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Atorvastatin inhibits miR-143 expression: A protective mechanism against oxidative stress in cardiomyocytes

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Statins are unequivocally the most widely prescribed drug for the primary and secondary prevention of coronary artery disease worldwide [1]. Atorvastatin is the most commonly prescribed statin drug due to its lower therapeutic dose compared to others. The well-tolerated and high benefit–risk ration of atorvastatin has been demonstrated in clinical trials [2]. Atorvastatin reduces the risk of myocardial infarction, stroke, and death, primarily inhibiting reactive oxygen species (ROS) [3].

The epigenetic mechanisms postulated to be responsible for these changes included DNA methylation and histone modification [4]. To date, there is limited evidence available on the effects of statins on a third, recently described arm of epigenetics: microRNA (miRNA) expression. It has been established that miRNAs coordinate cell death by directly affecting the transcriptome, modulate multiple target transcripts and thus heavily influence gene expression patterns [5–7]. Recent studies found that miR-143 may be key to controlling cell death via repression of multiple anti-apoptosis targets, such as Bcl-2 and Fas [8–10]. In a separate study miR-143 was found to influence phenotypic modulation of vascular smooth muscle cells regulated by cardiac transcriptional factor, SRF (serum response factor) and Nkx2–5 (cardiac NK-2 transcription factor) [11].

A growing number of evidence has demonstrated the existence and functional role of FoxO1 in the myocardium. FoxO1 is the main target of insulin signaling and regulates metabolic homeostasis in response to oxidative stress [12,13]. While the previous efforts focused on FoxO-dependent regulation of protein-coding genes, non-coding RNA genes have emerged as equally important targets of many transcription factors. Very recently, a research group supported this view by showing that knock-down FoxO1 using siRNA triggered a significant increase in miR-143 level [14]. However, the effect of statins on FoxO1/miR-143 axis in the heart has not been investigated to date. Limited data is available on the effects of atorvastatin on miRNAs involved in the regulation of specific biological pathways.

The use of animals was in accordance with the regulations of the ethic committees of Harbin Medical University. All experimental procedures were confirmed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Firstly, we established oxidative stress injury model in vitro by treating hydrogen peroxide (H_2O_2) in neonatal rat ventricular myocytes (NRVMs). The cardiomyocytes were isolated and treated with H_2O_2 (100 μM) for 4 h as previously described. MTT analysis showed that cell viability was significantly decreased in the H_2O_2 treated group (Fig. 1A). Interestingly, the effect of H_2O_2 was alleviated upon pre-treatment with atorvastatin (10 μM) for 0.5 h. Moreover, pre-treatment with atorvastatin also increased FoxO1 mRNA level compared with H_2O_2 alone treated group (Fig. 1B). In contrast, miR-143 expression was significantly lower in atorvastatin/H_2O_2 co-treated group than that in H_2O_2 alone treated group (Fig. 1C). These results suggest that atorvastatin protects cardiomyocytes from oxidative stress and may involve the regulation of FoxO1/miR-143 axis.

In line with previous studies [15], we confirmed that miR-143 expression was up-regulated during oxidative stress stimuli both in vitro (Fig. 2A) and in vivo (Fig. 2B). The result indicates that miR-143 is the key regulator of core apoptosis pathways in the heart. Our strategy of inhibited miR-143 expression using anti-microRNA oligonucleotides (AMO) resulted in significantly lower miR-143 level as compared with either H_2O_2 treated group or H_2O_2/negative control co-treated group (Fig. 3A). Down-regulation of miR-143 enhanced cell viability after H_2O_2 treated (Fig. 3B). Importantly, inhibition of endogenous miR-143 rescued cardiomyocytes from oxidative stress induced apoptosis (Fig. 3C,D). A plausible mechanism of atorvastatin protected against oxidative stress in cardiomyocytes is presented. So we proposed that atorvastatin promoted FoxO1 and then triggered a decreased in miR-143 expression, which alleviated miR-143-dependent apoptosis. It should be noted that there is no evidence to prove how atorvastatin directly or indirectly regulates FoxO1, which is interesting to research in future

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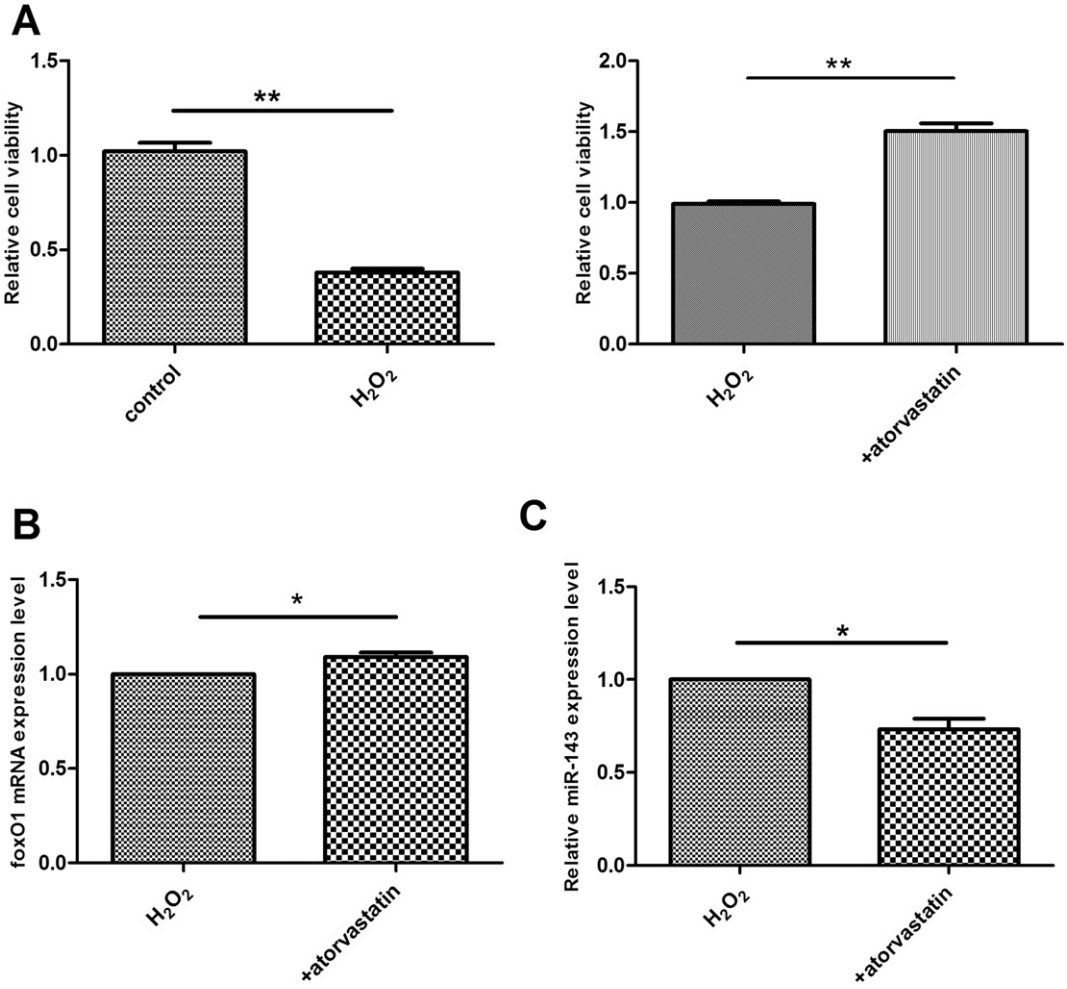

Fig. 1. Atorvastatin restored cell viability and regulated FoxO1 and miR-143 expression levels in H_2O_2-induced cardiomyocytes. (A) Cell viability was significantly increased in atorvastatin/H_2O_2 co-treated group (n = 4). (B) The level of FoxO1 mRNA expression was significantly increased in atorvastatin/H_2O_2 co-treated group (n = 4). (C) The level of miR-143 expression was significantly lower in atorvastatin/H_2O_2 co-treated group (n = 4). The data were represented as means ± SEM. *$p < 0.05$; **$p < 0.01$.

study. In summary, this is the first study to demonstrate a potential mechanism by which atorvastatin decreases miR-143 level which was regulated by FoxO1. The down-regulation of miR-143 level in cardiovascular disease patients on statins may thus be a compensatory mechanism to protect the heart.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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References

[1] R.S. Eidelman, G.A. Lamas, C.H. Hennekens, The new National Cholesterol Education Program guidelines: clinical challenges for more widespread therapy of lipids to treat and prevent coronary heart disease, Arch. Intern. Med. 162 (2002) 2033–2036.
[2] P.S. Sever, B. Dahlof, N.R. Poulter, H. Wedel, G. Beevers, M. Caulfield, et al., Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial—Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial, Lancet 361 (2003) 1149–1158.
[3] P. Amarenco, J. Bogousslavsky, A. Callahan III, L.B. Goldstein, M. Hennerici, A.E. Rudolph, et al., High-dose atorvastatin after stroke or transient ischemic attack, N. Engl. J. Med. 355 (2006) 549–559.
[4] A.B. Csoka, M. Szyf, Epigenetic side-effects of common pharmaceuticals: a potential new field in medicine and pharmacology, Med. Hypotheses 73 (2009) 770–780.
[5] H. Shan, X. Li, Z. Pan, L. Zhang, B. Cai, Y. Zhang, et al., Tanshinone IIA protects against sudden cardiac death induced by lethal arrhythmias via repression of microRNA-1, Br. J. Pharmacol. 158 (2009) 1227–1235.
[6] K.D. Taganov, M.P. Boldin, K.J. Chang, D. Baltimore, NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 12481–12486.
[7] T. Thum, C. Gross, J. Fiedler, T. Fischer, S. Kissler, M. Bussen, et al., MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts, Nature 456 (2008) 980–984.
[8] Y. Nakagawa, M. Iinuma, T. Naoe, Y. Nozawa, Y. Akao, Characterized mechanism of alpha-mangostin-induced cell death: caspase-independent apoptosis with release of endonuclease-G from mitochondria and increased miR-143 expression in human colorectal cancer DLD-1 cells, Bioorg. Med. Chem. 15 (2007) 5620–5628.
[9] H. Zhang, X. Cai, Y. Wang, H. Tang, D. Tong, F. Ji, microRNA-143, down-regulated in osteosarcoma, promotes apoptosis and suppresses tumorigenicity by targeting Bcl-2, Oncol. Rep. 24 (2010) 1363–1369.
[10] Y. Akao, Y. Nakagawa, A. Iio, T. Naoe, Role of microRNA-143 in Fas-mediated apoptosis in human T-cell leukemia Jurkat cells, Leuk. Res. 33 (2009) 1530–1538.
[11] M. Xin, E.M. Small, L.B. Sutherland, X. Qi, J. McAnally, C.F. Plato, et al., MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury, Genes Dev. 23 (2009) 2166–2178.
[12] X.C. Dong, K.D. Copps, S. Guo, Y. Li, R. Kollipara, R.A. DePinho, et al., Inactivation of hepatic Foxo1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation, Cell Metab. 8 (2008) 65–76.