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Research Article

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ERα promotes transcription of tumor suppressor gene *ApoA-I* by establishing H3K27ac-enriched chromatin microenvironment in breast cancer cells

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Abstract: Apolipoprotein A-I (ApoA-I), the main protein component of high-density lipoprotein (HDL), plays a pivotal role in reverse cholesterol transport (RCT). Previous studies indicated a reduction of serum ApoA-I levels in various types of cancer, suggesting ApoA-I as a potential cancer biomarker. Herein, ectopically overexpressed ApoA-I in MDA-MB-231 breast cancer cells was observed to have antitumor effects, inhibiting cell proliferation and migration. Subsequent studies on the mechanism of expression regulation revealed that estradiol (E2)/estrogen receptor α (ERα) signaling activates *ApoA-I* gene transcription in breast cancer cells. Mechanistically, our ChIP-seq data showed that ERα directly binds to the estrogen response element (ERE) site within the *ApoA-I* gene and establishes an acetylation of histone 3 lysine 27 (H3K27ac)-enriched chromatin microenvironment. Conversely, Fulvestrant (ICI 182780) treatment blocked ERα binding to ERE within the *ApoA-I* gene and downregulated the H3K27ac level on the *ApoA-I* gene. Treatment with p300 inhibitor also significantly decreased the *ApoA-I* messenger RNA (mRNA) level in MCF7 cells. Furthermore, the analysis of data from The Cancer Genome Atlas (TCGA) revealed a positive correlation between ERα and *ApoA-I* expression in breast cancer tissues. Taken together, our study not only revealed the antitumor potential of ApoA-I at the cellular level, but also found that ERα promotes the transcription of *ApoA-I* gene through direct genomic effects, and p300 may act as a co-activator of ERα in this process.

Key words: Apolipoprotein A-I (ApoA-I); Estrogen receptor α (ERα); Acetylation of histone 3 lysine 27 (H3K27ac); p300; Breast cancer

1 Introduction

According to the 2020 World Health Organization (WHO) report, the number of new breast cancer cases surpassed that of lung cancer for the first time, which means that breast cancer had become the world's most common cancer. Based on molecular characteristics, breast cancers are divided into hormone receptor (estrogen receptor (ER)/progesterone receptor (PR))-positive/Erb-B2 receptor tyrosine kinase 2 (ERBB2)-negative (70% of patients), ERBB2-positive (15%–20%), and triple-negative subtypes (15%) (Waks and Winer, 2019).

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Estrogen signaling modulates a variety of physiological processes through the direct or indirect regulation of target gene transcription (Vrtačnik et al., 2014). The abnormal overactivation of ERs may lead to the development of breast cancer, and the specific underlying mechanisms include: (1) amplification of ER coactivators (Anzick et al., 1997); (2) inhibition of ER corepressors (Mussi et al., 2006); (3) overexpressed bridge proteins promoting the recruitment of coactivators by ERs (Zwijsen et al., 1998; McMahon et al., 1999); (4) the mutations of ER enabling it to be activated at a lower concentration of estrogen (Herynk and Fuqua, 2004); and (5) ER localization on the plasma membrane and the subsequent signaling cascade activation (Levin and Pietras, 2008). Breast cancer patients with ERα-positive tumors are treated with endocrine therapy; however, most of them will develop endocrine resistance (Hanker et al.,

2020). This prompts us to conduct a deeper investigation about the complex role of ERα in breast cancer. It has been reported that ERα can inhibit programmed death-ligand 1 (*PD-L1*) expression in breast cancer cells (Liu et al., 2018), which suggests the double-edged sword role of ERα. Investigations of ERα regulation of cancer-related genes will contribute to the clinical treatment of breast cancer.

Apolipoprotein A-I (ApoA-I) is the main protein component of high-density lipoprotein (HDL) (Shao and Heinecke, 2018). In addition to its well-known function in reverse cholesterol transport (RCT) and protective effect against cardiovascular diseases, ApoA-I is involved in inflammatory and immune responses (Gordon et al., 2011). Many recent studies have discovered a reduction of serum ApoA-I in various types of cancer (Georgila et al., 2019). The decrease of serum ApoA-I level has been related to the progression and metastasis of breast cancer (Gonçalves et al., 2006). Besides, a positive correlation between the level of serum ApoA-I secreted in tumor tissue fluid and chemotherapy sensitivity was observed in breast cancer (Cortesi et al., 2009; Zhang et al., 2016). ApoA-I contains ten consecutive helical domains, which are essential for the biophysical properties of proteins that spontaneously dissolve lipids in an aqueous environment (Li et al., 1988). Based on the characteristics of these amphiphilic helical sequences, researchers have synthesized various peptides that do not share any sequence homology, but all mimic the function of ApoA-I (Reddy et al., 2014). The ApoA-I mimetic peptide D-4F has been proved effective in reducing proliferative response induced by oxidized low-density lipoprotein (oxLDL) in human breast adenocarcinoma cells (Cedó et al., 2016).

Although numerous studies have shown that ApoA-I is a potential biomarker with antitumor effects, the regulatory mechanism of its expression in breast cancer remains unclear. Exploring the regulation of *ApoA-I* expression will help us to better manipulate its level and function, which may contribute to prevention and treatment strategies for breast cancer. Herein, we explored the biological effect of ApoA-I on breast cancer cells and uncovered the effect of estradiol (E2)/ERα signaling on *ApoA-I* transcription in breast cancer.

2 Materials and methods

2.1 Vector construction and cell transfection

The human breast cancer cell lines MCF7 and MDA-MB-231 were purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, China). The human breast cancer cell line BT549 was obtained from Dr. Sulin LIU (Fudan University Cancer Hospital, Shanghai, China). The 293T cell line was kindly provided by Dr. Degui CHEN (Shanghai Institution of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China). All cell lines used in this study were cultured in Dulbecco's modified Eagle's medium (DMEM) high-glucose medium (HyClone, Marlborough, MA, USA) supplemented with 10% (volume fraction) fetal bovine serum (FBS; BI, Israel) and 1% (volume fraction) penicillin/streptomycin (Gibco, Carlsbad, CA, USA). The cells were placed in a humidified incubator at 37 °C under 5% CO_2 atmosphere. The following compounds were used in the corresponding experiments: 500 nmol/L ICI 182780 treatment for 7 d (Fulvestrant, Sigma-Aldrich, Darmstadt, Germany); 1 nmol/L E2 treatment for 2 d (Sigma-Aldrich) after 5 d of hormone deprivation; 20 μmol/L histone acetyltransferase inhibitor II (Selleck, Houston, Texas, USA).

2.2 Cell culture and treatment procedure

The coding sequences (CDSs) of *ApoA-I* and *ERα* amplified from the complementary DNAs (cDNAs) of MCF7 cells were separately inserted into a pLenti6.2/v5/TEV vector (Clonetech, Waltham, MA, USA). The recombinant plasmids and packaging plasmids were transfected into 293T cells using Lipofectamine™ 2000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After 48 h of transfection, the cell supernatant was collected and filtered. Virus-containing supernatant containing 0.1% (volume fraction) polybrene (Sigma-Aldrich) was added to 1.5×10^6 MDA-MB-231 cells seeded in a six-well plate. After 48 h of infection, cells were screened using 10 μg/mL Blasticidin S HCl (Gibco).

2.3 qRT-PCR analysis

Total RNA was extracted from breast cancer cells using the TRIzol® reagent (Ambion, Life Technologies, Carlsbad, CA, USA) and quantified by a NanoDrop 2000 spectrophotometer (Thermo Scientific,

Waltham, MA, USA). Total RNA was reverse-transcribed into cDNA using a Primescript™ RT Reagent Kit with genomic DNA (gDNA) eraser (TaKaRa, Kusatsu, Japan) according to the manufacturer's protocol. The cDNA was subjected to quantitative real-time polymerase chain reaction (qRT-PCR) amplification by using the SYBR Green Master Mix (Roche, Basel, Switzerland) and performing in triplicates on a Roche LightCycler 480 II system. Table 1 shows the primer sequences used in this study.

Table 1 Primer sequences of target gens in this study

Target gene	Primer sequence (5'→3')
GAPDH	F: CTGACTTCAACAGCGACACC
	R: GTGGTCCAGGGGTCTTACTC
ApoA-I	F: TGAGGCTCTCAAGGAGAACG
	R: CCTCACTGGGTGTTGAGCTT
GREB1	F: GACCAGCTTCTGATCACCCC
	R: TACCTAAAGCCGATGGTCGC
ERα	F: CCTCCTCATCCTCTCCCACA
	R: ATGCGATGAAGTAGAGCCCG
*ApoA-I*_ChIP-qPCR	F: GCCTTCAAACTGGGACACAT
	R: CATTTCTGGCAGCAAGATGA

GAPDH: glyceraldehyde-3-phosphate dehydrogenase; *ApoA-I*: apolipoprotein A-I; *GREB1*: growth regulation by estrogen in breast cancer 1; *ERα*: estrogen receptor α; F: forward; R: reverse.

2.4 Western blot

Cells were harvested and lysed using sodium dodecyl sulfate (SDS) lysis buffer containing protease inhibitors. Equal amounts of proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred onto nitrocellulose membrane using a wet-transfer system (Bio-Rad, Hercules, CA, USA). After 1 h of blocking, the membranes were placed in bovine serum albumin (BSA) solution at 4 °C overnight with the following primary antibodies: anti-β-actin (Proteintech, Rosemont, IL, USA; 66009-1-Ig), anti-vinculin (Cell Signaling Technology, Boston, MA, USA; 13901), anti-ApoA-I (Abcam, Cambridge, UK; ab52945), anti-ERα (Cell Signaling Technology; 8644), and anti-H3K27ac (Active Motif, Shanghai, China; 39133). After washing with Tris-buffered saline (TBS)/Tween buffer, the appropriate secondary antibodies (L3012: horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG) secondary antibody; L3032: HRP-conjugated goat anti-mouse IgG secondary antibody) were used for blocking at room temperature for 1 h. The expression levels of proteins were detected by the ChemiDoc Touch Imaging System (Bio-Rad).

2.5 CCK-8 and transwell assay

The cell counting kit-8 (CCK-8) experiments were carried out with 2000 cells in accordance with the kit instructions (Enhanced Cell Counting Kit-8; Beyotime, Shanghai, China). Specifically, 2000 cells were planted in each well of the 96-well plate. After 0, 1, 2, 3, and 4 d, the medium was removed, and 100 μL of medium containing 10 μL CCK-8 solution was added to react in the cell incubator for 2 h. The absorbance was subsequently measured at 450 nm.

For the transwell assay, 5×10^4 cells were placed on the upper layer of a cell culture insert with a cell permeable membrane (Millicell Hanging Cell Culture Inserts, 8.0 μm polyethylene terephthalate (PET); Millipore, Massachusetts, USA), and DMEM high-glucose medium containing 20% (volume fraction) FBS (BI) and 1% (volume fraction) penicillin/streptomycin (Gibco) was placed below the membrane. Following an 8-h incubation period, the cells that had migrated through the membrane were stained and counted.

2.6 ChIP-seq and ChIP-qPCR

This experiment was performed as previously described (Kong et al., 2016). In particular, the monolayer of cultured cells was fixed with 1% (volume fraction) formaldehyde for 8 min, which process was terminated by 2.5 mol/L glycine solution for 5 min. After washing with phosphate-buffered saline (PBS), cells were harvested and lysed using a cocktail containing high-salt and lysis buffer (50 mmol/L 4-(2-hydroxyerhyl) piperazine-1-erhanesulfonic acid (HEPES; pH 7.5), 500 mmol/L NaCl, 1 mmol/L ethylenediamine tetraacetic acid (EDTA), 0.001 g/mL Na-deoxycholate, 1% (volume fraction) Triton X-100, 0.001 g/mL SDS) (Roche). Next, the cell lysate was sonicated for 30 min (30 s on, 40 s off, 95% amplify) using a Bioruptor® sonicator (Diagenode, Belgium). The 10% (volume fraction) supernatant was taken out as the control group. The 2 μL of ERα or H3K27ac antibody (Active Motif) was added to the remaining sonicated chromatin for rotating overnight at 4 °C. Then, 15 μL protein A/G magnetic beads (Invitrogen) were added to bind to the antibody at 4 °C for 2 h. The beads were washed according to the following method: thrice with high-salt and lysis buffer, twice with low-salt buffer (10 mmol/L Tris-Cl

(pH 8.0), 250 mmol/L LiCl, 1 mmol/L EDTA, 0.001g/mL Na-deoxycholate, and 0.5% (volume fraction) NP-40), and once with TE buffer (2 mmol/L EDTA, 10 mmol/L Tris-Cl (pH 8.0)). The "beads" and the control group were resuspended in 100 μL elution and de-crosslink buffer (50 mmol/L Tris-Cl (pH 8.0), 10 mmol/L EDTA, and 0.01g/mL SDS) and incubated at 65 ℃ on the oscillator for 6 h. Chromatin was eluted by 60 μL of DNA elution buffer (10 mmol/L Tris-Cl, pH 8.0) containing 20 μg of proteinase A for 2 h at 37 ℃ and 20 μg of protease K for 1 h at 55 ℃. The DNA was purified using a MinElute® PCR purification kit (Qiagen, Valencia, CA, USA). A ChIP-seq library was constructed using KAPA HyperPrep Kits (KAPA, Boston, MA, USA) according to the manufacture's protocol. Sequencing was completed by Basepair Biotechnology Co., Ltd. (Suzhou, China). The ChIP-qPCR experiment was performed as described above.

Our own raw and processed nucleic acid sequencing data (ERα and H3K27ac ChIP-seq) were deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo) under the accession number GSE136673.

2.7 TCGA data analysis

The data analysis was carried out by the method described in the previous article published by our group (Liu et al., 2018). In short, The Cancer Genome Atlas (TCGA) data were downloaded from http://www.cbioportal.org. The expression of ERα in tumor samples was determined based on clinical data.

2.8 Statistical analysis

The results derived from three independent experiments were presented as mean±standard deviation (SD). The significance of the data was analyzed with GraphPad Prism 8.2.1 by a two-tailed unpaired *t*-test. The difference was considered significant when $P<0.05$.

3 Results

3.1 Proliferation and migration of MDA-MB-231 cells inhibited by ectopic overexpression of ApoA-I

In order to investigate the effect of ApoA-I on the biological behavior of breast cancer cells, we first determined the messenger RNA (mRNA) levels of *ApoA-I* in three breast cancer cell lines (Fig. 1a). Since the mRNA level of *ApoA-I* in MDA-MB-231 cells is the lowest, we constructed an ectopic ApoA-I-overexpression system in MDA-MB-231 cells (Fig. 1b). Then, we performed CCK-8 and transwell experiments to detect the effect of ApoA-I overexpression on MDA-MB-231 cells. The CCK-8 test result showed that the proliferation rate of ApoA-I-overexpressed MDA-MB-231 cells was significantly lower than that of the control group (Fig. 1c). Furthermore, ApoA-I inhibited the migration of MDA-MB-231 cells (Figs. 1d and 1e). This result indicated that ApoA-I may be a tumor suppressor that can inhibit the proliferation and migration of breast cancer cells.

3.2 ApoA-I transcription induced by E2/ERα signaling in breast cancer cells

In order to explore whether E2/ERα signaling modulates *ApoA-I* transcription, we exposed ERα-positive MCF7 cells to estrogen antagonist ICI 182780 to abolish the ERα functions. Accompanied by a decrease of ERα (Fig. 2a), the mRNA levels of growth regulation by estrogen in breast cancer 1 (*GREB1*; the classic ERα target gene) and *ApoA-I* in MCF7 cells were significantly downregulated by ICI 182780 (Fig. 2b).

Given that the ER inhibitor downregulated the *ApoA-I* mRNA level in MCF7 cells, we speculated that the activation of ERα signaling would promote the transcription of *ApoA-I*. We performed hormone deprivation on ER-positive MCF7 cells, and then added an equal volume of ethanol (EtOH, vehicle control) or E2. Our results showed that the transcription of both *GREB1* and *ApoA-I* was dramatically upregulated by E2 (Fig. 2c). Compared with ER-positive MCF7 cells, triple-negative BT549 and MDA-MB-231 cells had lower *ApoA-I* mRNA levels (Fig. 1a). We hypothesized that ectopic expression of ERα would also activate *ApoA-I* transcription in ER-negative breast cancer cells. As expected, the western blotting and qRT-qPCR data showed that the restoration of ERα expression in MDA-MB-231 cells upregulated the mRNA level of *ApoA-I* (Figs. 2d and 2e).

3.3 ERα directly binds to last exon of *ApoA-I* gene

In order to examine whether ERα has a direct role in regulating *ApoA-I* transcription, ChIP-seq was conducted using anti-ERα antibodies. We observed that

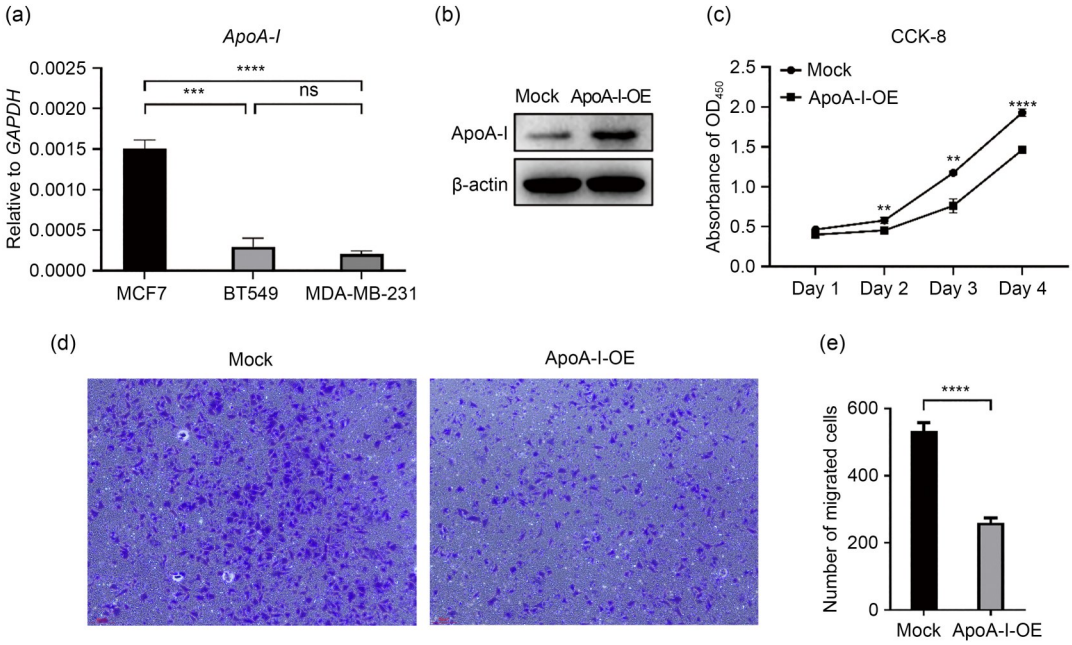

Fig. 1 Apolipoprotein A-I (ApoA-I) inhibits the proliferation and migration of MDA-MB-231 cells. (a) The *ApoA-I* messenger RNA (mRNA) levels in three different breast cancer cell lines were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). (b) The protein levels of ApoA-I in mock (control) and ApoA-I-overexpressed (OE) MDA-MB-231 cells were analyzed by western blotting. (c) The proliferation rates of mock and ApoA-I-OE MDA-MB-231 cells were analyzed by cell counting kit-8 (CCK-8). (d) Stained images of cells passing through the pore show the representative data of three independent transwell experiments. The scale bar is 50 μm. (e) Statistics of the number of cells passing through the pore in three independent transwell experiments. All data are derived from three independent experiments and presented as mean±standard deviation (SD). $^{ns}P\geqslant0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$. *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase; OD_{450}: optical density at 450 nm.

ERα was enriched in the last exon of the *ApoA-I* gene in both MCF7 and ERα-overexpressed MDA-MB-231 cells (Fig. 3a). Notably, when challenging hormone-depleted MCF7 cells with E2, the ChIP signal intensity of ERα on the *ApoA-I* gene was more significant than that of hormone-depleted MCF7 cells treated with EtOH (Fig. 3a). In addition, by analyzing the ERα-enriched region, we found that an estrogen response element (ERE) was located right in the center of the ERα peak (Fig. 3b).

3.4 p300-mediated H3K27ac is involved in the transcriptional regulation of *ApoA-I* through ERα

Aimed at exploring the mechanism by which ERα regulates *ApoA-I* transcription, we analyzed the epigenetic characteristics of the *ApoA-I* gene. The increased H3K27ac signal intensity was observed in hormone-depleted MCF7 cells when treated with E2 (Fig. 4a). To further investigate the relationship between ERα and H3K27ac on the *ApoA-I* gene, we performed ChIP-qPCR using anti-H3K27ac antibodies. Our data showed that ICI 182780 significantly reduced the abundance of H3K27ac on the *ApoA-I* gene in MCF7 cells (Fig. 4b). These data suggested that the binding of ERα on the *ApoA-I* gene is positively correlated with the abundance of H3K27ac modification.

Subsequently, we tested the role of acetyltransferase p300 in this process. With the intervention of p300 inhibitor (p300i), the mRNA level of *ApoA-I* decreased significantly along with the decline of H3K27ac level (Figs. 4c and 4d). Specifically, challenging MCF7 cells with p300i for 24 and 72 h made the *ApoA-I* mRNA level drop by more than 90% and 98%, respectively (Fig. 4d).

3.5 Positive correlation between mRNA expression levels of *ERα* and *ApoA-I* in breast cancer

In order to support our findings with clinical data, we analyzed the TCGA data for breast cancer (TCGA-BRCA) uploaded by Perou (Ciriello et al., 2015). According to the examined clinical data, we separated samples into ERα-negative and ERα-positive groups

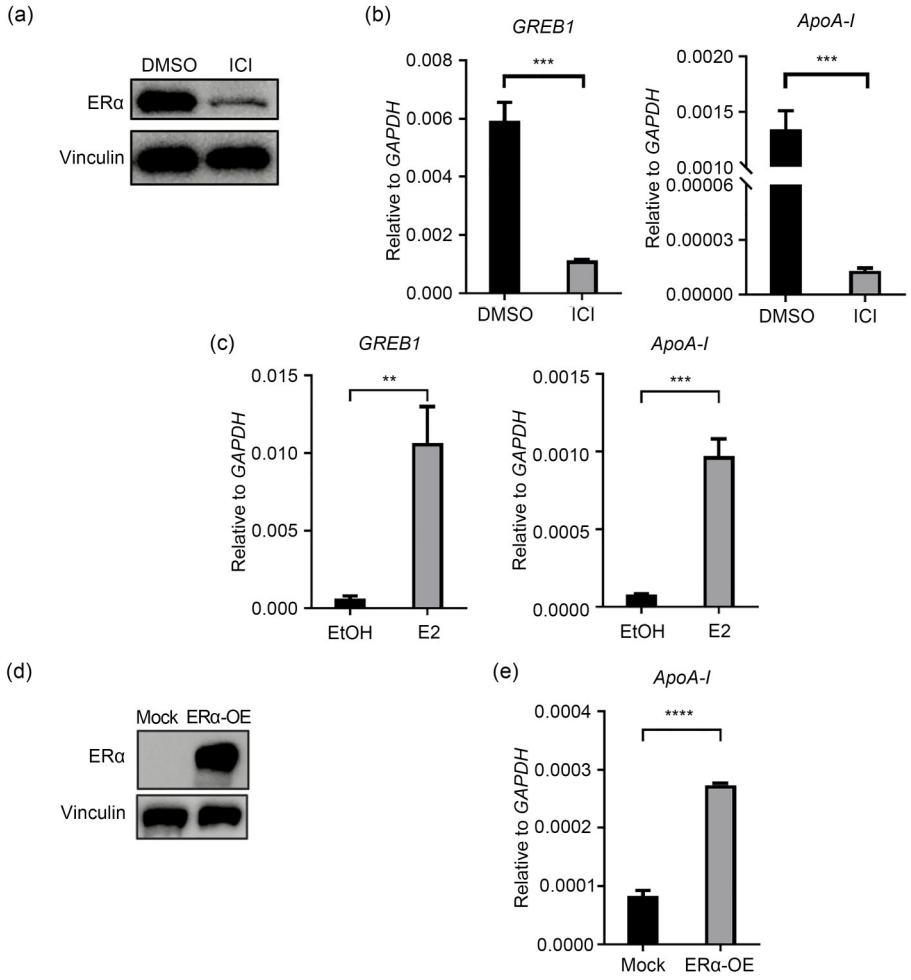

Fig. 2 Estradiol (E2)/estrogen receptor α (ERα) signaling induces apolipoprotein A-I (*ApoA-I*) transcription in breast cancer cells. (a) The ERα protein levels in dimethyl sulfoxide (DMSO) or ICI 182780-treated MCF7 cells were analyzed by western blotting. (b) Messenger RNA (mRNA) levels of growth regulation by estrogen in breast cancer 1 (*GREB1*) and *ApoA-I* in DMSO or ICI 182780-treated MCF7 cells were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). (c) *GREB1* and *ApoA-I* mRNA levels in hormone-depleted MCF7 cells treated with ethanol (EtOH) or E2 were analyzed by qRT-PCR. (d) The expression levels of ERα in mock (control) and ERα-overexpressed (OE) MDA-MB-231 cells were analyzed by western blotting. (e) The *ApoA-I* mRNA levels in mock and ERα-OE MDA-MB-231 cells were analyzed by qRT-PCR. All data are derived from three independent experiments and presented as mean±standard deviation (SD). ** *P*<0.01, *** *P*<0.001, **** *P*<0.0001. *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase.

(Fig. 5a). The average mRNA level of *ApoA-I* in the ERα-positive group was significantly higher than that in the ERα-negative group (Fig. 5b). Moreover, the regression analysis revealed a positive correlation between the mRNA expression levels of *ERα* and *ApoA-I* (Fig. 5c).

4 Discussion

Numerous studies have suggested the downregulation of serum and tissue ApoA-I in various cancer types (Zamanian-Daryoush and Didonato, 2015). Therefore, it is of high importance to understand the role of this phenomenon and the associated regulatory mechanism. In this study, we demonstrated that ApoA-I can inhibit the proliferation and migration of breast cancer cells. Moreover, ERα could directly bind to the last exon of *ApoA-I* gene to active its transcription, and p300 might be a co-activator of ERα by establishing an H3K27ac-enriched chromatin microenvironment in this process.

The ApoA-I mimetic peptide D-4F has been proven to inhibit the proliferation of human breast cancer cells (Peng et al., 2017). We found that the direct overexpression of human ApoA-I protein arrived at

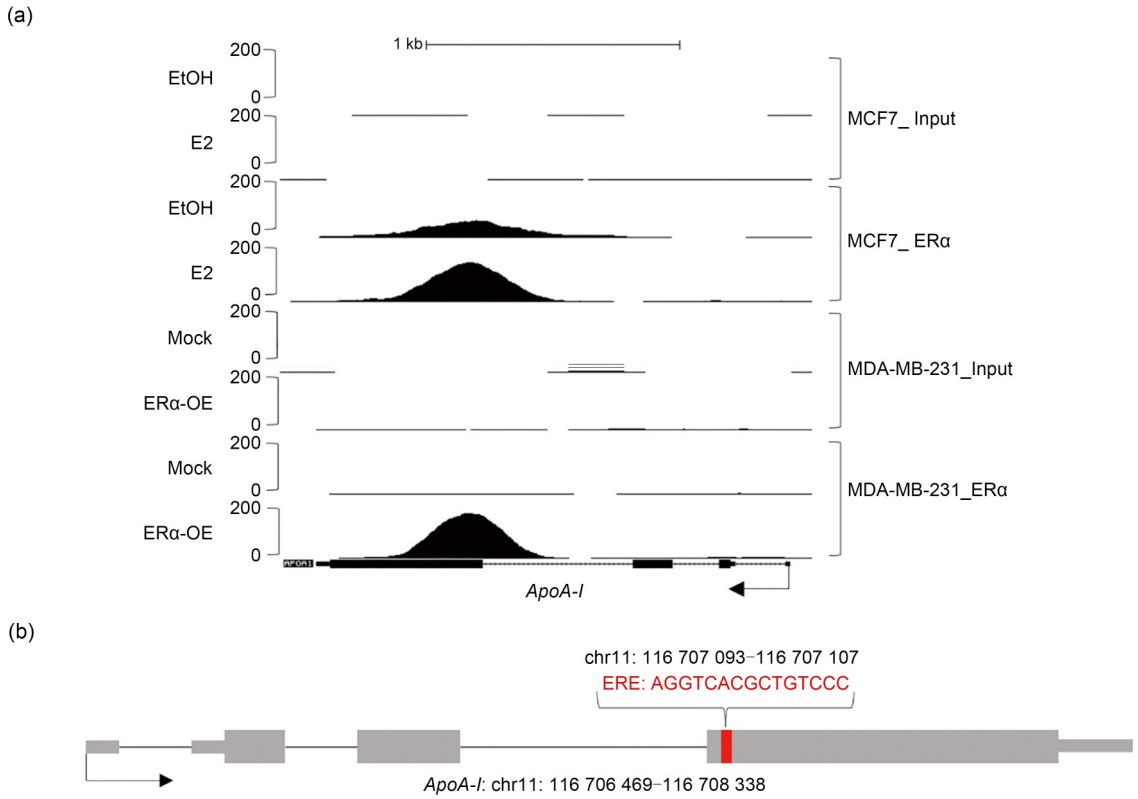

Fig. 3 Estrogen receptor α (ERα) directly binds to last exon of apolipoprotein A-I (*ApoA-I*) gene. (a) UCSC (University of California, Santa Cruz) Genome Brower tracks for *ApoA-I* from ChIP-seq analysis showing representative data of three independent experiments; (b) Schematic diagram of estrogen response element (ERE) sequence and its location on *ApoA-I* gene. ERE is marked in red. EtOH: ethanol; E2: estradiol; ERα-OE: ERα-overexpressed.

the same result. The antitumor effect of ApoA-I may be achieved through a cellular autonomic mechanism, or its impact on immune responses (Gordon et al., 2011), or both, which remains an elusive topic. In the cellular autonomic mechanism, ApoA-I can induce changes in the expression of cancer-related genes, thus producing anti-cancer effects. For example, ApoA-I induces the downregulation of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF)-1α expression, thereby inhibiting the proliferation and migration of ovarian cancer cells (Gao et al., 2011, 2012). In colorectal adenocarcinoma cells, ApoA-I inhibits the activity of cancer cells by downregulating cyclooxygenase-2 (COX-2) expression (Aguirre-Portolés et al., 2018). In our in vitro model, the anti-cancer effect is more likely to be achieved through the cellular autonomic mechanism due to the absence of an immune system. Nonetheless, the relevant specific mechanism remains to be further studied. Although most researches have indicated that ApoA-I levels are negatively correlated with the occurrence and progression of various types of cancer, there are also reports showing a positive correlation (Chen et al., 2013; Martin et al., 2015; Shi et al., 2018; Zografos et al., 2019). It is not clear whether the positive correlation between ApoA-I levels and cancer parameters as reported by a number of studies reflects the cancer-promoting effects of ApoA-I in these special cases.

Many transcription factors implicated in the regulation of *ApoA-I* promotor have been identified, including peroxisome proliferator-activated receptor γ (PPARγ), hepatocyte nuclear factor 4 (HNF4), liver receptor homologue-1 (LRH1), and ApoA-I regulatory protein 1 (ARP1)/nuclear receptor subfamily 2 group F member 2 (NR2F2) (Kardassis et al., 2014). Besides, an endogenously expressed long noncoding antisense transcript, *ApoA1-AS*, can also modulate *ApoA-I* transcription through recruiting histone methylation enzymes (Halley et al., 2014). Herein, for the first time, ERα was demonstrated to be an activator for *ApoA-I* gene transcription in breast cancer cells: we observed a positive correlation between the expression

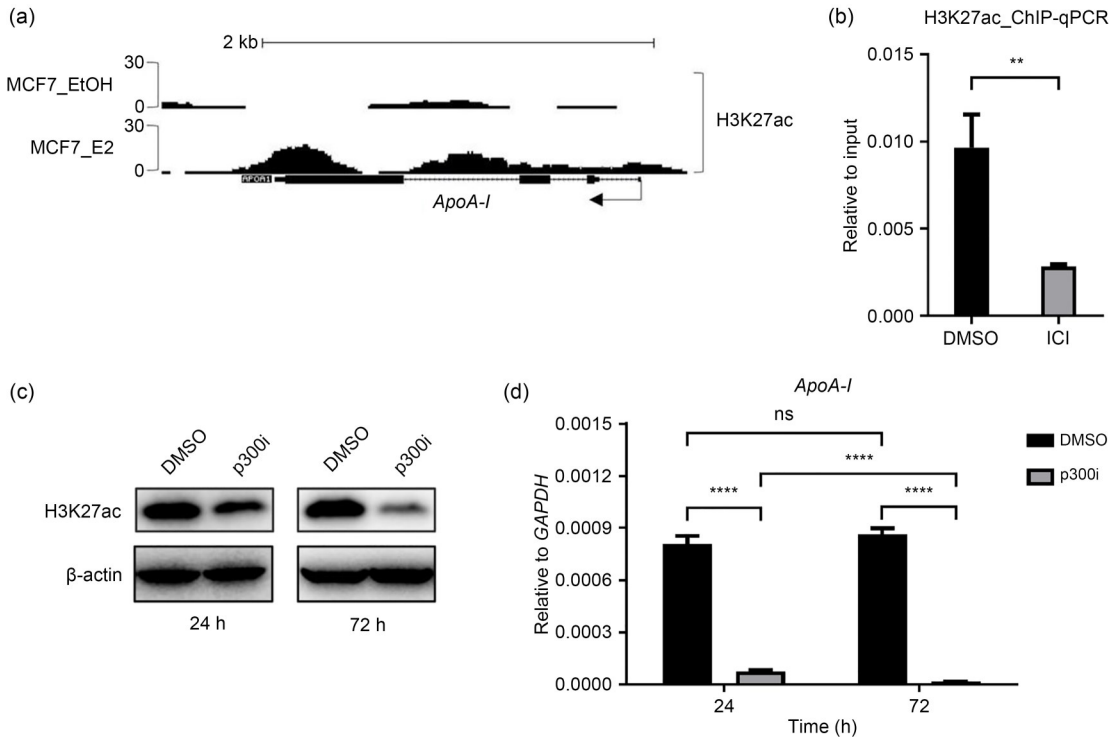

Fig. 4 p300-mediated acetylation of histone 3 lysine 27 (H3K27ac) is involved in the transcriptional regulation of apolipoprotein A-I (*ApoA-I*) by estrogen receptor α (ERα). (a) UCSC (University of California, Santa Cruz) Genome Brower tracks for *ApoA-I* from ChIP-seq analysis showing representative data of two independent experiments. Hormone-depleted MCF7 cells were treated with EtOH (MCF7_EtOH) or E2 (MCF7_E2) for 24 h. (b) ChIP-qPCR analysis of H3K27ac abundance on *ApoA-I* gene in MCF7 cells treated with dimethyl sulfoxide (DMSO) or ICI 182780 for 7 d. (c) Western blot analysis of the H3K27ac level in MCF7 cells treated with DMSO or p300 inhibitor (p300i) for 24 and 72 h. (d) Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of *ApoA-I* messenger RNA (mRNA) levels in MCF7 cells treated with DMSO or p300i for 24 and 72 h. All data are derived from three independent experiments and presented as mean±standard deviation (SD). $^{ns}P\geqslant0.05$, $^{**}P<0.01$, $^{****}P<0.0001$. EtOH: ethanol; E2: estradiol; *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase.

of *ERα* and *ApoA-I* mRNAs, in both breast cancer cells cultured in vitro and breast tumor samples.

Estrogen can exert its function through both genomic and non-genomic signaling. Our ChIP-seq data displayed an ERα enrichment signal within the *ApoA-I* gene, suggesting that ERα promoted the transcription of *ApoA-I* gene through genomic signaling. Genomic signaling can be divided into direct and indirect types. In direct genomic signaling, dimerized ERα binds directly to specific DNA sequences known as EREs (Klinge, 2001). By analyzing the sequence of ERα enrichment locus on the *ApoA-I* gene, we found the sequence AGGTCACGCTGTCCC that has proved to be an ERE (Bourdeau et al., 2004). ERα seems to modulate *ApoA-I* transcription through direct genomic signaling, while this is suggested by the experiment through the process focusing on the influence of ERE sequence mutation.

Recruiting various co-regulators to form an ER complex is the intrinsic mechanism of estrogen signaling (Heldring et al., 2007). The activity of ERs depends on co-regulators within this complex. We found that H3K27ac abundance on the *ApoA-I* gene is tightly dependent on ERα. Moreover, the inhibitor of acetyltransferase p300 could suppress *ApoA-I* mRNA expression. We speculate that histone acetyltransferase p300 may be a co-activator of ERα by making the chromatin region containing *ApoA-I* more accessible to transcriptional factors. A recent study revealed that Ajuba, the LIM protein in MCF7 and T47D breast cancer cells, could recruit deleted in breast cancer 1 (DBC1) and cAMP-regulated enhancer-binding protein (CREB)-binding protein (CBP)/p300 to enhance the regulatory activity of ERα on target genes by acetylating ERα (Xu et al., 2019). We hypothesize that p300 may combine with ERα to enhance the transcriptional

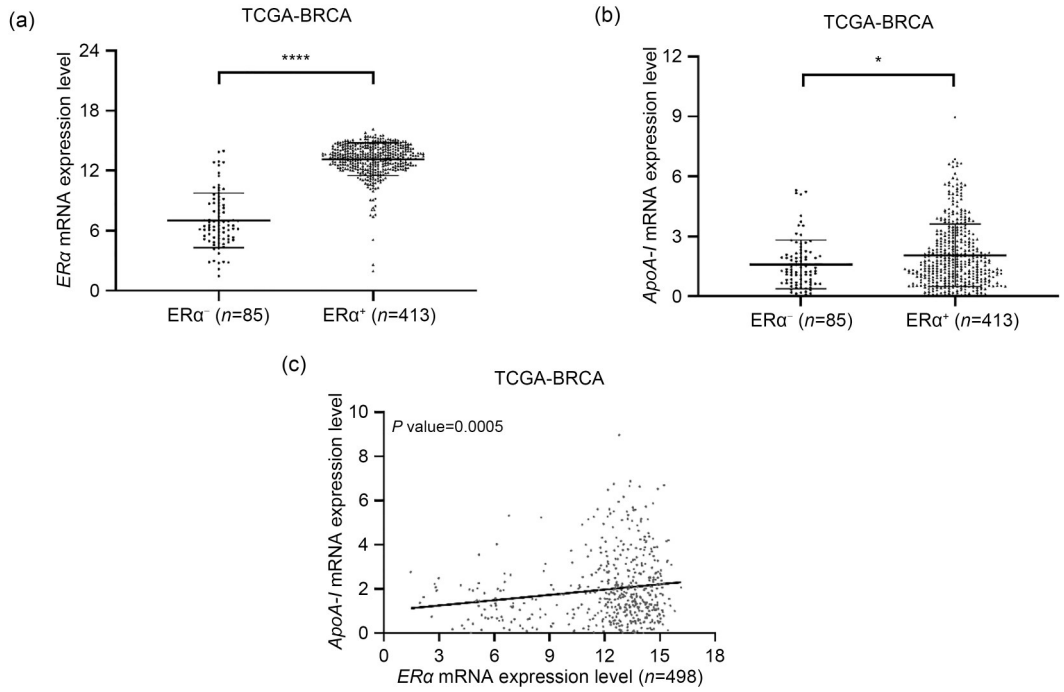

Fig. 5 Positive correlations between messenger RNA (mRNA) expression levels of estrogen receptor α (*ERα*) and apolipoprotein A-I (*ApoA-I*) in breast cancer. (a, b) The Cancer Genome Atlas (TCGA) data were analyzed for *ERα* (a) and *ApoA-I* (b) mRNA expression levels in ERα-negative (ERα$^-$) and ERα-positive (ERα$^+$) breast cancer tissues. (c) Regression analysis of the correlation between *ERα* and *ApoA-I* mRNA levels in breast cancer tissues. * *P*<0.05, **** *P*<0.0001. TCGA-BRCA: TCGA data for breast cancer.

activity of ERα on *ApoA-I*, which process requires further verification.

5 Conclusions

This study revealed the tumor suppressive role of ApoA-I in breast cancer cells and identified ERα as a new activator of *ApoA-I* gene transcription through directly binding to the last exon of the *ApoA-I* gene. This is the site where an ERE sequence is found, and p300 may serve as a co-activator of ERα in activating ApoA-I expression by establishing an H3K27ac-enriched chromatin microenvironment. Our study demonstrated the mechanism of ERα promoting *ApoA-I* gene transcription in breast cancer cells, providing further supporting evidence of the double-edged nature of the E2/ERα signaling in tumorigenesis. Moreover, given the positive regulatory effect of ERα on *ApoA-I* expression and the tumor suppressive role of ApoA-I in various types of cancer, such as melanoma (Zamanian-Daryoush et al., 2013), breast cancer (Cedó et al., 2016), colon cancer (Gkouskou et al., 2016), and pancreatic cancer (Peng et al., 2017), it is considered that the administration of ApoA-I mimetic peptides may enhance the efficacy of systemic therapy for luminal breast cancer.

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Author contributions

Bingjie WANG performed the experimental research and TCGA data analysis, wrote and edited the manuscript. Yinghui SHEN performed the ChIP-seq experiment. Tianyu LIU contributed to the data analysis, writing and editing of the manuscript. Li TAN contributed to the study design and editing of the

manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Bingjie WANG, Yinghui SHEN, Tianyu LIU, and Li TAN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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