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Effects of annexin A7 inhibitor-ABO on the expression and distribution of long noncoding RNA-CERNA1 in vascular endothelial cells apoptosis

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Abstract

More and more studies reported that diverse biological roles of long noncoding RNAs were usually dependent on their subcellular location. In our previous study, long noncoding RNA CERNA1 was identified both located in cytoplasm and nucleus of vascular endothelial cells (VECs). And CERNA1 in cytoplasm, which functioned as competitive endogenous RNA (ceRNA), alleviated the apoptosis of VECs. However, the function of CERNA1 in nucleus was still unclear. In this study, we found that nuclear CERNA1 positively regulated BCL2L10, which accelerated the serum and FGF-2 starvation-induced apoptosis of VECs, by enhancing the histone modification level of H3K9ac and H3K4me3 in BCL2L10 promoter region. Furthermore, due to the paradoxical function, we investigated the variation of CERNA1 subcellular location in VECs. The results showed that, as the change of apoptosis status, CERNA1 altered the cellular distribution in VECs. And the annexin A7 inhibitor, ABO (6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine), not only increased the expression of CERNA1 by TIA-1, but also specifically improved its cytoplasm distribution proportion so as to inhibit the apoptosis of VECs. This evidence suggested that the subcellular location of CERNA1 played an important role in the VECs apoptosis and ABO might be a potential chemical molecule for therapy of VECs apoptosis related cardiovascular diseases.

Keywords VECs · Apoptosis · CERNA1 · ABO · Nucleocytoplasmic distribution

Introduction

Apoptosis in vascular endothelial cells (VECs) is closely linked to cardiovascular diseases (CVD) such as arteriosclerosis, thrombus formation and plaque erosion etc. [1]. CVD accounts for about 30% of the world's total deaths each year and constitute a major cause of death and produce immense health and economic burdens in globally [2]. Therefore, it is necessary to identify novel apoptosis-related therapeutic target in VECs for CVD.

Long noncoding RNAs (lncRNAs) are defined as transcripts longer than 200 nucleotides without significant protein-coding potential. More and more studies reported that lncRNAs acted as new regulators in multiple biological processes such as differentiation, proliferation, apoptosis, invasion and reprogramming of induced pluripotent stem cell by interaction with DNA, RNA or proteins [3]. Accumulating data showed that the action mechanism of lncRNAs usually depends on its subcellular location. LncRNAs in nucleus are often involved in the regulation of gene transcription and epigenetic regulation, while lncRNAs in cytoplasm are

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involved in diverse post-transcriptional regulation, such as the ceRNA pathway [3, 4].

Recently, lncRNAs were found to be functionally important in VECs for CAD, including NRON, Tie-1 AS, Alien, Punisher, MALAT1, MIAT, TGFB2-OT1 and CERNA1 [5–11]. Among these lncRNAs, CERNA1, formerly known as LOC100129973, was first identified in ABO (6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine) inhibited the serum and FGF-2 starvation-induced VECs apoptosis [10]. Moreover, ABO could directly bound to Thr^{286} of annexin A7 (ANXA7) and inhibited its activity in VECs [12, 13]. In our previous study, CERNA1 functioned as a competing endogenous RNA (ceRNA) by sponging miR-4707-5p and miR-4767, thus upregulated two apoptosis-inhibitors API5 and BCL2L12. Besides, we also observed that CERNA1 located in both cytoplasm and nucleus [10]. Considering that ceRNAs usually act in cytoplasm, we aim to investigate the role of nuclear CERNA1 and the effect of ABO on CERNA1 cellular distribution in VECs. This study could provide a novel therapeutic target and a novel treatment strategy for VECs apoptosis related cardiovascular diseases.

Materials and methods

Cell culture

Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cords as described [14], and then cultured in M199 medium (Gibco) with 10% (v/v) fetal bovine serum (Gibco) and 10 IU/mL fibroblast growth factor 2 (FGF-2) in a humidified incubator at 37 °C with 5% CO_2. Cells at not more than passage 10 were used for experiments.

Quantitative real-time PCR

RNA was extracted from whole-cell/cytoplasmic fraction/nuclear fraction by the Trizol reagent method (Invitrogen) and underwent reverse transcription and quantitative PCR (Roche) with the primer pair sequences for genes (Supplementary Table S1). The reverse transcription step was used of the PrimeScript RT reagent kit with gDNA Eraser (TAKARA). PCR reactions involved use of SYBR Premix Ex Taq (Tli RNaseH Plus) and the levels of expressed genes were measured by the $2^{-\Delta\Delta Ct}$ method with MxPro 4.00 (Stratagene).

Transient transfection with siRNA in HUVECs

Specific siRNAs against lncRNA CERNA1/BCL2L10/TIA1 were designed and synthesized by Invitrogen (Supplementary Table S2). Scramble siRNA was used as a control (Santa Cruz, sc-37007). Cells at 70% confluence were transfected with siRNA by Lipofectamine 2000 (Invitrogen) transfection reagent according to the manufacturer's instructions.

Cell apoptosis assay

An Annexin V-FITC/PI Apoptosis Detection Kit (BestBio, Shanghai, China) was used to assess the cell apoptosis rate according to the manufacturer's instructions. In brief, the cells were collected using trypsin without EDTA, washed two times with cold PBS, resuspended with the binding buffer, and incubated with Annexin V-FITC staining solution for 15 min at 4 °C and PI staining solution for 5 min at 4 °C in the dark. After that, the apoptosis rate was immediately detected by a flow cytometer (BD FACS Calibur™ Flow Cytometer, BD Biosciences, San Jose, CA, USA). Three independent experiments were performed.

Western blot analysis

Treated HUVECs were lysed in protein lysis buffer (Beyotime). Protein content was determined by use of the BCA Protein Assay Kit (Beyotime). Proteins were separated by 12% or 9% SDS-PAGE and transferred to PVDF membrane (Millipore), which was incubated with primary antibodies for BCL2L10 (Abcam, ab96625), PARP (Cell Signaling, 9542L), Caspase 3 (Cell signaling, 9661), TIA1 (Santa Cruz Biotechnology, sc166247) and ACTB (Sigma, 122M4782) at 4 °C overnight and detected with corresponding horseradish peroxidase-conjugated secondary antibody (1:10,000) at room temperature for 1 h. The membranes were incubated with Immobilon Western Chemiluminescent HRP Substrate (Millipore) for 5 min at room temperature and exposed to X-ray film (Kodak). The relative protein content was analyzed by ImageJ software and normalized to loading controls.

Chromatin Immunoprecipitation (ChIP) assay

Chromatin Immunoprecipitation (ChIP) experiments were performed with the EZ-Magna ChIP A/G Kit (Millipore, 17-10086) following the manufacturer's instructions. The ChIPAb + Trimethyl-Histone H3 (Lys4) antibody (Millipore, 17–614) and the ChIPAb + Acetyl-Histone H3 (Lys9) antibody (Millipore, 17–614) were used in ChIP and mouse IgG was used as a negative control. The PCR primers are listed in Supplementary Table S1.

TIA1 phosphorylation detection

HUVECs were washed with ice-cold PBS and lysed in IP buffer with proteinase inhibitor mix (Beyotime, China). After centrifuging, the total protein supernatant was

incubated with TIA1 antibody (Santa Cruz Biotechnology, sc166247) or normal IgG (as control), followed by incubation with protein A/G agarose beads overnight at 4 °C. Then the beads were washed three times with IP buffer and then eluted with 3 × SDS loading buffer. Serine phosphorylation of TIA1 was detected by western blot assay with Serine phosphorylation antibody (Santa Cruz Biotechnology, sc-81514).

Statistical analysis

Data are presented as mean ± SEM and analysis involved use of GraphPad Prism 6. Images were processed by Adobe Photoshop CS5 (Adobe, USA). $P < 0.05$ was considered statistically significant. All experiments were repeated at least 3 times independently.

Results

Nearly 30% CERNA1 located in nucleus of vascular endothelial cells

In our previous work, RNA-FISH technology was used to detect the subcellular distribution of CERNA1 in VECs which cultured in M199 medium with fetal bovine serum (FBS) and fibroblast growth factor 2 (FGF-2). The result showed that most CERNA1 located in the cytoplasm and a small portion of CERNA1 located in the nucleus [10]. To further confirm that CERNA1 could localize in the nucleus and get the accurate cellular distribution proportion of CERNA1 in VECs, we respectively extracted CERNA1 from the cytoplasm and nucleus of normal cultured HUVECs. And the result showed that about 30% CERNA1 localized in nucleus of VECs (Fig. 1).

CERNA1 positively regulated the expression of BCL2L10

Considering that lncRNAs in nucleus usually regulate the transcription of its adjacent gene in genome, we speculated that nuclear CERNA1 of VECs could alter its adjacent gene expression. Since the apoptosis-related gene BCL2L10 locates in the neighboring region of CERNA1 gene in genome, we tested whether CERNA1 affect the expression of BCL2L10. HUVECs were transfected with siCERNA1 at 20, 40, 80 nM and the efficiency of knockdown was detected (Fig. 2a). qPCR and Western blot results showed that the mRNA and protein levels of BCL2L10 were significantly decreased after knockdown of CERNA1 (Fig. 2b–d). Therefore, nuclear CERNA1 induced the expression of its genome proximity gene-BCL2L10 which is an apoptosis-related gene.

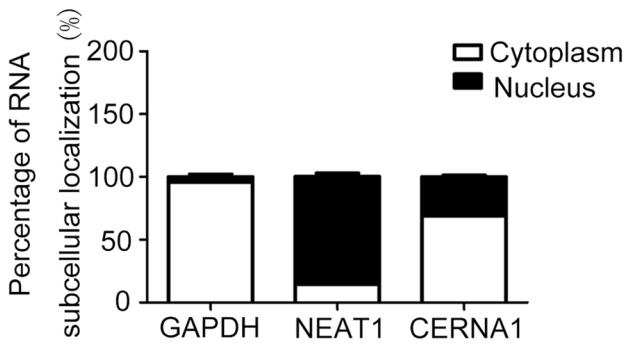

Fig. 1 The cellular distribution of CERNA1 in VECs. HUVECs were separated into two fractions, and then RNA was separately extracted from cytoplasm and nucleus; qPCR was used to detect CERNA1 level (GAPDH as cytoplasmic marker and NEAT1 as nuclear marker). (Data are mean ± SEM, n ≥ 3)

CERNA1 altered the epigenetic modification level of BCL2L10 promoter region

To investigate the underlying mechanism that CERNA1 enhanced BCL2L10 expression, we analyzed the histone modification of BCL2L10 promoter region by Epigenetics database of NCBI. As shown in Supplementary Fig.S1A, it suggested that, the expression of BCL2L10 could be regulated by the histone modification of H3K4me3 or H3K9ac in its promoter region in VECs.

To verify whether CERNA1 increased the BCL2L10 expression by altering histone modification level, chromatin immunoprecipitation assay (ChIP) and the specific antibodies to H3K4me3 and H3K9ac were used to detected the histone modification levels of H3K4me3 and H3K9ac in BCL2L10 promoter region after knockdown of CERNA1. The results of the two ChIP were similar. Both the histone modification levels of H3K4me3 and H3K9ac were significantly reduced (Fig. 3 and Supplementary Fig. S1B). Taken together, CERNA1 positively regulated the adjacent gene-BCL2L10 expression by enhancing histone modification levels of H3K4me3 and H3K9ac in BCL2L10 promoter region.

BCL2L10 acted as a pro-apoptotic regulator in VECs

Recent studies reported that BCL2L10 had the promotion or inhibition effects on apoptosis, which mainly based on cell type, protein interaction or apoptosis inducer [15–18]. However, the function of BCL2L10 in VEC apoptosis has not been identified. To figure out its role, siBCL2L10 were transfected into HUVECs and then induced to apoptosis by the starvation treatment for another 24 h. Annexin V-FITC/PI staining and flow cytometry assay were performed to determine the extent of apoptosis after BCL2L10 knockdown. It is revealed that the proportion of apoptotic cells was lower in BCL2L10 RNAi group than control group (20.71% vs. 38.96%) (Fig. 4a).

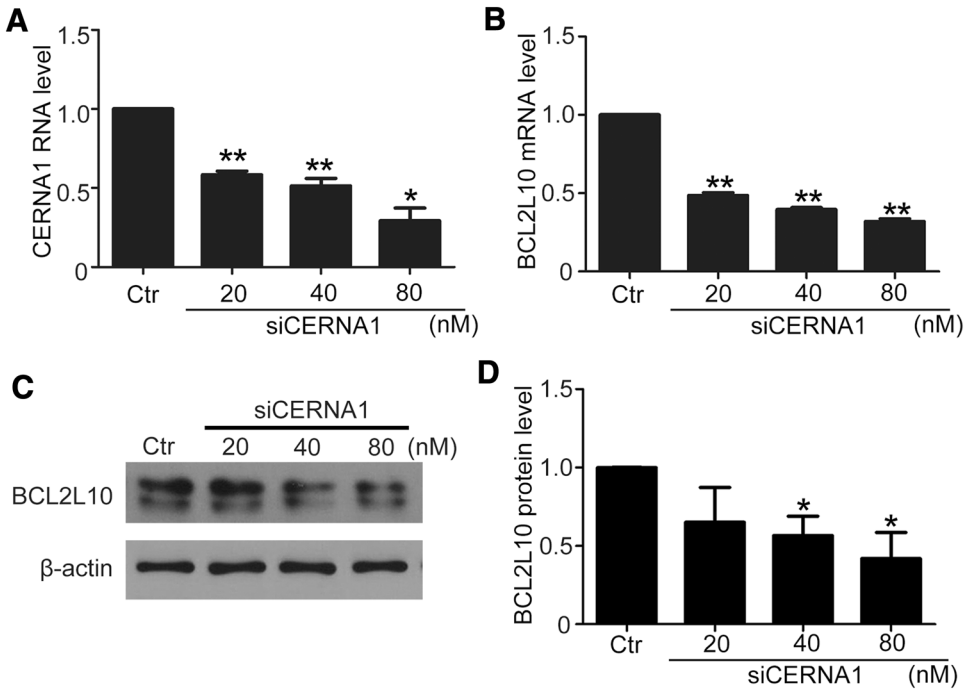

Fig. 2 Decreased RNA level of CERNA1 led to a reduction on RNA level and protein level of BCL2L10 in HUVECs. HUVECs were transfected with siCERNA1 or siCtr at 20, 40, 80 nM for 24 h. qPCR analysis of CERNA1 RNA level (**a**) and BCL2L10 mRNA level (**b**). Western bolt analysis of BCL2L10 protein level (**c** and **d**). (Data are mean ± SEM, $*p < 0.05$, $**p < 0.01$ vs. control (Ctr), n ≥ 3)

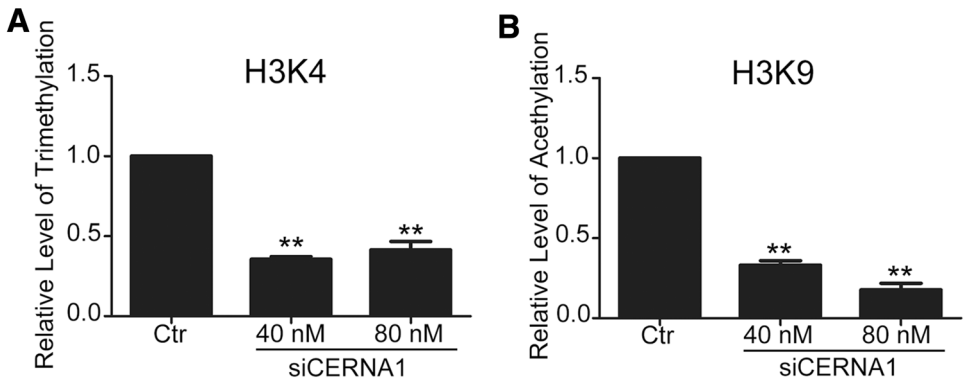

Fig. 3 Knockdown of CERNA1 altered the epigenetic modification level of BCL2L10 promoter region in HUVECs. HUVECs were transfected with siCERNA1 or siCtr at 40, 80 nM for 24 h. ChIP and qPCR analysis of histone modification level of H3K4me3 (**a**) and H3K9ac (**b**) in BCL2L10 promoter region (data are mean ± SEM, $**p < 0.01$ vs. control (Ctr), n ≥ 3)

Subsequently, different concentrations of siBCL2L10 were transfected into HUVECs and then induced to apoptosis by the starvation treatment for another 24 h. The western blot result of cleaved PARP suggested that BCL2L10 promoted the serum and FGF-2 starvation-induced VECs apoptosis (Fig. 4b). Since BCL2L10 can induce apoptosis through caspase-3 pathway in gastric cancer cells [16], we verified the role of BCL2L10 on promoting caspase-3 cleaving. Along with knockdown of BCL2L10, the cleaved caspase 3 level were significantly inhibited in a dose-dependently manner (Fig. 4c). These data indicated that BCL2L10 was an apoptosis facilitated factor in VECs.

The relative cellular distribution of CERNA1 altered along with changing the apoptosis state of VECs

Based on the results above, nuclear CERNA1 accelerated an apoptosis facilitated factor-BCL2L10 expression in VECs by epigenetic pathway. Meanwhile, we previously found that cytoplasmic CERNA1 alleviated the serum and FGF-2 starvation-induced apoptosis by ceRNA pathway [10]. Therefore, we speculated that the cellular distribution of CERNA1 play the key role in determining cell fate.

To validate this hypothesis, we respectively extracted CERNA1 from the cytoplasm and nucleus of HUVECs

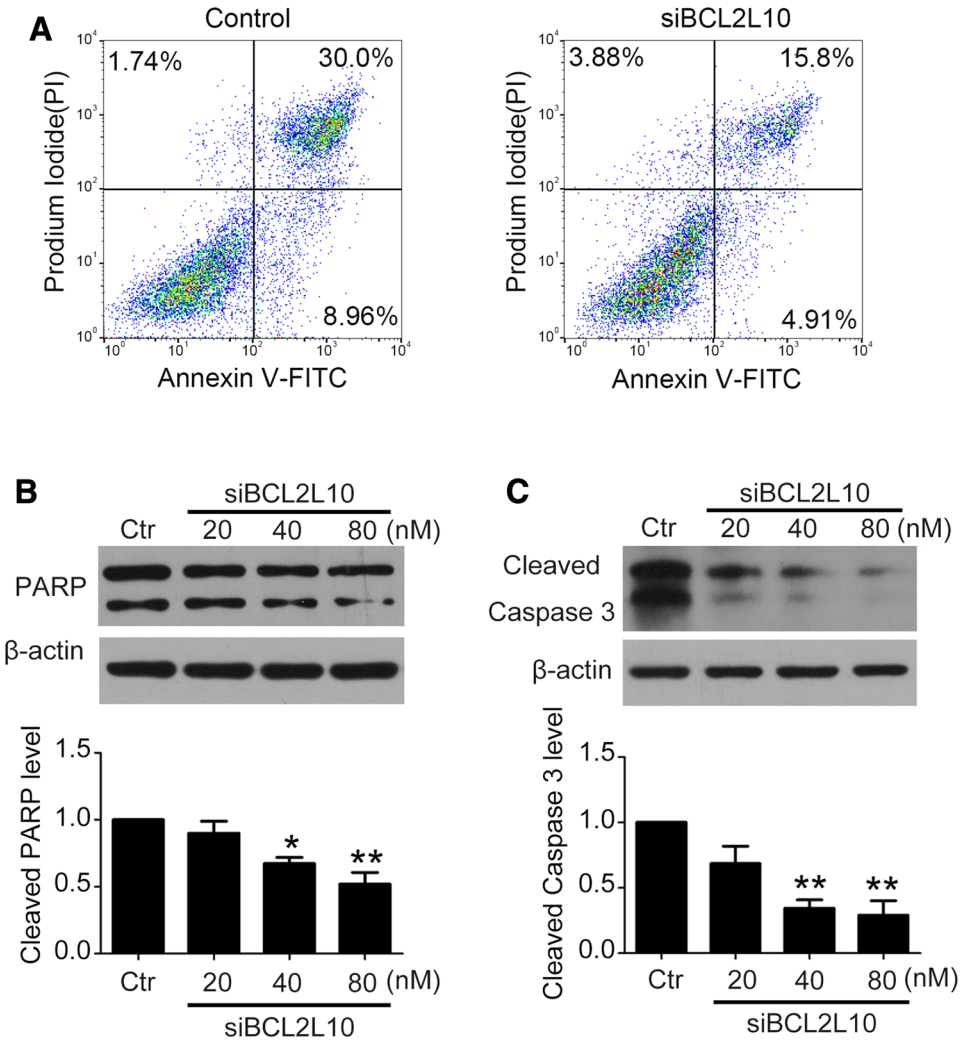

Fig. 4 BCL2L10 promoted the serum and FGF-2 starvation-induced apoptosis in HUVECs. HUVECs were transfected with 80 nM scramble siRNA (Control) or siBCL2L10 for 24 h, and then induced to apoptosis by the starvation treatment for another 24 h. AnnexinV-FITC/PI staining and flow cytometry assay to determine the proportion of apoptosis. **b, c** With transfected different concentration of siBCL2L10 or scramble siRNA (Ctr) for 24 h, HUVECs were induced to apoptosis by the starvation treatment for another 24 h. Protein levels of PARP (**b**) and cleaved caspase 3 (**c**) were analyzed by western blot (data are mean ± SEM, $*p<0.05$, $**p<0.01$ vs. control (Ctr), n≥3)

which treated in three ways as follows: (Nor) HUVECs were cultured in M199 medium with FGF-2 and serum; (Ctr) HUVECs were cultured in basal M199 medium, in which FGF-2 and serum are not included, with 0.05% DMSO for 24 h. This serum and FGF-2 starvation treatment could induce the apoptosis of VECs [10]; (ABO) HUVECs were cultured in basal M199 medium with 50 μM ABO (6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine) for 24 h, which reduced the serum and FGF-2 starvation-induced apoptosis [10]. Then, we calculated the nucleocytoplasmic distribution percentage of CERNA1 in the three group. Compared to Nor group, the cytoplasm distribution proportion of CERNA1 significantly shrank in Ctr group and the nucleus correspondingly elevated (Fig. 5a). In contrary, compared to Ctr group, the cytoplasm distribution proportion of CERNA1 significantly increased in ABO group, along with the nucleus decreased (Fig. 5a). These results indicated that, no matter the serum and FGF-2 starvation treatment which induced apoptosis or ABO treatment which inhibited apoptosis, CERNA1 participated in the regulation of VECs apoptosis by changing its relative cellular distribution.

ANXA7 inhibtor-ABO increased CERNA1 expression through TIA1 in VECs

We previously found that ABO could directly target ANXA7 and inhibited threonine phosphorylation along with GTPase activity of ANXA7 [13]. And the yeast two-hybrid identified TIA1 as an interaction protein of ANXA7. T-cell intracellular antigen-1 (TIA1) is a DNA/RNA binding protein broadly expressed in eukaryotic cells, participating in multiple aspects of cellular metabolism. Furthermore, ABO promoted the interaction between ANXA7 and TIA1, and this interaction significantly inhibited TIA1 phosphorylation in HUVECs cultured in M199 medium with FGF-2 and serum [12]. Hence, we speculated that ABO could regulate CERNA1 expression in TIA1 depended way.

To validate this hypothesis, different concentrations of siTIA1 were transfected into HUVECs and the RNA level

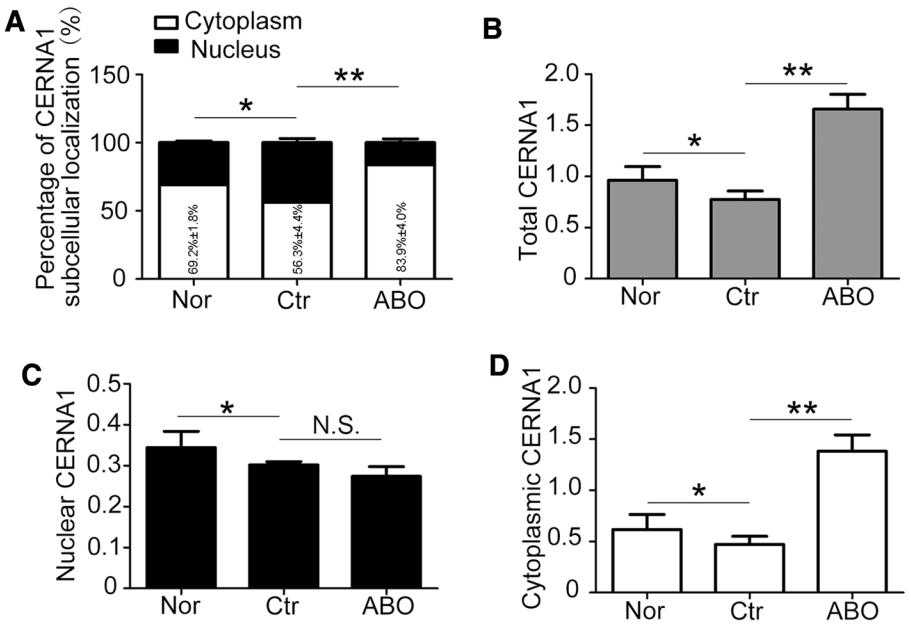

Fig. 5 ABO boosted CERNA1 expression and specially promoted its accumulation in cytoplasm in the serum and FGF-2 starvation-induced apoptosis of VECs. (Nor) HUVECs were cultured in M199 medium with FGF-2 and serum; (Ctr) HUVECs were cultured with 0.05% DMSO for 24 h in basal M199 medium which without FGF-2 and serum; (ABO) HUVECs were cultured with 50 μM ABO for 24 h in basal M199 medium which without FGF-2 and serum. As treated in the three ways above, cells were harvested and separated into two fractions, and then RNA was separately extracted from cytoplasm and nucleus; qPCR was used to detect CERNA1 level. **a** The relative cellular distribution of CERNA1 in HUVECs were analyzed. **b–d** The relative RNA levels of total (**b**), nuclear (**c**) and cytoplasmic (**d**) CERNA1 in HUVECs were detected under the three conditions above. Total CERNA1 level of Nor was set as 1 (data are mean ± SEM, $*p < 0.05$, $**p < 0.01$, n ≥ 3)

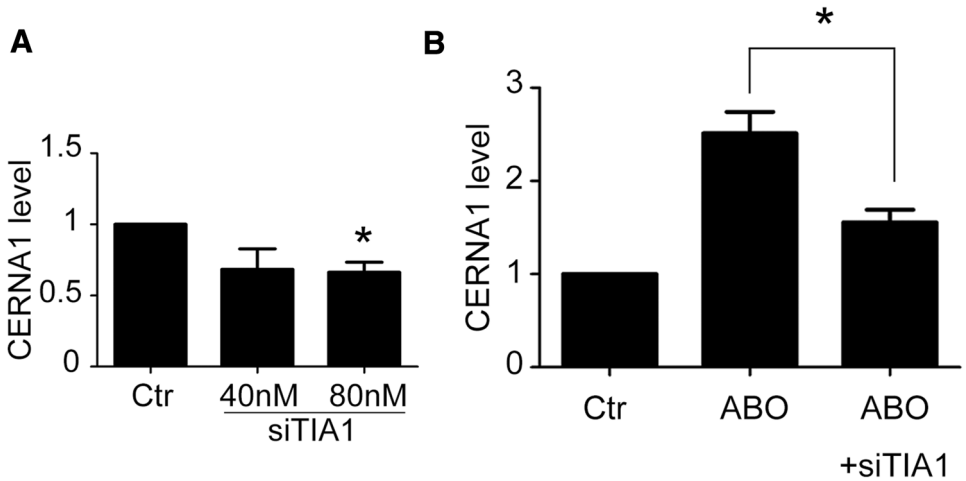

Fig. 6 TIA1 participated in the ABO-induced expression of CERNA1. **a** HUVECs were transfected by siTIA1 or siCtr at 40, 80 nM for 24 h. qPCR analysis of CERNA1 RNA level; **b** (Ctr) HUVECs were transfected with siCtr at 80 nM for 24 h and then cells were cultured in basal M199 with 0.05% DMSO for 24 h; (ABO) HUVECs were transfected with siCtr at 80 nM for 24 h and then cells were cultured in basal M199 medium with 50 μM ABO for 24 h; (ABO + siTIA1) HUVECs were transfected with siTIA1 at 80 nM for 24 h and then cells were cultured in basal M199 medium with 50 μM ABO for 24 h. qPCR analysis of CERNA1 RNA level (data are mean ± SEM, $*p < 0.05$ vs. control (Ctr), n ≥ 3)

of CERNA1 was detected by qPCR. After TIA1 knockdown, the expression of CERNA1 was significantly reduced (Fig. 6a). Then we analyzed the total protein levels and the serine phosphorylation levels of TIA1 after ABO reduced the serum and FGF-2 starvation-induced VECs apoptosis. The western results showed that, consistent with previous

research, ABO obviously increased the total TIA1 protein level and significantly decreased the TIA1 phosphorylation ratio (Supplementary Fig.S2). Furthermore, we investigated the role of TIA1 in ABO-induced upregulation of CERNA1 expression. The RNA level of CERNA1 was significantly boosted after ABO alone treatment. Whereas, compared with ABO alone treatment, reduced TIA1 in HUVECs with ABO treatment led to decreased the expression of CERNA1 (Fig. 6b). Taken together, the data suggested that ANXA7 inhibitor-ABO promoted CERNA1 expression in TIA1 depended way.

ABO specifically promoted the cytoplasm distribution of CERNA1 in VECs

For further investigation whether the alternation of the relative cellular distribution come from nuclear–cytoplasmic trafficking or specifically localizing in cytoplasm/nucleus of VECs, we respectively detected the total, cytoplasmic or nuclear CERNA level under different apoptosis states. As mentioned above, (Nor) HUVECs were normally cultured; (Ctr) The serum and FGF-2 starvation treatment induced the apoptosis of VECs [10]; (ABO) ABO treatment reduced the serum and FGF-2 starvation-induced apoptosis [10]. These results suggested that, compared to Ctr group, total CERNA1 level in ABO group was boosted (Fig. 5b); moreover, the elevated CERNA1 was mainly localized in cytoplasm, not in nucleus of VECs (Fig. 5c, d). Therefore, ABO inhibited the serum and FGF-2 starvation-induced apoptosis of VECs by specifically improving the abundance of CERNA1 in the cytoplasm.

Discussion

According to the subcellular location of long noncoding RNA (lncRNA), the molecular regulation mechanisms are various [3, 4]. Long noncoding RNA-CERNA1 located both in cytoplasm and nucleus of VECs. Our previous work showed that cytoplasmic CERNA1 suppressed VECs apoptosis through ceRNA pathway: CERNA1 promoted the expression of two apoptosis inhibitors, API5 and BCL2L12, by sponging miR-4707-5p and miR-4767 [10]. And this study suggested that CERNA1 promoted VECs apoptosis by epigenetic regulation pathway: CERNA1 increased H3K4me3 and H3K9ac modification on the promoter of apoptosis enhancer-BCL2L10, and then enhanced its expression (Fig. 7). We further proved that ANXA7 inhibitor-ABO determined the cell fate, not only by enhancing CERNA1 expression, but also by specifically promoted its cytoplasmic localization.

The molecular action mechanisms of lncRNAs have been classified into many categories such as guide molecule, signal molecule, scaffold molecule and so on [4, 19]. Yet, the basic biological chemistry mechanisms of lncRNAs are mainly dependent on the following three types: (1) the interaction between RNA and RNA; (2) the interaction between RNA and DNA; (3) the interaction between RNA and protein [4, 20].

In the cytoplasm, the main interactions are between lncRNAs and RNA or between lncRNAs and protein [20]. Uchl1 is associated with brain function and neurodegenerative disease. A lncRNA, which is antisense to Uchl1, promoted Uch1 translation by binding with Uch1 mRNA and

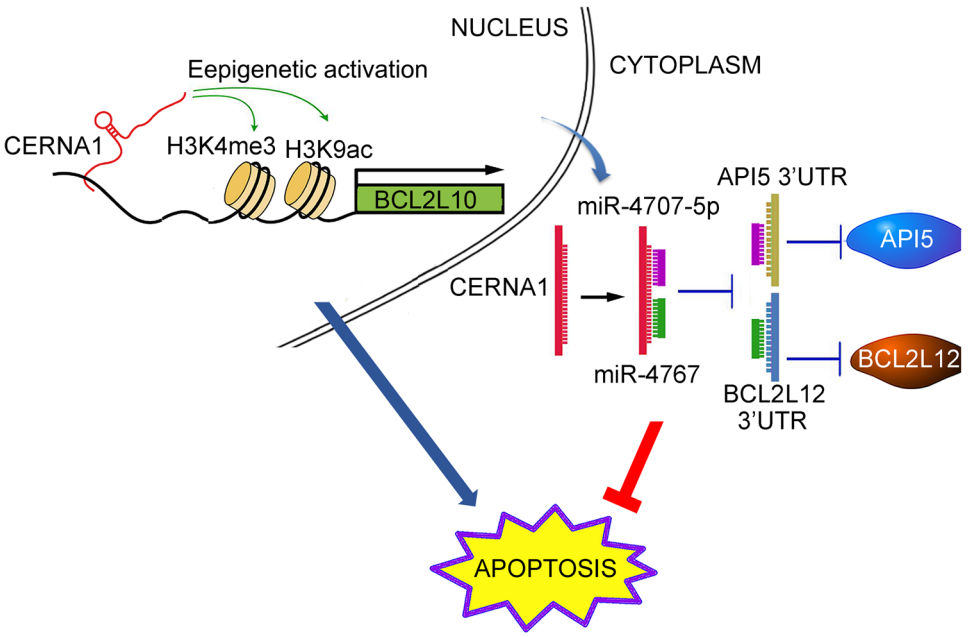

Fig. 7 Conceptual schematic of CERNA1-related regulatory mechanism of VEC apoptosis. ceRNA pathway: CERNA1, functioned as a ceRNA to bind with miR-4707-5p and miR-4767, regulates the expression of their targets (API5 and BCL2L12) and further suppresses apoptosis in HUVECs; Epigenetic pathway: CERNA1 positively regulated BCL2L10, which accelerates the serum and FGF-2 starvation-induced apoptosis of VECs, by increasing the histone modification level of H3K9ac and H3K4me3 in BCL2L10 promoter region

polysome in the cytoplasm [21]. The lincRNA-p21 interplayed with the target mRNA in the cytoplasm, thus inhibited its translation [22]. In addition, lincRNA-MD1, acted as ceRNA, regulated muscle differentiation by sponging miR-133 [23]. Hence, for cytoplasmic CERNA1, two types of interactions have been identified: one is that CERNA1 could sponge with miR-4707-5p and miR-4767; the interaction between CERNA1 and AGO2 is the other [10].

In the nucleus, lncRNAs usually work as a guide molecule or a skeleton molecule to recruit epigenetic modifiers, thus control gene expression. During this process, the main interactions are between lncRNAs and DNA or between lncRNAs and protein [20]. And a growing number of nucleoprotein involved in the interaction between lncRNAs and protein, including hnRNP (hnRNP-K and lincRNA-p21, hnRNP-A/B and lincRNA-Cox2, hnRNPL and lncRNA THRIL), transcriptional factor (CTCF and lncRNA Jpx, NF-κB and lncRNA Lethe/PACER) and epigenetic modifiers (WDR5 and lncRNA NeST, PRC2 and HOTAIR) [24–30]. Nuclear lncRNAs are either direct or indirect through some specific protein interact with their target sequence in genome [31–33]. Therefore, combined with our results, it is possible that the DNA target sequence of nuclear CERNA1 is BCL2L10 promoter region and CERNA1 acts as a scaffold molecule between BCL2L10 promoter region and epigenetic modifier. In our next investigation, we will verify the interaction between CERNA1 and BCL2L10 promoter region, and then identify the corresponding nucleoproteins.

The nucleocytoplasmic distribution of lncRNAs was affected by many factors, such as RNA motif and some regulators. It had been reported that SRm160, IBP160, and RNPS1 promoted nuclear speckle localization of MALAT-1 [34, 35]. In our study, we found ANXA7 inhibitor-ABO promoted the cytoplasmic localization of CERNA1 under the starvation of serum and FGF-2 in VECs. We firstly demonstrated that ABO inhibited the apoptosis of vascular endothelial cells by specifically improving the abundance of CERNA1 in the cytoplasm. It provided a powerful research tool for us to further study the regulation mechanism of CERNA1 cellular distribution.

Taken together, the role of CERNA1 in apoptosis regulation of VECs is a double-edged sword: cytoplasmic CERNA1 inhibited the serum and FGF-2 starvation-induced apoptosis by ceRNA pathway; while, nuclear CERNA1 enhanced it by epigenetic pathway. The paradoxical function of CERNA1 in apoptosis is very similar to the bi-directional regulation of p53 in autophagy [36–38]. Nuclear p53 stimulates the autophagy pathway and thereby sustaining the ability of cells to cope with stress; whereas, cytoplasmic p53 inhibits autophagy and hence facilitates cell death [36–38]. To distinguish the role of p53 in nucleus or cytoplasm, nuclear localization sequence or nuclear export signal was deleted from p53 DNA sequence [38, 39]. Unlike protein, lncRNAs do not possess the distinct nuclear localization sequence or nuclear export signal, so that it is hard to study on the distinct functions of CERNA1 in different subcellular location. However, it was fortunately found that ABO not only enhanced the expression of CERNA1 through TIA1, but also promoted the cytoplasm distribution of CERNA1. Since cytoplasmic CERNA1 is an attractive target to improve endothelial function, our finding also provided new clues for drug design of VECs apoptosis related cardiovascular diseases.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All experimental procedures were performed in accordance with the ARRIVE guidelines [40] and approved by the ethics committee in Shandong University.

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