European Review for Medical and Pharmacological Sciences 2020; 24: 1168-1176

LncRNA CHRF promotes cell invasion and migration *via* EMT in gastric cancer

J. GONG[1], Y. WANG[2], C. SHU[1]

[1]Department of Internal Medicine-Oncology, Huangshi Central Hospital, Pu'ai District, Affiliated Hospital of Hubei Polytechnic University, Edong Healthcare Group, Huangshi, China
[2]Department of Radiation Therapy Center, Huangshi Central Hospital, Pu'ai District, Affiliated Hospital of Hubei Polytechnic University, Edong Healthcare Group, Huangshi, China

Abstract. – OBJECTIVE: Long non-coding RNAs (lncRNAs) have been verified to involve in the development and progression of gastric cancer (GC). However, the expression of lncRNA CHRF level in GC has not been mentioned before. Here, we focused on the function of lncRNA CHRF played in GC.

PATIENTS AND METHODS: A total of 103 GC tissues and paired para-tumor tissues from GC patients were collected. The quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was applied to measure the lncRNA CHRF level in these samples and GC cell lines. The Wound-healing experiment, transwell assay, and Matrigel assay were employed to study the migration and invasion abilities of GC cells. The underlying molecular of lncRNA CHRF was measured using Western-blot.

RESULTS: LncRNA CHRF expression was significantly higher in 103 GC tissue samples compared with the adjacent para-tumor samples. In GC cells, lncRNA CHRF showed increased expression levels than the human fetal gastric epithelial cells (GES-1). Inhibition of lncRNA CHRF reduced the invasion and migration of MKN-7 cells while the over-expression of lncRNA CHRF promoted HGC-27 cells metastasis. Furthermore, we found that lncRNA CHRF could promote the progression of epithelial-mesenchymal transition (EMT) to promote the GC cell metastasis.

CONCLUSIONS: Our current study demonstrated that lncRNA CHRF functioned as an oncogene in GC and promoted cell invasion and migration *via* EMT. This might furnish a potential target for the GC biological diagnosis and therapy.

Key Words:
LncRNA CHRF, EMT, Gastric cancer, Invasion, Migration.

Introduction

Gastric cancer (GC), with a high incidence rate, remains one of the most common malignant tumors in the digestive system[1]. In recent years, with the improvement of treatment conditions, including surgical treatment, chemotherapy, and radiation therapy, the mortality of GC has significantly decreased. Furthermore, the targeted molecular drugs are also used as a new treatment method in the treatment of GC[2,3]. Although the medical technologies continue to advance, the 5-year survival rate of GC still remains low. Metastasis, drug resistance, and recurrence of GC are the main factors affecting the therapeutic effect of GC, so it is crucial to explore the mechanism of the GC development and progression.

Long non-coding RNA (lncRNA), as a type of RNA without protein-coding function, was over 200 nt long. It was recognized as a noisy transcription before[4]. However, in a few decades, reports[5] have proved that lncRNAs could participate in the regulation of cell progression, including proliferation, angiogenesis, cell distribution, apoptosis, invasion, and migration. In tumors, lncRNAs could function as oncogenes or tumor suppressors. Particularly, lncRNA ANRIL promotes cell growth but inhibits cell apoptosis by inhibiting KLF2 and P21 expression in non-small cell lung cancer[6]. Moreover, lncRNA CCAT1 accelerates the gallbladder cancer progression *via* negative regulation of miRNA-218-5p[7]. In addition, lncRNA UPAT inhibits UHRF1 degradation to promote colon cancer tumorigenesis[8]. However, lncRNA FER1L4, acting as a competing endogenous RNA, suppresses cancer cell proliferation *via* promoting the PTEN expression[9]. In breast cancer, LINC01355 suppresses cell growth by transcriptional repression of CCND1, which is mediated by FOXO3[10]. In GC, lncRNA GClnc1 could promote tumorigenesis and it might function as a scaffold for KAT2A and WDR5

Corresponding Author: Chang Shu, MM; e-mail: 81044964@qq.com

complexes, which regulate the histone modification pattern[11]. The upregulation of lncRNA GMAN is correlated with GC metastasis *via* competitively binding GMAN-AS to regulate the translation of Ephrin A1[12]. In addition, lncRNA HOXC-AS3 mediates GC tumorigenesis by binding to YBX1[13]. LINC01133 sponging miR-106a-3p inhibits GC progression by regulating the APC expression level and the Wnt/β-catenin axis, while lncRNA MT1JP binding to miR-92a-3p, slows down the GC development by regulating FBXW7[14,15]. However, the expression and role of lncRNA CHRF played in GC has not been mentioned before.

Here, we collected 103 paired GC tissue and para-tumor tissue samples, and then, detected the expression of lncRNA CHRF. The relative expression of lncRNA CHRF in GC tissues with lymph node metastasis to GC tissues without lymph node metastasis was also analyzed. Also, the expression of lncRNA CHRF in GC cell lines was compared to the human fetal gastric epithelial cell line GES-1. By using siRNA and lentivirus, we inhibited lncRNA CHRF expression in MKN-7 cells while overexpressed lncRNA CHRF level in HGC-27 cells. The abilities of the cell invasion and migration of the experimental MKN-7 and HGC-27 cells were displayed by using the Wound-healing and transwell assays. Furthermore, several EMT markers, including E-cadherin, Vimentin, and N-cadherin were confirmed to explain the role of lncRNA CHRF in EMT of GC cells. Taken all together, we expounded that lncRNA CHRF was overexpressed in GC tissues and cells, and promoted cell invasion and metastasis *via* EMT. Our study might provide a target for further biotherapy and diagnosis for GC.

Patients and Methods

Clinical Tissues

The GC and corresponding paracancerous specimens used were from GC patients hospitalized in the Department of Thoracic Surgery, Huangshi Central Hospital from 2014 to 2017, including 71 males and 32 females, aged 41-74 years, with a median age of 59.3 years. They received no radiotherapy or chemotherapy before the surgery. The study obtained the informed consent from the patients. This study was approved by the Ethics Committee of Huangshi Central Hospital.

Cell Lines and Maintain

Human gastric cancer-derived cell lines (HGC-27, BGC-823, SGC-7901, MKN-45, and MKN-7), and human fetal gastric epithelial cell line GES-1 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; HyClone, South Logan, UT, USA). The medium contained 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA) and 1% Penicillin-Streptomycin Solution (Gibco, Rockville, MD, USA). The cells were maintained in a humidified air incubator containing 5% carbon dioxide at 37°C.

Cell Transfection

MKN-7 and HGC-27 cells were placed in 6-well plates (5×10^4/well) and cultured for 24 h. siRNA-lncRNA CHRF and the corresponding control empty vector siRNA-NC were transfected with Lipofectamine 3000 transfection reagent (Invitrogen, Carlsbad, CA, USA), and LV-lncRNA CHRF and corresponding empty vector LV-Control were transfected with polybrene (Hanbio, Shanghai, China). The transfection operation was carried out according to the manufacturers' protocols.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted from the tissue samples by TRIzol reagent (Beyotime, Nanjing, China) and reverse transcribed into cDNA using the reverse transcription kit from TaKaRa (Otsu, Shiga, China). The CHRF gene was amplified using SYBR Kit (TaKaRa, Otsu, Shiga, China) with ABI 7500 (Applied Biosystems, Foster City, CA, USA) according to manufacturers' instructions. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control gene. The primer sequence of lncRNA CHRF is: forward: 5'-CTCCAAGCAGGGGTCATCAG-3', reverse: 5'-GCCTGCCTTACTGTGCTCTT-3'. The primer sequence of GAPDH is: forward: 5'-TGTGGGCATCAATGGATTTGG-3', reverse: 5'-ACACCATGTATTCCGGGTCAAT-3'. The relative expression level was analyzed by comparing the Ct value using the $2^{-\Delta\Delta Ct}$ method.

Transwell and Matrigel Assay

The transwell assay for cell invasiveness: MKN-7 or HGC-27 cells in the logarithmic growth phase were routinely digested, cen-

trifuged, and resuspended in the serum-free DMEM medium (a density of 2 x 10^5/mL). A total of 100 μL of the cell suspension was added to the upper chamber of the Transwell chamber (Millipore, Billerica, MA, USA) in which the Matrigel gel (BD Biosciences, San Jose, CA, USA) was pre-packaged, and 600 μL of DMEM medium containing 10% FBS was added into the lower chamber. After incubating at 37°C for 36 h, the chamber was removed, washed with PBS, and fixed with 4% paraformaldehyde for 30 min. After air-drying, the chamber was stained in 0.1% crystal violet for 30 min. The cells that did not pass through the upper surface of the chamber membrane were gently wiped off using the cotton swabs. Five high-power microscope fields were randomly selected under the microscope (Olympus, Tokyo, Japan), the number of the transmembrane cells was counted, and the average value was taken as the result. For cell migration, the top chamber was covered with nothing, and the other steps were the same as the invasiveness assay.

Western Blot

Western blot was used to detect the EMT-related indicators. The experimental cells were cultured for 48 h. The total protein sample from the cells was extracted using the radioimmunoprecipitation assay (RIPA) reagent (Beyotime, Shanghai, China). A total of 20 μg protein was electrophoresed using an 8% polyacrylamide gel (Beyotime, Shanghai, China), and transferred to the polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After the transfer was completed, 5% skim milk powder was used for blocking at room temperature for 2 h. The mouse anti-human N-cadherin antibody (1:1000), mouse anti-human E-cadherin antibody (1:1000), mouse anti-human Vimentin antibody (1:1000), and mouse anti-human GAPDH antibody (1:1000) were applied to immerse the membrane overnight at 4°C. Then, after being washed 3 times with Phosphate-Buffered Saline-Tween (PBS-T), the membrane was maintained in Horse Radish Peroxidase (HPR)-labeled mouse anti-mouse lgG secondary antibody for 1 to 2 h at room temperature. After the incubation of the secondary antibody was completed, the enhanced chemiluminescence (ECL) kit (Millipore, Billerica, MA, USA) was used to analyze the band, and the ratio of the absorbance value of the specific protein band to the internal reference protein band was calculated.

Statistical Analysis

Each set of experiments was repeated 5 times. The data were processed using the Statistical Product and Service Solutions (SPSS) 21.0 statistical software (IBM Corp, Armonk, NY, USA). The *t*-test was used to analyze the measurement data. The differences between the two groups were analyzed using the Student's *t*-test. The comparison between multiple groups was made using the One-way ANOVA test followed by the post-hoc test (Least Significant Difference). The comparative analysis of cell migration and invasion ability was performed by the One-way analysis. $p<0.05$ was considered statistically significant.

Results

LncRNA CHRF Was Overexpressed in GC Tissues, Especially Tissues with Lymph Node Metastasis

To study the expression level of lncRNA CHRF in GC, we collected 103 paired GC tissues and para-tumor tissues and measured the lncRNA CHRF expression level using qRT-PCR. As shown in Figure 1A, the expression of lncRNA CHRF in GC tissue samples was significantly higher than that in para-tumor tissue samples with a 2.608 folds elevation ($p<0.001$). Furthermore, we next measured the expression of lncRNA CHRF in 66 GC tissue samples without lymph node metastasis compared with 37 samples with lymph node metastasis and found that the expression of lncRNA CHRF in tissues with lymph node metastasis was markedly increased than in the group without lymph node metastasis (4.17 to 3.103, $p<0.01$) (Figure 1B). These results suggested that lncRNA CHRF might act as a promoter of GC progression.

LncRNA CHRF is Expressed Higher in GC-Derived Cell Lines

Next, we cultured 5 GC-derived cell lines, including HGC-27, BGC-823, SGC-7901, MKN-45, and MKN-7, and human fetal gastric epithelial cell line GES-1. The expression of lncRNA CHRF in these 6 cell lines was detected with qRT-PCR. All 5 GC cell lines performed elevated lncRNA CHRF expression level than the GES-1 cells (Figure 2A). This indicated that lncRNA CHRF was overexpressed in GC cells, as well as in GC tissues, and function as an oncogene. For further exploring the function of lncRNA CHRF

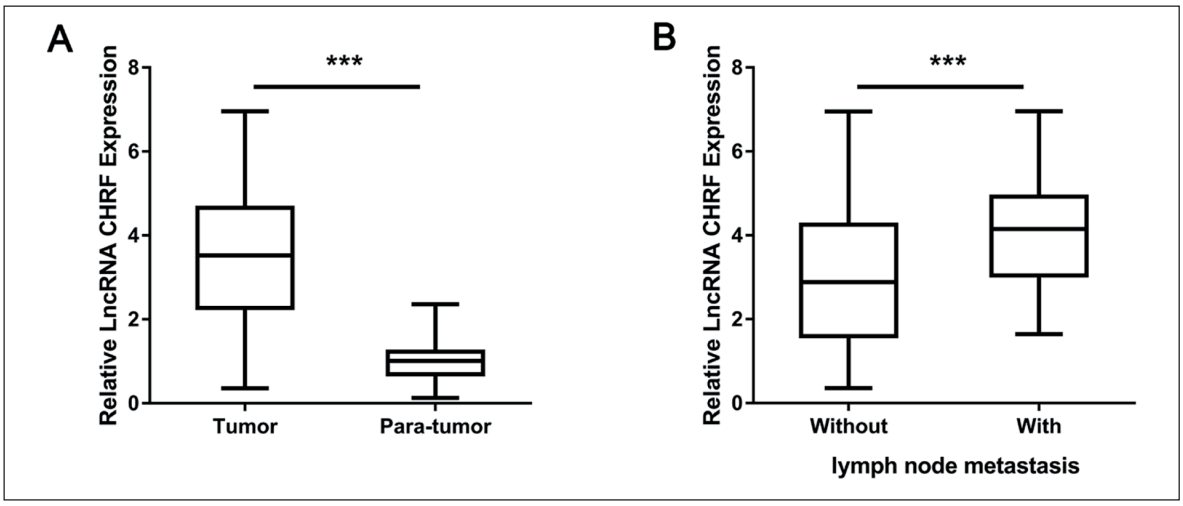

Figure 1. LncRNA CHRF was high-expressed in gastric cancer (GC) tissues. **A,** QRT-PCR showed the lncRNA CHRF expression level in a total of 103 PCa tissues and adjacent para-tumor tissues. **B,** QRT-PCR indicated the lncRNA CHRF expression level in a total of in 66 GC tissues without lymph node metastasis compared with 37 samples with lymph node metastasis. ***p<0.001, compared to control group.

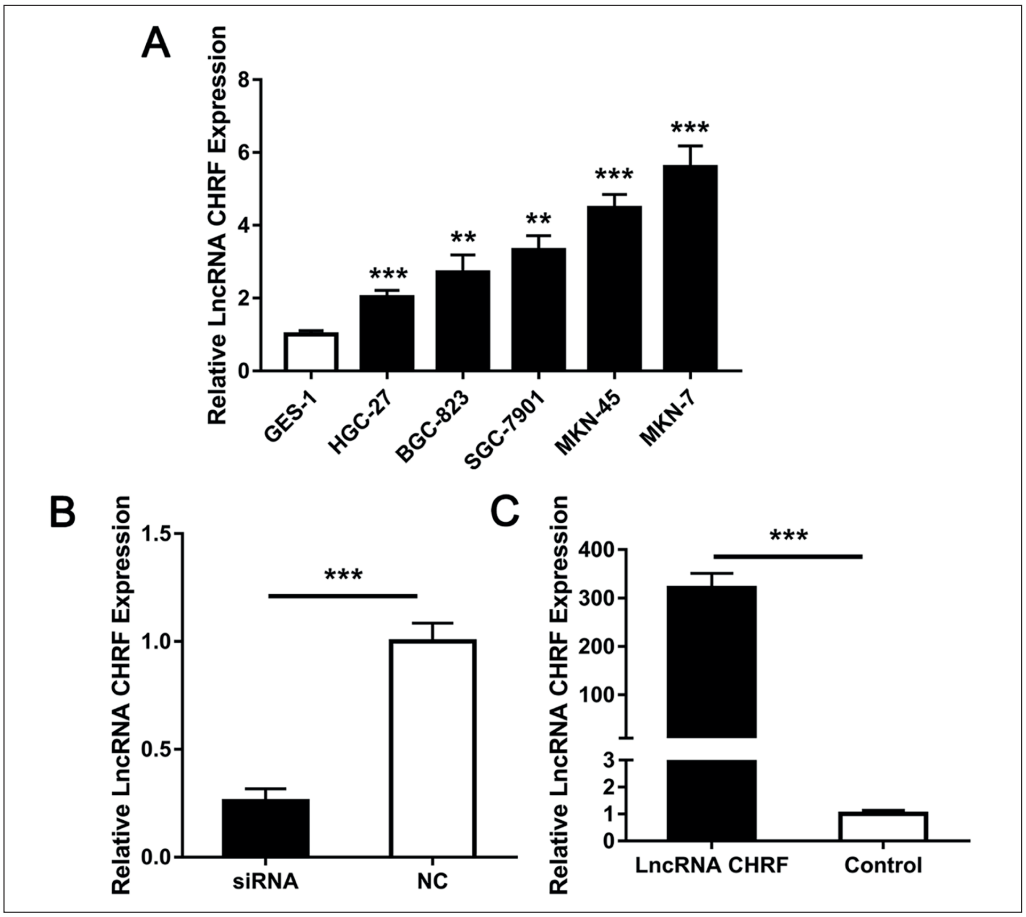

Figure 2. LncRNA CHRF was highly-expressed in gastric cancer (GC) cells. **A,** LncRNA CHRF expression level in GC cell linesHGC-27, BGC-823, SGC-7901, MKN-45, and MKN-7, and human fetal gastric epithelial cell line GES-1. **B,** SiRNA targeting LncRNA CHRF (siRNA) and negative controls (siRNA-NC) were transfected into MKN-7 cells. **C,** LV-CHRF and LV-Control were transfected into HGC-27 cells. ***p<0.001, **p<0.01 *p<0.05 compared to the control group.

in GC cells, we inhibited lncRNA CHRF expression using siRNA in MKN-7 cells and overexpressed it using lentivirus in HGC-27 cells. The expression of lncRNA CHRF in siRNA-lncRNA CHRF treated cells was significantly decreased than in the NC group, while in LV-lncRNA CHRF transfected cells it increased compared to the control group (Figures 2B, 2C).

Aberrant LncRNA CHRF Expression Affected Cell Migration and Invasion of GC Cells

To evaluate the influence of lncRNA CHRF in cell migration and invasion, we employed the Wound-healing assay and transwell assay. After siRNA treatment, the Wound-healing rate of MKN-7 cells was significantly lower than the NC group (Figures 3A, 3B). By contrast, HGC-27 cells over-expressed lncRNA CHRF showed an increased Wound-healing ability than the control group (Figures 3C, 3D). Also, the transwell Matrigel assay displayed that the inhibition of lncRNA CHRF restrained the MKN-7 cell invasion ability while the overexpression of lncRNA CHRF promoted HGC-27 cell invasion (Figure 4A-4C). Analogously, the transwell assay confirmed that siRNA slowed down the migration of MKN-7 cells, but LV-lncRNA CHRF accelerated HGC-27 cell migration, which was similar to the results of the Wound-healing assay. All these data suggested that lncRNA CHRF could promote cell migration and invasion of GC cells.

LncRNA CHRF Promoted GC Cell Metastasis Via EMT

EMT has been identified to be an important biological progression in promoting tumor metastasis. Also, the progression of EMT could be promoted by lncRNA CHRF in colorectal cancer and prostate cancer. Here, we measured several EMT markers, including N-cadherin, E-cadherin, and Vimentin in the established MKN-7 and HGC-27 cells. Clearly, an evident increase of the E-cadherin but a significant decrease of N-cadherin

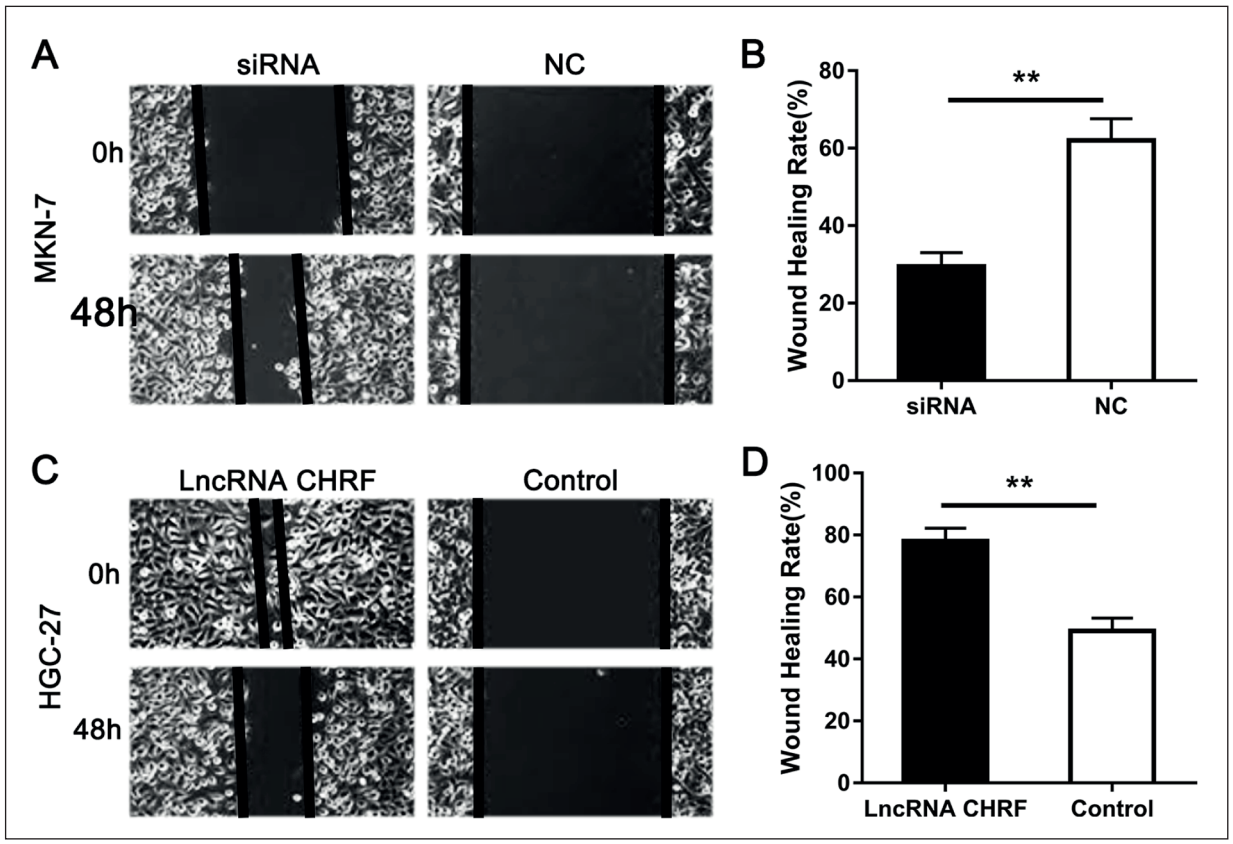

Figure 3. LncRNA CHRF affected the wound-healing ability of PCa cells. **A, B,** Wound-healing assay was used to detect the migration ability of siRNA treated MKN-7 cells compared with the siRNA-NC group (magnification: 10×). **C, D,** Wound-healing assay was used to detect the migration ability of LV-CHRF treated HGC-27 cells compared with the Control group (magnification: 10×). $^{**}p<0.01$, $^{*}p<0.05$ compared to the control group.

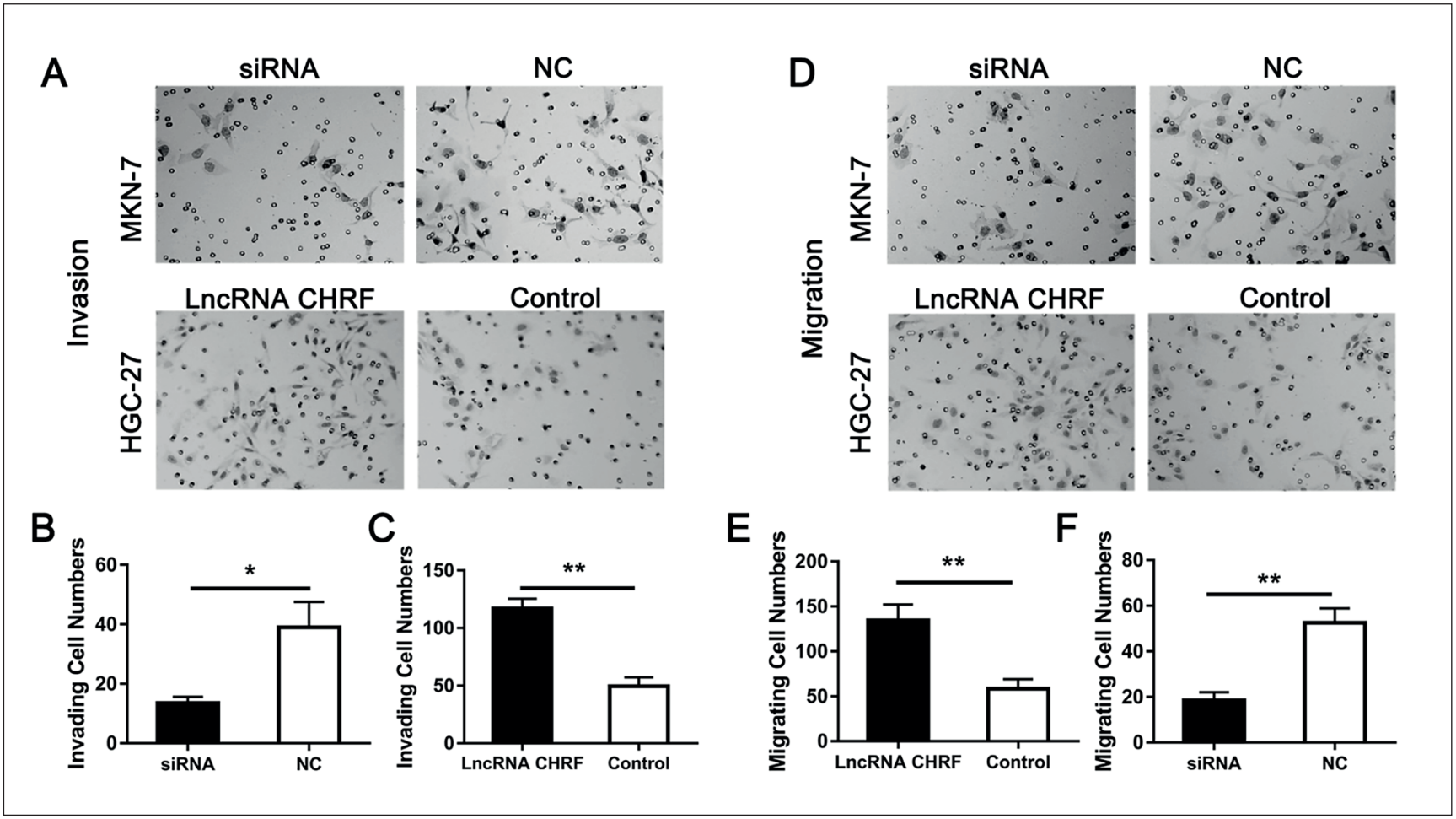

Figure 4. LncRNA CHRF affected the invasion and migration of PCa cells. **A, B, C,** Transwell invasion assay indicated the invaded cell number in established MKN-7 cells and HGC-27 cells (magnification: 40×). **D, E, F,** Transwell migration assay showed the migrated cell number in established MKN-7 cells and HGC-27 cells (magnification: 40×). $^{**}p<0.01$, $^{*}p<0.05$ compared to the control group.

and Vimentin was found in lncRNA CHRF inhibited MKN-7 cells compared to the NC group, which indicated a weakened EMT progression (Figures 5A, 5B). By contrast, the overexpression of lncRNA CHRF inhibited the expression of E-cadherin but promoted the expression of Vimentin and N-cadherin, which suggested an elevated EMT progression (Figures 5A, 5C). These demonstrated that lncRNA CHRF could promote the progression of EMT in GC.

Discussion

In this study, we demonstrated that lncRNA CHRF was overexpressed in GC tissues when compared with adjacent para-tumor tissues. In GC with lymph node metastasis, lncRNA CHRF is expressed significantly higher than without lymph node metastasis. Also, in GC cells, lncRNA CHRF expression increased as well. The inhibition of lncRNA CHRF significantly inhibited cell invasion and migration of GC cells while its overexpression had the opposite effect. Furthermore, lncRNA CHRF promoted EMT progression of GC cells. These indicated lncRNA CHRF participated in the progression of GC and acted as a promoting factor of GC metastasis.

The treatment strategy of GC is a combination of surgery, radiotherapy, and chemotherapy. However, some patients at advanced stage have a poor prognosis and poor therapeutic reflection. Recurrence and metastasis are the main causes of the reduction of the survival rate in patients with GC[2,3]. Therefore, the molecular mechanism of gastric carcinogenesis and progression needs to be explored in depth. The therapeutic targets and methods are the focus of GC research.

LncRNAs have been proved to contribute to the tumorigenesis, development, and progression

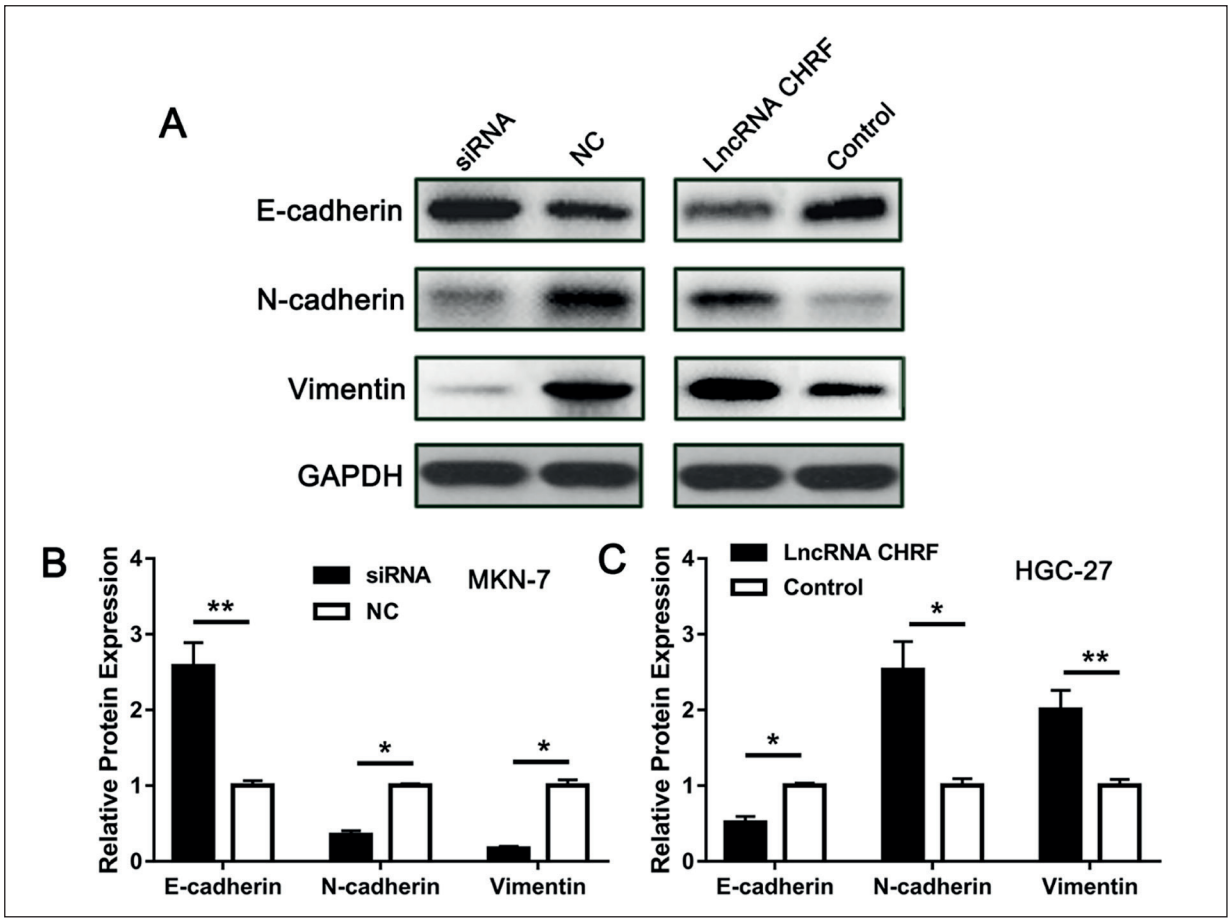

Figure 5. LncRNA CHRF promoted EMT of GC. **A,** Expression of EMT markers in experimental cells. **B, C,** The relative level of protein. The data are presented as the mean ± SD of three independent experiments. *p<0.05, **p<0.01 compared to the control group.

of GC. It could act as oncogenes or tumor suppressors in GC. Indeed, lncRNA HOXA11-AS increased cell proliferation and metastasis of GC by providing a scaffold for the chromatin modification factors DNMT1, PRC2, and LSD1[16]. LncRNA PVT1 activated the STAT3/VEGFA pathway to promote GC angiogenesis[17]. LncRNA MALAT1 is associated with chemoresistance of GC regulated by autophagy *via* miR-23b-3p[18]. However, LINC01939 regulates EGR2 expression to inhibit GC metastasis *via* function as a sponge of miR-17-5p[19]. LINC00628 also suppresses GC development *via* long-range modulating the expression of the cell cycle-related genes[20].

Our study focused on the function of lncRNA CHRF in GC and demonstrated that lncRNA CHRF can act as an oncogene in GC. LncRNA CHRF has been identified to promote the progression and EMT in prostate cancer cell line PC3 and induces miR-489 loss to promote the colorectal cancer metastasis[21,22]. Also, we found that the progression of EMT in GC could be accelerated by lncRNA CHRF. EMT refers to a process in which the epithelial phenotype transforms the chromosomal phenotype under certain conditions of epithelial cell carcinoma. EMT is a temporary occurrence of epithelial cells in order to adapt to changes in the surrounding environment. In the process of transformation, this process can be reversed. This transformation is closely related to the repair of wounds, the growth and development of embryos, and the development of tumors[23-29]. We found that lncRNA CHRF expressed was negatively correlated with epithelial marker E-cadherin but positively correlated with mesenchymal markers N-cadherin and Vimentin. This was familiar to the results of lncRNA CHRF, which promoted EMT in prostate cancer and colorectal cancer.

Conclusions

For the first time we elucidated that lncRNA CHRF expression was remarkedly increased in GC tissues and cells, and it promoted cell invasion and migration *via* EMT. However, a study on the underlying mechanism of lncRNA CHRF and an *in vivo* study were needed for the explanation of the role of LncRNA CHRF in GC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Kim YH, Shin SW. Helicobacter pylori and prevention of gastric cancer. N Engl J Med 2018; 378: 2244-2245.
2) Ding Z, Jiang L, Zhang K, Huang R. Short- and long-term outcomes of conversion in laparoscopic gastrectomy for gastric cancer. J BUON 2018; 23: 1004-1012.
3) Ren J, Liu J, Sui X. Correlation of COX-2 and MMP-13 expressions with gastric cancer and their effects on prognosis. J BUON 2018; 23: 665-671.
4) Gu C, Liao B, Li X, Cai L, Li Z, Li K, Yang J. Global network random walk for predicting potential human lncRNA-disease associations. Sci Rep 2017; 7: 12442.
5) Lin Y, Zhang CS, Li SJ, Li Z, Sun FB. LncRNA LOC554202 promotes proliferation and migration of gastric cancer cells through regulating p21 and E-cadherin. Eur Rev Med Pharmacol Sci 2018; 22: 8690-8697.
6) Nie FQ, Sun M, Yang JS, Xie M, Xu TP, Xia R, Liu YW, Liu XH, Zhang EB, Lu KH, Shu YQ. Long noncoding RNA ANRIL promotes non-small cell lung cancer cell proliferation and inhibits apoptosis by silencing KLF2 and P21 expression. Mol Cancer Ther 2015; 14: 268-277.
7) Ma MZ, Chu BF, Zhang Y, Weng MZ, Qin YY, Gong W, Quan ZW. Long non-coding RNA CCAT1 promotes gallbladder cancer development via negative modulation of miRNA-218-5p. Cell Death Dis 2015; 6: e1583.
8) Taniue K, Kurimoto A, Sugimasa H, Nasu E, Takeda Y, Iwasaki K, Nagashima T, Okada-Hatakeyama M, Oyama M, Kozuka-Hata H, Hiyoshi M, Kitayama J, Negishi L, Kawasaki Y, Akiyama T. Long noncoding RNA UPAT promotes colon tumorigenesis by inhibiting degradation of UHRF1. Proc Natl Acad Sci U S A 2016; 113: 1273-1278.
9) Xia T, Chen S, Jiang Z, Shao Y, Jiang X, Li P, Xiao B, Guo J. Long noncoding RNA FER1L4 suppresses cancer cell growth by acting as a competing endogenous RNA and regulating PTEN expression. Sci Rep 2015; 5: 13445.
10) Ai B, Kong X, Wang X, Zhang K, Yang X, Zhai J, Gao R, Qi Y, Wang J, Wang Z, Fang Y. LINC01355 suppresses breast cancer growth through FOXO3-mediated transcriptional repression of CCND1. Cell Death Dis 2019; 10: 502.
11) Sun TT, He J, Liang Q, Ren LL, Yan TT, Yu TC, Tang JY, Bao YJ, Hu Y, Lin Y, Sun D, Chen YX, Hong J, Chen H, Zou W, Fang JY. LncRNA GClnc1 promotes gastric carcinogenesis and may act as a modular scaffold of WDR5 and KAT2A complexes to specify the histone modification pattern. Cancer Discov 2016; 6: 784-801.
12) Zhuo W, Liu Y, Li S, Guo D, Sun Q, Jin J, Rao X, Li M, Sun M, Jiang M, Xu Y, Teng L, Jin Y, Si J, Liu W, Kang Y, Zhou T. Long noncoding RNA GMAN, up-regulated in gastric cancer tissues, is associated with metastasis in patients and promotes

translation of ephrin A1 by competitively binding GMAN-AS. Gastroenterology 2019; 156: 676-691. e11.

13) Zhang E, He X, Zhang C, Su J, Lu X, Si X, Chen J, Yin D, Han L, De W. A novel long noncoding RNA HOXC-AS3 mediates tumorigenesis of gastric cancer by binding to YBX1. Genome Biol 2018; 19: 154.

14) Yang XZ, Cheng TT, He QJ, Lei ZY, Chi J, Tang Z, Liao QX, Zhang H, Zeng LS, Cui SZ. LINC01133 as ceRNA inhibits gastric cancer progression by sponging miR-106a-3p to regulate APC expression and the Wnt/β-catenin pathway. Mol Cancer 2018; 17: 126.

15) Zhang G, Li S, Lu J, Ge Y, Wang Q, Ma G, Zhao Q, Wu D, Gong W, Du M, Chu H, Wang M, Zhang A, Zhang Z. LncRNA MT1JP functions as a ceRNA in regulating FBXW7 through competitively binding to miR-92a-3p in gastric cancer. Mol Cancer 2018; 17: 87.

16) Sun M, Nie F, Wang Y, Zhang Z, Hou J, He D, Xie M, Xu L, De W, Wang Z, Wang J. LncRNA HOXA11-AS promotes proliferation and invasion of gastric cancer by scaffolding the chromatin modification factors PRC2, LSD1, and DNMT1. Cancer Res 2016; 76: 6299-6310.

17) Zhao J, Du P, Cui P, Qin Y, Hu C, Wu J, Zhou Z, Zhang W, Qin L, Huang G. LncRNA PVT1 promotes angiogenesis via activating the STAT3/VEGFA axis in gastric cancer. Oncogene 2018; 37: 4094-4109.

18) YiRen H, YingCong Y, Sunwu Y, Keqin L, Xiaochun T, Senrui C, Ende C, XiZhou L, Yanfan C. Long noncoding RNA MALAT1 regulates autophagy associated chemoresistance via miR-23b-3p sequestration in gastric cancer. Mol Cancer 2017; 16: 174.

19) Chen M, Fan L, Zhang SM, Li Y, Chen P, Peng X, Liu DB, Ma C, Zhang WJ, Zou ZW, Li PD. LINC01939 inhibits the metastasis of gastric cancer by acting as a molecular sponge of miR-17-5p to regulate EGR2 expression. Cell Death Dis 2019; 10: 70.

20) Zhang ZZ, Zhao G, Zhuang C, Shen YY, Zhao WY, Xu J, Wang M, Wang CJ, Tu L, Cao H, Zhang ZG. Long non-coding RNA LINC00628 functions as a gastric cancer suppressor via long-range modulating the expression of cell cycle related genes. Sci Rep 2016; 6: 27435.

21) Liu C, Wang L, Li Y, Cui Y, Wang Y, Liu S. Long non-coding RNA CHRF promotes proliferation and mesenchymal transition (EMT) in prostate cancer cell line PC3 requiring up-regulating microRNA-10b. Biol Chem 2019. pii: /j/bchm.ahead-of-print/hsz-2018-0380/hsz-2018-0380.xml. doi: 10.1515/hsz-2018-0380. [Epub ahead of print].

22) Tao Y, Han T, Zhang T, Ma C, Sun C. LncRNA CHRF-induced miR-489 loss promotes metastasis of colorectal cancer via TWIST1/EMT signaling pathway. Oncotarget 2017; 8: 36410-36422.

23) Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S, Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med 2015; 21: 449-456.

24) Cui H, Hu Y, Guo D, Zhang A, Gu Y, Zhang S, Zhao C, Gong P, Shen X, Li Y, Wu H, Wang L, Zhao Z, Fan H. DNA methyltransferase 3A isoform b contributes to repressing E-cadherin through cooperation of DNA methylation and H3K27/H3K9 methylation in EMT-related metastasis of gastric cancer. Oncogene 2018; 37: 4358-4371.

25) Dong J, Wang R, Ren G, Li X, Wang J, Sun Y, Liang J, Nie Y, Wu K, Feng B, Shang Y, Fan D. HMGA2-FOXL2 axis regulates metastases and epithelial-to-mesenchymal transition of chemoresistant gastric cancer. Clin Cancer Res 2017; 23: 3461-3473.

26) Dongre A, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. Nat Rev Mol Cell Biol 2019; 20: 69-84.

27) Wang Y, Wen M, Kwon Y, Xu Y, Liu Y, Zhang P, He X, Wang Q, Huang Y, Jen KY, LaBarge MA, You L, Kogan SC, Gray JW, Mao JH, Wei G. CUL4A induces epithelial-mesenchymal transition and promotes cancer metastasis by regulating ZEB1 expression. Cancer Res 2014; 74: 520-531.

28) Wong IY, Javaid S, Wong EA, Perk S, Haber DA, Toner M, Irimia D. Collective and individual migration following the epithelial-mesenchymal transition. Nat Mater 2014; 13: 1063-1071.

29) Xu SH, Huang JZ, Xu ML, Yu G, Yin XF, Chen D, Yan GR. ACK1 promotes gastric cancer epithelial-mesenchymal transition and metastasis through AKT-POU2F1-ECD signalling. J Pathol 2015; 236: 175-185.