FEBS Letters 586 (2012) 137–142

FEBS Letters

journal homepage: www.FEBSLetters.org

Acetylation-dependent regulation of mitochondrial ALDH2 activation by SIRT3 mediates acute ethanol-induced eNOS activation

Li Xue [a,b], Feng Xu [a,b], Lujing Meng [a,b], Shujian Wei [a,b], Jiali Wang [a,b], Panpan Hao [a,b], Yuan Bian [a,b], Yun Zhang [a], Yuguo Chen [a,b,*]

[a] Department of Emergency, Qilu Hospital, Shandong University, Jinan, Shandong, China
[b] Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Public Health, Jinan, Shandong, China

ARTICLE INFO

Article history:
Received 21 October 2011
Revised 27 November 2011
Accepted 28 November 2011
Available online 7 December 2011

Edited by Stuart Ferguson

Keywords:
Aldehyde dehydrogenase 2
Acetylation
Endothelial nitric-oxide synthase
Ethanol
Reactive oxygen species
SIRT3

ABSTRACT

Moderate alcohol consumption has beneficial effects on endothelial nitric-oxide synthase (eNOS) activation, which can engender an array of anti-atherogenic actions. Here we show that in human aortic endothelial cells (HAECs), rapid activation of mitochondrial aldehyde dehydrogenase 2 (ALDH2) mediates ethanol-induced eNOS activation by preventing reactive oxygen species (ROS) accumulation. Furthermore, activation of ALDH2 by ethanol is due to its hyperacetylation by SIRT3 inactivation. These data suggest that ethanol-induced eNOS activation in HAECs may be dependent on ALDH2 hyperacetylation by SIRT3 inactivation.

1. Introduction

Moderate alcohol consumption has been found to reduce the risk of cardiovascular morbidity [1,2], partially because of its favorable effects on endothelial nitric-oxide synthase (eNOS) activation [3,4], which in turn engenders an array of anti-atherogenic actions. However, there exists individual difference in response to these benefits of moderate drinking. Understanding the molecular mechanisms underlying ethanol-induced eNOS activation may be helpful for guiding the correct clinical use of ethanol.

Ethanol can rapidly activate phosphatidylinositol 3-kinase (PI3K)/Akt pathway to increase eNOS activation by an adenosine receptor-dependent mechanism in human umbilical vein endothelial cells (HUVECs) [4]. In addition, ethanol, as a small exogenous liposoluble molecule, can be metabolized by mitochondrial enzyme systems, accompanied by reactive oxygen species (ROS) generation. ROS have detrimental or beneficial effects on eNOS activation depending on its concentrations [5,6]. During ethanol exposure, ROS level is dependent on the capability of antioxidant systems to antagonize ROS generation.

Mitochondrial aldehyde dehydrogenase 2 (ALDH2), a key enzyme in ethanol metabolism, has been found to possess the antioxidant property to resist ethanol or aldehyde-induced ROS formation [7,8]. In vitro and in vivo studies show that regulation of ALDH2 activity can affect cellular response to oxidative stress [8,9]. ALDH2 activity can be affected by gene regulation [10] or post-translational modifications [11–13]. For example, ALDH2 can be directly deacetylated by SIRT3 leading to enzymatic inactivation of ALDH2 [13].

SIRT3, a NAD^+-dependent class III histone deacetylase, is localized in the mitochondrial matrix where it triggers adaptive responses to a variety of metabolic stresses by regulating mitochondrial protein acetylation levels [14]. SIRT3KO mice have hyperacetylated mitochondrial proteins [15]. In addition, SIRT3 enzymatic activity is positively sensitive to the NAD^+/NADH redox status [16]. Interestingly, ethanol metabolism convert NAD^+ to NADH leading to the reduced NAD^+/NADH ratio [17], which may have the potential to negatively affect SIRT3 activity.

In this study, we examined the hypothesis that acetylation modification of ALDH2 may be involved in ethanol-induced eNOS activation in human aortic endothelial cells (HAECs). We further investigated the role of SIRT3 in the potential mechanisms involved.

Abbreviations: ALDH2, aldehyde dehydrogenase 2; DTT, dithiothreitol; eNOS, endothelial nitric-oxide synthase; HAECs, human aortic endothelial cells; HUVECs, human umbilical vein endothelial cells; NAC, *N*-acetylcysteine; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; siRNA, small interference RNA

* Corresponding author. Address: Department of Emergency, Qilu Hospital, Shandong University, No. 44 Wenhuaxi Road, Jinan, Shandong 250012, China. Fax: +86 531 82169524.

E-mail address: chen919085@126.com (Y. Chen).

0014-5793/$36.00
doi:10.1016/j.febslet.2011.11.031

2. Materials and methods

2.1. Cell culture, treatment and transfection

See Supplementary material.

2.2. eNOS activity assay

Cellular eNOS activity was measured by the conversion of L-arginine to NO by use of a nitric-oxide synthase assay kit (Beyotime Institute of Biotechnology).

2.3. Mitochondrial ALDH2 and SIRT3 activity assay

The mitochondria were isolated, then the mitochondrial ALDH2 and SIRT3 enzymatic activity were determined as described in Supplementary material.

2.4. Western blot analysis

See Supplementary material.

2.5. ALDH2 acetylation by immunoprecipitation

The mitochondrial lysate was used for ALDH2 immunoprecipitation. And the acetylation of ALDH2 was detected as described in Supplementary material.

2.6. Assessment of intracellular ROS levels

ROS level was measured by staining cells with 2′,7′-dichlorodihydro-fluorescein diacetate (Sigma–Aldrich). Detailed protocol is explained in Supplementary material.

2.7. Mitochondrial NAD^+/NADH assay

Mitochondrial NAD^+/NADH ratio were quantified using an NAD^+/NADH assay kit according to the assay instructions (Abcam, San Francisco, CA).

2.8. Statistical analysis

The data are expressed as mean ± S.E. More than two groups were compared by one-way ANOVA followed by Student–Newman–Keuls post-hoc analysis. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Dose- and time-dependent effects of ethanol on eNOS activation

Ethanol treatment dose-dependently increased then decreased eNOS activity, with peak activity at 20 mM (1.67-fold) (Fig. 1A). The elevated eNOS activity was due to increased Akt (ser 1177) and eNOS (ser 473) phosphorylation by ethanol (Fig. 1B). Additionally, time-course study showed that eNOS activity increased began at 5 min after ethanol incubation and got the peak at 30 min, attributable to the upregulated Akt and eNOS phosphorylation (Fig. 1C and D). Moreover, activation of Akt and eNOS by ethanol was completely antagonized by PI3K inhibitors, wortmannin and LY 294002 (Fig. 1 E and F). Taken together, acute ethanol can stimulate PI3K/Akt pathway to induce eNOS activation.

3.2. Involvement of ALDH2 in the effects of ethanol on eNOS activation

Ethanol treatment (20 mM) time-dependently increased then decreased ALDH2 activity, with peak activity at 30 min (1.65-fold) (Fig. 2A). However, ethanol had barely any impact on ALDH2 protein expression (Fig. 2B) and mRNA levels (data not shown), indicating that activation of ALDH2 induced by ethanol may not be related to gene regulation.

Then we examined the influence of ALDH2 siRNA on eNOS activation. ALDH2 siRNA treatment could decrease ALDH2 expression by 80% and ALDH2 activity by 81.7%, whereas it had no effects on phosphorylation of Akt and eNOS (Supplementary Figs. B and C). However, ALDH2 siRNA inhibited ethanol-induced Akt/eNOS phosphorylation and eNOS activity (Fig. 2C and D). Thus, ALDH2 activation may have a mediating role in ethanol-induced eNOS activation.

3.3. ALDH2 mediated ethanol-induced Akt and eNOS activation by inhibiting ROS accumulation

Ethanol (20 mM) had no effect on ROS level (Fig. 3A). However, ROS level was markedly increased by approximately 66% in cells expressing ALDH2 siRNA after ethanol treatment (Fig. 3A and B). Administration of ROS scavengers, NAC and DTT, into cells expressing ALDH2 siRNA inhibited ethanol-induced ROS accumulation and increased Akt/eNOS phosphorylation and eNOS activity (Fig. 3C and D). Taken together, ethanol-induced ALDH2 activation may prevent ROS accumulation to ensure ethanol-induced Akt and eNOS activation.

3.4. ALDH2 acetylation by ethanol and its regulation by SIRT3

ALDH2 acetylation peaked at 30 min after ethanol treatment (2.2-fold) (Fig. 4A). Conversely to this alteration in ALDH2 acetylation, SIRT3 activity decreased with time, and got the minimum value at 30 min (Fig. 4B). However, ethanol had no effect on SIRT3 protein expression (data not shown). We then determined the effect of SIRT3 on ALDH2 acetylation by overexpressing SIRT3. As shown in Fig. 4C and D, SIRT3 overexpression reversed ethanol-inhibited SIRT3 activity and ethanol-elevated ALDH2 acetylation and activity.

3.5. The effects of SIRT3 overexpression on ROS level and Akt/eNOS activation

Then we detected whether SIRT3 overexpression had influence on the downstream signals of ALDH2 involved in ethanol-induced eNOS activation. ROS level increased significantly in cells overexpressing SIRT3 after ethanol treatment (Fig. 5A and B). Subsequently, SIRT3 overexpression reversed ethanol-induced Akt/eNOS phosphorylation and eNOS activity (Fig. 5C and D).

3.6. The decrease in NAD^+/NADH mediated ethanol-induced SIRT3 inactivation

Mitochondrial NAD^+/NADH ratio decreased by 65% at 30 min after ethanol treatment (Fig. 6A). Moreover, NAD administration dose-dependently reversed the inhibitory effect of ethanol on SIRT3 activity (Fig. 6B). As well, NAD treatment reversed ethanol-induced ALDH2, Akt and eNOS activation (Fig. 6B–D).

4. Discussion

The findings of this study demonstrated that rapid activation of ALDH2 involved in ethanol-induced eNOS activation by preventing ROS accumulation in cultured HAECs. Moreover, ethanol-induced ALDH2 activation was dependent on its acetylation modification by SIRT3 inactivation.

Previous studies suggest that low dose of ethanol can induce eNOS activation [3–5], which contributes to ethanol protective effects on the cardiovascular system. Our results confirmed these

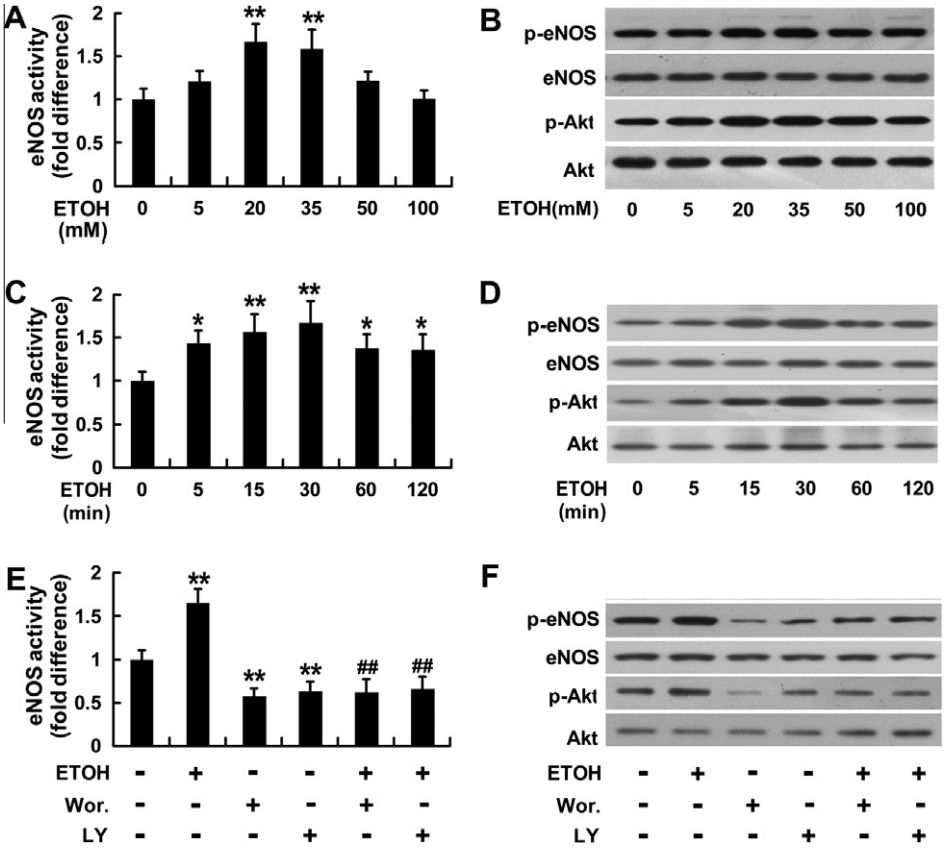

Fig. 1. Ethanol treatment modulated eNOS activation in human aortic endothelial cells (HAECs). (A and B) Dose-dependent activation of eNOS: (A) eNOS activity, (B) immunoblot analysis of eNOS and Akt phosphorylation. (C and D) Time-dependent activation of eNOS and Akt. (E and F) PI3K mediated ethanol-induced Akt and eNOS activation. PI3K inhibitors: Wortmannin (Wor.) and LY294002 (LY). Data represent the mean ± S.E. from three separate experiments. $^{*}P < 0.05$ vs. control, $^{**}P < 0.001$ vs. control, $^{\#\#}P < 0.001$ vs. ethanol group.

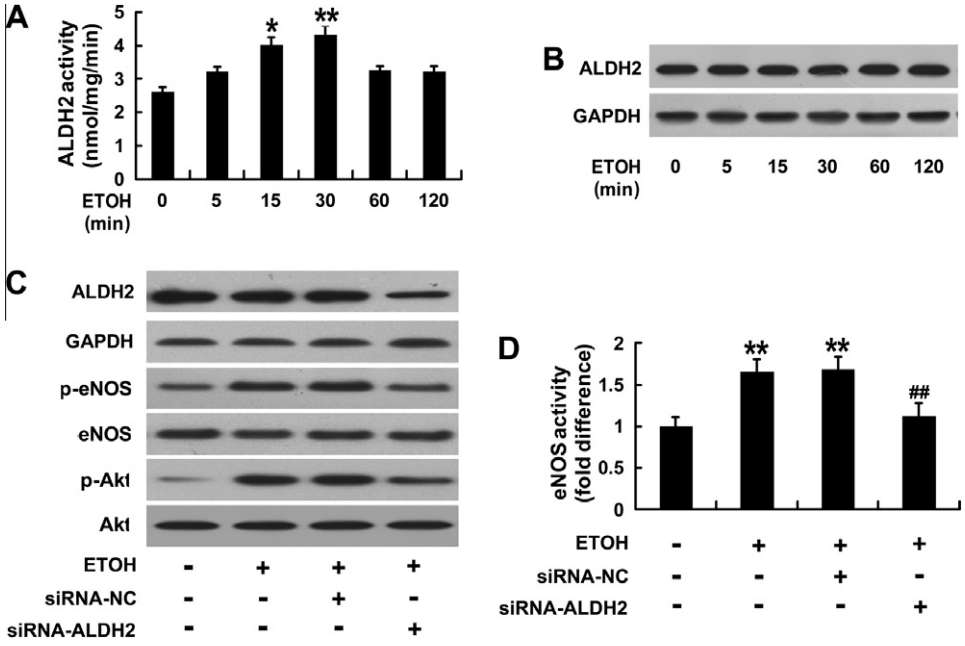

Fig. 2. Involvement of ALDH2 in ethanol-induced eNOS activation. (A and B) Time-dependent alterations in ALDH2 activation: (A) ALDH2 activity, (B) Western blot analysis of ALDH2 expression. (C) Immunoblot analysis of ALDH2 siRNA on ethanol-induced Akt and eNOS phosphorylation. (D) Effects of ALDH2 siRNA on ethanol-upregulated ALDH2 and eNOS activity. Data are the mean ± S.E. from three separate experiments. $^{*}P < 0.05$ vs. control, $^{**}P < 0.001$ vs. control, $^{\#\#}P < 0.001$ vs. ethanol group.

findings that ethanol treatment (20–35 mM) could elicit rapid eNOS phosphorylation and activation (<5 min) by activating PI3K/Akt pathway in HAECs. Acute ethanol may stimulate PI3K/Akt signaling by an adenosine receptor-dependent mechanism

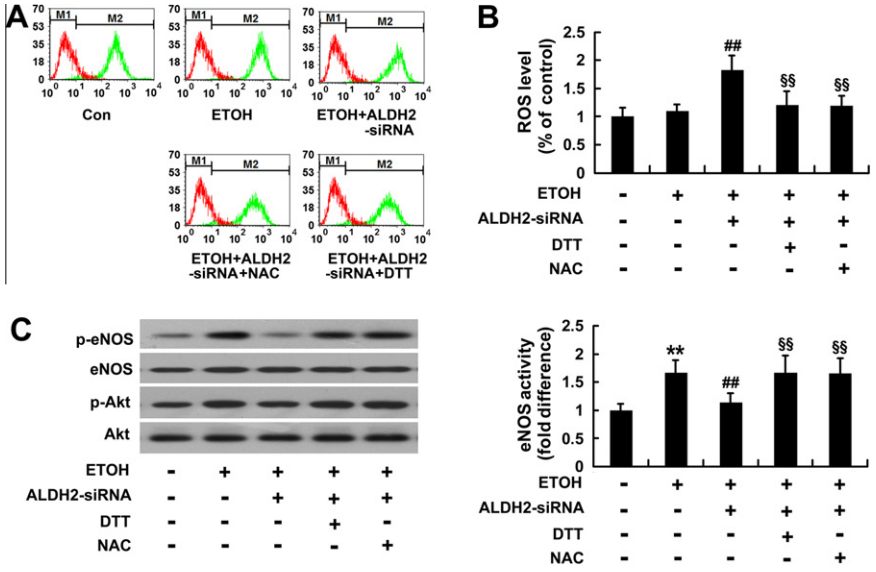

Fig. 3. Activated ALDH2 mediated ethanol-induced Akt-eNOS activation by inhibiting reactive oxygen species (ROS) accumulation. Cells expressing ALDH2 siRNA were incubated with ROS scavenger *N*-acetylcysteine (NAC) or dithiothreitol (DTT), before ethanol treatment. (A and B) ROS levels. (C) Immunoblot analysis of phospho-Akt and phospho-eNOS. (D) eNOS activity. Data are the mean ± S.E. from three separate experiments. $^{**}P < 0.001$ vs. control, $^{\#\#}P < 0.001$ vs. ethanol group, $^{\S\S}P < 0.001$ vs. ALDH2 siRNA + ethanol group.

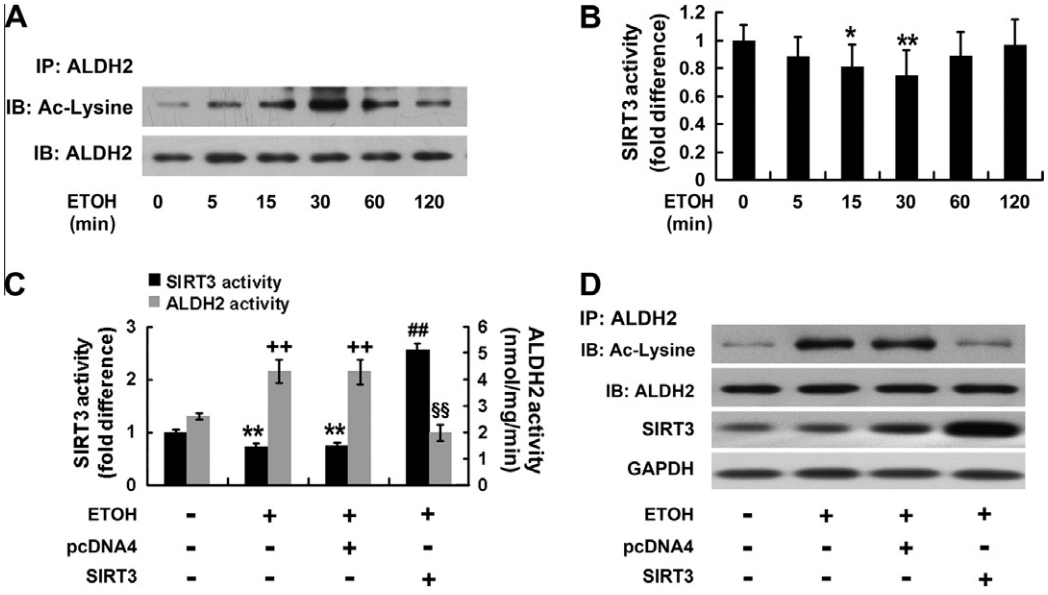

Fig. 4. Effect of ethanol on ALDH2 acetylation and its regulation by SIRT3 in HAECs. (A) Time-dependent acetylation of ALDH2. (B) Time-dependent inactivation of SIRT3. (C) The effect of SIRT3 overexpression on ethanol-upregulated SIRT3 and ALDH2 activity. (D) The effect of SIRT3 overexpression on ethanol-induced ALDH2 acetylation. Data are the mean ± S.E. from three separate experiments. SIRT3 activity: $^{*}P < 0.05$ vs. control, $^{**}P < 0.001$ vs. control, $^{\#\#}P < 0.001$ vs. pcDNA4 + ethanol group. ALDH2 activity: $^{++}P < 0.001$ vs. control, $^{\S\S}P < 0.001$ vs. pcDNA4 + ethanol group.

[5]. However, high-dose ethanol (50–100 mM) had little influence on eNOS activation in HAECs, as previously reported in HUVECs [5].

In addition to activation of PI3K/Akt signaling, ethanol metabolism can induce ROS generation, which may counteract or provoke eNOS activation. However, our findings showed that 20 mM ethanol had no effect on ROS levels, consistently with previous observation [5]. ROS accumulation occurs when generation exceeds the capacity of local antioxidants. In our study, low-dose ethanol could elicit rapid activation of ALDH2, an important antioxidant mitochondrial protein due to its detoxification of toxic aldehydes [18,19]. Moreover, the cells expressing ALDH2 siRNA showed increased ROS levels after ethanol treatment. These results suggest that low-dose ethanol can provoke ROS generation and activate the antioxidant ALDH2 at the same time to maintain the balance of redox state. Furthermore, ROS accumulation reversed ethanol-induce Akt and eNOS phosphorylation, which could be blocked by ROS scavengers, indicating that ROS may depress eNOS activity by inhibiting Akt phosphorylation. Taken together, ALDH2 activation may mediate ethanol-induced eNOS activation by preventing ROS accumulation.

A key finding of this study is the significant activation of ALDH2 by ethanol related to post-translational modification. Our results showed that ALDH2 activation is positively related to its acetylation level, consistently with a previous study [13]. ALDH2 has been reported to be a substrate of SIRT3 [13]. Indeed, our study found that ALDH2 could be reversibly acetylated by SIRT3 inactivation.

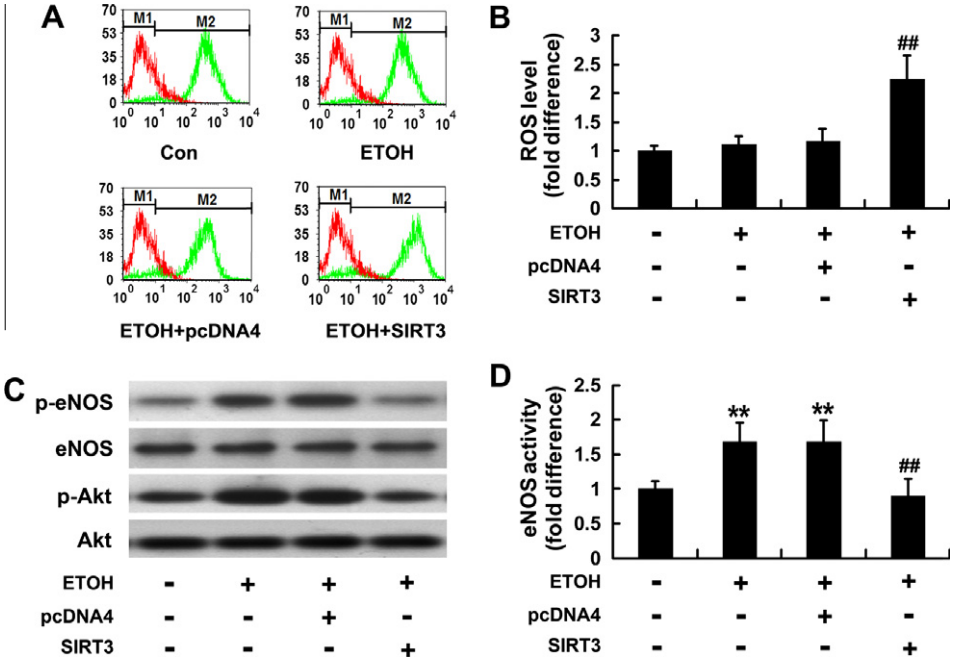

Fig. 5. Effects of SIRT3 overexpression on ROS level and Akt/eNOS activation in HAECs treated with ethanol. (A and B) ROS levels. (C) Immunoblot analysis of phospho-Akt and phospho-eNOS. (D) eNOS activity. Data are the mean ± SE from three separate experiments. $^{**}P < 0.001$ vs. control, $^{\#\#}P < 0.001$ vs. pcDNA4 + ethanol group.

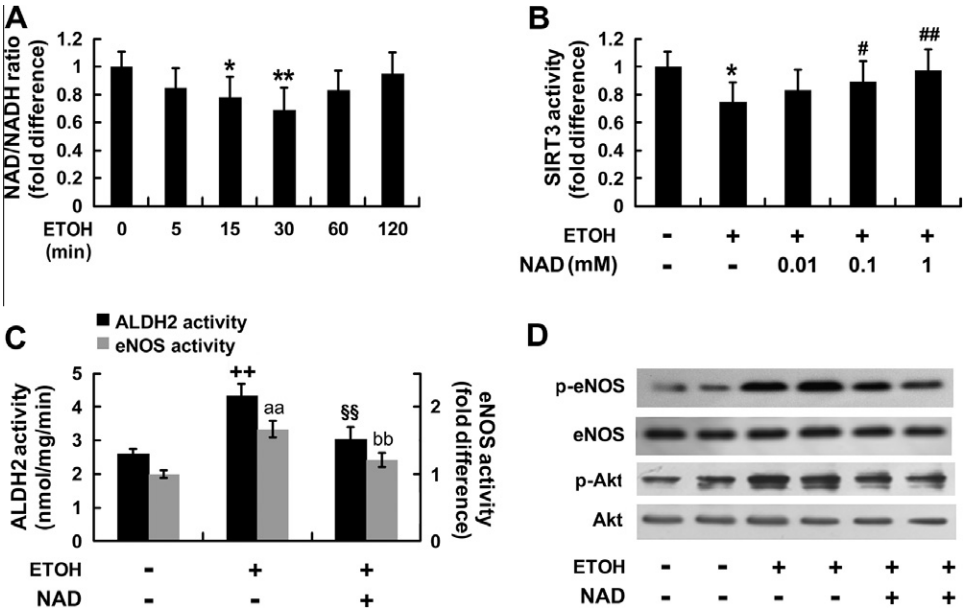

Fig. 6. The decrease in NAD^+/NADH mediated ethanol-induced SIRT3 inactivation. (A) Time-dependent shift in mitochondrial NAD^+/NADH ratio. HAECs were treated with NAD (0.01 mM, 0.1 mM and 1 mM) before ethanol incubation, then (B) SIRT3 activity, (C) ALDH2 and eNOS activity, (D) Akt and eNOS phosphorylation were detected. Data are the mean ± S.E. from three separate experiments. SIRT3 activity: $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. ethanol group, $^{\#\#}P < 0.001$ vs. ethanol group. ALDH2 activity: $^{++}P < 0.001$ vs. control, $^{\S\S}P < 0.001$ vs. ethanol group. eNOS activity: $^{aa}P < 0.001$ vs. control, $^{bb}P < 0.001$ vs. ethanol group.

These findings indicate that under acute ethanol exposure, inactivation of SIRT3 may have antioxidant effect through upregulating ALDH2 activity. However, other report shows that SIRT3 knockout can increase superoxide levels by inhibiting the acetylation and activation of manganese superoxide dismutase (MnSOD) in mice under ionizing radiation [20]. A possible explanation for this discrepancy could be that SIRT3 inactivation may have anti-oxidant and pro-oxidant effects through regulating different antioxidant enzymes acetylation under various stressors.

SIRT3 is a NAD^+-dependent deacetylase and its enzymatic activity is sensitive to NAD^+/NADH ratio. In this study, ethanol rapidly decreased the mitochondrial NAD^+/NADH ratio and then increased it to basal level. In ethanol metabolism, the key metabolic enzymes convert NAD^+ to NADH leading to the reduced NAD^+/NADH ratio [17], which may be the reason for this NAD^+/NADH fluctuation. Moreover, exogenous NAD administration could reverse ethanol-induced SIRT3 inactivation, verifying the positive association of SIRT3 with the NAD^+/NADH ratio as previously reported [16]. As

well, NAD reversed ethanol-induced ALDH2 and eNOS activation, the downstream signals of SIRT3. This dependence of ethanol effects on NAD^+/NADH ratio and the variant metabolic state in different person, may partially explain the individual discrepancy in response to moderate drinking.

In summary, our findings suggest that low-dose ethanol-induced decrease of NAD^+/NADH ratio may result in SIRT3 inactivation, leading to hyperacetylation and activation of ALDH2. ALDH2 activation can prevent ROS accumulation to maintain the balance of redox state to assure ethanol-induced Akt and eNOS activation. In addition, rapid activation of ALDH2 may also accelerate the clearance of ethanol, forming a positive feedback. Furthermore, we can speculate that activation of ALDH2 may be a potential therapeutic strategy for canceling out the individual difference in responding to moderate drinking.

Acknowledgments

We thank Prof. Zhang Ming-xiang (Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Misnistry of Public Health) for supplying HAECs. This study was supported by the National 973 Basic Research Program of China (2010CB732605 and 2009CB521900), and grants from Department of Science and Technology of Shandong Province (Y2007C075, ZR2010HQ017 and 2008RKB060), and Shandong Provincial Outstanding Medical Academic Professional Program, and 1020 Program (Excellent Medical Subject Leaders), a key program from Health Department of Shandong Province (2009HD011) and a program from Shandong University (2010TS082).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.11.031.

References

[1] Di Castelnuovo, A., Rotondo, S., Iacoviello, L., Donati, M.B. and De Gaetano, G. (2002) Meta-analysis of wine and beer consumption in relation to vascular risk. Circulation 105, 2836–2844.
[2] Mukamal, K.J., Conigrave, K.M., Mittleman, M.A., Camargo Jr., C.A., Stampfer, M.J., Willett, W.C. and Rimm, E.B. (2003) Roles of drinking pattern and type of 0 alcohol consumed in coronary heart disease in men. N. Engl. J. Med. 348, 108–118.
[3] Venkov, C.D., Myers, P.R., Tanner, M.A., Su, M. and Vaughan, D.E. (1999) Ethanol increases endothelial nitric oxide production through modulation of nitric oxide synthase expression. Thromb. Haemost. 81, 638–642.
[4] Liu, J., Tian, Z.G., Gao, B. and Kunos, G. (2002) Dose-dependent activation of antiapoptotic and proapoptotic pathways ethanol treatment in human vascular endothelial cells. J. Biol. Chem. 277, 20927–20933.
[5] Victor, V.M., Rocha, M., Sola, E., Banuls, C., Garcia-Malpartida, K. and Hernandez-Mijares, A. (2009) Oxidative stress, endothelial dysfunction and atherosclerosis. Curr. Pharm. Des. 15, 2988–3002.
[6] Lopez-Ongil, S., Hernandez-Perera, O., Navarro-Antolin, J., Perez de Lema, G., Rodriguez-Puyol, M., Lamas, S. and Rodriguez-Puyol, D. (1998) Role of reactive oxygen species in the signalling cascade of cyclosporine A-mediated up-regulation of eNOS in vascular endothelial cells. Br. J. Pharmacol. 124, 447–454.
[7] Ma, H., Yu, L., Byra, E.A., Hu, N., Kitagawa, K., Nakayama, K.I., Kawamoto, T. and Ren, J. (2010) Aldehyde dehydrogenase 2 knockout accentuates ethanol-induced cardiac depression: role of protein phosphatases. J. Mol. Cell. Cardiol. 49, 322–329.
[8] Li, S.Y., Gomelsky, M., Duan, J.H., Zhang, Z.J., Gomelsky, L., Zhang, X.C., Epstein, P.N. and Ren, J. (2004) Overexpression of aldehyde dehydrogenase-2 (ALDH2) transgene prevents acetaldehyde-induced cell injury in human umbilical vein endothelial cells. J. Biol. Chem. 279, 11244–11252.
[9] Wenzel, P., Schuhmacher, S., Kienhofer, J., Müller, J., Hortmann, M., Oelze, M., Schulz, E., Treiber, N., Kawamoto, T., Scharffetter-Kochanek, K., et al. (2008) Manganese superoxide dismutase and aldehyde dehydrogenase deficiency increase mitochondrial oxidative stress and aggravate age-dependent vascular dysfunction. Cardiovasc. Res. 80, 280–289.
[10] Kimura, Y., Nishimura, F.T., Abe, S., Fukunaga, T., Tanii, H. and Saijoh, K. (2009) A promote polymorphism in the ALDH2 gene affects its basal and acetaldehyde/ethanol-induced gene expression in human peripheral blood leukocytes and HepG2 Cells. Alcohol Alcohol. 44, 261–266.
[11] Chen, C.H., Budas, G.R., Churchill, E.N., Disatnik, M.H., Hurley, T.D. and Mochly-Rosen, D. (2008) Activation of aldehyde dehydrogenase-2 reduces ischemic damage to the heart. Science 321, 1493–1495.
[12] Choi, J.W., Kim, J.H., Cho, S.C., Ha, M.K., Song, K.Y., Youn, H.D. and Park, S.C. (2011) Malondialdehyde inhibits an AMPK-mediated nuclear translocation and repression activity of ALDH2 in transcription. Biochem. Biophys. Res. Commun. 404, 400–406.
[13] Lu, Z., Bourdi, M., Aponte, A.M., Chen, Y., Lombard, D.B., Gucek, M., Pohl, L.R. and Sack, M.N. (2011) SIRT3-dependent deacetylation exacebates acetaminophen hepatotoxicity. EMBO Rep. 12, 840–846.
[14] Ahn, B.H., Kim, H.S., Song, S., Lee, I.H., Liu, j., Vassilopoulos, A., Deng, C.X. and Finkel, T. (2008) A role for the mitochondrial deacetylase SIRT3 in regulating energy homeostasis. Proc. Natl. Acad. Sci. 105, 14447–14452.
[15] Lombard, D.B., Alt, F.W., Cheng, H.L., Bunkenborg, J., Streeper, R.S., Mostoslavsky, R., Kim, J., Yancopoulos, G., Valenzuela, D., Murphy, A., et al. (2007) Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. Mol. Cell. Biol. 27, 8807–8814.
[16] Yang, H., Yang, T., Baur, J.A., Perez, E., Matsui, T., Carmona, J.J., Lamming, D.W., Souza-Pinto, N.C., Bohr, V.A., Rosenzweig, A., de Cabo, R., Sauve, A.A. and Sinclair, D.A. (2007) Nutrient-sensitive mitochondrial NAD+ levels dictate cell survival. Cell 130, 1095–1107.
[17] Mantle, D. and Preedy, V.R. (1999) Free radicals as mediators of alcohol toxicity. Adverse Drug React. Toxicol. Rev. 18, 235–252.
[18] Ohta, S., Ohsawa, I., Kamino, K., Ando, F. and Shimokata, H. (2006) Mitochondrial ALDH2 deficiency as an oxidative stress. Ann. NY Acad. Sci. 1011, 36–44.
[19] Szocs, K., Lassegue, B., Wenzel, P., Wendt, M., Daiber, A., Oelze, M., Meinertz, T., Munzel, T. and Baldus, S. (2007) Increased superoxide production in nitrate tolerance is associated with NAD(P)H oxidase and aldehyde dehydrogenase downregulation. J. Mol. Cell. Cardiol. 42, 1111–1118.
[20] Tao, R., Coleman, M.C., Pennington, J.D., Ozden, O., Park, S.H., Jiang, H., Kim, H.S., Flynn, C.R., Hill, S., Hayes Mcdonald, W., et al. (2010) Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. Mol. Cell. 40, 893–904.