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Cervical carcinoma progression is aggravated by lncRNA ZNF281 by binding KLF15

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Abstract. – OBJECTIVE: This study aims to explore the biological roles of long non-coding RNA (lncRNA) ZNF281 and KLF15 in regulating cervical carcinoma progression.

PATIENTS AND METHODS: Differential expressions of ZNF281 in 58 collected cervical carcinoma and normal tissues were detected by quantitative real-time polymerase chain reaction (qRT-PCR). The relationship between ZNF281 and clinicopathologic characteristics in cervical carcinoma patients was analyzed. By generating ZNF281 knockdown model in HeLa and Si-Ha cells through the transfection of shZNF281, migratory ability changes were examined *via* transwell and wound healing assay. The role of ZNF281 in *in vivo* tumorgenicity of cervical carcinoma was examined by implanting xenografted cancers in nude mice. The downstream target of ZNF281 and their interaction were assessed by bioinformatics tool and Dual-Luciferase reporter assay, respectively. Finally, co-regulations of ZNF281 and KLF15 on cervical carcinoma progression were elucidated.

RESULTS: ZNF281 was upregulated in cervical carcinoma tissues and cell lines. It was correlated to TNM staging, and incidences of lymphatic metastasis and distant metastasis in cervical carcinoma patients, while it was unrelated to age and tumor size. The knockdown of ZNF281 effectively attenuated migratory ability in HeLa and SiHa cells. Besides, knockdown of ZNF281 also reduced tumorigenicity of cervical carcinoma in nude mice. KLF15 was the downstream gene binding ZNF281, and they were negatively correlated to each other in cervical carcinoma tissues. Notably, KLF15 was responsible for ZNF281-induced regulation on cervical carcinoma migration.

CONCLUSIONS: LncRNA ZNF281 is upregulated in cervical carcinoma samples, and it is correlated to lymphatic metastasis, distant metastasis, and poor prognosis in cervical carcinoma patients. By targeting KLF15, ZNF281 triggers migratory potential in cervical carcinoma. We believed that ZNF281 is a promising biomarker for cervical carcinoma.

Key Words:
LncRNA ZNF281, KLF15, Cervical carcinoma, Metastasis.

Introduction

Cervical carcinoma is one of the most prevalent and fatal malignant tumors in the world, and it is also the second most common malignant tumor in women. In recent years, the onset of cervical carcinoma has shown a younger trend. There are 528,000 new cases of cervical carcinoma each year, and about 266,000 people die from it. In developing countries, cervical carcinoma has become the leading cause of cancer deaths in women[1-3]. Therefore, it is urgent to clarify the pathogenesis of cervical carcinoma and to develop more effective prevention, diagnosis and treatment methods[4,5]. In the past few decades, a large number of epidemiological and functional studies have confirmed the causal relationship between human papillomavirus (HPV) infection and the carcinogenesis of cervical carcinoma. HPV-induced genomic instability and cumulative somatic mutations are risk factors for increasing the susceptibility to cervical carcinoma[5-7]. Nowadays, long non-coding RNAs (lncRNAs) have been well explored because of their involvement in tumor progression by regulating relevant pathways[8,9].

LncRNAs are non-coding RNAs containing more than 200 nucleotides long, and cannot encode proteins. LncRNAs are hot topics in epigenetic research since they are functional in chromosome remodeling, transcriptional and post-transcriptional regulations[10,11]. Increasing evidence has proven the relationship between abnormally expressed lncRNAs and human cancers[11,12]. It is suggested that lncRNAs create a favorable microenvironment for uncontrolled

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and progressive growth of tumor cells[13,14]. LncRNA ZNF281 has been detected to be differentially expressed in tumor profiling as an oncogene, which is capable of regulating tumor cell behaviors[15,16]. Using online bioinformatics analysis tool, it is predicted that lncRNA ZNF281 could specifically bind KLF15. This study aims to clarify the co-regulations of ZNF281 and KLF15 on the progression of cervical carcinoma, which provides new ideas for clinical diagnosis and treatment of cervical carcinoma.

Patients and Methods

Patients and Cervical Carcinoma Samples

A total of 58 cancer tissues and adjacent normal ones from cervical carcinoma patients were collected in Jinan Maternity and Child Care Hospital Affiliated to Shandong First Medical University. These collected samples were pathologically diagnosed and stored within 5 min to prevent RNA degradation. All patients with cervical cancer did not have radiotherapy and chemotherapy before surgery, and it was pathologically confirmed as cervical carcinoma. The following patients were excluded from this study: patients with other malignancies; mental disease; myocardial infarction; heart failure or other chronic diseases, or those previously exposed to radioactive rays of CC patients. Tumor node metastasis (TNM) staging of cervical carcinoma was determined based on the criteria proposed by The Union for International Cancer Control (UICC). All cervical carcinoma patients signed written informed consent. This study complied with the Helsinki Declaration and approved by the Medical Research Ethics Committee of Jinan Maternity and Child Care Hospital Affiliated to Shandong First Medical University.

Cell Lines and Reagents

Cervical carcinoma cell lines (HeLa, SiHa, Caski and C33-A) and the normal cervical epithelial cell line (HaCaT) were cultivated in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) in a 5% CO_2 incubator at 37°C.

Transfection

Cells in the logarithmic growth phase were inoculated in 6-well plates with serum-free medium. Until cells were grown to 70-80% density, shZNF281 or shCtrl (GeneCopoeia, Rockville, MD, USA) was transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Transfection efficacy was tested at 48 h.

Transwell Migration Assay

Transwell chambers (Millipore, Billerica, MA, USA) were inserted in each well of a 24-well plate. 200 μL of serum-free suspension (2×10^5 mL) was applied in the upper layer of the chamber, and 500 μL of medium containing 10% FBS was applied in the bottom. After 48-h incubation, migratory cells in the bottom were reacted with 15-min methanol, 20-min crystal violet and captured using a microscope. Migratory cells were counted in 5 randomly selected fields per sample.

Wound Healing Assay

Cells were prepared into suspension with 5×10^5 cells/mL, and implanted in 6-well plates. Until 90% of cell attachment, an artificial wound was made using a sterilized pipette tip. Cells were washed in phosphate-buffered saline (PBS) for 2-3 times and cultured in the medium containing 1% FBS. 24 hours later, wound closure was captured for calculating the percentage of wound healing.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used for isolating total cellular RNAs, which were reversely transcribed into complementary deoxyribose nucleic acids (cDNAs) (PrimeScript RT Reagent; TaKaRa, Otsu, Shiga, Japan). Using the SYBR®Premix Ex Taq™ kit (TaKaRa, Otsu, Shiga, Japan) and StepOne Plus Real-time PCR system (Applied Biosystems, Foster City, CA, USA), qRT-PCR was carried out. Relative level was calculated by $2^{-\Delta\Delta Ct}$ and normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH)[17]. Primers were synthesized using Primer 5.0 software, and their sequences were listed as follows: LncRNA ZNF281: forward: 5'-AGGTCCCAGGCTTGTCACT-3', reverse: 5'-CAGAAAGGCAGGCGAGTTAT-3'; KLF15: forward: 5'-GCCAAGTTCAGCCGCCA-3', reverse: 5'-CCAACCAGCCTATCACCCAG-3'; GAPDH: forward: 5'-ACCACAGTCCATGCCATCAC-3', reverse: 5'-TCCACCACCCTGTTGCTGTA-3'.

Western Blot

Cells were lysed in radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) on ice for 30 min, and centrifuged at 4°C, 14000×g for 15 min. The concentration of isolated protein was measured by bicinchoninic acid (BCA) method (Pierce Biotechnology, Rockford, IL, USA). Protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred on polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, membranes were soaked in 5% skim milk for 2 hours. Primary antibodies were applied for overnight incubation at 4°C. On the next day, horseradish peroxidase (HRP)-labeled secondary antibodies were used for 2 h incubation. Band exposure was achieved by enhanced chemiluminescence (ECL) with GAPDH as the internal reference.

Dual-Luciferase Reporter Assay

Based on the predicted binding sites in the 3'-UTR of ZNF281 and KLF15, wild-type and mutant-type ZNF281 vectors were constructed, and co-transfected into cells with NC or pcDNA-KLF15, respectively. Luciferase activity was finally measured at 48 h (Promega, Madison, WI, USA).

In Vivo Xenograft Model

This study was approved by the Animal Ethics Committee of Shandong First Medical University Animal Center. Ten male nude mice with 4 weeks old were randomly implanted with HeLa cells transfected with shZNF281 (n=5) or shCtrl (n=5), respectively by subcutaneous administration. The width and length of the xenografted tumor were weekly recorded. Six weeks later, mice were sacrificed for harvesting the tumor tissues and weighed. Tumor volume (mm^3) = ($width^2$×length)/2.

Statistical Analysis

Data were expressed as mean ± standard deviation and processed by Statistical Product and Service Solutions (SPSS) 22.0 (IBM, Armonk, NY, USA). Measurement data were compared using the Student's *t*-test, and categorical variables were analyzed by χ^2-test or Fisher's exact test. Chi-square analysis was conducted for analyzing the relationship between ZNF281 and clinical indicators in cervical carcinoma patients. Kaplan-Meier method was used for survival analysis, followed by log-rank test for comparing differences between curves. A significant difference was set at $p<0.05$.

Results

Upregulated ZNF281 in Cervical Carcinoma Cases

QRT-PCR results showed that ZNF281 was upregulated in cervical carcinoma tissues than normal ones (Figure 1A). Recruited cervical carcinoma patients were divided into high and low ZNF281 expression groups, respectively according ZNF281 level in the corresponding cancer tissues. As shown in Table I, ZNF281 level was positively correlated to TNM staging, lymphatic metastasis and distant metastasis in cervical carcinoma patients. Consistently, higher level of ZNF281 was detected in cervical carcinoma cases with advanced T stage (T3-4), lymphatic metastasis or distant metastasis (Figure 1B). We thereafter detected ZNF281 levels in cervical carcinoma cell lines. Compared with the normal cervical epithelial cell line, ZNF281 was consistently upregulated in cervical carcinoma cell lines (Figure 1C).

Knockdown of ZNF281 Suppressed Migratory Ability in Cervical Carcinoma

Transfection of shZNF281 remarkably downregulated ZNF281 in HeLa and SiHa cells, indicating the effective transfection (Figure 2A). Migratory potential in cervical carcinoma cells was assessed by both transwell and wound healing assay. Knockdown of ZNF281 markedly decreased migratory cell number in HeLa and SiHa cells (Figure 2B). Moreover, wound healing percentage decreased after transfection of shZNF281 (Figure 2C). Therefore, ZNF281 was able to stimulate cervical carcinoma migration.

ZNF281 Stimulated Tumorigenicity of Cervical Carcinoma in Nude Mice

In vivo functions of ZNF281 in cervical carcinoma were subsequently explored by generating xenograft model in nude mice. Compared with those of controls, tumor volume and tumor weight were lower in cervical carcinoma tissues harvested from mice administrated with HeLa cells transfected with shZNF281 (Figure 3A, 3B). ZNF281 was downregulated, while KLF15 was upregulated in xenografted cervical carcinoma

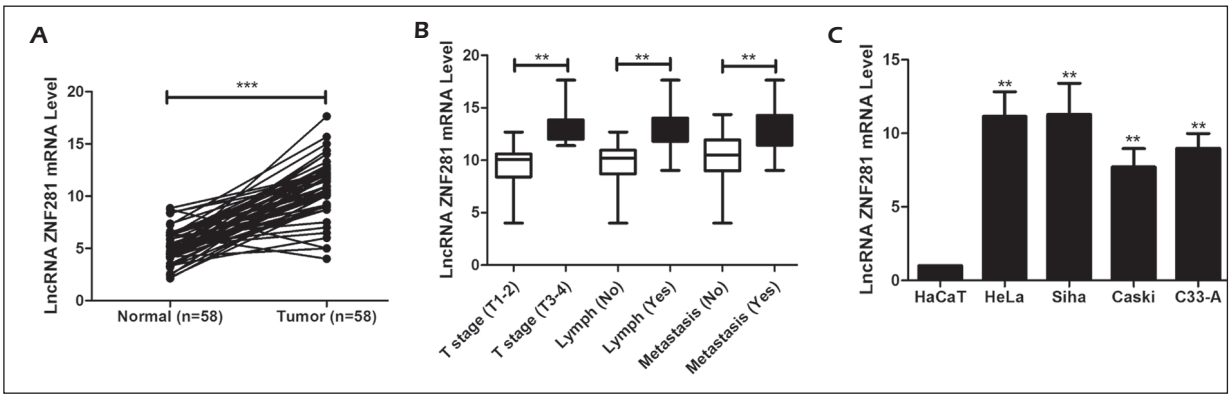

Figure 1. Upregulated ZNF281 in cervical carcinoma cases. **A,** Differential levels of ZNF281 in cervical carcinoma tissues (n=58) and normal tissues (n=58). **B,** Differential levels of ZNF281 in cervical carcinoma tissues classified by T stage, lymphatic metastasis and distant metastasis. **C,** Kaplan-Meier curves are depicted based on ZNF281 levels in cervical carcinoma patients. Data were expressed as mean±SD. $^{**}p < 0.01$, $^{***}p < 0.001$.

tissues harvested from mice with *in vivo* knockdown of ZNF281 (Figure 3C, 3D). It is suggested that ZNF281 stimulated the growth of cervical carcinoma *in vivo*.

ZNF281 Specifically Targeted KLF15

To explore the mechanism of lncRNA ZNF281 in regulating the progression of cervical carcinoma, we searched potential targets of lncRNA ZNF281. Based on miRDB database, the results of bioinformatics analysis suggested that KLF15 might be the target gene of lncRNA ZNF281. A binding sequence was discovered in the 3'-UTR of ZNF281 and KLF15 (Figure 4A, left). Overexpression of KLF15 only decreased Luciferase activity in the wild-type ZNF281 vector, rather than the mutant-type one, proving the binding between KLF15 and ZNF281 (Figure 4A, right). Western blot analysis uncovered that protein level of KLF15 was upregulated in HeLa and SiHa cells transfected with shZNF281 (Figure 4B). In addition, KLF15 was detected to be downregulated in cervical carcinoma tissues (Figure 4C).

KLF15 Reversed the Role of ZNF281 in Regulating Cervical Carcinoma Progression

Since KLF15 has been proven as the target gene binding ZNF281, its potential involvement

Table I. Clinicopathologic characteristics of the patients in low- and high-ZNF281 expression group.

Parameters	No. of of cases	ZNF281 expression		*p*-value
		Low (n = 35)	High (n = 23)	
Age (years)				0.245
< 60	23	16	7	
≥ 60	35	19	16	
Tumor size				0.867
< 4 cm	32	19	13	
≥ 4 cm	26	16	10	
T stage				0.015
T1-T2	34	25	9	
T3-T4	24	10	14	
Lymph node metastasis				0.018
No	36	26	10	
Yes	22	9	13	
Distance metastasis				0.031
No	44	30	14	
Yes	14	5	9	

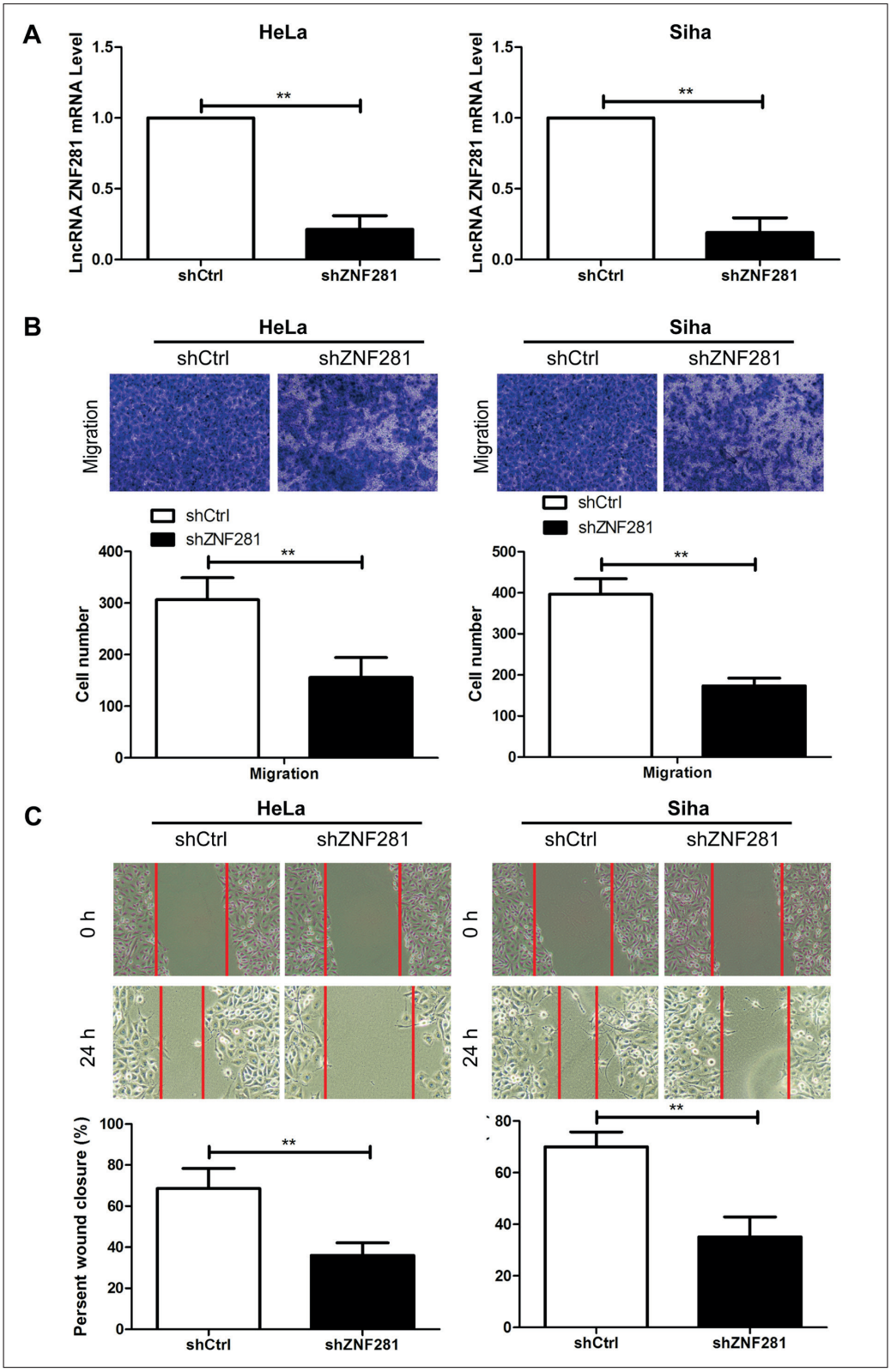

Figure 2. Knockdown of ZNF281 suppressed migratory ability in cervical carcinoma. **A,** Transfection efficacy of shZNF281 in HeLa and SiHa cells. **B,** Migration in HeLa and SiHa cells transfected with shCtrl or shZNF281 (magnification 40×). **C,** Wound closure in HeLa and SiHa cells transfected with shCtrl or shZNF281 (magnification 40×). Data were expressed as mean±SD. $^{**}p < 0.01$.

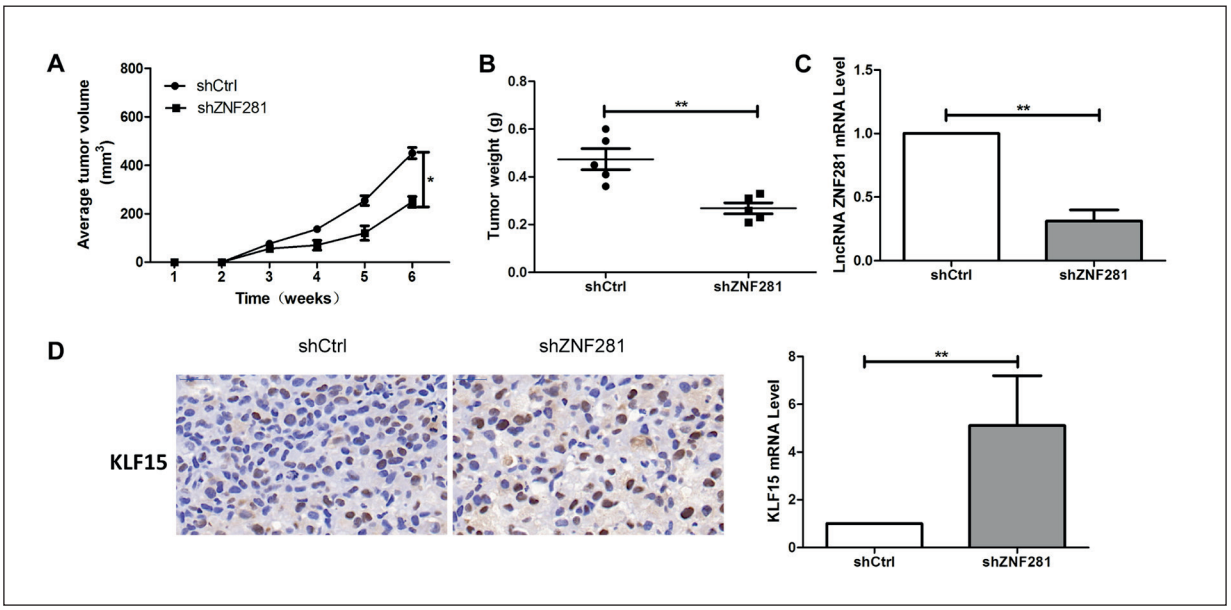

Figure 3. ZNF281 stimulated tumorigenicity of cervical carcinoma in nude mice. **A,** Tumor volume of xenografted cervical carcinoma harvested from nude mice administrated with HeLa cells transfected with shCtrl or shZNF281. **B,** Tumor weight of xenografted cervical carcinoma harvested from nude mice administrated with HeLa cells transfected with shCtrl or shZNF281. **C,** ZNF281 level in xenografted cervical carcinoma harvested from nude mice administrated with HeLa cells transfected with shCtrl or shZNF281. **D,** KLF15 level in xenografted cervical carcinoma harvested from nude mice administrated with HeLa cells transfected with shCtrl or shZNF281, (magnification: 40×). Data were expressed as mean±SD. *$p < 0.05$, **$p < 0.01$.

in cervical carcinoma progression was analyzed. Transfection efficacy of siKLF15 was examined in HeLa and SiHa cells at first (Figure 5A). Of note, compared with HeLa and SiHa cells with ZNF281 knockdown, migratory cell number and wound closure percentage were much higher in those with co-silenced ZNF281 and KLF15 (Figure 5B, 5C). Hence, KLF15 was involved in ZNF281-regulated cervical carcinoma migration.

Discussion

The latest research uncovered that HPV infection is detected in the majority of cervical carcinoma cases. In addition, epigenetic and genetic changes are closely linked to cervical carcinoma progression[5-7]. In human genomes, protein-encoding DNAs account for only 1.5% of chromosomal DNAs. The abundant non-coding RNAs are also functional and extensively transcribed. LncRNAs, non-coding RNAs containing more than 200 nucleotides[10,11], are of significance in maintaining normal functions in the body. Meanwhile, abnormally expressed lncRNAs are closely related to human diseases[12-15]. According to the specific functions, lncRNAs are classified into four subtypes as follows: (1) signal lncRNAs. LncRNAs directly bind target genes and spatio-temporally regulate their expressions as signals. (2) Decoy lncRNAs. Serving as molecule sponges, lncRNAs indirectly regulate gene expressions and functions by binding proteins and miRNAs. (3) Guide lncRNAs. This type of lncRNAs contain a domain that is capable of binding specific transcription regulatory proteins, and a spatial structure binding target gene DNAs. LncRNAs guide specific transcription regulatory factors to the target genes. (4) Scaffold lncRNAs. They usually have a complex spatial structure that is able to recruit multiple genes as a scaffold, thus forming a functional protein complex[18-20].

Previous studies[15,16] suggested that lncRNA ZNF281 was involved in the malignant progression of various malignant disorders, including hepatocellular carcinoma and glioma. However, the relationship between lncRNA ZNF281 and cervical carcinoma is not clear. The clinical samples of cervical carcinoma tissues and adjacent normal ones were collected, and qRT-PCR data showed that ZNF281 was upregulated in cancer tissues, compared to adjacent normal ones. By analyzing clinical data of recruited patients, it was found that ZNF281 was correlated to TNM staging, and

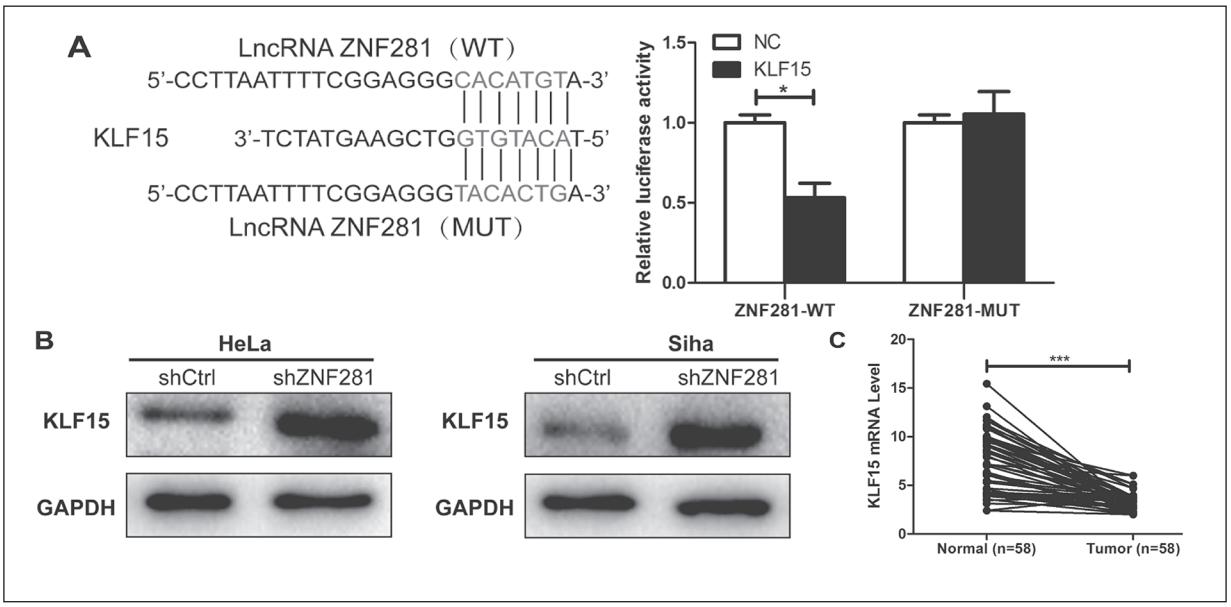

Figure 4. ZNF281 specifically targeted KLF15. **A,** Dual-luciferase reporter assay confirmed the binding between ZNF281 and KLF15. **B,** Protein level of KLF15 in HeLa and SiHa cells transfected with shCtrl or shZNF281. **C,** Differential levels of KLF15 in cervical carcinoma tissues (n = 58) and normal tissues (n = 58). Data were expressed as mean ± SD. *$p < 0.05$, ***$p < 0.001$.

incidences of lymphatic metastasis and distant metastasis in cervical carcinoma patients, while it was unrelated to age and tumor size. We believed that ZNF281 was an oncogene involved in the malignant progression of cervical carcinoma.

Local invasion and distant metastasis are the most typical characteristics of tumors. During the process of tumor metastasis, a series of changes, including cell polarity disappearance, cell-to-cell adhesion decline, increased adhesion to endothelial cell basement membrane, and cytoskeleton remodeling, lead to tumor cell infiltration. Detached tumor cells from primary foci gradually migrate to surrounding tissues, blood vessels, vascular system and eventually distant organs, thereafter developing metastases[21,22]. Therefore, great efforts should be made to prevent and alleviate tumor metastasis[23,24]. In this paper, ZNF281 was highly expressed in cervical carcinoma cell lines, especially HeLa and SiHa cells, which were used for the following *in vitro* experiments. Knockdown of ZNF281 in HeLa and SiHa cells greatly decreased migratory cell numbers and wound closure ability, suggesting the inhibited migratory ability. Moreover, *in vivo* knockdown of ZNF281 markedly slowed down the growth of cervical carcinoma in nude mice.

To clarify the molecular mechanism of ZNF281 in regulating cervical carcinoma migration, we searched its downstream gene. Based on the predicted binding sites in the 3'-UTR of ZNF281 and KLF15, wild-type and mutant-type ZNF281 vectors were constructed for dual-luciferase reporter assay. Overexpression of KLF15 decreased luciferase activity in the wild-type vector, whereas it did not influence that in the mutant-type one, confirming the binding between ZNF281 and KLF15. In addition, the protein level of KLF15 was upregulated in HeLa and SiHa cells with ZNF281 knockdown. Interestingly, the higher migratory cell number and wound closure percentage were observed in cervical carcinoma cell lines with co-knockdown of ZNF281 and KLF15 compared with those with solely knockdown of ZNF281, which indicated that KLF15 was able to abolish the carcinogenic effects of ZNF281 on the migration of cervical carcinoma cell lines. To sum up, our findings proposed that ZNF281/KLF15 axis was responsible for aggravating the malignant progression of cervical carcinoma, which could be utilized in the diagnosis and treatment of cervical carcinoma.

Conclusions

This study demonstrated that lncRNA ZNF281 is upregulated in cervical carcinoma samples,

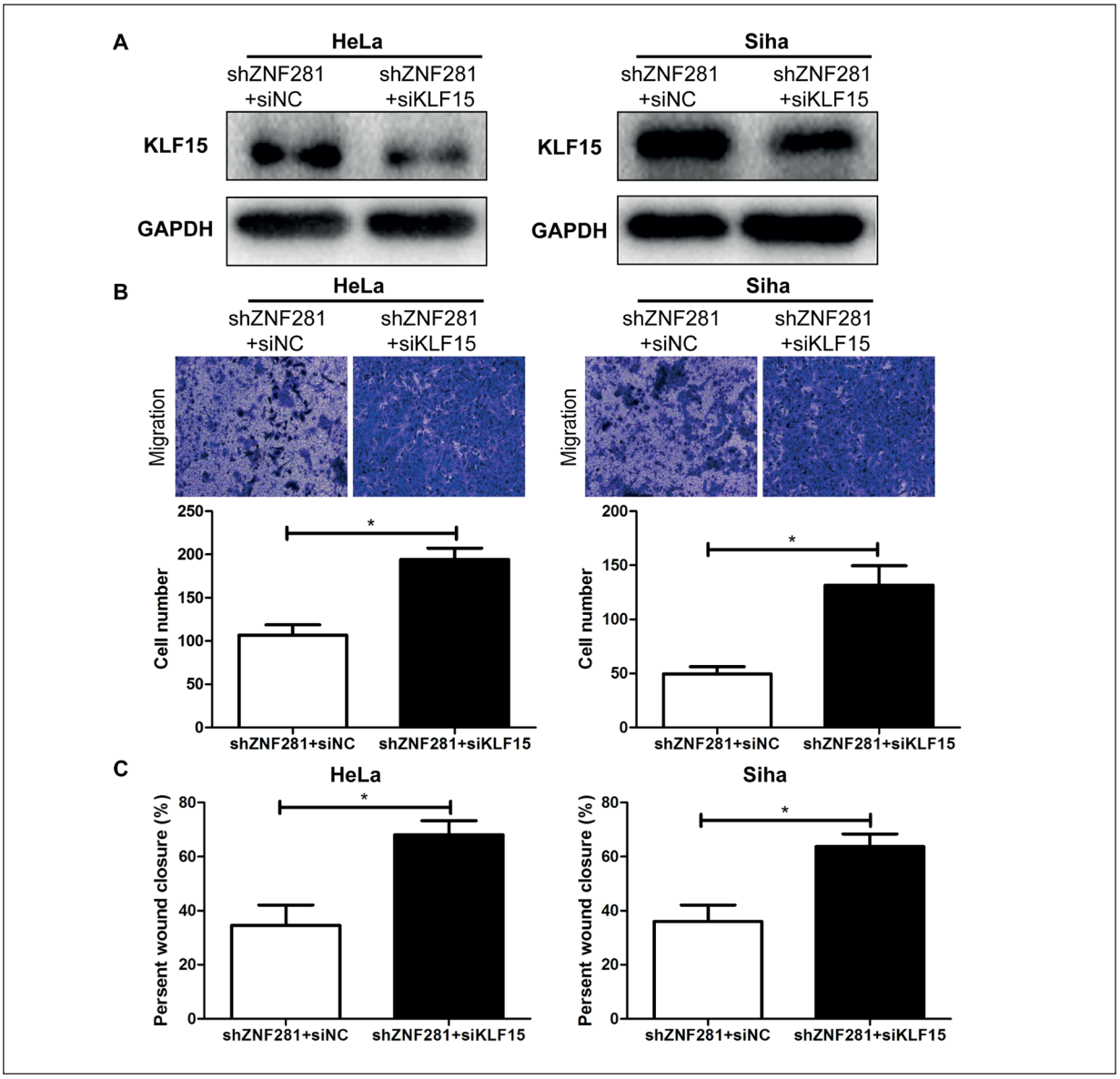

Figure 5. KLF15 reversed the role of ZNF281 in regulating cervical carcinoma progression. **A,** Protein level of KLF15 in HeLa and SiHa cells co-transfected with shZNF281+siNC or shZNF281+siKLF15. **B,** Migration in HeLa and SiHa cells co-transfected with shZNF281+siNC or shZNF281+siKLF15 (magnification 40×). **C,** Wound closure in HeLa and SiHa cells co-transfected with shZNF281+siNC or shZNF281+siKLF15 (magnification 40×). Data were expressed as mean±SD. $^{**}p<0.01$.

and it is correlated to lymphatic metastasis, distant metastasis in cervical carcinoma patients. By targeting KLF15, ZNF281 triggers migratory potential in cervical carcinoma. We believe that ZNF281 is a promising biomarker for cervical carcinoma.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Gottlieb SD. U.S. Cervical cancer mortality. J Adolesc Health 2016; 58: 372.
2) Di J, Rutherford S, Chu C. Review of the cervical cancer burden and population-based cervical cancer screening in China. Asian Pac J Cancer Prev 2015; 16: 7401-7407.
3) Jena R. Cervical cancer virtual special issue. Clin Oncol (R Coll Radiol) 2015; 27: 431-432.

4) Wuerthner BA, Avila-Wallace M. Cervical cancer: screening, management, and prevention. Nurse Pract 2016; 41: 18-23.
5) Park KJ. Cervical adenocarcinoma: integration of HPV status, pattern of invasion, morphology and molecular markers into classification. Histopathology 2020; 76: 112-127.
6) Fang J, Zhang H, Jin S. Epigenetics and cervical cancer: from pathogenesis to therapy. Tumour Biol 2014; 35: 5083-5093.
7) Derbie A, Mekonnen D, Woldeamanuel Y, Van Ostade X, Abebe T. HPV E6/E7 mRNA test for the detection of high grade cervical intraepithelial neoplasia (CIN2+): a systematic review. Infect Agent Cancer 2020; 15: 9.
8) Morelli E, Gulla A, Rocca R, Federico C, Raimondi L, Malvestiti S, Agosti V, Rossi M, Costa G, Giavaresi G, Azab KA, Cagnetta A, Cea M, Tagliaferri P, Neri A, Munshi NC, Viglietto G, Tassone P, Amodio N. The non-coding RNA landscape of plasma cell dyscrasias. Cancers (Basel) 2020; 12: 320.
9) Wang Y, Fang Z, Hong M, Yang D, Xie W. Long-non-coding RNAs (lncRNAs) in drug metabolism and disposition, implications in cancer chemo-resistance. Acta Pharm Sin B 2020; 10: 105-112.
10) Tsagakis I, Douka K, Birds I, Aspden JL. Long non-coding RNAs in development and disease: conservation to mechanisms. J Pathol 2020; 250: 480-495.
11) Peng L, Liu F, Yang J, Liu X, Meng Y, Deng X, Peng C, Tian G, Zhou L. Probing lncRNA-protein interactions: data repositories, models, and algorithms. Front Genet 2019; 10: 1346.
12) Jathar S, Kumar V, Srivastava J, Tripathi V. Technological developments in lncRNA biology. Adv Exp Med Biol 2017; 1008: 283-323.
13) Mishra K, Kanduri C. Understanding long noncoding RNA and chromatin interactions: what we know so far. Noncoding RNA 2019; 5: 54.
14) Sauvageau M. Diverging RNPs: toward understanding lncRNA-protein interactions and functions. Adv Exp Med Biol 2019; 1203: 285-312.
15) Zhang Z, Yang L, Yao X, Yang M, Li G. LncRNA-ZNF281 interacts with miR-539 to promote hepatocellular carcinoma cell invasion and migration. Cancer Biother Radiopharm 2020; 35: 137-142.
16) Li XT, Li JC, Feng M, Zhou YX, Du ZW. Novel lncRNA-ZNF281 regulates cell growth, stemness and invasion of glioma stem-like U251s cells. Neoplasma 2019; 66: 118-127.
17) Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.
18) Sun X, Zheng H, Sui N. Regulation mechanism of long non-coding RNA in plant response to stress. Biochem Biophys Res Commun 2018; 503: 402-407.
19) Wu Z, Liu X, Liu L, Deng H, Zhang J, Xu Q, Cen B, Ji A. Regulation of lncRNA expression. Cell Mol Biol Lett 2014; 19: 561-575.
20) Peng WX, Koirala P, Mo YY. LncRNA-mediated regulation of cell signaling in cancer. Oncogene 2017; 36: 5661-5667.
21) Beltraminelli T, De Palma M. Biology and therapeutic targeting of tumour-associated macrophages. J Pathol 2020; 250: 573-592.
22) Nikolaou S, Machesky LM. The stressful tumour environment drives plasticity of cell migration programmes, contributing to metastasis. J Pathol 2020; 250: 612-623.
23) Yeung KT, Yang J. Epithelial-mesenchymal transition in tumor metastasis. Mol Oncol 2017; 11: 28-39.
24) Pastushenko I, Blanpain C. EMT Transition states during tumor progression and metastasis. Trends Cell Biol 2019; 29: 212-226.