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MicroRNA-139-3p suppresses growth and metastasis of glioblastoma via inhibition of NIN1/RPNI2 binding protein 1 homolog

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Abstract. – OBJECTIVE: This study aimed to investigate the role of microRNA-139-3p (miR-139-3p) in glioblastoma, and further explored the underlying molecular mechanism.

PATIENTS AND METHODS: Gene Expression Omnibus (GEO) dataset (accession code GSE90603) was selected to identify differentially expressed microRNAs in glioblastoma. The level of miR-139-3p in glioblastoma tissues and cell lines was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl zolium bromide (MTT), colony formation, healing, and transwell invasion assays re applied to assess the role of miR-139-3p in blastoma cells growth and aggressiveness. direct target of miR-139-3p was confirmed usi luciferase reporter assay. NIN1/RPNI2 binding protein 1 homolog (NOB1) specific short hairpin RNA (shRNA), and lentiviral vector encoding NOB1 stable transfected cells were expression levels of NOB1 were detected by Western blotting and qRT-PCR. Glioblastoma cells were subcutaneously implanted into nude mice to determine the role of miR-139-3p in tumor growth and metastasis *in vivo*.

RESULTS: miR-139-3p was remarkably down-expressed in glioblastoma tissue compared to control normal tissues. Overexpression of miR-139-3p suppressed the growth and metastasis of glioblastoma cells. Moreover, miR-139-3p inhibited glioblastoma growth and lung metastasis *in vivo*. Whereas under-expression of miR-139-3p caused an opposite outcome. Furthermore, our work revealed that NOB1 expression was negatively associated with miR-139-3p and NOB1 knock-down mimicked the effect of miR-139-3p on glioblastoma cell proliferation and mobility phenotypes. Finally, overexpression of NOB1 neutralized the inhibition of miR-139-3p in glioblastoma cells.

CONCLUSIONS: Our findings indicated that miR-139-3p played a vital role in inhibiting glioblastoma growth and metastasis by targeting NOB1.

Key Words:
Glioblastoma, miR-139-3p, Growth, Metastasis.

List of abbreviations

GEO, gene expression omnibus; NOB1, NIN1/RPNI2 binding protein 1 homolog; qRT-PCR, quantitative real-time polymerase chain reaction; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; shRNA, short hairpin RNA; GBM, glioblastoma; FBS, fetal bovine serum; DMEM, Dulbecco modified Eagle's medium; TMZ, temozolomide; DMSO, dimethyl sulfoxide; WT, wild-type; MUT, mutant-type; OD, optical density; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; 3'-UTR, 3'-untranslated region, cDNA, complementary DNA; NSCLC, non-small cell lung cancer; TNM, tumor-node-metastatic.

Introduction

Glioma is a frequently occurring type of primary malignant cancer of the human brain[1]. The current therapeutic methods include maximal resection, followed by radiotherapy in combination with temozolomide (TMZ)[2]. Glioblastoma (GBM) is the most aggressive type of glioma and exhibits strong aggression properties[3]. The invasive phenotype is critical to the clinical progression of malignant GBM, and complicates complete surgical resection and permits postoperative tumor re-growth[4]. Therefore, there is an urgent need for the development of novel mechanistic methods to investigate the invasiveness and migration properties of cancer cells, which could be exploited to design more effective therapeutic strategies.

MicroRNAs are small non-coding RNAs that post-transcriptionally control the expression of their down-stream target genes[5]. Substantive

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studies demonstrate that altered expression of microRNA is related to human cancer progression, including growth, metastasis, angiogenesis, and chemotherapy resistance[6]. MicroRNAs commonly function as tumor suppressors or oncogenes and play vital roles in the diagnosis and treatment of cancer[7]. Previous researches have identified abnormal microRNA expression to be closely associated with the prognosis of patients across several cancers[8]. MiR-139-3p is markedly lower in patients with colorectal cancer than in control subjects and is a potential non-invasive biomarker for detection of colorectal cancer[9]. Furthermore, miR-139-3p acts as a tumor suppressor that restricts cervical cancer cell growth and aggressiveness, and induces cell apoptosis via inhibition of NIN1/RPNI2 binding protein 1 homolog (NOB1)[10]. NOB1 is a subunit of the 26 S proteasome, encoding a 50-kDa protein and contains a zinc ribbon domain and a PIN domain[11]. Wang et al[12] identified that NOB1 is up-regulated in epithelial cancers, including prostate, hepatocellular carcinoma, lung, and pancreatic cancers, and that down-regulation of NOB1 restrains growth and mobility in renal cancer cells. In add down-regulation of NOB1 inhibits the and cell colony formation ability of ovaria cer cells. However, how abnormal miR-13 expression regulates glioblastoma invasion a metastasis, has not yet been ful tigated.

In this investigation, we ted that miR-139-3p was remarka own-r lated in glioblastoma samples, i ing c imens and cell line Wit pressio f miR-139-3p, cellul owth, on, and metastasis were si antly inhi and the same effect w d on tumo owth *in vivo*, whereas own-e sion of miR-139-3p resulted i ompletely e ite outcomes. In additio e demonstrated NOB1, a miR-139- rget g played a very important role in roce

Mat ls and Methods

a Specimens and Cell Lines

Forty frozen paraffin-embedded specimens human glioblastoma samples and 5 pa- or tissue samples) were obtained from Binzhou Central Hospital. Informed consents of patients were obtained before surgery. The glioblastoma cell lines (U251, U-87MG, SHG-44, and TJ905) and normal human brain glial cells, HEB, were obtained from the Cobioer Biotechnology Co., Ltd (Nanjing, Jiangsu, China). The cell lines were cultured i MI-1640 or Dulbecco Modified E dium (DMEM) supplemented wi % feta bovine serum (FBS) (Wisent, ec, Cana-da), with 100 U/mL penicillin a ug/mL streptomycin.

Transfection of MiR -3p Precursors and In or

MiR-139-3p mi s miR -3p) and its positive rol (nam miR-NC), as well iR-139-3p miR-139-3p^{inhi}) and i e control (C^{inhi}), were provided Xmb eneCopoeia, Guangzhou, Guangdong, China). fection of miR-139-3p or i was carrie sing Lipofectamine R MAX transfection reagent (Invitrogen, sbad, CA, US)[13].

ient Tra ection an tivi onstructs

Tra of vectors or short hairpin RNA (shRNA) was conducted with Lipofectamine (Invitrogen, Carlsbad, CA, USA). The shR-A nst NOB1 was designed and purchased from Genepharma Co., Ltd (Shanghai, China). Lentiviral vector encoding NOB1 complementary DNA (cDNA) was constructed by Genepharma Co., Ltd (Shanghai, China) and designated as pLV-NOB1. The empty vector was used as control (pLV-vector).

Luciferase Reporter Assay

293T cells were seeded in a 96-well plate at 70 to 80 % confluence. After 24 h, the cells were transfected with miR-139-3p and wild-type (WT) or mutant-type (MUT) 3′-UTR of NOB1 gene. Forty-eight hours after transfection, the cells were collected and Renilla luciferase activity was assessed by using a dual-luciferase reporter system (Promega, Madison, WI, USA)[14].

Cell Proliferation Assay

Cells were plated into 96-well plates, and cultured for 1, 2, or 3 days. 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added into 96-well plates. After 4 h, the supernatant was removed and 200 µl dimethyl sulfoxide (DMSO) was added; the optical density (OD) value was determined at 490 nm[15].

Wound Healing Assay

Cells were seeded into a 6-well plate. After 24 h, the monolayer cells were created by using a 100 μl tip, and cells were continually cultured with serum-free medium. The wound closure at 0 h and 24 h was photographed, and the percentage of the wound closure was calculated[16].

Transwell Invasion Assay

Cells were seeded into a Matrigel-coated chamber (8 μm), and placed in a 24-well plate. After 24 h, the lower chamber cells were fixed and stained with 0.1% crystal violet. The invading cells were counted[17].

Colony Formation Assay

Cells were cultured in a 25-mm^3 dish and placed in an incubator for four weeks. The colonies were stained with 0.1% crystal violet and the number of cell colonies was calculated[18].

Quantitative Real-time RT-PCR (qRT-PCR) Assay

Total RNA was isolated using Trizol (TaKaRa, Otsu, Shiga, Japan). RNA (1 μg) was reverse-transcribed to cDNA using a Prim[illegible] RT reagent kit (TaKaRa, Otsu, Shiga, Japan). qRT-PCR was performed to detect the level of miR-139-3p or other genes using IQ™ SY[illegible] Green Supermix and Bio-Ra[illegible] real-time detection system. The form[illegible] relative expression value was thr[illegible] $2^{(-\Delta\Delta C[illegible])}$ method[19]. The reference gene, U6 [illegible] used [illegible]ing the level of miR-1[illegible]-3p [illegible]astoma tissues and para-tum[illegible] tissues. [illegible] primers used for PCR were [illegible] follows: GA[illegible] (forward primer): 5'-[illegible]TACACTG[illegible]ACC-3' and (reverse primer): 5'-AAGTGGTCGTTGAGGG[illegible]TG-3'; NO[illegible] (forward primer): 5'-TG[illegible]AACCCCTAA[illegible]GGAGACC-3' and [illegible]rse primer): 5'-CCTTGTCCGTGTCACA[illegible]TCT[illegible].

Western Blotting

[illegible]al pr[illegible]re collected in RIPA Lysis Bu[illegible] contai[illegible] protein inhibitors (Beyotime, G[illegible], Guangdong, China). Membranes [illegible]ed with anti-NOB1 or anti-GAPDH (1:1000, Abcam, Cambridge, MA, USA) at [illegible] overnight and hybridized with horseradish pe[illegible]dase-conjugated goat anti-rabbit IgG antibody. Target proteins were assessed by using the enhanced chemiluminescence (ECL) system (Millipore, Braunschweig, Germany) and visualized with the ChemiDoc XRS system (Bio-Rad, Hercules, CA, USA)[20].

Xenograft Model of Glioblastoma In Vivo

All nude mice were bred and house[illegible] LAC-accredited SPF rodent faciliti[illegible] the People's Hospital of Dezhou City. A[illegible] experiments were approved by the Animal Eth[illegible] mmittee of the People's Hospital of De[illegible]u City. [illegible]-139-3p or miR-NC transfected [illegible]s were su[illegible]ously injected into the [illegible]s of n[illegible]e mice. [illegible] tumor sizes were me[illegible]d each [illegible]k[21], and calculated as tumor v[illegible] × wid[illegible]2. To study metastasis [illegible]ivo, g[illegible]stoma[illegible]s were transfected w[illegible] miR-139-3p [illegible]9-3p inhibitor and [illegible] into tail v[illegible] nude mice. After six [illegible]ks, t[illegible]e were sacrificed and the lungs were excised. [illegible]ngs were stained with Bou[illegible]tion for 24 [illegible] then paraffin-embedded, sectioned, and stained with hematoxylin [illegible] eosin staining (H&E)[22].

Statistical Analysis

[illegible] are [illegible]sented as Mean ± SD for three repeate[illegible]ments and the difference was compared using the Student's *t*-test. $p < 0.05$ was [illegible]red statistically significant.

Results

MiR-139-3p is Down-Expressed in Glioblastoma

Gene expression datasets used for statistical analysis were acquired from the GEO database with the accession code GSE90603. The screening was performed in GEO datasets that contained both the glioblastoma samples and the matched normal tissues samples. As shown in Figure 1A, miR-139-3p was down-expressed in glioblastoma samples as compared to that in control samples. In order to compare miR-139-3p expression pattern in glioblastoma and control tissue, miR-139-3p levels were evaluated in 35 glioblastoma tissues and 5 para-tumor tissues. As shown in Figure 1B, miR-139-3p level in glioblastoma was significantly lower than that in the control tissues. MiR-139-3p levels were also determined in a panel of glioblastoma cell lines by qRT-PCR. Similar results were obtained in four glioblastoma cell lines, in which the levels of miR-139-3p were lower compared to those in normal human brain glial cells, HEB (Figure 1C). In order to investigate the role of miR-139-3p

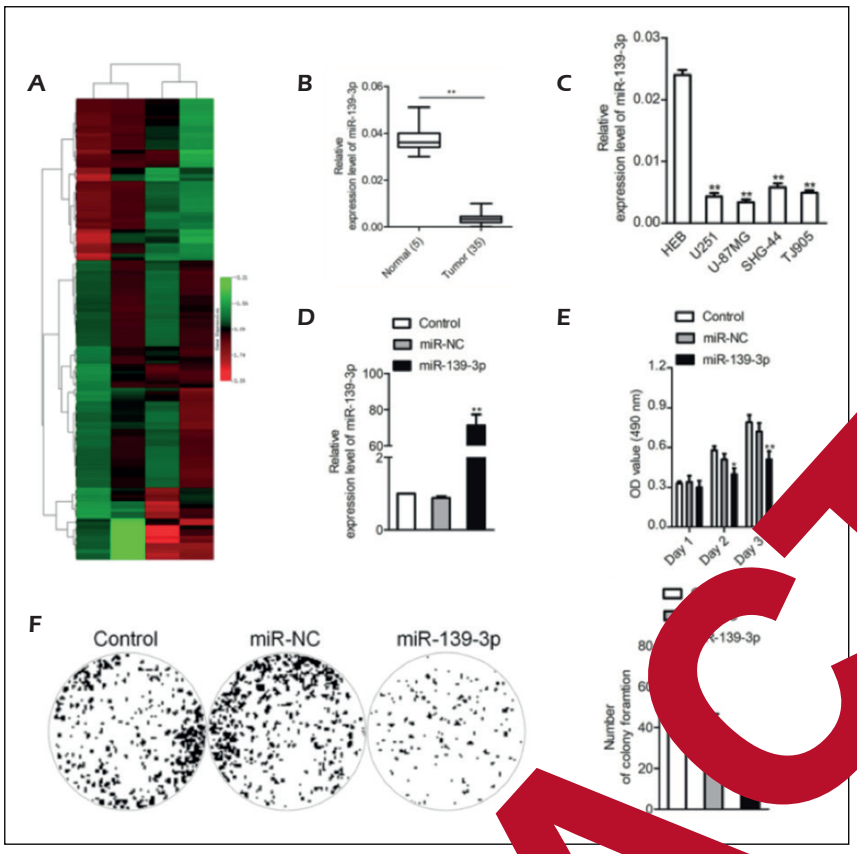

Figure 1. MiR-139-3p inhibits glioblastoma cells growth. **A**, Microarray analysis of miRNA expression in glioblastoma tissues and corresponding normal tissues. level of miR-139-3p fr tissues and tumor ti of patient with glioblastom determined by qRT-PCR assa <0.01, compared to norm al. **C**, -3p levels were r red in ferent glioblas cell lines by q **p<0 compar to HEB **D**, MG ce re transfe d wi -NC miR-139 p. The el 3p was mined y qRT-P . Cell e plated into 96-wel ultured for 2 or 3 days. ssay was per- ed and OD v e was measured nm. **F**, Cells were subjected to s r colony formation assay in a culture plate. The cell colonies were counted after four weeks. Data are presented as the ean ± SD from three independent easurements. **p<0.01, compared o control.

in the growth of glioblastoma nstructe the miR-139-3p overexpressio ste iR-139-3p was significantly up-re ted in -139-3p transfected glioblastoma 7MG re 1D). The MTT assay ult that th - duction of miR-139 signific nhibited the proliferation of astoma ce gure 1E). Colony forma g h analysis l led that miR-139-3p o expre inhibited the colony growth o mor cells (e 1F). These observati suggest that mi -3p is a tumor supp r in g lastoma.

MiR- Overe ession Inhibits l Mig on a nvasion

demo e effect of miR-139-3p on mig on, we formed wound closure assays, wh e observed that the distance between n miR-139-3p-transfected U-87MG lls was markedly longer than that in control cells ure 2A). Additionally, we observed shorter d e in the wound edges of miR-139-3p^{inhi}-transfected U-87MG cells compared with that in control (Figure 2B). To investigate the effect of altered miR-139-3p expression on glioblastoma cell invasion, we performed the transwell analysis, wherein we observed that miR-139-3p overexpression remarkably suppressed U-87MG cell invasion (Figure 2C). We found that miR-139-3p^{inhi} significantly accelerated glioblastoma cell invasion *in vitro* (Figure 2D).

MiR-139-3p Overexpression Inhibits Glioblastoma Cell Metastasis in vivo

In vitro, miR-139-3p markedly inhibited glioblastoma cell growth, migration, and invasion. Whether miR-139-3p regulated tumor growth and metastasis *in vivo* was needed to be further investigated. MiR-139-3p transfected U-87MG cells were subcutaneously implanted into the nude mice. Tumors were monitored and measured every week. MiR-139-3p overexpression remarkably inhibited growth and tumor volume of glioblastoma cells (Figure 3A). We dissected the mice to harvest the tumor, noting that the Ki67 staining was remarkably inhibited in miR-139-3p group than in the miR-NC group (Figure 3B), which confirmed that miR-139-3p inhibited glioblastoma growth *in vivo*. In addition, the occurrence of lung metastasis of glioblastoma cells in the

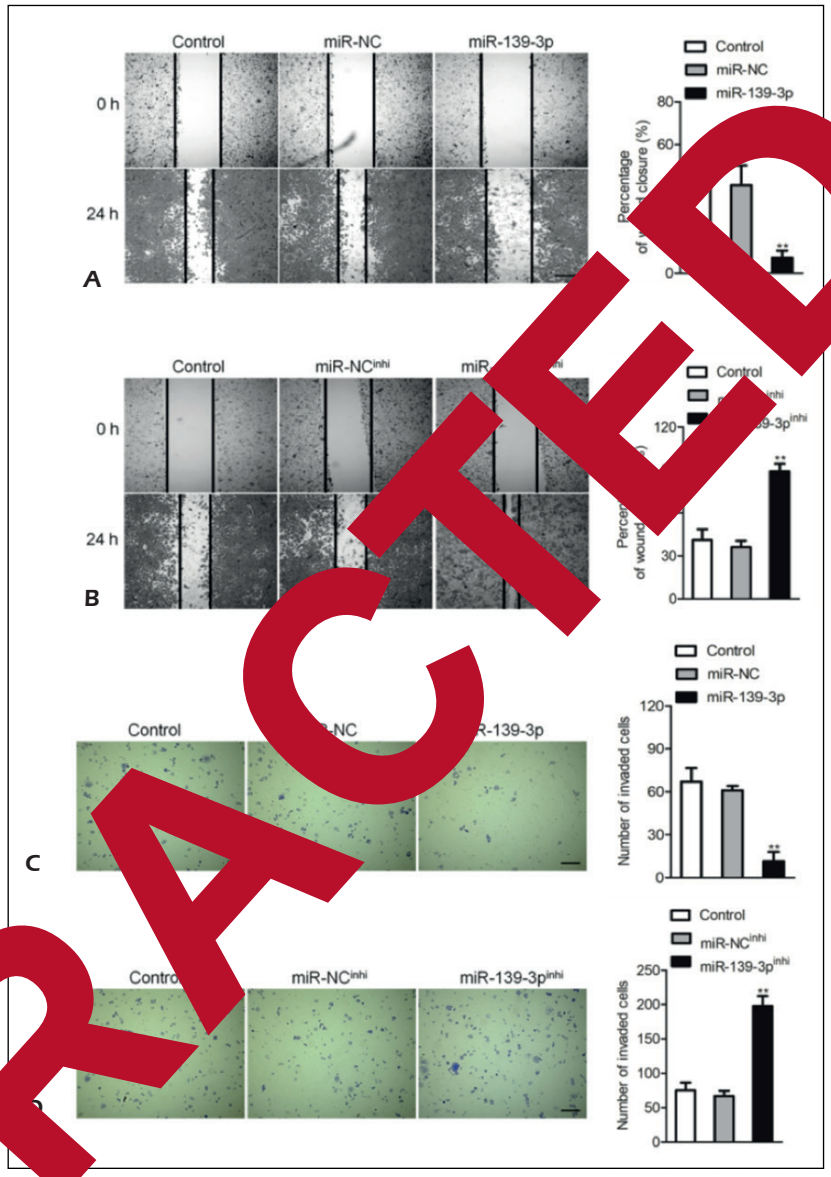

Figure 2. Effects of miR-139-3p on aggressiveness in glioblastoma cells. **A**, Wound healing analysis of U-87MG cells. A representative image was shown *(left panel)*. Histograms showed the percentage of wound closure *(right panel)*. **B**, Transwell analysis. A representative image was shown *(left panel)*. Histograms showed the number of invaded cells *(right panel)*. **C**, Wound healing analysis of U-87MG cells. A representative image was presented *(left panel)*. Histograms exhibited the percentage of wound closure *(right panel)*. **D**, Transwell assay. Photographs show cells that invaded through the membrane *(left panel)*. A representative image was shown *(left panel)*. Histograms showed the number of invaded cells *(right panel)*. $^{**}p<0.01$, compared to control.

miR-13[illegible] transfection gr[illegible] was remarkably supp[illegible]d, as [illegible]pared to that in control group (Fi[illegible]3C). [illegible] these results showed that miR-139-3[illegible] the [illegible]th and metastasis of [illegible]blast[illegible]ells i[illegible]*vo*.

Mi[illegible]39-3p [illegible]ctly Binds to [illegible]'UTR of NOB1

[illegible] microRNAs regulate their target [illegible]nes by binding to their 3'-UTR regions[23]. miR-[illegible] and TargetScan were selected to predict the ta[illegible] of miR-139-3p and we found that NOB1 may be a target of miR-139-3p (Figure 4A). To investigate whether miR-139-3p binds directly to the 3'-UTR of NOB1 and modulates NOB1 expression, 3'-UTR of NOB1 was cloned into the downstream of the luciferase reporter gene. Reduced luciferase activity revealed that NOB1 is indeed a target of miR-139-3p (Figure 4B). Taken together, the results indicated that miR-139-3p regulated NOB1 by binding to the 3'-UTR region directly. QRT-PCR assay analysis showed that glioblastoma cell lines exhibited high NOB1 expression as compared to the normal human brain glial cells, HEB (Figure 4C). In addition, NOB1 showed higher expression in glioblastoma samples compared to that in normal tissue samples (Figure 4D). We also investigated the relationship between miR-139-3p and NOB1 in glioblastoma specimens. As shown in Figure 4E, there was a negative correlation

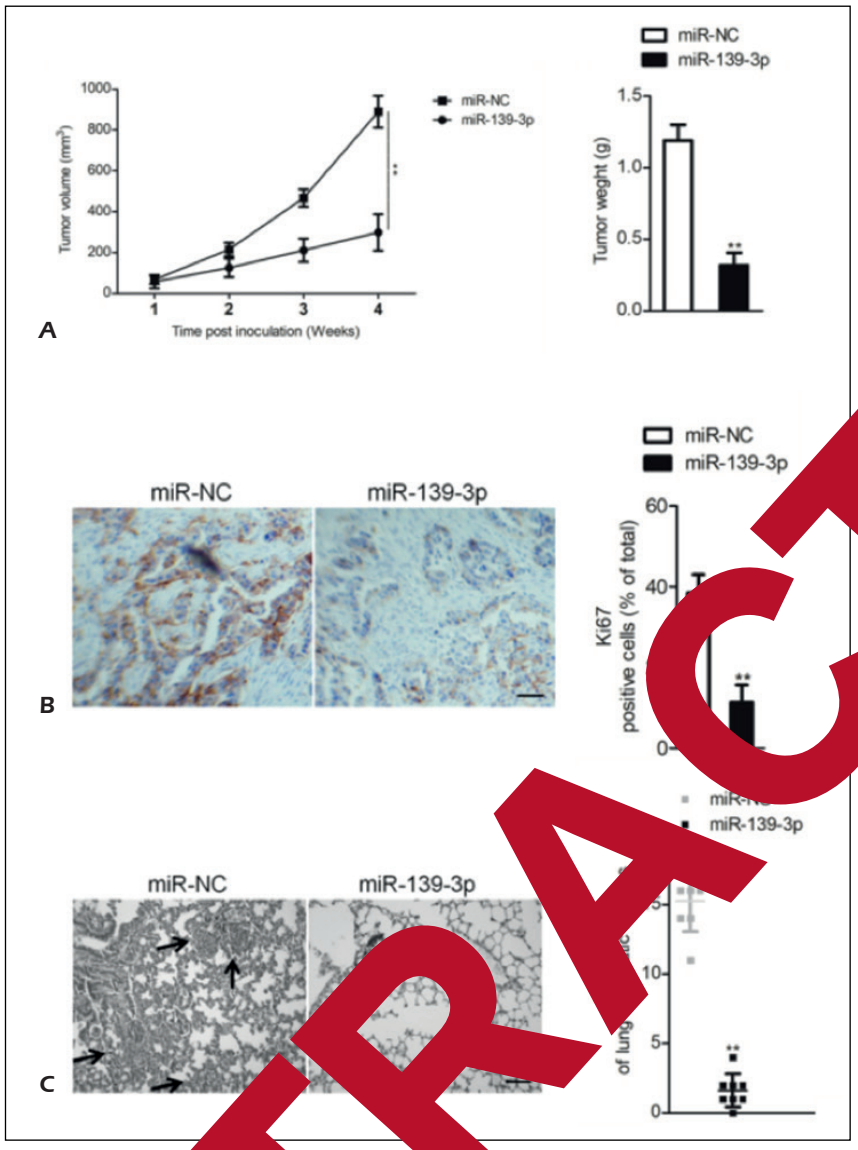

Figure 3. Effect of miR-139-3p on glioblastoma cells tumor growth and metastasis *in vivo*. **A**, MiR-NC or miR-139-3p U-87MG cells were implanted into the n... Measure the tumor ... week. **B**, Tumor w... moved an... embedded in par... or immuno-histochemical st... with Ki67. **C**, Metastatic lesio... e lungs of the glio... oma ce... tasis models ... shown after ... The ... ers of m...astatic lu... sio... the miR... 5p transfec...n gr... ere... less th... those m... up. ***p*... com-pared to ...C gro...

between ... 139-3p level... NOB1 levels. Additional... NOB1 was signifi... y down-regulated a... U-87M... as transfected with miR-139-3p ... re 4... whereas NOB1 was remarkably up-reg... ter tran... ion with miR-139-3p^{inhi} ...ure ... These ...ervations hint that NOB1 ... e a ta... of miR-139-3p.

Th... ct of MiR-139-3p Gl... oma is Rescued ... NOB1 Overexpression

...ittle is known about the role of NOB1 in g... stoma progression, and whether NOB1 knock-down could mimic the effect of miR-139-3p in glioblastoma. Short hairpin RNA (shRNA), specifically targeting NOB1 (shNOB1), was used to stably knock down the expression of NOB1 in the U-87MG cells (Figure 5A). To determine the role of NOB1 in glioblastoma growth and metastasis, the effect of NOB1 knock-down on U-87MG cell proliferation, colony formation, migration and invasion was examined (Figure 5B-5E). The stable knock-down of NOB1 in U-87MG had a similar effect as miR-139-3p overexpression on the growth, migration and invasion of U-87MG cells *in vitro*, which suggested that NOB1 might play an oncogenic role by promoting glioblastoma growth and metastasis. To investigate whether miR-139-3p suppresses the growth and mobility of glioblastoma cells by inhibiting NOB1, we constructed a U-87MG cell line that was transfected with miR-139-3p

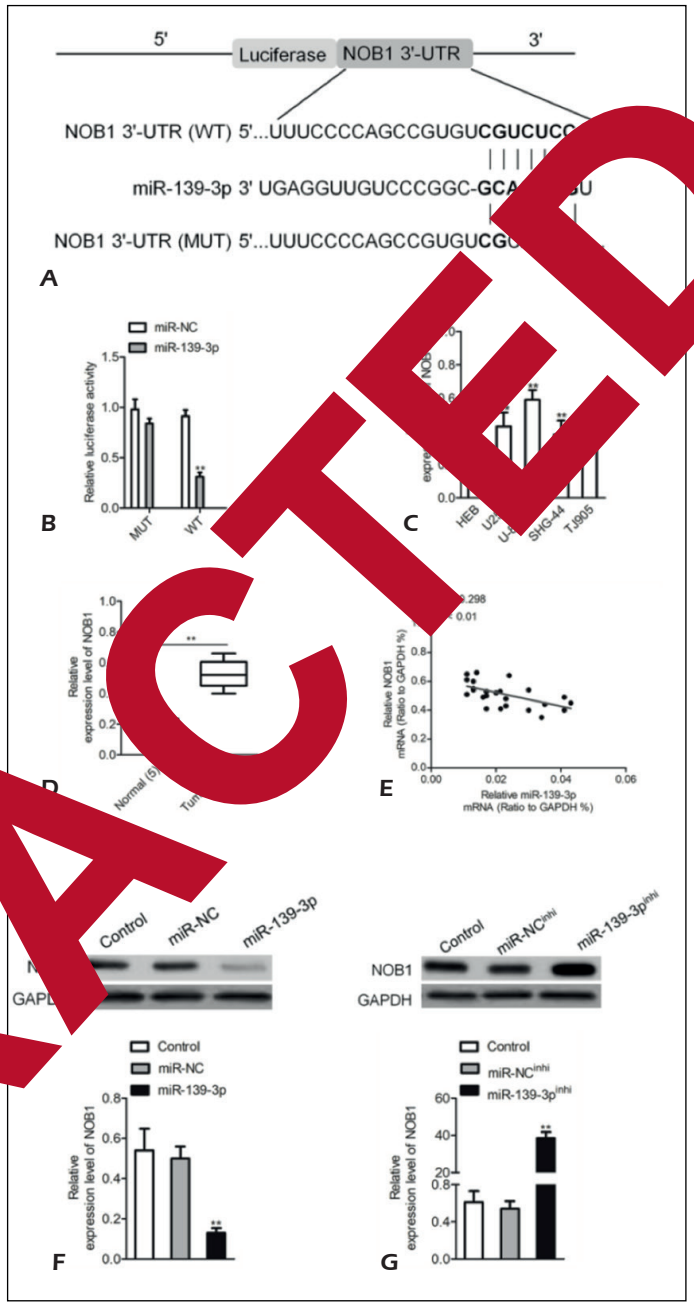

Figure 4. NOB1 is the target of miR-139-3p. **A**, Predicted miR-139-3p target sequences in 3'-UTR of NOB1. B, Luciferase activity of NOB1 (MT or MUT) was evaluated after miR-139-3p transfection into 293T cells. ***p*<0.01, compared to miR-NC. **C**, The expression level of NOB1 was detected by qRT-PCR assay in several glioblastoma cell lines. ***p*<0.01, compared to HEB cells. **D**, The level of NOB1 in glioblastoma tissues was measured by qRT-PCR. ***p*<0.01, compared to normal. **E**, The correlation analysis of miR-139-3p and NOB1 expressions. **F**, The level of NOB1 in U-87MG cells after cells transfected with miR-139-3p was measured by qRT-PCR and Western blotting assays. **G**, The expression level of NOB1 in U-87MG cells after cells transfected with miR-139-3pinhi was measured by qRT-PCR and Western blot analysis. ***p*<0.01, compared to control.

pLV 1. In noblotting and qRT-PCR is de d that ectopic expression of NO rescued expression after U-87MG cells w fected with miR-139-3p (Figure 6A). colony formation, wound closure d transwell assay exhibited that NOB1 overexion restored the inhibition of miR-139-3p in g stoma cell growth and metastasis (Figure 6B-6E). Altogether, these results demonstrated that miR-139-3p inhibits cell growth and metastasis of glioblastoma by targeting NOB1.

Discussion

Glioblastoma is one of the most aggressive tumors, and the occurrence of metastasis leads to a sharp decline in the survival rate of patients[24]. Thus, identification of metastasis-related biomarkers and therapeutic targets will help improve glioblastoma prognosis. There is increasing evidence to suggest that miRNAs have vital roles in tumor progression in controlling their target genes, and thus they might serve as biomarkers

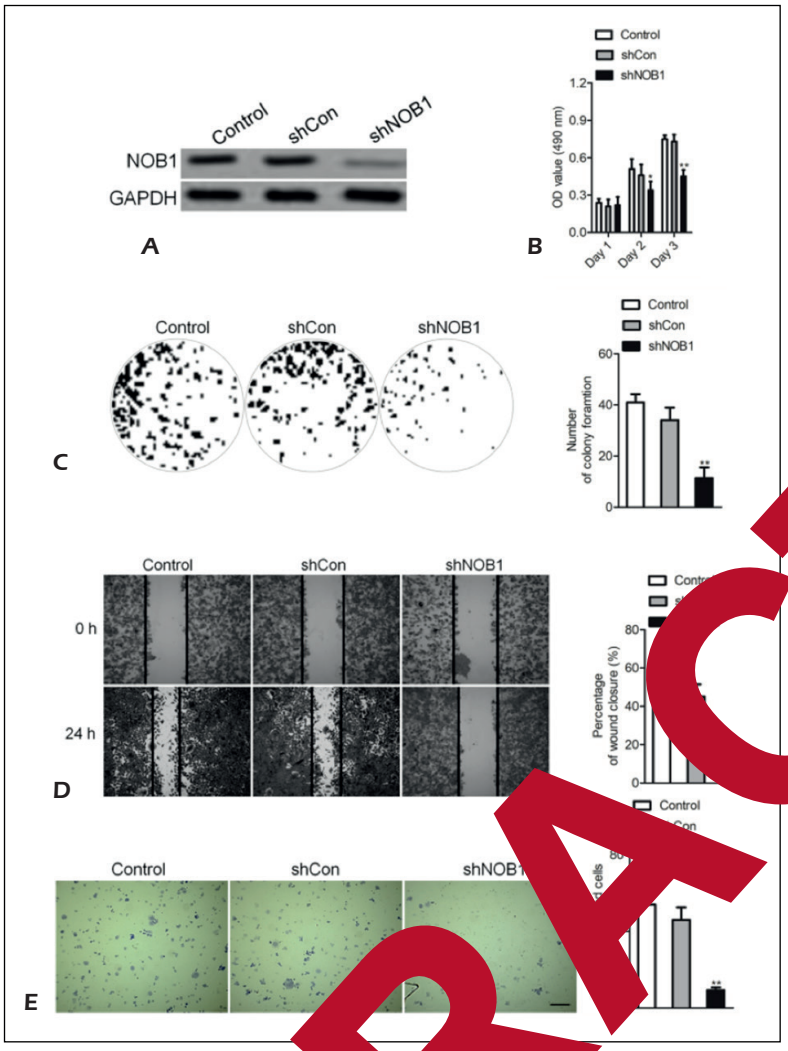

Figure 5. NOB1 knocks-down mimics the effects of miR-139-3p on U-87MG cells. **A**, To knock-down NOB1, shNOB1 or shCon were transfected into U-87MG cells. The NOB1 was detected by Wester... U-87MG cells proliferation ... measured by MTT. **C**, C... formation ability of U-87MG cells ... measured by the soft agar colony fo... assay. The image and quantitative ana... colonies were shown. **D**, ... MG cell... rans-fected with sh... or shCon, an... migration ab... of the ce... was inve... ed with t... ound he... assay. Qua...-tative ana... f th... centage ... wound closu... om... pendent ... ments w... own. **E**, ... nvasio... pacity of ... MG cells wa... d with the ... ll invasion ass... ntitative anal-ysis ... invasive cell... was shown. The data ar... nted as mean ± SD. *p<0.05, **p<0.01, ... ed to control.

for prediction an... gnosis of c...[25]. Here, we report tha... 3p is a no... metastasis-associated ... RNA ... lays a significant role in regula... glioblastom... wth and metastasis. We... nd a significant d... se in miR-139-3p l... in gli... lastoma and miR-139-3p was fou... pre... glioblastoma cell proliferation and p... by in... ing NOB1 expression. ... NOB... quen... xpressed in the liver, lung, a... leen, ... inly located in the nucleus. It was ... orted th... OB1 is abnormally expressed in in... tal carcinoma and may be involved in ... e and development of tumors[26]. The ... pression of NOB1 messenger RNA (mRNA) and ... ins in papillary thyroid carcinoma is signifi-ca... higher than that in normal thyroid tissues[27]. In non-small cell lung cancer (NSCLC) and prostate cancer, the NOB1 expression is correlated with tumor-node-metastatic (TNM) stages and lymph node metastasis[28]. Consistently, our results verified that NOB1 level was markedly increased in glioblastoma compared to controls.

In addition, the use of shRNA knock-down of NOB1 inhibits U-87MG cell growth, migration, and invasion, suggesting that NOB1 plays a crucial role in regulating glioblastoma cell proliferation and metastasis. It is acknowledged that miRNAs regulate gene expression by binding to the 3'-UTR of the target genes. In this study, a bioinformatics approach was used to predict NOB1 as a candidate target gene and luciferase reporter assay was performed, for *in vitro* confirmation. Meanwhile, the expression of NOB1 was down-regulated and up-regulated in U-87MG cells after transfection with miR-139-3p and miR-139-3p^{inhi}, respectively. In addition, overexpression of NOB1 neutralized the effects of miR-139-3p suppression. These results corrob-

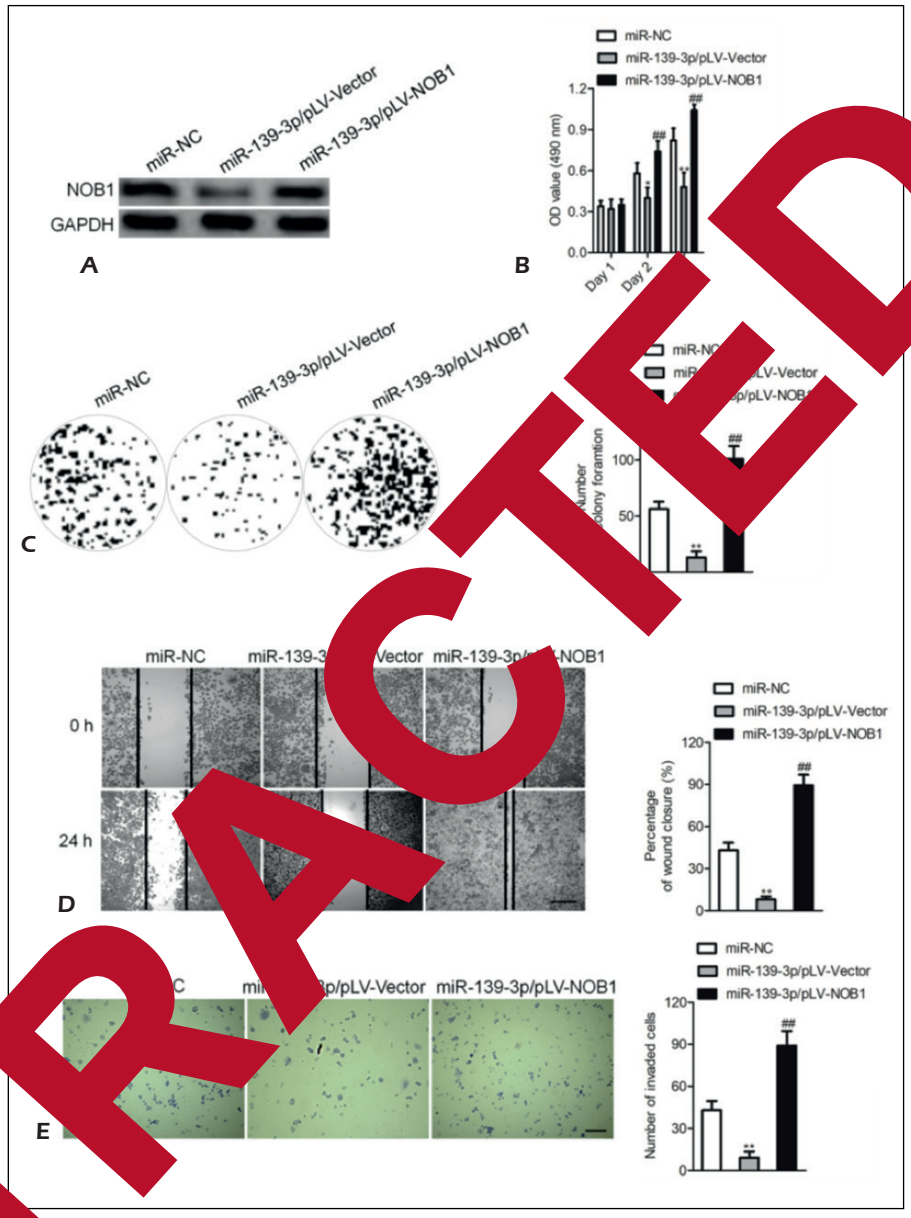

Figure 6. Effect of miR-139-3p on glioblastoma cells rescues by NOB1 overexpression. **A**, pLV-NOB1 was transfected into U-87MG. The expression level of NOB1 was detected by Western blot. **B**, Cell proliferation activity was measured by MTT assay. **C**, Growth ability was measured by the colony formation assay *in vitro*. The image and quantitative analysis of colonies were shown. **D**, U-87MG cells were co-transfected with pLV-NOB1 and miR-139-3p, and then the migration ability of the cells was investigated with the wound healing assay. **E**, The invasion ability of the cells was investigated with the transwell invasion assay. The data are presented as mean ± SD. *p<0.05, **p<0.01, compared to miR-NC. $^{\#\#}p$<0.01, compared to cell co-transfected with miR-139-3p and pLV-Vector.

orate[illegible] miR-[illegible]3p act as a tumor suppressor by [illegible]iting [illegible]B1 in glioblastoma progression.

[illegible]clusions

[illegible]erved the down-regulation of miR-139-[illegible] g[illegible]toma. Overexpression of miR-139-[illegible] inhibited the growth, invasion, and metastasis [illegible]lioblastoma, whereas inhibition of miR-139-3p[illegible]ulted in almost the opposite outcomes. NOB1, the direct target gene of miR-139-3p, plays a crucial role in this process. These findings may help us to investigate the mechanism of miR-139-3p regulation of glioblastoma better, and might provide a novel therapeutic target for the treatment of glioblastoma growth and metastasis.

Conflict of Interests

The authors declare that no conflicts of interest exist.

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