Original Article

Long noncoding RNA lncARSR promotes epithelial ovarian cancer cell proliferation and invasion by association with HuR and miR-200 family

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Abstract: Epithelial ovarian cancer (EOC) is the fifth leading cause of female cancer-related deaths worldwide. Long non-coding RNAs (lncRNAs) are emerging as crucial regulators in various biological processes through diverse mechanisms. Recently, lncRNA Activated in RCC with Sunitinib Resistance (lncARSR) has been reported to be upregulated and involved in sunitinib resistance of renal cell carcinoma cells. However, the functional roles in EOC have not yet been explored. In the current study, we detected the expression levels of lncARSR in 76 paired EOC tissues and adjacent normal tissues, and observed that lncARSR expression was significantly increased in EOC tissues and correlated with FIGO stage, histological grade, lymph nodes metastasis and worse survival. Loss- and gain-of-function assays demonstrated that lncARSR promoted EOC cell proliferation and invasion. Further investigations showed that lncARSR interacted with HuR, upregulated β-catenin expression and then activated Wnt/β-catenin signaling pathway to regulate cell proliferation. Moreover, lncARSR increased ZEB1 and ZEB2 expression by competitively binding the miR-200 family to induce EMT and invasion. Our findings suggest that the lncARSR may provide a novel therapeutic strategy for EOC treatment.

Keywords: lncARSR, HuR, Wnt/β-catenin, ceRNA, miR-200 family

Introduction

Epithelial ovarian cancer (EOC) is the fifth leading cause of female cancer-related deaths worldwide, which causes approximately 14,240 deaths each year [1]. Despite the great advances in surgery, chemotherapy and radiotherapy over the last decades, the prognosis of patients with EOC remains unsatisfactory [2]. Therefore, understanding the molecular mechanisms implicated in EOC initiation and progression and is urgently needed for the development of EOC diagnosis and treatment.

Long non-coding RNAs (lncRNAs) are emerging as crucial regulators in various biological processes. lncRNAs are often expressed in a disease-, tissue- or developmental stage-specific manner [3]. Recent evidences demonstrated that lncRNAs are frequently dysregulated in cancers and play critical roles in regulating

tumor growth and aggressiveness. For example, lncRNA LSINCT5 is overexpressed in EOC tissues, and affects the proliferation of EOC cells [4]. MALAT1 is a widely expressed oncogenic lncRNA indicating high-level metastatic potential and poor prognosis in lung cancer [5]. MEG3 is frequently silenced and functions as a tumor suppressor in some cancers, such as neuroblastomas, liver cancer and gastric cancer [6, 7]. Recently, lncRNA Activated in RCC with Sunitinib Resistance (lncARSR), locates on 9q82, was found to be upregulated and involved in sunitinib resistance of renal cell carcinoma [8]. However, the functional roles and underlying mechanisms of lncARSR in EOC development have not yet been investigated. In our present study, we detected the differential expression of lncARSR in EOC tissues and adjacent normal tissues. Moreover, we demonstrated that lncARSR promoted EOC cell prolifera-

tion and invasion through association with HuR and miR-200 family, respectively.

Materials and methods

Patients and tissue samples

Fresh EOC tissues and pair-matched adjacent normal tissues were obtained from 76 patients with the primary EOC between September 2010 and June 2011 at Department of Gynecology of the First Hospital of Jilin University. Tumor samples and respective normal tissues from resection surgery were immediately frozen and kept in liquid nitrogen until use. None of the patients receive preoperative treatment, such as radiotherapy, chemotherapy or hormonal therapy before collecting specimens. This study was approved by the Research Ethics Committee of the First Hospital of Jilin University. Written informed consents were obtained from all the subjects.

Cell culture

Human EOC cell lines (SKOV3, HO8910, ES-2, and CAOV3) and a normal human ovarian epithelial cell line (IOSE80) were purchased from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (China). Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS; HyClone), 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma) at 37°C in a humidified incubator with 5% CO_2.

Cell proliferation assay

CCK-8 assay was used to determine cell viability. Cells in different treatment groups were plated into 96-well plates at a density of $3×10^3$ cells per well. 10 µl CCK-8 was added to the wells at different time point. CCK-8 reagent was incubated for 1.5 h at 37°C. A spectrophotometer was used to measure the absorbance at 450 nm (Bio-Rad).

Lentiviral construction and cell transfection

To generate clones stably overexpressing lncARSR, SKOV3 cells were infected with a lentiviral vector expressing lncARSR or an empty lentiviral vector control. To generate clones stably silencing lncARSR, CAOV3 cells were infected with a lentiviral vector expressing lncARSR shRNA or a non-silencing shRNA control. The

target sequences of lncARSR shRNAs were shown as follow: KD1: GAGCATGAAGAACT-CCAACTT, KD2: GCATGAAGAACTCCAACTTCA. Stable cells were selected for 1 week by using puromycin.

Colony formation assays

3000-4000 cells were seeded in one 6-well culture dish and cultured for 2 weeks. The colonies were fixed and then stained with 1% crystal violet and counted.

Transwell invasion assay

Transwell invasion assay was performed using Matrigel-coated (BD Biosciences) 24-well transwell chambers (Costar). Cells were seeded onto the upper chambers of the transwell in serum-free DMEM. The lower chambers of the transwell were filled with DMEM supplemented with 10% FBS. After incubation for 24 h, cells on the upper surface of the filter were removed using a cotton swab. Cells migrating through the filter to the lower surface were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 10 min. The filters were then washed in PBS twice before observation.

Quantitative real-time PCR (qRT-PCR)

Total RNAs were isolated using TRIzol® Reagent (Invitrogen) according to the manufacturer's instructions. Real-time PCR was performed using SYBR Green Mixture (Takara, Dalian, China) on ABI7500.

Luciferase reporter assays

β-catenin/TCF firefly luciferase reporter construct (TOPflash) and pRL-TK reporter was purchased from Promega (Madison, WI, USA). $6×10^4$ cells were seeded onto 12-well plates and then transiently transfected with pTOP-flash and pRL-TK using Lipofectamine® 2000 (Invitrogen) for 48 hours. The luciferase activities were detected by using the Dual-Luciferase Assay System (Promega). The firefly luciferase activity was normalized to Renilla luciferase activity.

RNA pull-down assays

RNA pull-down assays were performed as described previously [9]. Briefly, RNAs were biotin-labeled with the Biotin RNA Labeling Mix
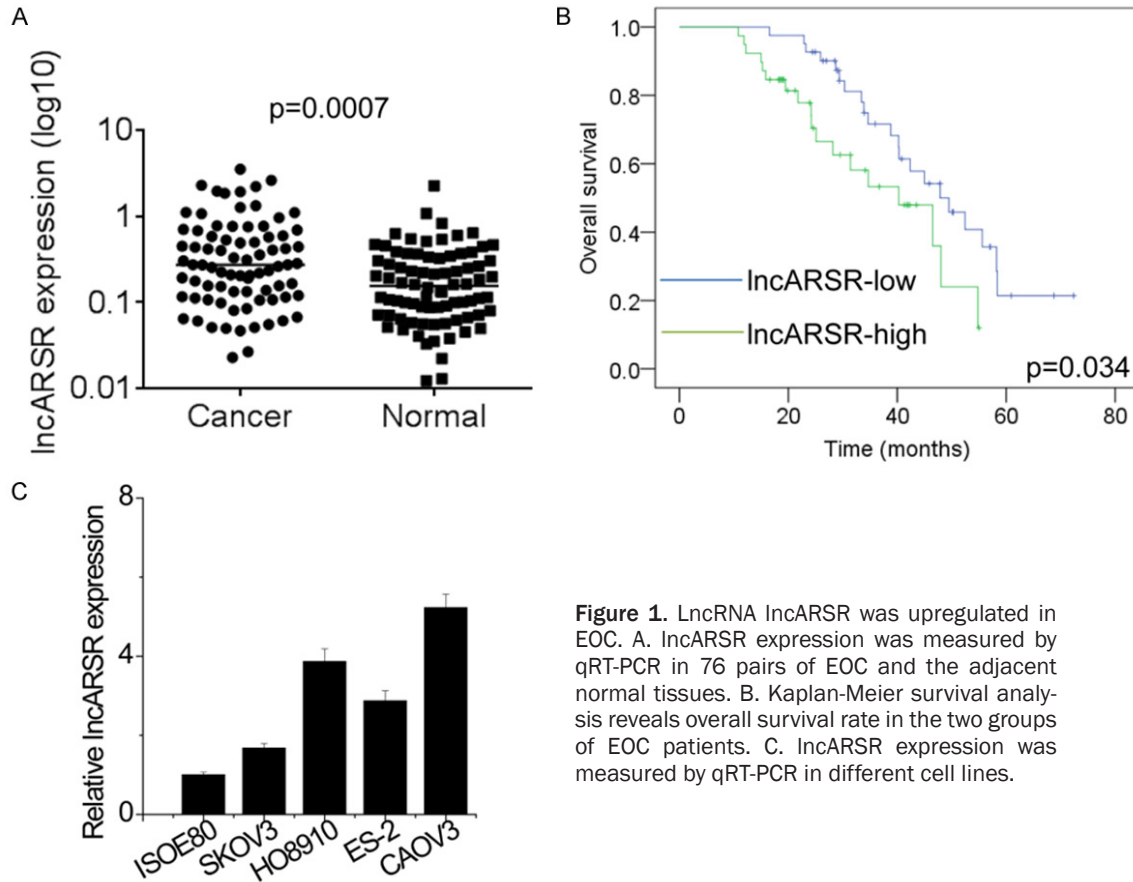
Figure 1. LncRNA lncARSR was upregulated in EOC. A. lncARSR expression was measured by qRT-PCR in 76 pairs of EOC and the adjacent normal tissues. B. Kaplan-Meier survival analysis reveals overall survival rate in the two groups of EOC patients. C. lncARSR expression was measured by qRT-PCR in different cell lines.

(Roche) and *in vitro* transcribed using T7 RNA polymerase (Roche). After purification, biotinylated RNAs were incubated with cell lysates. Streptavidin agarose beads (Life Technologies) were added to each binding reaction and incubated for 1 hour at room temperature. The eluted proteins were detected by western blot.

RNA immunoprecipitation (RIP)

RIP assays were performed according to the guidelines in the Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA).

Western blot

Cells were lysed in RIPA buffer (Beyotime, Beijing, China) supplemented with cocktail protease inhibitors (Roche, Mannheim, Germany). Samples were separated on SDS-PAGE, transferred onto PVDF (Millipore, Bedford, MA) membranes. After blocking, the membranes were incubated with primary antibodies and the corresponding secondary antibodies conjugated to horseradish peroxidase. The signals of the

membranes were detected by ECL Substrate (Pierce, Rockford, IL).

Statistical analysis

All statistical analyses were performed using SPSS software. Survival curves were calculated using Kaplan-Meier and log-rank tests. The Chi-square test was used to analyze the relationship between lncARSR expression and clinicopathological characteristics. Student's t-test and multi-way classification ANOVA tests were performed for results from qRT-PCR experiments, colony formation assays and CCK-8 assays. Correlation between lncARSR and β-catenin expression was analyzed by Spearman rank correlation. P < 0.05 was considered to be statistically significant.

Results

lncARSR is upregulated in EOC and predicts poor prognosis

Firstly, we performed qRT-PCR to examine the lncARSR expression in EOC tissues. We ob-

Table 1. The relationship between lncARSR expression and clinicopathological features in epithelial ovarian cancer patients

Variable	lncARSR expression		P value
	High	Low	
Age (years)			
≤ 50	16	18	0.645
> 50	22	20	
Size (cm)			
≥ 2	24	22	0.639
< 2	14	16	
FIGO stages			
I-II	12	27	0.001
III-IV	26	11	
Histological type			
Serous	27	25	0.622
Non-serous	11	13	
Histological grade			
G1-G2	12	24	0.006
G3	26	14	
Lymphatic metastasis			
Yes	24	15	0.039
No	14	23	
Distant metastasis			
Yes	24	13	0.012
No	14	25	
Serum CA125			
≤ 500	19	18	0.818
> 500	19	20	

served that lncARSR was significantly upregulated in EOC tissues than adjacent non-tumor tissues (**Figure 1A**). To reveal the correlation between clinicopathological characteristics and lncARSR expression, we classified EOC patients into two groups: high lncARSR group (lncARSR expression ≥ median expression) and low lncARSR group (lncARSR expression < median expression). Correlation analysis showed that high lncARSR expression positively correlated with advanced FIGO stage, histological grade, distant metastasis and lymph node metastasis (**Table 1**), but no associations were observed between lncARSR expression and other clinical features. Moreover, EOC patients with high lncARSR expression had a poorer overall survival than patients with low lncARSR expression (**Figure 1B**). We further determined lncARSR expression in EOC cell lines. Results showed that EOC cell lines (SKOV3, HO8910, ES-2, and CAOV3) expressed higher lncARSR

expression than normal human ovarian epithelial cells IOSE80 (**Figure 1C**). Thus, these data indicated that lncARSR may function as an oncogene for EOC development.

lncARSR promotes EOC cell proliferation and invasion

Next, we performed gain- and loss-of-function studies in EOC cells to investigate the functional roles of lncARSR in EOC. We developed SKOV3 cells with stably overexpressed lncARSR, and CAOV3 cells with stably silenced lncARSR expression (**Figure 2A** and **2B**). CCK-8 and colony formation assays showed that overexpression of lncARSR enhanced the proliferative ability of SKOV3 cells compared to that of control cells (**Figure 2C** and **2D**). On the contrary, knockdown endogenous lncARSR expression significantly suppressed the proliferation of CAOV3 cells (**Figure 2E** and **2F**). Moreover, transwell invasion assay showed that the number of invaded SKOV3 cells was obviously increased after lncARSR overexpression (**Figure 2G**), whereas lncARSR knockdown inhibited the cell invasion of CAOV3 cells (**Figure 2H**). These data suggest that lncARSR promotes the proliferation and invasion of EOC cells, and it may serve as a crucial regulator in EOC progression.

lncARSR activates Wnt/β-catenin signaling in EOC cells

Wnt/β-catenin signaling pathway is one of the most common dysregulated pathways implicated in EOC development. To determine the relationship between lncARSR and the Wnt/β-catenin signaling pathway, TOP/FOP luciferase reporter system of common WNT pathway activation was performed. Our results showed that WNT signaling was activated by lncARSR overexpression (**Figure 3A**), while WNT signaling was suppressed by lncARSR knockdown (**Figure 3B**). Western blot and qRT-PCR analysis was used to detect the β-catenin expression, the key components of Wnt/β-catenin signaling. We found that lncARSR overexpression significantly increased the expression of β-catenin (**Figure 3C**), while lncARSR inhibition exerted the opposite effect (**Figure 3D**). In addition, the correlation analysis by qRT-PCR in 60 EOC tissue samples showed that lncARSR expression was positively associated with β-catenin expression (r^2 = 0.6061, P =
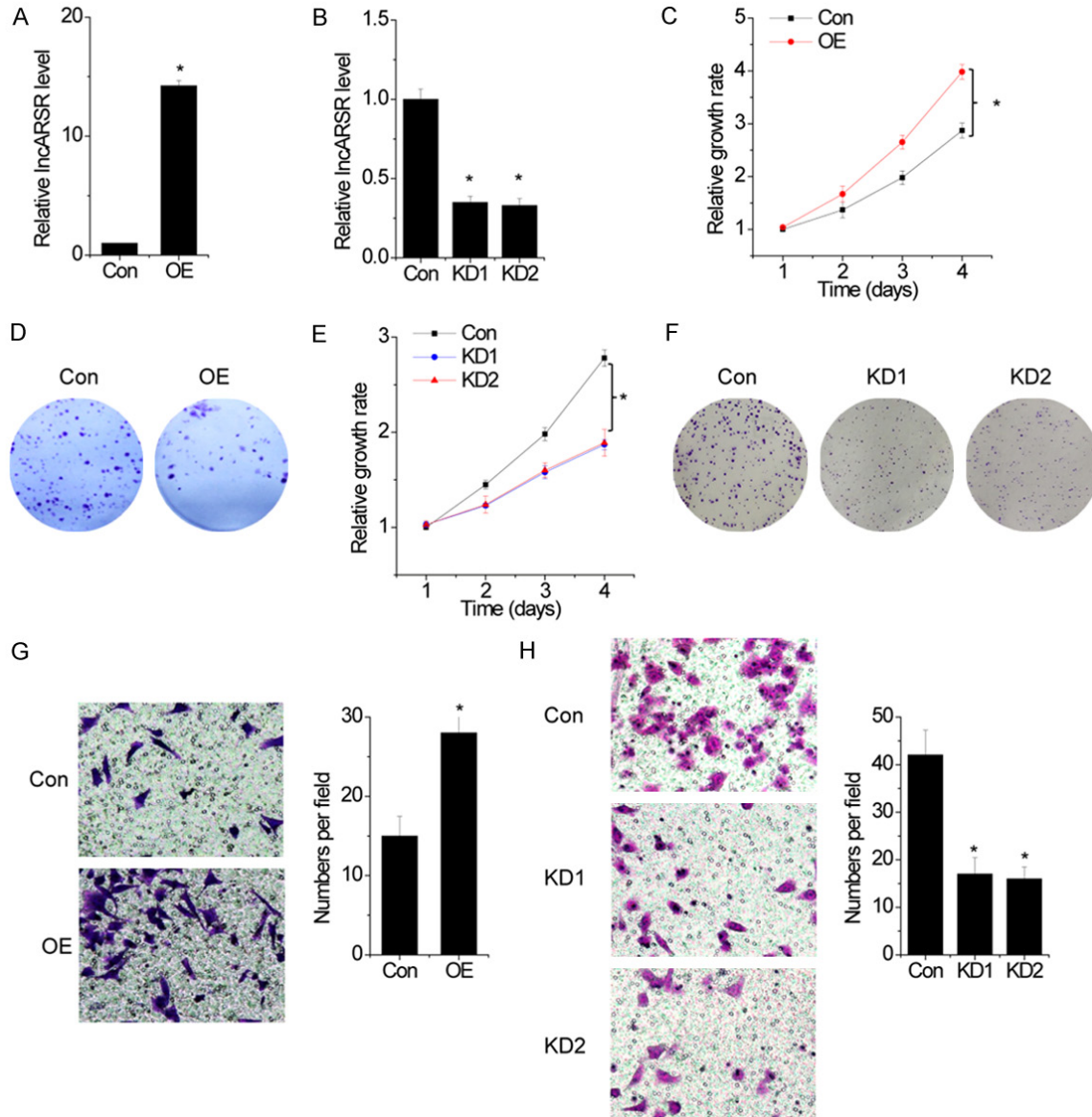
Figure 2. lncARSR promotes EOC cell proliferation and invasion. A. Efficacy of lncARSR overexpression (OE) as determined by qRT-PCR. B. Efficacy of lncARSR knockdown (KD) by shRNAs as determined by qRT-PCR. C. CCK-8 assays were performed to determine the cell proliferation of SKOV3 cells after lncARSR overexpression. D. Representative results of the colony formation of SKOV3 cells with lncARSR overexpression. E. CCK-8 assays were performed to determine the cell proliferation of CAOV3 cells after lncARSR knockdown. F. Representative results of the colony formation of CAOV3 cells with lncARSR overexpression. G. Cell invasion was analyzed using a transwell chamber with matrigel. Overexpression of lncARSR promotes SKOV3 cell invasion. H. Cell invasion was analyzed using a transwell chamber with matrigel. Knockdown of lncARSR suppresses CAOV3 cell invasion. Data are shown as means ± SEM, *$P < 0.05$.

0.0001, **Figure 3E**). lncARSR expression also affected the expression levels of downstream effectors of β-catenin, including c-myc and cyclin D1 (**Figure 3F** and **3G**), which varied with the lncARSR expression. Together, our findings indicate that Wnt/β-catenin signaling may be a downstream mechanism of lncARSR in EOC development.

lncARSR interacts with HuR and upregulates β-catenin expression

To investigate the mechanism by which lncARSR regulates β-catenin expression, we examined lncARSR localization. Cytoplasmic and nuclear RNA fractions results showed that lncARSR is mainly expressed in the cytoplasm (**Figure 4A**).
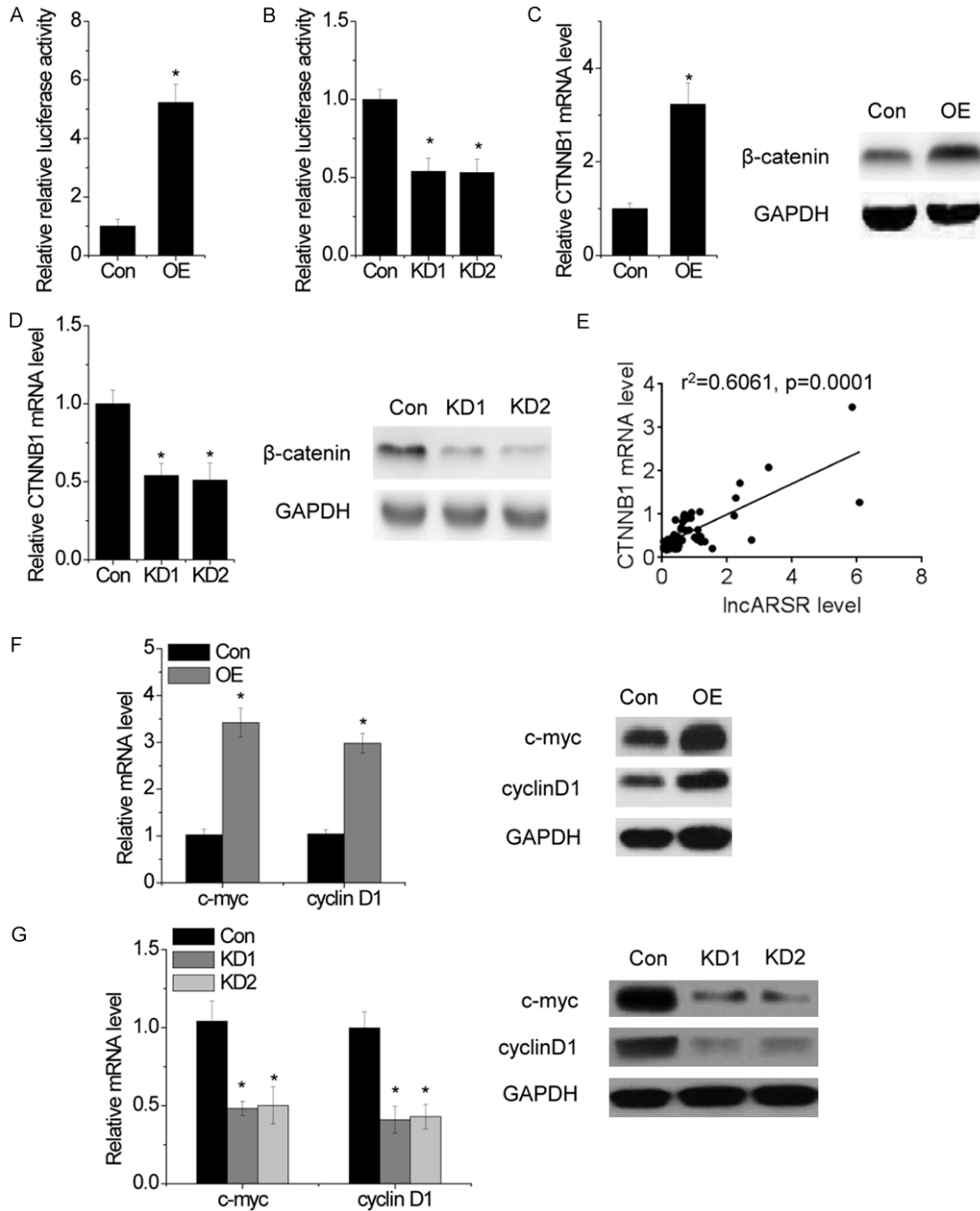
Figure 3. lncARSR activates Wnt/β-catenin signaling pathway in EOC cells. A. Luciferase activity of TOPflash reporter was evaluated in SKOV3 cells overexpressing lncARSR. B. Luciferase activity of TOPflash reporter was evaluated in CAOV3 cells with lncARSR knockdown. C. The mRNA and protein level of β-catenin was determined by qRT-PCR and western blot in SKOV3 cells overexpressing lncARSR, respectively. D. The mRNA and protein level of β-catenin was determined by qRT-PCR and western blot in CAOV3 cells with lncARSR knockdown, respectively. E. The correlation between lncARSR and β-catenin mRNA expression was determined in EOC tissue samples. F. The mRNA and protein level of c-myc and cyclin D1 was determined by qRT-PCR and western blot in SKOV3 cells overexpressing lncARSR, respectively. G. The mRNA and protein level of c-myc and cyclin D1 was determined by qRT-PCR and western blot in CAOV3 cells with lncARSR knockdown, respectively. Data are shown as means ± SEM, *$P < 0.05$.
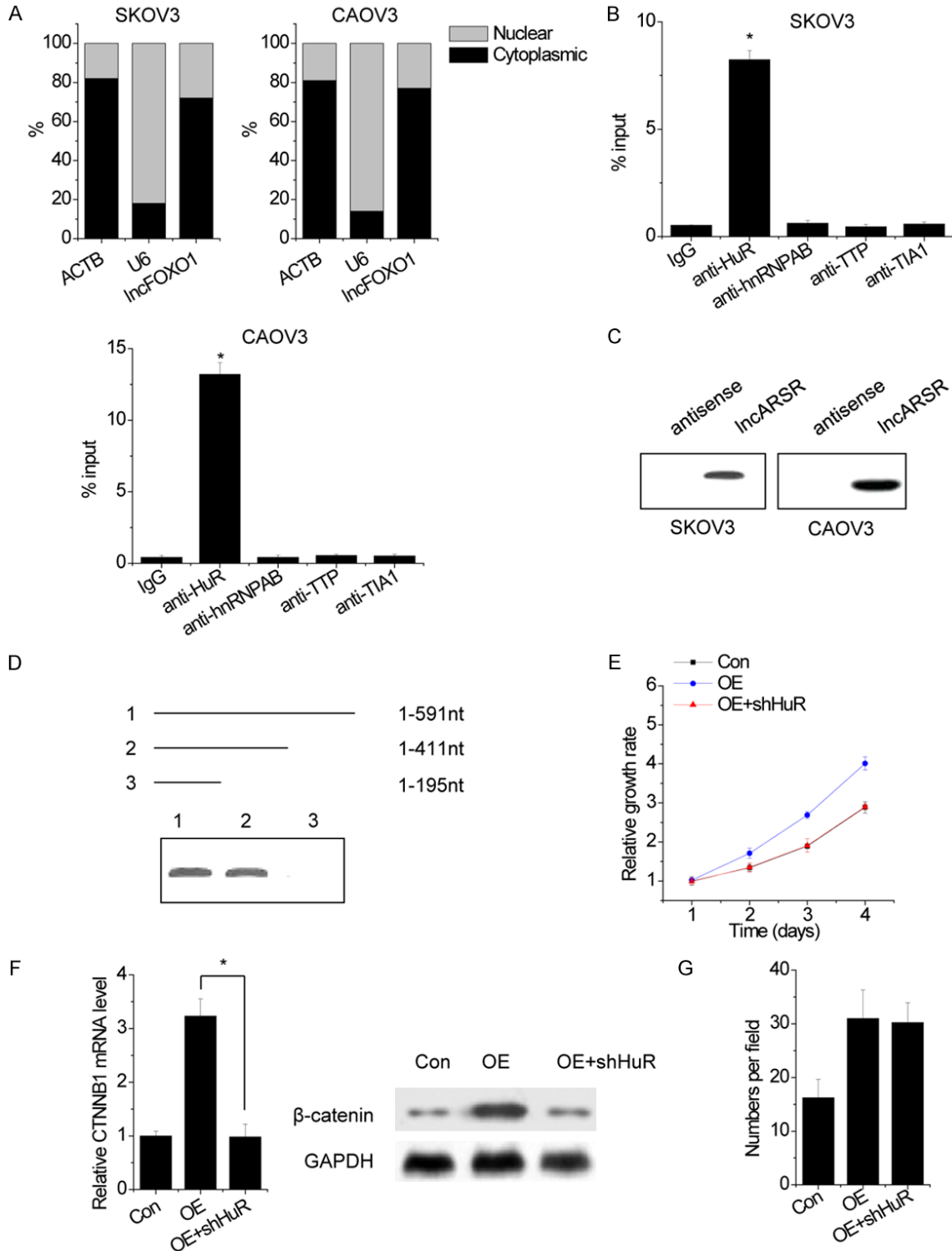
Figure 4. lncARSR interacts with HuR and upregulates β-catenin expression. (A) Relative lncARSR levels in cell cytoplasm or nucleus of EOC cell lines were detected by qRT-PCR. (B) RIP assays were performed in EOC cells and the co-precipitated RNA was subjected to qRT-PCR for lncARSR. (C) Biotinylated lncARSR or its antisense RNA were incubated with nuclear extracts (SKOV3 and CAOV3 cells), targeted with streptavidin beads, and washed, and associated proteins were resolved in a gel. Western blotting analysis of the specific association of HuR and lncARSR. (D) RNAs corresponding to different fragments of lncARSR were treated, and associated HuR was detected by RNA pull-down and followed by western blot analysis. (E-G) SKOV3 cells were transfected with HuR shRNA for 48 hours, and the cell proliferation (E), β-catenin level (F) and invasion (G) were detected. Data are shown as means ± SEM, *P < 0.05.
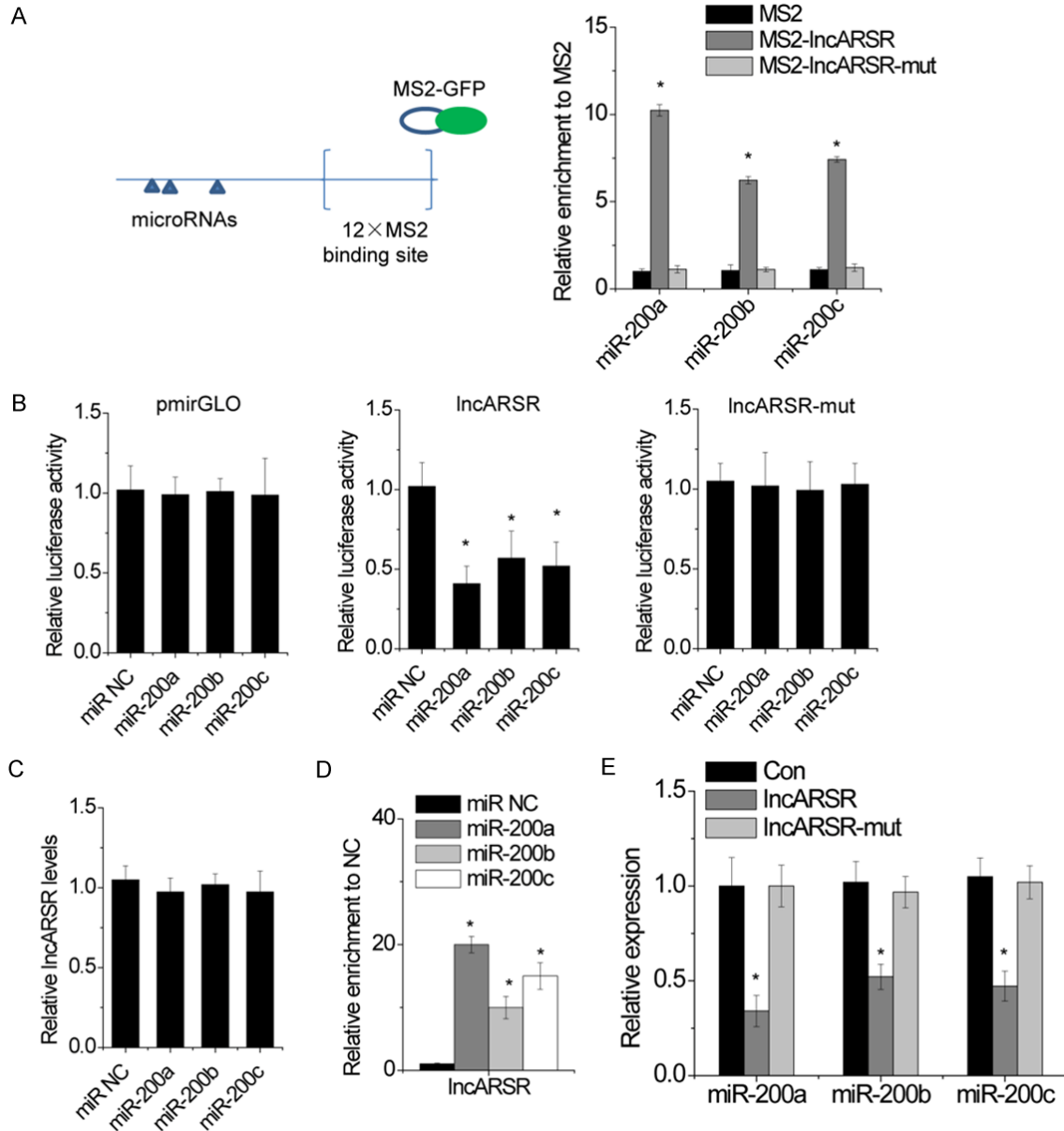
Figure 5. lncARSR interacts with miR-200 family. A. MS2-RIP followed by microRNA qRT-PCR to detect microRNAs endogenously associated with lncARSR. B. Luciferase activity in EOC cells co-transfected with miR-200s and luciferase reporters containing nothing, lncARSR or mutant transcript. Data are presented as the relative ratio of firefly luciferase activity to renilla luciferase activity. C. The relative expression of lncARSR in EOC cells transiently overexpressing miR-200s. D. Anti-AGO2 RIP was performed in EOC cells transiently overexpressing miR-200s, followed by qRT-PCR to detect lncARSR associated with AGO2. E. The relative expression of miR-200s in EOC cells transiently overexpressing lncARSR or lncARSR-mut. Data are shown as means ± SEM, *P < 0.05.

Cytoplasmic lncRNAs often functions through interaction with RNA-binding proteins (RBPs) [10]. Thus, we hypothesized that lncARSR may increase β-catenin mRNA expression through association with RBPs, such as HuR, hnRNPAB, TTP, and TIA1. The results of RIP assays demonstrated that HuR directly interacted with lncARSR and β-catenin mRNA in both SKOV3 and CAOV3 cells (**Figure 4B**). For further confirmation, we performed RNA pull-down assays and found that lncARSR associated with HuR (**Figure 4C**). Deletion analysis showed that the 3'-end (412-591nt) of lncARSR was essential for lncARSR-HuR interaction (**Figure 4D**). Moreover, knockdown of HuR attenuated the effect of lncARSR-induced proliferation and
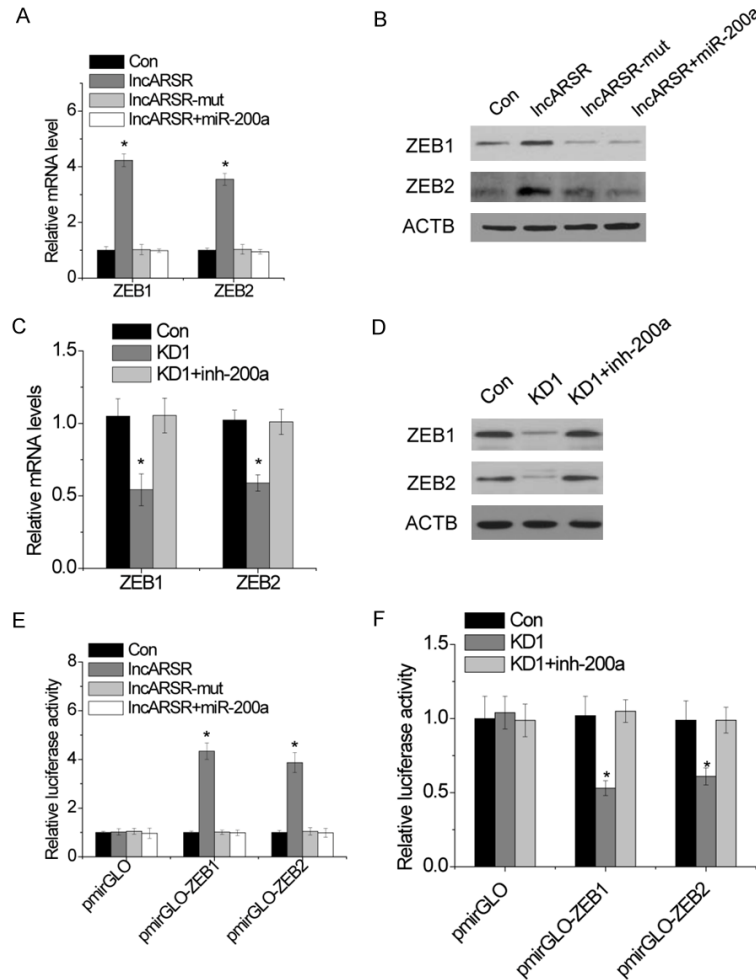
Figure 6. lncARSR upregulates ZEB1 and ZEB2 expression. (A and B) The mRNA (A) or protein (B) levels of ZEB1 and ZEB2 in stable SKOV3 cell clones. (C and D) The mRNA (C) or protein (D) levels of ZEB1 and ZEB2 in stable CAOV3 cell clones. (E and F) Luciferase activity in stable SKOV3 cell clones (E) or CAOV3 cell clones (F) transfected with luciferase reporters containing ZEB1 3'UTR, ZEB2 3'UTR or nothing. Data are shown as means ± SEM, *P < 0.05.

could function as competing endogenous RNAs (ceRNA) by competitively binding common microRNAs. The miR-200 family, including miR-200a, miR-200b and miR-200c, inhibits EMT and tumor invasion by suppression of ZEB1 and ZEB2. Interestingly, lncARSR has predicted miR-200s targeting site, indicating that lncARSR may exert as a ceRNA of ZEB1 and ZEB2. To confirm the direct association between lncARSR and miR-200s, we performed a MS2-RIP to pull down endogenous microRNAs interacted with lncARSR. We found that the lncARSR RIP in SKOV3 cells was significantly enriched for miR-200s compared to the empty vector (MS2), IgG, and lncARSR with mutations in miR-200s targeting sites (named lncARSR-mut) (**Figure 5A**). For further validation, we constructed luciferase reporters expressing the lncARSR and lncARSR-mut. We observed that miR-200s reduced the luciferase activities of the lncARSR but not empty vector or lncARSR-mut (**Figure 5B**). However, we found no significant difference in lncARSR levels after overexpression of miR-200s (**Figure 5C**). In addition, we performed anti-AGO2 RIP in SKOV3 cells transfected with miR-200s. Endogenous lncARSR was significantly pulled down by AGO2 in miR-200s-transfected cells (**Figure 5D**). Overexpression of lncARSR, but not lncARSR-mut, decreased the miR-200s expression (**Figure 5E**). All these data demonstrate that lncARSR directly interacts with the miR-200 family.

lncARSR upregulates ZEB1 and ZEB2 expression

We wondered whether lncARSR could modulate ZEB1 and ZEB2 and then EMT and invasion of EOC cells. For the rescue experiment, we over-

β-catenin upregulation in SKOV3 cells (**Figure 4E** and **4F**). However, knockdown of HuR did not reversed the cell invasion increased by lncARSR overexpression (**Figure 4G**), indicating that the lncARSR-mediated invasion did not depends on HuR. Together, our results indicate that lncARSR enhances EOC cell proliferation by upregulating β-catenin in a HuR-dependent manner.

lncARSR physically associates with miR-200 family

Next, we investigated the mechanisms by which lncARSR promoted EOC cell invasion. lncRNAs
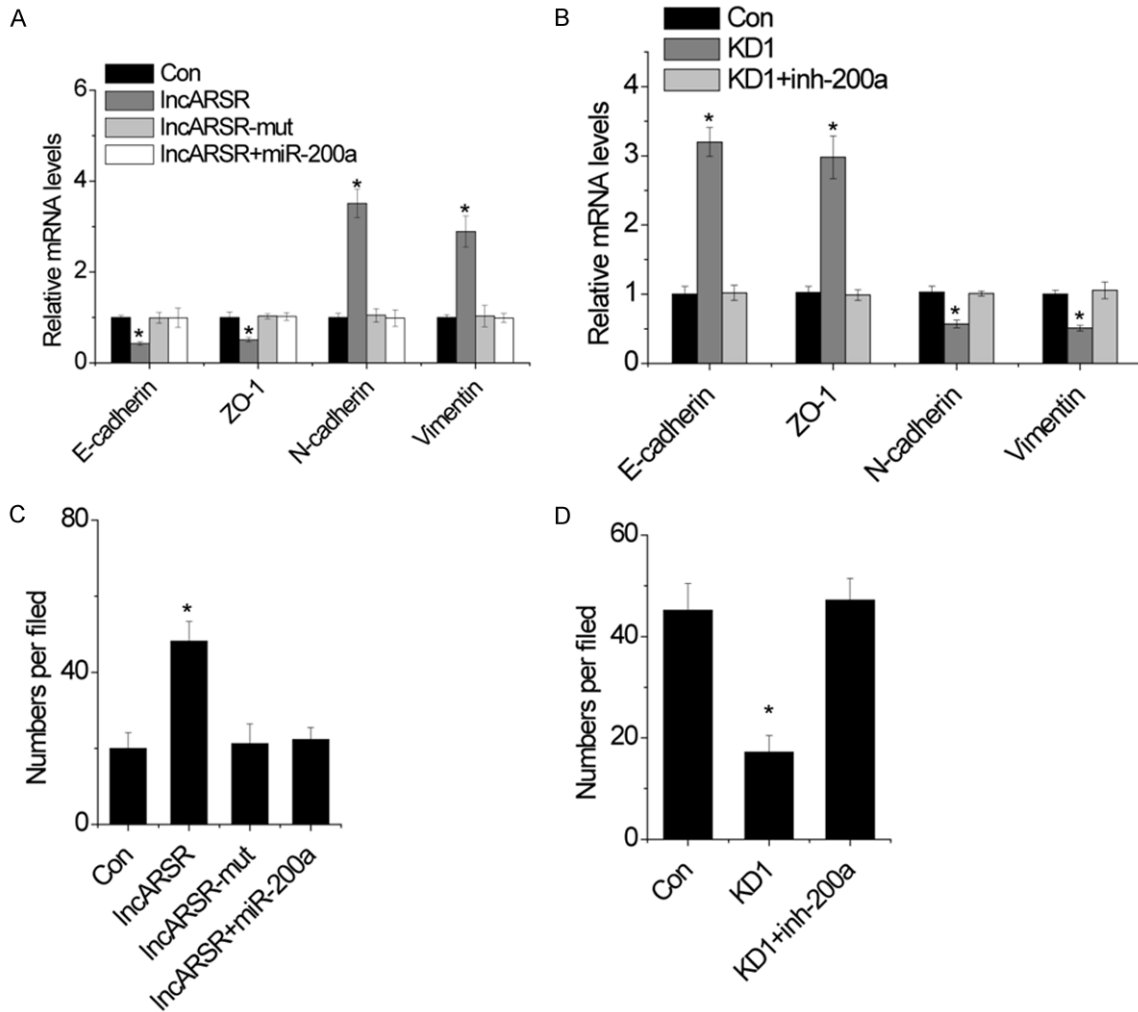
Figure 7. lncARSR induces EMT. (A and B) The mRNA levels of EMT markers in indicated SKOV3 (A) and CAOV3 (B) cell clones. (C and D) The cell invasion in indicated SKOV3 (A) and CAOV3 (B) cell clones was detected by Transwell assay. Data are shown as means ± SEM, *P < 0.05.

expressed miR-200a in lncARSR-overexpressing SKOV3 cells. Upregulation of lncARSR, but not lncARSR-mut, increased the mRNA and protein levels of ZEB1 and ZEB2. Ectopic expression of miR-200a abrogated this increase (**Figure 6A** and **6B**). Additionally, we inhibited miR-200a in lncARSR-silencing CAOV3 cells. The depletion of lncARSR reduced ZEB1 and ZEB2 expression. Silence of miR-200a overcame the decrease of ZEB1 and ZEB2 (**Figure 6C** and **6D**). To ascertain whether this observation depends on regulation of the ZEB1 and ZEB2 3'UTR, we constructed luciferase reporters containing the ZEB1 or ZEB2 3'UTR (pmirGLO-ZEB1 or pmirGLO-ZEB2). Luciferase plasmid (pmirGLO-ZEB1, pmirGLO-ZEB2, or the control reporter pmirGLO) was transfected into the

different SKOV3 and CAOV3 cell clones. Overexpression of lncARSR, but not the mutant, increased the luciferase activity of pmirGLO-ZEB1 and pmirGLO-ZEB2. Overexpression of miR-200a abolished this upregulation (**Figure 6E**). Reciprocally, the depletion of lncARSR suppressed the luciferase activity of pmirGLO-ZEB1 and pmirGLO-ZEB2, which were rescued by miR-200a inhibitor (**Figure 6F**). Taken together, we demonstrate an important role of lncARSR in modulating ZEB1 and ZEB2 by acting as a ceRNA.

lncARSR induces EMT

We speculated that lncARSR regulated invasion and EMT through modulating ZEB1 and ZEB2.

Analysis of the epithelial markers E-cadherin and ZO-1 and the mesenchymal markers N-cadherin and vimentin revealed that overexpression of lncARSR, but not the mutant, reduced E-cadherin and ZO-1 and increased N-cadherin and vimentin. Consistently, ectopic expression of miR-200a abolished these effects (**Figure 7A**). Conversely, lncARSR depletion upregulated E-cadherin and ZO-1 and downregulated N-cadherin and vimentin, which was abolished by miR-200a inhibition (**Figure 7B**). Together, these data suggest that lncARSR induces EMT through competitive binding of miR-200s.

To test whether lncARSR promotes invasive behavior by promoting EMT, we determined the invasion ability of different SKOV3 and CAOV3 cell clones using Matrigel-coated transwell experiments. We observed that overexpression of lncARSR, but not the mutant, significantly increased the invasion potential of SKOV3 cells, and this invasion potential was completely reversed when miR-200a was overexpressed (**Figure 7C**). On the contrary, silence of lncARSR reduced the invasion potential of CAOV3 cells, which was abolished by miR-200a inhibitor (**Figure 7D**). Altogether, these results demonstrate that lncARSR induces EMT and promotes a more invasive phenotype by competitively binding miR-200s.

Discussion

The main finding of our current study was that lncARSR is upregulated in EOC tissues and closely correlated with advanced FIGO stage, histological grade, lymph node metastasis and poor prognosis. Further investigation demonstrated that lncARSR interacted with HuR, upregulated β-catenin expression and then activated Wnt/β-catenin signaling pathway to regulate cell proliferation. Moreover, lncARSR increased ZEB1 and ZEB2 expression by competitively binding the miR-200 family to induce EMT and invasion. Thus, our results indicate that lncARSR functions as an oncogenic lncRNA in EOC progression and can be considered as a promising therapeutic target for EOC.

It has been reported that lncARSR is closely associated with chemo-resistance in renal cancer and hepatocellular carcinoma (HCC). Overexpression of lncARSR in HCC is associated with large tumor size and advanced BCLC stage, and indicates poor survival. Mechanistically, lncARSR physically associates with PTEN mRNA, promotes PTEN mRNA degradation, and then activates PI3K/Akt pathway [11]. In renal cancer, lncARSR enhances sunitinib resistance via competitively binding miR-34/miR-449 to upregulate AXL and c-MET expression. Moreover, lncARSR can be incorporated into exosomes to disseminate sunitinib resistance [8]. lncARSR also promotes the self-renewal, tumorigenicity and metastasis of renal tumour-initiating cells. Mechanistically, the binding of lncARSR to YAP suppresses YAP phosphorylation and promotes YAP nuclear translocation [12]. Our current study reveals a novel molecular mechanism by which lncARSR promotes EOC proliferation and invasion. We found that lncARSR is mainly expressed in the cytoplasm and interacts with RNA binding protein HuR. Moreover, a positive correlation between lncARSR and β-catenin mRNA expression was observed in EOC tissues, further supporting the regulatory relationship between lncARSR and β-catenin stability. Finally, our results showed that knockdown endogenous HuR expression abrogated lncARSR's effect in EOC proliferation and β-catenin expression, indicating that lncARSR functions in EOC in a HuR-dependent manner.

Knockdown of HuR did not reverse the cell invasion increased by lncARSR overexpression. We speculated that other mechanisms were involved in lncARSR-induced invasion. In this study, we found that lncARSR shares miR-200s response elements with ZEB1 and ZEB2, the critical regulator of EMT [13]. We observed that ectopic expression of lncARSR was sufficient to increase ZEB1 and ZEB2 and induce EMT. Notably, this role depends on the binding of miR-200s, indicating that lncARSR functions as a ceRNA. It is widely recognized that EMT facilitates tumor invasion and metastasis [14]. Taken together, our results revealed that lncARSR induced EMT and cell invasion.

Collectively, in the present study, we provided evidence that lncARSR was upregulated in EOC and promoted proliferation and invasion of EOC cells via activating Wnt/β-catenin signaling pathway and functioning as a ceRNA of ZEB1 and ZEB2. lncARSR may be a novel therapeutic target for EOC treatment.

Disclosure of conflict of interest

None.

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