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HYOU1 promotes cell growth and metastasis *via* activating PI3K/AKT signaling in epithelial ovarian cancer and predicts poor prognosis

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Abstract. – OBJECTIVE: Hypoxia upregulated 1 (HYOU1) has been reported to be abnormally expressed in different malignancies, especially in breast cancer. However, the role of HYOU1 in epithelial ovarian cancer (EOC) remains largely unclear. This study aimed to explore the expression and function of HYOU1 in EOC progression.

PATIENTS AND METHODS: HYOU1 levels in EOC tissues and cell lines were investigated by RT-PCR. The clinical and prognostic significance of HYOU1 in 127 cases of EOC was analyzed using the Chi-square analysis, Kaplan-Meier analysis, and the Cox proportional hazards regression model. We have also performed multiple cells experiments to evaluate the effects of HYOU1 on EOC cell proliferation, apoptosis, migration, and invasion. The protein levels of associated PI3K/Akt signaling pathway was detected using Western blot assay.

RESULTS: We found that the expression levels of HYOU1 were significantly upregulated in both EOC tissues and cell lines. A higher expression of HYOU1 was associated with advanced FIGO stage, LN metastasis, and shorter overall survival. In addition, univariate and multivariate analysis identified high HYOU1 expression as an unfavorable prognostic factor for overall survival. Functional assays revealed that the inhibition of HYOU1 suppressed the tumor proliferation and colony formation, as well as the migratory and invasive capacity. Finally, when HYOU1 was silenced, the results of Western blot showed that the levels of p-PI3K, p-Akt, as well as cell cycle and EMT genes, were respectively downregulated.

CONCLUSIONS: Our findings highlighted the targeting of HYOU1 as a novel therapeutic approach for the treatment of EOC.

Key Words:
HYOU1, Epithelial ovarian cancer, PI3K/AKT signaling pathway, Prognosis, Tumorigenesis.

Introduction

Ovarian cancer is a leading cause of death among gynecological cancers worldwide, and the fatality rate of ovarian cancer is the highest of all types of gynecological tumors, causing about 140,200 deaths in the world annually[1,2]. The epithelial ovarian cancer (EOC) is a common entity accounting for 80 to 90% of ovarian cancer cases[3]. The current standard of therapy is the debulking surgery combined with chemotherapy[4]. In spite of continuous efforts to improve the therapeutic response, the prognosis of EOC patients remains poor, with a 5-year survival rate of only 30%[5,6]. In addition, although growing researches have been published in recent years and have contributed to the understanding of the basic and clinical knowledge, the complex molecular mechanism of EOC is still insufficiently understood[7]. Thus, it is necessary to further study the molecular mechanisms involved in EOC carcinogenesis and to identify the prognostic markers for the design of the individual therapy, and the targeted treatment of EOC. Hypoxia upregulated 1 (HYOU1), also known as Grp170 and HSP12A, is encoded by this HYOU1 gene and belongs to the heat shock protein 70 family[8,9]. Up to date, the biological function of HYOU1 in cell progress remains largely unclear. Previously, evidence has shown that HYOU1 may be involved in the regulation of apoptosis and its inhibition is correlated with accelerated apoptosis[10,11]. In addition, there are cellular experiments which indicate that this gene has an important cytoprotective role in hypoxia-induced cellular perturbation. Recently, with the development of sequencing technology, the dysregulation of HYOU1 was reported in several tumors, which suggested that HYOU1 may play a

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functional effect in the development and progression of tumors[12,13]. Zhou et al[14] firstly reported that HYOU1 was highly expressed in nasopharyngeal carcinoma and associated with poor prognosis of the patient with nasopharyngeal carcinoma, suggesting that HYOU1 may serve as a tumor promoter in this disease, although *in vitro* and *in vivo* experiments were not been performed. However, whether HYOU1 was abnormally expressed in EOC, as well as its potential biological function in the progression of EOC, remains largely unclear. In this study, we analyzed microarray data from GEO datasets, finding that HYOU1 expression was significantly up-regulated in EOC tissues, which was consistent with the expression trend of HYOU1 in breast cancer[9] and nasopharyngeal carcinoma[14]. Furthermore, RT-PCR assays of clinical samples from our hospital also confirmed that HYOU1 was overexpressed in EOC tissues. Given the possibility of HYOU1 as a potential positive regulator in EOC, we further analyzed its prognostic value in EOC patients and performed a lost-function assay to explore its specific biological function in the progression of EOC. Finally, we also tried to explore the potential mechanism of HYOU1 involved in the proliferation and metastasis.

Patients and Methods

Clinical Specimens

A total of 127 EOC patients (relevant clinical features were summarized in Table I) were enrolled in this research, which was approved by the Ethics Committee of Qingdao Hiser Medical Group from April 2010 to March 2013. The EOC specimens and adjacent non-cancerous normal tissue samples were immediately frozen using liquid nitrogen and subsequently stored at -80°C for further use. No patients received any chemotherapy, immunotherapy or radiotherapy before the surgery. The informed consents were obtained from all patients.

Cell Lines and Cell Transfection

We obtained IOSE80 (ovarian epithelial cells) and three EOC cells, HEY, SKOV3, OVCAR-3, from Uban Biotechnology Co., Ltd. (Jiading, Shanghai, China). The cells were maintained in RPMI 1640 medium (BioSUN, Xuhui, Shanghai, China) supplemented with fetal bovine serum (FBS; 10% concentration) and penicillin-streptomycin antibiotics solution (1%; HY Stem Cells, Shapingba, Chongqing, China). For cell transfection, a LipoFiter 3 transfection reagent (Han-Bio, Jiading, Shanghai, China) was employed to transfect small interfering RNAs (siRNAs) into SKOV3 and OVCAR-3 cells. The siRNAs targeting HYOU1 (si-HYOU1-1 and si-HYOU1-2) and negative control siRNAs (si-NC) were purchased from ZoonBio Co., Ltd. (Nanjing, Jiangsu, China).

RNA Isolation and Quantitative Real Time-PCR

The total RNA from EOC tissues or cells was extracted using TRIzol reagent, and the quantity of the RNA was determined by NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Then, the cDNA was reverse-transcribed using Fermentas RevertAid first strand cDNA synthesis kit (Think-far, Haidian, Beijing, China). Afterward, qRT-PCR assays for HYOU1 determination were conducted using SYBR Premix Ex Taq real time-PCR kit (TaKaRa, Dalian, Liaoning, China). Glyceralde-

Table I. Correlation between HYOU1 expression with clinicopathologic features of EOC.

Parameters	Category	No.	HYOU1 expression		*p*-value
			High	Low	
Age (y)	≤50	63	30	33	0.657
	>50	64	33	31	
FIGO stage	I-II	80	34	46	0.037
	III-IV	47	29	18	
Differentiation	1/2	72	32	40	0.183
	3	55	31	24	
Tumor size	≤5 cm	89	45	44	0.384
	>5 cm	48	28	20	
LN Metastasis	No	87	37	50	0.019
	Yes	40	26	14	

Table II. Microorganism isolated from patients with PJI.

Genes	Primer sequences (5'-3')
HYOU1:	ForwardGAGGAGGCGAGTCTGTTGG
HYOU1:	ReverseGCACTCCAGGTTTGACAATGG
GAPDH:	ForwardTGGCCTTCCGTGTTCCTAC
GAPDH:	ReverseGAGTTGCTGTTGAAGTCGCA

hyde-3-phosphate dehydrogenase (GAPDH) was used as the reference gene. Relative HYOU1 expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers for HYOU1 are listed in Table II.

Western Blot Assays

HYOU1 siRNAs-transfected SKOV3 and OVCAR-3 cells were lysed using RIPA lysis buffer (SeeBio, Pudong, Shanghai, China), and a BCA assay kit (HuaMaiKe, Changping, Beijing, China) was applied to measure the concentration of proteins. Subsequently, 20 μg proteins (per lane) were separated by 8-10% sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE) using a Bio-Rad Western blot apparatus and then, transferred to Millipore polyvinylidene difluoride (PVDF) membranes. Next, non-fat milk (5%) was employed to block the membranes, and sequentially the membranes were probed by primary antibodies targeting GAPDH, vimentin, N-cadherin, phosphorylated-PI3K (p-PI3K), phosphorylated-AKT (p-AKT), PI3K, and AKT for 12 h at 4°C. On the second day, the membranes were washed with TBST and incubated with the corresponding secondary antibodies. Then, the proteins were detected using the NCM ECL Ultra assay kit (NCM Biotech, Suzhou, Jiangsu, China). All the antibodies were purchased from Univ-Bio Co., Ltd. (Pudong, Shanghai, China). The optical density of the protein bands was analyzed by Image J software (NIH, Bethesda, ML, USA).

Cell Counting Kit-8 Assays

The cellular growth curves of HYOU1 siRNAs or negative control siRNAs-transfected SKOV3 and OVCAR-3 cells were measured by the CCK-8 assay kit (BioTechWell, Qingpu, Shanghai, China). Briefly, at a density of 1×10^4 cells/well, the treated cells were first planted in 96-well plates. Subsequently, at the indicated time period (24 h, 48 h, 72 h, and 96 h), the cells were mixed with CCK-8 reagent (10 μl per well). After incubation for 2 h at 37°C, the optical density (450 nm) was determined by a microplate reader (NYW-MB96; NYAW, Daxing, Beijing, China).

EdU Assays

EdU (5-Ethynyl-2'-deoxyuridine; SolarBio, Tongzhou, Beijing, China) assays were applied to examine the proliferation of SKOV3 and OVCAR-3 cells. In short, after transfection with HYOU1 siRNAs or negative control siRNAs, the SKOV3, and OVCAR-3 cells were harvested and re-plated in 48 well plates. Forty-eight hours later, the EdU reagent (100 μl per well) was added into the cells, and the plates were continued to be cultured for 2 h. Then, the Phosphate-buffered saline (PBS) was used to wash the cells, and the DAPI reagent was added into the cells. After rinsing with PBS twice, a NIB100F fluorescence microscope (Micro-Science, Jinan, Shandong, China) was applied to observe the cellular fluorescence.

Cell Colony Formation Assays

For colony formation assays, the SKOV3 and OVCAR-3 cells were first transfected with HYOU1 siRNAs or negative control siRNAs. Then, the treated cells were harvested and re-placed into 6-well plates (500 cells per well). The transfections were re-conducted every 3 days. Two weeks later, the cell colonies were stained using 0.2% crystal violet solution (in 20% methanol) and subsequently photographed by a NIB100F microscope (Micro-Science, Jinan, Shandong, China).

Cell Apoptosis Analysis

Briefly, the HYOU1 siRNAs or negative control siRNAs-transfected SKOV3 and OVCAR-3 cells were collected and stained with Annexin V-FITC as well as propidium iodide solution. After incubation in the dark for 15-20 min, the cells were subjected to flow cytometry analysis using a Millipore Guava easyCyte 12 HT flow cytometer (Merck, Songjiang, Shanghai, China).

Caspase 3 and Caspase 9 Activity Examination

The relative activity of caspase 3 and caspase 9 was measured by a caspase 3/9 activity assay kit (Beyotime Biotech, Haimen, Jiangsu, China). In short, the treated cells were harvested and lysed using the lysis buffer in the kit. Then, the cell lysates were centrifuged at 4°C (16HYOU1g/min) for 15 min. The supernatant was then collected and subjected to caspase 3 and caspase 9 activity examination using the Ac-DEVD-pNA (2mM) on a microplate reader (NYW-MB96; NYAW, Daxing, Beijing, China) at the wavelength of 405 nm.

Wound Healing Assays

Firstly, the SKOV3 or OVCAR-3 cells after transfection with the HYOU1 siRNAs or negative control siRNAs were planted into 6-well plates. Subsequently, the cells were cultured at 37°C with 5% CO_2 until 90-100% cell confluence. Then, a linear scratch was generated by a pipette tip (200 μl). Finally, a NIB100F microscope (Micro-Science, Jinan, Shandong, China) was utilized to photograph the wounded areas at 0 h and 48 h.

Transwell Invasion Assays

To monitor the cell invasion, the transwell invasion assays were carried out. In brief, SKOV3 and OVCAR-3 cells were first transfected with HYOU1 siRNAs or negative control siRNAs. After 24 h, the cells were washed using PBS and harvested using trypsin. Afterward, 5×10^4 cells (in 250 μl medium without serum) were planted into the upper sides of the Matrigel-coated transwell inserts (pore size: 8 μm; Millipore, Jiading, Shanghai, China), and 20% FBS contained culture medium (600 μl) was added into the lower chambers. After incubation for 24 h, 0.1% crystal violet solution (in 20% methanol) was applied to stain the invaded cells on the lower sides of the insert membranes. After rinsing with PBS for three times, a NIB100F microscope (Micro-Science, Jinan, Shandong, China) was employed to take images of the invasive cells.

Statistical Analysis

The statistical analyses were carried out using the SPSS 19.0 software (IBM Corp., Armonk, NY, USA). The independent-samples *t*-test was employed for two-group analysis, while the One-way ANOVA test was applied when analyzing more than two groups. The Tukey's post-hoc test was used to validate the ANOVA for comparing measurement data between groups. The Kaplan-Meier curves for the overall survival rates were compared by a log-rank test. The hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using the Cox proportional hazards model. A value of $p<0.05$ was considered statistically significant.

Results

HYOU1 is Upregulated in Human EOC

To explore the dysregulated genes in EOC tissues, we first downloaded genes array expression profile datasets GSE26712 and GSE66957 from the open Gene Expression Omnibus (GEO) database. The results of the statistical analysis showed the abnormally expressed pattern of genes in the EOC tissues (Figure 1A). Then, we identified 345 genes that were significantly upregulated and 40 genes that were significantly downregulated by the Venn analysis (Figure 1B). Of note, the expression levels of HYOU1 were significantly upregulated in EOC tissues in both GSE26712 and GSE66957 (Figure 1C). In order to confirm the results of the online microarray data, we further performed RT-PCR to detect the expression of HYOU1 in 127 paired of ECO tissues and matched normal tissues, finding that HYOU1 was significantly overexpressed in EOC tissues, which was consistent with the online results (Figure 1D). Moreover, we also observed that the expression of HYOU1 was significantly upregulated in three EOC cell lines compared to IOSE80 (Figure 1E). Taken together, our results strongly suggested that HYOU1 was highly expressed in EOC tissues and may display a functional effect in the progression of EOC.

Increased HYOU1 Expression Correlates with Advanced Clinical Stage and Poor Clinical Outcomes

In order to explore the clinical significance of HYOU1 expression in EOC patients, we used HYOU1 levels as a cutoff point to divide all 127 EOC patients into two groups (High and Low). Then, we performed the Chi-square analysis to analyze the association between HYOU1 expression and clinicopathological features in 127 EOC patients, discovering that higher expression of HYOU1 was associated with advanced FIGO stage (p=0.037) and LN Metastasis (p=0.019) (Table I). However, the relative HYOU1 expression was not associated with other parameters such as age (p=0.657), differentiation (p=0.183) and tumor size (p=0.384). Then, the Kaplan-Meier survival analysis was performed to explore the association between the HYOU1 expression and the prognosis of EOC patients and we found that high expression of HYOU1 indicated the shorter overall survival of EOC patients (Figure 1F). In addition, by univariate analysis, three prognostic factors for overall survival were identified: FIGO stage (I-II *vs.* III-IV, p=0.014), LN Metastasis (No *vs.* Yes, p=0.007), and HYOU1 expression level (Higher *vs.* Lower, p=0.007). Moreover, the results of the multivariate analysis showed that HYOU1 expression was a significant independent predictor of poor survival in EOC patients

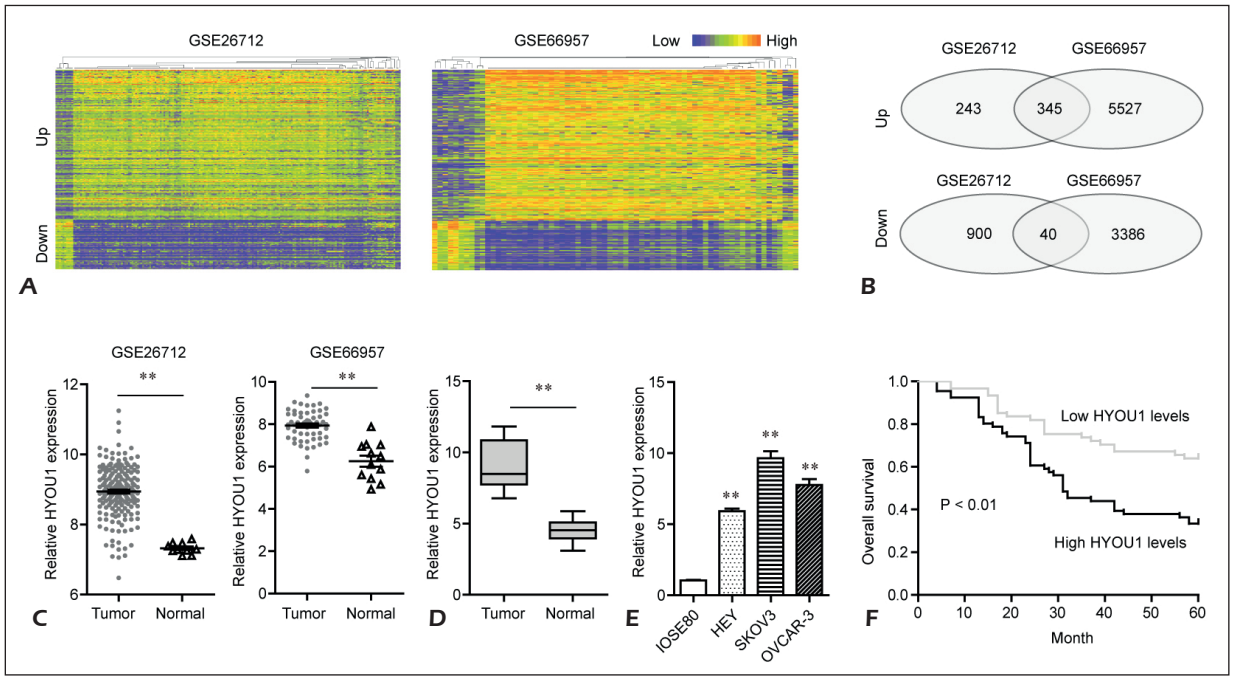

Figure 1. HYOU1 was upregulated in EOC and associated with poor prognosis. **A**, Heat map analysis of the mRNAs expression of the groups was created using a method of hierarchical clustering by GeneSpring GX, version 7.3. Microarray data were obtained from GEO (GSE26712 and GSE66957). **B**, The Venn analysis of the genes that are significantly upregulated or downregulated in GSE26712 and GSE66957. **C**, Expression of HYOU1 in the GSE26712 and GSE66957 cohorts. **D**, qRT-PCR analysis of HYOU1 expression in 127 pairs of EOC and corresponding normal tissues. **E**, qRT-PCR analysis of HYOU1 expression in three EOC cell lines and IOSE80 cells. **F**, The Kaplan-Meier overall survival analyses were used to investigate the relationship between HYOU1 expression and EOC patient survival. **p*<0.05, ***p*<0.01.

(HR=2.955, 95% CI: 1.327-4.451, *p*=0.017) as well as FIGO stage and LN Metastasis (Table III).

HYOU1 Knockdown Depressed Proliferation and Induced Apoptosis in SKOV3 and OVCAR-3 Cells

Given the above findings that HYOU1 was highly expressed in EOC, we next attempted to clarify the potential involvement of HYOU1 in malignant behavior of EOC. To achieve that, we first manipulated the expressing levels of HYOU1 in SKOV3 and OVCAR-3 cells with exogenous introduction of HYOU1 siRNAs (si-HYOU1-1 and si-HYOU1-2). The qRT-PCR assays were then performed to evaluate the knockdown efficiency and the data suggested that HYOU1 siRNAs caused a remarkable reduction of HYOU1 in both SKOV3 and OVCAR-3 cells (Figure 2A). We then investigated the effects of HYOU1 knockdown on the proliferation of SKOV3 and OVCAR-3 cells. The results of CCK-8 assays indicated that the introduction of HYOU1 siRNAs led to a notable decline of cellular viability of SKOV3 and OVCAR-3 cells (Figure 2B).

Table III. Univariate and multivariate analyses of prognostic factors in EOC patients.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age (y)	1.664	0.578-2.321	0.346	–	–	–
FIGO stage	3.321	1.338-4.631	0.014	3.105	1.155-4.127	0.033
Differentiation	1.466	0.788-2.138	0.114	–	–	–
Tumor size	1.579	0.642-2.447	0.217	–	–	–
LN Metastasis	3.554	1.462-5.217	0.008	3.069	1.216-4.325	0.021
HYOU1 expression	3.642	1.548-5.167	0.007	2.955	1.327-4.451	0.017

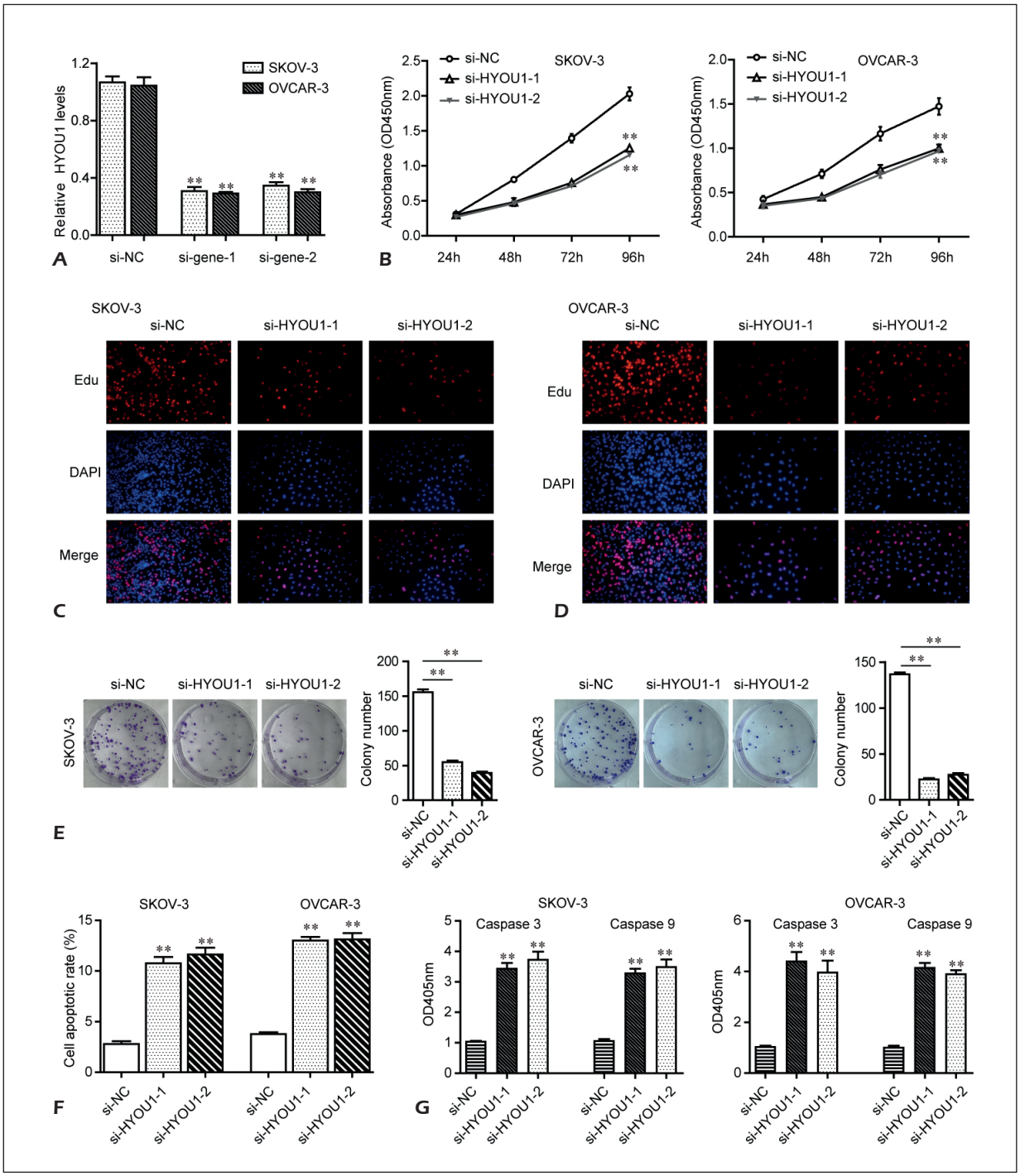

Figure 2. The cell proliferation of SKOV3 and OVCAR-3 cells was inhibited by the transfection of HYOU1 siRNAs. **A**, The qRT-PCR assays detected the expression of HYOU1 in SKOV3 and OVCAR-3 cells. **B**, The cellular growth of SKOV3 and OVCAR-3 cells after transfection with HYOU1 siRNAs was examined by the CCK-8 assays. **C**, and **D**, The cellular proliferation of HYOU1 siRNAs-transfected-SKOV3 and OVCAR-3 cells was evaluated by EdU assays (Magnification: 100×). **E**, The cell colony formation capacity of SKOV3 and OVCAR-3 cells was measured by colony formation assays (Magnification: 10×). **F**, The apoptosis of SKOV3 and OVCAR-3 cells was assessed by flow cytometry. **G**, The expression levels of caspase 3 and caspase 9 were evaluated by caspase 3 and caspase 9 activity examination assays. *p<0.05, **p<0.01.

In line with the inhibitory effects of HYOU1 siRNAs on cellular growth observed by the CCK-8 assays, the data of EdU assays also demonstrated that the silence of HYOU1 dramatically suppressed the cell proliferation (Figures 2C and D). In addition, the cell colony formation assays confirmed that the transfection of HYOU1 siRNAs significantly reduced the number of SKOV3 and OVCAR-3 cell colonies (Figure 2E). We next asked whether the knockdown of HYOU1 could affect the apoptosis of the EOC cells. According to the data of flow cytometry, the repression of the expression of HYOU1 markedly promoted the apoptosis of SKOV3 and OVCAR-3 cells (Figure 2F). Moreover, the molecular mechanism study revealed that HYOU1 siRNAs-transfected SKOV3 and OVCAR-3 cells expressed remarkably lower levels of caspase 3 and caspase 9 compared with the control cells (Figure 2G). Taken together, these data revealed an indispensable role of HYOU1 in maintaining cellular growth of EOC.

The Metastatic Potentials of SKOV3 and OVCAR-3 Cells Were Suppressed by Silence of HYOU1 Expression

Next, we set out to explore the influence of HYOU1 deficiency on EOC cell metastasis-related migratory and invasive capacity. To this purpose, we transfected HYOU1 siRNAs or NC siRNAs into SKOV3 and OVCAR-3 cells and sequentially performed wound healing and transwell invasion assays to evaluate the cell migratory and invasive abilities, respectively. The results of wound healing assays demonstrated that the silence of HYOU1 caused notable suppression of SKOV3 cell migration (Figure 3A). Also, the transwell invasion assays revealed that the introduction of HYOU1 siRNAs resulted in a significant reduction of invaded cell number of SKOV3 cells (Figure 3B). Similar results from wound healing and transwell invasion assays were also observed in OVCAR-3 cells when the cells were transfected with HYOU1 siRNAs (Figures 3C and D). In addition, the investigation of molecular mechanism indicated that the depression of the expression of HYOU1 dramatically reduced the protein levels of vimentin and N-cadherin, suggesting that HYOU1 had an impact on epithelial-mesenchymal transition. Collectively, our data provided insight that HYOU1 could modulate the metastatic potentials of EOC cells.

HYOU1 Deficiency Depressed the Activity of PI3K-AKT Signaling in SKOV3 and OVCAR-3 Cells

Upon finding that HYOU1 knockdown suppressed the tumor cell growth and mobility of EOC, we next aimed to unravel the possible molecular mechanisms behind these functions. As PI3K-AKT signaling pathway was widely involved in diverse functional regulation in many types of cancers we, therefore, focused this signaling and further conducted Western blot assays to determine the protein level changes of the relevant molecules. According to the results, after the transfection of HYOU1 siRNA, the protein expression of p-PI3K and p-AKT in SKOV3 cells were markedly decreased, while there was no impact on the expression of PI3K and AKT (Figure 4A). Similar results were also observed in OVCAR-3 cells transfected with HYOU1 siRNAs (Figure 4B). Therefore, our data suggested that the depletion of HYOU1 inhibited the activity of PI3K-AKT signaling in the EOC cells.

Discussion

EOC is a common malignant ovarian neoplasm with a poor 5-year survival rate (less than 30%)[15,16]. The high mortality rate in EOC reflects late-stage diagnosis, disease recurrence, and chemoresistance[17,18]. To improve the prognosis of EOC patients, various efforts are made. The prediction of prognosis after diagnosis is considered to be a very important direction. Thus, it is necessary to explore novel cancer biomarker for EOC. In this study, we firstly reported the role of HYOU1 on the progression of EOC. Our results showed that HYOU1 expression was significantly upregulated in both EOC tissues and cell lines, suggesting its potential function and diagnostic value in EOC patients. Then, by clinical assays, we found that high HYOU1 expression was associated with FIGO stage and LN Metastasis, indicating that this gene may contribute to the clinical progression of EOC. Moreover, the results of the Kaplan-Meier assays confirmed that a higher expression of HYOU1 predicted poorer prognosis of EOC patients. More importantly, the Cox regression analysis further demonstrated that HYOU1 was an independent risk factor for poor prognosis of EOC patients, suggesting the clinical application of HYOU1 as a novel biomarker for EOC patients. Previously, the oncogenic function of HYOU1 has been reported in breast cancer and

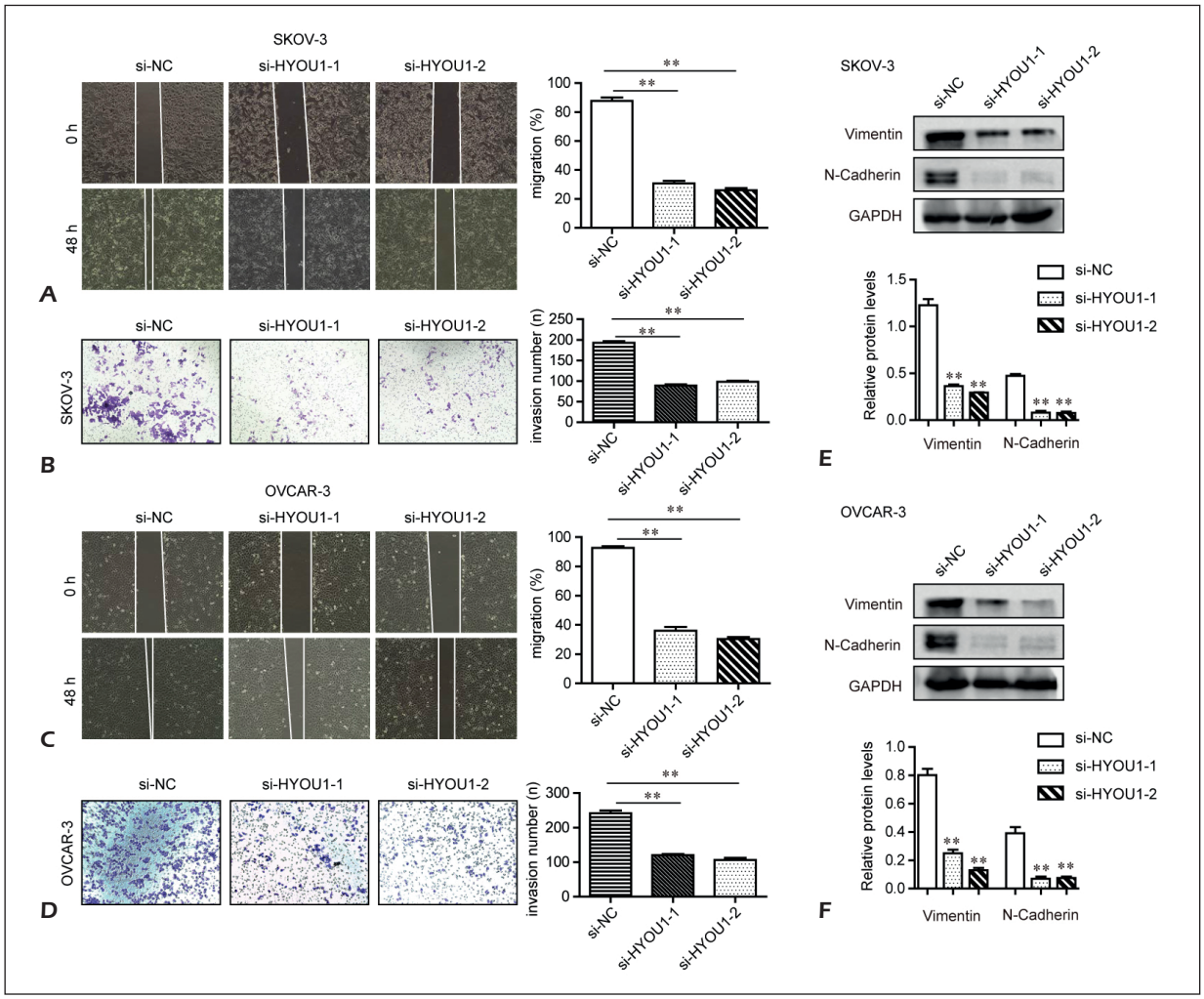

Figure 3. The invasion and migration of SKOV3 and OVCAR-3 cells were suppressed by HYOU1 siRNAs transfection. **A**, The migration of HYOU1 siRNAs-transfected-SKOV3 cells was assessed by wound healing assays (Magnification: 10×). **B**, The invasion of SKOV3 cells after treatment was determined by transwell invasion assays (Magnification: 40×). **C**, and **D**, Wound healing and transwell invasion assays were separately applied to detect the migratory and invasive abilities of OVCAR-3 cells after transfection with HYOU1 siRNAs or negative control siRNAs (si-NC) (Magnification: 10×) (Magnification: 40×). **E**, and **F**, Western blot assays examined the protein levels of N-cadherin and vimentin in SKOV3 and OVCAR-3 cells. **p*<0.05, ***p*<0.01.

nasopharyngeal carcinoma[9,14]. However, whether HYOU1 also displayed a similar role in EOC remains unknown. In this study, we performed *in vitro* assays to explore the function of HYOU1 in SKOV3 and OVCAR-3 cells, finding that the knockdown of HYOU1 decrease cell proliferation and colony formation by promoting cell cycle progression. As we know, metastasis is a leading cause of cancer-related death. When EOC patients were firstly diagnosed, some of them with metastasis may not accept surgical treatment and have a shorter five-year survival rate. According to our clinical data, we suggested that HYOU1 may be involved in the regulation of metastasis. In order to demonstrate this hypothesis, we performed wound healing assays and transwell invasion assays to study the effect of HYOU1 on the ability of migration and invasion, finding that the downregulation of HYOU1 significantly suppressed the migration and invasion of EOC cells. It has been known to us that EMT plays an important role in EOC migration and invasion. Then, we further detected the expression levels of EMT-related markers in EOC cells transfected with si-HYOU1 or si-NC, finding that the downregulation of HYOU1 inhibited the expression of N-Cadherin and Vimentin, suggesting that HYOU1 may influence the progress of EMT.

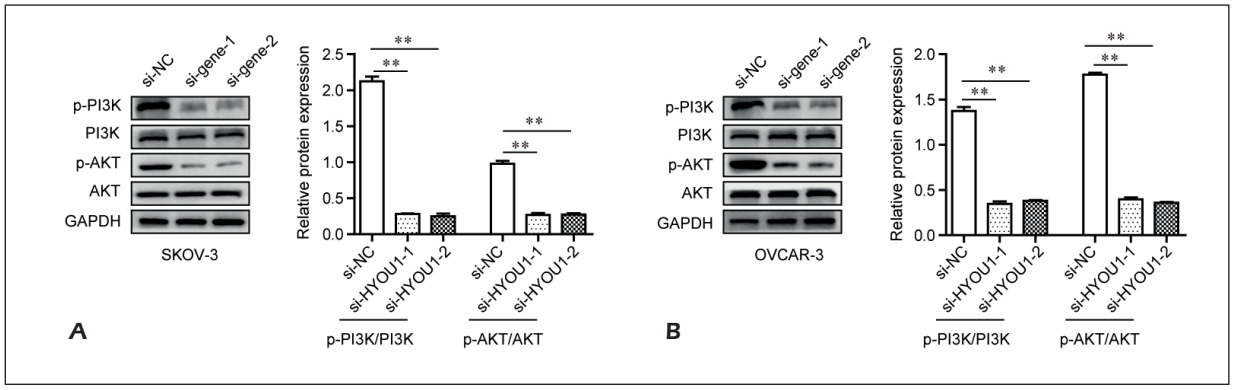

Figure 4. Knockdown of HYOU1 repressed the activity of the PI3K/AKT signaling pathway in SKOV3 and OVCAR-3 cells. **A**, The protein levels of PI3K, as well as its phosphorylated molecular (p-PI3K), AKT, phosphorylated-AKT (p-AKT) in SKOV3 cells was measured by Western blot assays. **B**, The same molecules involved in PI3K/AKT signaling pathway in OVCAR-3 cells were also examined by Western blot assays. *$p<0.05$, **$p<0.01$.

Growing researches have shown that the PI3K/Akt signaling pathway was widely involved in the regulation of EMT process[19,20]. For instance, Sonic hedgehog-Gli1 signals promoted EMT in EOC by mediating PI3K/AKT pathway[21]. Jin et al[22] reported that c-Yes was overexpressed in EOC and contributed to tumor migration and invasion via PI3K/AKT pathway in EOC. Recent findings by Zhang et al[23] showed that GRP137 was highly expressed in both EOC tissues and cell lines, and its overexpression promotes cell metastasis and EMT by modulating the PI3K/AKT pathway in EOC. Our present results also suggested that the downregulation of HYOU1 significantly inhibited the protein expression of p-AKT and p-AKT in EOC cells. Thus, our findings present the conclusion that HYOU1 may act as a positive regulator in the EMT process by PI3K/AKT signaling pathway, resulting in the metastasis of EOC patients.

Conclusions

For the first time, we revealed that HYOU1 was upregulated in EOC tissues and cell lines. HYOU1 expression was also significantly associated with poor prognosis in patients with EOC. Moreover, HYOU1 acted as a tumor promoter in EOC by modulating PI3K/AKT pathway. These findings add insight into our understanding of EOC metastasis. Future studies will be required to fully elucidate the molecular mechanisms by which HYOU1 may promote pathogenesis and progression in EOC.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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