The International Journal of Biochemistry & Cell Biology 57 (2014) 115–122

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

TIA1 interacts with annexin A7 in regulating vascular endothelial cell autophagy

Shuya Huang[a], Ning Liu[a], Haiying Li[a], Jing Zhao[a], Le Su[a], Yun Zhang[b], Shangli Zhang[a], Baoxiang Zhao[c,*], Junying Miao[a,b,*]

[a] Shandong Provincial Key Laboratory of Animal Cells and Developmental Biology, School of Life Science, Shandong University, Jinan 250100, China
[b] The Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Health, Shandong University Qilu Hospital, Jinan 250012, China
[c] Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, China

ARTICLE INFO

Article history:
Received 11 July 2014
Received in revised form
27 September 2014
Accepted 9 October 2014
Available online 18 October 2014

Keywords:
ANXA7
TIA1
Phosphorylation
Autophagy
Endothelial cell

ABSTRACT

T-cell intracellular antigen-1 (TIA1) is a DNA/RNA binding protein broadly expressed in eukaryotic cells, participating in multiple aspects of cellular metabolism. TIA1 phosphorylation was related with cell apoptosis and its RNA binding activity, however, the regulator and other functions of TIA1 phosphorylation were very little known. To find the modulator of TIA1 phosphorylation, we performed yeast two-hybrid screening and identified annexin A7 (ANXA7) as an interaction protein of TIA1. Recent study showed that a small molecule ABO could directly target ANXA7 and inhibit ANXA7 activity and its targets' phosphorylation. As a GTPase, ANXA7 was speculated to modulate TIA1 phosphorylation. Our results showed that ABO treatment promoted the interaction between TIA1 and ANXA7, and then greatly inhibited phosphorylation of TIA1 in HUVECs. Further results showed that ABO-increased interaction between ANXA7 and TIA1 significantly promoted the processing of a pro-autophagic factor *FLJ11812* and the expression of ATG13. Moreover, we found that ABO increased TIA1 protein level, co-localization of ANXA7 and TIA1, and ATG13 expression in the aortic endothelium of apoE$^{-/-}$ mice. These data highlighted the new role of TIA1 phosphorylation in autophagy.

1. Introduction

T-cell intracellular antigen-1 (TIA1) belongs to a large family of RNA-binding proteins and contains three RNA recognition motifs (RRMs) at its NH2 termini and a glutamine-rich (Q-rich) domain at its COOH termini. The previous studies have shown that TIA1 has two major isoforms induced by alternative mRNA splicing (Beck et al., 1996; Kawakami et al., 1994). TIA1a includes an 11-amino acid mini exon within RRM2, whereas TIA1b lacks this exon (Anderson and Kedersha, 2002). In normal cells, TIA1 is mostly distributed in nucleus and serves as a RNA-splicing regulator of a series of alternatively spliced pre-mRNAs (Forch et al., 2000). In the cytoplasm, TIA1 has been reported to regulate the translation of various mRNAs by binding to AU-rich elements (AREs) located in mRNA 3′untranslated regions (3′UTRs).

In addition to being a regulator in RNA splicing and translation, TIA1 was also shown to participate in apoptosis. In thymocytes subjected to Fas ligation, Fas-activated serine/threonine kinase (FASTK) was rapidly dephosphorylated and TIA1 was soon phosphorylated at serine residues, and then DNA fragmentation occurred (Tian et al., 1995). Phosphorylation of TIA1 by FASTK was also shown to result in higher U1 snRNP recruitment activity and modulate Fas mRNA alternative splicing (Izquierdo and Valcarcel, 2007). Furthermore, in our recent study, we revealed that TIA1 was responsible for the processing of a long noncoding RNA *FLJ11812*, and *FLJ11812* was involved in autophagy by regulating the expression of autophagy related 13 (ATG13) (Ge et al., 2014). So TIA1 phosphorylation plays a very important role in cellular metabolism, however, the modulator and other functions of TIA1 phosphorylation were little known.

To find modulator of TIA1 phosphorylation and its action mechanism, we perform yeast two-hybrid screening by screening a

Abbreviations: HUVEC, human umbilical vein endothelial cell; ANXA7, annexin A7; TIA1, T-cell intracellular antigen-1; ABO, 6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine; ApoE$^{-/-}$ mice, apolipoprotein E-deficient mice; LC3, microtubule-associated protein 1 light chain 3; lncRNA, long noncoding RNA; ATG13, autophagy related protein 13.

* Corresponding authors at: Institute of Developmental Biology, School of Life Science, Shandong University, Jinan 250100, China. Tel.: +86 531 88364929; fax: +86 531 88565610.
E-mail addresses: bxzhao@sdu.edu.cn (B. Zhao), miaojy@sdu.edu.cn (J. Miao).

http://dx.doi.org/10.1016/j.biocel.2014.10.015
1357-2725/

human cDNA library and identified annexin A7 (ANXA7) as an interaction protein of TIA1. ANXA7 is a member of annexin family, which contains calcium-dependent phospholipid binding proteins and codes for Ca^{2+}-dependent GTPase (Caohuy and Pollard, 2002). ANXA7 was reported to be a tumor suppressor and participated in tumorigenesis through a signaling pathway engaging other tumor suppressor genes, DNA-repair genes, senescence and apoptosis-related genes (Kriebardis et al., 2007; Srivastava et al., 2001, 2003). Previous studies showed that homozygous ANXA7(−/−) mutation is lethal by E10 (Herr et al., 2001), and ANXA7(+/−) mutation also has several unpredicted phenotypes, such as induction of islet hyperplasia, β-cell hypertrophy, abnormal regulation of islet gene expression by the fed/fasted state, and an alteration in the Ca^{2+}-dependence of glucose-induced insulin secretion (Mears et al., 2012). Therefore, traditional genetic method is very difficult for determining the role of ANXA7 GTPase in cell signaling.

In a recent study, we found that a small molecule 6-amino-2, 3-dihydro -3-hydroxymethyl-1, 4-benzoxazine (ABO) could directly target ANXA7 and inhibit ANXA7 activity and its targets' phosphorylation (Li et al., 2013b). Furthermore, our *in vivo* experiments showed that targeting ANXA7 by ABO could significantly inhibit atherosclerosis in apolipoprotein E-deficient ($apoE^{-/-}$) mice with increased autophagy and decreased apoptosis in aortic endothelium (Li et al., 2013a). In this study, we showed that ABO treatment could inhibit ANXA7 GTPase activity by a direct measurement (Fig. S2). Therefore, the chemical molecule ABO provides a novel and useful tool for investigating the role of ANXA7 GTPase in cell signaling compared to traditional genetic methods.

Therefore, the aim of our present study is to determine the role and action mechanism of ANXA7 in regulating TIA1 phosphorylation by using ABO. The results showed that ABO treatment promoted the interaction of ANXA7 and TIA1 and then inhibited TIA1 phosphorylation in human umbilical vein endothelial cells (HUVECs). Furthermore, ABO could not induce autophagy when TIA1 was knocked down. Moreover, ABO treatment significantly increased the level of *FLJ11812* and the expression of ATG13. The *in vivo* experiments also showed increased co-localization of ANXA7 with TIA1 and ATG13 protein level in the aortic endothelium of $apoE^{-/-}$ mice treated with ABO. Our findings revealed that the important role of ANXA7 and TIA1 phosphorylation in autophagy.

2. Materials and methods

2.1. Cell culture

HUVECs were obtained as described by Jaffe et al. (1973) and cultured on gelatin-coated plastic dishes in M199 medium (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone, USA) and 2 ng/mL FGF-2 at 37 °C in a humidified incubator with 5% CO_2. Passage number of HUVECs for experiments ranged from passage 5 to 10.

COS7 cells were grown in DMEM medium (Gibco, USA) with 10% FBS, penicillin (50 U/ml) and streptomycin (50 μg/mL) (Invitrogen, 10378-016) at 37 °C in a humidified incubator with 5% CO_2.

HEK293 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured on plastic dishes in DMEM medium supplemented with 10% FBS at 37 °C in a humidified incubator with 5% CO_2.

2.2. Plasmid transfection

The coding region of human ANXA7 and TIA1 C-terminal cDNAs was subcloned into pEGFP-C2 and pMyc-C2 expression vector to produce pEGFP-C2-ANXA7, pEGFP-C2-TIA1, pMyc-C2-ANXA7, and pMyc-C2-TIA1 constructs, respectively. Cells were plated onto 6 cm dish at a density 1×10^6 mL^{-1} and grown for 24 h. When cell density reached 70–80% confluence, cells were transfected with indicated expression vectors using Lipofectamine® 2000 Transfection Reagent (Invitrogen, 11668-019). After transfection for 48 h, cells were harvested and analyzed by western blot.

2.3. RNA interference

TIA1 siRNA (29504) and scramble siRNA (37007) were purchased from Santa Cruz Biotechnology. ANXA7 siRNA was designed and custom synthesized by Invitrogen as previously reported (Wang et al., 2010). When cell density reached 50–60% confluence, 80 nM siRNA against TIA1 or ANXA7 and scramble siRNA (negative control) were transfected into cells with RNAiFect Transfection Reagent according to the manufacturer's protocol (QIAGEN, 301605). We monitored the efficiency of gene silencing by western blot assay.

2.4. Immunoprecipitation

COS7 cells or HUVECs were washed with ice-cold PBS and lysed in IP buffer (Beyotime, China) containing 150 mM NaCl, 20 mM Tris–HCl (PH 7.5), 1% Triton X-100, and proteinase inhibitor mix. After centrifuging at 4 °C, the supernatant was collected and precleared with protein A/G agarose beads (Beyotime, China) for 1 h at 4 °C. The supernatant was collected after centrifuging and incubated with specific antibodies or normal IgG (as control), followed by incubation with protein A/G agarose beads overnight at 4 °C.

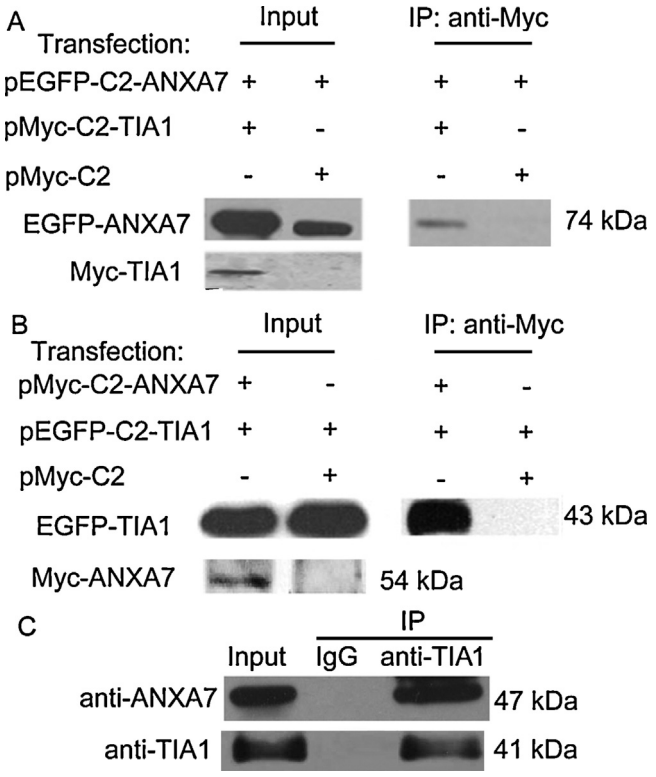

Fig. 1. ANXA7 interacted with TIA1 in COS7 cells and HUVECs. (A) Co-immunoprecipitation of GFP-tagged ANXA7 proteins with Myc-tagged TIA1 from COS7 cells co-transfected pEGFP-C2-ANXA7 with pMyc-C2-TIA1 or pMyc-C2. Co-immunoprecipitated ANXA7 was detected with anti-GFP antibody in western blot assay. (B) Co-immunoprecipitation of GFP-tagged TIA1 proteins with Myc-tagged ANXA7 from COS7 cells co-transfected pEGFP-C2-TIA1 with pMyc-C2-ANXA7 or pMyc-C2. Co-immunoprecipitated TIA1 was detected with anti-GFP antibody in western blot assay. (C) HUVECs were lysed, and the supernatant was incubated with anti-TIA1 antibody or normal mouse IgG. Co-immunoprecipitated ANXA7 was detected with anti-ANXA7 antibody in western blot assay.