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LncRNA MAGI2-AS3 suppresses the proliferation and invasion of non-small cell lung carcinoma through miRNA-23a-3p/PTEN axis

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Abstract. – OBJECTIVE: To uncover the biological role of long non-coding RNA (lncRNA) MAGI2-AS3 in the progression of non-small cell lung carcinoma (NSCLC) and its molecular mechanism.

PATIENTS AND METHODS: LncRNA MAGI2-AS3 level in NSCLC tissues and cell lines was determined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Chi-square test was conducted to analyze the correlation between MAGI2-AS3 level and pathological characteristics of NSCLC patients. Survival analysis was performed in NSCLC patients with high expression or low expression of MAGI2-AS3. *In vitro* influences of MAGI2-AS3 on viability and invasive ability of A549 and PC9 cells were evaluated. MicroRNA-23a-3p (miRNA-23a-3p), the target gene of MAGI2-AS3 was determined through the dual-luciferase reporter gene assay. In a similar way, the target gene of miRNA-23a-3p was identified. Finally, the regulatory effect of MAGI2-AS3/miRNA-23a-3p/PTEN (gene of phosphate and tension homology deleted on chromosome ten) axis on cellular behaviors of NSCLC cells was assessed.

RESULTS: LncRNA MAGI2-AS3 was downregulated in NSCLC tissues and cell lines. Its level was closely related to tumor size, Tumor Node Metastasis (TNM) stage and distant metastasis of NSCLC patients. The worse prognosis was identified in NSCLC patients with low expression of MAGI2-AS3 relative to those with a high expression. Overexpression of MAGI2-AS3 markedly attenuated viability and invasive ability of A549 and PC9 cells. MiRNA-23a-3p was verified to be the target gene of MAGI2-AS3, and furthermore, PTEN was the target of miRNA-23a-3p. Overexpression of miRNA-23a-3p could reverse the inhibited viability and invasion in NSCLC cells overexpressing MAGI2-AS3.

CONCLUSIONS: MAGI2-AS3 is downregulated in NSCLC. Overexpression of MAGI2-AS3 suppresses the proliferative and invasive abilities of NSCLC *via* miRNA-23a-3p/PTEN axis.

Key Words
MAGI2-AS3, MiRNA-23a-3p, PTEN, NSCLC.

Introduction

Lung carcinoma is the leading cause of cancer deaths. Its incidence and mortality are the highest in tumors worldwide[1]. Based on the histological classification, lung carcinoma is divided into two categories: small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). NSCLC accounts for about 80% of all lung carcinoma cases[2]. Although great strides have been made in the therapeutic strategies, the prognosis of NSCLC remains very poor. It is reported that the 5-year survival of NSCLC is about 15% and that is 4% in those with distant metastasis[3]. Therefore, it is urgent to search for effective diagnostic methods and therapeutic targets for NSCLC, thus improving the clinical outcomes of these patients.

Long non-coding RNA (lncRNA) is a type of non-coding RNA with a transcript of more than 200 nucleotide residues long. It lacks the open reading frame and regulates gene expressions at different levels[4]. Current researches[5-8] have illustrated the involvement of lncRNAs in regulating malignant phenotypes of tumor cells, thereafter influencing the progression of tumor diseases. Several abnormally expressed lncRNAs have been discovered in NSCLC. They are capable of mediating cellular behaviors of NSCLC cells and further affecting the disease progression. For example, lncRNA HOTAIR is upregulated in NSCLC, which is related to tumor stage and lymphatic metastasis of NSCLC patients[9]. Schmidt et al[10] pointed out that MALAT-1 is upregulated in NSCLC. Transfection of MALAT-1 siRNA in

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nude mice attenuates the tumorigenesis of NSCLC, suggesting its carcinogenic role in NSCLC. Therefore, determining the expression pattern of lncRNA and its mechanism may provide new ideas and targets for early screening, molecular diagnosis, and clinical treatment of NSCLC.

MAGI2-AS3 is a newly discovered lncRNA that is suggested to suppress the proliferative ability of breast cancer[11]. Wang et al[12] found that MAGI2-AS3 can alleviate the progression of bladder cancer through microRNA-15b-5p (miR-15b-5p)/CCDC19 axis. Nevertheless, the specific regulatory role of MAGI2-AS3 in NSCLC is unclear. This study mainly investigated the expression pattern and biological function of MAGI2-AS3 in NSCLC.

Patients and Methods

Patients

40 paired tumor tissues and matched adjacent tissues were surgically resected from NSCLC patients treated in Beijing Chaoyang District Sanhuan Cancer Hospital from December 2016 to March 2018. They did not receive preoperative anti-tumor therapy and were pathologically diagnosed. Clinical data of enrolled NSCLC patients were collected. All subjects volunteered to participate in the study and signed written informed consent. This investigation was approved by the Ethics Committee of Beijing Chaoyang District Sanhuan Cancer Hospital. Signed written informed consents were obtained from all participants before the study.

Cell Culture and Transfection

Bronchial epithelial cell line HBE and lung carcinoma cell lines A549, PC9, NCI-H441, and NCI-H1650 were provided by Cell Bank (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 μg/mL penicillin and 0.1 mg/mL streptomycin, in a 37°C, 5% CO_2 incubator.

Cells pre-seeded in a 6-well plate were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Transfected cells for 24-48 h were harvested for *in vitro* experiments.

Cell Counting Kit-8 (CCK-8)

cells were seeded in the 96-well plate and cultured overnight. The absorbance (A) at 450 nm was recorded at the established time points using the Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) for depicting the viability curves.

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

Extraction of total RNA in cells was performed using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and subjected to reverse transcription. The extracted complementary deoxyribose nucleic acid (cDNA) was applied for PCR using the SYBR Green method. Primer sequences were as follows: MAGI2-AS3: F: 5'-CACCTTGCTTGACACACTTGA-3', R: 5'-CATTACAGCTCGGCTACTGC-3'; MiRNA-23a-3p: F: 5'-GCGATCACATTGCCAGGG-3', R: 5'-CAGTGCGTGTCGTGGAGT-3'; PTEN: F: 5'-ACACGAC GGGAAGACAAGTT-3', R: 5'-TCCTCTGGTCCTGGTATGAAG-3'.

Transwell

Diluted Matrigel was used to pre-coat the transwell chamber overnight at 4°C. Cell density was adjusted to 2×10^5/mL in a serum-free medium. 500 μL of medium containing 10% FBS and 200 μL of cell suspension were added in the basolateral and apical chamber of the 24-well plate, respectively. 24 h later, cells were fixed in methanol for 30 min and stained with 0.1% crystal violet for another 30 min. Invasive cells were observed and photographed using an inverted microscope.

Western Blot

The total protein was extracted from cells using radioimmunoprecipitation assay (RIPA) and quantified by bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). The protein sample was loaded for electrophoresis and transferred on a polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Membranes were blocked in 5% skim milk for 2 h and subjected to incubation with primary and secondary antibodies. Bands were exposed by enhanced chemiluminescence (ECL) and analyzed by Image Software (NIH, Bethesda, MD, USA).

Dual-Luciferase Reporter Gene Assay

Cells were co-transfected with miR-NC/miRNA-23a-3p mimics and wild-type/mutant-type vectors (MAGI2-AS3/PTEN) using Lipofectamine 2000 for 48 h. Transfected cells were then lysed to. determine the relative luciferase activity (Promega, Madison, WI, USA).

Statistical Analysis

GraphPad Prism 7.0 (La Jolla, CA, USA) was used for data analyses. Data were expressed as mean ± SD. Intergroup differences were analyzed by the *t*-test. Chi-square test was performed to evaluate the correlation between MAGI2-AS3 level and pathological characteristics of NSCLC patients. Survival analysis was carried out using the Kaplan-Meier method. $p<0.05$ was considered as statistically significant.

Results

MAGI2-AS3 Was Downregulated in NSCLC

This study collected 40 cases of NSCLC tissues and matched adjacent normal tissues. QRT-PCR data revealed a lower abundance of MAGI2-AS3 in NSCLC tissues relative to controls (Figure 1A). Furthermore, the relation between MAGI2-AS3 level and pathological characteristics of NSCLC patients was analyzed. It is indicated that MAGI2-AS3 level was significantly correlated to tumor size, Tumor Node Metastasis (TNM) stage and distant metastasis of NSCLC patients (Table I). The lower level of MAGI2-AS3 was observed in NSCLC patients with stage III-IV relative to those with stage I-II (Figure 1B). MAGI2-AS3 was lowly expressed in lung carcinoma cell lines A549 and PC9 relative to bronchial epithelial cell line HBE (Figure 1C). In addition, Kaplan-Meier curves indicated a worse prognosis in NSCLC patients expressing a low level of MAGI2-AS3 (Figure 1D).

Overexpression of MAGI2-AS3 Suppressed the Proliferative and Invasive Abilities of NSCLC

Transfection efficacy of pcDNA3.1-MAGI2-AS3 was verified in A549 and PC9 cells, which sufficiently upregulated MAGI2-AS3 level (Figure 2A). After transfection of pcDNA3.1-MAGI2-AS3, the viability in NSCLC cells was markedly suppressed (Figure 2B). Similarly, the invasive ability of NSCLC cells was attenuated by the overexpression of MAGI2-AS2 (Figure 2C).

MAGI2-AS3 Sponged MiRNA-23a-3p as a ceRNA

A great number of studies have demonstrated that lncRNA could interact with miRNA and further influence disease progression. Through TargetScan analysis, the binding sequences between MAGI2-AS3 and miRNA-23a-3p were predicted (Figure 3A). Subsequently, the overexpression of miRNA-23a-3p was found to be able to decrease the luciferase activity in wild-type MAGI2-AS3 vector, but failed to influence the mutant-type MAGI2-AS3 vector (Figure 3B). After transfection of pcDNA3.1-MAGI2-AS3, miRNA-23a-3p was downregulated in PC9 cells (Figure 3C). Besides, miRNA-23a-3p was upregulated in NSCLC tissues relative to controls (Figure 3D). A negative correlation was identified between expression levels of MAGI2-AS3 and miRNA-23a-3p in NSCLC tissues (Figure 3E). Hence, it is suggested that MAGI2-AS3 sponged miRNA-23a-3p and negatively regulated its level in NSCLC.

Table I. The correlation between MAGI2-AS3 level and pathological characteristics of NSCLC patients (n=40).

Clinicopathologic features	Number of cases	MAGI2-AS3 expression		*p*-value
		Low (n=20)	High (n=20)	
Age (years)				0.525
≤45	18	8	10	
>45	22	12	10	
Gender				0.327
Male	25	11	14	
Female	15	9	6	
Tumor size				0.010*
≤5CM	16	4	12	
>5CM	24	16	8	
TNM stage				0.025*
I-II	17	5	12	
III-IV	23	15	8	
Distant metastasis				0.043*
Yes	21	13	8	
No	19	7	12	

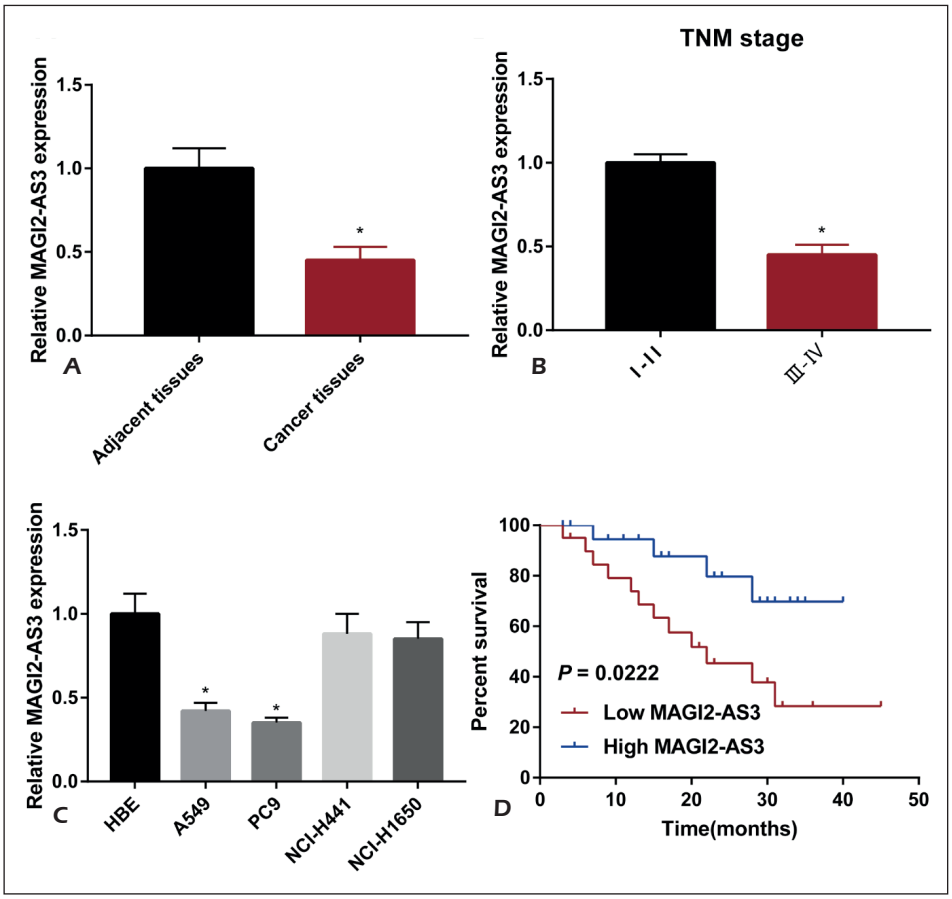

Figure 1. MAGI2-AS3 was downregulated in NSCLC. ***A,*** Relative level of MAGI2-AS3 in NSCLC tissues and matched adjacent normal tissues. ***B***, Relative level of MAGI2-AS3 in NSCLC patients with stage III-IV and stage I-II. ***C,*** Relative level of MAGI2-AS3 in bronchial epithelial cell line HBE and lung carcinoma cell lines A549, PC9, NCI-H441, and NCI-H1650. ***D,*** Survival analysis of NSCLC patients with high level and low level of MAGI2-AS3.

MAGI2-AS3 Interacted with MiRNA-23a-3p to Mediate PTEN Level in NSCLC

Furthermore, the potential target of miRNA-23a-3p was predicted. Binding sequences between miRNA-23a-3p and PTEN were depicted in Figure 4A. Similarly, the dual-luciferase reporter gene assay verified the binding relation between miRNA-23a-3p and PTEN in PC9 cells (Figure 4B). Transfection of pcDNA3.1-MAGI2-AS3 markedly upregulated PTEN level in PC9 cells, but was further downregulated after the co-transfection of miRNA-23a-3p mimics (Figure 4C, D). We speculated that PTEN was involved in the progression of NSCLC influenced by MAGI2-AS3 and miRNA-23a-3p. As the viability curves illustrated, the inhibited viability in PC9 cells overexpressing MAGI2-AS3 was partially reversed after co-transfection of miRNA-23a-3p mimics (Figure 4E). Identically, the inhibitory effect of MAGI2-AS3 on the invasive ability of PC9 cells was reversed after overexpression of miRNA-23a-3p (Figure 4F). Collectively, MAGI2-AS3 exerted its tumor-suppressor role in NSCLC *via* mediating miRNA-23a-3p/PTEN axis.

Discussion

The morbidity and mortality of lung carcinoma account for 20-25% of all malignancies[13]. NSCLC is the majority of all lung carcinoma subtypes[14]. Invasion and metastasis are the leading causes for the poor prognosis of NSCLC. The lack of effective early-stage diagnostic strategies and tumor hallmarks significantly limits the therapeutic efficacy of NSCLC. Increasing evidence[15,16] has shown the crucial role of lncRNAs in tum-

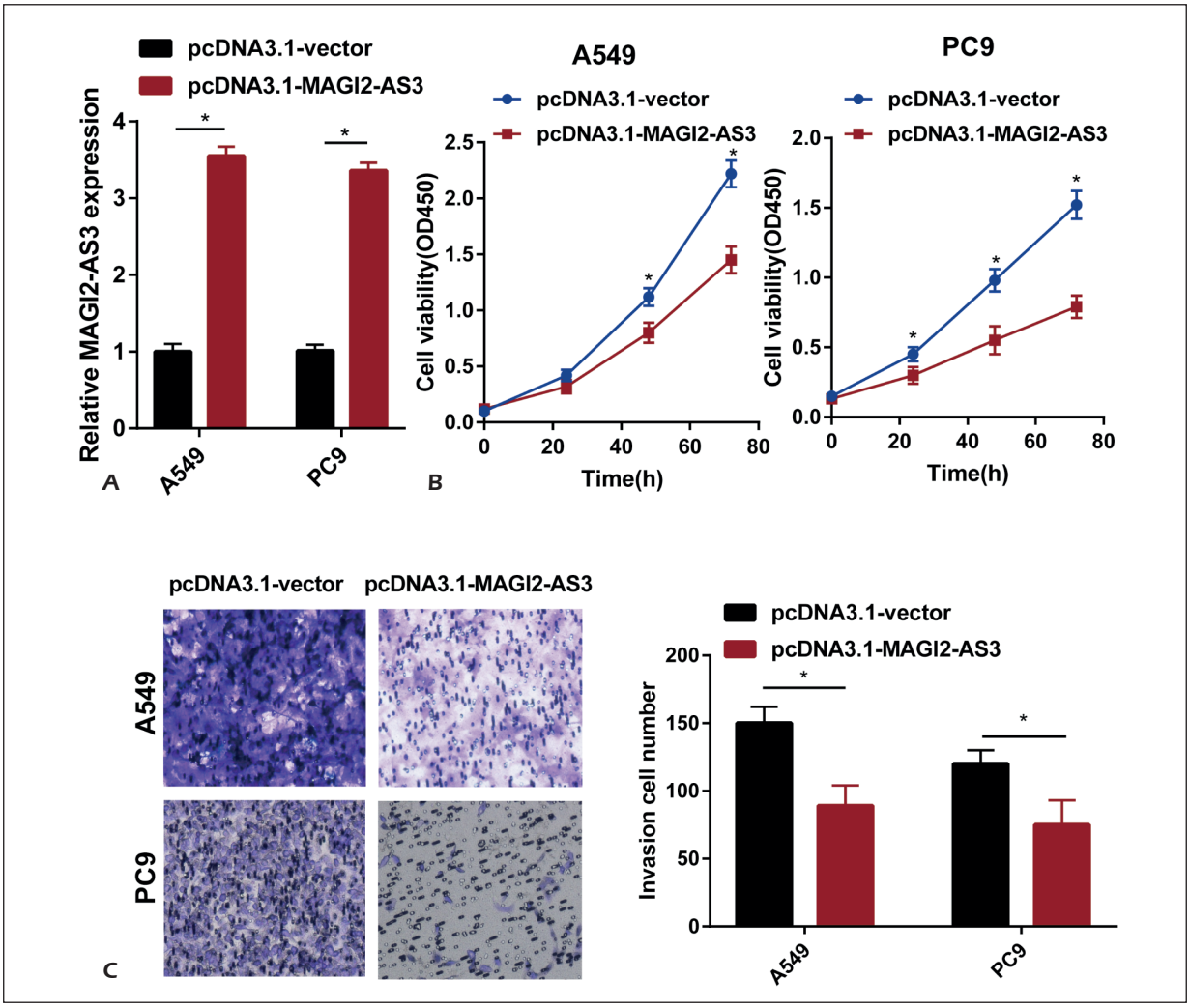

Figure 2. Overexpression of MAGI2-AS3 suppressed the proliferative and invasive abilities of NSCLC. ***A,*** Transfection efficacy of pcDNA3.1-MAGI2-AS3 in A549 and PC9 cells. ***B,*** CCK-8 assay showed the viability in A549 and PC9 cells transfected with pcDNA3.1-vector or pcDNA3.1-MAGI2-AS3. ***C,*** Transwell assay showed the invasion in A549 and PC9 cells transfected with pcDNA3.1-vector or pcDNA3.1-MAGI2-AS3 (magnification: 40×).

origenesis. Research of abnormally expressed lncRNAs contributes to improving diagnostic efficacy and prognosis. This study illustrated MAGI2-AS3 downregulation in NSCLC tissues and cell lines. *In vitro* experiments further suggested that overexpression of MAGI2-AS3 could attenuate the proliferative and invasive abilities of NSCLC cells.

Recent studies[17,18] have proposed that lncRNA serves as a ceRNA in tumors. In 2011, Salmena et al[19] pointed out a novel ceRNA hypothesis that a complex post-transcriptional network, including lncRNAs, mRNAs and other RNAs, inhibits RNA functions through the competition with a single or multiple miRNA. This ceRNA network has proved to be vital in many types of tumors[20-22]. LncRNA NEAT1 sponges miR-377-3p to inhibit E2F3 and thus attenuates the progression of NSCLC[23]. As a tumor suppressor in NSCLC, TINCR upregulates FBXW7 by sponging miR-544a[24]. MIAT is upregulated in NSCLC, which inhibits miR-150 level to mediate ZEB1 expression, thus accelerating lung carcinoma cell invasion[25]. In this study, miRNA-23a-3p was verified to bind to MAGI2-AS3. Previous studies[26-28] have shown that miRNA-23a-3p was able to influence multiple types of tumors. However, its biological role in NSCLC is unclear. Our results demonstrated that miRNA-23a-3p was highly expressed in NSCLC. Notably, the overexpression

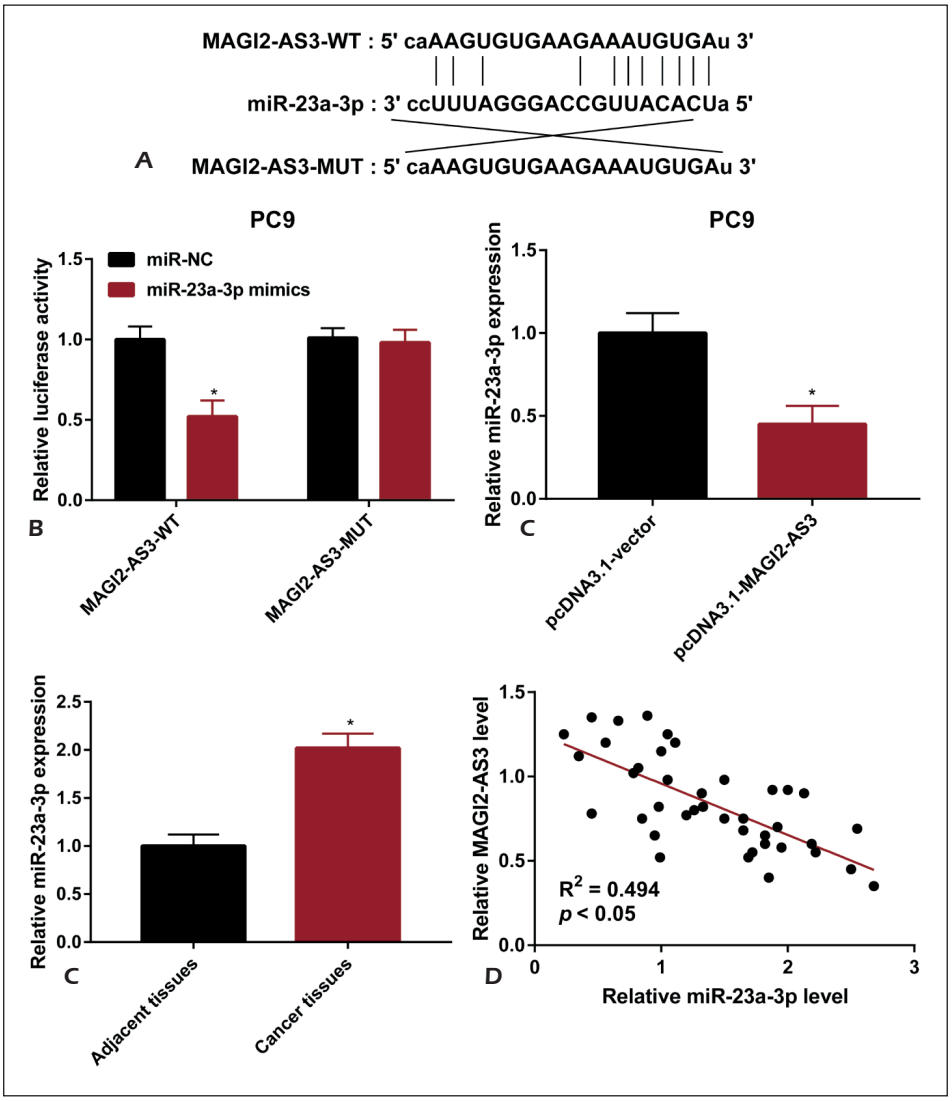

Figure 3. MAGI2-AS3 sponged miR-23a-3p as a ceRNA. ***A,*** Predicted binding sequences between MAGI2-AS3 and miR-23a-3p. ***B,*** Relative luciferase activity in PC9 cells co-transfected with miR-NC/miR-23a-3p mimics and MAGI2-AS3-WT/MAGI2-AS3-MUT. ***C,*** Relative level of miR-23a-3p in PC9 cells transfected with pcDNA3.1-vector or pcDNA3.1-MAGI2-AS3. ***D,*** Relative level of miR-23a-3p in NSCLC tissues and matched adjacent normal tissues. ***E,*** Negative correlation between expression levels of MAGI2-AS3 and miR-23a-3p in NSCLC tissues.

of miRNA-23a-3p could reverse the regulatory effect of MAGI2-AS3 on cellular behaviors of NSCLC cells. Furthermore, PTEN was identified to be the downstream gene of miRNA-23a-3p. As a well-known tumor-related gene, PTEN is important in maintaining cellular homeostasis through mediating various cell phenotypes[29]. PTEN deficiency is believed to participate in the EMT of lung carcinoma[30,31]. Here, PTEN was negatively correlated to miRNA-23a-3p, but positively correlated to MAGI2-AS3. Our conclusions illustrated that MAGI2-AS3 sponged miRNA-23a-3p to downregulate PTEN level, thus alleviating the progression of NSCLC.

Conclusions

LncRNA MAGI2-AS3 is downregulated in NSCLC. Overexpression of MAGI2-AS3 suppresses the proliferative and invasive abilities of NSCLC *via* miRNA-23a-3p/PTEN axis. MAGI2-AS3 could be utilized as a molecular target for the treatment of NSCLC.

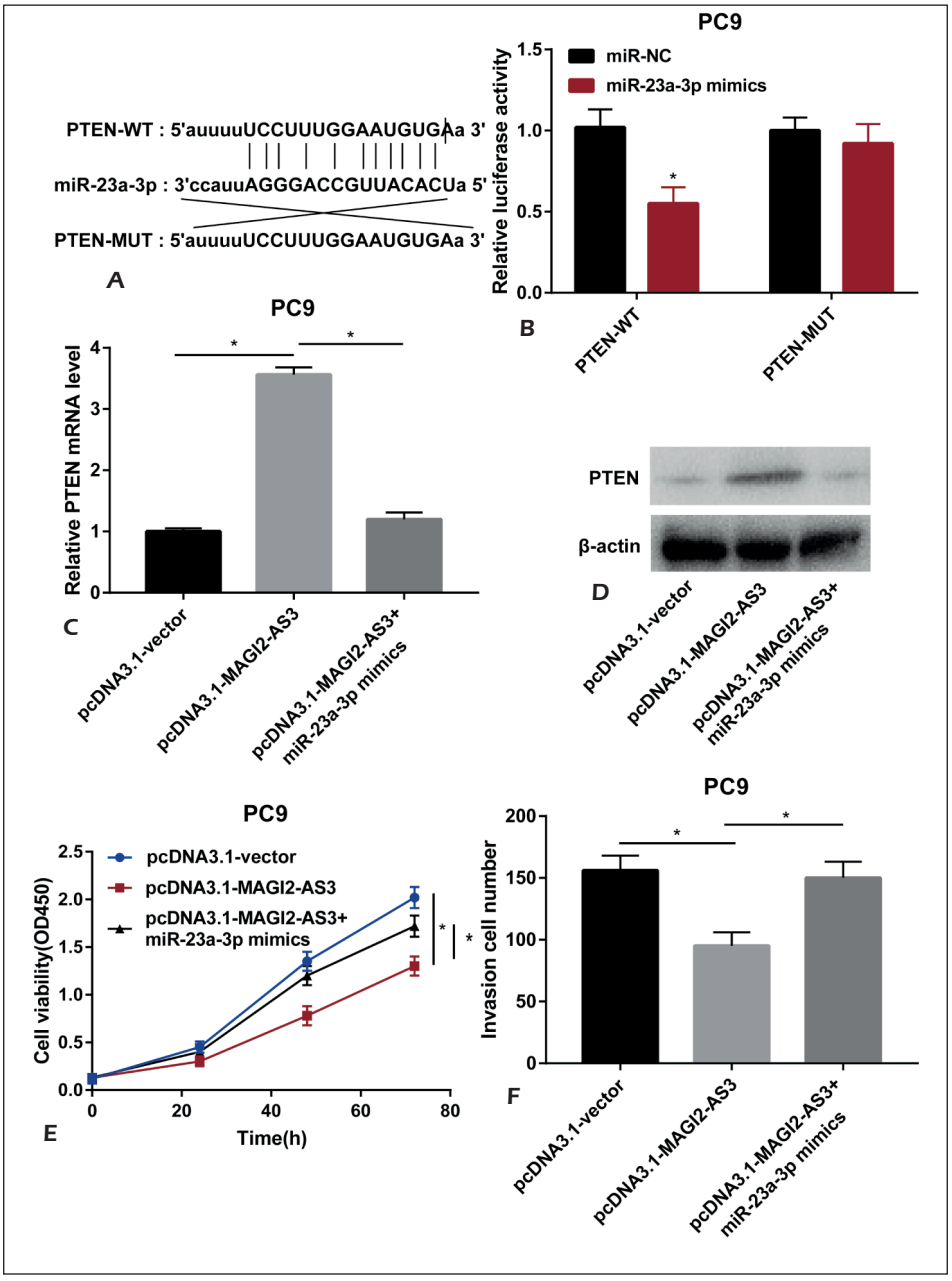

Figure 4. MAGI2-AS3 interacted with miR-23a-3p to mediate PTEN level in NSCLC. ***A,*** Predicted binding sequences between miR-23a-3p and PTEN. ***B,*** Relative luciferase activity in PC9 cells co-transfected with miR-NC/miR-23a-3p mimics and PTEN-WT/PTEN-MUT. ***C,*** mRNA level of PTEN in PC9 cells transfected with pcDNA3.1-vector, pcDNA3.1-MAGI2-AS3 or pcDNA3.1-MAGI2-AS3+miR-23a-3p mimics. ***D,*** Protein level of PTEN in PC9 cells transfected with pcDNA3.1-vector, pcDNA3.1-MAGI2-AS3 or pcDNA3.1-MAGI2-AS3+miR-23a-3p mimics. ***E,*** CCK-8 assay showed the viability in PC9 cells transfected with pcDNA3.1-vector, pcDNA3.1-MAGI2-AS3 or pcDNA3.1-MAGI2-AS3+miR-23a-3p mimics. ***F,*** Invasion in PC9 cells transfected with pcDNA3.1-vector, pcDNA3.1-MAGI2-AS3 or pcDNA3.1-MAGI2-AS3+miR-23a-3p mimics.

Conflict of Interests

The authors declared that they have no conflict of interests.

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