning. *Redox Biol* 2014; 2: 702–714.