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Long non-coding RNA DLEU1 contributes to the development of endometrial cancer by sponging miR-490 to regulate SP1 expression

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This study aimed to investigate the role of long non-coding RNA DLEU1 in endometrial cancer (EC) development. The DLEU1 expression in EC tissues and cells (HHUA, KLE, Ishikawa, and ECC-1) were detected. The expression of DLEU1 was suppressed by transfection with sh-DLEU1 and the effects of DLEU1 suppression on the malignant behaviors of Ishikawa cells, including cell viability, apoptosis, migration and migration were then detected. In addition, the interaction of DLEU1 and miR-490 as well as between miR-490 and SP1 in EC were investigated. Furthermore, the regulatory relationship between DLEU1 and PI3K/AKT/GSK-3β pathway was explored. DLEU1 was upregulated in EC tissues and cells. Suppression of DLEU1 significantly inhibited Ishikawa cell viability, promoted cell apoptosis, decreased BCL-2 expression and increased the expression of Bax, cleaved-caspase-3 and cleaved-caspase-3, suppressed cell migration and invasion, and inhibited EMT via increasing the expression of E-cadherin and decreasing the expression of N-cadherin, Snail and Vimentin. In addition, DLEU1 could sponge miR-490 and miR-490 inhibition significantly reversed the effects of DLEU1 suppression on the malignant behaviors of Ishikawa cells. Furthermore, SP1 was verified as a target of miR-490, and SP1 knockdown could reverse the effects of miR-490 inhibition on the malignant behaviors of Ishikawa cells. Besides, suppression of DLEU1 inhibited PI3K/AKT/GSK-3β pathway, while miR-490 inhibition activated this pathway that could be neutralized by SP1 knockdown. Our findings indicate that DLEU1 contributes to EC development by sponging miR-490 to regulate SP1 expression. DLEU1/miR-490/SP1 axis may provide a new strategy for EC therapy.

1. Introduction

Endometrial cancer (EC) is a common gynecologic malignancy with increasing incidence (Burke et al. 2014; Morice et al. 2016). Despite great advances in the surgical treatment and chemoradiotherapy, many patients will develop high risk of recurrence and distal metastasis after treatments (Vale et al. 2012) and the prognosis of these patients is poor (Jurcevic et al. 2014). Therefore, it is imperative to elucidate the key mechanism underlying EC tumorigenesis and progression and consequently develop new therapeutic strategies for EC therapy.

Long non-coding RNAs (lncRNAs) are a spectrum of RNAs with longer than 200 nucleotides (nt) in length and have been found to be involved in various fundamental biological and pathophysiological processes (Kornienko et al. 2013; Martin and Chang 2012). Aberrant expression of some lncRNAs can function as proto-oncogenes or tumor suppressor genes in cancer biology (Lheureux and Oza 2016; Silva et al. 2010; Gutschner and Diederichs 2012). In EC development, lncRNA colon cancer-associated transcript 2 can promote cancer cell growth and metastasis (Xie et al. 2017). Moreover, lncRNA growth arrest-specific 5 can act as a tumor suppressor to inhibit tumor pathogenesis (Guo et al. 2015). LncRNA HOX transcript antisense RNA (HOTAIR) is found to regulate autophagy and thus promote the cisplatin-induced resistance in EC cells (Sun et al. 2017). Recently, lncRNA deleted in lymphocytic leukemia 1 (DLEU1), located on chromosome 13q14.3 (Yin et al. 2013), has been found to be highly expressed in breast cancer (Wu et al. 2016). Moreover, DLEU1 is reported to play a key role in tumorigenesis and cancer development, such as ovarian cancer (Zhang et al. 2018) and gastric cancer (Li et al. 2017). However, the roles of DLEU1 in EC development are rarely reported, let alone the corresponding molecular mechanism.

The current study investigated the effects of aberrant expression of DLEU1 on the malignant behaviors of EC cells and explored the regulatory mechanism of DLEU1 by investigating the interaction between DLEU1 and miR-490 as well as the regulatory relationship between DLEU1 and PI3K/AKT/GSK-3β pathway. Our study will provide a new sight for developing novel strategies for the treatment of EC.

2. Investigations and results

2.1. DLEU1 was upregulated in EC

As shown in Fig. 1A, DLEU1 expression in EC tissues was higher than that in non-tumor tissues ($P<0.01$). Moreover, we found that DLEU1 was also highly expressed in EC cells (HHUA, KLE, Ishikawa, and ECC-1) compared with that in normal endometrium (NE) cells (all $P<0.01$, Fig. 1B). Ishikawa cells were then selected for subsequent experiments because the expression of DLEU1 was the highest (Fig. 1B).

2.2. Suppression of DLEU1 inhibited the malignant behaviors of Ishikawa cells

To investigate the role of DLEU1 in EC development, the expression of DLEU1 was suppressed by transfection with sh-DLEU1. As shown in Fig. 2, DLEU1 expression was successfully suppressed because DLEU1 expression in sh-DLEU1 group was markedly lower than that in sh-NC group ($P<0.01$). The effects of DLEU1 suppression on the malignant behaviors of Ishikawa cells were then detected. The results showed that suppression of DLEU1 significantly inhibited cell viability ($P<0.05$, Fig. 2B), promoted cell apoptosis ($P<0.001$, Fig. 2C), decreased BCL-2 expression

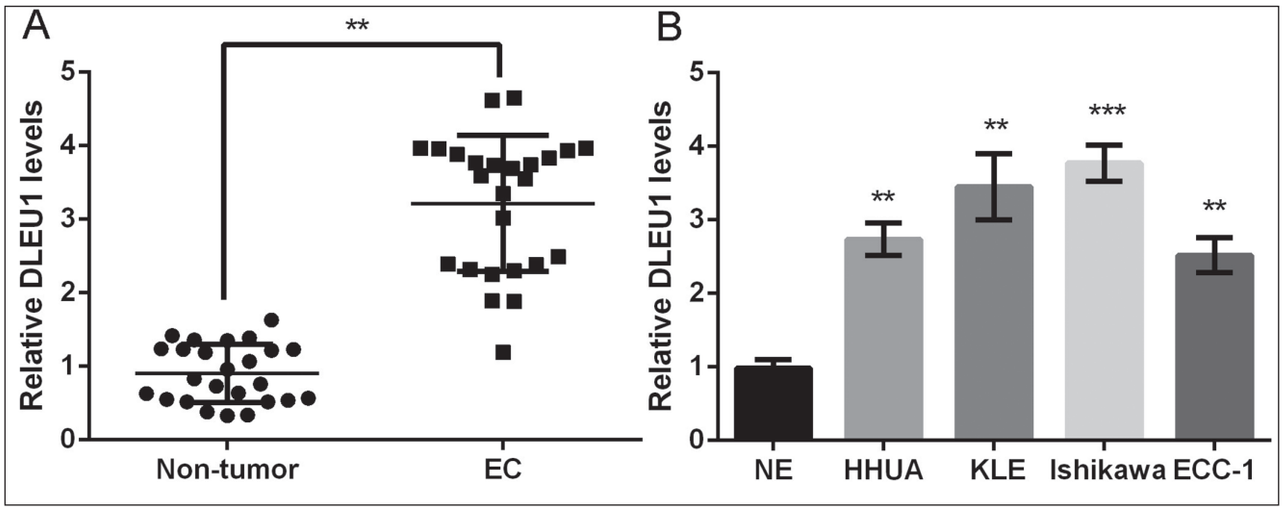

Fig. 1: DLEU1 expression was upregulated in endometrial cancer (EC). A: DLEU1 expression in EC tissues and non-tumor tissues; B: DLEU1 expression in EC cells (HHUA, KLE, Ishikawa, and ECC-1) and normal endometrium (NE) cells. Data are presented as the means±standard deviation (SD). ** P<0.01 and *** P<0.001 compared to control.

and increased the expression of Bax, cleaved-caspase-3 and cleaved-caspase-3 (Fig. 2D), suppressed cell migration (P<0.05, Fig. 2E) and invasion (P<0.05, Fig. 2F), and inhibited EMT via increasing the expression of E-cadherin and decreasing the expression of N-cadherin, Snail and Vimentin (Fig. 2G).

2.3. DLEU1 regulated the malignant behaviors of Ishikawa cells through sponging miR-490

It has been reported that DLEU1 plays a key role in ovarian carcinoma development by sponging miR-490-3p (Wang et al. 2017), we thus investigated the interaction of DLEU1 and miR-490 in EC. The results showed that miR-490 expression in sh-DLEU1 group was significantly higher than that in sh-NC group (P<0.001, Fig. 3A). To confirm this result, we also measured the miR-490 expression in EC tissues and cells. Expected results were obtained that miR-490 expression was down-regulated in EC tissues and cells in comparison with that in normal tissues and cells (P<0.05, Fig. 3B and 3C). To further investigate whether DLEU1 played a key role in EC development by sponging miR-490, we overexpressed and suppressed the expression of miR-490 in Ishikawa cells (P<0.01, Fig. 3D). The combined effects of DLEU1 suppression and miR-490 inhibition were then investigated. The results showed that miR-490 inhibition significantly reversed the effects of DLEU1 suppression on cell viability (P<0.05, Fig. 3E), apoptosis (P<0.01, Fig. 3F), the expression changes of apoptosis-related proteins (Fig. 3G), migration (P<0.05, Fig. 3H) and invasion (P<0.05, Fig. 3I), and the expression changes of EMT-markers (Fig. 3J).

2.4. SP1 was verified as a target of miR-490

To elucidate the possible mechanism of miR-490, the interaction between miR-490 and SP1 was investigated. The results of luciferase reporter assay showed that miR-490 could target SP1 3'UTR (P < 0.05, Fig. 4A), indicating that SP1 was a target of miR-490. Moreover, we found that the expression levels of SP1 mRNA and protein in miR-490 mimic group was significantly lower than that in mimic control group, while SP1 expression in miR-490 inhibitor group was markedly higher than that in inhibitor control group (P<0.05, Fig. 4B and 4C). These data indicated that SP1 was a target of miR-490 and its expression was negatively regulated by miR-490.

2.5. miR-490 regulated the malignant behaviors of Ishikawa cells by targeting SP1

In order to explore whether SP1 was a functional target of miR-490 to mediate the role of miR-490 in EC development, SP1 was successfully knocked down in Ishikawa cells (P<0.001, Fig. 5A). Moreover, we found that miR-490 inhibition significantly enhanced cell viability (P<0.05, Fig. 5B), inhibited cell apoptosis (P<0.001, Fig. 5C), increased the expression of BCL-2 and decreased the expression of Bax, cleaved-caspase-3 and cleaved-caspase-3 (Fig. 5D), promoted cell migration (P<0.05, Fig. 5E) and invasion (P<0.05, Fig. 5F), and inducing EMT via decreasing the expression of E-cadherin and increasing the expression of N-cadherin, Snail and Vimentin (Fig. 5G). Moreover, the above effects were reversed after miR-490 inhibition and SP1 knockdown at the same time (Fig. 5B-G), indicating miR-490 regulated the malignant behaviors of Ishikawa cells by targeting SP1.

2.6. Effects of DLEU1 on EC development exerted through the PI3K/AKT/GSK-3β pathway

To further elucidate the possible mechanism of DLEU1 in EC, we investigated the regulatory relationship between DLEU1 and the PI3K/AKT/GSK-3β pathway. The results showed that suppression of DLEU1 resulted in the significant decrease in the expression levels of p-PI3K, p-AKT and p-GSK-3β, while miR-490 inhibition had opposite effects (Fig. 6). Moreover, the increased expression levels of p-PI3K, p-AKT and p-GSK-3β caused by miR-490 inhibition were significantly neutralized by SP1 knockdown (Fig. 6). These data indicated that the effects of DLEU1 on EC development might be through regulating PI3K/AKT/GSK-3β pathway.

3. Discussion

In the present study, we investigated the effects of DLEU1 on EC development. DLEU1 was upregulated in EC tissues and cells. Suppression of DLEU1 significantly inhibited Ishikawa cell viability, promoted cell apoptosis, suppressed cell migration and invasion, and inhibited EMT. In addition, DLEU1 could sponge miR-490 and miR-490 inhibition significantly reversed the effects of DLEU1 suppression on the malignant behaviors of Ishikawa cells. Furthermore, SP1 was verified as a target of miR-490, and SP1 knockdown could reverse the effects of miR-490 inhibition on

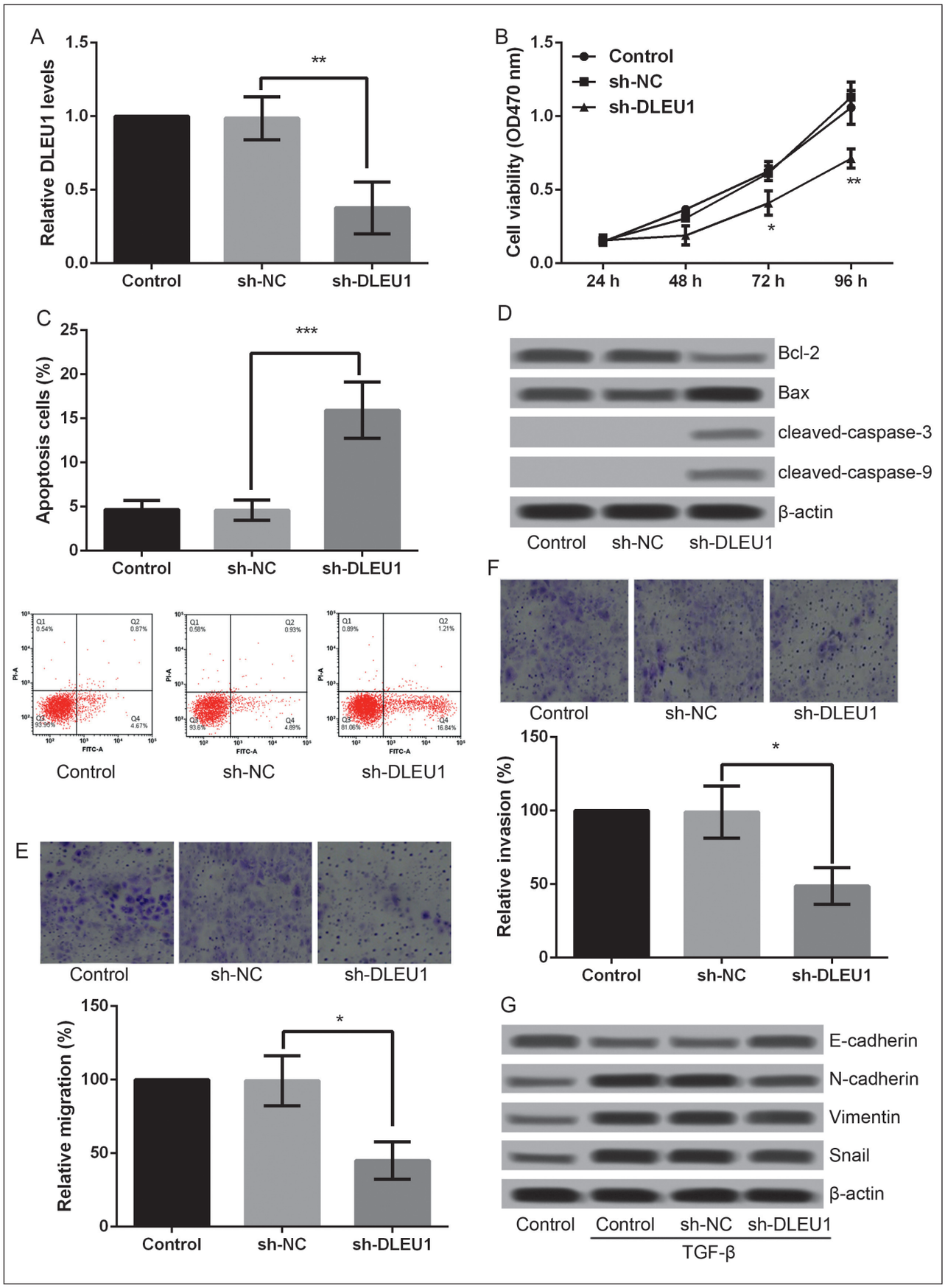

Fig. 2: Suppression of DLEU1 inhibited the malignant behaviors of Ishikawa cells. A: DLEU1 expression in Ishikawa cells transfected with sh-DLEU1 and sh-NC; B: Cell viability of different groups; C: Cell apoptosis of different groups; D: The expression of apoptosis-related proteins; E: Cell migration of different groups; F: Cell invasion of different groups; G: The expression of epithelial-mesenchymal transition (EMT)-markers. Data are presented as the means±SD. * P<0.05, ** P<0.01 and *** P<0.001 compared to control.

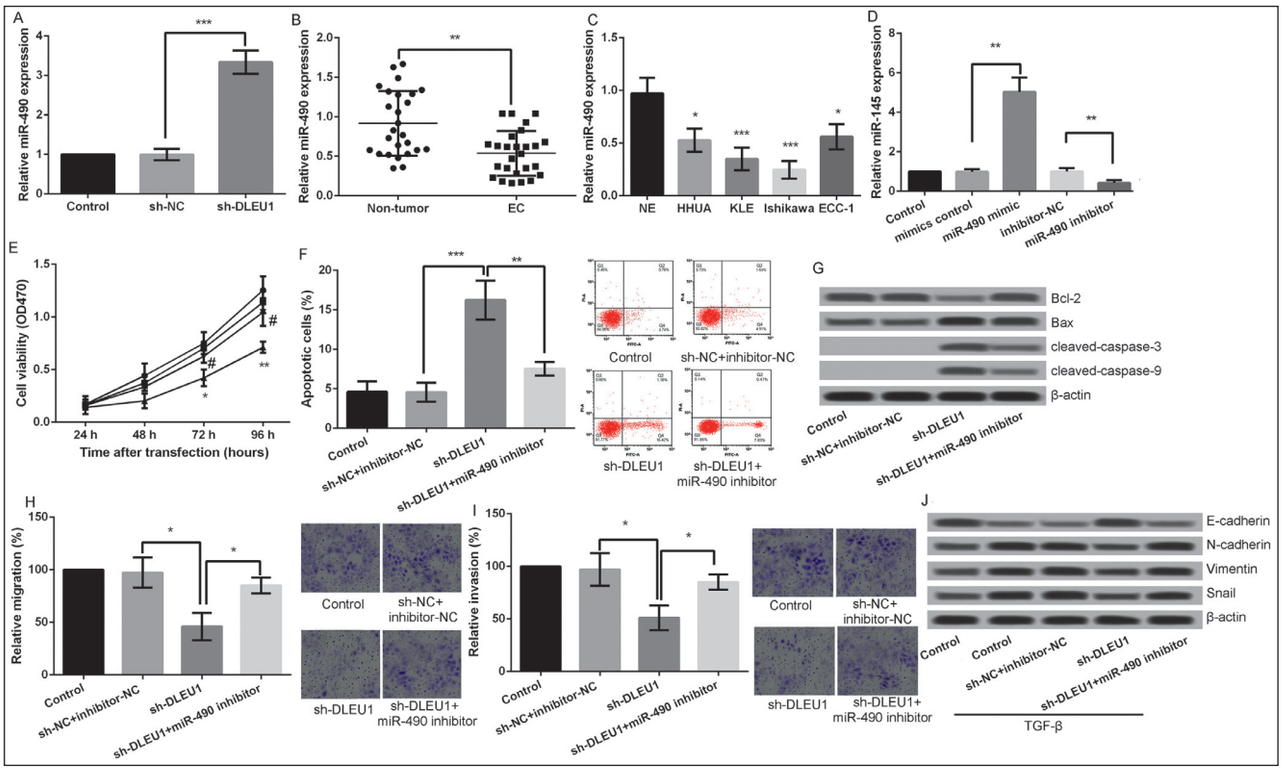

Fig. 3: DLEU1 regulated the malignant behaviors of Ishikawa cells through sponging miR-490. A: The miR-490 expression in Ishikawa cells transfected with sh-DLEU1 and sh-NC; B: The miR-490 expression in EC tissues and non-tumor tissues; C: The miR-490 expression in EC cells (HHUA, KLE, Ishikawa, and ECC-1) and normal endometrium (NE) cells; D: The miR-490 expression in Ishikawa cells transfected with miR-490 mimic, miR-490 inhibitor and their controls. E: Cell viability of different groups; F: Cell apoptosis of different groups; G: The expression of apoptosis-related proteins; H: Cell migration of different groups; I: Cell invasion of different groups; J: The expression of EMT-markers. Data are presented as the means± SD. * P<0.05, ** P<0.01 and *** P<0.001 compared to control.

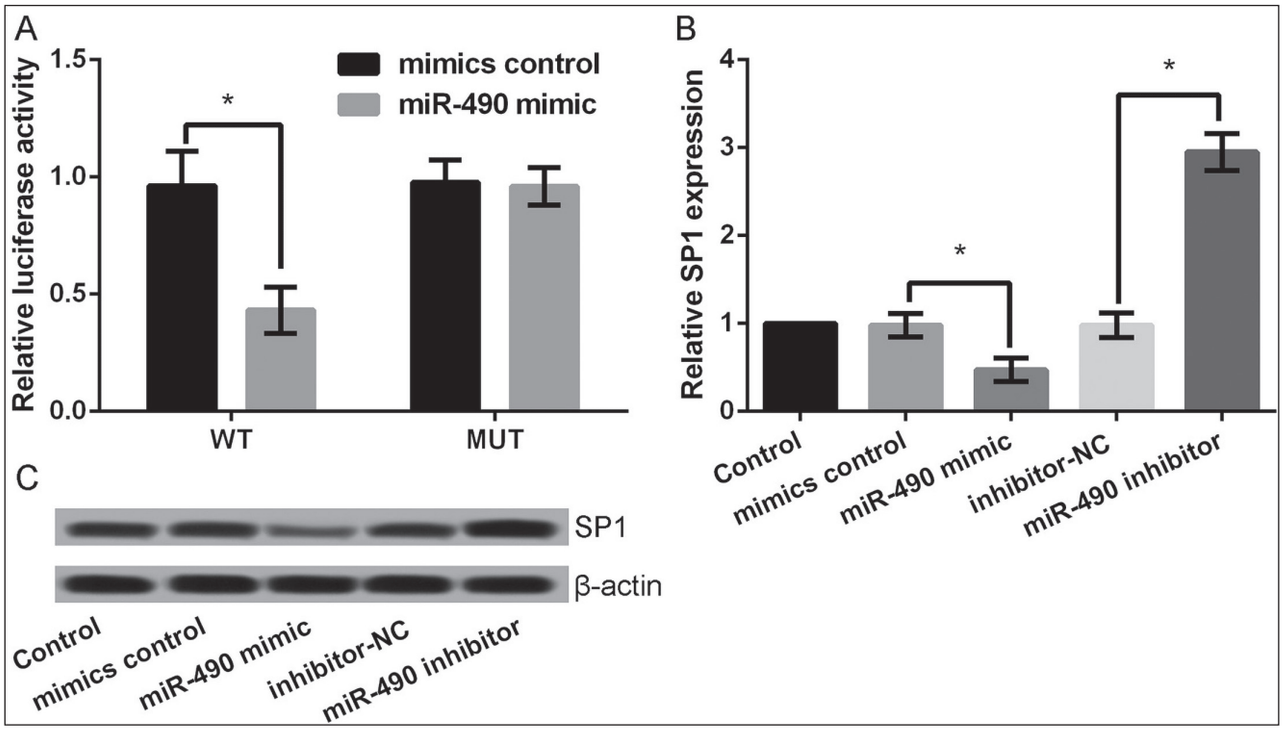

Fig. 4: SP1 was verified as a target of miR-490. *A:* The results of luciferase reporter assay showed that miR-490 could target SP1 3'UTR. B: The expression of SP1 mRNA in Ishikawa cells transfected with miR-490 mimic, miR-490 inhibitor and their controls. C: The expression of SP1 protein in Ishikawa cells transfected with miR-490 mimic, miR-490 inhibitor and their controls. Data are presented as the means ± SD. * P<0.05, ** P<0.01 and *** P<0.001 compared to control.

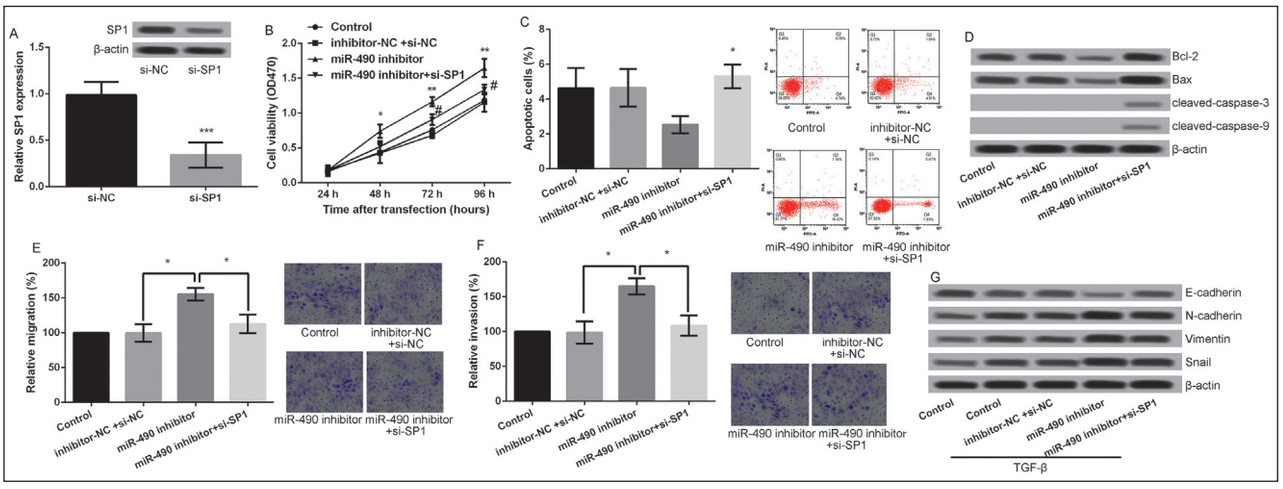

Fig. 5: miR-490 regulated the malignant behaviors of Ishikawa cells by targeting SP1. *A:* The expression of SP1 in Ishikawa cells transfected with si-SP1 and si-NC. B: Cell viability of different groups; C: Cell apoptosis of different groups; D: The expression of apoptosis-related proteins; E: Cell migration of different groups; F: Cell invasion of different groups; G: The expression of EMT-markers. Data are presented as the means ±SD. * P<0.05, ** P<0.01 and *** P<0.001 compared to control.

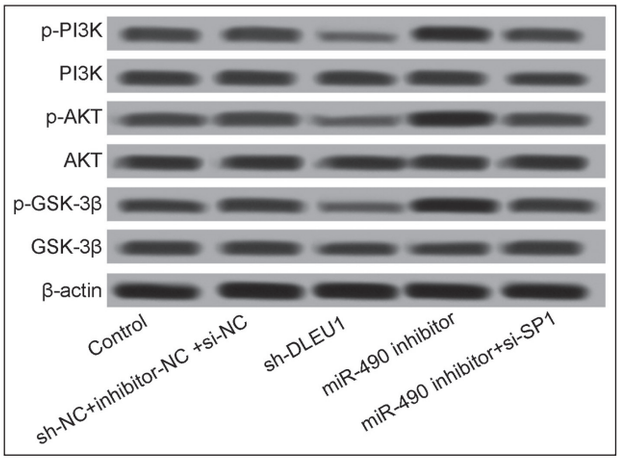

Fig. 6: Protein expression of PI3K, p-PI3K, AKT, p-AKT, GSK-3β and p-GSK-3β proteins in different groups.

the malignant behaviors of Ishikawa cells. Besides, suppression of DLEU1 inhibited PI3K/AKT/GSK-3β pathway, while miR-490 inhibition activated this pathway that could be neutralized by SP1 knockdown. These data elucidated the roles of DLEU1/miR-490/SP1 axis in EC development and merit further discussion.

Consistent with previous findings that DLEU1 contributes to cancer development via sponging miR-490-3p (Wang et al. 2017), we also found that DLEU1 could sponge miR-490 and miR-490 inhibition significantly reversed the effects of DLEU1 suppression on the malignant behaviors of Ishikawa cells. Accumulating evidence has supported that miR-490 contributes to the development of several cancers: Jia et al. demonstrated that miR-490 could suppress the tumorigenesis and progression of breast cancer (Jia et al. 2016; Zhao and Zheng 2016); Li et al. revealed that miR-490 could regulate the metastasis of lung cancer cells (Li et al. 2016); Chen et al. suggested that miR490-5p could inhibit tumor growth in renal cell carcinoma (Chen et al. 2016); Zhang et al. (2013) confirmed that miR-490-3p promoted cell growth and EMT of hepatocellular carcinoma cells; and Chen et al. (2015) indicated that miR-490-3p inhibited the tumorigenesis and progression of ovarian epithelial carcinoma. Notably, a previous study has confirmed that miR-490-3p could function as a suppressor to inhibit tumorigenesis and progression of EC (Sun et al. 2016). Given the key role of miR-490 in EC development, we hypothesize that DLEU1 may promote the development of EC via down-regulating miR-490.

Furthermore, SP1 was identified as a functional target of miR-490. Dysregulation of SP1 has been found to be involved in many cancers (Li and Davie 2010). SP1 inhibition can suppress stem cell growth and induce apoptosis in colon cancer (Zhao et al. 2013). In addition, miR-375 is found to suppress cell migration and invasion in squamous cervical cancer via targeting SP1 (Wang et al. 2011). miR-330 can suppress cell motility in prostate cancer by targeting SP1 (Mao et al. 2013). Based on our results, we believe that miR-490 may regulate the development of EC by targeting SP1.

Obviously, the PI3K/Akt pathway is involved in tumorigenesis and angiogenesis (Jiang and Liu 2008; Karar and Maity 2011; Slomovitz and Coleman 2012). Also, overactivation of the PI3K/AKT/mTOR pathway are found implicated in EC pathogenesis (Slomovitz and Coleman 2012), and PI3K/AKT/mTOR pathway has been shown to enhance progestin resistance in EC cells through inhibiting autophagy (Hua et al. 2017). Besides, Kang et al. (2012) demonstrated that thioridazine could induce apoptosis of EC cells by regulating the PI3K/Akt/mTOR pathway. In this study, suppression of DLEU1 inhibited PI3K/AKT/GSK-3β pathway, while miR-490 inhibition activated this pathway that could be neutralized by SP1 knockdown. It can therefore be assumed that the PI3K/AKT/GSK-3β pathway may be a regulatory mechanism to mediate the role of DLEU1/miR-490/SP1 axis in EC.

Taken together, our findings indicate that DLEU1 may promote EC cell viability, migration and invasion and induce apoptosis by sponging miR-490 to regulate SP1 expression. DLEU1/miR-490/SP1 axis may provide a new strategy for EC therapy.

4. Experimental

4.1. Patients and samples

Between April 2013 and December 2017, 25 EC patients who underwent an initial hysterectomy were enrolled in this study. The characteristics of EC patients are summarized in the Table. Following surgical excision, the EC tissues and matched normal endometrial tissues were obtained from these patients and then stored at -80 °C after immediately frozen in liquid nitrogen.

Table 1: Characteristics of patients with endometrial cancer

Total no.	25
Median age, years (range)	53.6 (39-75)
Pathological tumor stage	
I	13 (52%)
II	5 (20%)
III	4 (16%)
IV	3(12%)

Total no.	25
Differentiation	
G1	7 (28%)
G2	9 (36%)
G3	9 (36%)
Lymphatic metastasis	
(+)	8 (32%)
(−)	16 (64%)
Unknown	1 (4%)

4.2. Cell lines and cell culture

The human EC cell lines HHUA, KLE, Ishikawa, and ECC-1 were acquired from American Type Culture Collection (ATCC). They were cultured in Minimum Essential Medium Eagle (Sigma-Aldrich, UK) containing 15 % of fetal bovine serum (Gibco, Darmstadt, Germany) and then maintained at a 37 °C incubator with a humidified atmosphere of 5 % CO_2.

4.3. Transient transfection and treatment

For the transfection of the short hairpin RNA (shRNA) directed against DLEU1, miR-490 mimics/inhibitor, small interfering RNA (siRNA) targeting SP1 or their corresponding controls, Ishikawa cells (2×10^5) were seeded in 6-well plates and cell transfection was then performed using Lipofectamine 2000 Reagent (Life Technologies). In addition, the medium was added with 10 ng/mL of TGF-β1 (Sigma, St. Louis, MO) to induce epithelial-mesenchymal transition (EMT).

4.4. MMT assay for assessing cell viability

Cells were plated in 96-well plates at either 10^4 cells/well after 24 h of transfection. The cells were then incubated with MTT (0.5 mg/ml, Sigma) for another 4 h at a 37 °C incubator. Next, the medium was removed, and the precipitated formazan was dissolved in 100 µl of dimethyl sulfoxide (DMSO, Sigma) for 15 min. Finally, the absorbance at 570 nm (A570) was measured to assess cell viability using a µQuant universal microplate spectrophotometer (BioTekInstruments, Winooski, VT).

4.5. Flow cytometry for detecting cell apoptosis

To measure apoptosis, cells were collected after 24 h of transfection. After washed with PBS, cells were stained with fluorescein isothiocyanate (FITC)-labelled annexin V and propidium iodide (PI) using the Annexin V-FITC kit (Biosea Biotechnology Co., Beijing, China), and this was followed by flow cytometry analysis on a flow cytometry (FACSCalibur, Becton-Dickinson, San Jose, CA).

4.6. Transwell assay for measuring cell migration and invasion

Cell migration and invasion were measured by a Transwell assay, but the Transwell chambers (8-µm pore size; Corning co. Ltd., USA) were precoated with Matrigel (Becton-Dickinson, Bedford, MA) for invasion assays. Briefly, 24 h after transfection, cells were cultured in serum-free RPMI 1640 medium for 24 h and then added into the upper layer of Transwell chamber. After that, RPMI 1640 medium mixed with 10% FBS was then added into the lower layer of Transwell chamber. After 48 h of incubation, Transwell chambers were fixed with methanol, and stained with Giemsa. The migrated and invaded cells in 10 random fields were counted under a microscope (IX83, Olympus).

4.7. Luciferase reporter assay

The full-length SP1 3'-UTR containing the miR-490 binding site was amplified and the inserted into the Xba1-site of pGL3 vector (Promega, WI), which was named as pGL3-SP1 wide-type (WT) reporter construct. Using a site-directed mutagenesis kit (Stratagene, CA), the pGL3-SP1-mutated (MUT) reporter construct was synthesized with point mutations in the seed sequence. After that, cells (1×10^6) were cotransfected with 1 µg of pGL3-SP1-WT (or pGL3-SP1-MUT) reporter constructs, 50 pmol of miR-490 mimic (or mimic control) , and 1 µg of a Renilla luciferase expression construct pRL-TK (Promega, WI) using Lipofectamine 2000. Renilla luciferase activity was used as the internal control. After 36 h of transfection, luciferase activities of pGL3-SP1-WT and pGL3-SP1-MUT reporter constructs were measured using the dual luciferase assay system (Promega, WI).

4.8. Quantitative real-time PCR (qRT-PCR) analysis

Using TRIzol Reagent (Life Technologies), total RNA was extracted and its RNA quantity was then measured with a SmartSpec Plus spectrophotometer (Bio-Rad). The qRT-PCR analyses for detecting the expression of mRNAs were conducted using a GoTaq 2-Step RT-qPCR System (Promega, Madison, WI, USA). GAPDH was used as a control qRT-PCR analyses for detecting the expression of miRNAs was carried out using miScript II RT Kits (Qiagen, Hilden, Germany), miScript SYBR Green PCR Kits (Qiagen) and miScript Primer Assays. All these assays were all performed on a Mx3005P QPCR System (Stratagene, La Jolla, CA, USA). Finally, the relative expression of targets was quantified by the $2^{-\Delta\Delta Ct}$ method.

4.9. Western blot

Total proteins were extracted from cells by cell lysis buffer (Beyotime, Haimen, China). Equal amount of protein extracts (30 µg per lane) were separated on 12% SDS-polyacrylamide gels and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were then immunoblotted by incubation with the primary antibodies to Bcl-2, Bax, cleaved-caspased-3, cleaved-caspased-9, E-cadherin, N-cadherin, Snail, Vimentin, SP1, PI3K, p-PI3K, AKT, p-AKT, GSK-3β, p-GSK-3β and β-actin (1:1,000, Abcam, Cambridge, UK). β-actin was used as the control. After incubation with the recommended secondary antibodies, the protein signals were revealed using an Odyssey Infrared Imaging System (LI-COR, Lincoln, NE, USA). β-Actin was used as the control.

4.10. Statistical analysis

The data are presented as the means ± standard deviation (SD) and considered to be statistically significant at $P<0.05$. Statistical analysis for data was performed with Student's t-tests or one-way ANOVA using SPSS Statistics 20.0 software (IBM, Armonk, NY, USA).

Conflicts of interest: None declared.

References

Burke WM, Orr J, Leitao M, Salom E, Gehrig P, Olawaiye AB, Brewer M, Boruta D, Herzog TJ, Shahin FA (2014) Endometrial cancer: A review and current management strategies: Part II. Gynecol Oncol 134: 393-402.

Chen K, Zeng J, Tang K, Xiao H, Hu J, Huang C, Yao W, Yu G, Xiao W, Guan W (2016) miR-490-5p suppresses tumour growth in renal cell carcinoma through targeting PIK3CA. Biol Cell 108: 41-50.

Chen S, Chen X, Xiu YL, Sun KX, Zhao Y (2015) MicroRNA-490-3P targets CDK1 and inhibits ovarian epithelial carcinoma tumorigenesis and progression. Cancer Lett 362: 122-130.

Guo C, Song W, Sun P, Jin L, Dai H (2015) LncRNA-GAS5 induces PTEN expression through inhibiting miR-103 in endometrial cancer cells. J Biomed Sci 22: 100.

Gutschner T, Diederichs S (2012) The hallmarks of cancer: a long non-coding RNA point of view. RNA Biol 9: 703-709.

Hua L, Zhang L, Zhang X, Cui Z (2017) PI3K/AKT/mTOR pathway promotes progestin resistance in endometrial cancer cells by inhibition of autophagy. Oncotargets Ther 10: 2865-2871.

Jia Z, Liu Y, Gao Q, Han Y, Zhang G, Xu S, Cheng K, Zou W (2016) miR-490-3p inhibits the growth and invasiveness in triple-negative breast cancer by repressing the expression of TNKS2. Gene 593: 41-47.

Jiang B-H, Liu L-Z (2008) PI3K/PTEN signaling in tumorigenesis and angiogenesis. Biochim Biophys Acta 1784: 150-158.

Jurcevic S, Olsson B, Klinga-Levan K (2014) MicroRNA expression in human endometrial adenocarcinoma. Cancer Cell Int 14: 1-8.

Kang S, Dong SM, Kim BR, Mi SP, Trink B, Byun HJ, Rho SB (2012) Thioridazine induces apoptosis by targeting the PI3K/Akt/mTOR pathway in cervical and endometrial cancer cells. Apoptosis 17: 989-997.

Karar J, Maity A (2011) PI3K/AKT/mTOR pathway in angiogenesis. Frontiers Mol Neurosci 4: 51.

Kornienko AE, Guenzl PM, Barlow DP, Pauler FM (2013) Gene regulation by the act of long non-coding RNA transcription. BMC Biol 11: 59.

Lheureux S, Oza AM (2016) Endometrial cancer-targeted therapies myth or reality? Review of current targeted treatments. Eur J Cancer 59: 99-109.

Li J, Feng Q, Wei X, Yu Y (2016) MicroRNA-490 regulates lung cancer metastasis by targeting poly r(C)-binding protein 1. Tumour Biol 37: 15221-15228.

Li L, Davie JR (2010) The role of Sp1 and Sp3 in normal and cancer cell biology. Ann Anat 192: 275-283.

Li X, Li Z, Liu Z, Xiao J, Yu S, Song Y (2017) Long non-coding RNA DLEU1 predicts poor prognosis of gastric cancer and contributes to cell proliferation by epigenetically suppressing KLF2. Cancer Gene Ther doi: 10.1038/s41417-017-0007-9.

Mao Y, Chen H, Lin Y, Xu X, Hu Z, Zhu Y, Wu J, Xu X, Zheng X, Xie L (2013) microRNA-330 inhibits cell motility by downregulating Sp1 in prostate cancer cells. Oncol Rep 30: 327-333.

Martin L, Chang HY (2012) Uncovering the role of genomic "dark matter" in human disease. J Clin Invest 122: 1589-1595.

Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E (2016) Endometrial cancer. Lancet 387: 1094-1108.

Silva JM, Perez DS, Pritchett JR, Halling ML, Tang H, Smith DI (2010) Identification of long stress-induced non-coding transcripts that have altered expression in cancer. Genomics 95: 355-362.

Slomovitz BM, Coleman RL (2012) The PI3K/AKT/mTOR pathway as a therapeutic target in endometrial cancer. Clin Cancer Res 18: 5856-5864.

Sun KX, Chen Y, Chen S, Liu BL, Feng MX, Zong ZH, Zhao Y (2016) The correlation between microRNA490-3p and TGFα in endometrial carcinoma tumorigenesis and progression. Oncotarget 7: 9236-9249.

Sun MY, Zhu JY, Zhang CY, Zhang M, Song YN, Rahman K, Zhang LJ, Zhang H (2017) Autophagy regulated by lncRNA HOTAIR contributes to the cisplatin-induced resistance in endometrial cancer cells. Biotechnol Lett 96: 1-8.

Vale CL, Tierney J, Bull SJ, Symonds PR (2012) Chemotherapy for advanced, recurrent or metastatic endometrial carcinoma. Cochrane Database Sytem Rev CD003915

Wang F, Li Y, Zhou J, Xu J, Peng C, Ye F, Shen Y, Lu W, Wan X, Xie X (2011) miR-375 is down-regulated in squamous cervical cancer and inhibits cell migration and invasion via targeting transcription factor SP1. Am J Pathol 179: 2580-2588.

Wang LL, Sun KX, Wu DD, Xiu YL, Chen X, Chen S, Zong ZH, Sang XB, Liu Y, Zhao Y (2017) DLEU1 contributes to ovarian carcinoma tumourigenesis and

development by interacting with miR-490-3p and altering CDK1 expression. J Cell Mol Med 21: 3055-3065.

Wu Q, Guo L, Jiang F, Li L, Li Z, Chen F (2016) Analysis of the miRNA-mRNA-lncRNA networks in ER+ and ER- breast cancer cell lines. J Cell Mol Med 19: 2874-2887.

Xie P, Cao H, Li Y, Wang J, Cui Z (2017) Knockdown of lncRNA CCAT2 inhibits endometrial cancer cells growth and metastasis via sponging miR-216b. Cancer Biomark 21: 123-133.

Yin C, Chung TH, Ayello J, Park TH, van de Ven C, Cairo MS, Lee S (2013) Overexpression of deleted in lymphocytic leukemia 1 (DLEU1) significantly induces programmed cell death and inhibits cell proliferation in primary mediastinal B-cell lymphoma (PMBL): DLEU1 may be a tumor suppressor gene in a subset of patients with PMBL. Blood 122: 3852.

Zhang LY, Liu M, Li X, Tang H (2013) miR-490-3p modulates cell growth and epithelial to mesenchymal transition of hepatocellular carcinoma cells by targeting endoplasmic reticulum-Golgi intermediate compartment protein 3 (ERGIC3). J Biol Chem 288: 4035-4047.

Zhang J, Tao Z, Wang Y (2018) Long non-coding RNA DANCR regulates the proliferation and osteogenic differentiation of human bone-derived marrow mesenchymal stem cells via the p38 MAPK pathway. Int J Mol Med 41: 213-219.

Zhao L, Zheng XY (2016) MicroRNA-490 inhibits tumorigenesis and progression in breast cancer. Onco Targets Ther 9: 4505-4516.

Zhao Y, Zhang W, Guo Z, Ma F, Wu Y, Bai Y, Gong W, Chen Y, Cheng T, Zhi F (2013) Inhibition of the transcription factor Sp1 suppresses colon cancer stem cell growth and induces apoptosis in vitro and in nude mouse xenografts. Oncol Rep 30: 1782.