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FoxO regulates expression of ABCA6, an intracellular ATP-binding-cassette transporter responsive to cholesterol

Q1 Junfang Gai, Meiling Ji, Chenxi Shi, Wenli Li, Simin Chen, Yeyu Wang, Hao Li*

Key Laboratory of Cardiovascular Disease and Molecular Intervention, Department of Pathophysiology, Nanjing Medical University, Nanjing, China

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ABSTRACT

ATP-binding-cassette (ABC) proteins have been recognized as key players in cellular physiological transport processes. ABC transporter A6 (ABCA6) is a member of the ABC subfamily A. Although it was cloned more than 10 years ago, its expression regulation, subcellular localization, and physiologic function remain largely unknown. We here demonstrated that expression of ABCA6 was Forkhead box O (FoxO)-dependent in human endothelial cell line EA.hy926 and human umbilical vein endothelial cells. Two functional FoxO-responsive elements were identified in ABCA6 promoter and characterized in detail. ABCA6 mRNA was suppressed by insulin-like growth factor-1 which stimulates the phosphorylation and inactivation of FoxOs while inhibitor of phosphatidylinositol 3-kinase had the opposite effect. By immunofluorescence and confocal microscopy, ABCA6 protein is localized primarily in an intracellular compartment, likely representing the Golgi apparatus. ABCA6 mRNA was demonstrated to be responsive to cholesterol loading as well as 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors in human endothelial cells. Our data provide evidence for an essential role of FoxO proteins in the transcription of ABCA6 in human vascular endothelial cells. Based on its cholesterol responsiveness, a potential involvement of ABCA6 in intracellular lipid transport processes may be anticipated.

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1. Introduction

ATP-binding-cassette (ABC) transporters constitute a family of evolutionarily highly conserved multispan transmembrane proteins which use the energy of ATP hydrolysis to translocate a variety of substrates across membrane barriers (Dean and Annilo, 2005). Substrates that are transported by ABC molecules include lipids, peptides, amino acids, carbohydrates, vitamins, ions, and xenobiotics (Dean and Annilo, 2005; Kaminski et al., 2006). Based on their structural relatedness, ABC transporters are subdivided into seven subfamilies, designated ABC A–G (Dean and Annilo, 2005). The human ABCA subfamily of ABC proteins consists of 12 full-size transporters, which are characterized by the presence of a highly hydrophobic segment located between the two transmembrane domains (Klein et al., 1999). Some members of this group mediate the transport of a variety of physiologic lipid compounds. For example, ABCA1 has been reported to participate in reverse cholesterol transport (Luciani and Chimini, 1996). ABCA3 has been postulated to contribute to the biogenesis of surfactant lamellar bodies in human lung alveolar type II cells (Yamano et al., 2001). ABCA4 transports N-retinyli-dene-phosphatidyl-ethanolamine in the retina (Weaver et al., 2002). ABCA12 is implicated in intracellular translocation processes of sphingolipids in keratinocytes (Akiyama et al., 2005). ABCA2 and ABCA7 have also been implicated in lipid translocation or homeostasis (Ile et al., 2004; Linsel-Nitschke et al., 2005). Mutations of these ABCA proteins are known to be related to various diseases (Kaminski et al., 2006).

Five genes of the ABCA subfamily (ABCA5, ABCA6, ABCA8, ABCA9, and ABCA10) are arranged in a chromosome cluster on mouse chromosome 11 and human chromosome 17 (Annilo et al., 2003). First to be characterized in completeness was ABCA6. Because of their high sequence homology and unique genomic organization, these genes are referred to as “ABCA6-like transporters” (Piehler et al., 2002). ABCA5 was detected in lysosomes and late endosomes and may serve critical functions in the endolysosomal system (Kubo et al., 2005). ABCA8 has been implicated in acting as a multi-specific drug transporter with a substrate spectrum including ochratoxin A, oestradiol-β-glucuronide, taurocholate and leukotriene C4 (Tsuruoka et al., 2002). ABCA6, ABCA9 and ABCA10 were demonstrated to be cholesterol-responsive in human macrophages (Kaminski et al., 2001; Piehler et al., 2002;

Abbreviations: IGF-1, insulin-like growth factor-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PI3K, phosphatidylinositol 3-kinase; CHO, Chinese hamster ovary; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate; DTT, dithiothreitol.

* Corresponding author at: 140 Hanzhong Road, Department of Pathophysiology, Nanjing Medical University, Nanjing, China. Tel.: +86 25 86862886; fax: +86 25 86862888.
E-mail address: haoli@njmu.edu.cn (H. Li).

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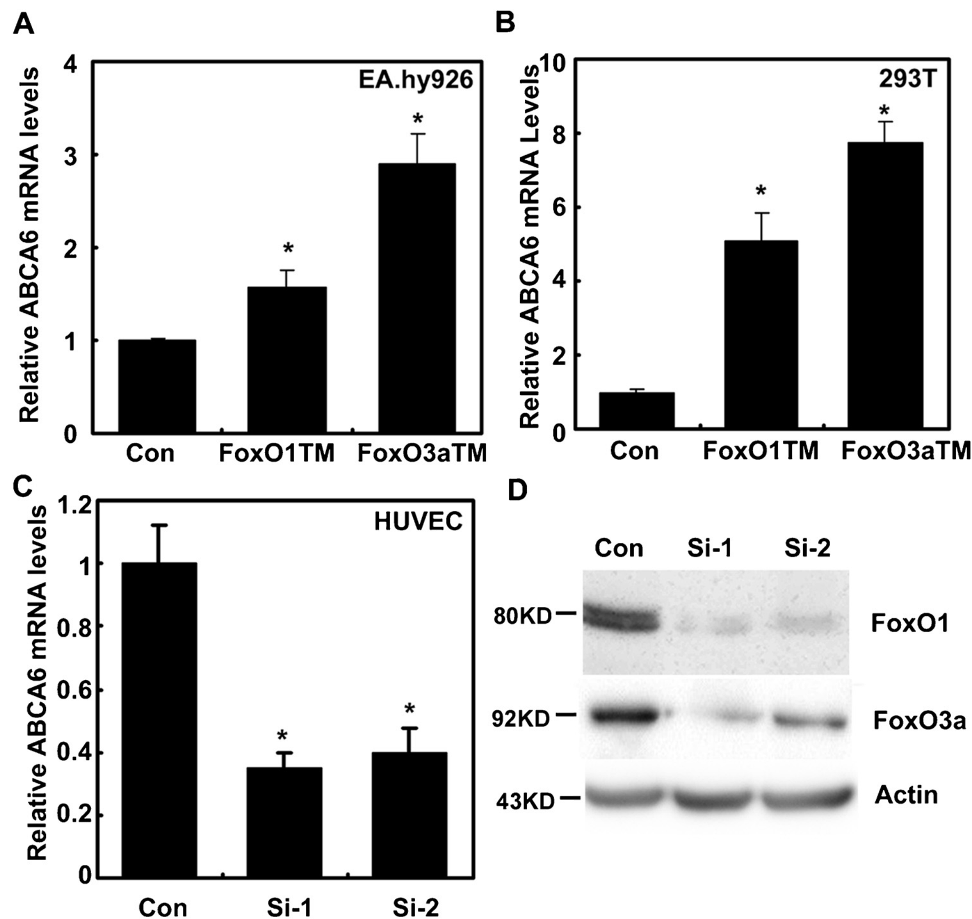

Fig. 1. FoxO-dependent expression of ABCA6. (A and B) EA.hy926 cells or 293T cells were transfected as indicated and ABCA6 mRNA was quantified using real-time PCR. Data are mean ± SD from three experiments. $^{*}P < 0.05$ *versus* control. (C) ABCA6 mRNA in HUVECs was quantified after transfection with siRNA. Data are mean ± SD from three experiments. $^{*}P < 0.05$ *versus* control. (D) HUVECs were transfected with siRNA and harvested for western blot after 48 h.

Wenzel et al., 2003). The substrate spectrum and biological significance of ABCA6-like transporters largely remain unknown.

Human ABCA6 consists of 1617 amino acids with a calculated molecular weight of approximately 180 kDa and a classical ABC full-transporter structure (Kaminski et al., 2001). The human ABCA6 gene is ubiquitously expressed in the organism, with high levels found in liver, lung, heart, brain, and ovaries (Kaminski et al., 2001). In mouse liver, ABCA6 is exclusively (>99%) expressed in parenchymal cells (Ye et al., 2008). ABCA6 mRNA is not detected in any tumors (Ohtsuki et al., 2007). ABCA6 has been described to be up-regulated during macrophage differentiation and down-regulated by acLDL loading (Klucken et al., 2000). Further characterization of the ABCA6 protein such as its expression regulation, subcellular localization, and physiologic function has not been reported to date.

Forkhead box O (FoxO) transcription factor family consists of FoxO1, FoxO3a, and FoxO4 (also known as FKHR, FKHRL1, and AFX, respectively). FoxOs regulate hormonal, nutrient, and stress responses (Salih and Brunet, 2008). Activation of the PI3K pathway blocks the function of FoxO factors by Akt-dependent phosphorylation of three conserved residues, which leads to inhibition of DNA binding, nuclear exclusion, and subsequent sequestration in the cytoplasm. Dephosphorylation of FoxOs in turn stimulates nuclear entry, leading to the activation or repression of target genes (Pierrou et al., 1994; Woods and Rena, 2002).

In a microarray analysis of expression profile in a human endothelial cell line transfected with FoxO plasmids, we found that ABCA6 mRNA increased with FoxO overexpression. In the present study, we investigated molecular mechanisms by which FoxO regulates ABCA6 transcription in vascular endothelial cells. In addition, we determined the subcellular localization of ABCA6 and reported its responsiveness to cellular cholesterol levels.

2. Materials and methods

2.1. Cell culture and reagents

Human umbilical vein endothelial cells (HUVEC) were isolated from freshly delivered umbilical cords and grown in endothelial cell medium (Sciencell) supplemented with endothelial cell growth supplement (ECGS), 5% FBS and penicillin/streptomycin, at 37 °C in a humidified atmosphere with 5% (v/v) CO_2. In all experiments, HUVEC passages 2–5 were used. The endothelial cell line EA.hy926, which is derived from HUVEC, and human embryonic kidney-293T (HEK-293T) cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin. Human lung cancer cell line NCI-H1299 was obtained from American Type Culture Collections (Manassas, VA) and maintained in RPMI-1640 supplemented with 10% FBS and penicillin/streptomycin.

IGF-1, lovastatin, and mevastatin were purchased from Sigma. PI3K inhibitor LY294002 was purchased from Cell Signaling (Beverly, MA). AcLDL was purchased from Yiyuan Biotechnology (Guangzhou, China).

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