NORAD regulates epithelial-mesenchymal transition of non-small cell lung cancer cells via miR-422a

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Abstract. The poor prognosis of non-small cell lung cancer (NSCLC) is related to epithelial-mesenchymal transition (EMT). Recent studies demonstrated that non-coding RNA activated by DNA damage (NORAD) displays a carcinogenic effect and targets microRNA (miR)-422a, which may be involved in tumor cell migration and invasion. The aim of the present study was to investigate the effect of NORAD on NSCLC cell EMT and the underlying mechanism. Reverse transcription-quantitative PCR and western blotting were performed to detect the expression levels of long non-coding RNAs, miRNAs and mRNAs. Cell viability, migration and invasion were detected by conducting Cell Counting Kit-8, wound healing and Transwell assays, respectively. The target of NORAD was predicted using starBase and further confirmed by conducting a dual-luciferase reporter assay. The results indicated that NORAD expression was significantly increased in lung cancer tissues and cells compared with adjacent healthy tissues and cells. Compared with the control groups, NORAD overexpression promoted SK-MES-1 cell viability, migration and invasion, whereas NORAD knockdown resulted in the opposite effects in A549 cells. Moreover, miR-422a, which was predicted to be a target of NORAD, displayed lower expression levels in lung cancer tissues compared with adjacent healthy tissues. In addition, miR-422a overexpression partially reversed NORAD overexpression-induced increases in SK-MES-1 cell viability, migration, invasion and EMT. In addition, miR-422a knockdown partially reversed the effects of NORAD knockdown. The present study suggested that NORAD regulated lung cancer cell EMT by regulating the expression of miR-422a, providing a potential therapeutic target for the intervention of the development of NSCLC.

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Abbreviations: NSCLC, non-small cell lung cancer; EMT, epithelial-mesenchymal transition

Key words: non-coding RNA activated by DNA damage, microRNA-422a, NSCLC, EMT

Introduction

The difficulty of early diagnosis, the diversity and complexity of tumorigenesis and the progression of non-small cell lung cancer (NSCLC) contribute to the poor prognosis of the disease (1,2). A number of patients with NSCLC die as a result of tumor metastasis, and until the last decade, the 5-year overall survival rate of patients with metastatic NSCLC was <5% (3,4). Moreover, as the mechanism underlying the recurrence and metastasis of NSCLC is not completely understood, exploring the molecular mechanisms underlying the metastasis and progression of the disease may aid with the development of therapeutic strategies for NSCLC.

During epithelial-mesenchymal transition (EMT), epithelial cells transform into cells with a mesenchymal phenotype via a specific process, which is accompanied by alterations in cell structure, adhesion, morphology and migration (5). A number of studies have demonstrated that EMT serves an important role in the invasion and metastasis of various malignant tumors, such as colorectal (6), pancreatic (7), prostate (8), breast (9) and lung (10) cancer. According to Otsuki *et al* (11), EMT signaling promotes epithelial tumor malignancy, such as lung cancer, indicating that inhibiting EMT signaling may suppress tumor metastasis, recurrence or drug resistance. Therefore, developing EMT-targeted therapy may serve as a promising therapeutic strategy for lung cancer.

Long non-coding RNAs (lncRNAs) are non-protein-coding transcripts that are >200 nucleotides in length (12). Although the mechanisms of action underlying lncRNAs are not completely understood, increasing evidence has suggested that lncRNAs are important regulator in a number of biological processes, such as cell proliferation and apoptosis (13). Non-coding RNA activated by DNA damage (NORAD) is an lncRNA that is downregulated in breast and lung cancer, and low NORAD expression levels in the two types of cancer are correlated with lymph node metastasis and poor patient prognosis (14). Moreover, previous studies have indicated that NORAD acts as a sponge for miRNAs (miRs) to affect the occurrence and development of NSCLC (15,16).

However, whether NORAD affects EMT during the development of NSCLC is not completely understood; therefore, the present study aimed to investigate whether NORAD was involved in NSCLC EMT and its underlying mechanism, with the aim of identifying a novel therapeutic target for NSCLC.

Materials and methods

Tissue collection. NSCLC cancer tissues and healthy adjacent tissues (≥5 cm from the tumor margin) were obtained from 50 patients (age, 32-70 years; mean age, 45±7 years; 32 male patients and 18 female patients) who were diagnosed with NSCLC in Jingmen No. 1 People's Hospital between February 2018 and April 2019. In all patients with NSCLC, diagnosis was histopathologically confirmed. Written informed consent was obtained from all patients. The present study was approved by Jingmen No. 1 People's Hospital Ethics Committee (approval no. JM2018010232).

Cell transfection and grouping. Human bronchial epithelium 16HBE cells were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. NSCLC lines (A549, SK-MES-1, H1975 and SK-LU-1) were purchased from American Type Culture Collection. Cells were cultured in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc.) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) in humidified air with 5% CO_2 at 37˚C.

To explore the effects of NORAD on lung cancer cells, cells were divided into the following groups: i) Control (cells without treatment); ii) pc-control (cells transfected with pc-control); iii) pc-NORAD (cells transfected with pc-NORAD); iv) small interfering RNA (si)-control (cells transfected with si-control); and v) si-NORAD (cells transfected with si-NORAD). To explore the potential mechanisms underlying NORAD in lung cancer cells, cells were divided into the following groups: i) Control (cells without treatment); ii) mimic-control (cells transfected with miR-422a mimic-control); iii) mimic (cells transfected with miR-422a mimic); iv) pc-NORAD; v) mimic + pc-NORAD; vi) inhibitor-control (cells transfected with miR-422a inhibitor-control); vii) inhibitor (cells transfected with miR-422a inhibitor); viii) si-NORAD; and ix) inhibitor + si-NORAD.

SK-MES-1 cells were transfected with pc-NORAD, pc-control, miR-422a mimic (5'-ACUGGACUUAGGGUC AGAAGGC-3'; Shanghai GenePharma Co., Ltd.) or mimic control (5'-UUUGUACUACACAAAAGUACUG-3'; Shanghai GenePharma Co., Ltd.). A549 cells were transfected with si-NORAD (5'-AAGCCACCTTTGTGAACAGTA-3'), si-control (5'-TTCTCCGAACGTGTCACGT-3'), miR-422a inhibitor (5'-GCCUUCUGACCCUAAGUCCAGU-3') or inhibitor control (5'-CAGUACUUUUGUGUAGUACAAA-3'). Cell transfection ($2x10^5$ cells/well) was performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). At 48 h post-transfection, subsequent experiments were performed.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from cells using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed into cDNA using a High-Capacity cDNA Reverse Transcription kit (Invitrogen; Thermo Fisher Scientific, Inc.). The following temperature protocol was used for reverse transcription: 37˚C for 40 min and 85˚C for 5 min. Subsequently, NORAD expression levels were determined by qPCR using a 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) and SYBR Green PCR Master Mix (Toyobo Life Science). The sequences of the primers used to measure NORAD expression levels were as follows: NORAD forward, 5'-CCTGGAAGGTGAGCG AAGT-3' and reverse, 5'-AGAGGGTGGTGGGCATTT-3'; and GAPDH forward, 5'-TGGTCACCAGGGCTGCTT-3' and reverse, 5'-AGCTTCCCGTTCTCAGCC-3'. To detect miR-422a expression levels, qPCR was performed using the SYBR Premix Ex Taq kit (Takara Bio, Inc.). The sequences of the primers used to measure miR-422a expression levels were as follows: miR-422a forward, 5'-ACUGGACUUAGG GUCAGAAGGC-3' and reverse, 5'-GCCUUCUGACCCUAA GUCCAGU-3'; and U6 forward, 5'-CTTCGGCAGCACATA TAC-3' and reverse, 5'-GAACGCTTCACGAATTTGC-3'. The following thermocycling conditions were used for qPCR: Pre-degeneration at 95˚C for 30 sec; 39 cycles at 95˚C for 10 sec and 60˚C for 30 sec; and final extension at 72˚C for 30 sec. mRNA and miRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (17) and normalized to the internal reference genes GAPDH and U6, respectively.

Cell Counting Kit-8 (CCK-8) assay. Cells ($1x10^3$/well) were plated in 96-well plates for 24, 48 or 72 h at 37˚C. Subsequently, 10 μl CCK-8 reagent was added to each well and incubated for 1 h 37˚C. Cell viability was measured by detecting the absorbance at a wavelength of 450 nm using a microtiter plate.

Wound healing assay. Cell migration was assessed by performing a wound healing assay. During the wound healing assay, cells ($2x10^5$ cells/well) were cultured in RPMI-1640 medium supplemented with 1% FBS at 37˚C with 5% CO_2. At 90% confluence, a single scratch was made in the cell monolayer using a medium-sized pipette tip. The monolayer was washed with PBS to remove cell debris. At 0 and 24 h, images of the cells were captured using a light microscope to measure the scratch width (magnification, x100). Migration rate=(scratch width at 0 h-scratch width at 48 h)/scratch width at 0 h.

Transwell assay. At 48 h post-transfection, cells ($3x10^5$) were cultured in medium without serum. A Transwell assay (pore size, 8 μm; Corning, Inc.) was performed to detect cell invasion. Cells ($2x10^5$ cells/ml) were plated into the upper chamber with medium containing 1% FBS, which was pre-coated with Matrigel® at 37˚C for 30 min. Medium supplemented with 10% FBS (500 μl) was plated in the lower chambers. Following incubation for 24 h at 37˚C, cells on the upper chamber surface were removed. Invading cells were fixed with 50% methanol for 30 min at 4˚C and stained with 0.1% crystal violet for 30 min at room temperature. Stained cells were visualized under a light microscope (magnification, x100) and the number of invading cells was calculated to determine the relative invasion rate.

Dual-luciferase reporter assay. starBase (starbase.sysu.edu.cn) was used to predict the target gene of NORAD, and the binding sites were verified by performing adual-luciferase

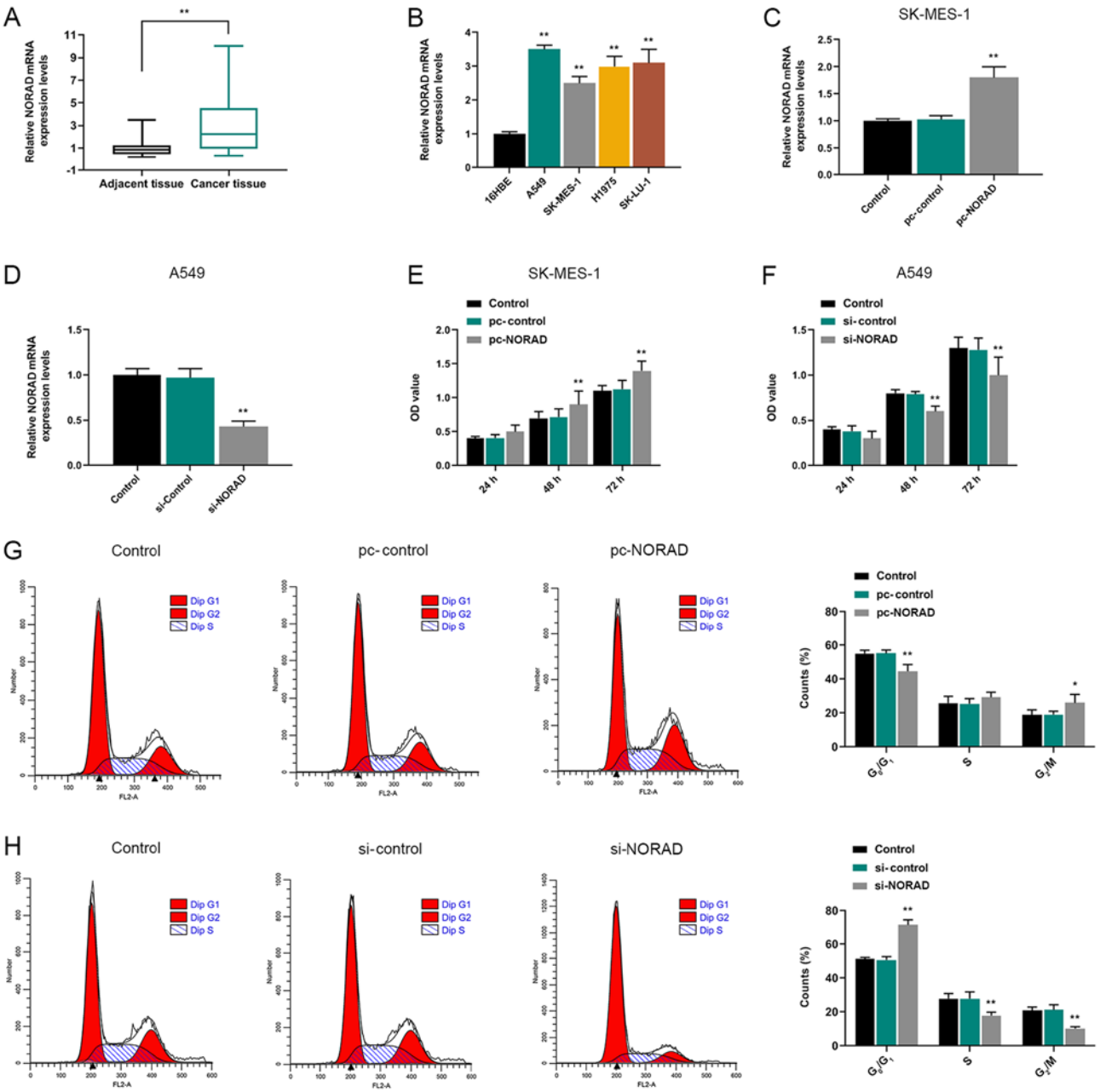

Figure 1. Effects of NORAD in NSCLC tissues and on NSCLC cell viability. (A) NORAD expression in NSCLC tissues. **P<0.001 vs. Adjacent tissue. (B) NORAD expression in NSCLC cell lines. **P<0.001 vs. 16HBE. Transfection efficiency of (C) pc-NORAD in SK-MES-1 and (D) si-NORAD in A549 cells. **P<0.001 vs. pc-Control or si-Control. Effect of NORAD on (E) SK-MES-1 and (F) A549 cell viability. **P<0.001 vs. pc-Control or si-Control. Effect of NORAD on (G) SK-MES-1 and (H) A549 cell cycle distribution. *P<0.05 and **P<0.001 vs. pc-Control or si-Control. NORAD, non-coding RNA activated by DNA damage; NSCLC, non-small cell lung cancer; si, small interfering RNA.

reporter assay. pGL3-NORAD 3'-UTR wild-type (wt) plasmid and pGL3-NORAD 3'-UTR mutated (mut) plasmid were obtained from Guangzhou RiboBio Co., Ltd. In brief, the wt 3'-UTR of NORAD containing the miR-422a binding sequences was obtained by PCR amplification from human genomic DNA, and mutated (mut) NORAD obtained using the Quick-Change Site-Directed Mutagenesis kit (Stratagene). The wt and mut 3'-UTRs of NORAD were inserted into the pGL3 luciferase reporter plasmids (Promega Corporation) to obtain pGL3-NORAD 3'-UTR wt plasmid and pGL3-NORAD 3'-UTR mut plasmid, respectively. 293T cells ($2x10^5$ cells; The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences) were co-transfected with NORAD-wt plasmid (100 ng) or NORAD-mut plasmid (100 ng), and 50 nM miR-422a mimic or 50 nM miR-422a mimic-control using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. At 48 h post-transfection, luciferase activity was measured using the dual-luciferase reporter assay system (Promega Corporation). Firefly luciferase activities were normalized to *Renilla* luciferase activities.

Western blotting. Total protein was extracted from cells using RIPA lysis buffer (Santa Cruz Biotechnology, Inc.) and cell lysates were centrifuged at 20,000 x g for 10 min at 4˚C. Equal amounts of protein (30 μg) were separated via 10%

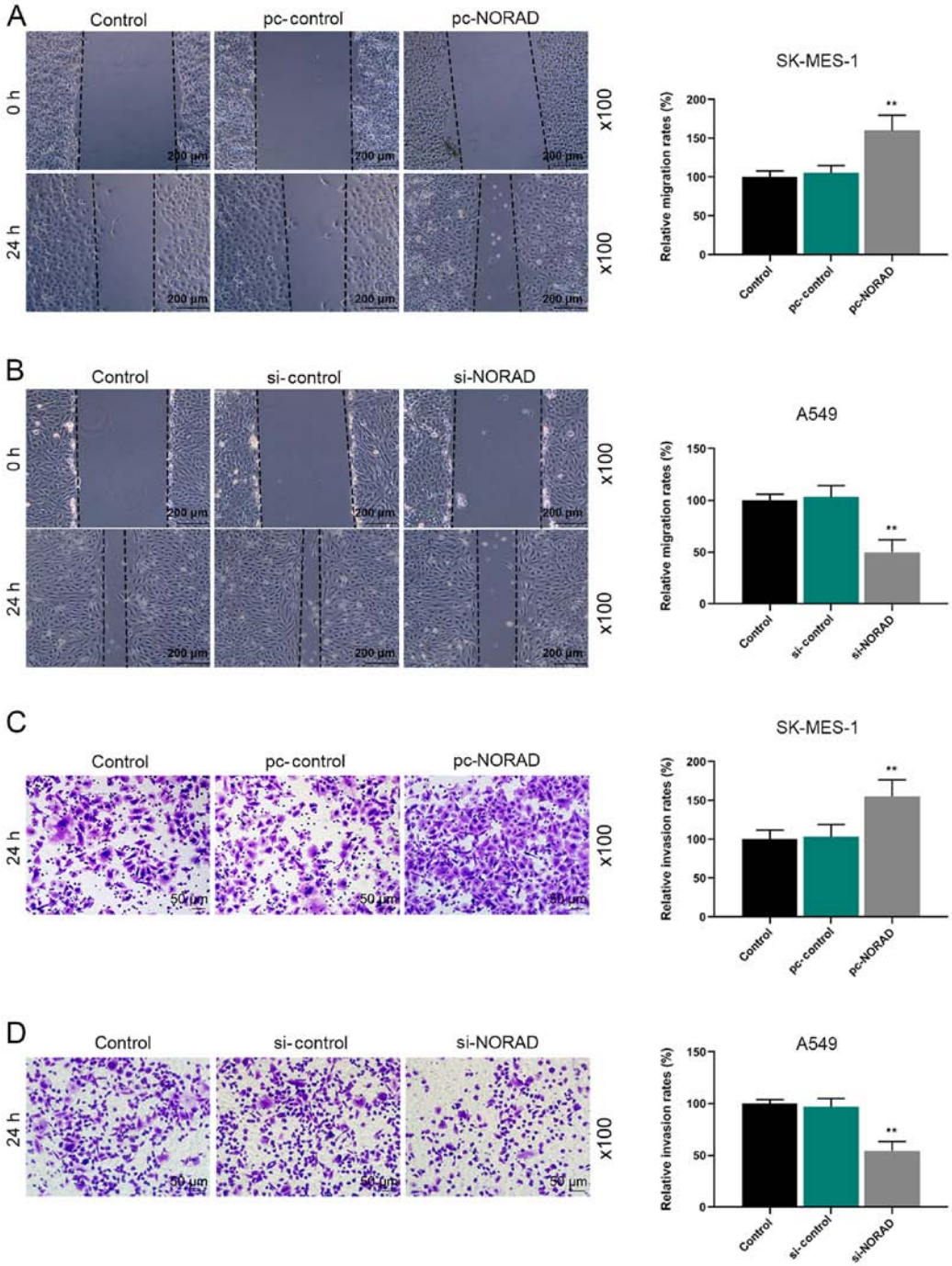

Figure 2. Effects of NORAD on NSCLC cell biological behaviors, such as migration and invasion. Effect of NORAD on (A) SK-MES-1 and (B) A549 cell migration. Effect of NORAD on (C) SK-MES-1 and (D) A549 cell invasion. **P<0.001 vs. pc-Control or si-Control. NORAD, non-coding RNA activated by DNA damage; NSCLC, non-small cell lung cancer; si, small interfering RNA.

SDS-PAGE and transferred onto PVDF membranes at 100 V for 1.5 h. The membranes were blocked with 5% non-fat milk for 1 h at room temperature and incubated overnight at 4˚C with primary antibodies targeted against: Matrix metallopeptidase (MMP)2 (1:1,000; 72 kD; cat. no. ab37150; Abcam), MMP9 (1:1,000; 95 kD; cat. no. ab73734; Abcam), E-cadherin (1:10,000; 97 kD; cat. no. ab40772; Abcam), N-cadherin (1:1,000; 130 kD; cat. no. ab18203; Abcam) and GAPDH (1:10,000; 36 kD; cat. no. ab181602; Abcam). Following primary incubation, the membranes were incubated with a secondary horseradish peroxidase-conjugated antibody (goat anti-rabbit IgG; 1:10,000; cat. no. ab205718; Abcam) for 1 h at room temperature. Protein bands were visualized using enhanced chemiluminescence detection reagent (Thermo Fisher Scientific, Inc.). Protein expression was quantified using Image-Pro Plus software (version 6.0; Media Cybernetics, Inc.) with GAPDH as the loading control.

Statistical analysis. Statistical analyses were performed using SPSS software (version 20.0; IBM Corp). Data are presented as the mean ± standard deviation. Comparisons between two groups were analyzed using the paired or unpaired Student's

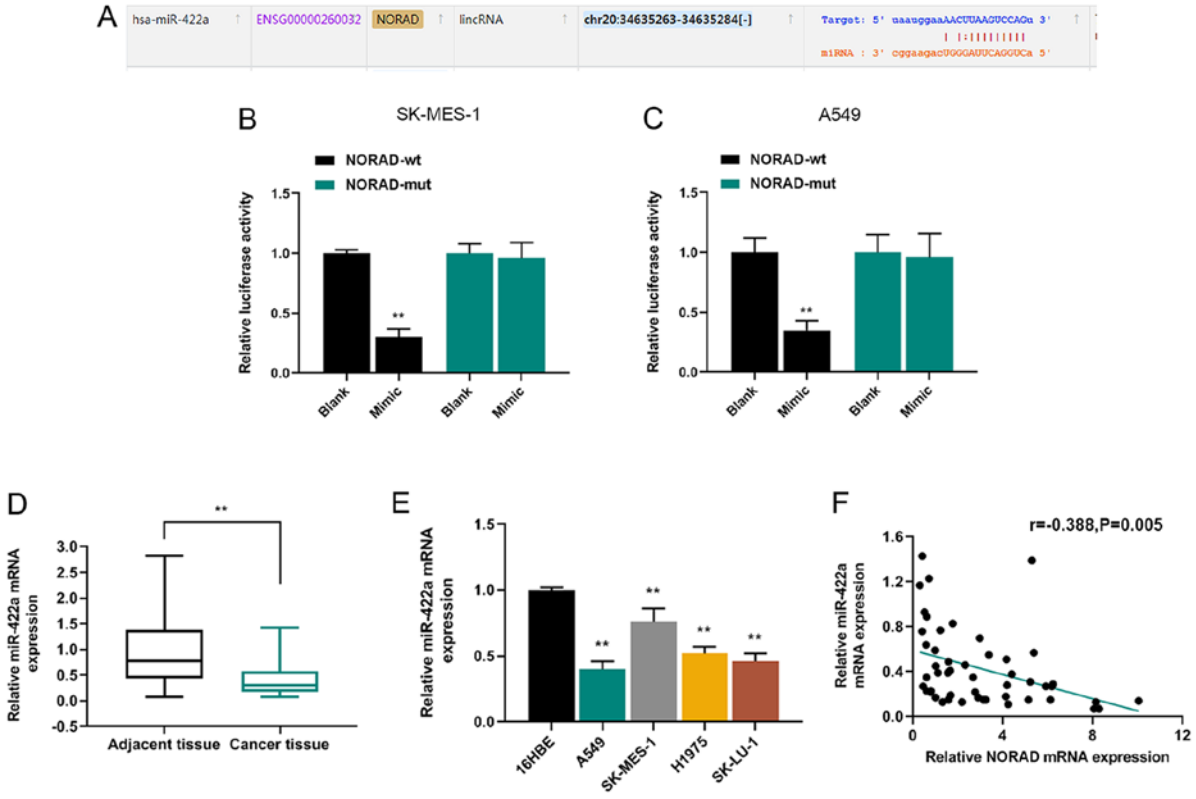

Figure 3. Interaction between NORAD and miR-422a in NSCLC tissues. (A) Binding site between miR-422a and NORAD. Effect of miR-422a mimic on the luciferase activity of NORAD-wt and NORAD-mut in (B) SK-MES-1 and (C) A549 cells. **P<0.001 vs. Blank. (D) miR-422a expression levels in NSCLC tissues. **P<0.001 vs. adjacent tissue. (E) miR-422a expression levels in NSCLC cell lines. **P<0.001 vs. 16HBE. (F) miR-422a expression was negatively correlated with NORAD expression in NSCLC tissues. NORAD, non-coding RNA activated by DNA damage; miR, microRNA; NSCLC, non-small cell lung cancer; wt, wild-type; mut, mutant.

t-test. Comparisons among multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. The correlation between continuous variables was analyzed using Pearson's correlation analysis. P<0.05 was considered to indicate a statistically significant difference. All experiments were performed at least three times.

Results

NORAD is highly expressed in NSCLC tissues and cells. The expression levels of NORAD in NSCLC tissues and cells were detected via RT-qPCR. The results indicated NORAD expression was significantly higher in NSCLC tissues compared with healthy adjacent tissues (Fig. 1A). Moreover, NORAD expression levels in NSCLC cell lines (A549, SK-MES-1, H1975 and SK-LU-1) were significantly higher compared with the normal human bronchial epithelial 16HBE cell line (Fig. 1B).

Effects of NORAD on NSCLC cell biological behaviors. The RT-qPCR results indicated that pc-NORAD and si-NORAD were successfully transfected into SK-MES-1 and A549 cells, respectively (Fig. 1C and D). CCK-8, wound healing and Transwell assays were performed to detect the effects of NORAD on NSCLC cell viability, migration and invasion, respectively. At 48 and 72 h, pc-NORAD-transfected cells displayed higher viability compared with the pc-control group, whereas si-NORAD-transfected cells displayed significantly lower viability compared with the si-control group (Fig. 1E and F). Moreover, the results indicated that NORAD overexpression significantly reduced the number of cells in G_1 phase and increased the number of cells in G_2/M phase compared with the pc-control group. By contrast, NORAD knockdown increased the number of cells in G_1 phase and reduced the number of cells in G_2/M phase compared with the si-control group (Fig. 1G and H). In addition, the wound healing assay demonstrated that pc-NORAD significantly increased cell migration compared with the pc-control group, whereas si-NORAD displayed the opposite results compared with the si-control group (Fig. 2A and B). Similarly, the results of the Transwell assay indicated that NORAD-overexpression cells displayed a significantly higher invasion rate compared with the pc-control group, whereas NORAD-knockdown cells displayed a significantly decreased invasion rate compared with the si-control group (Fig. 2C and D).

NORAD interacts with miR-422a and the expression of miR-422a in NSCLC tissues. The potential mechanisms underlying NORAD in NSCLC cells were explored, and the relationship between NORAD and miRNA was predicted. starBase was used to identify the binding sites between NORAD and miR-422a (Fig. 3A). A dual-luciferase reporter assay indicated that the luciferase activity of NORAD-wt was significantly reduced by miR-422a mimic compared with the blank group, which verified the relationship between NORAD and miR-422a (Fig. 3B and C). Furthermore, the expression of miR-422a in NSCLC tissues was detected via RT-qPCR. The

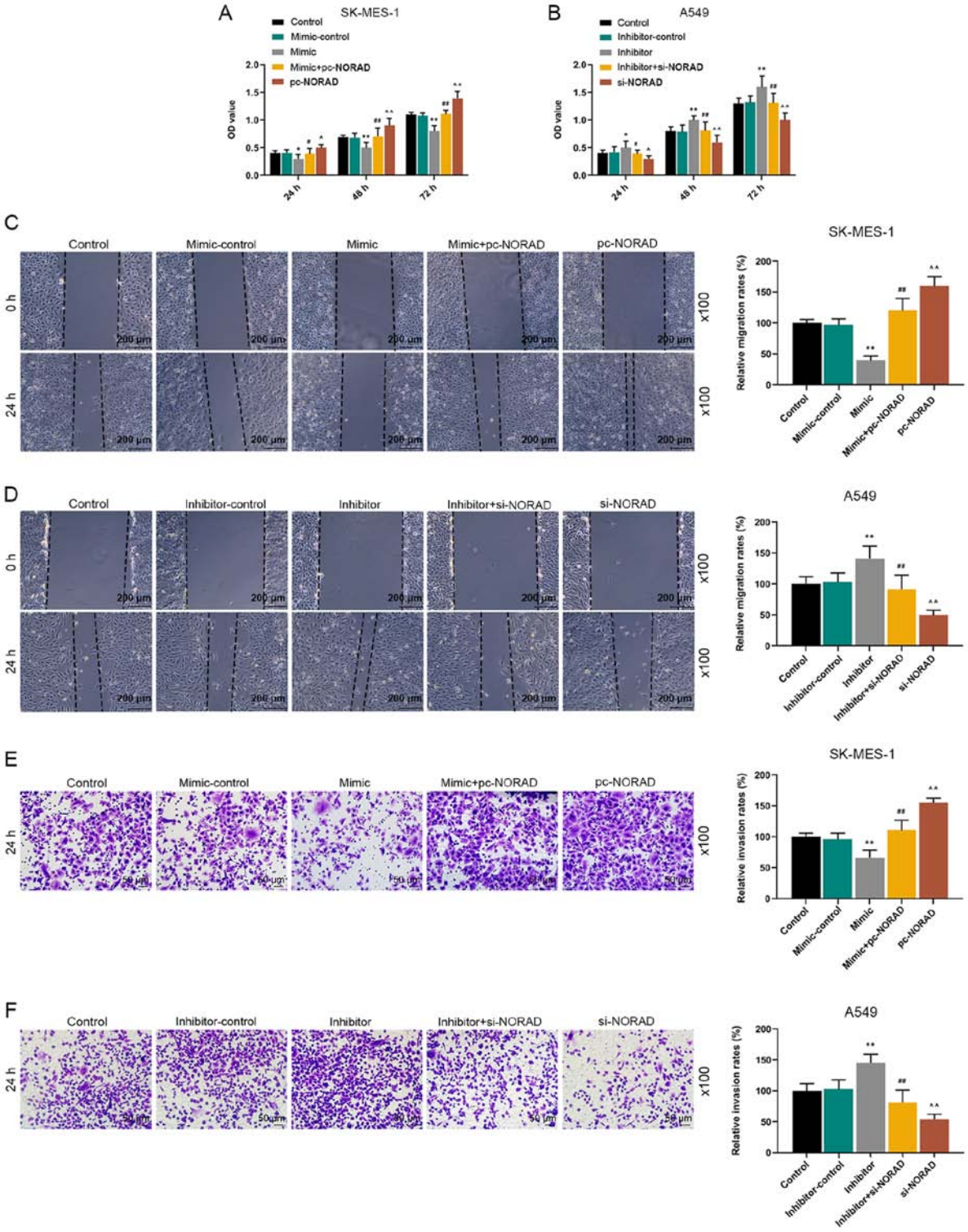

Figure 4. NORAD regulates NSCLC cell viability, migration and invasion via miR-422a. Effect of miR-422a and NORAD on (A) SK-MES-1 and (B) A549 cell viability. Effect of miR-422a and NORAD on (C) SK-MES-1 and (D) A549 cell migration. Effect of miR-422a and NORAD on (E) SK-MES-1 and (F) A549 cell invasion. *P<0.05 and **P<0.001 vs. mimic-Control or inhibitor-Control; $^{\#}$P<0.05 and $^{\#\#}$P<0.001 vs. mimic or inhibitor; $^{\wedge}$P<0.05 and $^{\wedge\wedge}$P<0.001 vs. mimic + pc-NORAD or inhibitor + si-NORAD. NORAD, non-coding RNA activated by DNA damage; NSCLC, non-small cell lung cancer; miR, microRNA; si, small interfering RNA.

results demonstrated that miR-422a expression levels were significantly lower in NSCLC tissues compared with healthy adjacent tissues (Fig. 3D). In addition, miR-422a expression levels in the NSCLC cell lines (A549, SK-MES-1, H1975 and SK-LU-1) were significantly reduced compared with 16HBE cells (Fig. 3E). Pearson's correlation analysis indicated that miR-422a expression was negatively correlated with NORAD expression in NSCLC tissues (Fig. 3F).

NORAD regulates the biological behaviors of NSCLC cells via miR-422a. CCK-8, wound healing and Transwell assays were performed to detect the effects of NORAD and miR-422a on

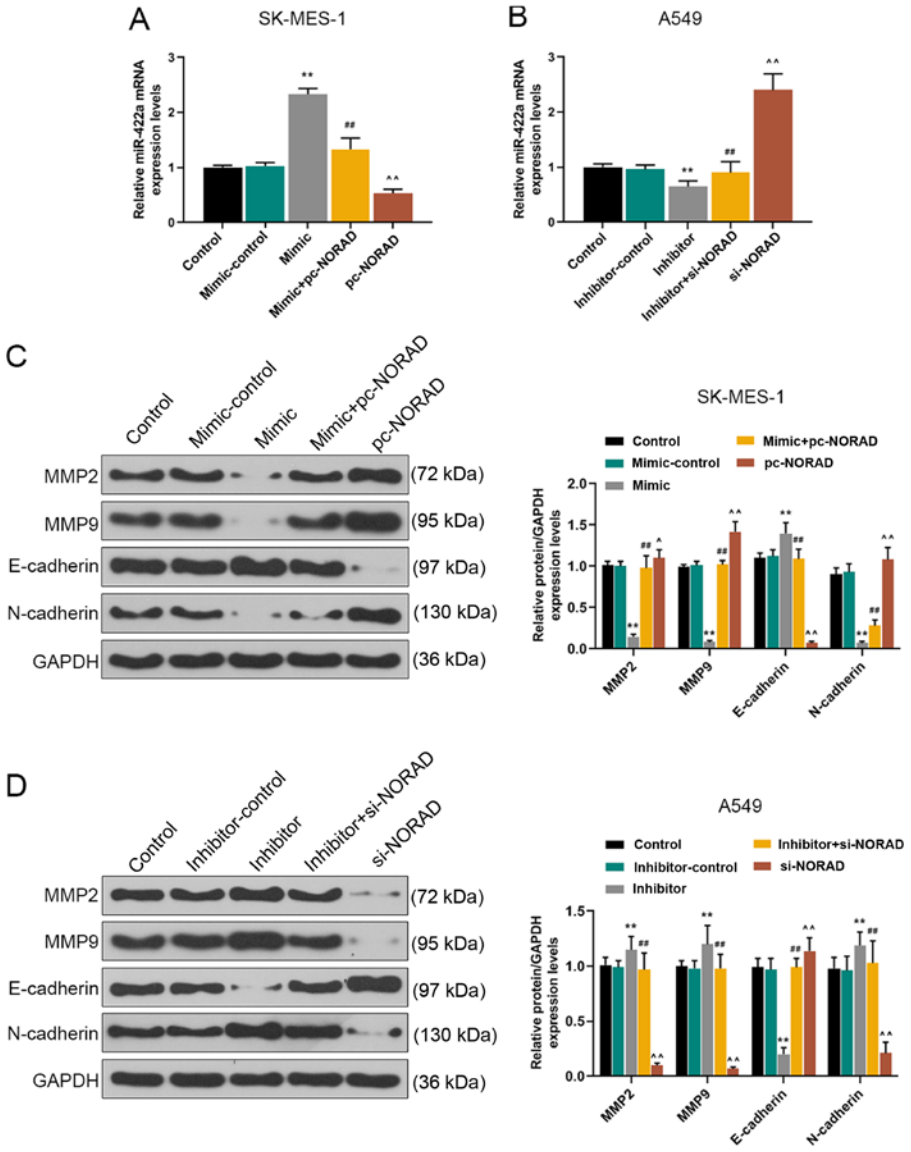

Figure 5. NORAD regulates EMT by regulating miR-422a. Effects of miR422a and NORAD on miR422a expression in (A) SK-MES-1 and (B) A549 cells. EMT-related protein expression levels in (C) SK-MES-1 and (D) A549 cells. **P<0.001 vs. mimic-Control or inhibitor-Control; $^{\#\#}$P<0.001 vs. mimic or inhibitor; $^{\wedge}$P<0.05 and $^{\wedge\wedge}$P<0.001 vs. mimic + pc-NORAD or inhibitor + si-NORAD. NORAD, non-coding RNA activated by DNA damage; EMT, epithelial-mesenchymal transition; miR, microRNA; si, small interfering RNA; MMP, matrix metallopeptidase.

cell viability, migration and invasion. The CCK-8 assay results indicated that miR-422a overexpression significantly reduced cell viability compared with the mimic-control, but cells transfected with miR-422a mimic and pc-NORAD displayed significantly increased cell viability compared with the mimic group. However, miR-422a knockdown significantly increased cell viability compared with the inhibitor-control, which was reversed by co-transfection with si-NORAD (Fig. 4A and B). Moreover, the wound healing assay results suggested that miR-422a mimic significantly decreased cell migration compared with the mimic-control group, whereas miR-422a inhibitor displayed the opposite effect compared with the inhibitor-control group. Furthermore, cells co-transfected with miR-422a mimic and pc-NORAD displayed significantly increased cell migration compared with the mimic group, whereas cells co-transfected with miR-422a inhibitor and si-NORAD displayed decreased cell migration compared with the inhibitor group (Fig. 4C and D). The results of the Transwell assay revealed that miR-422a overexpression significantly inhibited cell invasion compared with the mimic-control group, but miR-422a knockdown displayed the opposite effects compared with the inhibitor-control group. However, alterations to NORAD expression levels partly reversed the effects of miR-422a on cell invasion (Fig. 4E and F).

NORAD regulates EMT by regulating miR-422a expression. The RT-qPCR results indicated that the expression levels of miR-422a in SK-MES-1 cells were significantly decreased by NORAD overexpression compared with the mimic + pc-NORAD group, whereas NORAD knockdown displayed the opposite effects compared with the inhibitor + si-NORAD group (Fig. 5A and B). To explore the effect of NORAD on NSCLC cell EMT, western blotting was performed to measure the expression levels of EMT-related proteins, such as MMP2, MMP9, E-cadherin and N-cadherin. The results demonstrated that miR-422a overexpression significantly reduced the protein

expression levels of MMP2, MMP9 and N-cadherin, but significantly increased the protein expression levels of E-cadherin compared with the mimic-control group. NORAD overexpression reversed miR-422a overexpression-mediated effects on protein expression (Fig. 5C). However, miR-422a knockdown significantly increased the protein expression levels of MMP2, MMP9 and N-cadherin, and significantly reduced the expression levels of E-cadherin compared with the inhibitor-control group. NORAD knockdown reversed miR-422a knockdown-mediated effects on protein expression (Fig. 5D).

Discussion

NSCLC is one of the most frequently diagnosed types of cancer, with a poor prognosis and high mortality rate (18). Moreover, developing effective strategies to improve the prognosis of NSCLC remains a challenge. NORAD is an important lncRNA that serves a key role in cancer cell migration and invasion (19,20). lncRNAs have critical functions during cancer progression (21,22). For example, lncRNA small nucleolar RNA host gene 1 (SNHG1) functions as an oncogene in colorectal cancer, promoting colorectal cancer cell proliferation via epigenetic silencing of Kruppel like factor 2 and cyclin dependent kinase inhibitor 2B in the nucleus (23). Knockdown of HOXD antisense growth-associated long non-coding RNA inhibited cell proliferation and migration, and promoted cell apoptosis in bladder cancer cells (24). The present study investigated the role of NORAD in NSCLC cell viability, migration, invasion, as well as the underlying mechanisms. The results revealed that NORAD expression was significantly higher in NSCLC tissues and cells compared with adjacent healthy tissues and cells. In addition, compared with the control groups, NORAD overexpression promoted NSCLC cell viability, migration and invasion, whereas NORAD knockdown displayed the opposite effects. Furthermore, the results indicated that NORAD regulated NSCLC cell EMT by serving as a sponge for miR-422a.

Previous studies have indicated that NORAD serves a vital role in promoting the development of a number of different types of cancer. For instance, Zhou *et al* (25) reported that NORAD expression is increased in breast cancer tissues, which promotes cell proliferation, migration and invasion, and is related to a poor prognosis. According to Tong *et al* (26), NORAD knockdown can inhibit cell proliferation, reduce bufalin's chemical resistance, and inhibit cell cycle transition and xenograft growth, suggesting that it displays an anticancerous effect on epithelial ovarian cancer. Another study demonstrated that low expression of NORAD in osteosarcoma significantly inhibits cell proliferation and invasion *in viv*o (27). Moreover, it has been reported that NORAD promotes the development of other types of cancer, such as gastric (28), prostate (29) and ovarian cancer (26,30), as well as osteosarcoma (27). Similarly, Chen *et al* (15) indicated that NORAD increases the expression of AKT1 by adsorbing miR-656-3p to promote NSCLC cell proliferation and migration. The results of the aforementioned studies were consistent with the results of the present study. The present study indicated that NORAD was associated with the occurrence and development of NSCLC, and that the expression of NORAD was increased in NSCLC tissues and cell lines compared with adjacent healthy tissues and cells. In addition, NORAD expression was associated with A549 and SK-MES-1 cell viability, migration and invasion. Based on the results, it was hypothesized that NORAD may serve as a novel therapeutic target for NSCLC.

lncRNAs can regulate cell biological behaviors via miRNAs (31). In the present study, the relationship between NORAD and miR-422a was investigated using a dual-luciferase reporter assay. In addition, a negative correlation between NORAD expression and miR-422a expression was identified in NSCLC. Moreover, compared with the control group, miR-422a overexpression reduced NSCLC cell viability, migration and invasion, which was reversed by NORAD overexpression. By contrast, compared with the inhibitor-control group, miR-422a knockdown increased NSCLC cell viability, migration and invasion, which was reversed by NORAD knockdown. Therefore, the results suggested that NORAD served as an oncogene during NSCLC progression by regulating miR-422a. A previous study reported that miR-422a regulates metabolism and malignant tumors of gastric cancer cells by targeting pyruvate dehydrogenase kinase 2 (32). In addition, it was also reported that LINC00313, an oncogene in papillary thyroid cancer, regulates cell proliferation, invasion, migration and EMT by regulating miR-422a expression (33). Another previous study demonstrated that miR-422a also functions as a tumor suppressor in lung cancer cells by regulating sulfatase 2-mediated transforming growth factor-β/SMAD signaling pathway (34). LINC00858 facilitates NSCLC cell proliferation by sponging miR-422a and activating kallikrein related peptidase 4 (35). However, the specific signaling pathways involved in the procancer effects of NORAD in NSCLC require further investigation.

EMT is associated with cancer metastasis and drug resistance to treatment (36). Various mechanisms, especially EMT, which is related to pathogenesis of chronic obstructive pulmonary disease, are involved in the progression and metastasis of NSCLC (37). EMT can increase cell migration and invasion to induce cancer metastasis, and MMPs such as MMP2 and MMP9 can promote the carcinogenesis of EMT by degrading barriers such as the extracellular matrix (38). E-cadherin and N-cadherin are two representative EMT markers (39). Noh *et al* (40) indicated that N-cadherin expression may contribute to the biological aggression of glioma. Liao *et al* (41) indicated that lncRNA H19 imprinted maternally expressed transcript overexpression mediates lung cancer cell proliferation and invasion by blocking the expression of N-cadherin and inducing the expression of E-cadherin. A recent study demonstrated that NORAD knockdown inhibits gastric cancer cell migration and invasion by regulating EMT-related genes (42). Wu *et al* (43) reported that miR-422a is associated with lymphatic metastasis in lung cancer. It has also been reported that miR-422a overexpression inhibited NSCLC cell EMT progression (34), which is consistent with the results of the present study. The present study indicated that miR-422a overexpression reduced the expression levels of MMP2, MMP9 and N-cadherin, and increased the expression levels of E-cadherin compared with the mimic-control group, but NORAD overexpression reversed miR-422a overexpression-mediated effects. By contrast, miR-422a knockdown increased the expression levels of MMP2, MMP9 and N-cadherin and reduced the expression levels of E-cadherin

compared with the inhibitor-control group, whereas NORAD knockdown reversed miR-422a knockdown-mediated effects. Therefore, it was speculated that NORAD may serve as a novel therapeutic target for NSCLC. However, the present study had a number of limitations. For example, *in vivo* experiments need to be conducted to confirm the results of the present study. In addition, the target genes and signaling pathways associated with miR-422a-mediated regulation of NSCLC development, and other potential mechanisms underlying the role of NORAD in NSCLC cells require further investigation.

In conclusion, the present study indicated that NORAD displayed a procancer role in NSCLC by sponging miR-422a. NORAD promoted NSCLC cell viability, migration and invasion via downregulating miR-422a. Therefore, NORAD may serve as a novel therapeutic target for NSCLC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZC and QC contributed to the conception and design of the study. ZC, QC and CX acquired, analyzed and interpreted the data. ZC and QC drafted and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all patients. The present study was approved by Jingmen No. 1 People's Hospital Ethics Committee (approval no. JM2018010232).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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