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LncRNA LOC554202 promotes proliferation and migration of gastric cancer cells through regulating p21 and E-cadherin

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Abstract. – OBJECTIVE: To explore whether long noncoding RNA (lncRNA) LOC554202 could regulate proliferative and migratory abilities of gastric cancer (GC) cells.

MATERIALS AND METHODS: Expression level of LOC554202 in GC cell lines HGC-27 and MGC-803, as well as normal gastric mucosal cell line GES-1 was detected by quantitative real-time polymerase chain reaction (qRT-PCR). LOC554202 knockdown or overexpression in HGC-27 and MGC-803 cells was achieved by transfection of LOC554202-siRNA or pcDNA-LOC554202, respectively. Cell cycle in GC cells was accessed by flow cytometry. Migratory ability of GC cells was determined by wound healing assay and transwell assay. Finally, protein expressions of p21 and E-cadherin in GC cells were detected by Western blot.

RESULTS: LOC554202 expression was higher in GC cells than that of gastric mucosal cells ($p<0.01$). Overexpression of LOC554202 in MGC-803 cells enhanced proliferative and migratory abilities, but decreased protein expressions of p21 and E-cadherin ($p<0.01$). On the contrary, LOC554202 overexpression in HGC-27 cells decreased proliferative and migratory abilities, but increased protein expressions of p21 and E-cadherin ($p<0.01$).

CONCLUSIONS: LncRNA LOC554202 is highly expressed in GC cells. It could promote proliferative and migratory abilities by downregulating expression levels of p21 and E-cadherin in GC cells.

Key Words:
LncRNA LOC554202, GC, Proliferation, Migration, P21, E-cadherin.

Introduction

In China, gastric cancer (GC) manifests as high mortality and poor prognosis, seriously threating human health[1]. RNA has gradually become one of the hotspots in the post-genome researches. More than 98% of the genomic transcripts are noncoding RNAs (ncRNAs). Among them, long noncoding RNAs (lncRNAs) are a class of RNAs with a transcript length of more than 200 nt that do not encode proteins. LncRNA participates in the development of various diseases by regulating gene expressions at epigenetic, transcriptional or post-transcriptional levels[2-4]. Researches have shown that lncRNA exerts an important role in the occurrence and development of tumor diseases, which has gradually been well recognized. Researchers have found out some lncRNAs that are related to GC. Some certain lncRNAs in GC participate in the occurrence and progression of GC, which are served as potential diagnostic hallmarks[5-7]. It is reported that lncRNA00152, OR3A4, MALAT1, NEAT1, SNHG17 and others are greatly involved in pathogenic progression of GC[8-12]. For example, SNHG15 is capable of promoting proliferation and metastasis of GC cells by regulating MMP2 and MMP9. LncRNA BC032469 upregulates hTERT expression by sponging miR-1207-5p, thereby promoting proliferative capacity of GC cells[13]. HOXA-As2 stimulates proliferation of GC cells by epigenetically downregulating P2PLK3/DD3 axis[14]. It is reported that LOC554202 expression in breast cancer is

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closely correlated to tumor size and clinical stage. Highly expressed LOC554202 may influence cell cycle, proliferation and metastasis of breast cancer cells[15]. This study aims to investigate whether LOC554202 could regulate pathogenic progression of GC, providing new therapeutic targets for clinical treatment of GC.

Materials and Methods

Reagent and Antibodies

TRIzol, PrimeScript RT reagent Kit with gDNAEraser and SYBR Premix Ex Taq™ were provided by TaKaRa (Otsu, Shiga, Japan); Lipofectamine 2000 was provided by Invitrogen (Carlsbad, CA, USA); LOC554202 primers, siRNA and negative control were constructed by Ribobio (Guangzhou, China); GAPDH primer was constructed by Sangon (Shanghai, China); pcDNA-LOC554202 and pcDNA3.1 were provided by Generay (Shanghai, China); transwell chambers were provided by Corning (Wuhan, China); Cell counting kit-8 (CCK-8) reagent kit was provided by Beyotime (Shanghai, China); GC cell lines MGC-803 and HGC-27 were provided by Boster (Wuhan, China); Gastric mucosal cell line GES-1 was preserved in our laboratory; Anti-p21, anti-E-cadherin were provided by Abcam (Cambridge, MA, USA); GAPDH was provided by Bioworld (St. Louis Park, MN, USA).

Cell Culture

MGC-803 and HGC-27 cells were cultured in RPMI-1640 (Roswell Park Memorial Institute-1640) (HyClone, South Logan, UT, USA) containing 10% FBS (fetal bovine serum) (Gibco, Rockville, MD, USA) and maintained in a 5% CO_2 incubator at 37°C. GES-1 cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS).

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

The TRIzol kit was used to extract the total RNA, which was then reversely transcribed into cDNA. After the complementary Deoxyribose Nucleic Acid (cDNA) was amplified, qRT-PCR was performed to detect the expressions of related genes using SYBR Premix Ex Taq II kit. Relative gene expression was detected using ABI Prism 7900HT system. Primers used in the study were: LOC554202, F: 5′-TCTCTGGTGCTTCCCTCCTT-3′, R: 5′-GATCTAAGCTTGAGCCCCCA-3′; E-cadherin, F: 5′-GGTCTGTCATGGAAGGTGCT-3′, R: 5′-CGTAGGGAAACTCTCTCGGTC-3′; p21, F: 5′-CCGAAGTCAGTTCCTTGTGG-3′, R: 5′-CATGGGTTCTGACGGACAT-3′.

Cell Transfection

MGC-803 cells were pre-seeded in the 6-well plates and transfected with pcDNA3.1 or pcDNA-LOC554202, respectively. HGC-27 cells were pre-seeded in the 6-well plates at a density of 3.0×10^5 cells per well. Until confluence of 60%, HGC-27 cells were transfected with LOC554202-siRNA1, LOC554202-siRNA2 or si-NC, respectively, according to the instructions of Lipofectamine 2000. Culture medium was replaced 6 hours later.

CCK-8 Assay

Transfected cells were seeded into 96-well plates at a density of 3.0×10^3 cells per well. 10 μL of CCK-8 solution (cell counting kit-8, Dojindo, Kumamoto, Japan) were added in each well after cell culture for 24 h, 48 h and 72 h, respectively. The absorbance at 450 nm of each sample was measure by a microplate reader.

Flow Cytometry

Transfected cells were centrifuged and resuspended in 2 mL of pre-cooled 70% ethanol. After overnight fixation, cells were centrifuged again and incubated with 10 μL of Annexin V-FITC in dark for 15 min. Finally, 300 μL of binding buffer and 5 μL of PI were added, followed by apoptosis detection using flow cytometry within 1 h.

Wound Healing Assay

Cells were seeded into 6-well plates at a density of 3.0×10^5 cells per well. A sterile 10 μL micropipette tip was used to vertically scratch the cell plate until 80% of cell confluence. After removing the exfoliated cells with phosphate-buffered saline (PBS), serum-free medium was placed for 48 h incubation. Migratory cells were observed and captured under an inverted microscope, and the width of the scratch was measured and photographed.

Transwell Assay

Cells were centrifuged and resuspended in serum-free medium at a density of 3.0×10^5/mL. 100 μL of cell suspension and 600 μL of medium containing 10% fetal bovine serum (FBS) were ad-

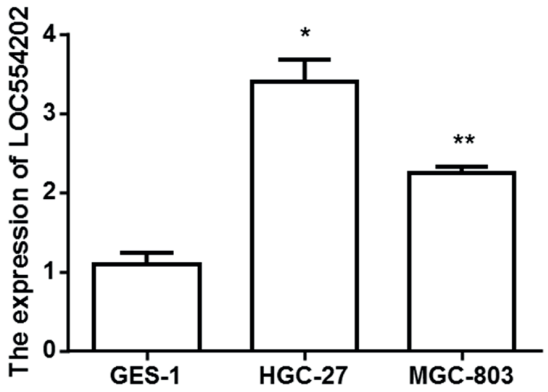

Figure 1. LOC554202 expression in GC cells and gastric mucosal cells (*p<0.05, **p<0.01, compared with GES-1 cells).

ded in the upper and lower chamber, respectively. After cell culture for 48 h, cells were fixed with 4% paraformaldehyde for 15 min and stained with crystal violet for 15 min. Inner cells were carefully cleaned. Migratory cells in 5 randomly selected fields of each sample were captured.

Western Blot

Cells were lysed for protein extraction. The concentration of each protein sample was determined by a BCA (bicinchoninic acid) kit (Abcam, Cambridge, MA, USA). Protein sample was separated by gel electrophoresis and transferred to PVDF (polyvinylidene difluoride) membranes (Millipore, Billerica, MA, USA). After incubation with primary and secondary antibody, immunoreactive bands were exposed by enhanced chemiluminescence method.

Statistical Analysis

We used Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) for statistical analysis. The quantitative data were represented as mean ± standard deviation ($\bar{x}$±s). Differences between two groups were compared using the t test and those among multiple groups were compared using one-way ANOVA, followed by post-hoc test. p<0.05 was considered statistically significant.

Results

LOC554202 Expression in GC Cells and Gastric Mucosal Cells

QRT-PCR data showed that LOC554202 expression is higher in GC cell lines (HGC-27 and MGC-803) than that of gastric mucosal cell line (GES-1). In particular, HGC-27 cells expressed the highest level of LOC554202 (Figure 1).

Transfection Efficacies of pcDNA-LOC554202 and LOC554202-siRNA in GC Cells

After transfection for 48 h, expression level of LOC554202 in GC cells was detected by qRT-PCR. MGC-803 cells transfected with pcDNA-LOC554202 expressed higher level of LOC554202 than those transfected with pcDNA3.1 (p<0.01, Figure 2A). Besides, HGC-27 cells transfected with LOC554202-siRNA1 or LOC554202-siRNA2 showed higher level of LOC554202 than those transfected with si-NC (p<0.01, Figure 2B).

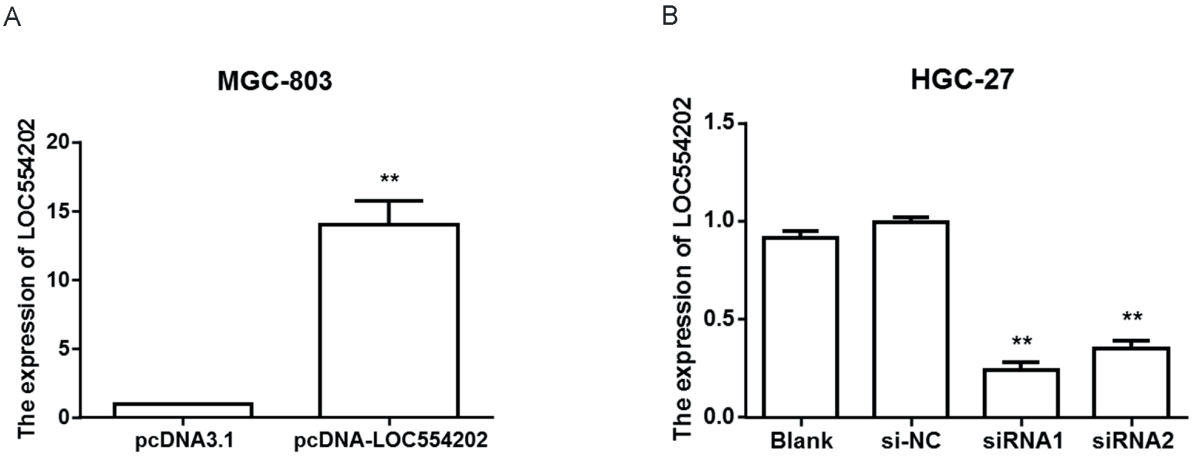

Figure 2. ***A,*** Transfection efficacies of pcDNA-LOC554202 and ***B,*** LOC554202-siRNA in GC cells (**p<0.01, compared with pcDNA3.1 or si-NC group).

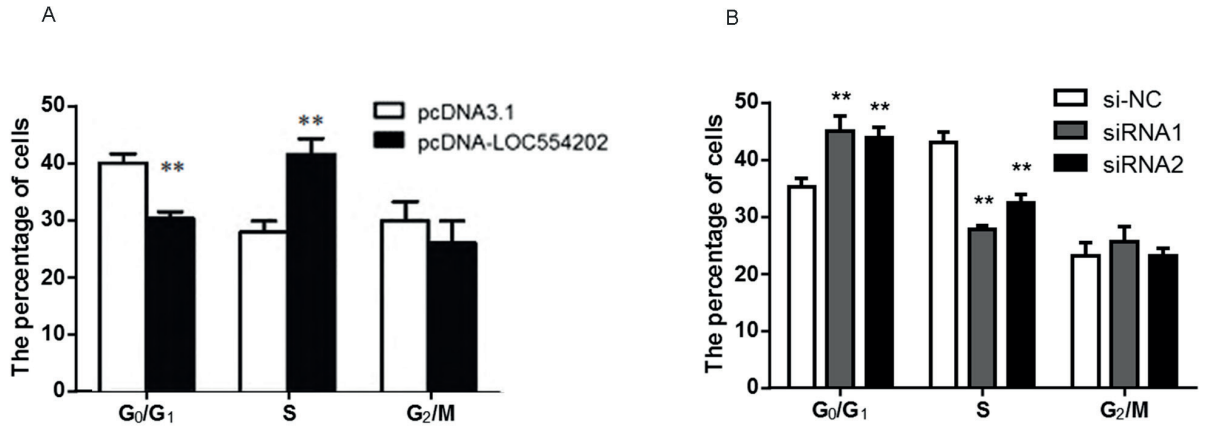

Figure 3. Effects of overexpression or knockdown of LOC554202 on cell cycle of GC cells. ***A,*** Cell cycle in MGC-803 cells after LOC554202 overexpression. ***B,*** Cell cycle in HGC-27 cells after LOC554202 knockdown (**p<0.01, compared with pcDNA3.1 or si-NC group).

Effects of Overexpression or Knockdown of LOC554202 on Cell Cycle of GC Cells

Flow cytometry showed that after transfection of pcDNA-LOC554202 for 48 h, the proportion of GC cells in G0/G1 phase significantly decreased, whereas the proportion in S phase increased than those of controls (p<0.01, Figure 3A). On the contrary, after transfection of LOC554202-siRNA in GC cells for 48 h and 72 h, the proportion in G0/G1 phase remarkably increased, whereas the proportion in S phase decreased compared with those of controls (p<0.01, Figure 3B). The above data showed that overexpression of LOC554202 could promote the proliferation of GC cells. Silencing LOC554202 could arrest GC cells in G0/G1 phase, thus inhibiting the proliferative ability.

Effects of Overexpression or Knockdown of LOC554202 on Migratory Ability of GC Cells

Wound healing assay was performed to determine the migratory ability of GC cells. Compared with GC cells transfected with pcDNA3.1, those transfected with pcDNA-LOC554202 showed increased migratory ability (p<0.01, Figure 4A). By comparison, LOC554202 knockdown decreased migratory ability of GC cells (p<0.01, Figure 4B). Subsequently, transwell assay was conducted to further evaluate the role of LOC554202 in regulating migration of GC cells. 48 hours after pcDNA-LOC554202 or pcDNA3.1 transfection, the amount of migratory cells was higher in MGC-803 cells overexpressing LOC554202 (p<0.01, Figure 5A). On the contrary, LOC554202 knockdown in HGC-27 cells remarkably decreased the amount of migratory cells (p<0.01, Figure 5B). It is suggested that high expression of LOC554202 in GC cells may contribute to the promotion of cell migration.

LOC554202 Regulated Expressions of p21 and E-Cadherin in GC Cells

Western blot results elucidated protein expressions of p21 and E-cadherin in MGC-803 cells decreased after LOC554202 overexpression (p<0.01). However, protein expressions of p21 and E-cadherin were upregulated in HGC-27 cells after LOC554202 knockdown (p<0.01, Figure 6A). QRT-PCR data showed the similar trends in mRNA levels of p21 and E-cadherin after overexpression or knockdown of LOC554202 in GC cells (p<0.01, Figure 6B).

Discussion

GC is one of the common digestive system tumors, and its pathogenesis has been widely researched in recent years. A variety of differentially expressed lncRNAs have been discovered during the development and progression of GC, such as H19, MEG3, and MALAT1[16-18]. So far, there are still many lncRNAs that have not yet been discovered, nor as their biological functions. It is of great significance to clarify the molecular mechanism of GC-related lncRNAs, so as to develop new therapeutic targets for GC. In order to investigate the role of LOC554202 in GC, we first detected its expression in GC cells and gastric mucosal cells. The data showed that LOC554202 is highly

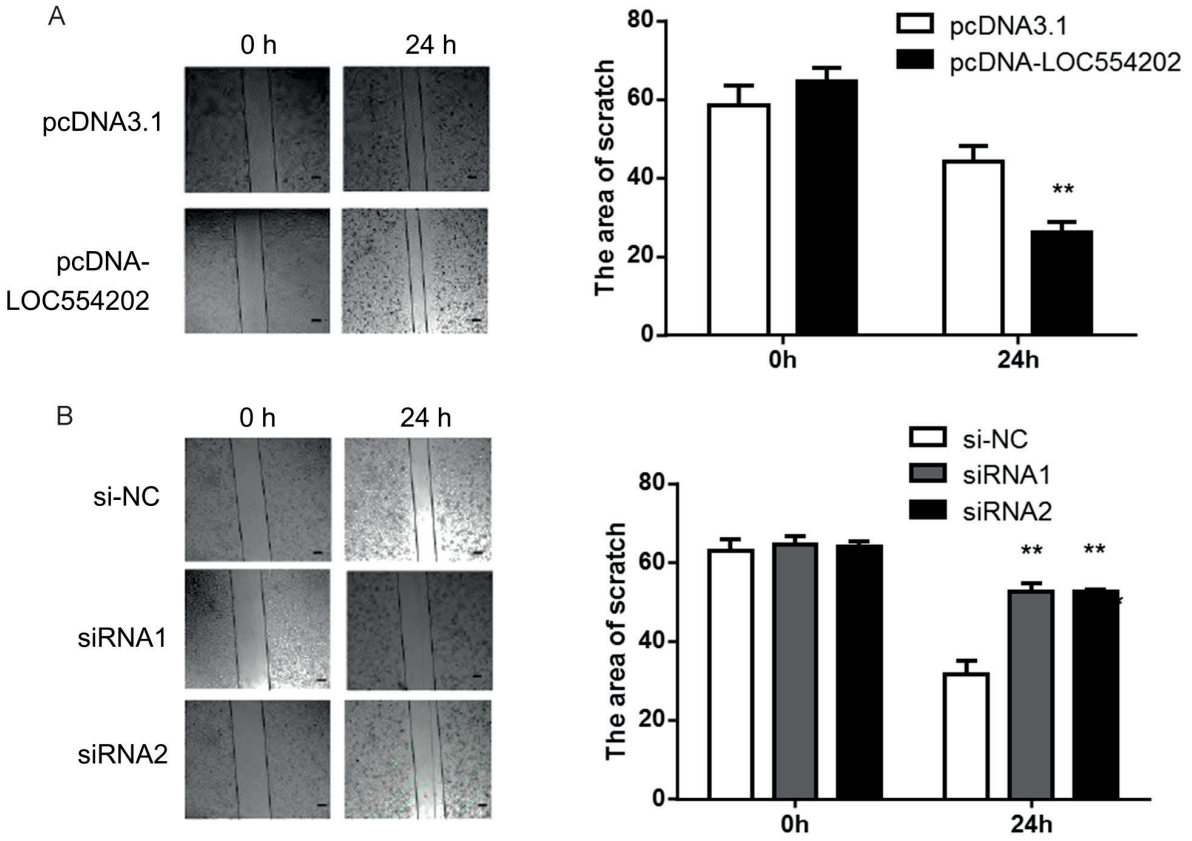

Figure 4. Effects of overexpression or knockdown of LOC554202 on migratory ability of GC cells. ***A,*** Cell migratory in MGC-803 cells after LOC554202 overexpression detected by wound healing assay. ***B,*** Cell migratory in HGC-27 cells after LOC554202 knockdown (**p<0.01, compared with pcDNA3.1 or si-NC group).

expressed in GC cells, which was consistent with the previous study[15]. Subsequently, LOC554202 was knocked down in GC cells using RNAi interference technology. CCK-8 assay demonstrated that LOC554202 knockdown decreases proliferative ability of GC cells. Flow cytometry results indicated shortened S phase and prolonged G0/G1 phase in GC cells after LOC554202 knockdown. It is suggested that LOC554202 is greatly involved in regulating the proliferation of GC cells. Previous studies pointed out that p21 could regulate cell cycle by inhibiting cyclinD1-CDK4 and cyclinE-CDK2[19, 20]. Multiple lncRNAs are found to be regulated by p21. For instance, SNHG20 regulates proliferative ability of GC cells by targeting p21[21]. PVT1 mediates proliferation and migration of pancreatic cancer cells by inhibiting p21 expression[22]. In the present study, LOC554202 knockdown upregulated p21 expression in GC cells. We considered that LOC554202 arrested GC cells in G0/G1 phase by upregulating p21 expression. Migratory ability of GC cells in this study was accessed by wound healing and transwell assay. It is elucidated that LOC554202 knockdown decreases migratory ability of GC cells, suggesting the potential role of LOC554202 in regulating cell migration. To further elucidate the molecular mechanism of LOC554202 in the regulation of GC cell migration, we determined protein expression of E-cadherin. E-cadherin is one of calcium-dependent adhesion molecules. The deletion of E-cadherin can promote the migration and invasion of tumor cells[23,24]. Relative studies[25,26] have found that some certain lncRNAs could regulate cell migration and invasion through mediating E-cadherin expression, such as lncRNA01133 and H19. Our study suggested that E-cadherin expression in GC cells is upregulated by LOC554202 knockdown. We believed that LOC554202 knockdown decreases migratory ability of GC cells mainly by upregulating E-cadherin expression. Taken together, our study found that LOC554202 is highly expressed in GC cells. Silencing of LOC554202 in GC cells inhibited the proliferative and migratory abilities. Meanwhile, protein expressions of p21 and E-cadherin were

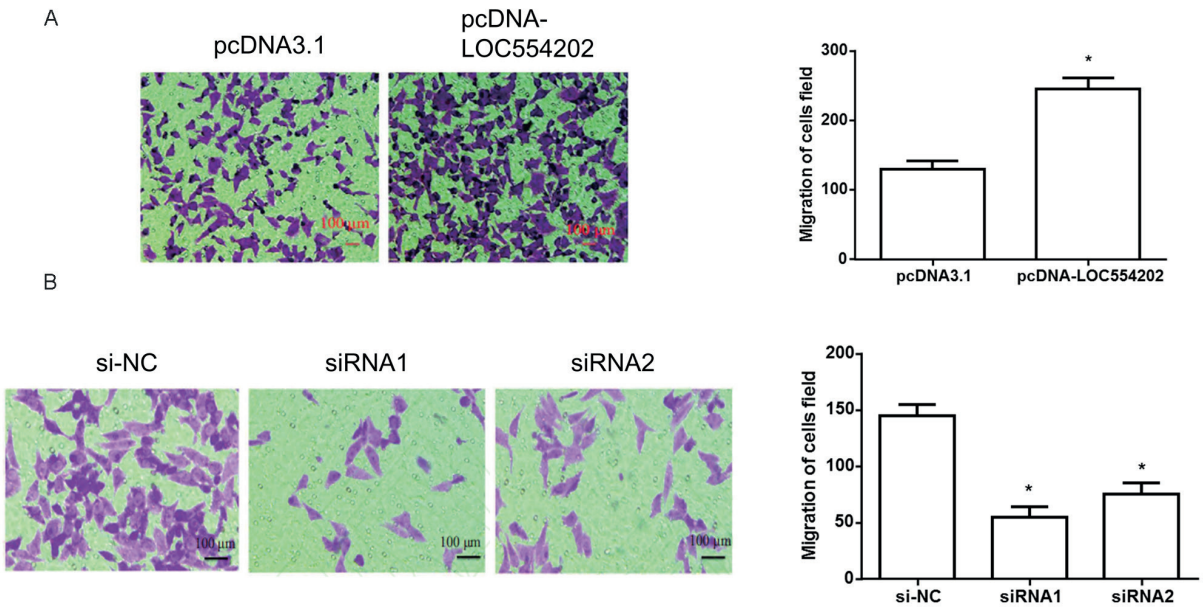

Figure 5. Effects of overexpression or knockdown of LOC554202 on migratory ability of GC cells. ***A,*** Cell migratory in MGC-803 cells after LOC554202 overexpression detected by transwell assay. ***B,*** Cell migratory in HGC-27 cells after LOC554202 knockdown (**p<0.01, compared with pcDNA3.1 or si-NC group).

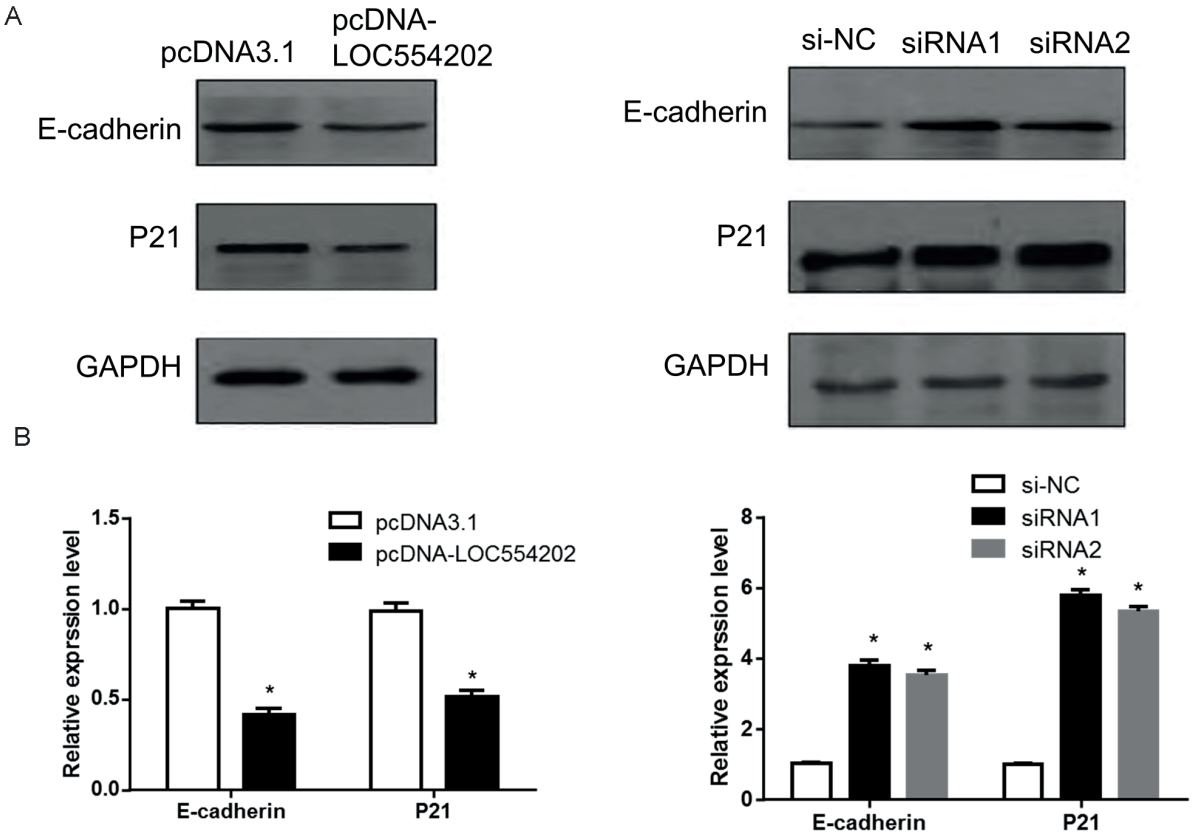

Figure 6. LOC554202 regulated expressions of p21 and E-cadherin in GC cells. ***A,*** Protein expressions of p21 and E-cadherin after overexpression or knockdown of LOC554202 in GC cells. ***B,*** The mRNA levels of p21 and E-cadherin after overexpression or knockdown of LOC554202 in GC cells (**p<0.01, compared with pcDNA3.1 or si-NC group).

upregulated after LOC554202 knockdown, suggesting that LOC554202 may affect the proliferation and migration of GC cells by regulating the expressions of p21 and E-cadherin. However, how LOC554202 regulates the expressions of p21 and E-cadherin in GC cells remains to be further studied.

Conclusions

We showed that LncRNA LOC554202 is highly expressed in GC cells. It could promote proliferative and migratory abilities by downregulating p21 and E-cadherin in GC cells.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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