Silencing of Long Non-Coding RNA (LncRNA) Non-Coding RNA Activated by DNA Damage (NORAD) Inhibits Proliferation, Invasion, Migration, and Promotes Apoptosis of Glioma Cells via Downregulating the Expression of AKR1B1

Authors' Contribution:
Study Design **A**
Data Collection **B**
Statistical Analysis **C**
Data Interpretation **D**
Manuscript Preparation **E**
Literature Search **F**
Funds Collection **G**

ABE **Lun Luo**
ACD **Chuan Chen**
DFG **Haiyong He**
BCFG **Meiqin Cai**
BCE **Cong Ling**

Department of Neurosurgery, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, P.R. China

Corresponding Author: Lun Luo, e-mail: luolunshine@163.com
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Background: We aimed to investigate the functions of long non-coding RNA (lncRNA) non-coding RNA activated by DNA damage (NORAD) in glioma and identify the potential mechanisms.

Material/Methods: The expression of NORAD and AKR1B1 in human glioma cell lines were examined using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Then, cell proliferation, invasion, and migration were tested by Cell Counting Kit-8 (CCK-8), colony formation assay, Transwell, and scratch wound healing assay after NORAD silencing. Meanwhile, western blotting was utilized to measure the expression of migration-related proteins. Apoptosis of glioma cells was detected using flow cytometry and apoptosis-related proteins expression was determined. Moreover, the correlation between NORAD and AKR1B1 was verified by RNA-binding protein immunoprecipitation (RIP assay). After co-transfection with AKR1B1 overexpressed plasmid and NORAD siRNA, cell proliferation, invasion, migration, and apoptosis were examined again. Furthermore, the expression of proteins in extracellular signal-regulated kinase (ERK) signaling was tested using western blotting.

Results: The results revealed that NORAD and AKR1B1 were highly expressed in glioma cells. NORAD silencing inhibited proliferation, invasion and migration but promoted apoptosis of glioma cells, accompanied by the expression changes of migration- and apoptosis-related proteins. However, after co-transfection with AKR1B1 pcDNA3.1 in NORAD silencing cells, the effects of NORAD silencing on proliferation, invasion, migration, and apoptosis were attenuated. Consistently, the expression of phosphorylated ERK (p-ERK) was decreased after NORAD silencing, which were reversed following AKR1B1 overexpression.

Conclusions: These findings demonstrated that NORAD silencing suppressed proliferation, invasion, and migration and boosted apoptosis of glioma cells via downregulating the AKR1B1 expression, which may provide a potential therapeutic target for glioma treatment.

MeSH Keywords: Apoptosis • Cell Migration Assays • Cell Proliferation • Neoplasm Invasiveness

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Background

Glioma is known as one of the most prevalent and aggressive brain tumors and it is well-known for its unfavorable prognosis, which accounts for about 80% of malignant neoplasia of the central nervous system [1,2]. Compelling evidence indicates that thousands of new patients are diagnosed with glioma each year worldwide [3]. Despite some progress in the diagnosis and treatment strategies in recent years, the overall survival rate of patients with glioma is markedly poor, which is attributed to tumor invasion and metastasis [4,5]. Therefore, a deeper understanding of the molecular mechanisms implicated in the development and progression of glioma is crucial for the prevention and treatment of glioma, which is a major task for researchers.

Long non-coding RNAs (lncRNAs) are acknowledged as one subtype of endogenous non-protein-coding transcripts, which consist of >200 nucleotides [6,7]. LncRNAs are newly identified regulators in multiple cancers. A growing body of literature has shown that aberrant levels of lncRNAs are involved in multiple biological processes of cancers through regulating the expression of genes at the transcriptional or posttranscriptional level [8,9]. It has been well documented that lncRNA non-coding RNA activated by DNA damage (NORAD) is dramatically upregulated in various malignant tumors, contributing to cancer progression [10,11]. Reports have also demonstrated that NORAD silencing can suppress proliferation, invasion, and migration but promote apoptosis in gastric cancer cells via regulation of the RhoA/ROCK1 pathway [12]. However, the functions of NORAD in glioma remain to be elucidated.

In the current study, we concentrated on the functions of NORAD on proliferation, invasion, migration, and apoptosis of glioma cells, and probed the underlying mechanisms involved in the progress of gliomas. Our findings may offer new insights into the diagnosis and strategies of glioma.

Material and Methods

Cell culture

Human glioma cell lines (GSC11, M059J, U251, and T98G) and normal human astrocytes A735 cells were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific) added with 10% fetal bovine serum (FBS; Sigma-Aldrich, St Louis, MO, USA) at 37°C in a humidified incubator containing 5% CO_2.

Cell transfection

For the transfection, U251 cells were plated in a 24-well plate. When cells reached 70% confluence, small interfering RNAs (siRNAs) against NORAD (si-NORAD-1 and si-NORAD-2), negative control for NORAD siRNA (si-NC), AKR1B1 overexpressed plasmid pcDNA3.1 (pcDNA-AKR1B1) and empty vector plasmid (pcDNA-NC) were transfected into cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) following manufacturer's recommendations. The sequences used in this study were as follows:
si-NORAD-1, 5'-AAGCCACCTTTGTGAACAGTA-3';
si-NORAD-2, 5'-AATAGAATGAAGACCAACCGC-3';
si-NC, 5'-GCGCGATAGCGCGAATATA-3'.
All the aforementioned transfection complexes were purchased from GenePharma (Shanghai, China). At 48 hours post-transfection, cells were collected and the transfection efficacy was detected using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis or western blot analysis.

Cell proliferation assay

Cell Counting Kit-8 (CCK-8) assay was used to test proliferation of U251 cells after transfection. In brief, cells were grown in 96-well plates. At 24, 48, and 72 hours after transfection, 10 μL CCK-8 solution was appended for 2 hours of incubation. The optical density was determined at 450 nm using a microplate reader.

Transwell assay

The ability of cell invasion was examined utilizing Transwell assay. 24-well Transwell plates (Corning, Inc.) with 8-μm pore inserts coated with Matrigel (BD Biosciences) was used in this experiment. U251 cells were re-suspended in 200 μL serum-free DMEM (5×10^4 cells/well) and put into the upper compartment. Then, 600 μL DMEM containing 10% FBS was added to the lower chamber. Following incubation for 24 hours, a cotton-tipped swab was employed to remove cells remaining on the upper surface of the filter. Cells transferred to the basolateral chamber were fixed with 4% paraformaldehyde and stained with 1% crystal violet solution. The invasive cells in 5 random fields (magnification 200x) were counted under an inverted microscope (Olympus Corporation, Tokyo, Japan).

Scratch wound healing assay

The migratory ability of U251 cells was determined using scratch wound healing assay. Briefly, cells were plated in a 6-well plate and cultured at 37°C in a humidified incubator until 80% confluence. Subsequently, the cells had been serum-starved in serum-free medium for 24 hours. A 200 μL of pipette tip was then applied to create a wound on the surface
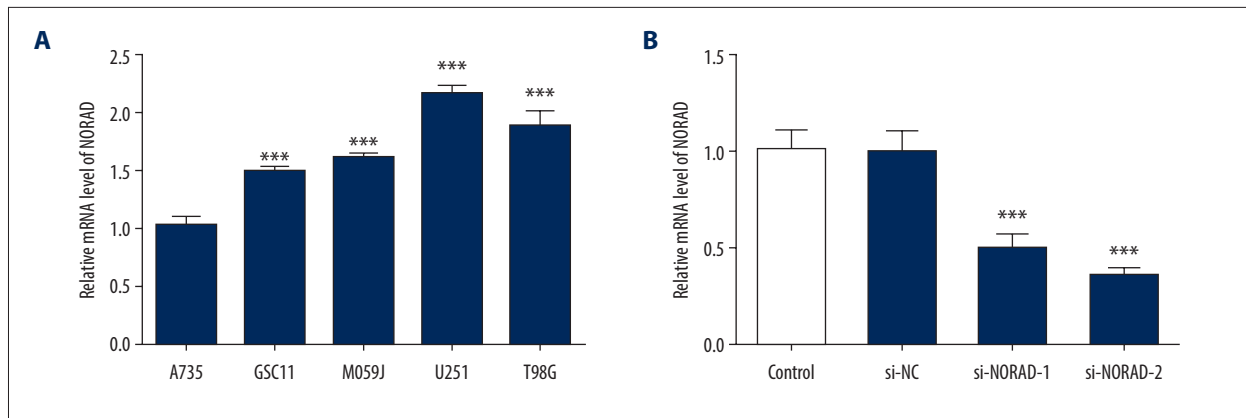
Figure 1. NORAD was highly expressed in glioma cell lines. (**A**) The expression of NORAD in glioma cell lines (GSC11, M059J, U251, and T98G) and normal human astrocytes A735 cells was detected using RT-qPCR. Three independent experiments were carried out (n=3). *** $P<0.001$ *vs.* A735. (**B**) The expression of NORAD was tested using RT-qPCR after transfection with si-NORAD-1 or si-NORAD-2. Three independent experiments were carried out (n=3). *** $P<0.001$ *vs.* si-NC. NORAD – noncoding RNA activated by DNA damage; RT-qPCR – reverse transcription-quantitative polymerase chain reaction; si-NORAD – small interfering RNAs (siRNAs) against NORAD.

of monolayers. The cells were washed twice with phosphate-buffered saline (PBS) in order to remove debris and then maintained in serum-free DMEM for 48 hours. Wound healing was observed using microscopy (magnification 100×; Olympus Corporation, Tokyo, Japan), the percentage of which was calculated using ImageJ software (NIH, MA, USA). The average distance of cells migrated into wound surface was detected 48 hours later under an inverted microscope.

Cell apoptosis assay

Transfected U251 cells were collected and then resuspended in the 1x binding buffer to a concentration of 1×10⁴ cells/well. An Annexin V-FITC Staining Cell Apoptosis Detection kit (KeyGEN BioTECH, China) was adopted for assessing cell apoptosis following manufacturer's recommendations. Briefly, cells were stained with Annexin V-FITC and propidium iodide (PI) and incubated in the dark. Cell apoptosis was analyzed with FlowJo software (Becton-Dickinson-San Jose, CA, USA).

RT-qPCR

Total RNA extraction was conducted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Then, cDNA was synthesized by use of a Reverse Transcription kit (Beijing TransGen Biotech, Beijing, China) in accordance with the manufacturer's guidelines. Samples were prepared for PCR using iTaq™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, Inc.) on an ABI 7500 instrument (Applied Biosystems; Foster City, CA, USA). GAPDH was considered as internal reference gene. Relative expression was normalized against GAPDH and calculated based on the $2^{-\Delta\Delta Cq}$ method. The qPCR primers were as follows:

NORAD forward, 5'-AAGCTGCTCTCAACTCCACC-3' and reverse, 5'-GGACGTATCGCTTCCAGAGG-3';
AKR1B1 forward, 5'-TTTTCCCATTGGATGAGTCGG-3' and reverse, 5'-CCTGGAGATGGTTGAAGTTGG-3';
GAPDH forward, 5'-ACAACTTTGGTATCGTGGAAGG-3' and reverse, 5'-GCCATCACGCCACAGTTTC-3'.

RNA immunoprecipitation (RIP) assay

The starBase database predicated that AKR1B1 could target NORAD. Therefore, RIP assay was employed to verify the binding between them. The experiment was performed using a RNA-Binding Protein Immunoprecipitation kit (Sigma-Aldrich; Merck KGaA). In brief, cells were lysed in lysis buffer and the cell lysates were incubated with magnetic beads conjugated to human anti-argonaute2 (Ago2) antibody (Abcam) or anti-IgG (negative control, EMD Millipore). The enrichment of NORAD and AKR1B1 was tested using RT-qPCR.

Western blot analysis

Cellular total protein was extracted from U251 cells using a protein lysis buffer in the presence of a protease inhibitor cocktail (Beyotime, Shanghai, China). A BCA Protein Quantitation kit (Beyotime, Shanghai, China) was executed for detecting the concentration of protein in each group. Equal amounts (40 µg) of protein samples were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore, USA). Subsequently, the membranes were blocked in 5% skimmed milk and then incubated with primary antibodies. After washing, proteins on the PVDF membranes were incubated with horseradish peroxidase-conjugated secondary
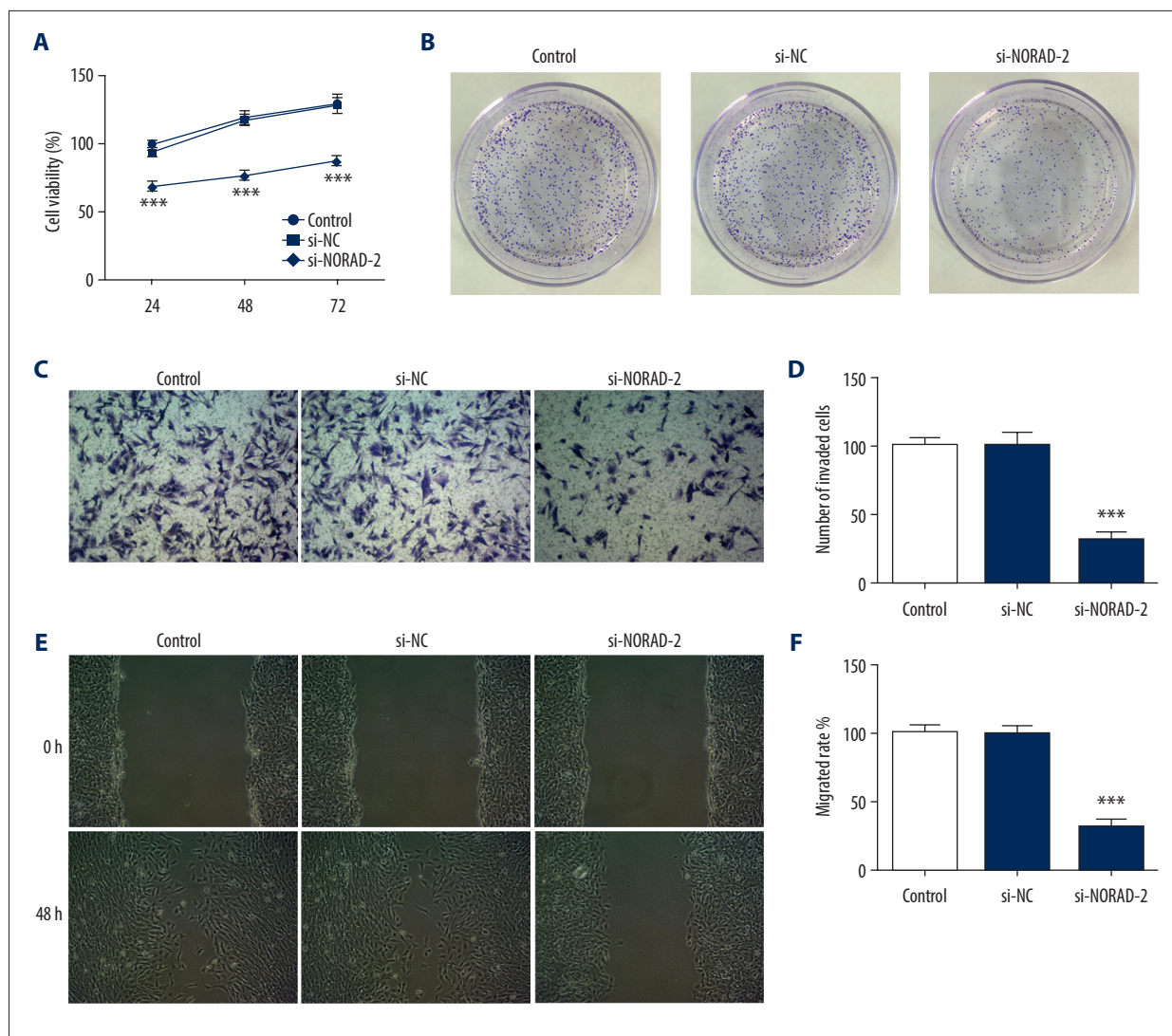
Figure 2. NORAD silencing inhibited proliferation, invasion, and migration of glioma cells. Cell proliferation was examined using (**A**) CCK-8 and (**B**) colony formation assay. (**C**) The invasion ability of U251 cells was evaluated using Transwell assay. (magnification 200×). (**D**) Quantification of cell invasion. (**E**) Representative images and (**F**) relative quantification of cell migration, as measured using wound healing assay. (magnification 100×). All experiments were repeated 3 times independently (n=3). *** $P<0.001$ *vs.* si-NC. NORAD – noncoding RNA activated by DNA damage; CCK-8 – Cell Counting Kit-8; si-NC – negative control for NORAD siRNA.

antibodies and visualized using a chemiluminescence kit (Pierce Biotechnology; Thermo Fisher Scientific, Inc). Intensity of bands were quantified by using ImageJ software (NIH, MA, USA). GAPDH was used as internal control.

Statistical analysis

The data in the present study were presented as mean±standard deviation (SD). All data were compared using Student's *t*-test or analysis of variance (ANOVA) followed by Turkey's post hoc test with GraphPad Prism 6.0 software. Differences were considered significant at P less than 0.05.

Results

The expression of NORAD was highly expressed in glioma cell lines

To explore the function of NORAD in glioma, the level of NORAD in several glioma cell lines (GSC11, M059J, U251, and T98G) were tested using RT-qPCR. Results suggested that NORAD was highly expressed in glioma cell lines relative to normal human astrocytes A735 cells, with the highest NORAD level in U251 cells (Figure 1A). Therefore, the U251 cell line was used to perform the following experiments in the present study. Then, si-NORAD-1
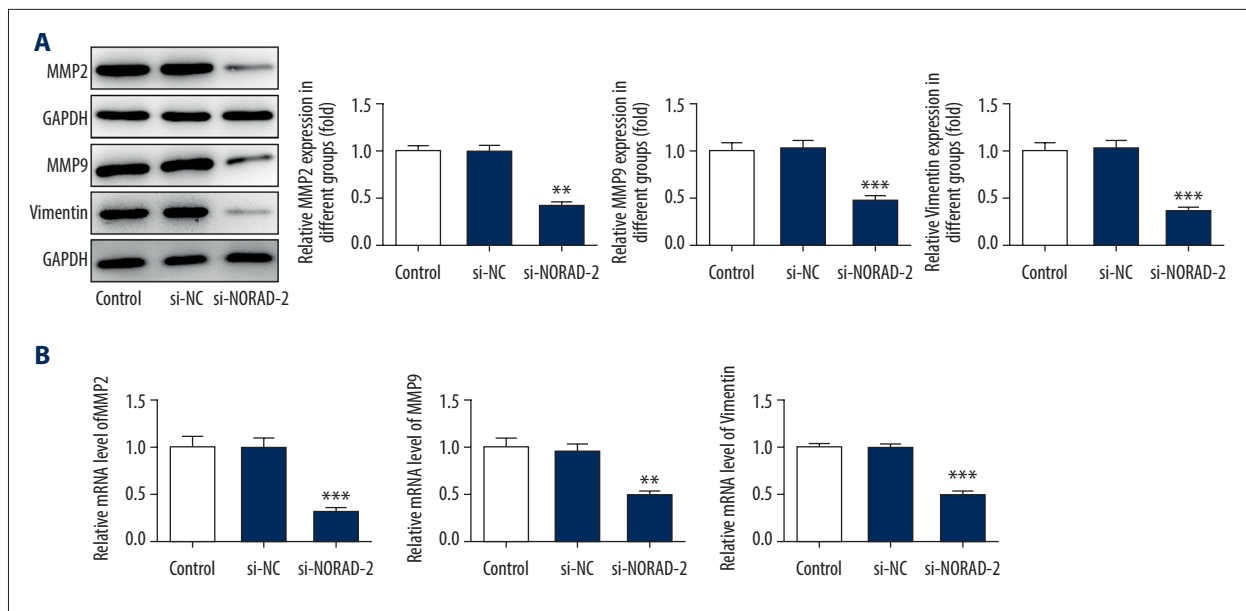
Figure 3. NORAD silencing reduced the expression of migration-related genes in glioma cells. The MMP2, MMP9, and vimentin (**A**) protein and (**B**) mRNA expression was detected using western blotting and RT-qPCR, respectively. All experiments were repeated 3 times independently (n=3). ** P<0.01, *** P<0.001 versus si-NC. NORAD – noncoding RNA activated by DNA damage; mRNA – messenger RNA; RT-qPCR – reverse transcription-quantitative polymerase chain reaction; si-NC – negative control for NORAD siRNA.

or si-NORAD-2 were transfected into U251 cells and the transfection efficiency was affirmed using RT-qPCR (Figure 1B). Cells transfected with si-NORAD-2 were selected for further assays due to its higher inference efficacy. The results indicated that NORAD might possess a prominent role in glioma.

NORAD silencing hampered proliferation, invasion, and migration of glioma cells

The roles of NORAD silencing on proliferation, invasion, and migration of glioma cells were investigated subsequently. The results of CCK-8 (Figure 2A) and colony formation assay (Figure 2B) demonstrated that proliferation was obviously suppressed in si-NORAD-2 group compared with the si-NC group. In addition, the ability of cell invasion and migration were assessed by Transwell assay and scratch wound healing assay, respectively. As exhibited in Figure 2C and 2D, transfection with si-NORAD-2 restrained glioma cell invasion in contrast with transfection with si-NC. The changes of cell migratory ability (Figure 2E, 2F) and expression of migration-related genes MMP2, MMP9, and vimentin (Figure 3A, 3B) had the same trend with cell invasion. Taken together, these results implicated that NORAD silencing suppressed proliferation, invasion, and migration of glioma cells.

NORAD silencing promoted apoptosis of glioma cells

To confirm the effect of NORAD silencing on apoptosis of glioma cells, flow cytometry analysis was performed in the study. Significant increase of apoptotic rate was observed in U251 cells after transfection with si-NORAD-2 relative to the si-NC group (Figure 4A, 4B). Meanwhile, the expression of apoptosis-related proteins was measured using western blotting. Results demonstrated that NORAD inhibition lowered the expression of Bcl-2, accompanied by increased expression of Bax, caspase-3, and caspase-9 (Figure 4C), hinting that cell apoptosis was accelerated after NORAD was silenced. These observations revealed that NORAD downregulation activated apoptosis of glioma cells.

AKR1B1 could bind to NORAD

To further explore the NORAD-mediated molecular mechanism, the starBase database was employed to predict the potential targets of NORAD, and AKR1B1 was predicted as a target of NORAD, which was verified by RIP assay (Figure 5A). Then, the protein and messenger RNA (mRNA) expression of AKR1B1 in glioma cell lines was evaluated using western blotting and RT-qPCR, respectively. As presented in Figure 5B and 5C, the protein and mRNA levels of AKR1B1 were notably upregulated in glioma cell lines, especially in U251 cells. Also, obviously downregulated expression of AKR1B1 protein and mRNA was
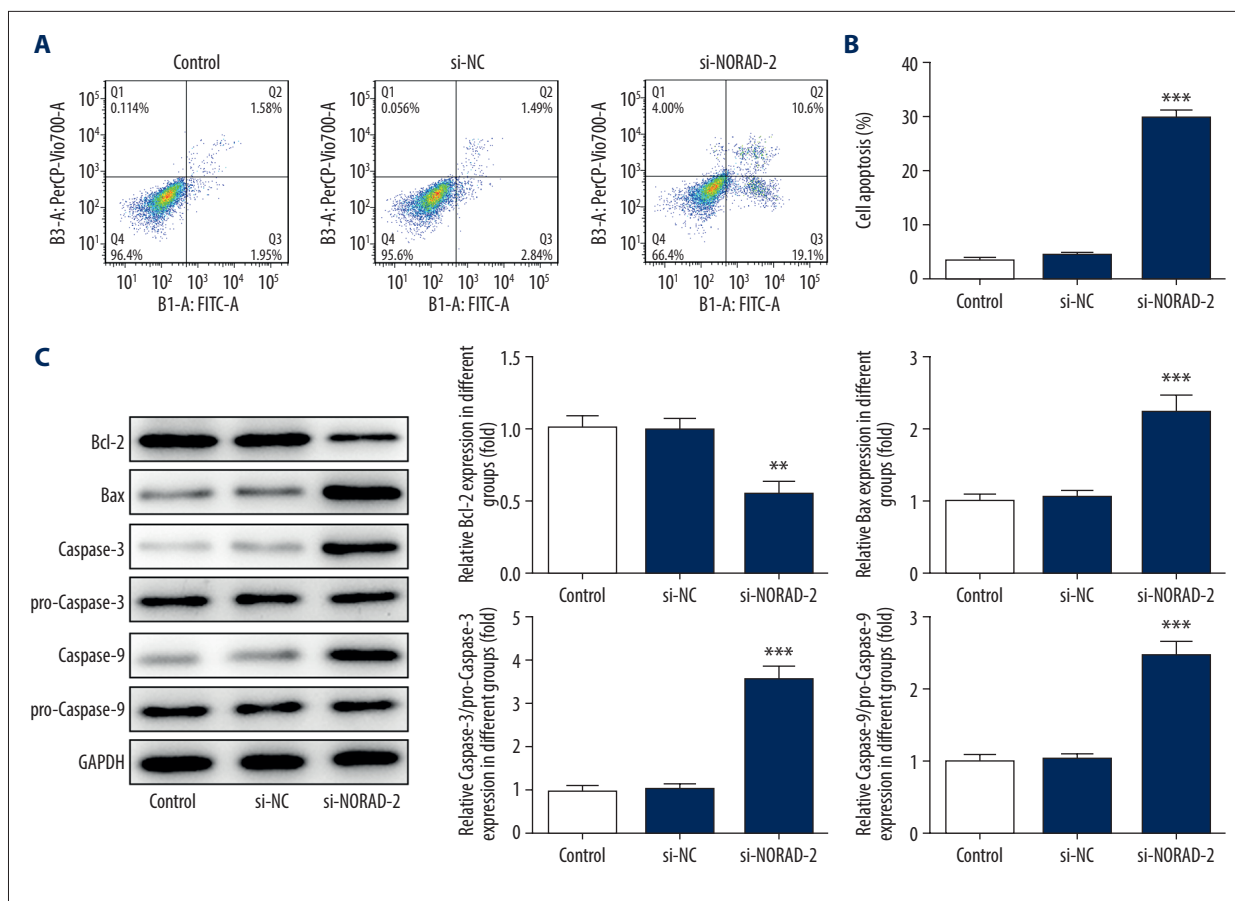
Figure 4. (**A–C**) NORAD silencing promoted apoptosis of glioma cells. (**A**) Flow cytometry analysis was used to assess the apoptotic rate of U251 cells. (**B**) The expression of apoptosis-related proteins was examined using western blotting. All experiments were repeated 3 times independently (n=3). ** *P*<0.01, *** *P*<0.001 versus si-NC. NORAD – noncoding RNA activated by DNA damage; mRNA – si-NC, negative control for NORAD siRNA.

observed after NORAD silencing (Figure 5D, 5E). Overall, these data suggested that AKR1B1 could bind to NORAD.

AKR1B1 overexpression reversed the inhibitory effects of NORAD downregulation on proliferation, invasion, and migration of glioma cells

To confirm the molecular mechanism of NORAD and AKR1B1 in glioma, AKR1B1 overexpressed plasmid pcDNA3.1 was transfected into U251 cells. As shown in Figure 6A, the expression of AKR1B1 was significantly upregulated in pcDNA-AKR1B1 relative to the pcDNA-NC group. Results of CCK-8 assay (Figure 6B) and colony formation assay (Figure 6C) demonstrated that AKR1B1 overexpression reversed the inhibition of NORAD silencing on proliferation of U251 cells. Concurrently, compared with the si-NORAD-2+pcDNA-NC group, the invasiveness and migration of U251 cells were increased after co-transfection of si-NORAD-2 and pcDNA-AKR1B1 (Figure 6D–6G). Moreover, changes of MMP2, MMP9, and vimentin expression presented the same results with the aforementioned findings (Figure 7A, 7B).

These findings indicated that AKR1B1 overexpression restored the effects of NORAD silence on proliferation, invasion, and migration of glioma cells.

AKR1B1 overexpression attenuated the promotive effect of NORAD downregulation on apoptosis of glioma cells

Apoptosis of glioma cells was tested using flow cytometry analysis. As determined in Figure 8A and 8B, the rate of apoptotic cells was dramatically decreased after AKR1B1 overexpression in U251 cells with NORAD silencing. Meanwhile, co-transfection with si-NORAD-2 and pcDNA-AKR1B1 upregulated the expression of Bcl-2 as well as downregulated the expression of Bax, caspase-3, and caspase-9 in comparison to co-transfection with si-NORAD-2 and pcDNA-NC (Figure 8C). Taken together, the data suggested that AKR1B1 overexpression relieved the promotion of NORAD silencing on apoptosis of glioma cells.
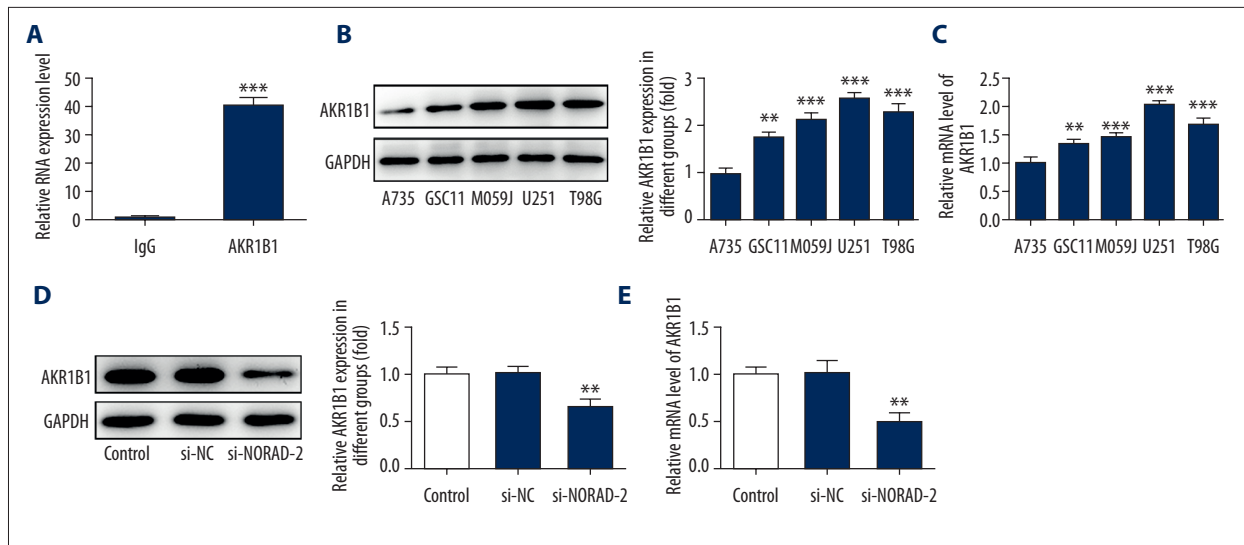
Figure 5. AKR1B1 interacted with NORAD. (**A**) The interplay between NORAD and AKR1B1 was detected by RIP assay. The expression of AKR1B1 in glioma cell lines (GSC11, M059J, U251, and T98G) was measured by (**B**) western blotting and (**C**) RT-qPCR, respectively. Three independent experiments were carried out (n=3). ** $P<0.01$, *** $P<0.001$ versus A735. The level of AKR1B1 in U251 cells was examined using (**D**) western blotting and (**E**) RT-qPCR after transfection with si-NORAD-2. All experiments were repeated 3 times independently (n=3). ** $P<0.01$ versus si-NC. NORAD – noncoding RNA activated by DNA damage; RIP – RNA-binding protein immunoprecipitation; RT-qPCR – reverse transcription-quantitative polymerase chain reaction; si-NORAD – small interfering RNAs (siRNAs) against NORAD; si-NC – negative control for NORAD siRNA.

NORAD could regulate extracellular signal-regulated kinase (ERK) signaling via targeting AKR1B1

To further explicit the molecular mechanism of NORAD and AKR1B1 in glioma, the expression of extracellular signal-regulated kinase (ERK) signaling was examined using western blotting. Result in Figure 9 shows that the expression of p-ERK was remarkably reduced after NORAD was decreased, which was abolished following co-transfection with si-NORAD-2 and pcDNA-AKR1B1. Overall, the finding illustrated that NORAD could regulate ERK signaling via targeting AKR1B1 in glioma.

Discussion

A growing body of evidence suggests that an increasing number of lncRNAs have been proven to be implicated in the progression of glioma [13,14]. The findings of this current study delineated that lncRNA NORAD was highly expressed in glioma cells, and silencing of NORAD suppressed the proliferation, invasion, and migration while promoting the apoptosis of glioma cells through downregulating the expression of AKR1B1.

Previous studies in the last several decades have indicated that NORAD serves a pivotal regulator in the occurrence and progression of a variety of malignancies, including gastric cancer, prostate cancer, and osteosarcoma [10,15,16]. Overexpression of NORAD is closely related to poor prognosis in various patients

with cancer, such as esophageal squamous cell carcinoma, gastric cancer, and ovarian cancer [17–19]. In the present study, highly expressed NORAD was identified in several glioma cell lines, including U251. We transfected NORAD siRNA into U251 cells to successfully knock-down NORAD expression for exploring the function of NORAD in glioma. Mounting evidence has highlighted the importance of NORAD in proliferation, invasion, migration, and apoptosis of cancer cells [12,20]. Our results consistently found that silencing of NORAD caused significant declines in proliferation, invasion, migration, and resistance to apoptosis, accompanied by inhibition of migration-related proteins MMP2, MMP9, and vimentin, downregulation of anti-apoptosis protein Bcl-2 and upregulation of pro-apoptosis proteins Bax, caspase-3, and caspase-9. Our study, for the first time, uncovered the aberrant expression and regulatory function of NORAD in glioma, suggesting a novel diagnostic and therapeutic target for glioma.

To identify the potential mechanism underlying NORAD in glioma, we searched on starBase database and found that NORAD could target AKR1B1, which was further verified by RIP assay. It has been reported that overexpression of AKR1B1 is highly correlated with basal-like breast cancer and predicts poor prognosis of breast cancer patients [21]. It is noteworthy that upregulated expression of AKR1B1 can promote proliferation and suppress apoptosis of pancreatic cancer cells via the ERK1/2 pathway [22]. Moreover, numerous studies have unveiled that activation of ERK signaling promotes the
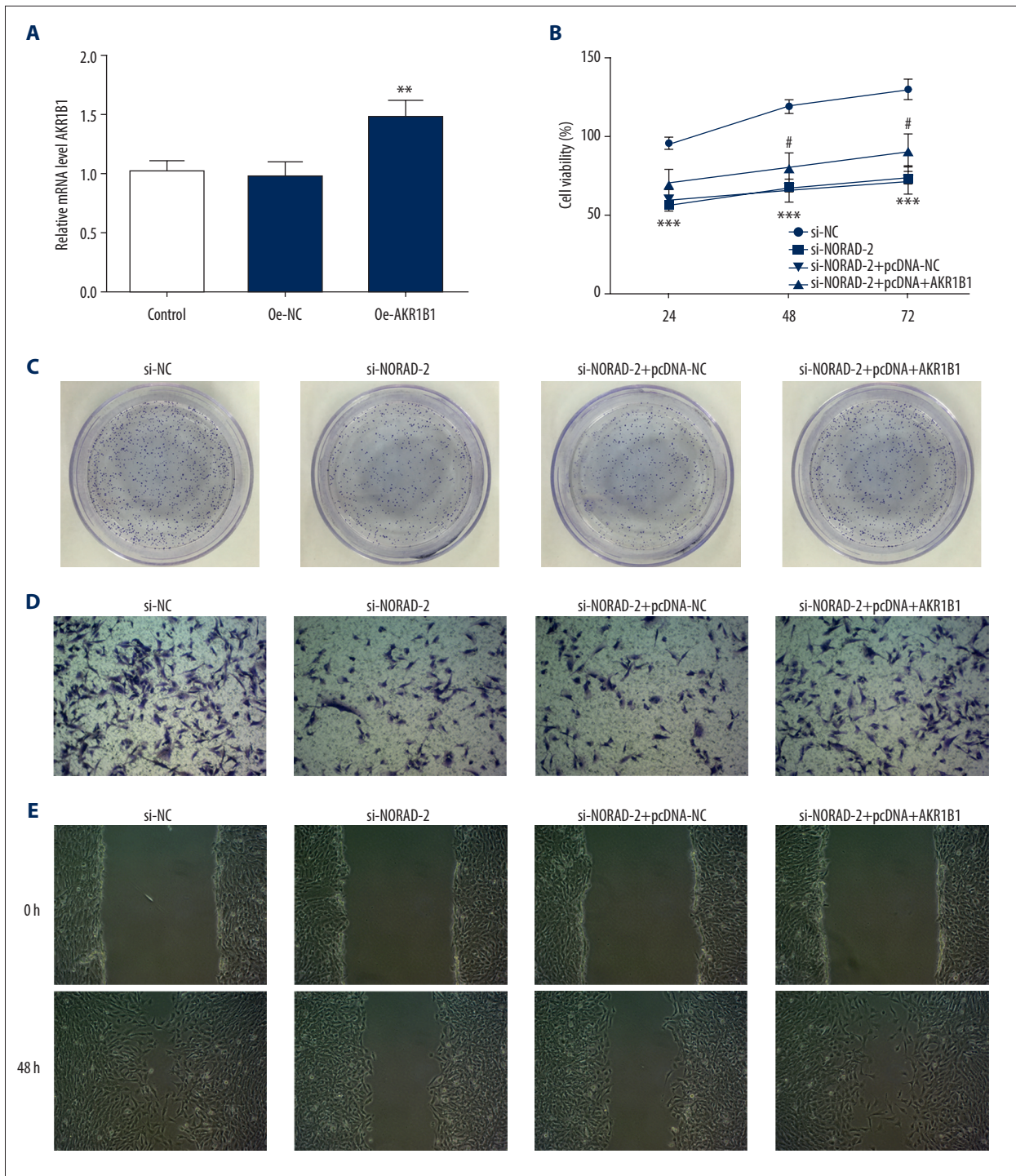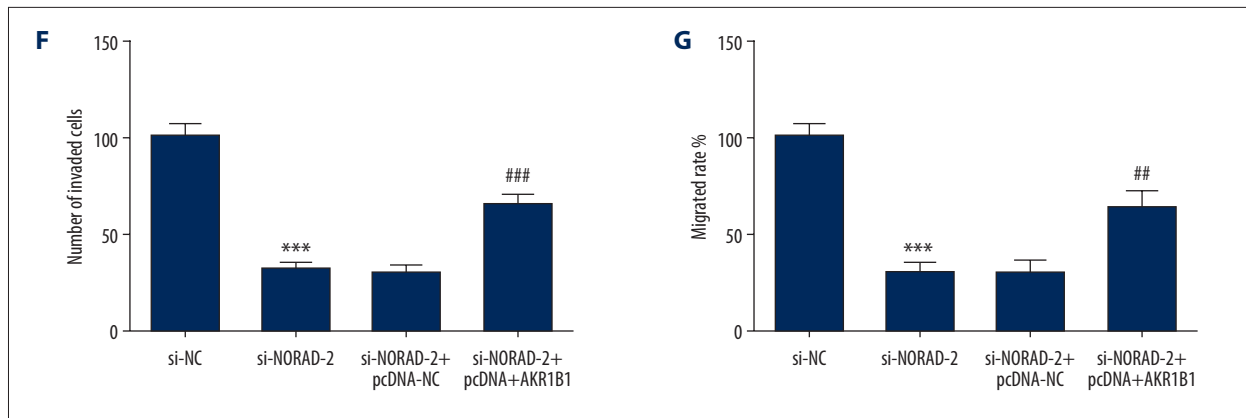
Figure 6. AKR1B1 overexpression reversed the effects of NORAD silencing on proliferation, invasion, and migration of glioma cells. (**A**) The expression of AKR1B1 was evaluated using RT-qPCR after transfection with AKR1B1 pcDNA3.1. Three independent experiments were carried out (n=3). ** *P*<0.01 versus pcDNA-NC. (**B**) CCK-8 assay and (**C**) colony formation assay tested the proliferation of U251 cells. (**D**) Invasion of U251 cells was evaluated using Transwell assay (magnification 200×). (**E**) Representative images of cell migration (magnification 100×). (**F**) Quantification of cell invasion. (**G**) Relative quantification, as examined using wound healing assay. All experiments were repeated 3 times independently (n=3). *** *P*<0.001 *vs.* si-NC; # *P*<0.05, ## *P*<0.01, ### *P*<0.001 *vs.* si-NORAD-2+pcDNA-NC. NORAD – noncoding RNA activated by DNA damage; RT-qPCR – reverse transcription-quantitative polymerase chain reaction; pcDNA-AKR1B1 – AKR1B1 overexpressed plasmid pcDNA3.1; pcDNA-NC – empty vector plasmid; CCK-8 – Cell Counting Kit-8; si-NC – negative control for NORAD siRNA; si-NORAD – small interfering RNAs (siRNAs) against NORAD.
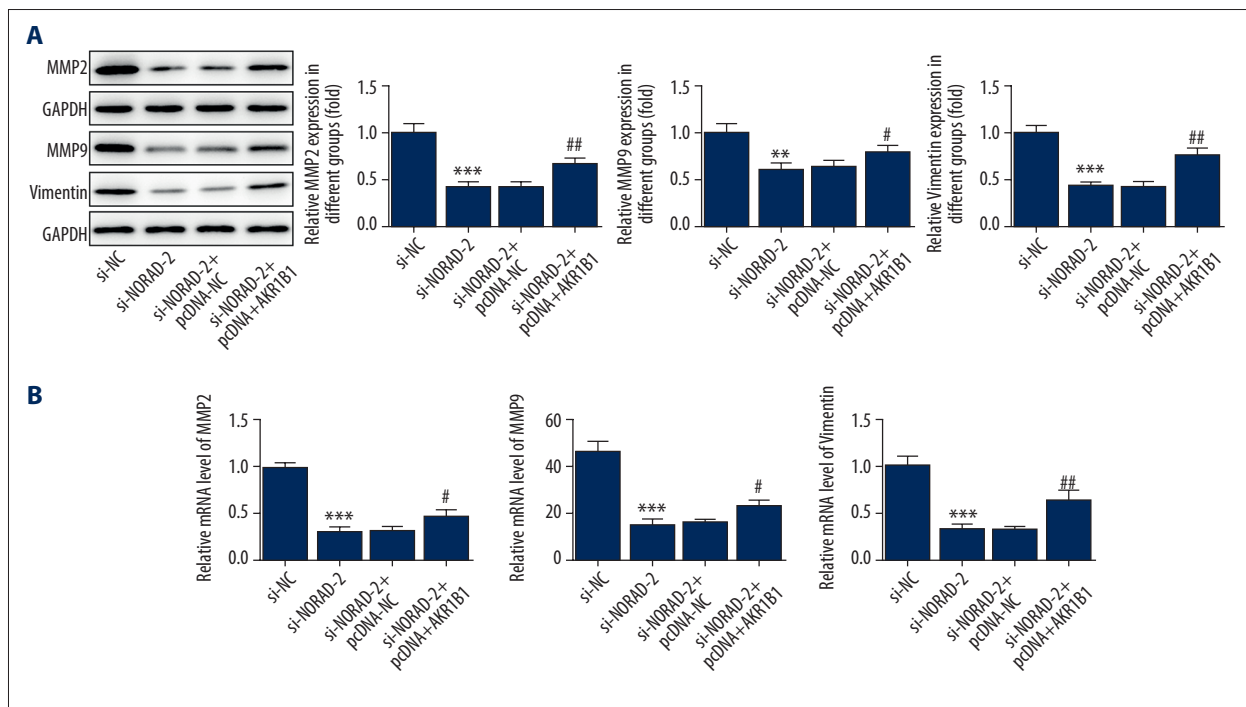

Figure 7. NORAD silencing decreased the expression of migration-related genes in glioma cells. The (**A**) proteins and (**B**) mRNA expression of MMP2, MMP9, and vimentin was detected using western blotting and RT-qPCR, respectively. All experiments were repeated three times independently (n=3). ** *P*<0.01, *** *P*<0.001 versus si-NC; # *P*<0.05, ## *P*<0.01 *vs.* si-NORAD-2+pcDNA-NC. NORAD – noncoding RNA activated by DNA damage; mRNA – messenger RNA; RT-qPCR – reverse transcription-quantitative polymerase chain reaction; si-NC – negative control for NORAD siRNA; si-NORAD – si-NORAD, small interfering RNAs (siRNAs) against NORAD; pcDNA-NC – empty vector plasmid.

Figure 8. (**A–C**) AKR1B1 overexpression attenuated the effects of NORAD silencing on apoptosis of glioma cells. (**A**) Flow cytometry analysis detected the apoptotic rate of U251 cells. (**B**) The expression of apoptosis-related proteins was examined using western blotting. Three independent experiments were carried out (n=3). ** *P*<0.01, *** *P*<0.001 *vs.* si-NC; # *P*<0.05, ### *P*<0.001 versus si-NORAD-2+pcDNA-NC. NORAD – noncoding RNA activated by DNA damage; si-NC – negative control for NORAD siRNA; si-NORAD – si-NORAD, small interfering RNAs (siRNAs) against NORAD; pcDNA-NC – empty vector plasmid.
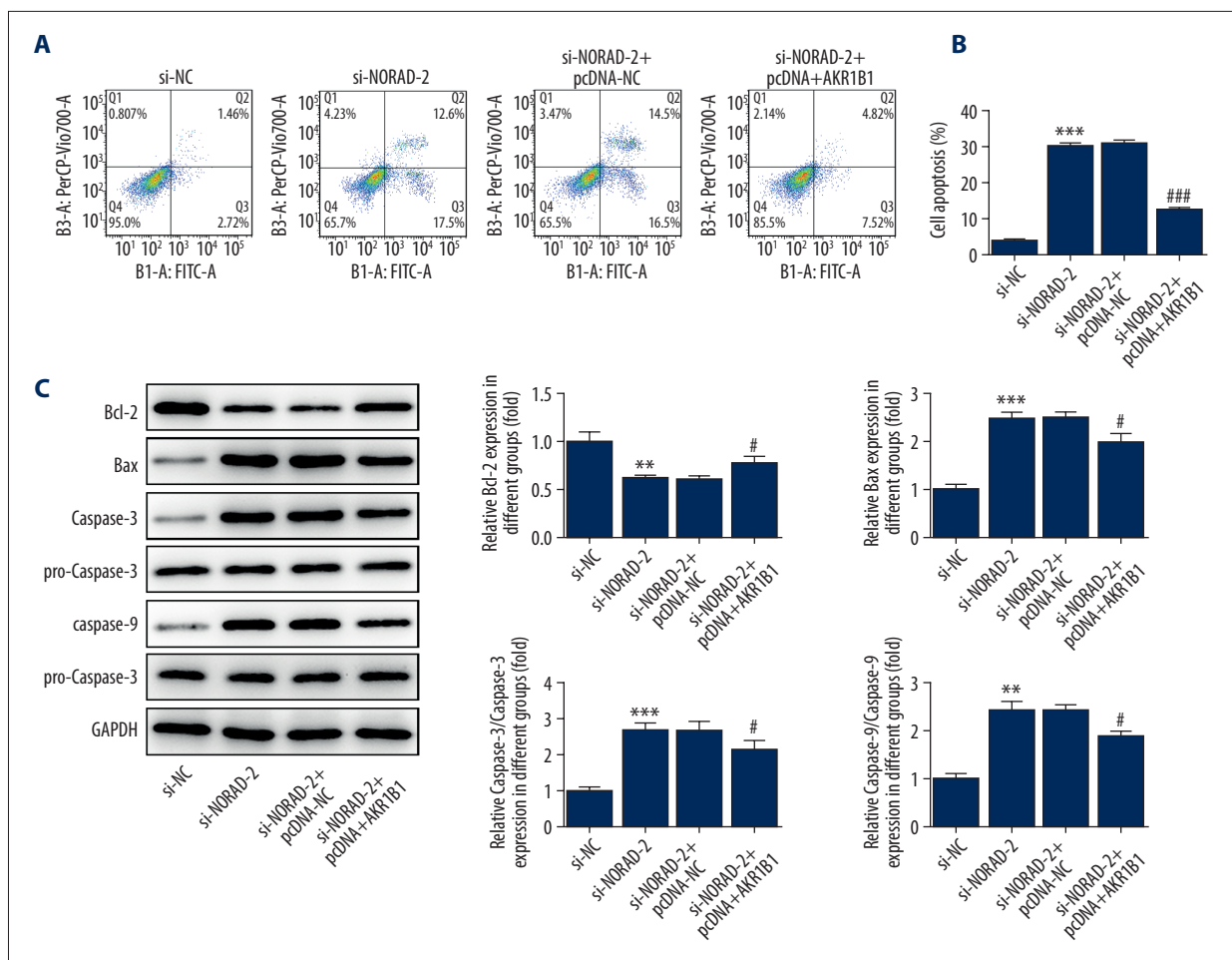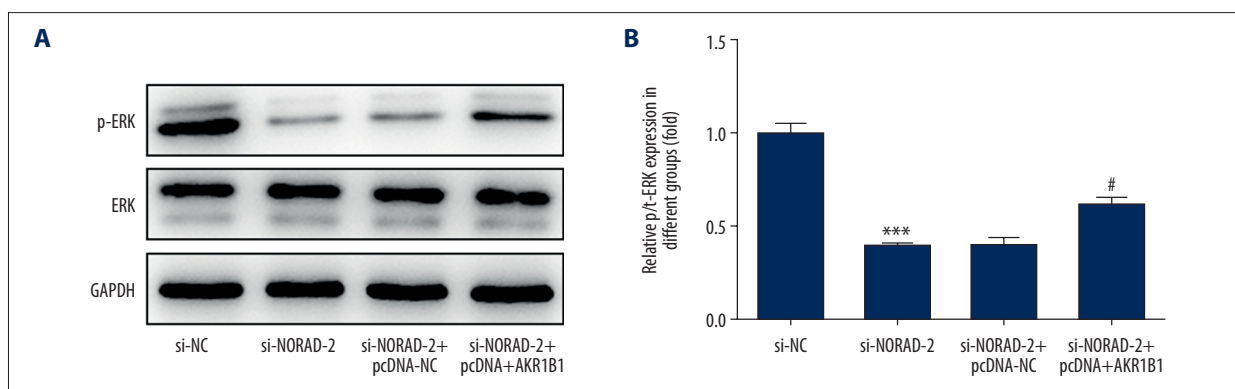

Figure 9. (**A, B**) NORAD could regulate ERK signaling via targeting AKR1B1. The expression of p-ERK was measured using western blotting. All experiments were repeated 3 times independently (n=3). *** *P*<0.001 *vs.* si-NC; # *P*<0.05 *vs.* si-NORAD-2+pcDNA-NC. NORAD – noncoding RNA activated by DNA damage; ERK – extracellular signal-regulated kinase; p-ERK – phosphorylated ERK; si-NC – negative control for NORAD siRNA; si-NORAD – small interfering RNAs (siRNAs) against NORAD; pcDNA-NC – empty vector plasmid.

progression of glioma [23,24]. Therefore, we deduced that NORAD might upregulate the expression of AKR1B1 to activate the ERK pathway, thus developing its carcinogenic role in glioma cells. In the current paper, obviously increased expression of AKR1B1 was observed in glioma cells. Importantly, AKR1B1 overexpression reversed the effects of NORAD silencing on proliferation, invasion, migration, and apoptosis of glioma cells, coupled with changes of migration- and apoptosis-related proteins. Besides, silencing of NORAD remarkably restrained the expression of phosphorylated ERK (p-ERK), which was restored by AKR1B1 overexpression.

Conclusions

Taken together, these findings elucidated that lncRNA NORAD was upregulated in glioma. NORAD silencing suppressed cellular proliferation, invasion, migration, and promoted apoptosis, whereas these impacts were rescued after AKR1B1 overexpression via activation of the ERK signaling. The present findings demonstrated that NORAD might be a novel therapeutic target for the treatment of glioma. However, the usage of a single glioma cell line and lack of studies *in vivo* or using patient-derived glioma models were limitations of the present research and therefore, a comprehensive analysis is required in the future.

Conflict of interest

None.

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