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ORIGINAL ARTICLE

HULC functions as an oncogene in ovarian carcinoma cells by negatively modulating miR-125a-3p

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Abstract

The aberrant expression of highly upregulated in liver cancer (HULC) has been reported to participate in ovarian cancer development. A recent research has revealed that HULC-modulated microRNAs (miRNAs) in tumorigenesis. To confirm the functions of HULC on tumorigenesis of ovarian, we explored the effects of HULC expression on ovarian cancer cell development, as well as the underlying mechanism. We transfected SKOV3 cells with pEX-HULC, sh-HULC, and miR-125a-3p mimic as well as their corresponding negative controls (pEX-3, sh-NC, and NC) to alter the expression of HULC and miR-125a-3p, which were analyzed by quantitative reverse transcription PCR (qRT-PCR). Expression of proteins associated with cell cycle, apoptosis, and signaling pathways was determined by Western blot assay. The proliferation, apoptosis, migration, and invasion were explored by bromodeoxyuridine (BrdU) incorporation assay, Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) method, and transwell migration and invasion assays, respectively. HULC overexpression promoted proliferation, migration, and invasion, while inhibited apoptosis of SKOV3 cells. In addition, HULC negatively regulated the expression of miR-125a-3p. Besides, miR-125a-3p mimic reversed the effects of HULC on proliferation, migration, and invasion as well as apoptosis of SKOV3 cells. Moreover, we found that HULC enhanced phosphorylated expression of regulatory factors in phosphatidylinositol 3 kinase/protein kinase B/mammalian targets of rapamycin (PI3K/AKT/mTOR) signaling pathway by downregulating expression of miR-125a-3p. Overexpression of HULC promoted ovarian carcinoma development by activating PI3K/AKT/mTOR signaling pathway via downregulating miR-125a-3p.

Keywords Ovarian carcinoma · HULC · miR-125a-3p · PI3K/AKT/mTOR signaling pathway

Introduction

Ovarian cancer has high mortality. It has been reported that there were approximately 184,799 ovarian cancer deaths and the age-standardized mortality of ovarian cancer was 3.9 per 100,000 all over the world. Meanwhile, the proportion of deaths died from ovarian cancer in all cancers deaths was 2.08%, ranking 15th in 2018 according to the report from international agency for research on cancer (IARC) [1]. The difficulties in diagnosing early stage are the primary leading to its high mortality [19]. Therefore, novel effective therapies are required to improve the survival of patients, for instance, target therapy, especially through signaling pathways [10, 18].

Long non-coding RNA (lncRNA) highly upregulated in liver cancer (HULC) is most upregulated in hepatocellular carcinoma and characterized as a novel mRNA-like non-coding RNA reported by Panzitt and colleagues [21]. In addition, aberrant expression of HULC has been revealed to be involved in malignancy development and progression. In the recent research, abnormal expression of HULC is associated with several tumors, for example, it has been reported that elevated HULC expression is associated with poor clinical outcomes among patients with osteosarcoma [25]. Moreover, HULC-modulated abnormal lipid metabolism

Highlights 1. HULC negatively mediates miR-125a-3p expression in ovarian carcinoma cells SKOV3.
2. HULC overexpression promotes ovarian carcinoma development by downregulating miR-125a-3p in SKOV3 cells.
3. Upregulated miR-125a-3p blocks PI3K/AKT/mTOR pathway activated by HULC overexpression.

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facilitates hepatoma cell growth [3]. A recent study has also reported that aberrant expression of HULC occurs in ovarian cancer and overexpression of HULC might promote ovarian carcinoma tumorigenesis [2]. However, the regulatory mechanisms of HULC on ovarian carcinoma are not entirely distinct.

More and more evidence has found that microRNAs (miRNAs) are regulated by lncRNAs in the tumorigenesis [16], such as liver cancer [27], gallbladder cancer [17], and osteosarcoma [11]. The regulatory function of lncRNAs on ovarian cancer via miRNAs has already reported [5, 31]. Nevertheless, it has been rarely reported that HULC participates in modulating ovarian cancer via a mechanism involving miR-125a-3p. miR-125a-3p, as a crucial member of miR-125a family, has been reported to suppress cell proliferation, migration, and invasion, while enhance apoptosis in malignancies, such as non-small cell lung cancer [9], gastric cancer [7], prostate cancer [20], glioma [30], and colon cancer [26]. Although the downregulated expression of miR-125a-5p has been examined and found to modulate viability and migration in ovarian cancer tissues [12], it still remains unclear whether miR-125a-3p also functions in the development of ovarian carcinoma, especially through a mechanism associated with HULC. Strikingly, it has revealed that miR-125a-3p blocks phosphatidylinositol 3 kinase/protein kinase B/mammalian targets of rapamycin (PI3K/AKT/mTOR) signaling pathway in cancer process since PI3K/AKT/mTOR pathway could be a potential target for ovarian cancer therapy [18, 24].

Therefore, we hypothesized that there might be a correlation between HULC and miR-125a during ovarian cancer development. Meanwhile, HULC-evoked activation of PI3K/AKT/mTOR signaling pathway might participate in the oncogenesis of ovarian cancers. In the present study, we illustrated the role of HULC in ovarian cancer development. The results obtained in our study demonstrated that miR-125a-3p was a crucial mediator between HULC and signaling transduction cascade. Furthermore, we elucidated miR-125a-3p and induced the inactivation of PI3K/AKT/mTOR pathway which accounted for inhibited proliferation, migration, and invasion as well as enhanced apoptosis of ovarian cancer cells.

Materials and methods

SKOV3 cell in vitro culture and treatment

SKOV3 ovarian cancer cells were obtained from American Type Culture Collection (ATCC; Rockville, MD, USA). All SKOV3 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM; Life Technologies Corporation, Carlsbad, CA, USA), which was further supplemented with 10% fetal bovine serum (FBS; Life Technologies Corporation); 100 IU/mL penicillin (Invitrogen, Carlsbad, CA, USA); and 100 μg/mL streptomycin (Invitrogen). SKOV3 cells were incubated at 37 °C in a humidified incubator containing 5% CO_2.

Cell transfection

Short-hairpin RNA (sh-RNA) oligonucleotides and its corresponding negative control were cloned into the pGPU6/Neo plasmid (GenePharma, Shanghai, China) for targeting HULC, which named sh-HULC and sh-NC. The plasmid vector (pEX-3) expressing the open reading frame of HULC was obtained from GenePharma named pEX-HULC. sh-HULC, sh-NC, pEX-HULC, and pEX were all transfected into SKOV3 cells with lipofectamine 3000 reagent (Life Technologies Corporation) according to the manufacturer's instructions. The stable transfectants were selected with G418 (0.5 mg/mL; Sigma-Aldrich, St Louis, MO, USA) for follow-up experiments. miR-125a-3p mimic and its corresponding negative control were synthesized by Life Technologies Corporation for the following transfection which were named miR-125a-3p mimic and NC.

Proliferation analysis based on bromodeoxyuridine (BrdU) incorporation assay

The proliferation of SKOV3 cells was visualized by colorimetric immunoassay. SKOV3 cells were seeded into 96-well plates (5×10^3) and incubated at 37 °C overnight. Then, cells were labeled with 1 mg/mL BrdU (Sigma-Aldrich) for 3 h. The BrdU-labeled cells were analyzed with a microplate reader (Bio-Rad, Hercules, CA, USA) at 450/550 nm. At least 1000 cells were counted in each group at least five replicates.

Apoptotic assay

Apoptosis was evaluated with a cell cycle and apoptosis analysis kit (C1052) (Beyotime, Shanghai, China). SKOV3 cells were stained by propidium iodide (PI)/fluorescein isothiocyanate (FITC)-conjugated Annexin V staining and observed with flow cytometry. After transfection or not, SKOV3 cells were washed with phosphate-buffered saline (PBS) (Sigma-Aldrich) and fixed in 70% ethanol. Subsequently, the fixed cells were washed twice with PBS and stained in PI/FITC-Annexin V containing 50 μg/mL RNase A (Sigma-Aldrich). Labeled SKOV3 cells were analyzed with FACScan (Beckman Coulter, Fullerton, CA, USA). The data was subsequently analyzed with FlowJo software.

Migration and invasion assay

Migration and invasion capacity of SKOV3 cells was measured with a transwell cell culture chambers (Corning, NY, USA). In migration assay, the cells suspended with 200 μL

of serum-free medium were transferred on the upper compartment of the 24-well transwell culture chamber, and 600 μL of complete medium was added into the lower compartment. The cells were fixed in the methanol after incubation at 37 °C. The cells on the upper surface of the filter were non-traversed, which carefully removed with a cotton swab. The traversed cells were stained with crystal violet and counted on the lower side of the filter. The invasion capacity of cells was determined with 24-well millicell hanging cell culture inserts with 8 mm PET membranes (Millipore, Bedford, Massachusetts, USA). The 5.0×10^4 cells in 200 μL of serum-free DMEM medium were plated onto BD BioCoat™ Matrigel™ Invasion Chambers (8-μM pore size polycarbonate filters; BD Biosciences, Woburn, MA, USA). The complete medium supplemented with 10% FBS was added to the lower chamber. After the invasion chambers were cultured for 48 h at 37 °C in an atmosphere containing 5% CO_2 according to the manufacturer's protocol, the non-invading cells were removed with a cotton swab; the invading cells were fixed in 100% methanol and then stained with crystal violet solution and counted with a microscope. The cells were quantified on the bottom surface from five randomly chosen fields.

Western blot assay

The SKOV3 cells in different groups were lysed with RIPA lysis buffer (Beyotime) containing protease inhibitors (Roche, Guangzhou, China) to extract total protein used for Western blot analysis. The concentration of total protein was determined with the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). The Western blot analysis was conducted in a Bio-Rad Bis-Tris Gel system referring to manufacturer's instructions. The separated proteins were transferred to polyvinylidene difluoride (PVDF) membrane (Millipore) which was subsequently blocked with 5% non-fat milk or bovine serum albumin (BSA; Millipore). Primary antibodies were prepared with 5% blocking buffer in 1:1000 used for incubating with membrane at 4 °C overnight. Primary antibodies were obtained from Abcam (Cambridge, UK) and Cell Signaling Technology (Danvers, MA), and the corresponding item number were shown as following: anti-p21 antibody (ab109199), anti-Cyclin D1 antibody (ab134175), anti-Bcl-2 antibody (ab196495), anti-Bax antibody (ab32503), anti-pro-Caspase-3 antibody (ab44976), anti-cleaved-Caspase-3 antibody (ab2302), anti-pro-Caspase-9 antibody (ab2013), anti-cleaved-Caspase-9 antibody (ab2324), anti-PI3K p110α antibody (C73F8), anti-p-PI3K antibody (#4228), anti-AKT antibody (#4228), anti-p-AKT antibody (#4060), anti-p-mTOR antibody (#5536), anti-mTOR antibody (#2983), anti-p-S6 antibody (#4858), anti-t-S6 antibody (#2317), and anti-β-actin antibody (ab8227). Afterwards, the secondary antibodies (ab205718, Abcam; #7056, #7054, Cell Signaling Technology) conjugated with horseradish peroxidase (HRP) were successively incubated with primary antibodies for 1 h at room temperature. After transferred into the Bio-Rad ChemiDoc™ XRS system (Bio-Rad), the membrane was covered with 200 μL Immobilon Western Chemiluminescent HRP Substrate (Millipore). The signals were analyzed by Image Lab™ Software (Bio-Rad).

Quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted from SKOV3 cells using a standard method with Trizol reagent (Life Technologies Corporation) according to the manufacturer's instruction. The concentration of RNA in the extract was assessed using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). RNA was reverse-transcribed to generate cDNA with the TaqMan microRNA reverse transcription kit (Thermo Fisher Scientific). The expression of HULC was quantified with a real-time PCR system (Applied Biosystems, Grand Island, NY, USA) using RT-RNA PCR kit (TaKaRa Biotechnology, Dalian, China) according to the manufacturer's recommendations. The reaction was performed referring to Uzan's program [25]. The expression of miR-125a-3p was determined with TaqMan Universal Master mix II (Applied Biosystems). The expression of HULC or miR-125 was normalized to the expression of β-actin or U6 with the $2^{-\Delta\Delta Ct}$ method described previously [15]. All reactions were performed in triplicate.

Statistical analyses

Each experiment was repeated at least three times. Data are presented as mean ± standard deviation (SD). All statistical analyses were performed with GraphPad Prism 7.0 software (Graph Pad Software, La Jolla, CA, USA). Comparisons between two groups were conducted by unpaired or paired (for matched comparisons) two-tailed Student's t test or non-parametric Mann-Whitney U test. Multiple comparisons were evaluated by one-way analysis of variance (ANOVA) and then validated by Tukey's test. A statistically significant result was considered at $P < 0.05$.

Results

HULC-modulated SKOV3 cells proliferation and apoptosis

In order to explore the role of HULC in proliferation and apoptosis of ovarian carcinoma cells, we manipulatively promoted or repressed HULC by transfection pEX-HULC or sh-HULC into SKOV3 cells. HULC was effectively upregulated or downregulated in pEX-HULC- or sh-HULC-transfected SKOV3 cells compared with their corresponding controls (pEX-3/sh-HULC) ($P < 0.05$; Figs. 1 a and b). Further, we

found that HULC overexpression obviously promoted proliferation of SKOV3 cells, while HULC knockdown could markedly lead to an inhibiting effect ($P < 0.05$ or $P < 0.01$; (Fig. 2 a). Additionally, we observed decreased expression of p21 and increased expression of Cyclin D1 in SKOV3 cells transfected with pEX-HULC in comparison with pEX-3, while the inverse results were shown in SKOV3 cells transfected with sh-HULC compared with sh-NC ($P < 0.05$ or $P < 0.01$; Figs. 2 b and c). In addition, the increased apoptotic rate of SKOV3 cells transfected with sh-HULC confirmed that downregulated expression of HULC statistically promoted apoptosis ($P < 0.01$; Fig. 2 d). Specifically, we analyzed the alterative expression of apoptosis-related proteins, including Bcl-2, Bax, pro-Caspase-3, cleaved-Caspase-3, pro-Caspase-9, and cleaved-Caspase-9 by Western blot assay, which indicated that the activation of Caspase-3/9 and the expression of Bax were promoted as well as Bcl-2 expression was repressed by HULC silence (Fig. 2 e).

Migration and invasion of ovarian carcinoma cell were associated with HULC

The migration of SKOV3 cells transfected with pEX-HULC significantly presented a higher rate compared with that transfected with pEX-3 ($P < 0.05$), while the migration of SKOV3 cells transfected with sh-HULC presented a lower rate in comparison with that transfected with sh-NC ($P < 0.05$) (Fig. 3 a). Similar results were observed in the invasion. The invasion of pEX-HULC-transfected-SKOV3 cells presented a higher rate compared with that transfected with pEX-3 ($P < 0.05$), while the invasion of SKOV3 cells which were transfected with sh-HULC presented a lower rate in comparison with that transfected with sh-NC ($P < 0.05$) (Fig. 3 b). These findings indicated that SKOV3 cells with HULC overexpression had more powerful migration and invasion capability compared with those in which HULC were silenced.

HULC negatively modulated the expression of miR-125a-3p

Results from qRT-PCR indicated that the relative miR-125a expression was reduced in SKOV3 cells transfected with pEX-HULC compared with pEX-3 ($P < 0.05$) (Fig. 4). On the contrary, the expression of miR-125a-3p was upregulated in SKOV3 cells transfected with sh-HULC in comparison with sh-NC ($P < 0.05$) (Fig. 4). These data showed that the expression of miR-125a-3p was negatively modulated by HULC.

miR-125a-3p was involved in HULC-modulated proliferation, apoptosis, migration, and invasion of SKOV3 cells

We have ascertained that HULC overexpression stimulated proliferation, migration, and invasion of SKOV3 cells. In our further research, we explored whether miR-125a-3p participated in HULC-mediated proliferation, apoptosis, migration, and invasion.

The elevated expression of miR-125a-3p was promoted by transfecting miR-125a-3p mimic into SKOV3 cells. The results indicated high transfection efficiency ($P < 0.05$) (Fig. 5 a). We observed that the proliferation of SKOV3 was attenuated by the upregulated miR-125a-3p in ovarian carcinoma cells transfected with pEX-HULC and miR-125a-3p mimic compared with those transfected with pEX-HULC and NC ($P < 0.01$) (Fig. 5 b). The varying expression of cell cycle-related regulatory proteins in SKOV3 cells transfected with pEX-HULC and miR-125a-3p mimic, such as the upregulated p21 and downregulated Cyclin D1, further validated that HULC regulated the proliferation by inhibiting the expression of miR-125a-3p ($P < 0.05$ or $P < 0.01$) (Figs. 5 c and d).

Furthermore, we distinctly noticed that the apoptotic rate of SKOV3 cells transfected with pEX-HULC and miR-125a-3p mimic was markedly increased ($P < 0.05$) (Fig. 5 e). From the results of Western blot assay, we observed that the activation of

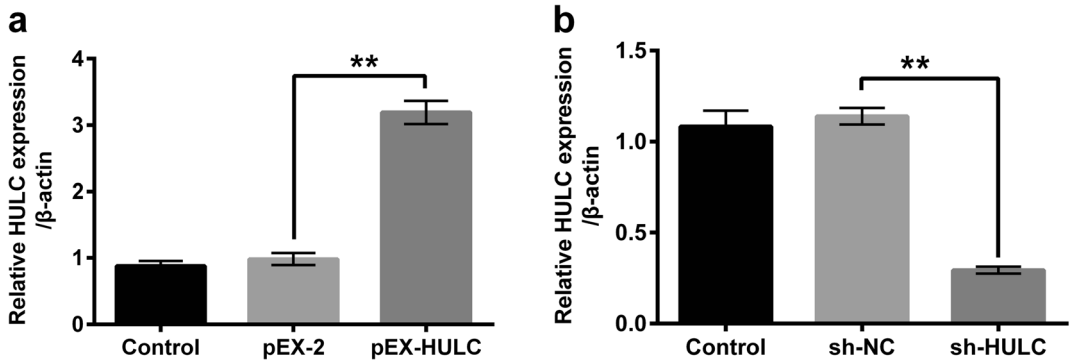

Fig. 1 The mRNA expression of HULC in ovarian carcinoma cells after upregulation (**a**) or downregulation (**b**) by plasmid (pEX-3 and pEX-HULC) or sh-RNA (sh-NC and sh-HULC) transfection. Results were representative of three separate experiments; data were expressed as the mean ± standard deviation (SD) ($n = 3$); HULC, long non-coding RNA highly upregulated in liver cancer; sh, small interfering RNA; NC, negative control. Double asterisks indicate versus pEX-3 or sh-NC; $P < 0.01$

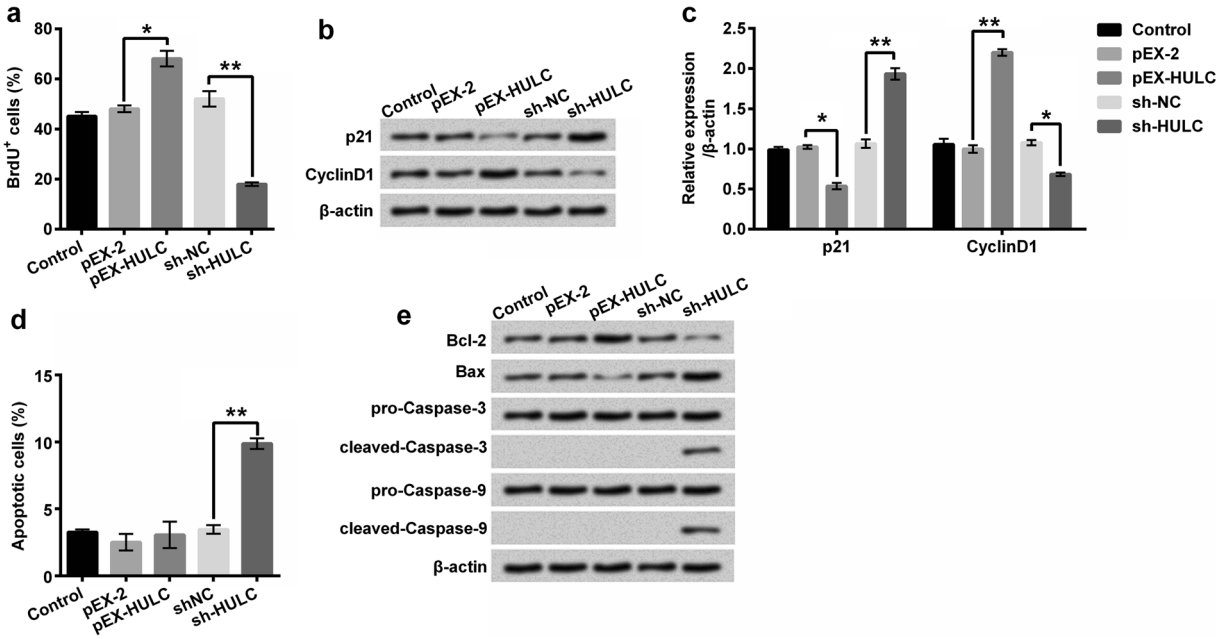

Fig. 2 HULC had an impact on the proliferation and apoptosis of ovarian carcinoma cell. **a** Upregulated/downregulated HULC induced ovarian carcinoma cell proliferation; **b**, **c** suppressed/promoted the expression of p21, while promoted/suppressed the protein expression of Cyclin D1; **d** inhibited/induced ovarian carcinoma cell apoptosis; and **e** altered the expression of apoptosis-related proteins compared with those of pEX-3/sh-NC-transfected cells. Results were representative of three separate experiments; data were expressed as the mean ± standard deviation (SD) ($n = 3$); HULC, long non-coding RNA highly upregulated in liver cancer; sh, small interfering RNA; NC, negative control. A single asterisk or double asterisks indicates versus pEX-3 or sh-NC; $P < 0.05$ or $P < 0.01$

Caspase-3/9 and the expression of Bax were promoted by miR-125a-3p mimic compared with those in SKOV3 cells transfected with pEX-HULC and NC, while Bcl-2 was suppressed (Fig. 5 f). Accordingly, we could verify that upregulated HULC might reduce apoptosis by inhibiting miR-125a-3p expression.

As for cells migration and invasion, we have validated that HULC overexpression promoted ovarian cancer cells migration and invasion. For exploring whether HULC regulate migration and invasion through a mechanism including containing miR-125a-3p, we enforced expression of miR-125a-3p by transfection SKOV3 cells with miR-125a-3p mimic (Figs. 5 g and h). The results revealed cells migration and invasion were repressed by miR-125a-3p mimic compared with that in pEX-HULC + NC group, suggesting that HULC might promote migration and invasion though downregulating miR-125a-3p expression.

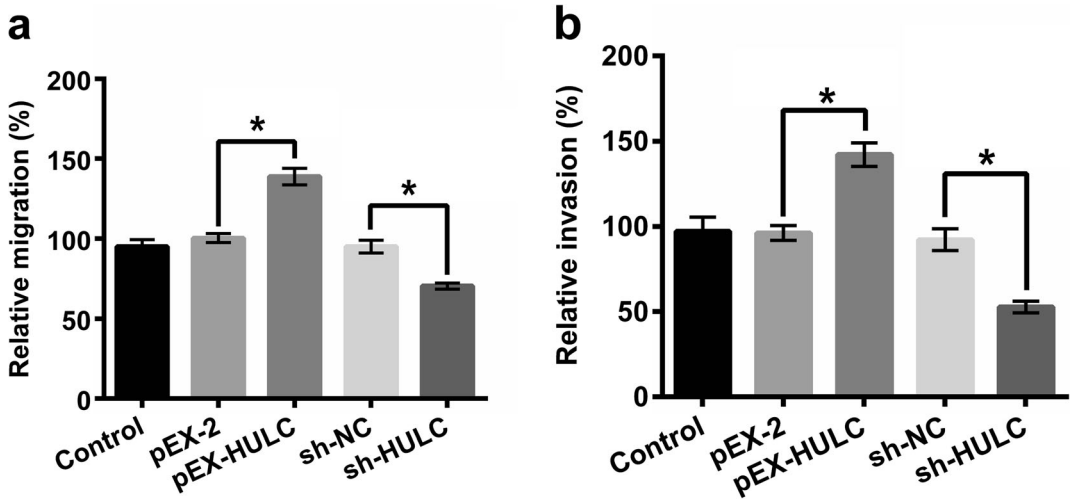

Fig. 3 The ovarian carcinoma cell migration and invasion potential were modulated by HULC expression. **a** Upregulated/downregulated HULC promoted/inhibited ovarian carcinoma cell migration and **b** invasion in comparison with those of pEX-3/sh-NC-transfected cells. Results were representative of three separate experiments; data were expressed as the mean ± standard deviation (SD) ($n = 3$); HULC, long non-coding RNA highly upregulated in liver cancer; sh, small interfering RNA; NC, negative control. A single asterisk indicates versus pEX-3 or sh-NC; $P < 0.05$

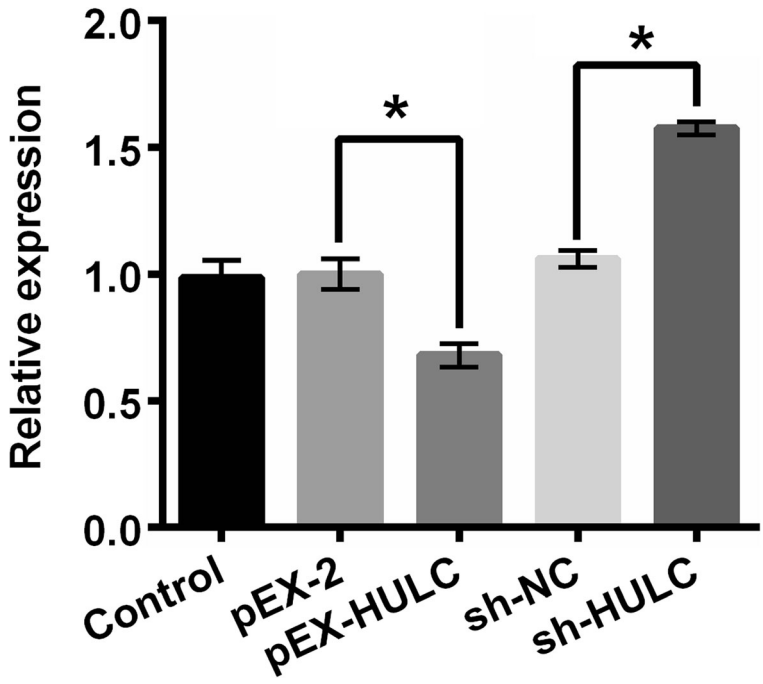

Fig. 4 HULC regulated the expression of miR-125a-3p in the ovarian carcinoma cells. Upregulated/downregulated HULC suppressed/promoted the miR-125a-3p expression compared with those of pEX-3/sh-NC-transfected cells. Results were representative of three separate experiments; data were expressed as the mean ± standard deviation (SD) ($n = 3$); HULC, long non-coding RNA highly upregulated in liver cancer; sh, small interfering RNA; NC, negative control. A single asterisk indicates versus pEX-3 or sh-NC; $P < 0.05$

HULC activated PI3K/AKT/mTOR signaling pathway by downregulating the expression of miR-125a-3p

In the last study, we focused on PI3K/AKT/mTOR signaling pathway regulated by HULC via miR-125a-3p. The phosphorylated expression of PI3K and AKT involved in the PI3K/AKT pathway was quantified by Western blot assay. Our results showed that HULC overexpression promoted phosphorylation of PI3K and AKT by inhibiting miR-125a-3p expression which was proved by transfection SKOV3 cells with pEX-HULC and miR-125a-3p mimic ($P < 0.01$ or $P < 0.001$) (Figs. 6 a and b). As for mTOR signaling pathway, the phosphorylation of mTOR and S6 induced by HULC was blocked by upregulated miR-125a-3p in the SKOV3 cells of

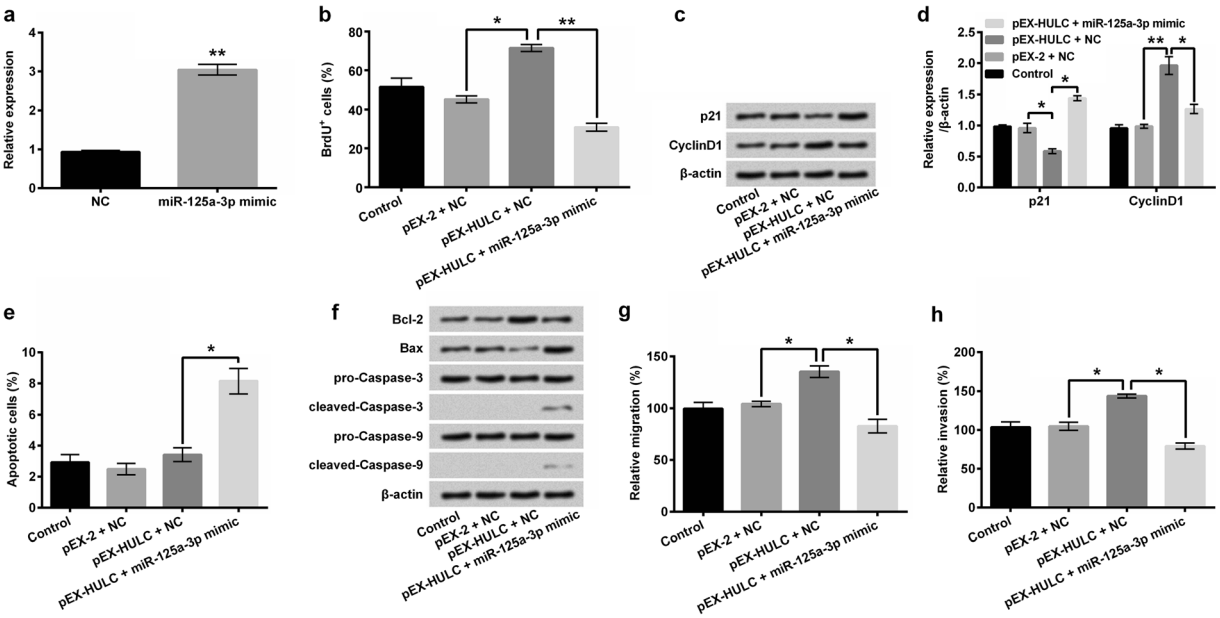

Fig. 5 HULC-modulated ovarian carcinoma cell proliferation, apoptosis, migration, and invasion via regulating miR-125a-3p. Overexpression of miR-125a-3p by miR-125a-3p mimic transfection (**a**) reversed the regulation of HULC on proliferation (**b**), expression of p21 and Cyclin D1 (**c**, **d**), apoptosis (**e**), expression of apoptosis-related proteins (**f**), migration (**g**), and invasion of ovarian carcinoma cells (**h**). Results were representative of three separate experiments; data were expressed as the mean ± standard deviation (SD) ($n = 3$); HULC, long non-coding RNA highly upregulated in liver cancer; sh, small interfering RNA; NC, negative control. A single asterisk or double asterisks indicates versus pEX-3 + NC or pEX-HULC + NC; $P < 0.05$ or $P < 0.01$

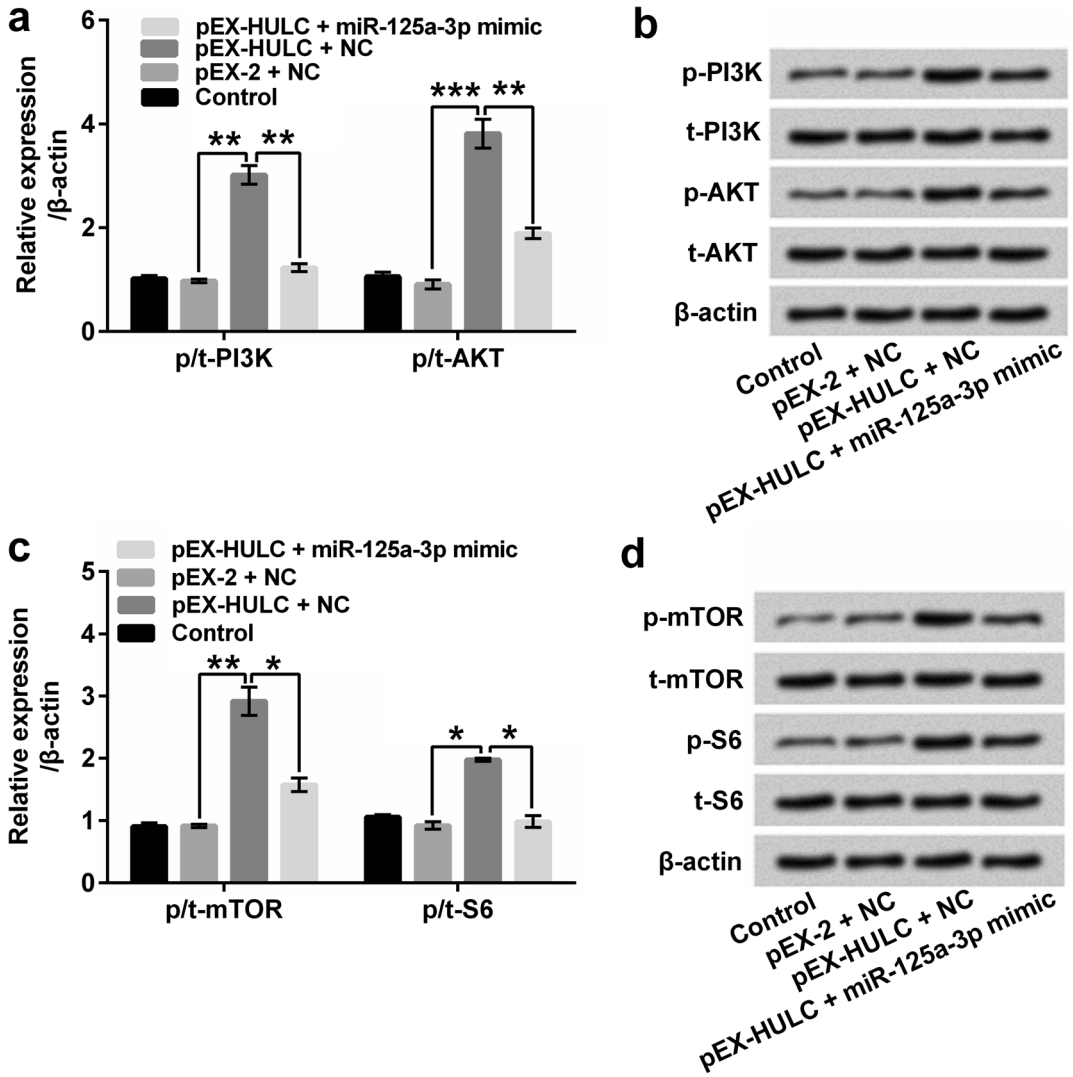

Fig. 6 HULC overexpression activated PI3K/AKT and mTOR signaling pathway via downregulating miR-125a-3p in ovarian carcinoma cells. HULC overexpression-induced phosphorylation of **a**, **b** PI3K and AKT in PI3K/AKT signaling pathway and **c**, **d** mTOR and S6 in mTOR was blocked by upregulated miR-125a-3p compared with those of ovarian carcinoma cells transfected with pEX-HULC and NC. Results were representative of three separate experiments; data were expressed as the mean ± standard deviation (SD) ($n = 3$); HULC, long non-coding RNA highly upregulated in liver cancer; sh, small interfering RNA; NC, negative control. A single asterisk, double asterisks, or triple asterisks indicates versus the pEX-3 + NC or pEX-HULC + NC; $P < 0.05$, $P < 0.01$, or $P < 0.001$

pEX-HULC + miR-125a-3p mimic group ($P < 0.05$ or $P < 0.01$) (Figs. 6 c and d). All the results revealed that HULC might activate PI3K/AKT/mTOR signaling pathway by downregulating the expression of miR-125a-3p.

Discussion

In the current study, we designed experiments to assess whether HULC is involved in the development of ovarian carcinoma cell growth. Of note, we elucidated a novel potential mechanism that HULC overexpression promoted ovarian carcinoma development by triggering PI3K/AKT/mTOR signaling pathway via downregulating miR-125a-3p. Our study may provide a new direction for investigating the oncogenic mechanism of HULC in ovarian cancers.

It has been previously observed that HULC is highly upregulated in hepatocellular carcinoma [21], osteosarcoma [25], and gastric cancer [32]. Similarly, the higher expression of HULC was observed in epithelial ovarian carcinoma tissues than normal samples in a recent research [2]. Accordingly, to definitely confirm the roles of HULC in regulating ovarian carcinoma tumorigenesis, we explored the effect of upregulated/downregulated HULC on the cell proliferation, apoptosis, migration, and invasion, as well as the expression of proteins involved in modulating cell cycle and apoptosis. Specifically, overexpression of p21 inhibits the proliferation of mammalian cells [28] and Cyclin D1 serves as an active factor

to regulate the continued cell cycle progression [23]. Besides, Bcl-2 interferes with programmed cell death according to Hockenbery's report [8]. Similarly, Caspase-3 and Caspase-9 are crucial for the formation of apoptotic bodies [6, 22].

In accordance with a previous study, that upregulated HULC induces ovarian carcinoma cell proliferation, migration, and invasion [2], our results also revealed that HULC overexpression contributed to the development of ovarian carcinoma indicated by the increased Cyclin D1 and reduced p21 protein expression. Besides, HULC overexpression restrained the apoptosis via mediating the formation of apoptosis-related protein. Accordingly, HULC silence advanced the apoptosis of SKOV3 cells. Similarly, the expression of Bcl-2 and Bax was downregulated by HULC siRNA in the glioma cells, while the expression of Caspase-3 and Caspase-8 was upregulated [33]. An emerging evidence suggests that upregulated HULC results in an increase in the ratio of LC3-II/LC3-I to activate autophagy [2] while inhibits apoptosis [32]. As a consequence, HULC overexpression accelerated the progression of ovarian cancer.

Growing evidences have noted that miR-125a-3p has emerged as a tumor suppressive effect on cancers [7, 30]. Moreover, a low level of miR-125a-3p was correlated with indicators of enhanced malignant potential in gastric cancer [7] and restoration of miR-125a-3p inhibits glioblastoma cellular migration, invasion and proliferation while induces apoptosis [30]. A recent study has implicated that miRNA as a target of lncRNA participates in the gallbladder cancer development [17]. In addition, HULC exerts indispensable functions on the proliferation, migration, and invasion by sponging miRNAs in malignancies [11, 13]. In our previous study, the expression of miR-125a-3p was negatively modulated by HULC. Further, we verified that HULC might promote ovarian carcinoma cell proliferation, migration, and invasion, while inhibited apoptosis by downregulating miR-125a-3p. In addition, the expression of p21 and Cyclin D1 was modulated by HULC via downregulating miR-125a-3p. The decreased protein expression of Bcl-2 and increased protein expression of Bax, cleaved-Caspase-3 and cleaved-Caspase-9 regulated by HULC were all reversed by upregulated miR-125a-3p.

Although increased expression of HULC has been proposed to play essential roles in the tumor growth and development, the mechanisms of regulating the signaling pathway remain to be elucidated. AKT is a proto-oncogene activated by PI3K, and PI3K signaling pathway appears to mediate the cell survival [29]. It has already been reported that PI3K is crucial in medicating proliferation and Cyclin expression through activating the AKT/mTOR signaling pathway in the ovarian carcinoma development [4]. Our studies indicated that overexpression of HULC activated the PI3K/AKT/mTOR signaling pathway by promoting the phosphorylation of PI3K, AKT, mTOR, and S6. Our results are consistent with previous studies, suggesting that HULC promotes the phosphorylation of AKT and mTOR to activate the PI3K/AKT/mTOR signaling pathway in the glioma cells [33]. In addition, the research provided some evidences that miR-125a-3p mediates the cell proliferation, apoptosis, migration, and invasion via PI3K/AKT/mTOR signaling pathway in colorectal cancer and liver cancer [14, 24, 26]. In accordance with above researches, our results confirmed that overexpression of miR-125a-3p blocked the PI3K/AKT/mTOR signaling pathway which was originally activated by HULC.

In conclusion, HULC functioned as an oncogene in ovarian carcinoma cell line SKOV3, and the potential mechanism might be associated with miR-125a-3p which was negatively modulated by HULC. Subsequently, PI3K/AKT/mTOR signaling pathway was activated to mediate the proliferation, apoptosis, migration, and invasion. Notwithstanding, we have elucidated the regulatory relationship between HULC and miR-125a-3p on the ovarian carcinoma development, the alignment between HULC and miR-125a-3p is still expected to be illuminated. In addition, the antineoplastic activity of miR-125a-3p regulated by HULC shows potential for application as a therapeutic target in ovarian carcinoma treatment.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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