

lncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway

G.-H. WEI¹, X. WANG²

¹Department of Pathology, Jinzhou Medical University, Jinzhou, Liaoning Province, China

²Physical Examination Centre, The Third Affiliated Hospital of Chongqing Medical University, Chongqing, China

Abstract. – OBJECTIVE: lncRNA MEG3 has been reported as a tumor suppressor gene in many different kinds of cancer, but its role in gastric cancer has not been fully understood. Then, we would like to explore its mechanism in gastric cancer.

PATIENTS AND METHODS: We first used qRT-PCR to detect the expression of lncRNA MEG3, then CCK8 and wound healing assay were used to detect the effect of lncRNA MEG3 on gastric cancer cells. Western blot assay was used to measure the expression of p53 when lncRNA MEG3 was overexpressed.

RESULTS: lncRNA MEG3 was highly expressed in the adjacent tissue, compared to the one in gastric cancer tissue. What's more, we also found that overexpression of lncRNA MEG3 could decrease the proliferation and metastasis of gastric cancer cells. Finally, overexpression of lncRNA MEG3 could also increase the expression of p53.

CONCLUSIONS: lncRNA MEG3 could inhibit the proliferation and metastasis of gastric cancer.

Key Words:

MEG3, lncRNA, Gastric cancer, Metastasis.

Introduction

According to a recent study¹, there were 951600 new gastric cancer cases and 723,100 deaths due to gastric cancer occurred in 2012. The incidence of gastric cancer is the highest in Eastern Asia, such as China, Japan and Korea. Torre et al¹ showed that the incidence of gastric cancer in China has ranked the first in the whole world. What's more, gastric cancer has caused 498,000 deaths in China². These investigations indicated that gastric cancer has become a serious threat to the whole world. Despite great advance has been made in the clinical treatment of gastric cancer, the effects were far from

satisfaction, suggesting that new therapy or target should be found in the treatment of gastric cancer. lncRNAs are a kind of non-coding RNA which are more than 200 nt. Recent works³⁻⁷ have shown that lncRNA played as crucial regulators in various kinds of cancer. Zhang et al⁸ found that lncRNA Linp1 was overexpressed in human triple-negative breast cancer, which could enhance repair of DNA double-strand breaks by serving as a scaffold linking Ku80 and DNA-PKcs, thereby coordinating the non homologous end joining pathway, promoting metastasis of breast cancer. Similarly, Wan et al⁹ also observed that lncRNA Jade could transcriptionally activate the expression of Jadel, a key component of human histone acetylation complex, inducing histone H4 acetylation in DNA damage response, promoting breast tumorigenesis. Peter et al¹⁰ showed that lncRNA was crucial to corresponding more balanced changes for mRNAs in urothelial cancer; they identified 32 lncRNAs with potential roles in disease progression. Especially, they found lncRNA AB074278 was upregulated and maintained to a pro-proliferative state in cancer through potential interaction with EMP1. Takahashi et al¹¹ reported that lncRNA ROR was highly expressed in malignant liver cancer cells, especially in hypoxic regions within tumor cell xenografts *in vivo*, and suppression of lncRNA ROR could lead to the decreased phosphorylation of p70S6K1, PDK1 and HIF-1 α , suggesting that lncRNA ROR was a hypoxia-responsive lncRNA that is functionally related to hypoxia signaling in liver cancer. These studies have shown that lncRNA played various roles in the progression of different kinds of tumors, but the mechanism of lncRNA in cancer are far from being clear yet. Accumulating evidence has shown that lncRNA could play as either oncogenic or tumor suppressor gene¹². Among these tumor suppressor lncRNAs, lncRNA MEG3

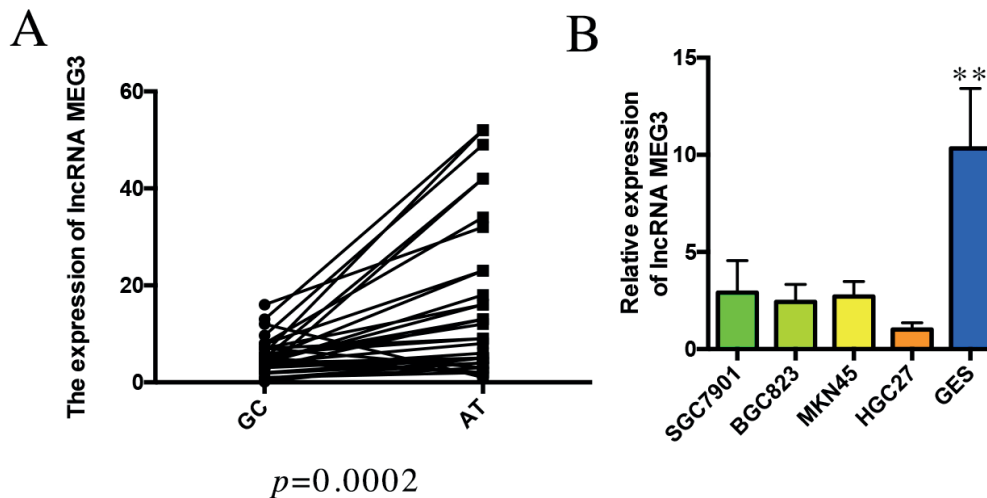


Figure 1. lncRNA MEG3 was low expressed in gastric cancer tissue and cell lines. (A) The expression of lncRNA MEG3 in 31 gastric cancer tissues (GC) and adjacent tissue (AT); (B) qRT-PCR was used to detect the expression of lncRNA MEG3 in four gastric cancer cell lines and one normal epithelial cell line. $p < 0.05$.

(maternally expressed gene 3) has attracted a lot of attention¹³. Scholars have shown that lncRNA MEG3 could regulate as tumor suppressor gene via regulating the expression of p53. Lu et al¹⁴ reported that MEG3 could regulate cell proliferation and apoptosis by activating p53 in meningioma and NSCLC. Zhu et al¹⁵ also showed that lncRNA MEG3 could inhibit the proliferation of liver cancer via regulating the expression of p53. However, investigations have found that MEG3 could act as tumor suppressor gene in gastric cancer, but the relation between lncRNA MEG3 and p53 remained largely unclear in gastric cancer. In our paper, we focused on the role of lncRNA MEG3 in gastric cancer. We showed that the expression of lncRNA MEG3 was lower in gastric cancer tissue. Importantly, the expression of lncRNA MEG3 was related to the tumor size of gastric cancer tissue. Suppression of lncRNA MEG3 could reduce the proliferation and metastasis ability of gastric cancer cells. At last, we found that lncRNA MEG3 could induce suppression in gastric cancer by regulating the expression of p53.

Patients and Methods

Patients, Specimens and Clinical Data Collection

Clinical samples (gastric cancer tissue and adjacent tissue) were collected from the General Surgery Department, the First Affiliated Hospital of Jinzhou Medical University (Jinzhou City, Liao-

ning Province) from Feb 2013 to Oct 2014 (n=31). None of these patients received any surgery operation, chemical or radiation treatment before. Their clinical data, such as gender, age, tumor size, tumor location, clinical stage and distant metastasis, were collected for future analysis. The specimens were divided into small pieces and stored in the liquid nitrogen for further experiments. All of the patients were well informed about the use of samples and informed consents were also signed. This study followed institutional ethical guidelines which were reviewed and approved by the Research Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University.

Total RNA Preparation and qRT-PCR Reactions

Total RNA samples from cell and tissue were obtained by using RNAiso Plus (TaKaRa, Dalian, China) following the manufacturer's protocol. The concentration of RNA was detected and reverse transcription polymerase chain reaction was performed using PrimeScript™ RT reagent Kit according to the manufacturer's instructions. The levels of mRNA expression were quantified by real-time PCR with SYBR Premix Ex Taq (TaKaRa, Dalian, China).

Cell Culture

Human gastric cancer cell lines (SGC7901, BGC823, MKN45, HGC27) and one normal gastric epithelial cell line (GES) were obtained from the Institute of Biochemistry and Cell Biology at

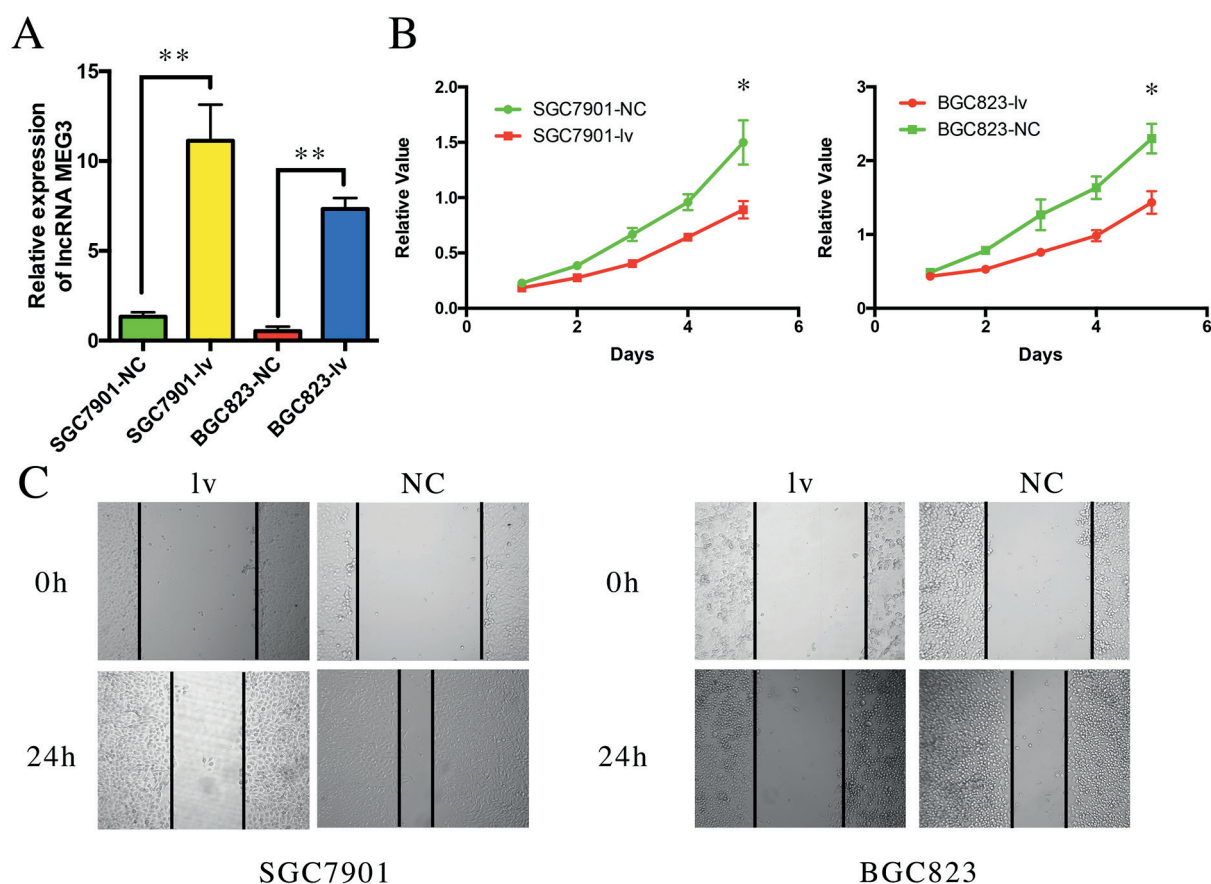


Figure 2. IncRNA MEG3 was closely related with the proliferation and metastasis ability of gastric cancer cell lines. (A) Overexpression of IncRNA MEG3 in SGC7901 and BGC823. $p < 0.01$; (B) CCK8 assay was applied to detect the proliferation ability of SGC7901 and BGC823 cells, after IncRNA MEG3 was overexpressed. $p < 0.05$; (C) Wound Healing assay was used to detect the effect of IncRNA MEG3 on the metastasis of gastric cancer cells.

the Chinese Academy of Sciences (Shanghai, China). SGC7901, GES and HGC27 were cultured in Dulbecco minimum essential medium (DMEM) (Hyclone, CA, America), while BGC823 and MKN45 were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Hyclone, South Logan, UT, USA). The medium contained 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 2.5% penicillin and streptomycin (Beyotime Biotechnology, Beijing, China). These cells were kept in flasks at 37°C with 5% CO₂.

Overexpression of IncRNA MEG3 in Gastric Cancer Cell Lines

We got overexpression of IncRNA MEG3 plasmid from GeneChem Company (Shanghai, China). The cells were seeded at 2×10⁵ cells/well in 2 ml of medium (RPMI 1640) in 6-well plates. Two hours before transfection, cells were washed twice with phosphate buffered saline (PBS) and placed

in Opti-MEM I Reduced Serum Medium (Invitrogen, Carlsbad, CA, USA). 1 µg of plasmid and 2 µl of lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) were added into 250 µl opti-MEM respectively. Five minutes later, diluted plasmid and lipofectamine 2000 were mixed and incubated for 30 minutes. Then, the 500 µl of plasmid-lipid complex and another 500 µl Opti-MEM were added to the each well containing cells. After 12 h of incubation, 2 µl RPMI 1640 with 10% serum was added to each well.

CCK8 Assay

Target cells were seeded into 96-well plates with at the density of 2000 cells per well. Five replicates were set for each group. Each well contained 100 µl DMEM with 10% FBS and 10 ml Cell Counting Kit 8 (Dojindo Laboratories, Kumamoto, Japan). The plate was cultured for 2 hours at 37°C. The absorbance value of each well

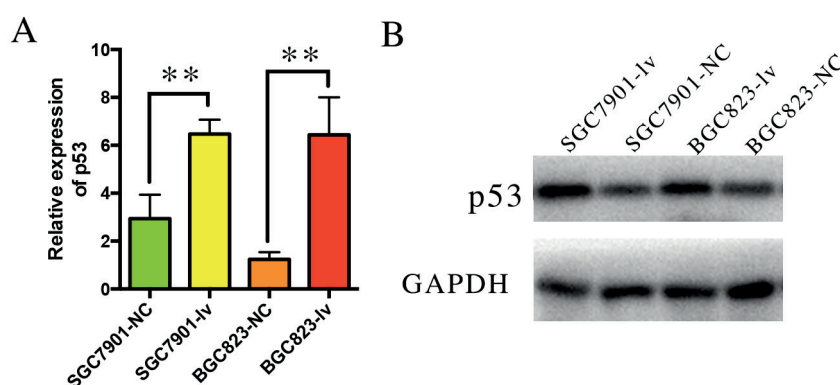


Figure 3. lncRNA MEG3 controlled the expression of p53 in gastric cancer cell lines. (A) qRT-PCR was used to detect the change of p53 at mRNA level, $p < 0.01$; (B) The expression of p53 was detected at protein level.

was measured and collected at 450 nm. The data were collected for 3 days, and the whole experiment was repeated 3 times.

Wound Healing Assay

Target cells were seeded into 6-well plate. When cells grew to 90% confluent, cells monolayers were scraped with a sterile micropipette tip. The wounded monolayers were washed with phosphate buffer solution (PBS) to remove cell debris. The distance between the two edges of the wound was calculated at three different positions. And 24 hours later, the distance between the two edges should be measured again.

Western Blot Assay

Cells were washed 3 times with phosphate buffer saline (PBS) to remove the rest cell culture media. Total cell proteins were extracted using RIPA lysis buffer containing protein inhibitor PMAF (Beyotime Biotechnology, Beijing, China). The concentration of protein was detected by standard bovine serum albumin protein quantitation assay. 50 mg of proteins were added to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). These membranes were blocked in 5% non-fat milk for 1 hour at room temperature and then were treated as antibody protocol described overnight at 4°C. Moreover, respective secondary antibody was used to incubate these membranes according to protocol. The protein bands were quantified with ECL system (Thermo Fisher Scientific, Waltham, MA, USA) and were analyzed by GraphPad Prism software.

Statistical Analysis

All of the experiments were performed at least three times and all the data were presented as means \pm standard deviation (SD). The difference between two groups was analyzed by the Student's *t*-test, and one-way ANOVA was used to analyzed data from more than two groups. We used "Brown-Forsythe test" to validate ANOVA. p -value < 0.05 was considered statistically significant. All statistical analyses were performed using the GraphPad Prism 5.

Results

lncRNA MEG3 was Low Expressed in Gastric Cancer Tissue and Cell Lines

To explore the possible role of lncRNA MEG3 in gastric cancer, we first compared the expression of lncRNA MEG3 in gastric cancer tissue with that of the adjacent tissue by using qRT-PCR. We found that the expression of lncRNA MEG3 was lower in gastric cancer tissue, while the expression of lncRNA MEG3 was higher in normal gastric epithelia, when compared with gastric cancer tissue (Figure 1 A). Then, we detected the expression of lncRNA MEG3 in four gastric cancer cell lines (SGC7901, BGC823, MKN45, HGC27) and one normal gastric cell line.

lncRNA MEG3 was Closely Related to the Proliferation and Metastasis Ability of Gastric Cancer Cell Lines

To understand the role of lncRNA MEG3 in gastric cancer, we then overexpressed lncRNA MEG3 in SGC7901 and BGC823 cells (Figure 2A). We then used the CCK8 assay to detect the ef-

fect of lncRNA MEG3 on the proliferation ability of gastric cancer cells. We found that overexpression of lncRNA MEG3 in gastric cancer cell line (SGC7901 and BGC823) could suppress the proliferation ability of gastric cancer cells (Figure 2B). What's more, we also found that metastasis ability of gastric cancer cells was suppressed when lncRNA MEG3 was overexpressed (Figure 2C). Taken together, we found that lncRNA MEG3 could inhibit proliferation ability of gastric cancer cells, but the mechanism remained unclear.

lncRNA MEG3 Controlled the Expression of p53 in Gastric Cancer Cell Lines

Studies^{13,14} have found that lncRNA MEG3 acted as a tumor suppressor gene in various tumors via regulating p53 signaling pathway. We wondered whether lncRNA MEG3 could regulate the expression of p53 in gastric cancer. We found that expression of p53 was increased at both mRNA and protein level (Figure 3A and Figure 3B), when lncRNA MEG3 was overexpressed, suggesting that lncRNA MEG3 could regulate the expression of p53 in gastric cancer cells.

lncRNA MEG3 Could be Used as a Potential Target for Predicting Prognosis of Gastric Cancer Patients

We found that the expression of lncRNA MEG3 was low in gastric cancer tissue and cell lines, and overexpression of lncRNA MEG3 could suppress

the proliferation and metastasis ability of gastric cancer cells. We then we tried to detect the relation between the expression of lncRNA MEG3 and clinical pathological characteristics. Interestingly, we found that the expression of lncRNA MEG3 was negatively related to the tumor size of gastric cancer (Figure 4A), but no significance was found with metastasis of gastric cancer, which might be due to the number of clinical samples (Figure 4A). In addition, we found that lncRNA MEG3 was closely related with the survival time of patients (Figure 4B), suggesting that lncRNA MEG3 may be used as a potential biomarker for gastric cancer.

Discussion

Gastric cancer has been for a long time the leading cause of cancer related death in the world, especially in Asian countries¹. Radiotherapy and chemotherapy are the most common methods of treating gastric cancer, after surgery. Despite great advances have been made in the treatment of gastric cancer, the mortality remains high in patients with advanced gastric cancer, suggesting that resistance exists under present treatments. To improve the effectiveness of treatments in gastric cancer, new therapeutic strategies and targets should be further studied. Recently scholars have shown that lncRNAs could act as a critical regulator in the development of gastric cancer. Ma et

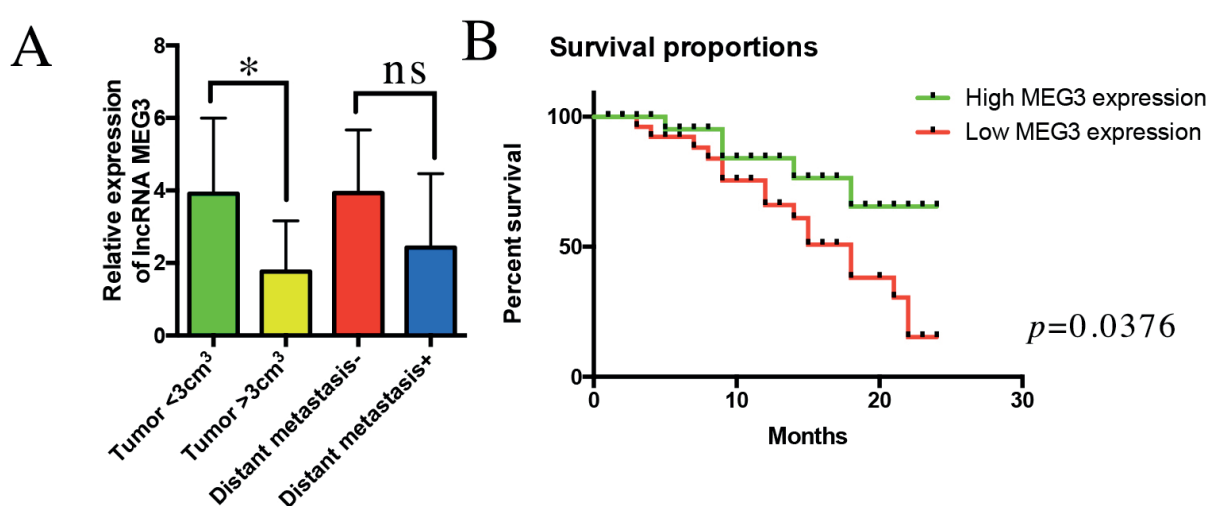


Figure 4. lncRNA MEG3 could be used as a potential target for predicting prognosis of gastric cancer patients. (A) Clinical analysis showed that the expression of lncRNA MEG3 was closely related with size of gastric cancer, but no significance was found in metastasis. *p* < 0.05. (B) Survival study showed that patients with high expression of lncRNA MEG3 had a longer survival time.

al¹⁶ reported that lncRNA XIST could promote cell growth and invasion by acting as a ceRNA to sponge miR-497, which controlled its down-stream target MACC1. Shao et al¹⁷ showed that lncRNA RMRP could sponge miR-206 to promote carcinogenesis of gastric cancer, and it could also be used as an effective biomarker for gastric cancer. These works showed that lncRNAs may be used as potential targets and biomarkers for gastric cancer. Notably, most lncRNAs in gastric cancer act as an oncogene, according to present studies. lncRNAs act as tumor suppressor gene in gastric cancer are rarely reported. lncRNA MEG3 is one of the tumor suppressor genes in various kinds of cancers, including gastric cancer. In our paper, we firstly detected the expression of lncRNA MEG3 in 31 patients with gastric cancer, and we found that the expression of lncRNA MEG3 was much lower in gastric cancer tissue, compared with the adjacent tissue. We then also found that the expression of lncRNA MEG3 was negatively related with the tumor size of the gastric cancer and positively related with the survival time of patients. But no significance in metastasis was observed according to the clinical pathological characteristics. Our data suggested that lncRNA MEG3 could be used as a potential biomarker to predict prognosis of gastric cancer patients. However, there are some differences between clinical results and cytology experiments in our work. Our results showed that overexpression of lncRNA MEG3 could inhibit proliferation and metastasis of gastric cancer. Wang et al¹⁸ found that lncRNA MEG3 could suppress migration and invasion of thyroid carcinoma. Yin et al¹⁹ found that MEG3 was remarkably correlated with deep tumor invasion and advanced tumor node metastasis. However, there was no significance found in the clinical results in our paper. It might be due to the small number of clinical samples. According to present investigation and our results, we assumed that MEG3 may play as a tumor suppressor gene in gastric cancer. Accumulating evidence showed that lncRNA MEG3 could inhibit the proliferation and metastasis of tumors through different pathways. Studies showed that MEG3 could act as a ceRNA which sponges different miRNAs, such as miR-21, miR-141, miR-181 and miR-29 to regulate the malignant activity in tumors. Recent works²⁰⁻²³ also revealed that lncRNA MEG3 could regulate different factors to achieve suppression role in tumors. Luo et al²⁴ showed that MEG3 could reduce Bcl-2 expression and enhance Bax and caspase 3 in prostate cancer. What's more,

MEG3 could also reduce the expression of Myc at both transcriptional and translational levels in lung cancer²⁵. Among these researches, we found that p53 signaling pathway was reported to interact with lncRNA MEG3. Studies^{14,15,26} showed that MEG3 could enhance stability and transcriptional activity of p53 to influence p53 target genes in suppressing the progression of tumors. However, whether MEG3 could regulate p53 in gastric cancer remained largely unclear. Then, we mainly focused on the relation between p53 and MEG3 in gastric cancer. We observed that overexpression of MEG3 promoted the expression of p53 in gastric cancer cell lines, suggesting that MEG3 may suppress the proliferation and metastasis of gastric cancer via regulating the expression of p53.

Conclusions

We showed that expression of lncRNA MEG3 was decreased in the gastric cancer tissue. The expression of lncRNA MEG3 was negatively related with the size of the gastric cancer, and positively related to the survival time of patients. We also found that MEG3 could inhibit both the proliferation and the metastasis of gastric cancer cells *in vitro*. Finally, we observed that MEG3 might regulate the expression of p53 in gastric cancer.

Conflict of interest

The authors declare no conflicts of interest.

References

- 1) TORRE LA, BRAY F, SIEGEL RL, FERLAY J, LORTET-TIEULENT J, JEMAL A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; 65: 87-108.
- 2) CHEN W, ZHENG R, BAADE PD, ZHANG S, ZENG H, BRAY F, JEMAL A, YU XO, HE J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; 66: 115-32.
- 3) DENG H, ZHANG J, SHI J, GUO Z, HE C, DING L, TANG JH, HOU Y. Role of long non-coding RNA in tumor drug resistance. *Tumour Biol* 2016; 37: 11623-11631.
- 4) SERGHIU S, KYRIAKOPOULOU A, IOANNIDIS JP. Long non-coding RNAs as novel predictors of survival in human cancer: a systematic review and meta-analysis. *Mol Cancer* 2016; 15: 50.
- 5) BARTONICEK N, MAAG JL, DINGER ME. Long noncoding RNAs in cancer: mechanisms of action and technological advancements. *Mol Cancer* 2016; 15: 43.
- 6) JIANG XM, LI ZL, LI JL, ZHENG WY, LI XH, CUI YF, SUN DJ. lncRNA CCAT1 as the unfavorable prognos-

- stic biomarker for cholangiocarcinoma. *Eur Rev Med Pharmacol Sci* 2017; 21: 1242-1247.
- 7) WU ZJ, LI Y, WU YZ, WANG Y, NIAN WQ, WANG LL, LI LC, LUO HL, WANG DL. Long non-coding RNA CCAT2 promotes the breast cancer growth and metastasis by regulating TGF- β signaling pathway. *Eur Rev Med Pharmacol Sci* 2017; 21: 706-714.
 - 8) ZHANG Y, HE Q, HU Z, FENG Y, FAN L, TANG Z, YUAN J, SHAN W, LI C, HU X, TANYI JL, FAN Y, HUANG Q, MONTONE K, DANG CV, ZHANG L. Long noncoding RNA LINP1 regulates repair of DNA double-strand breaks in triple-negative breast cancer. *Nat Struct Mol Biol* 2016; 23: 522-530.
 - 9) WAN G, HU X, LIU Y, HAN C, SOOD AK, CALIN GA, ZHANG X, LU X. A novel non-coding RNA lincRNA-JADE connects DNA damage signalling to histone H4 acetylation. *EMBO J* 2013; 32: 2833-2847.
 - 10) PETER S, BORKOWSKA E, DRAYTON RM, RAKHIT CP, NOON A, CHEN W, CATTO JW. Identification of differentially expressed long noncoding RNAs in bladder cancer. *Clin Cancer Res* 2014; 20: 5311-5321.
 - 11) TAKAHASHI K, YAN IK, HAGA H, PATEL T. Modulation of hypoxia-signaling pathways by extracellular linc-RoR. *J Cell Sci* 2014; 127: 1585-1594.
 - 12) QI P, ZHOU XY, DU X. Circulating long non-coding RNAs in cancer: current status and future perspectives. *Mol Cancer* 2016; 15: 39.
 - 13) ZHOU Y, ZHANG X, KLIBANSKI A. MEG3 noncoding RNA: a tumor suppressor. *J Mol Endocrinol* 2012; 48: R45-53.
 - 14) LU KH, LI W, LIU XH, SUN M, ZHANG ML, WU WQ, XIE WP, HOU YY. Long non-coding RNA MEG3 inhibits NSCLC cells proliferation and induces apoptosis by affecting p53 expression. *BMC Cancer* 2013; 13: 461.
 - 15) ZHU J, LIU S, YE F, SHEN Y, TIE Y, ZHU J, WEI L, JIN Y, FU H, WU Y, ZHENG X. Long noncoding RNA MEG3 interacts with p53 protein and regulates partial p53 target genes in hepatoma cells. *PLoS One* 2015; 10: e0139790.
 - 16) MA L, ZHOU Y, LUO X, GAO H, DENG X, JIANG Y. Long non-coding RNA XIST promotes cell growth and invasion through regulating miR-497/MACC1 axis in gastric cancer. *Oncotarget* 2017; 8: 4125-4135.
 - 17) SHAO Y, YE M, LI Q, SUN W, YE G, ZHANG X, YANG Y, XIAO B, GUO J. LncRNA-RMRP promotes carcinogenesis by acting as a miR-206 sponge and is used as a novel biomarker for gastric cancer. *Oncotarget* 2016; 7: 37812-37824.
 - 18) WANG C, YAN G, ZHANG Y, JIA X, BU P. Long non-coding RNA MEG3 suppresses migration and invasion of thyroid carcinoma by targeting of Rac1. *Neoplasia* 2015; 62: 541-549.
 - 19) YIN DD, LIU ZJ, ZHANG E, KONG R, ZHANG ZH, GUO RH. Decreased expression of long noncoding RNA MEG3 affects cell proliferation and predicts a poor prognosis in patients with colorectal cancer. *Tumour Biol* 2015; 36: 4851-4859.
 - 20) ZHANG J, YAO T, WANG Y, YU J, LIU Y, LIN Z. Long noncoding RNA MEG3 is downregulated in cervical cancer and affects cell proliferation and apoptosis by regulating miR-21. *Cancer Biol Ther* 2016; 17: 104-113.
 - 21) PENG W, SI S, ZHANG Q, LI C, ZHAO F, WANG F, YU J, MA R. Long non-coding RNA MEG3 functions as a competing endogenous RNA to regulate gastric cancer progression. *J Exp Clin Cancer Res* 2015; 34: 79.
 - 22) ZHOU X, JI G, KE X, GU H, JIN W, ZHANG G. MiR-141 inhibits gastric cancer proliferation by interacting with long noncoding RNA MEG3 and down-regulating E2F3 expression. *Dig Dis Sci* 2015; 60: 3271-3282.
 - 23) BRACONI C, KOGURE T, VALERI N, HUANG N, NUOVO G, COSTINEAN S, NEGRINI M, MIOTTO E, CROCE CM, PATEL T. microRNA-29 can regulate expression of the long non-coding RNA gene MEG3 in hepatocellular cancer. *Oncogene* 2011; 30: 4750-4756.
 - 24) LUO G, WANG M, WU X, TAO D, XIAO X, WANG L, MIN F, ZENG F, JIANG G. Long non-coding RNA MEG3 Inhibits cell proliferation and induces apoptosis in prostate cancer. *Cell Physiol Biochem* 2015; 37: 2209-2220.
 - 25) YAN-HUA L, XIANG-LEI L, HONG L, JIAN-JUN W. Long noncoding ribonucleic acids maternally expressed gene 3 inhibits lung cancer tumor progression through downregulation of MYC. *Indian J Cancer* 2015; 52 Suppl 3: E190-193.
 - 26) LV D, SUN R, YU Q, ZHANG X. The long non-coding RNA maternally expressed gene 3 activates p53 and is downregulated in esophageal squamous cell cancer. *Tumour Biol* 2016; Oct 24. [Epub ahead of print].