Original Article
Lidocaine inhibits cervical cancer cell proliferation and induces cell apoptosis by modulating the lncRNA-MEG3/miR-421/BTG1 pathway

Jinming Zhu[1], Shichao Han[2]

[1]Affiliated Zhongshan Hospital, Dalian University, Dalian 116000, China; [2]Department of Gynecology, The 2nd Affiliated Hospital of Dalian Medical University, Dalian 116021, China

Abstract: This study aimed to explore the effect of lidocaine on the growth of cervical cancer cells (HeLa) and the underlying molecular mechanisms. Cell counting kit-8 (CCK-8) and flow cytometry (FCM) were used to detect the cell viability and apoptosis of cervical cancer cells after lidocaine treatment. Lidocaine inhibited cell viability and promoted apoptosis in HeLa cells. Long noncoding RNA maternally expressed gene 3 (lncRNA-MEG3) was significantly downregulated in cervical cancer cells, and lidocaine increased the expression of lncRNA-MEG3 in HeLa cells. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR), CCK-8, and FCM assays were used to test indicators. MEG3-shRNA promoted the cell viability and inhibited apoptosis, while the effect of lidocaine was the opposite. The effects of lidocaine on HeLa cells were reversed by MEG3-shRNA. The level of miR-421 in cervical cancer and normal cervical cells was detected using qRT-PCR. The MEG3-plasmid could inhibit cell viability and induce cell apoptosis, but these effects were reversed by miR-421 upregulation. Hence, lidocaine suppressed tumor growth by regulating cell viability and inducing apoptosis. The results indicated that BTG anti-proliferation factor 1 (BTG1) was a direct target of miR-421. HeLa cells were transfected with inhibitor control, miR-421 inhibitor, control-shRNA, or BTG1-shRNA. The negative effects of the miR-421 inhibitor or knockdown BTG1 on cell viability and apoptosis were identified using CCK-8 assay and FCM. The miR-421 inhibitor improved cervical cancer progression by regulating BTG1 expression. The results suggested that lidocaine inhibited the growth of cervical cancer cells by modulating the lncRNA-MEG3/miR-421/BTG1 signaling pathway, providing opportunities for treating cervical cancer.

Keywords: BTG1, cervical cancer cells, lidocaine, lncRNA-MEG3, miR-421

Introduction

Cervical cancer is one of the most common malignancies among women worldwide, with more than 500,000 new cases globally each year statistically [1]. Although the incidence of cervical cancer has declined over the past 10 years, the 5-year survival rate of patients is still low [2, 3]. Therefore, new and effective treatment strategies targeting cervical cancer are urgently needed. Lidocaine is a common local anesthetic mainly used for anesthesia and analgesia [4]. In recent years, it has been shown to have anti-neoplastic effects on several cancers [5, 6]; however, whether it has an inhibitory effect on cervical cancer has not been reported. This study investigated the effects of lidocaine on cervical cancer cell growth and the relative mechanism of action.

MicroRNAs (miRNAs) are a class of small RNAs with 20-22 nucleotides. They are involved in the development of tumors as oncogenes or tumor suppressor genes [7, 8]. miR-421, one of the miRNAs, has been shown to play critical roles in several cancers [9-13]. However, the role of miR-421 in cervical cancer remains unknown.

Long noncoding RNAs (lncRNAs) are usually defined as the RNA molecules that are longer than 200 nucleotides and that lack biological functions [14]. Up to date, lncRNAs have been identified to participate in cancer processes, such as cell proliferation, apoptosis, differentiation, migration, and invasion by targeting oncogenes or tumor suppressors [15-17], indicating that they may play important roles in the regulation of the eukaryotic genome. Maternally expressed gene 3 (MEG3) represents a tumor

suppressor gene located on chromosome 14-q32 [18]. The MEG3 gene has been found to suppress the progression of cervical cancer via regulating cell proliferation and apoptosis [19, 20]. However, very little is known about the biological role of MEG3 in cervical cancer treatment using lidocaine.

This study aimed to explore the regulatory effect of lidocaine on the growth of cervical cancer cells and the relationship between lidocaine treatment and lncRNA-MEG3/miR-421 signaling pathway. First, the inhibitory effect of lidocaine on cervical cancer cells and the promotion of lncRNA-MEG3 were examined. The study confirmed that miR-421 directly bound to lncRNA-MEG3. The overexpression of MEG3 suppressed cell proliferation and induced the cell apoptosis of human cervical cancer cells, while these effects were reversed by a miR-421 mimic. Moreover, BTG1 was found to be a target of miR-421 in cervical cancer cells. The downexpressed miR-421 could inhibit proliferation and increase cell apoptosis, while the effect could be reversed by BTG1-shRNA. Taken together, the study demonstrated the inhibitory effect of lidocaine on cervical cancer cells and its related mechanisms of action, providing new ideas for treating cervical cancer. The study also demonstrated the role of MEG3 and miR-421 in the growth of cervical cancer cells, thus providing a new perspective for the treatment of cervical cancer.

Materials and methods

Cell culture

Human cervical cancer cells HeLa and the normal cervical cell line H8 and 293T cells were purchased from the American Type Culture Collection (VA, USA). All cell lines were cultured in Dulbecco's modified Eagle medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco), 1% penicillin/streptomycin and incubated at 37°C in a humidified atmosphere of 5% CO_2.

Cell transfection assay

HeLa cells were seeded at a concentration of 5 × 10^4 cells/mL in six-well plates and incubated overnight. According to the manufacturer's protocol, control-shRNA, MEG3-shRNA (GenePharma, Shanghai, China), MEG3-plasmid, control-plasmid, miR-421 mimic, mimic control, MEG3-plasmid+miR-421 mimic (GenePharma,

Shanghai, China), inhibitor control, miR-421 inhibitor, BTG1-shRNA, control-shRNA, or miR-421 inhibitor+BTG1-shRNA (GenePharma) was transfected into HeLa cells using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). The cells were transfected for 48 h at 37°C, following which they were collected for further experiments. The cell transfection efficiency was detected using quantitative real-time polymerase chain reaction (qRT-PCR) and/or Western blot analysis.

miRNA target analysis and dual-luciferase reporter assay

The relationship between miR-421 and lnc-MEG3 or BTG1 was identified using Starbase (http://starbase.sysu.edu.cn/) and TargetScan Release7.2 (www.targetscan.org/vert_72). For miR-421 and lncMEG3, the 3'-UTR products of lncMEG3 containing the target sequence of miR-421 were obtained by qRT-PCR and fused to a pmirGLO vector (Promega, Madison, MI, USA) to construct the reporter vector MEG3-wild-type (MEG3-wt). Similarly, the vector MEG3-mutated-type (MEG3-mut) was formed. The 293T cells cultured for 24 h were co-transfected with MEG3-wt or MEG3-mut, and mimic control or miR-421 mimic, using Lipofectamine 2000 reagent (Invitrogen) for 48 h. The luciferase activity was analyzed using a dual-luciferase reporter assay system (Promega) following the manufacturer's manual. The consistency was tested to investigate whether BTG1 was a direct target of miR-421. The experiment was performed at least three times.

RNA extraction and qRT-PCR

Total cellular RNA was extracted from the cells using TRIzol reagent (Invitrogen) and reverse transcribed to first-strand cDNA using a cDNA Synthesis Kit (Invitrogen). All reactions were performed to quantify the relation expression of mRNA/miRNA using a Prism 7000 Real-Time PCR system with SYBR qPCR Master Mix (Vazyme, NJ, USA) according to the protocol. The primers were provided by Sangon Biotech (China). The amplification conditions included a step of 5 min at 95°C followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. GAPDH and U6 were used as the internal controls. The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method [21]. All the experiments were performed in triplicate at least.
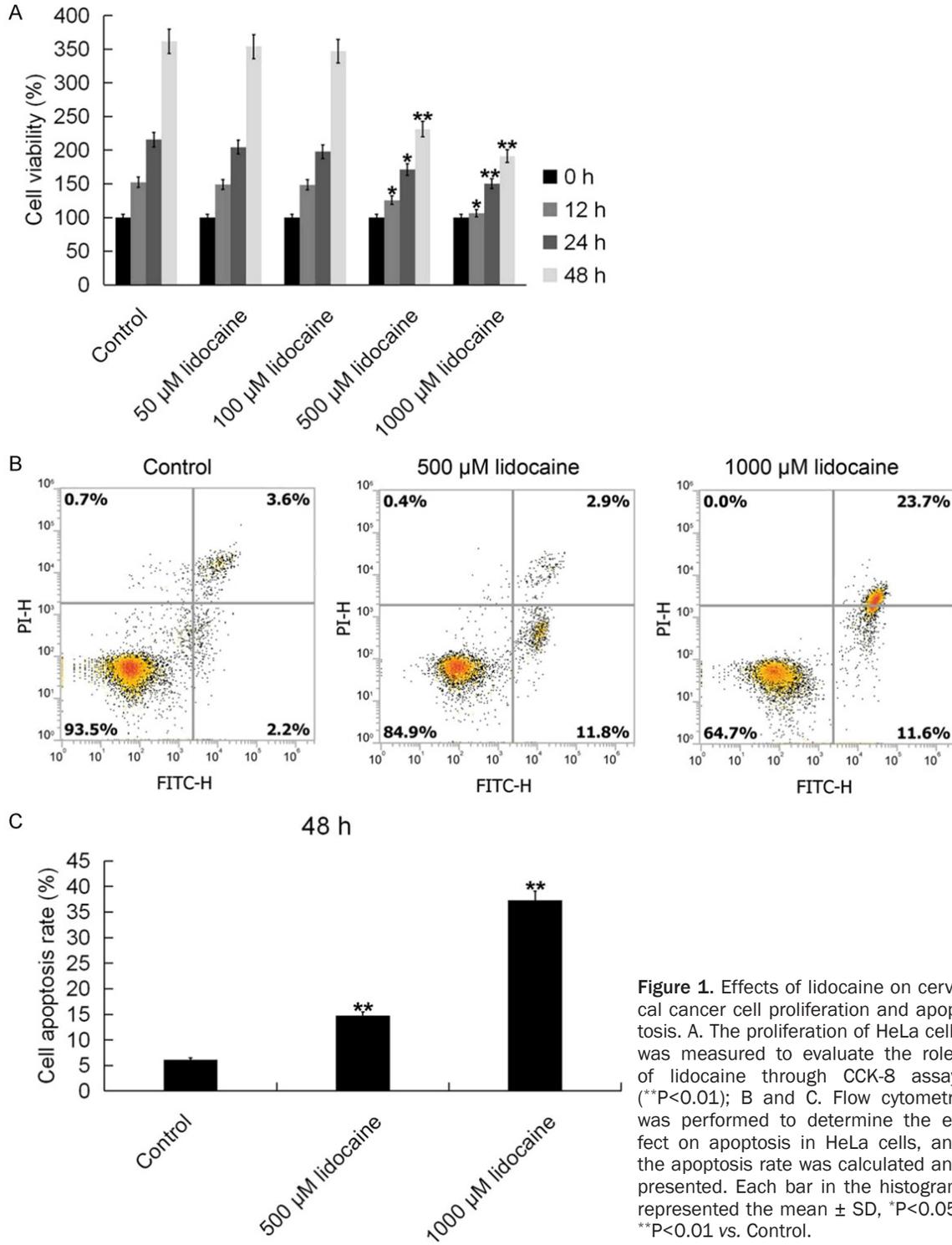
Figure 1. Effects of lidocaine on cervical cancer cell proliferation and apoptosis. A. The proliferation of HeLa cells was measured to evaluate the roles of lidocaine through CCK-8 assay. (**P<0.01); B and C. Flow cytometry was performed to determine the effect on apoptosis in HeLa cells, and the apoptosis rate was calculated and presented. Each bar in the histogram represented the mean ± SD, *P<0.05; **P<0.01 vs. Control.

Cell counting kit-8 (CCK-8) assay

Cell viability was assayed by CCK-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's protocols. Briefly, HeLa cells were seeded into 96-well plates in triplicate and incubated overnight. After specific treatment, 10 µL of CCK-8 solution was added to each well and incubated for 2 h at 37°C. Further, 100 µL of dimethyl sulfoxide (DMSO) was added to each well to solubilize the formazan product. The absorbance was then recorded at 450 nm us-
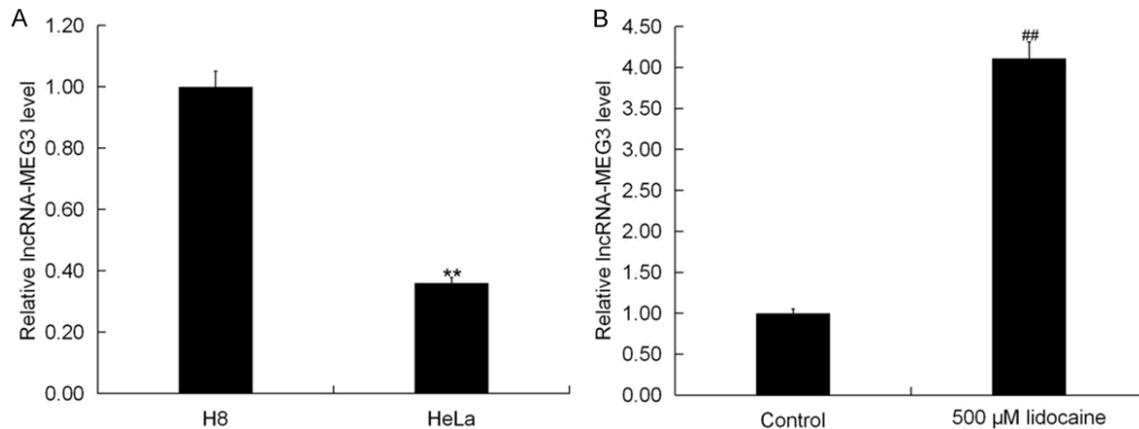
Figure 2. Lidocaine up-regulated lncRNA-MEG3 expression in cervical cancer cells. A. The expression of lncMEG3 in HeLa cells and H8 normal cervical cells was detected by qRT-PCR assay. B. Lidocaine treatment (500 μM) enhanced the expression of lncRNA-MEG3 in HeLa cells. The data were expressed as the mean ± SD. **P<0.01 vs. H8; ##P<0.01 vs. Control.

ing a microplate reader (Bio-Rad, CA, USA). The relative cell viability was normalized with the control group using optical density values.

Apoptosis assay

HeLa cells were seeded into six-well plates and incubated overnight. After specific treatment, the cells were collected by trypsinization, washed once with phosphate-buffered saline (PBS), and then resuspended in 1 × binding buffer at a density of $1 × 10^6$ cells/mL. Next, 100 μL of cell suspension was transferred to a 5-mL tube, and 5 μL of fluorescein isothiocyanate (FITC)-Annexin V and 5 μL of propidium iodide (Cat no. 70-AP101-100; MultiSciences, Hangzhou, China) were added according to the manufacturer's protocol. The stained cells were analyzed by flow cytometry (BD FACS Aria; BD Biosciences, MA, USA) within 1 h. Data were analyzed using FlowJo software (version 7.6.1; FlowJo LLC). All of the samples assayed were in triplicate.

Western blot analysis

HeLa cells were lysed with Radio Immuno Precipitation Assays (RIPA) buffer (Beyotime, Shanghai, China) after washing with cold PBS twice. The cracking products were centrifuged at 12,000 rpm and 4°C for 15 min to get the total protein, which was quantified using a bicinchoninic acid protein kit (Pierce, Rockford, IL, USA). The equal amount of protein was separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and then transferred to polyvinylidene difluoride membrane. The membrane was blocked for 1 h with PBST containing 5% nonfat milk. Then, it was incubated with BTG1 (Cat no. 151740; dilution rate: 1:1000; Abcam, MA, USA), p-Akt (Cat no. 4060; dilution rate: 1:1000; Cell Signaling Technology, MA, USA), Akt (Cat no. 4585; dilution rate: 1:1000; Cell Signaling Technology), or β-actin (Cat no. 4970; dilution rate: 1:1000; Cell Signaling Technology) antibody at 4°C overnight. The membrane was washed three times with PBST and incubated with horseradish peroxidase-conjugated secondary antibody anti-rabbit IgG (1:2,000; cat. no. 7074; Cell Signaling Technology) for 1 h at 37°C. The protein bands were visualized using an enhanced chemiluminescence (ECL) luminescent substrate (Pierce, Rockford, IL, USA) according to the manufacturer's protocol. The experiment was repeated three times at least.

Statistical analysis

Data were presented as the mean ± standard deviation of at least three independent experiments. Statistical analyses between groups were estimated by the Student *t* test or one-way analysis of variance followed by the Tukey's post-hoc test using SPSS 18.0 software package (SPSS Inc, IBM, Armonk, NY, USA). A *P* value less than 0.05 was considered as significant.

Results

*Lidocaine inhibited cell proliferation and promoted apoptosis in human cervical cancer cells*

The study investigated the effects of lidocaine on cell proliferation and apoptosis using a
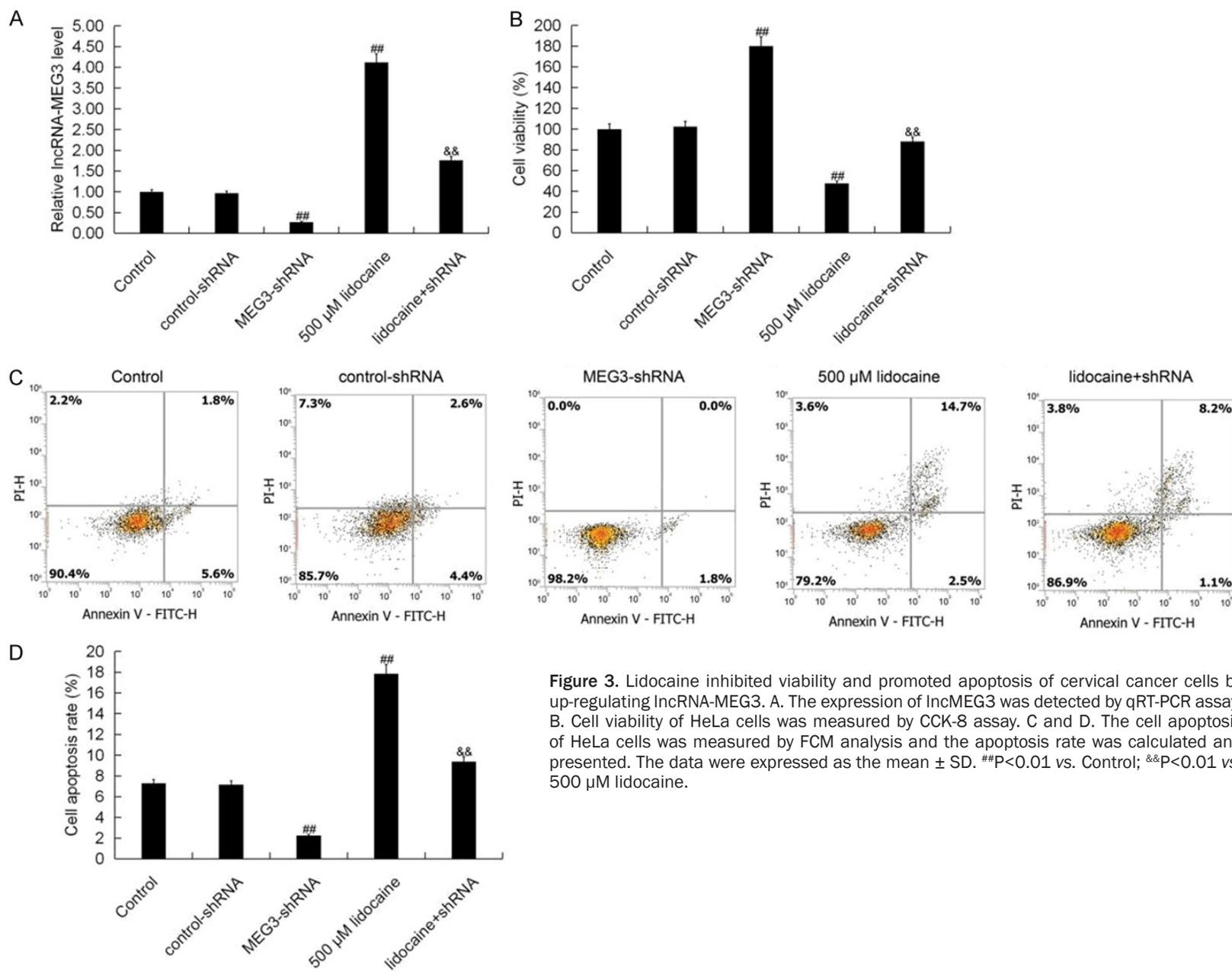
Figure 3. Lidocaine inhibited viability and promoted apoptosis of cervical cancer cells by up-regulating lncRNA-MEG3. A. The expression of lncMEG3 was detected by qRT-PCR assay. B. Cell viability of HeLa cells was measured by CCK-8 assay. C and D. The cell apoptosis of HeLa cells was measured by FCM analysis and the apoptosis rate was calculated and presented. The data were expressed as the mean ± SD. ##$P<0.01$ vs. Control; &&$P<0.01$ vs. 500 μM lidocaine.
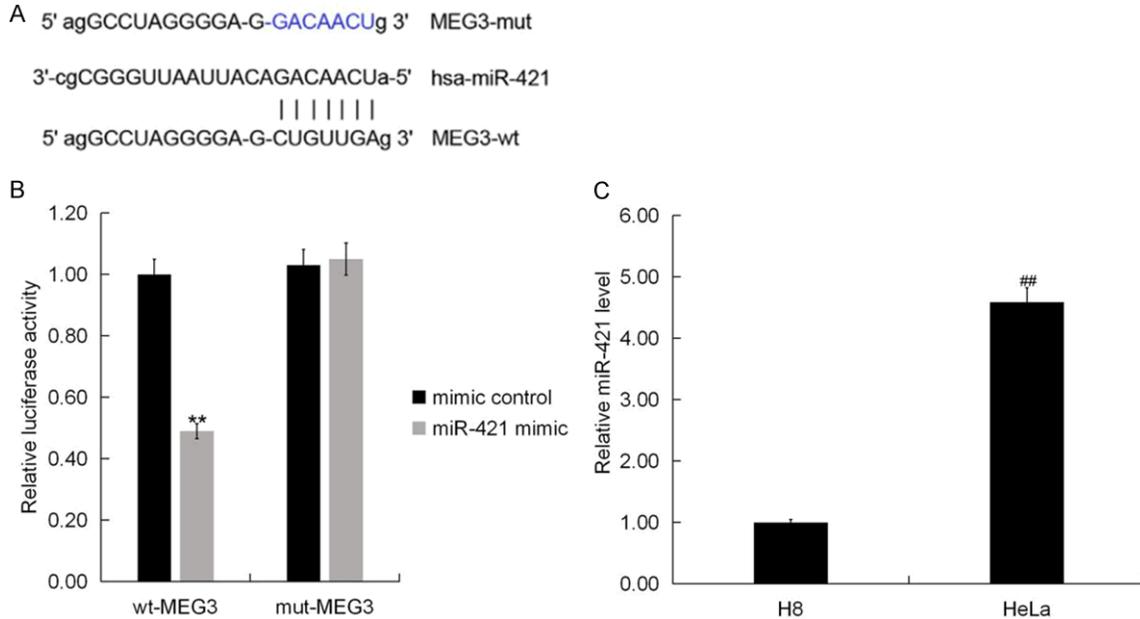
Figure 4. miR-421 bint to the 3'UTR of lncRNA-MEG3. A. The position of miR-421 target site in 3'-UTR of lncRNA-MEG3. B. Dual-luciferase reporter assay was performed to reveal the binding sites between LncRNA-MEG3 and miR-421. C. The expression of miR-421 in cervical cancer cells (HeLa) and H8 normal cervical cells was detected by qRT-PCR. The data were expressed as the mean ± SD. **P<0.01 vs. mimic control; ##P<0.01 *vs.* H8.

CCK-8 and an Annexin V-PE apoptosis detection kit, respectively. HeLa cells were treated with 50, 100, 500, or 1000 µM lidocaine for 12, 24, and 48 h. The results indicated that 500 and 1000 µM lidocaine significantly decreased HeLa cell proliferation in 12, 24, and 48 h (**Figure 1A**). Next, the increased apoptotic rate of HeLa cells was measured by flow cytometry analysis when the cells were cultured with 500 and 1000µM lidocaine for 24 h (**Figure 1B** and **1C**). The cells were treated with 500 µM lidocaine for 24 h in the following experiments.

Lidocaine increased the expression level of lncRNA-MEG3 in human cervical cancer cells

In advance, the expression level of lncRNA-MEG3 in human cervical cancer cell line HeLa and normal cervical cell line H8 was detected by qRT-PCR. The results showed that the expression of lncRNA-MEG3 was obviously downregulated in HeLa cells compared with H8 normal cervical cells (**Figure 2A**). Then, the relative gene expression of lncRNA-MEG3 after the cells were treated with 500 µM lidocaine for 24 h was examined using qRT-PCR. The treatment group had higher lncRNA-MEG3 ex-

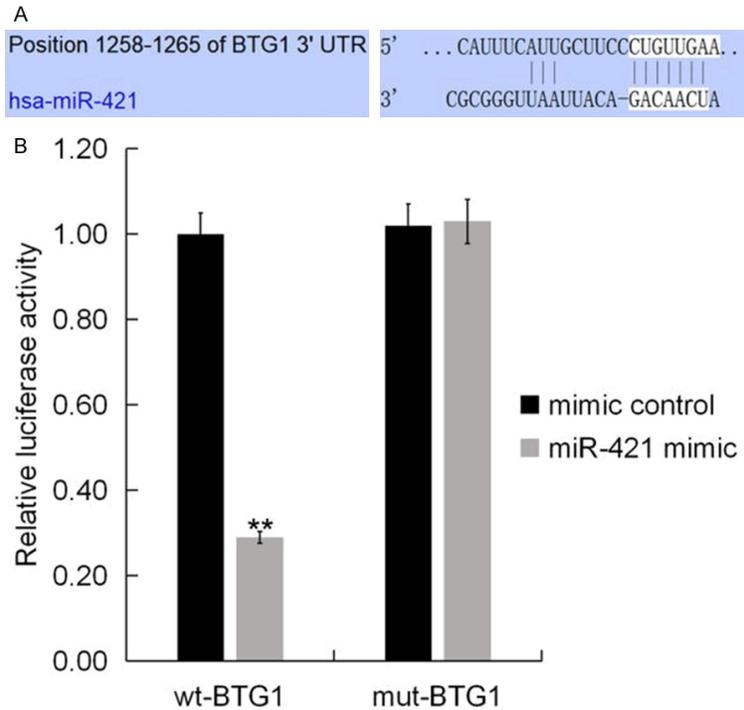pression in HeLa cells compared with the control group (**Figure 2B**).

Lidocaine influenced cell proliferation and apoptosis by upregulating lncRNA-MEG3 in human cervical cancer cells

HeLa cells were transiently transfected with control-shRNA or MEG3-shRNA and then treated with or without lidocaine (500 µM) for 24 h. Compared with the control group, the expression of lncRNA-MEG3 was significantly downregulated in the MEG3-shRNA transfection group, and 500 µM lidocaine significantly upregulated the level of lncRNA-MEG3 in HeLa cells, while lncRNA-MEG3 expression was significantly downregulated in the MEG3-shRNA + lidocaine group compared with the lidocaine-treatment-alone group (**Figure 3A**). According to the results of CCK-8 and apoptosis assays, MEG3-shRNA promoted the cell viability and inhibited the apoptosis of cervical cancer cells (HeLa) compared with the control group. Rather, lidocaine inhibited the HeLa cell viability and promoted apoptosis, and MEG3-shRNA + lidocaine (500 µM) promoted the cell vitality and inhibited apoptosis more markedly compared with lidocaine treatment alone (**Figure 3B-D**).
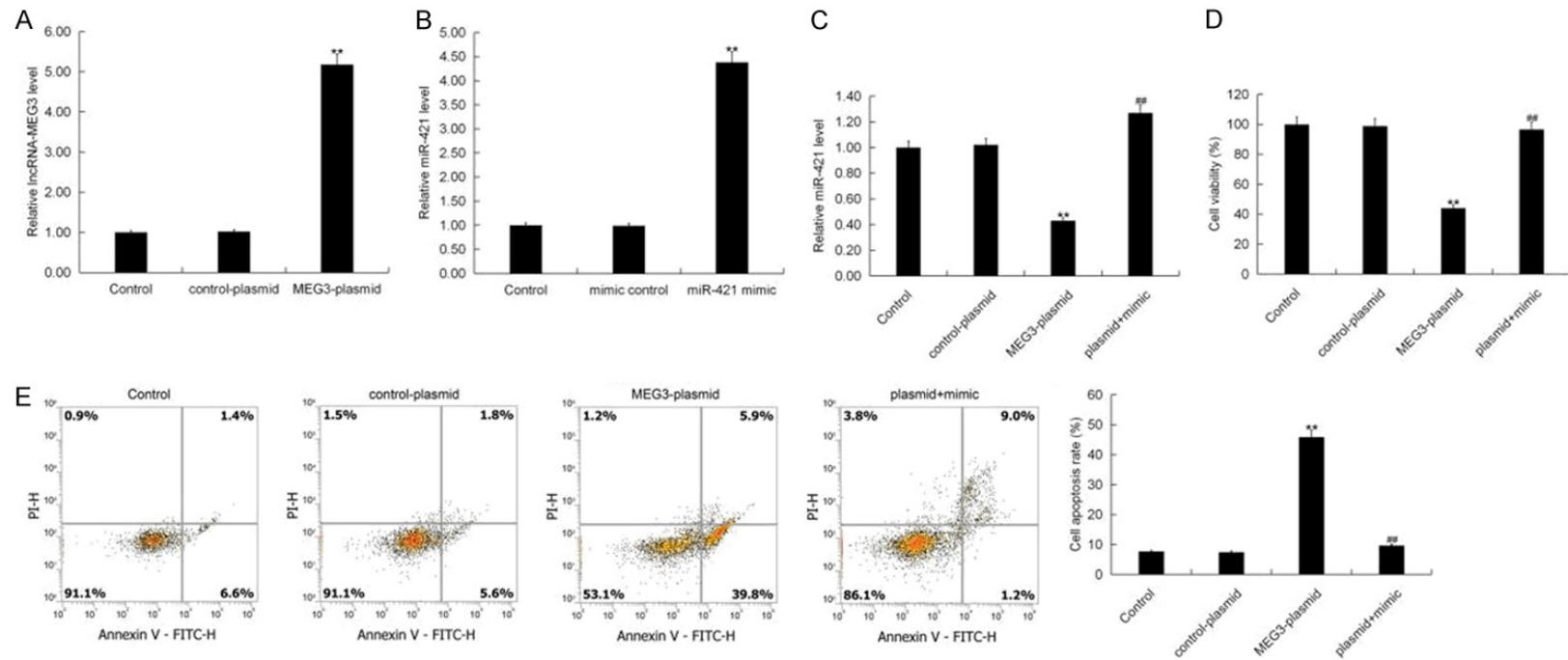
Figure 5. LncMEG3 regulated cervical cancer cell functions by regulating miR-421. A. HeLa cells were transfected with MEG3-plasmid or control-plasmid for 48 h. The mRNA expression of MEG3 in HeLa cells was detected by qRT-PCR analysis. B. HeLa cells were transfected with miR-421 mimic or mimic control for 48 h. The expression of miR-421 in HeLa cells was detected by qRT-PCR analysis. C. HeLa cells were transfected with control-plasmid, MEG3-plasmid, or MEG3-plasmid + miR-421 mimic for 48 h. The expression of miR-421 in HeLa cells was detected by qRT-PCR analysis. D. The cell viability of HeLa cells was measured through CCK-8 assay. E. Flow cytometry was performed to determine the influence on apoptosis in HeLa cells. The data were expressed as the mean ± SD. **$P < 0.01$ vs. Control; ##$P < 0.01$ vs. MEG3-plasmid.

A

Position 1258-1265 of BTG1 3' UTR   5'   ...CAUUUCAUUGCUUCCCUGUUGAA...
                                            ||| |||||||
hsa-miR-421   3'   CGCGGGUUAAUUACA−GACAACUA

B

Figure 6. BTG1 was a target of miR-421. A. The binding sites between miR-421 and BTG1 were predicted using TargetScan. B. Dual-luciferase reporter assay was performed to reveal the binding sites between miR-421 and BTG1. **$P<0.01$ vs. mimic control.

miR-421 directly bound to lncRNA-MEG3 and was upregulated in human cervical cancer cells

To study the molecular mechanisms of action of lidocaine in cervical cancer cells, the direct target of lncRNA-MEG3 was identified using the bioinformatics tool. The predicted results showed the binding sites of miR-421 and lncRNA-MEG3 (**Figure 4A**). In addition, a luciferase reporter assay was performed in 293T cells co-transfected with a luciferase vector plasmid to demonstrate the predicted binding sites of miR-421 on lncRNA-MEG3 3'-UTR. The miR-421 mimic significantly decreased the luciferase activity of lncRNA-MEG3 wild-type 3'-UTR but had no obvious effect on lncRNA-MEG3 3'UTR mut-type compared with the control group (**Figure 4B**). These results indicated that lncRNA-MEG3 was a direct target of miR-421. Then, the expression of miR-421 was explored in cervical cancer and normal cervical cells using qRT-PCR analysis. The results showed that the expression of miR-421 was significantly upregulated in HeLa cells compared

with the normal cervical cells (**Figure 4C**).

*MEG3-plasmid inhibited the miR-421 expression*

MEG3-plasmid, control-plasmid, miR-421 mimic, or mimic control was transfected into HeLa cells for 48 h to examine the effect of miR-421 and MEG3 on cervical cancer cells. After transfection, the cells were divided into six groups: control, control-plasmid, MEG3-plasmid, mimic control, miR-421 mimic, and MEG3-plasmid and miR-421 mimic co-transfection. The transfection efficiency was detected using the qRT-PCR assay. The expression level of MEG3 remarkably improved in the MEG3-plasmid group (**Figure 5A**) compared with the control group. The level of miR-421 significantly increased in the miR-421 mimic group compared with the control group (**Figure 5B**). In addition, the MEG3-plasmid significantly decreased the level of miR-421 in HeLa cells; however, the effects were reversed by the miR-421 mimic (**Figure 5C**).

MEG3-plasmid reduced the cell viability and induced apoptosis in human cervical cancer cells by downregulating miR-421 expression

The CCK-8 assay was used to detect the viability of HeLa cells after transfection with MEG3-plasmid, control-plasmid, or MEG3-plasmid + miR-421 mimic. The results indicated that the MEG3-plasmid significantly reduced the viability of HeLa cells, and the effect was reversed by co-transfection with the miR-421 mimic (**Figure 5D**). Next, the apoptotic rate of cervical cancer cells was measured by flow cytometry analysis. The MEG3-plasmid significantly increased HeLa cell apoptosis compared with the control group, and the effect was reversed by the miR-421 mimic (**Figure 5E**). The results confirmed that lncMEG3 inhibited cell viability and enhanced cell apoptosis by inhibiting the expression level of miR-421.
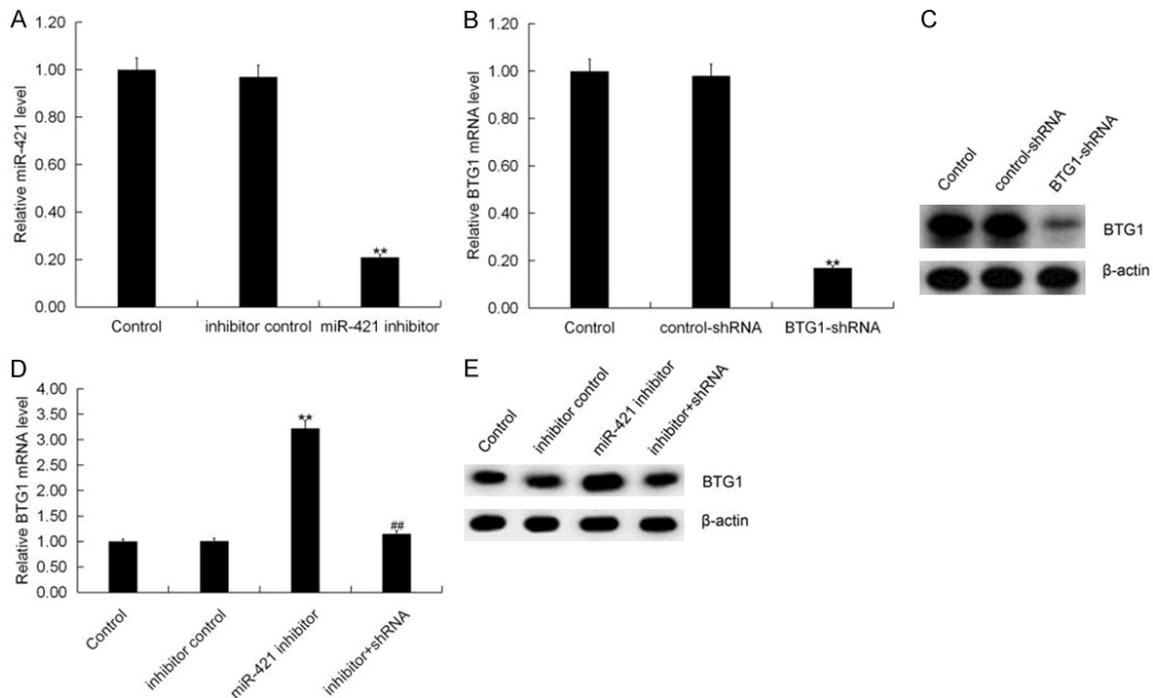
Figure 7. miR-421 negatively regulated the expression of BTG1 in cervical cancer cells. A. The level of miR-421 in HeLa cells transfected with inhibitor control or miR-421 inhibitor was detected using qRT-PCR; B and C. The mRNA and protein level of BTG1 in HeLa cells transfected with control-shRNA or BTG1-shRNA was detected using qRT-PCR and western blotting; D and E. The mRNA and protein level of BTG1 in HeLa cells transfected with inhibitor control, miR-421 inhibitor, or miR-421 inhibitor+BTG1-shRNA was detected using qRT-PCR and western blotting; The data were expressed as the mean ± SD. **$P<0.01$ vs. Control; ##$P<0.01$ *vs.* miR-421 inhibitor.

BTG1 was a direct target of miR-421

TargetScan was used to explore the relationship between BTG1 and miR-421. The results showed that BTG1 was a potential target of miR-421 (**Figure 6A**). Therefore, the luciferase reporter assay was used in 293T cells co-transfected with a luciferase vector plasmid containing wild-type or mutated 3'-UTR of BTG1 together with the miR-421 mimic or mimic control. The miR-421 mimic significantly decreased the luciferase activity of BTG1 wild-type 3'-UTR but had no obvious effect on BTG1 3'UTR mut-type compared with the control group (**Figure 6B**). The results illustrated the fact that BTG1 was a direct target of miR-421.

*miR-421 downexpression upregulated the expression of BTG1 in human cervical cancer cells*

To determine the role of miR-421 and BTG1 in cervical cancer cells, HeLa cells were transfected with inhibitor control, miR-421 inhibitor, BTG1-shRNA, control-shRNA, or miR-421 inhibi-

tor + BTG1-shRNA for 48 h. The qRT-RCR assay and/or Western blot assay were performed to detect the cell transfection efficiency. The results showed that the miR-421 inhibitor significantly reduced the expression of miR-421 in HeLa cells compared with the control group (**Figure 7A**). Further, the mRNA and protein expression levels of BTG1 more markedly decreased in the BTG1-shRNA group than in the control group (**Figure 7B** and **7C**). Compared with the control group, the mir-421 inhibitor significantly promoted the expression of BTG1 at the mRNA and protein levels, and this improvement was reversed by BTG1-shRNA co-transfection (**Figure 7D** and **7E**).

Knockout of BTG1 reversed the inhibitory effect of the miR-421 inhibitor on human cervical cancer cells

To explore the biological behavior in cervical cancer cells co-regulated by BTG1 and miR-421, experiments on cell viability and apoptosis were performed using CCK-8 assay and flow cytometry. The results revealed that the miR-
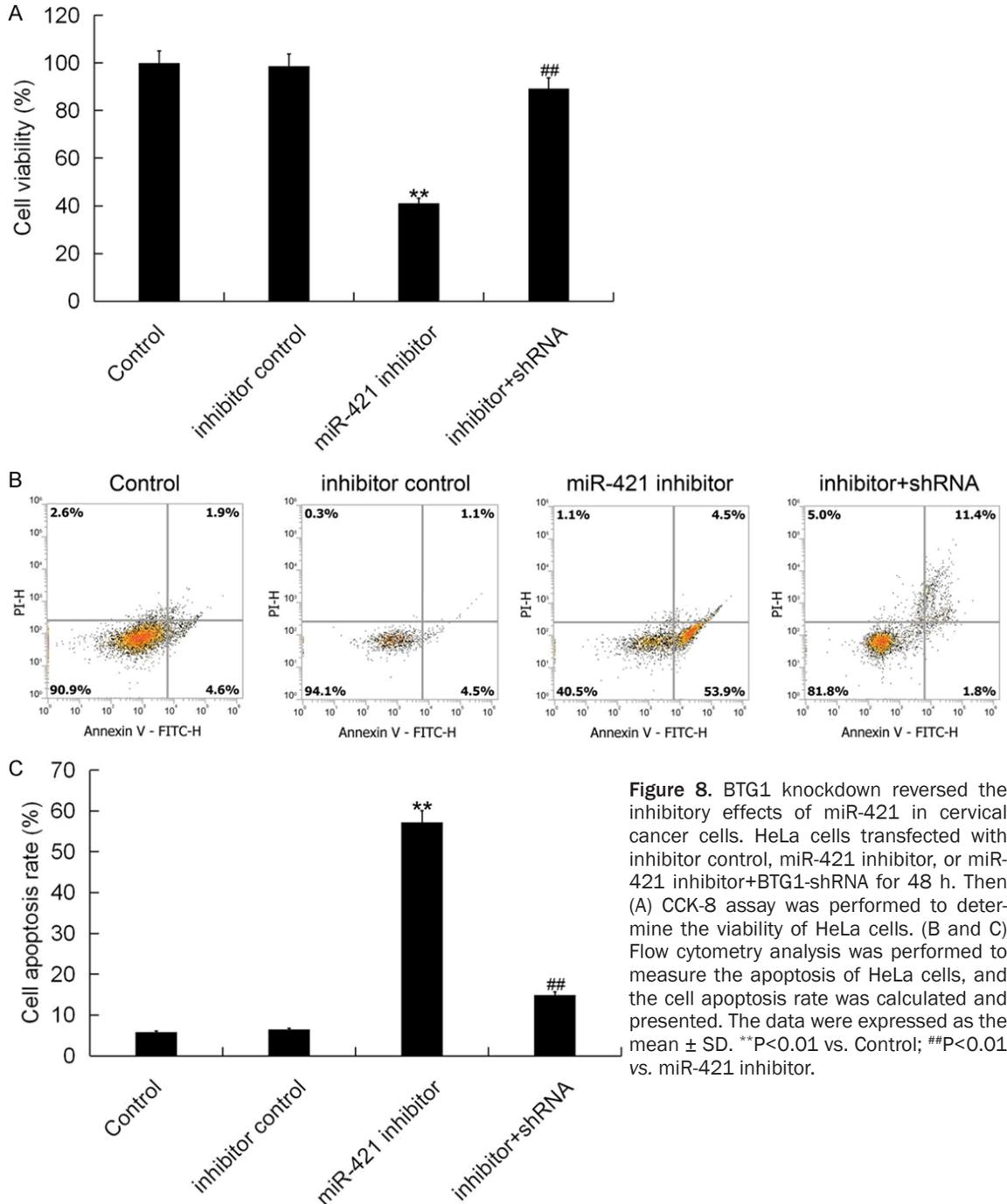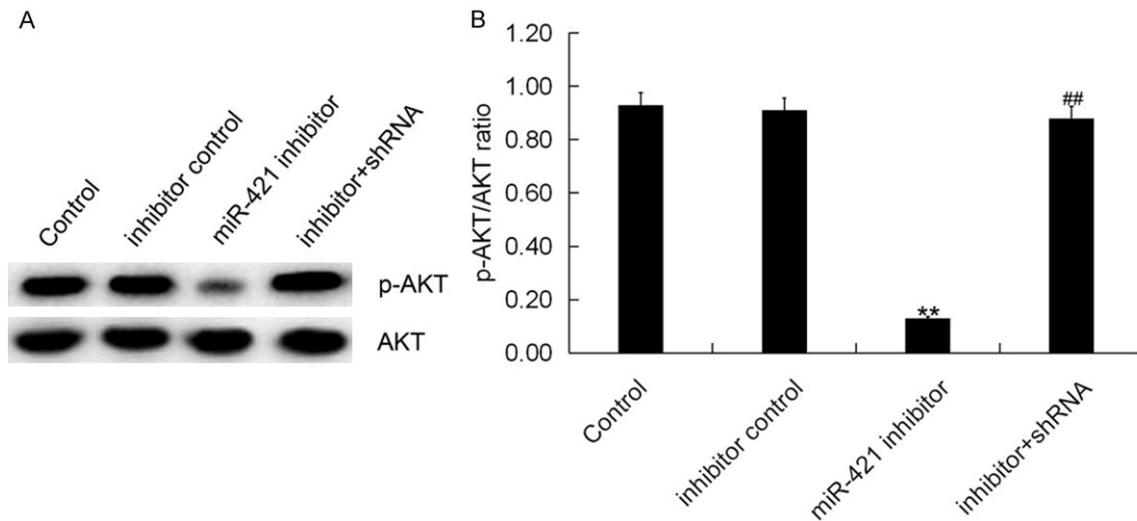
Figure 8. BTG1 knockdown reversed the inhibitory effects of miR-421 in cervical cancer cells. HeLa cells transfected with inhibitor control, miR-421 inhibitor, or miR-421 inhibitor+BTG1-shRNA for 48 h. Then (A) CCK-8 assay was performed to determine the viability of HeLa cells. (B and C) Flow cytometry analysis was performed to measure the apoptosis of HeLa cells, and the cell apoptosis rate was calculated and presented. The data were expressed as the mean ± SD. **P<0.01 vs. Control; ##P<0.01 vs. miR-421 inhibitor.

421 inhibitor significantly reduced the cell viability in HeLa cells, while the effect was reversed by BTG1-shRNA (**Figure 8A**). The results of the apoptosis assay demonstrated that compared with the control group, the downregulation of miR-421 significantly induced HeLa cell apoptosis, and the change was markedly eliminated by co-transfection with BTG1-shRNA (**Figure 8B**).

miR-421 inhibitor restrained the activation of the PI3K/Akt pathway

To further investigate the signaling pathway underlying the role of the miR-421 inhibitor in cervical cancer cells, the protein expression levels of p-Akt and Akt in HeLa cells was detected by Western blot analysis after transfection. The results showed that the miR-421 inhibitor

Figure 9. miR-421 inhibitor inhibited the activation of PI3K/AKT signal pathway in HeLa cells. HeLa cells transfected with inhibitor control, miR-421 inhibitor, or miR-421 inhibitor+BTG1-shRNA for 48 h. Then, the protein expression of AKT and p-AKT (A) was detected by Western blot analysis. (B) The ratio of p-AKT/AKT was calculated and presented. The data were expressed as the mean ± SD. **P<0.01 vs. Control; ##P<0.01 *vs.* miR-421 inhibitor.

significantly reduced the p-Akt protein (**Figure 9A**) expression level and p-Akt/Akt ratio (**Figure 9B**) compared with the control group. Moreover, the reduction effect on p-Akt protein expression and p-Akt/Akt ratio disappeared in the miR-421 inhibitor + BTG1-shRNA group (**Figure 9**).

Discussion

Lidocaine is a widely used amide local anesthetic. In recent years, many studies reported on its anti-tumor activity *in vitro*, such as in breast cancer, neuroblastoma, liver cancer, and oral cancer [22-25], but few reports are available on the effect of lidocaine on cervical cancer cell growth. The present study found that lidocaine significantly inhibited proliferation and induced apoptosis in cervical cancer cells.

Several studies found lncRNAs dysregulation had an influence on epigenetic information, promoted cellular growth, and led to uncontrolled tumor growth [26], but the molecular mechanisms of lncRNAs affecting drug therapy in cancers are still unknown. Previous studies showed that lncRNA-MEG3 acted as a tumor suppressor in cervical cancer, and the present study proved that a certain dose of lidocaine repressed the cervical cancer cell growth by upregulating the expression of lncRNA-MEG3 in

cervical cancer cells. miRNAs have attracted increasing attention because they are involved in tumor progression. A large number of studies showed that miRNAs acted as tumor suppressors or oncogenes and participated in the post-translational regulation of gene expression [27]. The present study demonstrated the MEG3-miR-421 activity using bioinformatics software analysis combined with dual-luciferase reporter assay. Further, miR-421 was found to be upregulated in cervical cancer cells. The upregulation of MEG3 inhibited cell activity and promoted cell apoptosis in HeLa cells, which proved its anti-tumor effect and further revealed that MEG3 led to the downregulation of the expression of miR-421 in HeLa cells. In addition, the miR-421 mimic could reverse the effects on HeLa cells induced by the MEG3-plasmid.

It was speculated that miR-421 might be a target gene for lidocaine in the treatment of cervical cancer. The results of bioinformatics tools analysis demonstrated that BTG1 was the target mRNA of miR-421 in cervical cancer cells. Then, qRT-PCR and Western blot analysis were used to measure the BTG1 mRNA and protein expression levels, respectively. The results indicated that BTG1 expression level was upregulated by miR-421 inhibitor treatment, and this increase was reversed by BTG1-shRNA. The miR-421 inhibitor significantly inhibited HeLa

cell viability and induced cell apoptosis, and all these effects were significantly eliminated by BTG1 silencing. Finally, the effect of the miR-421 inhibitor on the PI3K/AKT pathway in HeLa cells was investigated by detecting the protein expression of Akt and p-Akt using Western blot assay. The miR-421 inhibitor significantly decreased the p-Akt protein expression level and p-Akt/Akt ratio, while the impact of these reductions was reversed by knocking down BTG1.

In conclusion, the present study found that lidocaine repressed the growth of cervical cancer cells by modulating the lncRNA-MEG3/miR-421/BTG1 pathway. These findings suggested that the anti-tumor efficacy of lidocaine was positive, providing new opportunities for the clinical therapies for cervical cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Shichao Han, Department of Gynecology, The 2nd Affiliated Hospital of Dalian Medical University, No. 467 Zhong Shan Road, Dalian 116021, China. E-mail: han-shichao190509@163.com

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