ORIGINAL ARTICLES

Department of Radiotherapy[1]; Department of Oncology[2], The First Affiliated Hospital of Anhui, Medical University, Hefei, Anhui, China

# Long non-coding RNA NEAT1 regulates the proliferation, migration and invasion of gastric cancer cells via targeting miR-335-5p/ROCK1 axis

Hao Wang[1], Mingxia Zhang[1], Guoping Sun[2,*]

*Received September 20, 2017, accepted October 23, 2917*

**Corresponding author: Guoping Sun, Department of Oncology, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui 230022, China*
*guopingsun08@sohu.com*

*Pharmazie 73: 150–155 (2018)* *doi: 10.1691/ph.2018.7877*

This study aimed to elucidate the roles of long non-coding RNA Nuclear Paraspeckle Assembly Transcript 1 (NEAT1) in gastric cancer. The expression of NEAT1 in gastric cancer tissues and cells was determined. NEAT1 was then overexpressed and suppressed in BGC-823 cells *in vitro* to further explore the effects of NEAT1 on cell proliferation, migration, and invasion. In addition, the regulatory relationship among NEAT1, miR-335-5p and Rho Associated Coiled-Coil Containing Protein Kinase 1 (ROCK1) was investigated. LncRNA NEAT1 was upregulated in gastric cancer tissues and cells. Upregulated NEAT1 significantly promoted the proliferation, migration and invasion of BGC-823 cells, while suppression of NEAT1 exhibited contrary results. In addition, lncRNA NEAT1 inhibited the expression of miR-335-5p, and miR-335-5p targeted ROCK1 in BGC-823 cells. miR-335-5p overexpression significantly inhibited cell proliferation, migration and invasion, which was counteracted by ROCK1 overexpression concurrently. Our findings indicate that upregulation of NEAT1 may promote proliferation, migration and invasion of gastric cancer cells via targeting miR-335-5p/ROCK1 axis. NEAT1-miR-335-5p-ROCK1 axis may be a potential therapeutic strategy for gastric cancer therapy.

## 1. Introduction

Gastric cancer, a heterogeneous disease, is estimated as the second-leading cause of cancer-related deaths (Jemal et al. 2011). Gastric cancer reaches an advanced stage in most patients due to of its asymptomatic character or nonspecific symptoms in the early stage (Wadhwa et al. 2013), thus resulting in poor prognosis and a daunting impact on global health (Carcas 2013; Gryko et al. 2014). Regrettably, there is still no efficacious therapeutic strategy for advanced gastric cancer (Lordick et al. 2013; Shi and Zhou 2010). Therefore, a better understanding of molecular mechanisms underlying gastric cancer is urgently needed.

Long non-coding RNAs (lncRNAs) are RNAs with more than 200 nucleotides in length (Vemuganti 2013; Yin et al. 2014). Their dysregulation and specific roles in a diverse range of diseases, in particular cancers, have evoked increasing attention (Chen et al. 2016a; Martens-Uzunova et al. 2014; Prensner et al. 2014; Shi et al. 2013). Moreover, lncRNAs are considered as promising diagnostic biomarkers or therapeutic targets for a variety of cancers (Fatima et al. 2015; Yan et al. 2015). Recently, lncRNA Nuclear Paraspeckle Assembly Transcript 1 (NEAT1) was found to play an important role in cancer development. For instance, upregulation of NEAT1 can promote the proliferation of esophageal squamous cell carcinoma cells (Chen et al. 2015). NEAT1 is also upregulated in ovarian cancer tissues, and its increased expression is positively correlated with disease progression and poor prognosis of patients with ovarian cancer (Chen et al. 2016b). Highly expressed NEAT1 in breast cancer is observed related to the tumor size and lymph node metastasis, and NEAT1 inhibition can result in decreased proliferation and metastasis of breast cancer cells (Zhang et al. 2017). Unfortunately, there is limited report about the emerging functional role of lncRNA NEAT1 in gastric cancer.

In the present study, we investigated whether NEAT1 was dysregulated in gastric cancer tissues and cells. NEAT1 was then overexpressed and suppressed in BGC-823 cells *in vitro* to further explore the roles of NEAT1 in regulate cell proliferation, migration, and invasion. In addition, NEAT1 is found to regulate the growth, invasion and migration of pancreatic cancer cells though inhibiting miR-335-5p (Cao et al. 2016). miR-335 can suppress the migration and invasion of osteosarcoma cells via targeting Rho Associated Coiled-Coil Containing Protein Kinase 1 (ROCK1) (Yong et al. 2013). Thus, the regulatory relationship among NEAT1, miR-335-5p and ROCK1 was detected to elucidate the possible regulatory mechanism of NEAT1 in the progression of gastric cancer.

## 2. Investigations and results

### 2.1. *LncRNA NEAT1 was upregulated in gastric cancer and promoted gastric cancer cell proliferation, migration and invasion*

As shown in Fig. 1A, NEAT1 expression in gastric cancer tissues and adjacent normal controls was investigated. NEAT1 expression in gastric cancer tissues was significantly higher than that in adjacent normal controls ($P < 0.05$). Moreover, we found that NEAT1 expression was significantly increased in gastric cancer BGC-823 cells relative to nonmalignant gastric mucosa epithelial GES-1 cells ($P < 0.05$, Fig. 1B). Thus, to further observe the effects of NEAT1 on regulating the malignant behaviors of gastric cancer cells, GES-1 cells were transfected with pc-NEAT1 or its blank control, while BGC-823 cells were transfected with si-NEAT1 or si-control. After 48 of different transfection, NEAT1 expression in pc-NEAT1-transfected GES-1 cells was markedly higher than GES-1 cells transfected with blank control, while NEAT1 expression was obviously decreased in si-NEAT1-transfected BGC-823 cells compared with si-control-transfected BGC-823 cells ($P < 0.05$, Fig. 1C), indicating that NEAT1 was successfully overexpressed in GES-1 cells and suppressed in BGC-823 cells after transfection. Then, MTT assay was performed to assess cell viability of different transfected cells. The results showed that NEAT1 overexpression caused a significantly increase of GES-1 cell viability after 48 and 72 h of transfection, while opposite effects were obtained after NEAT1 suppression in BGC-823 cells ($P < 0.05$, Fig. 1D). Moreover, the results of colony assay were in consistent with MTT assay that the colony forming efficiency (CEF) of pc-NEAT1-

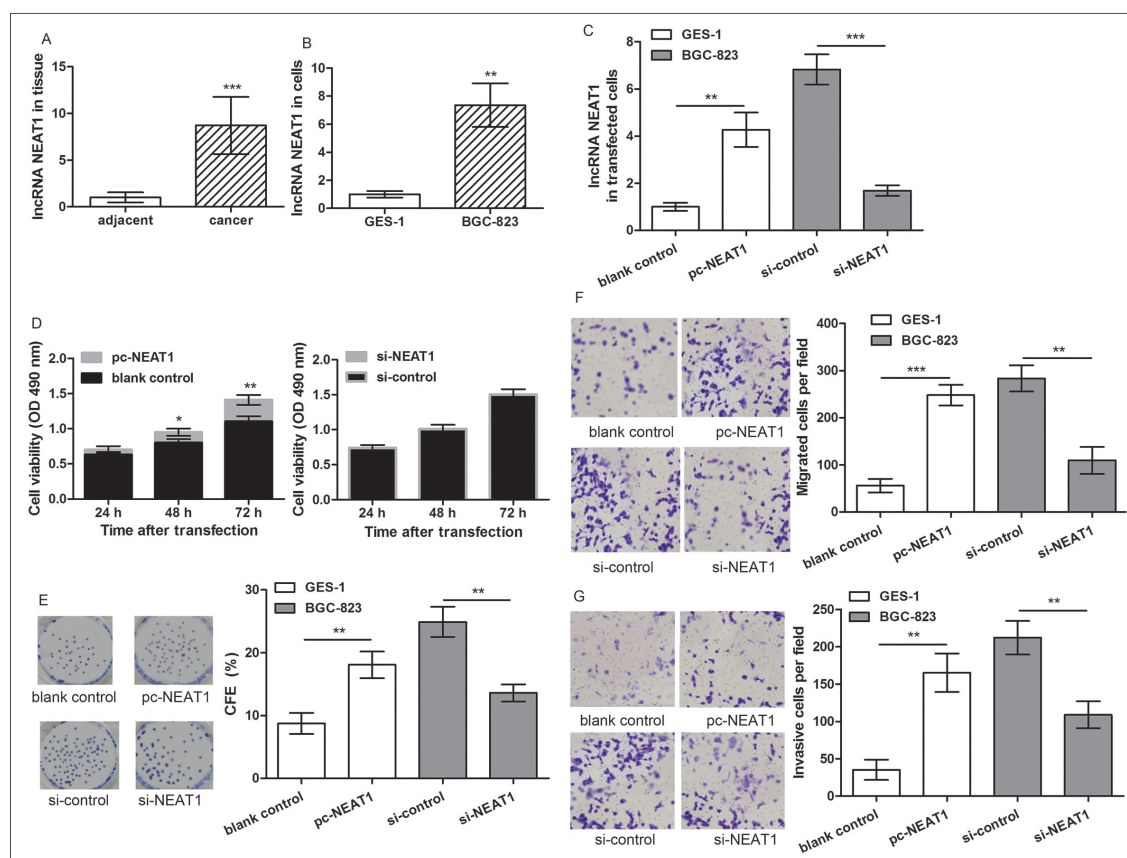


Fig. 1: LncRNA NEAT1 was upregulated in gastric cancer and promoted gastric cancer cell proliferation, migration and invasion. A: qRT-PCR showed NEAT1 expression in gastric cancer tissues and adjacent normal controls. B: qRT-PCR showed NEAT1 expression in gastric cancer BGC-823 cells and nonmalignant gastric mucosa epithelial GES-1 cells. C: qRT-PCR showed NEAT1 expression in GES-1 cells transfected with pc-NEAT1 or its blank control and BGC-823 cells transfected with si-NEAT1 or si-control. D: MTT assay showed cell viability of different transfected groups. E: Colony assay showed the colony forming efficiency (CEF) of different transfected cells. F: Transwell assay showed the migration of different transfected cells. G: Transwell assay showed the invasion of different transfected cells. * P<0.05, ** P<0.01 and *** P<0.001 compared with corresponding control.

transfected GES-1 cells was significantly stronger than that of GES-1 cells transfected with blank control, while the CEF of si-NEAT1-transfected BGC-823 cells were lower than si-control-transfected BGC-823 cells (P < 0.05, Fig. 1E). Besides, Transwell assay showed that overexpression of NEAT1 significantly promoted the migration and invasion of GES-1 cells, while knockdown of NEAT1 markedly inhibited the migration and invasion of BGC-823 cells (P < 0.05, Figs. 1F and 1G). These data indicated that NEAT1 overexpression promoted gastric cancer cell proliferation, migration and invasion.

### 2.2. *LncRNA NEAT1 inhibited miR-335-5p expression in gastric cancer cells*

It is reported that NEAT1 regulates the growth, invasion and migration of pancreatic cancer cells by inhibiting miR-335-5p (Cao et al. 2016). This prompted us to investigate the regulatory relationship between NEAT1 and miR-335-5p. As shown in Figs. 2A and 2B, miR-335-5p expression in gastric cancer tissues and BGC-823 cells was significantly lower than that in adjacent normal controls and GES-1 cells, respectively (all P < 0.05). In addition, miR-335-5p expression was markedly downregulated in pc-NEAT1-transfected GES-1 cells relative to GES-1 cells transfected with blank control, while obviously up-regulated in si-NEAT1-transfected BGC-823 cells in comparison to that in si-control-transfected BGC-823 cells (P < 0.05, Fig. 2C). These data indicated that lncRNA NEAT1 might inhibit the expression of miR-335-5p in gastric cancer cells.

### 2.3. *ROCK1 is a target of miR-335-5p*

ROCK1 is found as a direct target of miR-335 to mediate the tumour-suppressive function of miR-335 in osteosarcoma cells (Yong et al. 2013). We thus detected ROCK1 expression and the target relationship between ROCK1 and miR-335-5p. Expected results were obtained that ROCK1 expression in gastric cancer tissues and BGC-823 cells was significantly upregulated compared to adjacent normal controls and GES-1 cells, respectively (all P < 0.05, Figs. 3A and 3B). In addition, Targetscan tool predicted that miR-335-5p could target ROCK1 mRNA 3'UTR (Fig. 3C). The fluorescent reporter assay showed that miR-335-5p overexpression resulted in a significantly decrease of the relative luciferase activities containing the ROCK1 3'-UTR-WT (P < 0.05), but not the relative luciferase activities containing the ROCK1 3'-UTR-MUT (Fig. 3D). Therefore, GES-1 cells were transfected with miR-335-5p inhibitor or inhibitor control, while BGC-823 cells were transfected with miR-335-5p mimic or mimic control. After 48 of different transfection, miR-335-5p expression was successfully suppressed in miR-335-5p inhibitor-transfected GES-1 cells and was obviously overexpressed in miR-335-5p mimic-transfected BGC-823 cells (P < 0.05, Fig. 3E). Oppositely, ROCK1 expression in miR-335-5p inhibitor-transfected GES-1 cells was markedly decreased compared to that of inhibitor control-transfected GES-1 cells, while ROCK1 expression was obviously increased in miR-335-5p mimic-transfected BGC-823 cells relative to mimic control-transfected BGC-823 cells (P < 0.05, Fig. 3F). There data indicated that miR-335-5p could target and inhibits ROCK1 in gastric cancer cells.

### 2.4. *miR-335-5p may mediate gastric cancer cell proliferation, migration and invasion via regulating ROCK1*

To further investigate whether miR-335-5p may mediate the malignant behaviors of gastric cancer cells *via* regulating ROCK1, BGC-823 cells were transfected with miR-335-5p mimic and/or pc-ROCK1. As

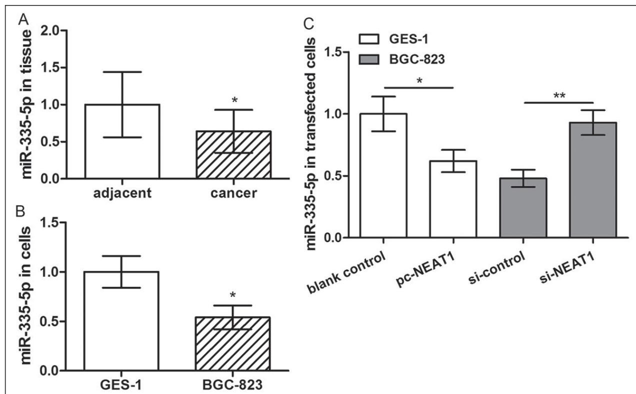


Fig. 2: LncRNA NEAT1 inhibited miR-335-5p expression in gastric cancer cells. A: qRT-PCR showed miR-335-5p expression in gastric cancer tissues and adjacent normal controls. B: qRT-PCR showed miR-335-5p expression in gastric cancer BGC-823 cells and nonmalignant gastric mucosa epithelial GES-1 cells. C: qRT-PCR showed NEAT1 expression in GES-1 cells transfected with pc-NEAT1 or its blank control and BGC-823 cells transfected with si-NEAT1 or si-control. * P<0.05 and ** P<0.01 compared with corresponding control.

mimic and pc-ROCK1 (P < 0.05, Figs. 4B and 4C). Similar effects were obtained in a Transwell assay that the migration and invasion of BGC-823 cells were significantly suppressed after miR-335-5p overexpression, which were markedly promoted after cotransfection with miR-335-5p mimic and pc-ROCK1 (P < 0.05, Figs. 4D and 4E). These data indicated that miR-335-5p might mediate gastric cancer cell proliferation, migration and invasion via regulating ROCK1.

## 3. Discussion

In the present study, we performed a comprehensive analysis of NEAT1-miR-335-5p-ROCK1 axis in the development of gastric cancer. The results showed that NEAT1 was upregulated in gastric cancer tissues and cells. Upregulated NEAT1 significantly promoted the proliferation, migration and invasion of BGC-823 cells, while suppression of NEAT1 exhibited contrary results. In addition, lncRNA NEAT1 inhibited the expression of miR-335-5p and miR-335-5p overexpression significantly inhibited cell proliferation, migration and invasion. Besides, ROCK1 was a target of miR-335-5p and effects of miR-335-5p overexpression on cell proliferation, migration and invasion were counteracted by ROCK1 overexpression concurrently. The regulatory mechanism of NEAT1-miR-335-5p-ROCK1 axis is shown in Fig. 5. These findings imply the roles of NEAT1-miR-335-5p-ROCK1 axis in the development of gastric cancer and merit further discussion.

It has been reported that NEAT1 contributes to the development of several cancer *via* regulation of miRNAs and their targets: Lu et al. (2016) demonstrated that NEAT1 regulated epithelial to

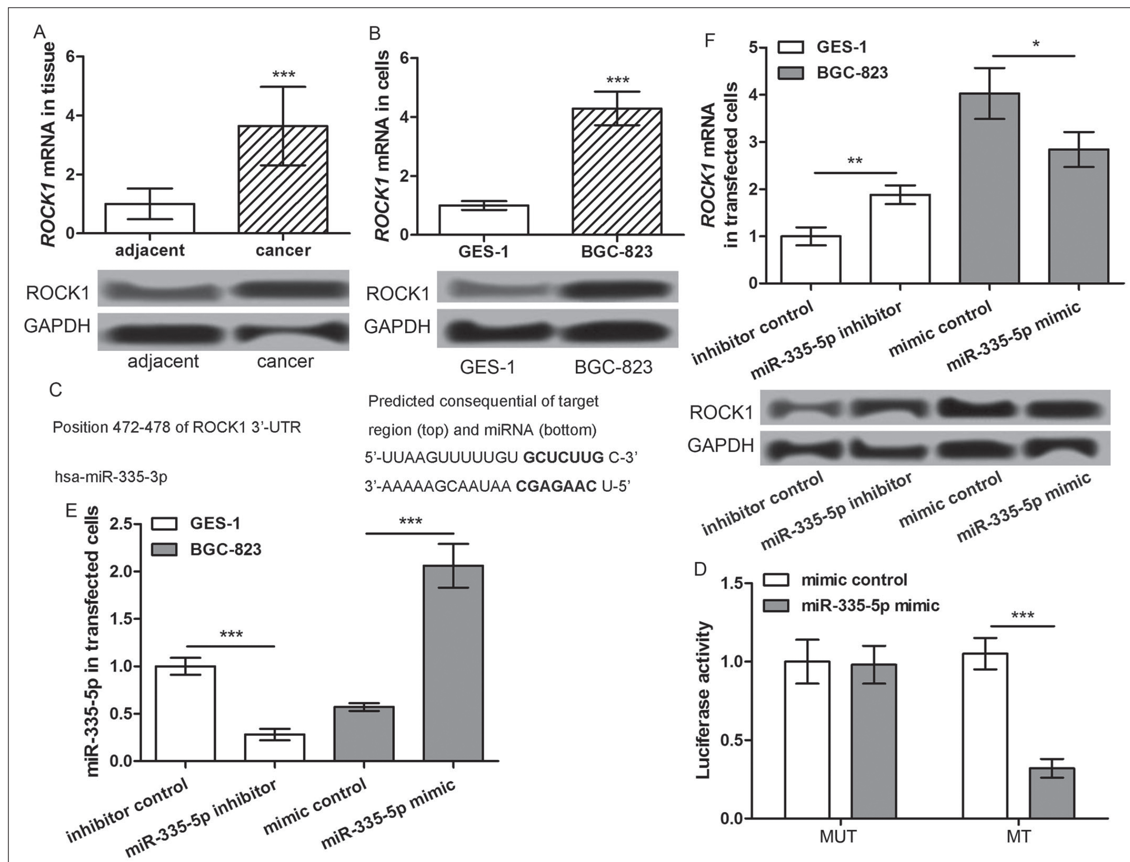


Fig. 3: miR-335-5p targeted and inhibited ROCK1 in gastric cancer cells. A: ROCK1 expression in gastric cancer tissues and adjacent normal controls. B: ROCK1 expression in gastric cancer BGC-823 cells and nonmalignant gastric mucosa epithelial GES-1 cells. C: Targetscan tool predicted the target sequence between miR-335-5p and ROCK1. D: Fluorescent reporter assay showed that the relative luciferase activities containing the ROCK1 3'-UTR-WT or ROCK1 3'-UTR-MUT after transfection with miR-335-5p mimic or mimic control. E: miR-335-5p expression in GES-1 cells transfected with miR-335-5p inhibitor or inhibitor control, and BGC-823 cells transfected with miR-335-5p mimic or mimic control. F: ROCK1 expression in GES-1 cells transfected with miR-335-5p inhibitor or inhibitor control, and BGC-823 cells transfected with miR-335-5p mimic or mimic control. * P<0.05, ** P<0.01 and *** P<0.001 compared with corresponding control.

shown in Fig. 4A, ROCK1 expression was significantly decreased in the miR-335-5p mimic group compared to that of mimic control group, while significantly increased in miR-335-5p mimic + pc-ROCK1 group relative to miR-335-5p mimic +blank control group (P < 0.05, Fig. 4A). MTT assay showed that BGC-823 cell viability and CEF were significantly inhibited after miR-335-5p overexpression, which was markedly increased after cotransfection with miR-335-5p

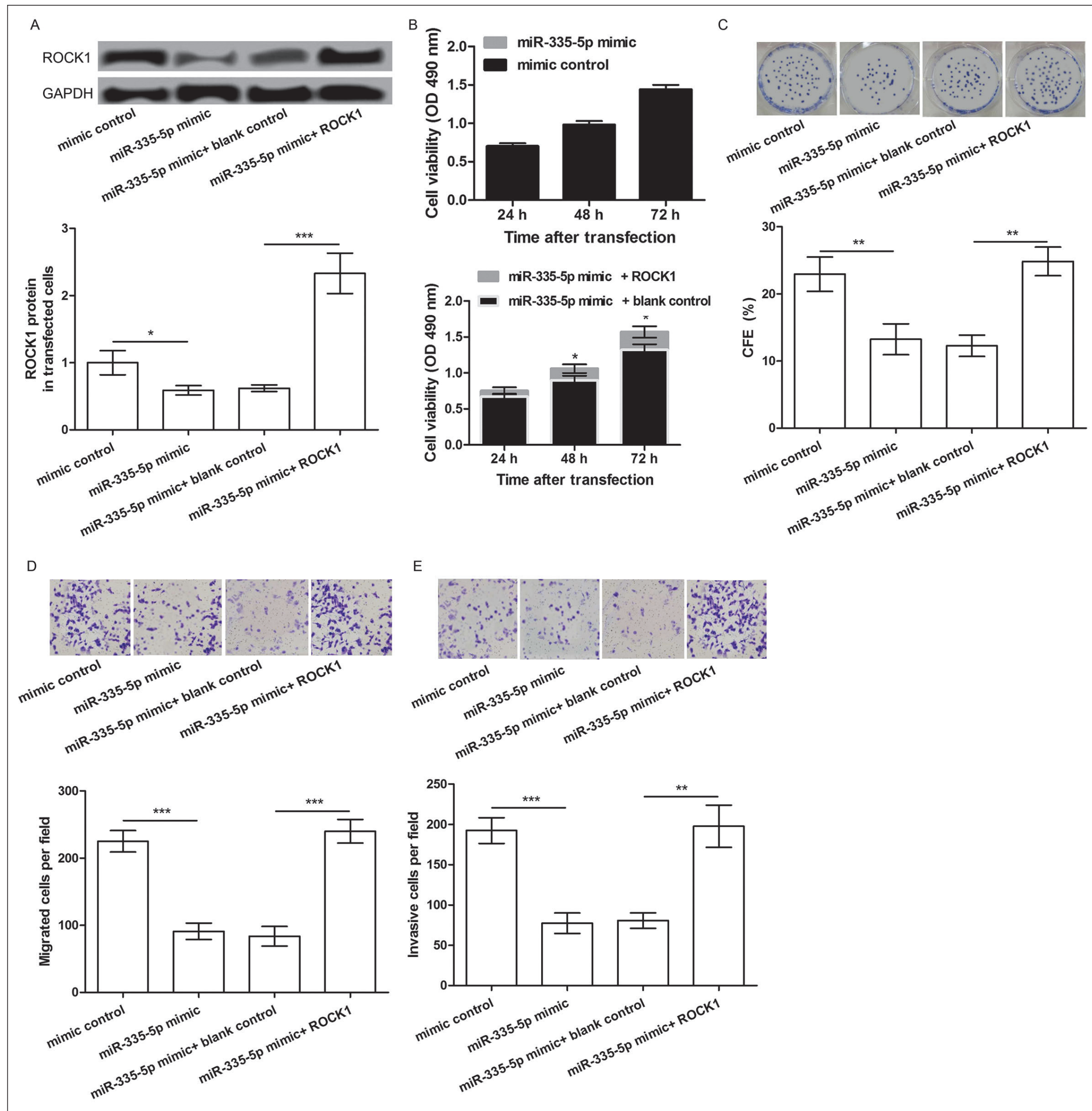


Fig. 4: miR-335-5p may mediate gastric cancer cell proliferation, migration and invasion via regulating ROCK1. BGC-823 cells were transfected with mimic control, miR-335-5p mimic and/or pc-ROCK1 or blank control. A: ROCK1 expression in different transfected groups. B: MTT assay showed that BGC-823 cell viability in different transfected groups. C: Colony assay showed the colony forming efficiency (CEF) of different transfected cells. D: Transwell assay showed the migration of different transfected cells. E: Transwell assay showed the invasion of different transfected cells. * P<0.05, ** P<0.01 and *** P<0.001 compared with corresponding control.

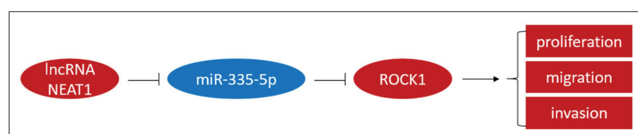


Fig. 5: The regulatory mechanism of NEAT1-miR-335-5p-ROCK1 axis in gastric cancer cells. Red nodes indicated up-regulated while blue indicated down-regulated.

mesenchymal transition and radioresistance of nasopharyngeal carcinoma cells by regulation of the miR-204/ZEB1 axis; Sun et al. (2016) confirmed that NEAT1 accelerated non-small cell lung cancer cell growth and metastasis through modulating the miR-377-3p/E2F3 axis; Peng et al. (2016) reported that NEAT1 promoted proliferation and inhibited apoptosis of laryngeal squamous cell cancer cells through targeting the miR-107/CDK6 pathway. In this study, NEAT1 was upregulated in gastric cancer tissues and cells. Upregulated NEAT1 significantly promoted the proliferation, migration and invasion of BGC-823 cells. Moreover, NEAT1 inhibited the expression of miR-335-5p and ROCK1 was a target of miR-335-5p. These finding imply that the roles of NEAT1 in gastric cancer may be mediated by miR-335-5p/ROCK1 axis. Notably, NEAT1 is found to regulate the malignant behaviors of pancreatic cancer cells though inhibiting miR-335-5p (Cao et al. 2016). miR-335-5p can suppress the proliferation and invasion of epithelial ovarian carcinoma cells (Wang et al. 2016). miR-335-5p is found to be a key regulator to mediate the tumor-promoting function of lncRNA taurine upregulated gene 1 (TUG1) in osteosarcoma cells *via* targeting ROCK1 (Yong et al. 2013). MicroRNA-335 was also found to suppress bladder cancer cell growth, migration and invasion *via* regulating ROCK1 (Wu et al. 2016). ROCK1 is required for anchorage-independent growth and invasion of non-small cell lung cancer cells (Vigil et al. 2012). In prostate and colorectal cancer cells, the activation of ROCK1 by RhoA can promote cell invasion and motility (Lin et al. 2008; Wilkinson et al. 2005). Furthermore, Cai et al. (2015) reported that miR-144 inhibited rectal cancer cell migration and proliferation through targeting ROCK1. Zheng et al. (2016) revealed that miR-145 could inhibit breast cancer cell growth and migration via targeting ROCK1. In this study, ROCK1 was a target of miR-335-5p and effects of miR-335-5p overexpression on cell proliferation, migration and inva-

sion were counteracted by ROCK1 overexpression concurrently. These finding imply that ROCK1 may be a downstream factor of NEAT1- miR-335-5p to control the development of gastric cancer. In conclusion, our findings indicate that upregulation of NEAT1 may promote proliferation, migration and invasion of gastric cancer cells *via* targeting the miR-335-5p/ROCK1 axis. NEAT1-miR-335-5p-ROCK1 axis may be a potential therapeutic strategy for gastric cancer therapy.

## 4. Experimental

### 4.1. Tissue samples and cell lines

Tissue samples and adjacent normal controls were obtained by surgical resection from 12 individuals diagnosed as gastric cancer patients at our hospital. Those patients had not been treated by radiotherapy or chemotherapy before surgery. All samples were frozen in liquid nitrogen immediately and stored at -80 °C. The study was approved and supervised by the local ethics committee.

Human gastric cancer cell line BGC-823 and nonmalignant gastric mucosa epithelial cell line GES-1 were obtained from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). All cells were cultured in RPMI1640 (Invitrogen, USA) supplemented with 10% fetal bovine serum (Invitrogen, US) at 37 °C in an humidified 5% $CO_2$ atmosphere.

### 4.2. Cell transfection

Cells were cultured in a 6-well cell culture plate for 24 h. GES-1 cells were transfected with pc-NEAT1, blank control, miR-335-5p inhibitor, and inhibitor control. BGC-82 cells were transfected with si-NEAT1, si-control, miR-335-5p mimic, mimic control, and/or pc-ROCK1. Cell transfection was proceeded using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's protocol.

### 4.3. MTT assay

The viability of cells was measured using 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) assay. In brief, transfected cells were seeded in a 96-well plate, and then cultured with 50 μL of MTT solution at 37 °C for 4 h. Then, 150 μL of dimethylsulfoxide was added into each well to lyse the formazan crystals formed in viable cells at room temperature for 15 min. The number of viable cells was measured at 490 nm with a spectrophotometer (Eppendorf, Germany).

### 4.4. Colony assay

Cell proliferation was performed using colony assay. Single-cell suspension was prepared using trypsin treatment. Cells, seeded into cell culture plates at a density of 200 cells/well (6-well), were cultured for 14 days at 37 °C. Afterwards, cells were washed with phosphate buffer twice and stained with a mixture of 0.5% crystal violet and 6.0% glutaraldehyde for 1 h at 37 °C. The plates were air dried at room temperature. Colonies was counted under microscope (Leica, Germany).

### 4.5. Cell migration and invasion assay

Cell migration and invasion were determined by Transwell assay. For cell invasion, the Transwell chambers (8 μm pore size; Corning Costar, NY, USA) were precoated with a diluted extra cellular matrix (ECM) solution. Briefly, single-cells were suspended in serum-free medium with 5% FBS and added into each well of the upper chamber. In the lower chamber, medium contained 10% FBS was added to be considered as a chemoattractant. After incubation for 24 h at 37 °C in 5% $CO_2$, cells that had not migrated and invaded upper chamber were wiped by cotton swabs. Then, the migrated or invaded cells were fixed, stained with 2% crystal violet, ultimately, counted under microscope (Olympus, Tokyo, Japan).

### 4.6. Luciferase reporter assay

Dual-luciferase activity assay was performed to evaluate the relationship of miR-335-5p and ROCK1. The full-length 3'UTR of ROCK1 mRNA, which contains miR-335-5p binding site, was amplified by PCR, and then inserted into the Xba1-site of pGL3 vector (Promega, WI, USA). The pGL3-ROCK1-3'-UTR-mut reporter containing point mutations in the seed sequence was synthesized using a site-directed mutagenesis kit (Stratagene, CA, USA). Subsequently, $1 \times 10^6$ cells were cotransfected with 50 pmol of miR-335-5p mimic (or mimic control), 1 μg of pGL3-ROCK1-3'-UTR-wt (or pGL3-ROCK1-3'-UTR-mut) plasmid, and 1 μg of Renilla luciferase expression vector using Lipofectamine 2000 (Invitrogen Inc., Carlsbad, CA, USA). Cell luciferase activity was detected according to the Dual-Luciferase® Reporter Assay System (Promega, WI, USA) after transfection for 36 h. Renilla luciferase activity was considered as an internal control. The experiment was repeated at least three times.

### 4.7. Western blotting

Total cellular extracts were obtained after the cells were washed twice using Hanks's balanced salt solution and lysed in RIPA buffer. Equal amounts of protein extracts (30 μg protein per lane) were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Polyvinylidene Fluoride (PVDF) membranes (Millipore, Boston, USA). The membranes were blocked with 5% skim milk in Tris Buffered Saline With Tween (TBST) for 1 h. The membranes were incubated overnight at 4 °C with primary antibodies to ROCK1 (1:1000, ab45171, Abcam) and GAPDH (1:1000, ab181603, Abcam), and then a secondary antibody conjugated with horseradish peroxidase (1:5000, ab7090, Abcam) was added to culture the membrane continuously for 1 h at 37 °C. The bands in membranes were developed using the enhanced chemiluminescence reagent (Santa Cruz) after washing for 3 times with 1× Tris Buffered Saline Tween (TBST). GAPDH was used as an internal control.

### 4.8. qRT-PCR

Total RNA was extracted from tissue samples and cells with Trizol reagent (Invitrogen, US) according to the manufacturer's instructions. The concentration and purity of isolated RNA were measured with Ultraviolet spectrophotometer (Merinton, Shanghai, China). Then RNA was reverse transcribed into cDNA using PrimeScript RT Reagent Kit (Applied Biosystems, Foster City, CA, USA) in accordance with the supplier's protocol. The qRT-PCR reactions were performed in the ABI 7500 system (Applied Biosystems, CA, USA). Three independent experiments were performed for each sample. GAPDH was used as an internal control and the relative expression levels of interest gene were calculated with the $2^{-\Delta\Delta CT}$ method.

### 4.9. Statistical analysis

Data are presented as mean±standard deviation (SD). Statistical significance was analyzed with graph prism 5.0 software (GraphPad Prism, San Diego, CA). The difference between two groups was performed using Student's t-test. The difference among groups was calculated by Post-hoc Turkey-test in one-way ANOVA. A value of $P < 0.05$ was considered to indicate statistically significant.

Acknowledgement: This work was supported by Anhui Medical University Clinical Research Project of 2015, Grant No: 2015xkj081.

Conflicts of interest: None declared.

### References

Cai SD, Chen JS, Xi ZW, Zhang LJ, Niu ML, Gao ZY (2015) MicroRNA-144 inhibits migration and proliferation in rectal cancer by downregulating ROCK-1. Mol Med Rep 12: 163-167.

Cao J, Zhang Y, Yang J, He S, Li M, Yan S, Chen Y, Qu C, Xu L (2016) NEAT1 regulates pancreatic cancer cell growth, invasion and migration though microRNA-335-5p/c-met axis. Am J Cancer Res 6: 2361-2374.

Carcas LP (2013) Gastric cancer review. J Carcinog 13: 14.

Chen X, Kong J, Ma Z, Gao S, Feng X (2015) Up regulation of the long non-coding RNA NEAT1 promotes esophageal squamous cell carcinoma cell progression and correlates with poor prognosis. Am J Cancer Res 5: 2808-2815.

Chen X, Fan S, Song E (2016a) Noncoding RNAs: new players in cancers. Adv Exp Med Biol 927: 1-47.

Chen ZJ, Zhang Z, Xie BB, Zhang HY (2016b) Clinical significance of up-regulated lncRNA NEAT1 in prognosis of ovarian cancer. Eur Rev Med Pharmacol Sci 20: 3373-3377.

Fatima R, Akhade VS, Pal D, Rao SM (2015) Long noncoding RNAs in development and cancer: potential biomarkers and therapeutic targets. Mol Cell Ther 3: 5.

Gryko M, Prycznicz A, Dawidziuk T, Zińczuk J, Cepowicz D, Kamocki Z, Kedra B, Guzińskaustymowicz K (2014) Comparison of expression of selected proteins in the cells of intestinal and diffuse type gastric cancer – immunohistochemical analysis. Progress Health Sci 4: 158-164.

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61: 69-90.

Lin SL, Chiang A, Chang D, Ying SY (2008) Loss of mir-146a function in hormone-refractory prostate cancer. RNA 14: 417-424.

Lordick F, Kang Y-K, Chung H-C, Salman P, Oh SC, Bodoky G, Kurteva G, Volovat C, Moiseyenko VM, Gorbunova V (2013) Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): a randomised, open-label phase 3 trial. Lancet Oncol 14: 490-499.

Lu Y, Tao L, Wei G, Liu L, Chen Q, Xu L, Zhang K, Zeng D, Liao R (2016) The long non-coding RNA NEAT1 regulates epithelial to mesenchymal transition and radioresistance in through miR-204/ZEB1 axis in nasopharyngeal carcinoma. Tumor Biol 37: 1-9.

Martens-Uzunova ES, Böttcher R, Croce CM, Jenster G, Visakorpi T, Calin GA (2014) Long noncoding RNA in prostate, bladder, and kidney cancer. Eur Urol 65: 1140-1151.

Peng W, Wu T, Han Z, Jin Q, He G, Yu H, Xuan L, Xin W, Tian L, Sun Y (2016) Long noncoding RNA NEAT1 promotes laryngeal squamous cell cancer through regulating miR-107/CDK6 pathway. J Exp Clin Cancer Res 35: 22.

Prensner JR, Chen W, Iyer MK, Cao Q, Ma T, Han S, Sahu A, Malik R, Wilderromans K, Navone N (2014) PCAT-1, a long noncoding RNA, regulates BRCA2 and controls homologous recombination in cancer. Cancer Res 74: 1651-1660.

Shi X, Sun M, Liu H, Yao Y, Song Y (2013) Long non-coding RNAs: a new frontier in the study of human diseases. Cancer Lett 339: 159-166.

Shi Y, Zhou Y (2010) The role of surgery in the treatment of gastric cancer. J Surg Oncol 101: 687-692.

Sun C, Li S, Zhang F, Xi Y, Wang L, Bi Y, Li D (2016) Long non-coding RNA NEAT1 promotes non-small cell lung cancer progression through regulation of miR-377-3p-E2F3 pathway. Oncotarget 7: 51784-51814.

Vemuganti R (2013) All's well that transcribes well: non-coding RNAs and post-stroke brain damage. Neurochem Int 63: 438-449.

Vigil D, Tai YK, Plachco A, Garton AJ, Castaldo L, Pachter JA, Dong H, Xin C, Tokar B, Campbell SL (2012) ROCK1 and ROCK2 are required for non-small cell lung cancer anchorage-independent growth and invasion. Cancer Res 72: 5338-5347.

Wadhwa R, Taketa T, Sudo K, Blum MA, Ajani JA (2013) Modern oncological approaches to gastric adenocarcinoma. Gastroenterol Clin North Am 42: 359-369.
Wang S, Li J, Ouyang L, Zhang S (2016) MiRNA-335-5p inhibits cell proliferation and invasion through regulating DKK1 in epithelial ovarian carcinoma. Int J Clin Exp Pathol 9: 8083-8090.
Wilkinson S, Paterson HF, Marshall CJ (2005) Cdc42-MRCK and Rho-ROCK signalling cooperate in myosin phosphorylation and cell invasion. Nature Cell Biol 7: 255-261.
Wu D, Niu X, Pan H, Zhou Y, Qu P, Zhou J (2016) MicroRNA-335 is downregulated in bladder cancer and inhibits cell growth, migration and invasion via targeting ROCK1. Mol Med Rep 13: 4379-4385.
Yan X, Hu Z, Feng Y, Hu X, Yuan J, Zhao SD, Zhang Y, Yang L, Shan W, He Q (2015) Comprehensive genomic characterization of long non-coding RNAs across human cancers. Cancer Cell 28: 529-540.
Yin KJ, Hamblin M, Chen YE (2014) Non-coding RNAs in cerebral endothelial pathophysiology: Emerging roles in stroke. Neurochem Int 77: 9-16.
Yong W, Wei Z, Qin F (2013) miR-335 suppresses migration and invasion by targeting ROCK1 in osteosarcoma cells. Mol Cell Biochem 384: 105-111.
Zhang M, Wu WB, Wang ZW, Wang XH (2017) lncRNA NEAT1 is closely related with progression of breast cancer via promoting proliferation and EMT. Eur Rev Med Pharmacol Sci 21: 1020-1026.
Zheng M, Xu S, Li Y, Zuo W (2016) MicroRNA-145 inhibits growth and migration of breast cancer cells through targeting oncoprotein ROCK1. Tumor Biol 37: 8189-8196.