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Verification of expressions of lncRNA FOXCUT in gastric adenocarcinoma patients and its effects on cell biological function based on TCGA database

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Abstract. – OBJECTIVE: This study aimed to investigate expressions of lncRNA FOXCUT in gastric adenocarcinoma patients and its effects on the cell biological function.

PATIENTS AND METHODS: Expressions and survival of lncRNA FOXCUT in gastric adenocarcinoma patients (GA) in the Cancer Genome Atlas (TCGA) database were collected. Fifty patients with GA treated in our hospital (patient group) and another 50 contemporaneous normal people (normal group) were collected. Expressions of lncRNA FOXCUT in GES1, SNU-5, HGC-27, SGC-7901, and AGS cells were detected. Also, si-lncRNA FOXCUT and si-NC sequences were transfected to SGC-7901. Si-RNA and si-NC groups were constructed in AGS cells. QRT-PCR was used to detect expressions of lncRNA FOXCUT in samples. MTT, transwell, and flow cytometry were used to detect the proliferation, invasion, and apoptosis of transfected cells. Patients were followed up for 5 years to observe their survival.

RESULTS: Expressions of lncRNA FOXCUT in cancer tissues of GA patients in TCGA database were significantly increased ($p<0.001$). The survival rate of patients with low expressions of lncRNA FOXCUT was significantly increased ($p=0.017$, $p=0.047$). LncRNA FOXCUT is closely related to patients' tumor diameter, lymph node metastasis, TNM staging, and differentiation degree ($p<0.05$). LncRNA FOXCUT has high clinical value in disease diagnosis. Multivariate Cox regression analysis found that tumor diameter, lymph node metastasis, and lncRNA FOXCUT were independent prognostic factors. Compared with GES1, expressions of lncRNA FOXCUT in GA cells increased significantly ($p<0.05$), the proliferation and invasion ability of si-RNA group decreased significantly ($p<0.05$) compared with si-NC group, and the apoptosis rate of si-RNA group was significantly lower than that of si-NC group ($p<0.05$).

CONCLUSIONS: We showed that the inhibition of the expressions of lncRNA FOXCUT can reduce the proliferation and invasion of GA cells and increase apoptosis, which can be used as a potential therapeutic target for GA.

Key Words:
LncRNA FOXCUT, Gastric adenocarcinoma, TCGA, Biological function.

Introduction

Gastric cancer (GC) is the most common malignant tumor in the world[1]. 2018 global cancer statistics show that[2] there have been more than 1 million new GC patients in the world, with an estimated death of more than 783,000. Its mortality and morbidity are respectively the 5th and 3rd of major cancers, and are more common in men, with a morbidity ratio of 2:1. GC can be divided into adenocarcinoma, adenosquamous carcinoma, squamous carcinoma, carcinoid and so on according to histopathological types, with gastric adenocarcinoma (GA)[3,4] representing the highest proportion. At present, the best clinical treatment for GC is mainly surgical. However, due to the relatively hidden onset of GC, most patients are in the advanced stage of disease after admission and tumor metastasis occurs. The opportunity for surgical treatment has been missed[5]. At present, early diagnosis, comprehensive treatment, and detection of GC disease are clinically lacking molecular markers, which is also one of the important obstacles for the improvement of GC prognosis[6]. Therefore, it is particularly important

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to find a key molecular marker. Researches[7] on the long non-coding RNA (lncRNA) have been investigated. LncRNA is a kind of non-coding single-stranded RNA with a length of more than 200 nt, which can regulate gene expression after transcription. Several studies[8,9] have shown that lncRNA is closely related to tumors. Feng et al[10] found that up-regulating expressions of lncRNA MEG3 can inhibit the migration and invasion of bladder cancer cells and enhance the chemosensitivity of bladder cancer cells to cisplatin. Zhao et al[11] showed that lncRNA-PVT1 can promote proliferation and migration of pancreatic cancer cells by regulating miR-448. FOXCUT is located on chromosome 6p25.3 as a member of lncRNA family. Pan et al[12] found that FOXCUT is closely related to the progress of esophageal cancer, but there are no relevant researches to prove whether there is a link with GC. Therefore, this work verifies the clinical value of FOXCUT in GA and its influences on the biological function of GA cells through the TCGA database to find new potential diagnosis and treatment targets for clinic.

Patients and Methods

Extraction of TCGA Data

The TCGA database (https://portal.gdc.cancer.gov/) was visited, the repositories to enter data browsing and downloading pages were selected, the RNA samples obtained from GA high-throughput sequencing and its corresponding Metadata, Manifest, and Cart files were downloaded, of which 373 were GA samples, 343 were cancer samples and 30 were paraneoplastic samples. The MRNA_merge.pl script was used to synthesize the matrix file, the ensemblToSymbol_incRNA.pl script was used to convert the ID, and FOXCUT expression quantity of all patients was extracted from the matrix file. http://gdac.broadinstitute.org/runs/stddata __2016 _ 01 _ 28/data/STAD/20160128/ was logged in, clinical data of GA patients were downloaded, age, survival time, survival status, missing samples were deleted, samples with survival time less than 1 month were deleted, and 295 cases remained.

Collection of Clinical Data

Fifty GA patients treated in our hospital from March 2013 to May 2014 were collected as patient group in this study, including 35 male patients and 15 female patients, with an average age of 55.0±4.3. Also, 50 normal people who underwent a physical examination in our hospital during the same period were collected as normal group, including 30 male patients and 20 female patients, with an average age of 54.1±4.5. Among them, clinical laboratory indexes and imaging examinations of patients in normal group were normal. This research was approved by the Medical Ethics Committee of our hospital. Inclusion criteria for patients were as follows: patients met the TNM staging criteria issued by the American Joint Committee on Cancer (ACJJ) in 2017[13]. Patients were diagnosed as GA through imaging and pathological examination. Patients and his family were informed of the purpose of the study and signed informed consent. Patients cooperated with the follow-up. Exclusion criteria for patients were as follows: patients' age was less than 18 years old and patients were complicated with other tumors. Needle cancer treatment was carried out before this study. Patients' expected survival time was less than 1 month and patients have congenital liver, kidney, and heart functional defects. There were no statistical differences in gender and age of patients between the two groups ($p>0.05$).

Sources of Reagents, Instruments, and Cells

GES1, SNU-5, HGC-27, SGC-7901, and AGS cells (Beina Biological Company, Beijing, China, BNCC337970, CRL-5973, BNCC100716, BNCC100674, CRL-1739); Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA, 11668019); MTT kit, dimethyl sulfoxide (DMSO) reagent (Beyotime Biotechnology Company, Shanghai, China, C0009, ST038); transwell kit, Roswell Park Memorial Institute-1640 (RPMI-1640), phosphate-buffered saline (PBS), bovine fetal serum (FBS), penicillin streptomycin double-antibody (Gibco, Grand Island, NY, USA, A1142802, 61870044, 14190250, 10437028, 15070063); radioimmunoprecipitation assay (RIPA), bicinchoninic acid (BCA) Protein Kit, enhanced chemiluminescence (ECL) Kit, Trypsin (Thermo Scientific, Austin, TX, USA, 89900, 23250, 35055, 90058); Transscript II Green Two-STEP QRT-PCR Supermix (TransGen Biotech, Beijing, China, AQ202-01, AQ301-01); Annexin V/PI Apoptosis Detection Kit (Shanghai Yisheng Biotechnology Co., Ltd., 40302ES20); Enzyme Labelling Instrument (BioTek Company, Northern VT, USA); PCR Instrument (ABI, Waltham, MA, USA, 7500); Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The primer sequences were synthesized by Shanghai Shenggong Biology Company. More details are shown in Table I.

Table I. FOXCUT, si FOXCUT, and GAPDH primer sequences.

Gene	Upstream primer	Downstream primer
FOXCUT	5'-TCCGATCATCTATCCCTTTACGA-3'	5'-CCCGGCTTCAAAAGACTCA-3'
si FOXCUT	5′-GGGAUUCUGGUCUAAGCAATT-3′	5′-UUGCUUAGACCAGAAUCCCTT-3′
GAPDH	5′-AGGGCTGCTTTTAACTCTGGT-3′	5′-CCCCACTTGATTTTGGAGGGA-3′

Cell Culture and Transfection

Repurchased SNU-5, HGC-27, SGC-7901, AGS cells, and normal gastric mucosa cells GES1 were transferred to RPMI-1640 medium (penicillin-streptomycin double antibody, 10% FBS), and cultured in 37°C, 5% CO_2 incubator. Si-LncRNA FOXCUT (si-RNA) and lncRNA were under negative control (si-NC). Cells were transfected with Lipofectamine™ 2000 kit and operation steps were strictly carried out in accordance with instructions of kit. All primers were transfected into cells with the greatest expression difference.

Methods

Detection of QRT-PCR

Collected cells and serum were extracted with TRIzol kit for total RNA and the extracted total RNA was detected for purity, concentration, and integrity by ultraviolet spectrophotometer and agarose gel electrophoresis. 5X TransScript® II All-in-one SuperMix for qPCR and gDNA Remover kits were used to carry out reverse transcription and operation procedures were in strict accordance with kits of manufacturers. Then, PCR amplification experiment was carried out. The PCR reaction system was as follows: cDNA 1 μL, upstream and downstream primers 0.4 μL each, 2X TransScript® Tip Green qPCR SuperMix 10 μL, Passive Reference Dye (50X). Finally, Nuclease-free Water was added to make up to 20μL. PCR reaction conditions were as follows: 94°C pre-denaturation for 30 s, 94°C denaturation for 5 s, 60°C annealing extension for 30 s, for a total of 40 cycles. Each sample was provided with 3 repeated holes, and the experiment was carried out 3 times. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as internal reference and the $2^{-\Delta\Delta ct}$ method was used to analyze the data.

Detection of Cell Proliferation

Cells harvested 24 h after transfection were collected, and the cell density was adjusted to 3*104 cells/hole. Cells were inoculated on 96-hole plates and incubated at 37°C for 24, 48, 72, and 96 h respectively. 20 μL MTT solution (5 μ mg/ml) was added at each time point. Cells were cultured at 37°C for 4 h and 150μL DMSO was added to each hole. Then, the OD value of each group of cells was measured under 490nm absorbance by an enzyme reader.

Detection of Cell Invasion Ability

Cells transfected for 24 h were collected and the cell density was adjusted to 3*104 cells/hole. Cells were inoculated on 24-hole plates respectively. Trypsin digestion was carried out and transferred to the upper chamber. 200 μL RPMI-1640 culture solution was added to the chamber. 500 mL RPMI-1640 (containing 10% FBS) was added to the lower chamber. The substrate and cells that did not pass through the membrane surface in the upper chamber were wiped off at 37°C for 48 h. PBS was used for washing 3 times, paraformaldehyde was used for fixing for 10min, and double-distilled water was used for washing 3 times. After it was dried, 0.5% crystal violet staining was used to observe the cell invasion with a microscope.

Detection of Cell Apoptosis

Cells transfected for 24 h were digested with 0.25% trypsin, washed twice with PBS after digestion, added with 100 μL of binding buffer, prepared into $1*10^6$/mL suspension, sequentially added with AnnexinV-FITC and propidium iodide (PI), incubated in dark at room temperature for 5 min, detected with FC500MCL flow cytometer system, and the experiment was repeated for 3 times to take the average value.

Follow-Up of Patients

Patients were followed up for 5 years and their survival was recorded by telephones and outpatient medical records. The follow-up time was 3, 6, 9, 12 months a year.

Statistical Analysis

SPSS20.0 software package was used to carry out statistical analysis on the collected data, GraphPad 7 software package was used to draw the required pictures, and K-S test was used to analyze the dose

data distribution. The normal distribution data was expressed by mean±standard deviation (Meas±SD), the comparison between the groups was conducted by independent-samples *t*-test, and data not conforming to the normal distribution were expressed by quartile (Meas (P25-P75). The non-parametric test was used for analysis, expressed as Z, one-way ANOVA was used for comparison among multiple groups, expressed as F, LSD *t*-test was used for post-event two-two comparison, repeated measurement ANOVA was used for multi-time point expression, expressed as *F*, Bonferroni was used for backtesting, ROC was used to plot the diagnostic value of lncRNA FOXCUT in GA, K-M survival curve was used to plot the 5-year survival of patients, Log-rank test was used for analysis, and multivariate Cox regression was used to analyze independent risk factors affecting the prognosis of patients. When $p<0.05$, there were statistical differences.

Results

Relationship Between Expressions of LncRNA FOXCUT in TCGA Database and Survival of Patients

Expressions of lncRNA FOXCUT in GA patients in TCGA database were extracted. Through log (x+1) 2, the analysis found that expressions of lncRNA FOXCUT in cancer tissue were significantly higher than those in paraneoplastic group, with differences ($p<0.001$). Moreover, by extracting the clinical data of patients, patients were divided into high and low expression groups according to the median value of lncRNA FOXCUT, and the survival curve was drawn. It was found that the survival rate of patients in low expression group was significantly higher than that of patients in high expression group, with differences ($p=0.017$), as shown in Figure 1.

Clinical Value of LncRNA FOXCUT in GA Patients

Detection of expressions of lncRNA FOXCUT in the serum of patient group and normal group showed that expressions of lncRNA FOXCUT in patient group were significantly higher than those in normal group ($p<0.001$). Analysis of the relationship between lncRNA FOXCUT and pathological data showed that lncRNA FOXCUT was related to the tumor diameter, lymph node metastasis, and TNM staging of patients. The degree of differentiation was closely related ($p<0.05$). Further drawing ROC curve showed that lncRNA FOXCUT had been followed up for 5 years in disease diagnosis, tumor diameter, lymph node metastasis, TNM staging, and the area under the curve area was respectively 0.958, 0.765, 0.732, 0.859, and 0.826. The total survival rate for 5 years was 36.0%. According to the median value of lncRNA FOXCUT, the 5-year survival rate of patients in low expressiaon group was significantly higher than that in high expression group ($p=0.047$). Multivariate Cox regression analysis found that tumor diameter, lymph node metastasis, and lncRNA FOXCUT were independent prognostic factors. See Figure 2 and Tables II, III and IV for details.

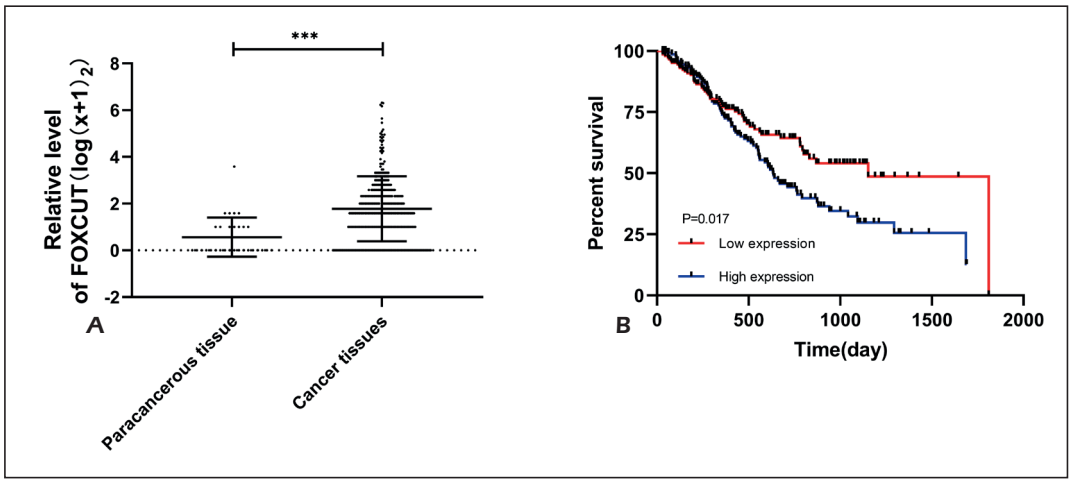

Figure 1. Expressions and survival analysis of lncRNA FOXCUT in patients with cancer tissues and adjacent tissues. ***A***, Expressions of LncRNA FOXCUT in cancer tissues were significantly higher than those in adjacent tissues. ***B***, The survival condition of patients in low expression group was higher than that in high expression group. *** indicates that $p<0.001$.

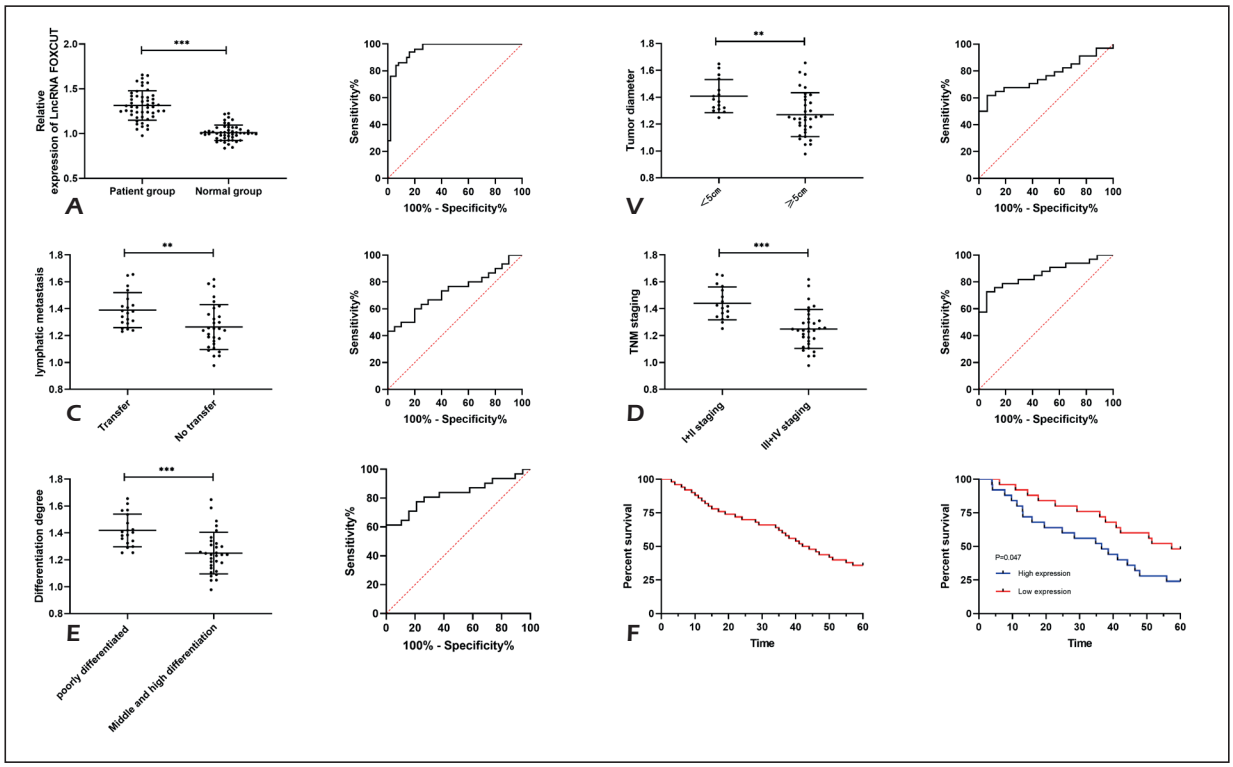

Figure 2. Clinical value of lncRNA FOXCUT in GA patients. ***A***, Expressions and Diagnostic Value of LncRNA FOXCUT in GA Patients. ***B***, Expressions and Diagnostic Value of LncRNA FOXCUT in Tumor Diameter of GA Patients. ***C***, Expressions and Diagnostic Value of LncRNA FOXCUT in Lymph Node Metastasis of GA Patients. ***D***, Expressions and Diagnostic Value of LncRNA FOXCUT in TNM Staging of GA Patients. ***E***, Expressions and Diagnostic Value of LncRNA FOXCUT in Differentiation Degree of GA Patients. ***F***, Patients' 5-year Survival and the Relationship Between High and Low Expressions of LncRNA FOXCUT and Patients' Survival. **indicates that $p<0.01$, and ***indicates that $p<0.001$.

Expressions of Lnc RNA FOXCUT in Cells and Its Effects on Cell Biological Function

By detecting the relative expressions of lncRNA FOXCUT of cells in each group, it was found that expressions of lncRNA FOXCUT in SNU-5, HGC-27, SGC-7901, and AGS cells were significantly increased ($p<0.05$) compared with GES1. AGS and SGC-7901 cells were selected for transfection. Detection showed that expressions of lncRNA FOXCUT in si-RNA group of AGS and SGC-7901 cells were significantly lower than

Table II. The Relationship between lncRNA FOXCUT and pathological treatment of patients.

Factor		Relative expressions of LncRNA FOXCUT	*T*-value	*p*-value
Gender	Male (n=35)	1.332±0.158	0.497	0.622
	Female (n=15)	1.307±0.168		
Age	< 55 years old (n=22)	1.327±0.178	0.484	0.631
	≥ 55 years old (n=28)	1.304±0.154		
Tumor diameter	<5 cm (n=34)	1.270±0.164	3.001	0.004
	≥5 cm (n=16)	1.408±0.123		
Lymph node metastasis	Transfer (n=20)	1.264±0.167	2.850	0.006+
	Not transferred (n=30)	1.390±0.130		
TNM staging	I+II (n=33)	1.249±0.145	4.638	<0.001
	III+IV (n=17)	1.440±0.122		
Degree of differentiation	Low differentiation (n=19)	1.419±0.122	4.048	<0.001
	Medium+High Differentiation (n=31)	1.250±0.155		

Table III. ROC parameters.

Parameters	AG diagnosis	Tumor diameter	Lymph node metastasis	TNM staging	Degree of differentiation
AUC	0.958	0.765	0.732	0.859	0.826
Std. Error	0.019	0.065	0.070	0.051	0.058
95CI%	0.921-0.994	0.636-0.893	0.595-0.869	0.758-0.960	0.715-0.942
Specificity	92.00%	93.75%	100.00%	94.12%	100.00%
Sensitivity	86.00%	61.76%	43.33%	72.72%	61.29%
Youden index	78.00%	55.51%	43.33%	66.84%	61.29%
Cut-off	< 1.154	< 1.274	< 1.221	< 1.299	< 1.252

those in si-NC group ($p<0.05$). MTT experiment showed that the proliferation ability of si-RNA group of AGS and SGC-7901 cells was significantly lower than that in si-NC group ($p<0.05$). Transwell experiment showed that the cell invasion rate of si-RNA group of AGS and SGC-7901 cells was significantly lower than that of si-NC group ($p<0.05$), and flow cytometry experiment showed that the apoptosis rate of si-RNA group of AGS and SGC-7901 cells was significantly lower than that of si-NC group ($p<0.05$), as shown in Figure 3.

Discussion

GA, as a common malignant tumor in clinic, is a heterogeneous and multifactorial cancer with poor prognosis[14]. In 2015, China's cancer statistics showed that[15] GC had 679,000 new patients and 498,000 dead patients. The morbidity and mortality were second only to lung cancer. Monitoring the global cancer survival trend from 2000 to 2014 by Allemani et al[16] found that the 5-year survival rate of GA has increased from 30.2% to 35.9% in the past ten years. Although it has been improved, it is far from clinically expected effects. Since GA is difficult to detect, the initial stage of the disease is similar to other stomach diseases, and there is a lack of highly specific diagnostic markers in clinical practice, it is of great significance to find an effective molecular marker for the diagnosis and prognosis of GA.

In this report, we chose lncRNA FOXCUT for research. LncRNA FOXCUT is a long-chain non-coding RNA. Previous studies[17,18] showed that lncRNA FOXCUT was closely related to nasopharyngeal carcinoma and oral squamous cell carcinoma, and in a literature review by Hou et al[19], lncRNA FOXCUT was up-regulated in esophageal carcinoma and was closely related to differentiation and metastasis. Esophageal cancer and GA belong to digestive tract tumor. There are no related researches on whether lncRNA FOXCUT has the same effect in GA. Therefore, in this work, we first analyzed expressions of lncRNA FOXCUT in GA patients through the TCAG database. Results showed that expressions of lncRNA FOXCUT in GA patients were significantly higher than those in paraneoplastic group, which indicated that lncRNA FOXCUT might become a potential diagnostic and therapeutic target for GA. For this reason, we further carried out tests in clinical patients. In this study, we chose serum

Table IV. Cox regression analysis.

Factor	Single factor			Multi-factor		
	HR	*p*-value	HR (95CI%)	HR	*p*-value	HR (95CI%)
Gender (male vs. female)	0.758	0.458	0.365-1.575			
Age (< 55 years vs. ≥55 years)	0.509	0.071	0.245-1.058			
Tumor diameter (< 5 cm vs. ≥ 5 cm)	5.174	0.002	1.798-14.891	5.539	0.003	1.796-17.082
Lymph node metastasis (metastasis vs. non-metastasis)	3.510	0.000	1.731-7.115	5.649	<0.001	2.405-13.268
TNM staging (I+II SV III+IV)	0.382	0.008	0.188-0.777	0.573	0.166	0.260-1.260
Degree of differentiation (low vs. medium+high)	3.705	0.000	1.787-7.681	1.131	0.782	0.473-2.708
LncRNA FOXCUT (High vs. Low)	6.951	0.000	3.116-15.507	9.315	<0.001	3.108-27.914

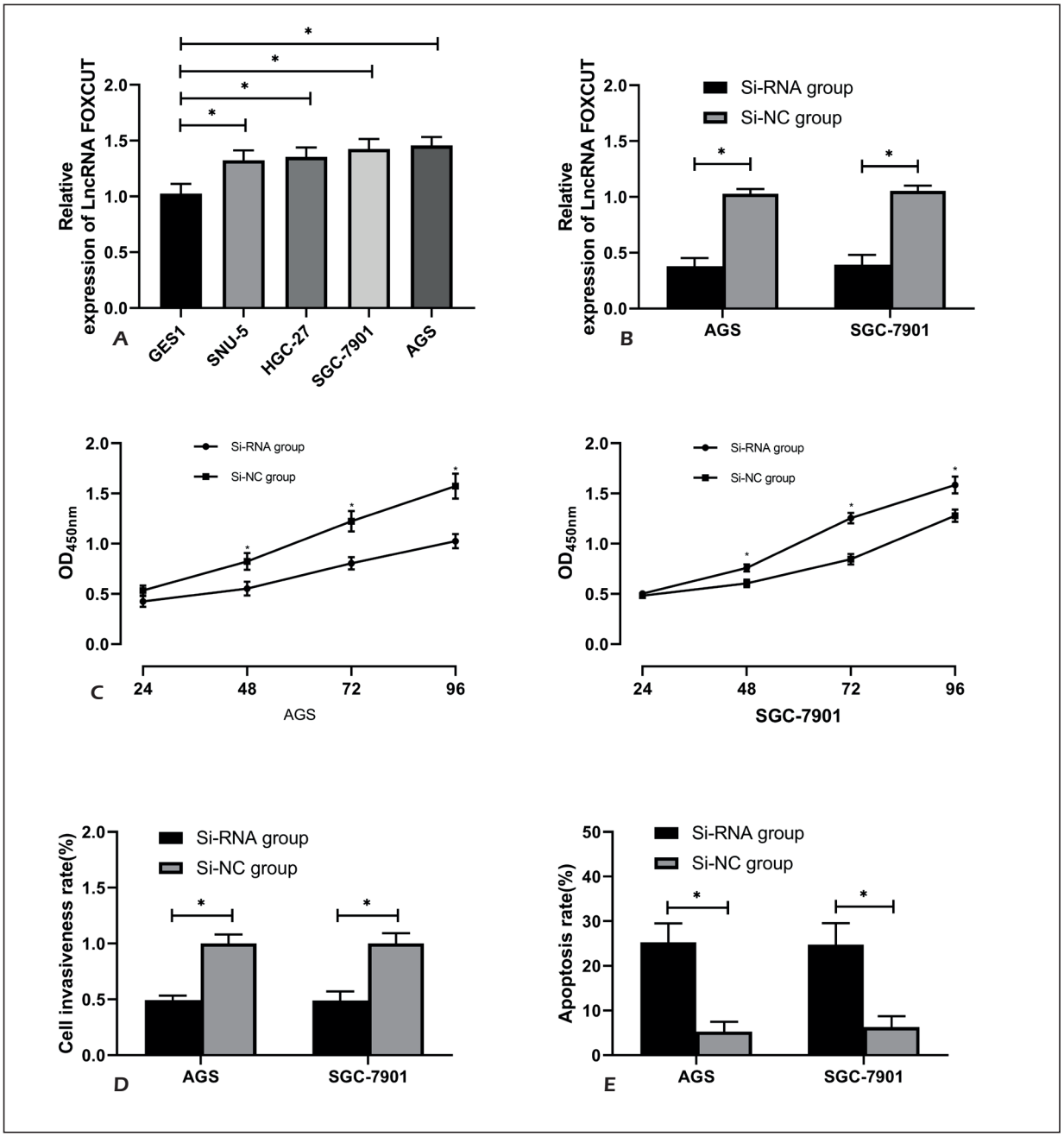

Figure 3. Expressions of lncRNA FOXCUT in cells and their effects on cell biological function. ***A***, Expressions of lncRNA FOXCUT in cells of each group. ***B***, Expressions of AGS and SGC-7901 cells after transfer inhibition primer. ***C***, Proliferation ability of transfected AGS and SGC-7901 cells. ***D***, Detection of invasiveness of transfected AGS and SGC-7901 cells. ***E***, Detection of apoptosis of AGS and SGC-7901 cells after transfection. *indicates that $p<0.05$.

as the test sample, mainly considering that it is easier to obtain than pathological tissues and has less invasive injury. By detecting expressions of lncRNA FOXCUT in serum of normal group and patient group and in GC cells and normal gastric tissue, it was found that expressions of lncRNA FOXCUT in serum of patient group were significantly higher than those in normal group, and by detecting expressions of lncRNA FOXCUT in GC cells were significantly higher than those in normal gastric tissue, which are consistent with database data. ROC curve analysis showed that the area under the lncRNA FOXCUT curve was 0.958, with high specificity and sensitivity, which

was a potential clinical diagnostic index for GA. Furthermore, we also analyzed the relationship between lncRNA FOXCUT and pathological data of GA patients, and found that lncRNA FOXCUT expression in GA patients is closely related to tumor diameter (≥5 cm), lymph node metastasis (metastasis), TNM stage (III+IV), and differentiation degree (low differentiation). Therefore, we further proved that lncRNA FOXCUT has good diagnostic value in tumor diameter, lymph node metastasis, TNM stage, and differentiation degree by drawing ROC curve, and is expected to become a potential molecular marker in the clinical diagnosis of GA. In the end, we also carried out a 5-year follow-up visit to patients, and the 5-year overall survival rate of patients was 36.0%, which was basically in line with relevant foreign literature reports[20,21]. According to the median value of lncRNA FOXCUT expression, patients were divided into high and low expression groups to analyze the relationship between lncRNA FOXCUT and the 5-year survival of patients. It was found that the 5-year survival rate of patients in high expression group was significantly lower than that in low expression group. We have preliminarily proved the clinical value of lncRNA FOXCUT through the above research.

LncRNA has relatively little research on short-chain and medium-chain non-coding RNA. At present, there are more and more researches on lncRNA and GA, such as HOTAIR, H19, etc.[22,23]. The relevant mechanism between lncRNA and GA has been partially proved. However, the biological function of lncRNA FOXCUT in GA cells is still unclear. In this study, we chose to inhibit expressions of lncRNA FOXCUT in AGS and SGC-7901 gastric adenocarcinoma cells to observe its effects on GA cells. Results showed that the proliferation and invasion ability of AGS and SGC-7901 cells transfected with si-RNA sequence were significantly inhibited, and the apoptosis rate was significantly increased, which indicated that lncRNA FOXCUT was expected to be a potential target for GA therapy.

However, we still have certain limitations in this study. First of all, we have only preliminarily studied effects of inhibition of lncRNA FOXCUTGA on the biological function of GA cells. It is not clear about its specific mechanism, through which channels, whether changes in cell biological function are regulated by target genes and related proteins. Second, we have not conducted nude mice tumor-forming experiments. It is not clear how much concentration of lncRNA FOXCUT can achieve the best tumor-inhibiting effect, and if it can become a potential GA target indicator of GA needs further verification. Therefore, we hope that in future research, we can further construct its relationship map through the ceRNA network, predict and verify its target genes, carry out animal experiments, and further supplement our research results.

Conclusions

We showed that inhibiting lncRNA FOXCUT expressions can reduce GA cell proliferation and invasion and increase cell apoptosis. Also, it can be used as a potential diagnostic index and therapeutic target for GA.

Conflict of Interests

The Authors declared that they have no conflict of interests.

References

1) Sitarz R, Skierucha M, Mielko J, Offerhaus GJA, Maciejewski R, Polkowski WP. Gastric cancer: epidemiology, prevention, classification, and treatment. Cancer Manag Res 2018; 10: 239-248.
2) Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
3) Ajani JA, Lee J, Sano T, Janjigian YY, Fan D, Song S. Gastric adenocarcinoma. Nat Rev Dis Primers 2017; 3: 17036.
4) Akce M, Jiang R, Alese OB, Shaib WL, Wu C, Behera M, El-Rayes BF. Gastric squamous cell carcinoma and gastric adenosquamous carcinoma, clinical features and outcomes of rare clinical entities: a National Cancer Database (NCDB) analysis. J Gastrointest Oncol 2019; 10: 85-94.
5) Wagner AD, Syn NL, Moehler M, Grothe W, Yong WP, Tai BC, Ho J, Unverzagt S. Chemotherapy for advanced gastric cancer. Cochrane Database Syst Rev 2017; 8: CD004064.
6) Shin VY, Ng EK, Chan VW, Kwong A, Chu KM. A three-miRNA signature as promising non-invasive diagnostic marker for gastric cancer. Mol Cancer 2015; 14: 202.
7) Engreitz JM, Haines JE, Perez EM, Munson G, Chen J, Kane M, McDonel PE, Guttman M, Lander E. Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature 2016; 539: 452-455.
8) Yao X M, Tang J H, Zhu H, Jing Y. High expression of LncRNA CASC15 is a risk factor for gastric cancer prognosis and promote the proliferation of gastric cancer. Eur Rev Med Pharmacol Sci 2017; 21: 5661-5667.

9) Liu X J, Li S L, Li J S, Lu H, Yin LL, Zheng W F, Wang W C. Long non-coding RNA ZEB1-AS1 is associated with poor prognosis in gastric cancer and promotes cancer cell metastasis. Eur Rev Med Pharmacol Sci 2018; 22: 2624-2630.
10) Feng SQ, Zhang XY, Fan HT, Sun QJ, Zhang M. Up-regulation of LncRNA MEG3 inhibits cell migration and invasion and enhances cisplatin chemosensitivity in bladder cancer cells. Neoplasma 2018; 65: 925-932.
11) Zhao L, Kong H, Sun H, Chen Z, Chen B, Zhou M. LncRNA-PVT1 promotes pancreatic cancer cells proliferation and migration through acting as a molecular sponge to regulate miR-448. J Cell Physiol 2018; 233: 4044-4055.
12) Pan F, Yao J, Chen Y, Zhou C, Geng P, Mao H, Fang X. A novel long non-coding RNA FOXCUT and mRNA FOXC1 pair promote progression and predict poor prognosis in esophageal squamous cell carcinoma. Int J Clin Exp Pathol 2014; 7: 2838-2849.
13) Kim SG, Seo HS, Lee HH, Song KY, Park CH. Comparison of the differences in survival rates between the 7th and 8th Editions of the AJCC TNM Staging System for Gastric Adenocarcinoma: a single-institution study of 5,507 patients in Korea. J Gastric Cancer 2017; 17: 212-219.
14) Tan P, Yeoh KG. Genetics and molecular pathogenesis of gastric adenocarcinoma. Gastroenterology 2015; 149: 1153-1162.
15) Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ7,8, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.
16) Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, NikšiĐ M, Bonaventure A, Valkov M, Johnson CJ, Estève J, Ogunbiyi OJ, Azevedo E Silva G, Chen WQ, Eser S, Engholm G, Stiller CA, Monnereau A, Woods RR, Visser O, Lim GH, Aitken J, Weir HK, Coleman MP; CONCORD WORKING GROUP. Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 2018; 391: 1023-1075.
17) Kong XP, Yao J, Luo W, Feng FK, Ma JT, Ren YP, Wang DL, Bu RF. The expression and functional role of a FOXC1 related mRNA-lncRNA pair in oral squamous cell carcinoma. Mol Cell Biochem 2014; 394: 177-186.
18) Guo H, Huang S, Li S, Yu H, Wu S, Zhou X. Prognostic significance of the long noncoding RNAs in nasopharyngeal carcinoma: a systematic review and meta-analysis. Cancer Manag Res 2018; 10: 1763-1779.
19) Hou X, Wen J, Ren Z, Zhang G. Non-coding RNAs: new biomarkers and therapeutic targets for esophageal cancer. Oncotarget 2017; 8: 43571-43578.
20) Katai H, Ishikawa T, Akazawa K, Isobe Y, Miyashiro I, Oda I, Tsujitani S, Ono H, Tanabe S, Fukagawa T, Nunobe S, Kakeji Y, Nashimoto A; Registration Committee of the Japanese Gastric Cancer Association. Five-year survival analysis of surgically resected gastric cancer cases in Japan: a retrospective analysis of more than 100,000 patients from the nationwide registry of the Japanese Gastric Cancer Association (2001-2007). Gastric Cancer 2018; 21: 144-154.
21) Jung KW, Won YJ, Kong HJ, Oh CM, Cho H, Lee DH, Lee KH. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2012. Cancer Res Treat 2015; 47: 127-141.
22) Zhao W, Dong S, Duan B, Chen P, Shi L, Gao H, Qi H. HOTAIR is a predictive and prognostic biomarker for patients with advanced gastric adenocarcinoma receiving fluorouracil and platinum combination chemotherapy. Am J Transl Res 2015; 7: 1295-1302.
23) Zhou X, Yin C, Dang Y, Ye F, Zhang G. Identification of the long non-coding RNA H19 in plasma as a novel biomarker for diagnosis of gastric cancer. Sci Rep 2015; 5: 11516.