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Inhibition of ANXA7 GTPase activity by a small molecule promotes HMBOX1 translation of vascular endothelial cells in vitro and in vivo

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ABSTRACT

Homeobox containing 1 (HMBOX1) is essential for the survival of human umbilical vein endothelial cells (HUVECs). However, the regulatory mechanism of HMBOX1 expression is still unclear. We recently found that a small molecule 6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine (ABO) directly targeted annexin A7 (ANXA7) and inhibited its GTPase activity. In addition, both HMBOX1 and ANXA7 participated in the autophagy and apoptosis of HUVECs. But, their relationship in the regulation of HMBOX1 expression is unknown. In this study, we found that ABO could elevate HMBOX1 at translation level through inhibiting ANXA7 GTPase activity. ABO failed to increase HMBOX1 protein level in ANXA7-deficient HUVECs. TGFB2 overlapping transcript 1 (*TGFB2-OT1*) that was increased by ABO facilitated HMBOX1 expression by increasing La-related protein 1 (LARP1) expression. Furthermore, the protein level of HMBOX1 was decreased under oxidized low-density lipoprotein (oxLDL) treatment in HUVECs and in the aortic endothelium of apolipoprotein E-deficient (apoE$^{-/-}$) mice, which could be reversed by ABO in vitro and in vivo. In conclusion, ANXA7 was an endogenous regulator of HMBOX1, and ABO promoted HMBOX1 translation by inhibiting ANXA7 GTPase activity and enhancing *TGFB2-OT1* expression. Besides, our data suggested that HMBOX1 might be a novel diagnostic marker and therapeutic target of atherosclerosis.

1. Introduction

Homeobox containing 1 (HMBOX1) is first identified and isolated from human pancreatic cDNA library as a transcription repressor (Chen et al., 2006). Until now, only a few reports focus on the functions of HMBOX1 in vitro. In natural killer cells, HMBOX1 is a negative regulator of cell function via suppressing the NKG2D/DAP10 signaling pathway (Wu et al., 2011a). Dennis Kappei et al. report that HMBOX1 could support telomerase-dependent telomere elongation as a direct telomere repeat binding protein (Kappei et al., 2013). In addition, HMBOX1 is found to participate in telomere maintenance in alternative lengthening of telomeres (ALT) cancer cells (Feng et al., 2013). A recent study about exercise-induced cardiac growth and pathological cardiac remodeling provides ample evidences of the link between HMBOX1 and miRNA-222 in cardiomyocyte (Liu et al., 2015).

Our previous studies showed that HMBOX1 was essential for rat bone marrow mesenchymal stem cells (BMSCs) and mouse embryonic stem cells (ESCs) differentiation into endothelial cells (ECs) induced by a small molecule 6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine (ABO). ABO induced ECs formation through elevating HMBOX1 level in BMSCs and ESCs, which could be blocked by knockdown of HMBOX1 (Han et al., 2012; Su et al., 2010). Furthermore, HMBOX1 was indispensable for the survival of human umbilical vein endothelial cells (HUVECs). Knockdown of HMBOX1 induced apoptosis and inhibited autophagy in HUVECs, leading to apoptotic cell death (Ma et al., 2015). However, the regulatory mechanism of HMBOX1 expression in HUVECs is still unknown. Besides, considering the vital role of HMBOX1 in HUVECs, we speculate that HMBOX1 is also important for the maintenance of endothelial function in vivo.

ANXA7 is a member of annexin family of calcium-dependent phospholipid binding proteins, possessing Ca^{2+}-dependent GTPase activity(Caohuy and Pollard, 2002). ANXA7 knockout mouse (−/−)

Abbreviations: HMBOX1, homeobox containing 1; HUVECs, human umbilical vein endothelial cells; ANXA7, annexin A7; TIA1, T cell intracellular antigen-1; ABO, 6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine; LARP1, La-related protein 1; oxLDL, oxidized low-density lipoprotein; *TGFB2-OT1*, TGFB2 overlapping transcript 1; ApoE$^{-/-}$ mice, apolipoprotein E-deficient mice.

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is lethal by E10 (Herr et al., 2001) and heterozygous ANXA7 (+/−) mutation mouse also has several unsatisfactory phenotypes, such as β-cell hypertrophy, islet hyperplasia, an alteration in the Ca^{2+} dependence of glucose-induced insulin secretion, and aberrant regulation of islet gene expression by the fed/fasted state(Mears et al., 2012). We previously found that ABO could directly target annexin A7 (ANXA7) and inhibit threonine phosphorylation along with GTPase activity of ANXA7 (Huang et al., 2014; Li et al., 2013b). In addition, ANXA7 was also involved in ABO-induced autophagy and inhibited apoptosis in cellular level or in aortic endothelium of apolipoprotein E-deficient ($apoE^{-/-}$) mice (Li et al., 2013a; Wang et al., 2010). These results showed that ABO, a small molecular regulator of ANXA7, could be a useful tool to investigate the complex cellular functions of ANXA7. As mentioned above, HMBOX1 also participated in HUVEC autophagy and apoptosis, suggesting that there may be an interaction between ANXA7 and HMBOX1.

In this study, we determined the relationship between ANXA7 and HMBOX1 in HUVECs, and ascertained HMBOX1 level in the aortic endothelium of $apoE^{-/-}$ mice using ABO. The results showed that ANXA7 was an endogenous regulator of HMBOX1, and ABO increased the expression of HMBOX1 by inhibiting ANXA7 GTPase activity and promoting *TGFB2-OT1* expression. Moreover, ABO could reverse the decreased HMBOX1 level in the aortic endothelium of $apoE^{-/-}$ mice, indicating that HMBOX1 participated in the development of atherosclerosis and might be a novel therapeutic target of atherosclerosis.

2. Materials and methods

2.1. Antibodies and reagents

Antibodies for HMBOX1 (sc-87768), CD31/PECAM-1 (sc-1506), GAPDH (sc-47724), and horseradish peroxidase-conjugated secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). ANXA7 (SAB1405462), β-actin (A5441), and cycloheximide (01810) all purchased from Sigma-Aldrich (USA), antibody for

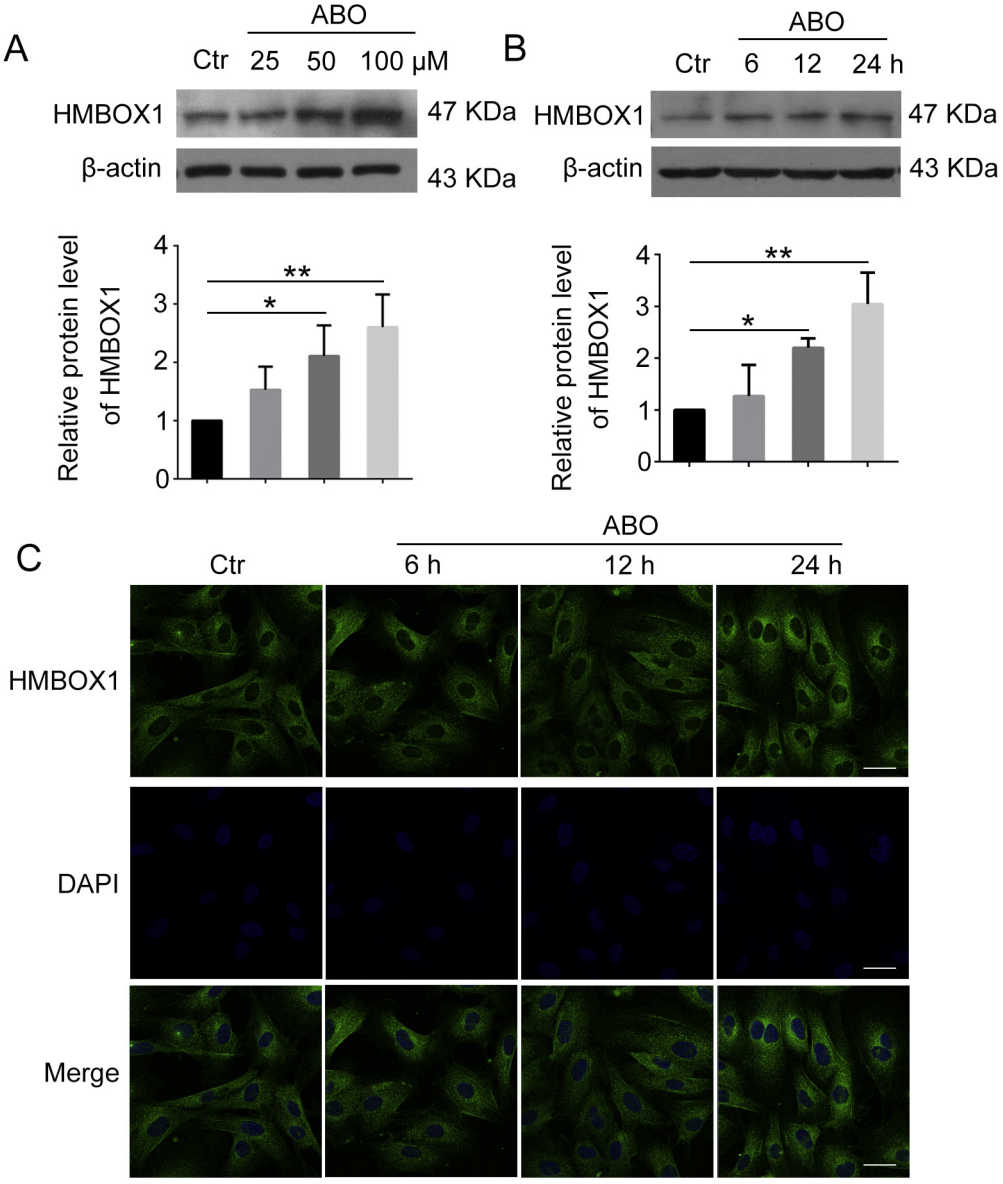

Fig. 1. ABO increases HMBOX1 protein level in HUVECs.
HUVECs of 60–70% confluence were treated with 25, 50, 100 μM ABO for 12 h (A) or 50 μM ABO for 6, 12, 24 h (B), cell lysates were subjected to western blotting. Bar graph showed the corresponding levels of HMBOX1 compared with those of β-actin. (C) Immunostaining of HMBOX1 subcellular distribution in HUVECs treated with 50 μM ABO for 6, 12, 24 h. Scale bar: 16 μm. (Data are mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, n = 3)