lncRNA DLEU2 promotes gastric cancer progression through ETS2 via targeting miR-30a-5p

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Research

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Abstract

Background

Gastric cancer (GC) is the fourth common and the second lethal malignancy. Further understanding of the molecular mechanisms of underlying gastric carcinogenesis will enhance the diagnosis and treatment of GC.

Methods

The expression of lncRNA DLEU2 and ETS2 was analysed using GEPIA online analyze, qRT-PCR and immunohistochemistry. siRNAs targeting to lncRNA DLEU2 were designed to reduce the expression of lncRNA DLEU2. miR-30a-5p mimics were purchased to up-regulate the level of miR-30a-5p. The pcDNA3.1-ETS2 vector was synthesized to up-regulate the expression of ETS2. The biological function of GC cells was detected by CCK8, clone formation, transwell, wound healing, western blot, and flow cytometry assay. More in-depth mechanisms were studied.

Results

LncRNA DLEU2 was significantly up-regulated in GC tissues. The expression of lncRNA DLEU2 was significantly associated with pathological grading and TNM stage of GC patients. Furthermore, knockdown of lncRNA DLEU2 inhibited the proliferation, migration and invasion of AGS and MKN-45 cells, and induced cell apoptosis. We also found that miR-30a-5p could directly bind to the 3’ UTR region of ETS2. Moreover, lncRNA DLEU2 bound to miR-30a-5p through the same binding site, which facilitated the expression of ETS2. Knockdown of lncRNA DLEU2 reduced the protein level of intracellular ETS2 and inhibited the AKT phosphorylation. ETS2 was highly expressed in GC tissues. The expression of ETS2 was significantly associated with age, pathological grading and TNM stage. ETS2 overexpression promoted cell proliferation and migration of AGS and MKN-45 cells. Furthermore, ETS2 overexpression rescued cell proliferation and migration inhibition induced by lncRNA DLEU2 down-regulation and miR-30a-5p up-regulation in AGS and MKN-45 cells. These results showed that lncRNA DLEU2 may regulate GC cells process through miR-30a-5p/ETS2 axis. In addition, hypoxic microenvironment resulted in the accumulation and nucleation of HIF-1α in gastric cancer cells, and up-regulated the expression of DLEU2.

Conclusions

LncRNA DLEU2 was a potential molecular target for GC treatment.

Introduction

Gastric cancer (GC) is the fourth common and the second lethal malignancy(1). Due to the lack of effective diagnostic markers, most GC patients miss the most appropriate diagnosis and treatment time, leading to advanced stage and metastatic tumors(2). The 5-year survival rate of advanced GC patients is less than 25%(2). Further understanding of the molecular mechanisms of underlying gastric carcinogenesis will enhance the diagnosis and treatment of GC.

Long-chain non-coding RNA (lncRNAs) are transcripts longer than 200 nucleotides with non-protein coding functions(3). The number of lncRNAs is large, and its regulatory mechanism is relatively more extensive and diverse. LncRNAs can interact with proteins, DNA and RNA through epigenetic modification, transcription and post-transcriptional regulation, thereby regulating tumor proliferation, mutation, cell cycle, invasion and metastasis(4–6). More studies have shown that lncRNAs play the role of competitive endogenous RNAs (ceRNAs), which regulate the expression of target genes through competitive binding of miRNAs and is closely related to tumor progression(7, 8). The host gene of lncRNA DLEU2 is located at chromosome 4995852–50125541 on chromosome 13, which is a frequent deletion mutation in leukemia and solid tumors, and is a hot gene locus for tumor research(9). One study reports that DLEU2 gene encodes a lncRNA (1.0-1.8 kb) - lncRNA DLEU2, which is polyadenylated and cleaved(10). However, little is known about the function of this lncRNA; and its sequence does not show homology to any other non-coding RNA. Our previous study found that lncRNA DLEU2 is highly expressed in GC tissues infected with H. pylori(11). Therefore, this study aims to reveal the specific function of lncRNA DLEU2 in GC.

In recent years, several studies have reported that miR-30a-5p exerts a tumor suppressor effect in various human tumors such as colon, breast, gallbladder and lung cancer(12–15). At the same time, the function of miR-30a-5p in GC has gradually been unveiled. MiR-30a-5p is thought to be involved in the development of GC, proliferation and migration of GC cells, and drug resistance, and is closely related to the recurrence-free and overall survival rate of GC patients(16–23). These studies suggest the enormous potential of miR-30a-5p for the treatment and diagnosis of GC. The present study found that lncRNA DLEU2 can act as a ceRNA to "adsorb" miR-30a-5p, hinder its targeting of the downstream target ETS2, thereby promoting the expression of ETS2, and ultimately promoting the malignant phenotype of GC cell.

ETS2 is a representative member of the transcription factor ETS family, which has a DNA-binding domain at the C-terminus required for the recognition of the consensus core sequence GGAA/T(24). Expression of ETS2 is observed in a variety of cell types(25). ETS2 has been shown to play important roles in embryonic development(26), immunity(27), osteogenesis(28) and tumorigenesis. ETS2 is over-expressed in breast, prostate and renal cell carcinomas, and its deletion inhibits the survival and metastasis of these cells(29–31). However, the roles of ETS2 in GC is not known.

Currently, we aimed to investigate the role of lncRNA DLEU2 in GC progression. We observed lncRNA DLEU2 was increased in GC tissues while ETS2 was decreased. lncRNA DLEU2 downregulation inhibited GC cells proliferation, invasion and migration, and induced cell apoptosis. In addition, ETS2 was identified as a target of miR-30a-5p. Taken these together, we speculated that lncRNA DLEU2 modulate miR-30a-5p/ETS2 axis in GC development.

Methods

Tissue collection

75 pairs of GC tissues and matched adjacent normal tissues were collected from patients who were diagnosed and underwent surgical resection at the Jinan Central Hospital. None of patients had any serious diseases other than GC, and did not receive chemotherapy or radiotherapy before operation. All collected samples were immediately snap-frozen in liquid nitrogen and stored until needed. The Histological grade was staged according to the seventh TNM staging of the International Union against Cancer/American Joint Committee on Cancer system. All research complied with the principles of the Declaration of Helsinki, and was approved by the Medical Ethics Committee of the Jinan Central Hospital. Enrolled patients had given written informed consent for publication.

Cell culture and transfections

Human GC cell lines AGS and MKN-45 were purchased from the American Type Culture Collection (ATCC, Rockville, MD) and cultured in Dulbecco's modified Eagle medium (DMEM; 4.5 g/L D-glucose) supplemented with 10% FBS (Invitrogen, Grand Island, NY) and 1% antibiotic/antimycotic in a humidified incubator at 37 °C containing 5% CO_2.

siRNAs targeting to lncRNA DLEU2 (si-DLEU2) were designed and synthetized (RiboBio, Guangzhou, China), the sequence of si-DLEU2 was used as follows: 5'-CUCAUUGAAUACUAUCAAAAAGGAA-3'. miR-30a-5p mimics were purchased from RiboBio (Guangzhou, China). The cDNA of ETS2 was synthesized by GENEWIZ and cloned into the pcDNA3.1 expression vector (pcDNA3.1-ETS2) (GenePharma, Shanghai, China). si-DLEU2, miR-30a-5p mimics and pcDNA3.1-ETS2 vector were transfected using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions.

Isolation of RNA and real-time PCR

Total RNA was extracted from tissues samples or cells with TRIzol reagent (Invitrogen, Carlsbad, CA) and purified according to the manufacturer's instructions. RNA concentration was measured spectrophotometrically at an optical density of 260 nm. The Reverse Transcription Kit (Takara, Dalian, China) was used to generate cDNA. Real-time PCR was performed using SYBR® Green (Takara, Dalian, China). PCR primers were used as follows: DLEU2-Forward, 5'-GGCGGCGGGTACTTATCTC-3'; DLEU2-Reverse, 5'- CCAGGGAAGGATGTAGCTGTG-3'; GAPDH-Forward, 5'- GGTGTGAACCATGAGAAGTATGA-3'; GAPDH-Reverse, 5'- GAGTCCTTCCACGATACCAAAG-3'. Relative fold expression was calculated by $2^{-\Delta\Delta Ct}$ using GAPDH as an endogenous control.

CCK8 assays

2000 cells were cultured in a 96-well plate for 0 h, 24 h, 48 h and 72 h. Then, the medium was replaced with 100 μl fresh DMEM containing 10 μl CCK8 solution (Dojindo Molecular Technologies, Japan). Incubate at 37 °C for 2 h. Absorbance at 450 nm was detected by a microplate reader (BioTek Instruments, USA)

Colony formation assays

2000 cells were planted into 6-well plates and cultured for 10 days. Then, cells were fixed with methanol and stained with 0.1% crystal violet solution. Colonies were counted and images were obtained. Colonies with at least 50 cells were considered significant.

Transwell assays

1×10^5 cells in 200 μl of FBS-free DMEM were seeded into the upper chamber of transwells precoated with Matrigel (BD Bioscience), and 600 μl of DMEM containing 10% FBS was added to the lower chamber. After 24 h of incubation, non-migrating or non-invasive cells remaining on the upper surface were removed with a cotton swab. Then the membranes were fixed with 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet for 15 min. Three random fields were counted in each chamber using an inverted microscope (Olympus).

Wound healing assays

A sterile plastic was used to create a wound in a single cells layer. After washing with PBS, the cells were cultured in FBS-free medium for 24 h. Five random fields of each wound was measured for quantification and images were obtained.

Western blot

Extraction of proteins from tissues samples or cells using RIPA buffer and protein concentration was measured by the BCA method (Tiangen, Beijing, China). 15 μg proteins were separated by SDS–PAGE and transferred to PVDF membranes (Millipore). Membranes were blocked with 5% skim milk and immunoblotted with primary antibodies, followed by incubation with matched second antibodies. Then blots were visualized using an enhanced chemiluminescence kit (Amersham, Little Chalfont, UK) and detected using the GelCapture version software (DNR Bio-Imaging Systems, Jerusalem, Israel). GAPDH was employed as endogenous control.

Apoptosis assay

1×10^6 cells were harvested after transfection for 24 h and resuspended in Annexin V binding buffer. 5 μl FITC-Annexin V and 5 μl PI were added to stain using the Apoptosis Detection Kit (BD Biosciences, San Jose, CA). Then, 400 μl PBS was added to the cells, which were analyzed using a FACScan flow cytometry system (BD Biosciences, San Jose, CA). Cell apoptosis was analyzed using FlowJo V7 software (Tree Star, Ashland, OR).

Luciferase reporter assay

Luciferase reporter assay was performed using psiCHECK2 vector (Promega). To construct psiCHECK2-ETS2 recombinant vector, the complete 3'UTR of human ETS2 mRNA containing the putative or mutative miR-30a-5p binding sites, was amplified and cloned into the psiCHECK2 vector. For the lncRNAs, wild or mutative full-length sequences of lncRNA DLEU2 was amplified and cloned into the psiCHECK2 vector.

AGS cells were co-transfected with one of the psiCHECK2 recombinant vector and miR-30-5p mimics, or miR-NC by Lipofectamine 2000 according to the manufacturer's guidelines. The relative luciferase activity was valued using the Dual-Luciferase Reporter Assay System (Promega) and Infinate M200 PRO microplate reader (Tecan, Shanghai, China).

Immunohistochemistry staining

Tissue samples were fixed in formalin for 24 h at 4 °C, and embedded in paraffin. The paraffin block was cut into 4 μm sections. Tissue sections were deparaffinized in Van-Clear (Hongci., Shanghai, China) and concentration gradient ethanol, then microwaved in 0.01 M citrate buffer for 10 min. After blocking with 5% goat serum for 1 h at room temperature, the sections were incubated with the primary antibody and then incubated with enzyme-labeled goat anti-mouse/rabbit IgG polymer (160101405L, Maixin., Shanghai, China). The immune response was visualized by the enhanced DAB chromogenic kit (1705252031, Maixin., Shanghai, China), and hematoxylin was used for counterstaining. The immunostaining score was evaluated blindly by two independent investigators as the product of positive staining cell ratio (R) and staining intensity score (S). R was divided into four levels: 0 (< 5%, negative), 1 (5–25%, sporadic), 2 (25–50%, focus), 3 (> 51%, diffuse). S was also divided into four levels: 0 (negative), 1 (weak), 2 (middle), 3 (strong). A total of 0–3 was considered to be low expression, while 4–9 was considered to be high expression. Finally, images were collected by a vertical microscope system (Nikon, Japan).

Immunofluorescence

After the cells were grown on the coverslip for 24 h, the medium was removed, and cells were washed twice with pre-chilled PBS buffer. Add 3% paraformaldehyde to fix the cells at 4 ℃ for 30 min, then wash the cells with 50 mM NH4Cl and permeate with 0.1% Triton100 at room temperature for 15 min. Cells were incubated with anti-HIF-1α antibody at room temperature for 1 h and labeled with secondary antibody. Immunolabeled cells were detected in a laser confocal microscope.

Statistical analysis

Statistical analyses were performed with SPSS software version 22.0 (IBM Corp., Armonk, NY). The mean ± standard deviation was used to present the experimental results. The differences between groups were calculated with using the Student's t-test or one-way ANOVA. The correlation among lncRNAs, miRNAs and mRNAs were analyzed by Pearson's correlation analysis. A statistically significant threshold was defined as $P < 0.05$.

Results

LncRNA DLEU2 is up-regulated in GC tissues

GEPIA collected the mRNA expression profile of 408 stomach adenocarcinoma (STAD) tissues and 211 normal tissues. As shown in Fig. 1A, lncRNA DLEU2 was highly expressed in STAD tissues compared into

normal tissues. We further examined the expression of lncRNA DLEU2 in 75 paired GC tissues and matched normal tissues using qRT-PCR, and results showed that lncRNA DLEU2 was significantly up-regulated in GC tissues (Fig. 1B). In addition, the expression of lncRNA DLEU2 was significantly associated with pathological grading ($P = 0.0087$) and TNM stage ($P = 0.0382$) of GC patients. A significant correlation with other features, such as age, gender and tumor diameter were not observed.

Knockdown of lncRNA DLEU2 inhibits cell proliferation, migration and invasion of GC cells.

To explore the biological functions of lncRNA DLEU2 in GC, we knocked down the expression of lncRNA DLEU2 using siRNA in AGS and MKN-45 cells. The expression of lncRNA DLEU2 was down-regulated approximately 60% after treated with siRNA targeting lncRNA DLEU2 (si-DLEU2) compared to the negative control (NC) (Fig. 1C). Knockdown of lncRNA DLEU2 significantly inhibited cell proliferation, as evaluated by CCK-8 assays (Fig. 1D). In parallel, the results of colony formation assay showed that colony formation ability of si-DLEU2 transfected cells was significantly suppressed, compared into NC (Fig. 1E). Moreover, cell migration and invasion were evaluated by transwell and wound healing assays, and we observed that the transfection of si-DLEU2 obviously inhibited the migration and invasion of AGS and MKN-45 cells (Fig. 1F). Furthermore, expression of E-cadherin was up-regulated, while N-cadherin, Vimentin, Snail and Slug were down-regulated in AGS and MKN-45 cells transfected with si-DLEU2 (Fig. 2A). In addition, apoptosis analysis revealed that lncRNA DLEU2 down-regulation could induce GC cell apoptosis (Fig. 2B). And, western blotting analysis revealed increased expression of Bax, cleaved caspase 3 and cleaved caspase 9, alongside decreased expression of Bcl2 in AGS and MKN-45 cells transfected with si-DLEU2 (Fig. 2C).

LncRNA DLEU2 acts as a tumor promoter through the AKT signaling pathway

To comprehensively elucidate the mechanisms of lncRNA DLEU2 in regulating GC cell proliferation, migration, invasion and apoptosis, we detected the activation of AKT signaling pathway. We found that AKT phosphorylation was reduced and expression of AKT was not significantly changed following treatment with si-DLEU2 compared to NC (Fig. 2D).

LncRNA DLEU2 acts as a ceRNA targeting ETS2 via miR-30a-5p

The algorithm predicted that lncRNA DLEU2 could act as a ceRNA to target ETS2 via miR-30a-5p and luciferase assay validated this (Fig. 4A-D). As shown in Fig. 4B, AGS cells co-transfected with miR-30a-5p mimics and lncRNA DLEU2-WT showed less luciferase activity than the other groups. In parallel, it was observed that AGS cells co-transfected with miR-30a-5p mimics and ETS2-WT revealed less luciferase activity than the other groups (Fig. 4D). In addition, the expression of ETS2 was down-regulated in GC cells transfected with si-DLEU2 (Fig. 4E). Moreover, we detected the expression of ETS2 in GC tissues. As shown in Fig. 4F and Table 2, ETS2 was highly expressed in GC tissues (58/75, 77.3%), compared into

normal tissues (15/75, 20%, $P < 0.001$). In addition, we analyzed the association between ETS2 expression and clinical characteristics of patients. As shown in Table 3, the expression of ETS2 was significantly associated with age ($P = 0.041$), pathological grading ($P < 0.001$) and TNM stage ($P = 0.023$). A significant correlation with other features, such as gender and tumor diameter were not observed.

Table 1
DLEU2 expression associated with the clinicopathological parameters in GC

Clinicopathological parameters	n	DLEU2 expression	P
Gender			
Male	55	1.437 ± 0.0617	0.5541
Female	20	1.500 ± 0.0764	
Age (years)			
< 60	33	1.507 ± 0.0731	0.8911
≥ 60	42	1.493 ± 0.0549	
Tumor diameter (cm)			0.1748
< 5	31	1.473 ± 0.0727	
≥ 5	44	1.623 ± 0.0549	
Pathological grading			0.0087*
I-II	23	1.406 ± 0.0632	
III-IV	52	2.067 ± 0.1004	
TNM staging			
T1-T2	15	1.997 ± 0.1695	0.0382*
T3-T4	60	1.417 ± 0.0869	

Table 2
ETS2 expression in GC compared with para-carcinoma tissue

Group	n	ETS2 expression		P
		Low (n%)	High (n%)	
GC	75	17 (22.7)	58 (77.3)	0.001**
para-carcinoma	75	60 (80.0)	15 (20.0)	

Table 3
ETS2 expression associated with the clinicopathological parameters in GC

Clinicopathological parameters	n	ETS2 Low (n%)	ETS2 High (n%)	P
Gender				
Male	55	11 (20.0)	44 (80.0)	0.800
Female	20	6 (30.0)	14 (70.0)	
Age (years)				
< 60	33	11 (33.3)	22 (66.7)	0.041*
≥ 60	42	5 (11.9)	37 (88.1)	
Tumor diameter (cm)	31	9 (29.0)	22 (71.0)	0.409
< 5	44	8 (18.2)	36 (81.8)	
≥ 5				
Pathological grading	23	12 (52.2)	11 (47.8)	0.001**
I-II	52	5 (9.6)	47 (90.4)	
III-IV				
TNM staging				
T1-T2	15	7 (46.7)	8 (53.3)	0.023*
T3-T4	60	10 (16.7)	50 (83.3)	

LncRNA DLEU2 exerts its role through regulating miR-30a-5p/ETS2 axis

Finally, to test whether lncRNA DLEU2 exerted its role through regulating miR-30a-5p/ETS2 axis, cells were transfected with pcDNA3.1-ETS2 overexpression plasmid, pcDNA3.1-ETS2 plasmid and miR-30a-5p mimics, pcDNA3.1-ETS2 plasmid and si-DLEU2, respectively. The results showed that ETS2 overexpression promoted cell proliferation and migration of AGS and MKN-45 cells (Fig. 4). Furthermore, ETS2 overexpression rescued cell proliferation and migration inhibition induced by lncRNA DLEU2 down-regulation and miR-30a-5p up-regulation in AGS and MKN-45 cells (Fig. 4). These results showed that lncRNA DLEU2 may regulate GC cells process through miR-30a-5p/ETS2 axis.

Hypoxia induces the expression of DLEU2 in gastric cancer cells

Hypoxia is an important feature of the tumor microenvironment. The rapid growth of tumors can lead to widespread hypoxia, leading to increased invasiveness and resistance to chemotherapy(32). Hypoxia can

change many cell characteristics, including transcriptome, proteome, metabolome, DNA repair and apoptosis. Therefore, at the end we examined the effect of hypoxia on the expression of DLEU2 in gastric cancer cells. As shown in Fig. 5A, hypoxia caused the accumulation of hypoxia-inducible factor-1α (HIF-1α) level in AGS and MKN45 cells and merged into the nucleus. In addition, as shown in Fig. 5B, hypoxia 12 and 24 h induced the expression of DLEU2.

Discussion

Many studies have shown that lncRNAs are involved in the GC progression as ceRNAs, and their abnormal expression is closely related to the biological process of GC(4, 33, 34). This study revealed the expression pattern of lncRNA DLEU2 in GC and its regulation on GC cell processes for the first time.

The results of GEPIA online analyze and qRT-PCR showed that lncRNA DLEU2 was significantly up-regulated in GC tissues. LncRNA DLEU2 is found to be highly expressed in non-small cell lung cancer(35), acute myeloid leukemia(31), glioma(36), hepatocellular carcinoma(37, 38), esophageal cancer(39, 40), osteosarcoma(41), and pancreatic cancer(42) tissues and cell lines, and its expression level is related to the patient's prognosis and overall survival closely related. Furthermore, knockdown of lncRNA DLEU2 inhibited the proliferation of AGS and MKN-45 cells, and induced cell apoptosis. And, western blotting analysis revealed increased expression of Bax, cleaved caspase 3 and cleaved caspase 9, alongside decreased expression of Bcl2 in AGS and MKN-45 cells transfected with si-DLEU2. These results suggested a potential cancer-promoting effect of lncRNA DLEU2 in GC.

The EMT process is a critical step in tumor progression, which increases cell infiltration and promotes the occurrence of distant metastases(43). One of its important features is the loss of epithelial markers and the acquisition of mesenchymal markers(44). In this study, we found that down-regulation of lncRNA DLEU2 inhibited the migration and invasion of AGS and MKN-45 cells using transwell and wound healing assays. Furthermore, increased E-cadherin expression, and decreased N-cadherin, Vimentin, Snail and Slug expression were observed in si-DLEU2 transfected cells, indicating the inhibition of down-regulation of lncRNA DLEU2 in EMT processes.

In the present study, we found that miR-30a-5p could directly bind to the 3’ UTR region of ETS2, thereby inhibiting the translation and protein stability of ETS2. Moreover, lncRNA DLEU2 bound to miR-30a-5p through the same binding site, which facilitated the expression of ETS2. Knockdown of lncRNA DLEU2 reduced the protein level of intracellular ETS2. The *ETS2* gene is located on chromosome 21q22.1-q22.3 with a span of 17.6 kb, which has no TATA box or CAAT box in its promoter and has a major CpG island at its 5’ untranslated region(45). ETS2 protein consists of 469 amino acid with an N-terminal pointed domain and a C-terminal DNA-binding domain. It also has a MAPK phosphorylation site at Thr72, which may mediate transcriptional regulation(46). As a transcription factor, ETS2 is involved in various tumor-associated gene promoter regulation, such as Cyclin D1(30). In addition, mutations in the core promoter of the telomerase reverse transcriptase (TERT) gene create a *de novo* binding site for ETS2, providing a mechanism for cancer-specific telomerase reactivation(47, 48). In most cancer cells, telomerase

reactivation is a ubiquitous process and one of the main features(49). In human cancers, TERT promoter mutations have been shown to define a subpopulation of patients with poor prognosis(47, 48). Moreover, the preferential binding of ETS2 to gain-of-function mutant p53 (mut-p53) has been suggested, raising the tumor promoting role of mut-p53(50). The above results indicated that ETS2 is involved in the malignant progression of tumor cells through a variety of mechanisms. Furthermore, lncRNA DLEU2 exerts a cancer-promoting function by regulating the expression of ETS2.

ETS2 is representative members of the ETS family of transcription factors and plays crucial roles in cell proliferation, differentiation, development, and transformation. In present study, we found that ETS2 was highly expressed in GC, which was consistent with the expression of ETS2 in esophageal squamous cell cancer(51). One study has shown that down-regulation of ETS2 significantly reduces the level of p-AKT in renal cell carcinoma cells(31). Consistent with the results of this study, knockdown of lncRNA DLEU2 obviously inhibited the AKT phosphorylation. We speculate that lncRNA DLEU2 likely affect the phosphorylation of AKT by regulating ETS2 expression.

In this study, we found that the hypoxic microenvironment resulted in the accumulation and nucleation of HIF-1α in gastric cancer cells, and up-regulated the expression of DLEU2. HIF1 is the core component of hypoxia signaling pathway(52). In experimental models, the activation of this pathway has been shown to promote the progression of gastric cancer(53). HIF-1 can up-regulate EMT-related transcription factors and is closely related to the prognosis of gastric cancer(54). Those results led us to speculate on the key role of DLEU2 in tumor hypoxia. Our results also revealed for the first time the correlation between DLEU2 and tumor hypoxia.

Conclusions

In conclusion, our data revealed that lncRNA DLEU2 was highly expressed in GC tissues and its expression was related with clinicopathological characteristics of GC patients. Down-regulation of lncRNA DLEU2 inhibited the proliferation, migration and invasion of GC cell as well as suppressed the EMT process and induced apoptosis. We also found that lncRNA DLEU2 acted as a ceRNA targeting ETS2 via miR-30a-5p. Furthermore, the AKT signaling pathway was required. In addition, DLEU2 may also play a key role in tumor hypoxia. Taken together, we suggested that lncRNA DLEU2 is a potential molecular target for GC treatment.

Abbreviations

Gastric cancer GC

Long-chain non-coding RNA lncRNAs

competitive endogenous RNAs ceRNAs

American Type Culture Collection ATCC

Dulbecco’s modified Eagle medium DMEM

siRNAs targeting to lncRNA DLEU2 si-DLEU2

pcDNA3.1-ETS2 expression vector pcDNA3.1-ETS2

stomach adenocarcinoma STAD

telomerase reverse transcriptase TERT

mutant p53 mut-p53

Declarations

Ethics approval and consent to participate

All research complied with the principles of the Declaration of Helsinki, and was approved by the Medical Ethics Committee of the Jinan Central Hospital.

Consent for publication

Enrolled patients had given written informed consent for publication.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All author generated the hypothesis, designed and performed the experiments, analyzed the data, provided conceptual advice and technical expertise, and edited the manuscript. Xiangdong Liu conceived and supervised the study. All authors have reviewed and approved the final version of the manuscript.

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No.

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Figures

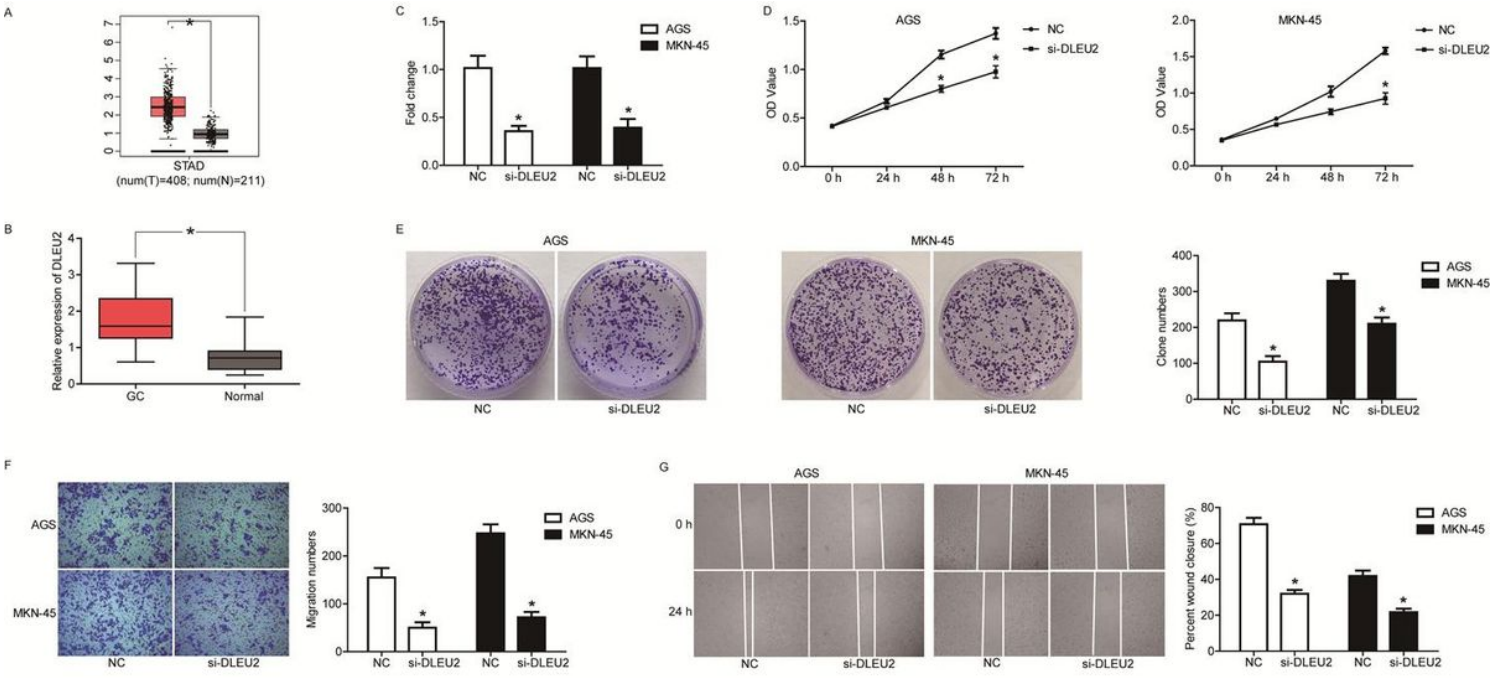

Figure 1

LncRNA DLEU2 is up-regulated in GC tissues and knockdown of lncRNA DLEU2 inhibits cell proliferation, migration and invasion of GC cells. (A)The boxplot of lncRNA DLEU2 level. Red and gray boxes represent stomach adenocarcinoma (STAD) tissues and normal tissues, respectively. The data came from the GEPIA database. (B) LncRNA DLEU2 levels in gastric cancer (GC) tissues and adjacent normal tissues (n = 75) were evaluated via qRT-PCR. (C) LncRNA DLEU2 levels were evaluated via qRT-PCR. (D) The

proliferation of AGS and MKN-45 cells was determined via CCK8 assay. (E) The clonogenicity of AGS and MKN-45 cells was detected by clony formation assay. (F) The invasion of AGS and MKN-45 cells were determined by transwell assay. (G) The migration of AGS and MKN-45 cells were determined by wound healing assay. *P < 0.05.

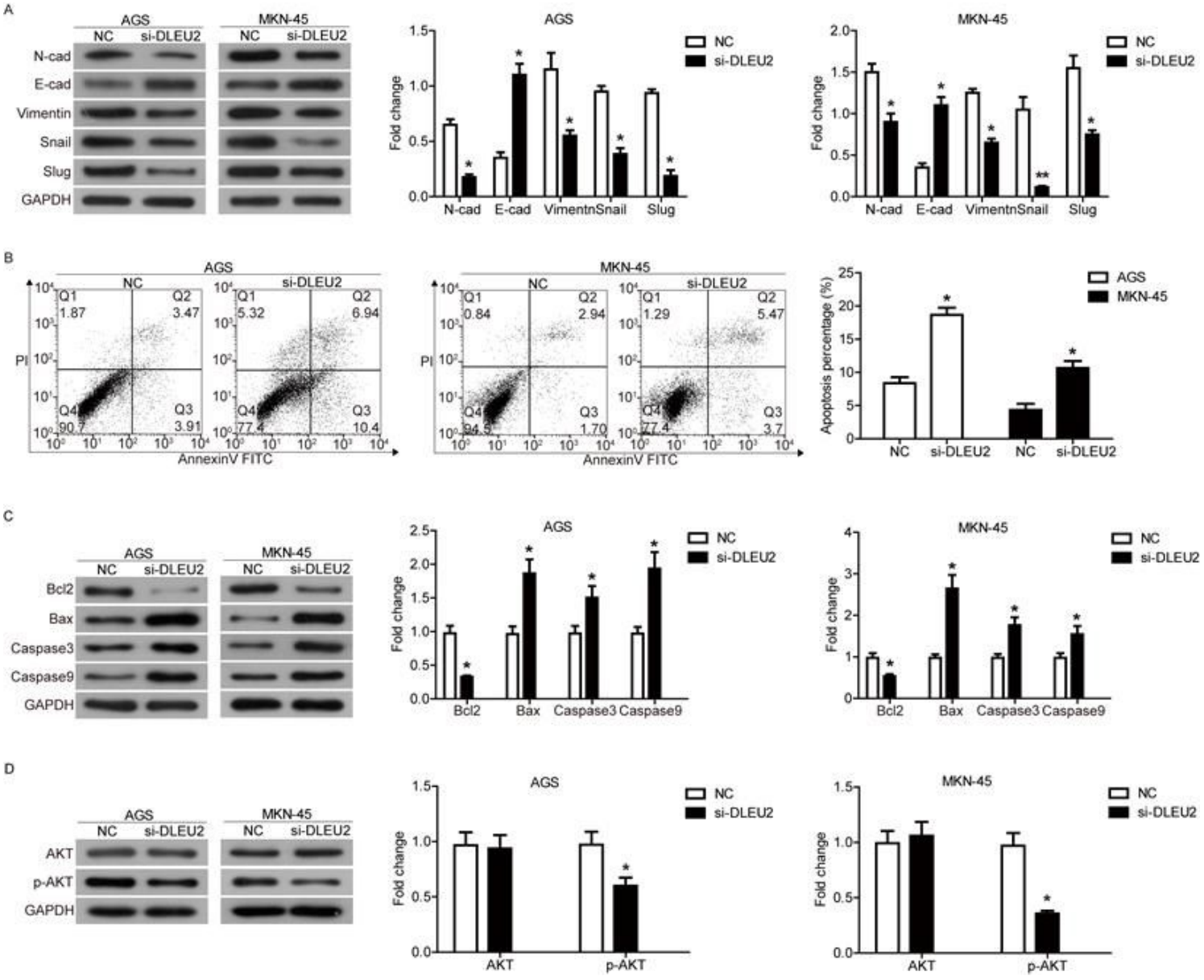

Figure 2

Knockdown of lncRNA DLEU2 suppresses the EMT process and induces apoptosis of GC cells. (A) The expression of EMT-related proteins was detected by western blot. (B) The apoptosis of AGS and MKN-45 cells was detected by flow cytometry. (C) The expression of apoptosis-related proteins was detected by western blot. (D) The activation of AKT signaling pathway was detected by western blot. *P < 0.05.

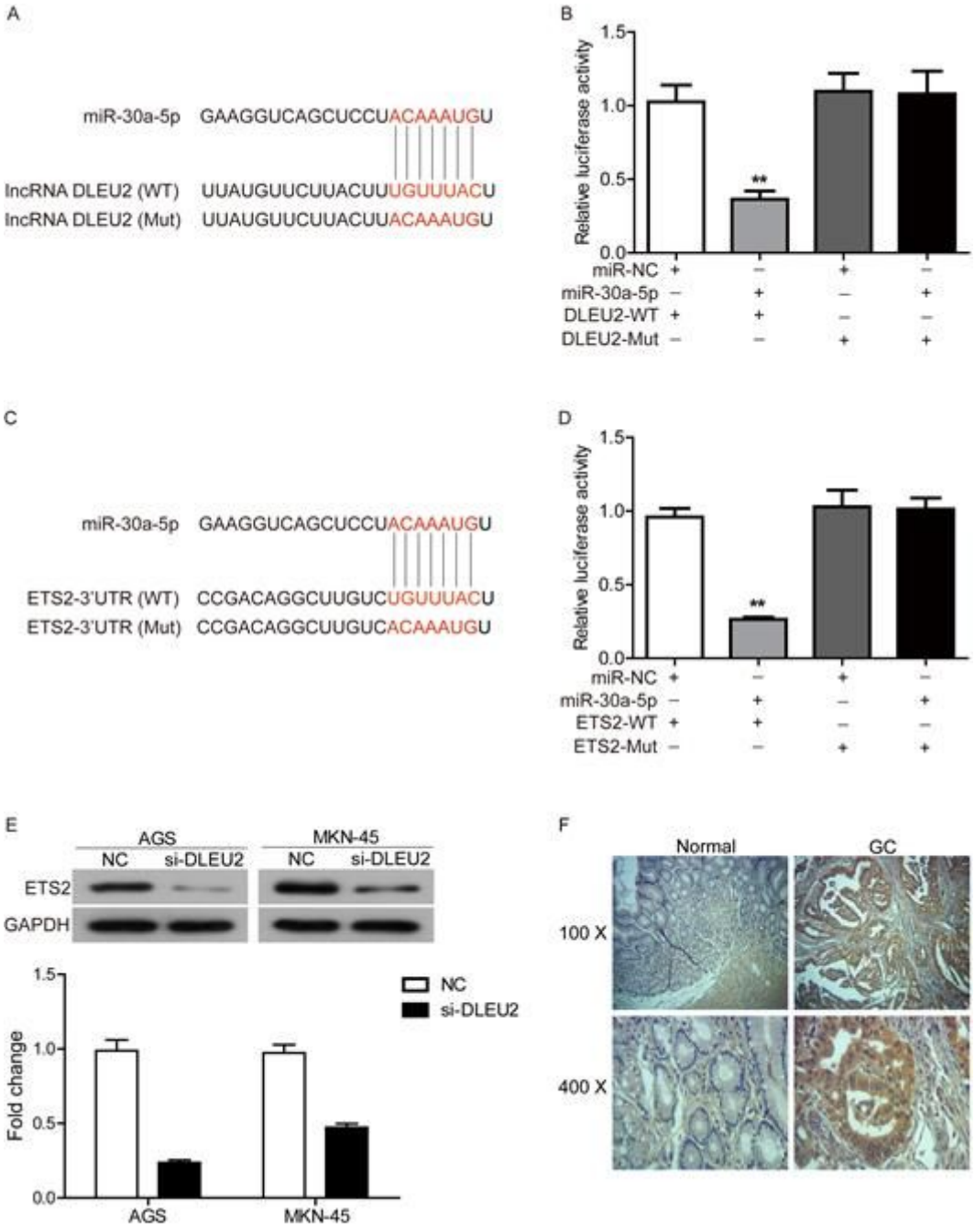

Figure 3

lncRNA DLEU2 acts as a ceRNA targeting ETS2 via miR-30a-5p. (A) The sequences of miR-30a-5p, wide type of lncRNA DLEU2 (WT) and mutated lncRNA DLEU2 (Mut). (B) The expression levels of luciferase of AGS cells transfected with wild-type (WT) or mutated (Mut) lncRNA DLEU2 reporters plus miR-30a-5p mimic or miR-NC were determined. (C) The sequences of miR-30a-5p, wide type of ETS2 (WT) and mutated ETS2 (Mut). (D) The expression levels of luciferase of AGS cells transfected with wild-type (WT) or mutated (Mut) ETS2 reporters plus miR-30a-5p mimic or miR-NC were determined. (E) The expression of ETS2 in AGS and MKN-45 cells transfected with si-DLEU2. (F) The expression of ETS2 in GC tissues and adjacent normal ovarian tissues were evaluated via immunohistochemistry. *P < 0.05.

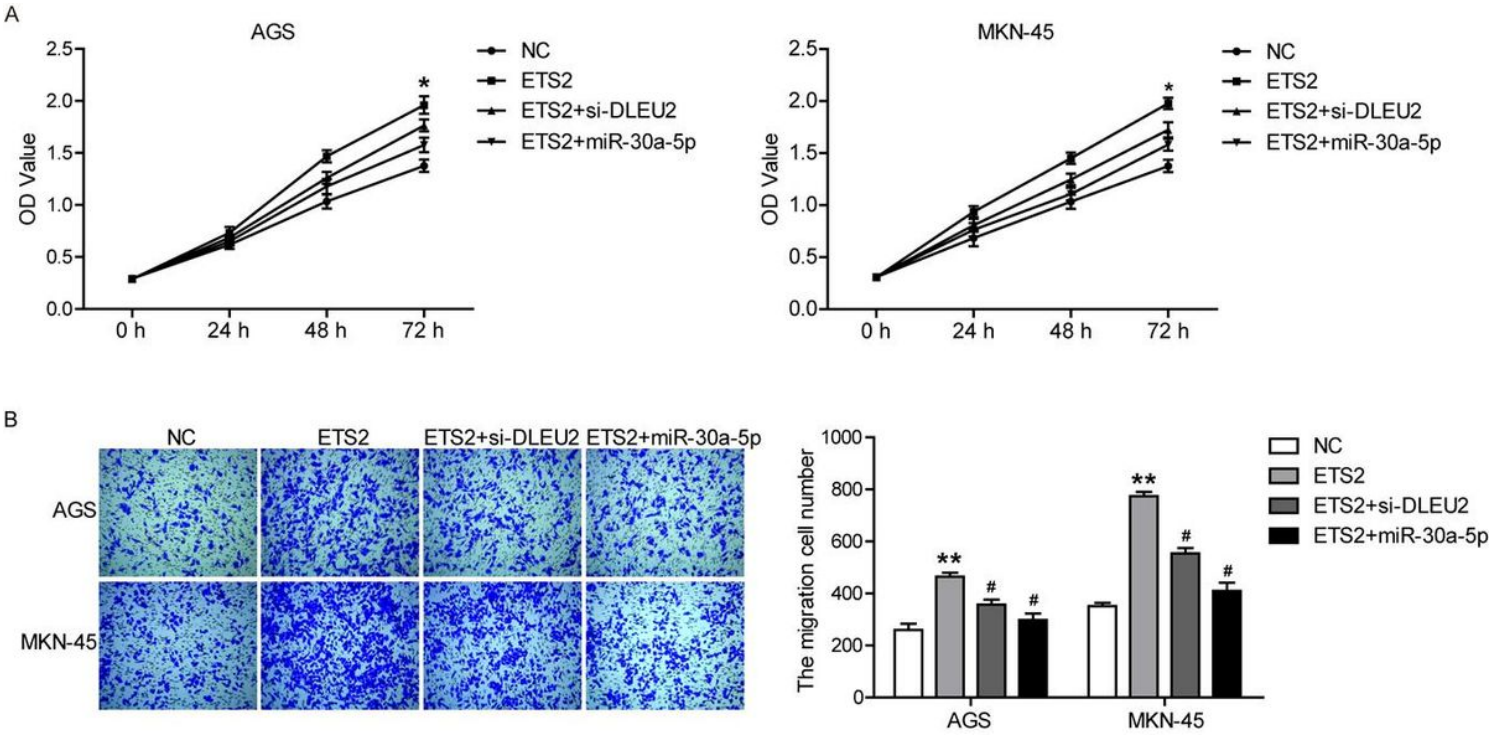

Figure 4

lncRNA DLEU2 exerts its role through regulating miR-30a-5p/ETS2 axis. (A) The proliferation of AGS and MKN-45 cells was determined via CCK8 assay. (B) The invasion of AGS and MKN-45 cells were determined by transwell assay. *P < 0.05.

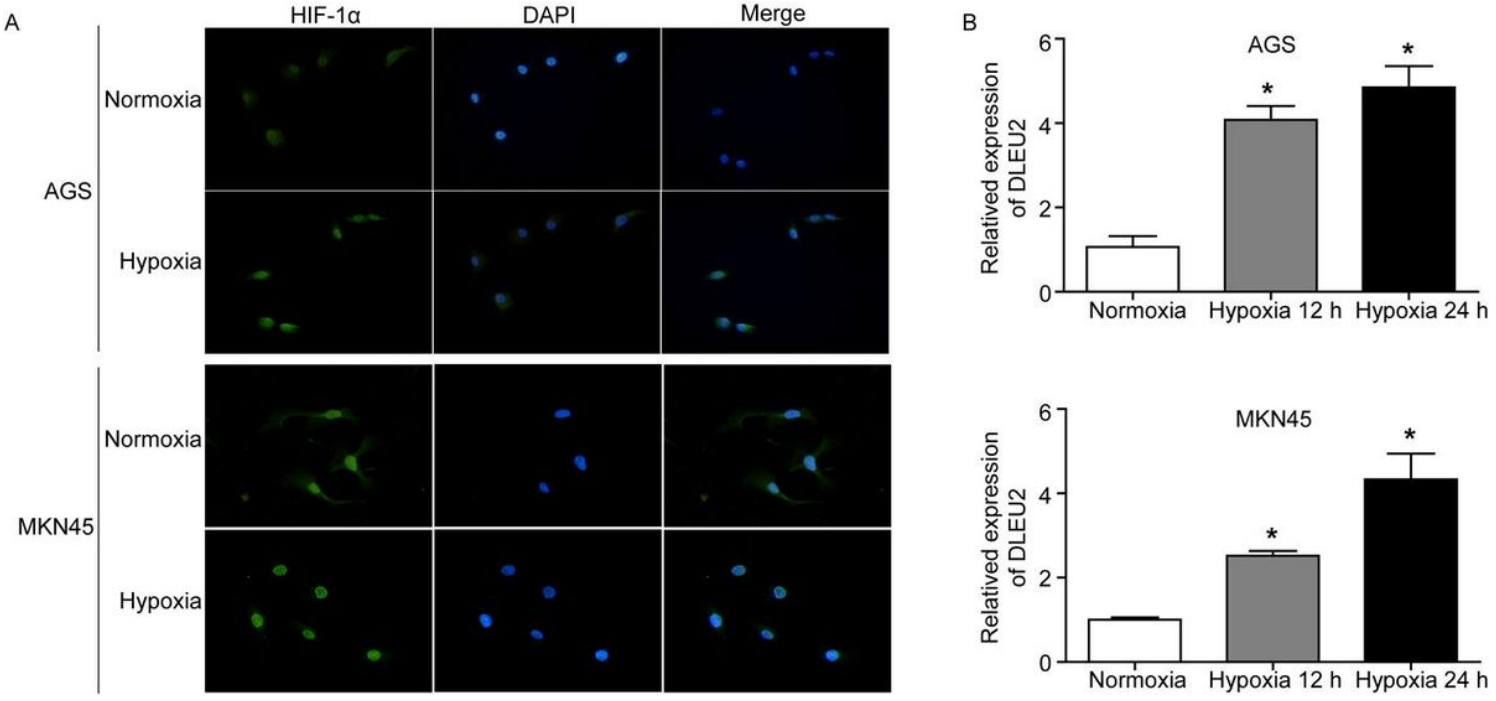

Figure 5

Hypoxia induces DLEU2 expression in gastric cancer cells. (A) Immunofluorescence detection of HIF-1α expression and subcellular localization in normoxic and hypoxic cells. (B) qRT-PCR detection of DLEU2

expression levels in normoxic and hypoxic cells for 12 h or hypoxia 24 h. *P < 0.05.