European Review for Medical and Pharmacological Sciences 2020; 24: 3818-3828

Mettl3 regulates the proliferation, migration and invasion of glioma cells by inhibiting PI3K/Akt signaling pathway

J.-W. JI[1,2], Y.-D. ZHANG[2], Y.-J. LAI[2], C.-G. HUANG[1]

[1]Department of Neurosurgery, Changzheng Hospital Affiliated to Naval Military Medical University, Shanhai, P.R. China
[2]Department of Neurological Center, The Third Affiliated Hospital of Chongqing Medical University (Gener Hospital), Chongqing, P.R. China

Abstract. – OBJECTIVE: Methyltransferase-like 3 (Mettl3), one of "writers" for N6-methyladenosine RNA methylation is determined to participate in a variety of cell biological functions. However, the functions of Mettl3 on tumor growth of glioma remain unknown. Here, we conducted a research to explore the contribution of Mettl3 in the progression of glioma.

PATIENTS AND METHODS: To detect the expression level of RNAs, quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed. To access the relative level of proteins, Western blot was conducted. The proliferative ability of glioma cells was detected by CCK-8 assay and colony formation assay. The migration and invasion of glioma cells were determined by wound healing assay and transwell invasion assay.

RESULTS: The expression of Mettl3 was significantly downregulated in tumor tissues compared to the adjacent normal tissues. The downregulation of Mettl3 led to the enhancement of glioma cell proliferation, migration, and invasion in vitro, and promoted the tumor growth of glioma cells in vivo. In addition, further investigation confirmed that Mettl3 plays critical roles in the development of glioma by targeting PI3K/Akt pathway.

CONCLUSIONS: Our study proves that Mettl3 plays a critical role in the proliferation, migration, and invasion of glioma cells by inactivating PI3K/Akt signaling pathway, providing a novel mechanism of glioma tumorigenesis and raising a new target for the treatment of glioma.

Key Words:
Mettl3, Glioma, Proliferation, Migration, Invasion, PI3K/Akt.

Introduction

As the most frequently diagnosed brain tumor among adults, human malignant glioma is characterized by a high fatality rate and poor cure rate[1-3]. In accordance with the standards of World Health Organization (WHO) , diffuse glioma was classified on a scale of II to IV and the higher was the grade, the worse the condition[1]. Although there were significant progresses in glioma treatments, including surgery, chemotherapy, and radiotherapy, the malignant glioma is still a serious public health problem due to its high mobility and poor median survival[4]. Thus, it is necessary to perform further investigation on the molecular mechanism of malignant glioma. N6-methyladenosine (m^6A) is a reversible modification in virtually all eukaryotic mRNA[5]. Wang et al[6] suggested that m6A acts as a regulatory role in the generation, proliferation, and differentiation of embryotic stem cells. In the last few years, many researchers have paid attention to its function on cancer. Results of some studies showed that the downregulation of m6A promoted tumorigenesis[7-9]. It has already been detected that the biological functions of m6A modification are mediated by "writer", "reader", and "eraser" proteins[10,11]. Mettl3 is one of the "writer" proteins which was defined as a catalyticase of the m6A modification. It was demonstrated that downregulation of the Mettl3 could remarkably decrease m6A modification of the mRNA[6,12].

As indicated above, m6A plays a significant role in the proliferation of stem cells and cancer cells. However, how m6A affects the growth of these cells and which pathway or molecular mechanism mediating the affection need further investigations. In our study, Mettl3 was found downregulated in glioma tissues compared with the normal tissues, and the downregulation of the Mettl3 could promote the proliferation of the

Corresponding Author: Chengguang Huang, MD; e-mail: naokeyisheng@163.com

glioma cells *in vitro* and enhance the tumorigenesis *in vivo*. Moreover, our study showed that the downregulation of Mettl3 enhanced the expression of the key enzyme of PI3K/Akt pathway. The inhibition of PI3K or MERK could abolish the abnormal proliferation of glioma cells caused by the reduction of Mettl3. The results of our study define that Mettl3 has regulatory functions on the proliferation, migration, and invasion of glioma cells *via* targeting PI3K/Akt pathway, providing a novel target for the treatment of glioma.

Patients and Methods

Tissue Specimens

Glioma tissues and the neighboring normal tissues were collected from 45 patients who were diagnosed with glioma cancer and treated in surgery at Changzheng Hospital Affiliated to Naval Military Medical University. The research was approved by the Medical Ethics Committee of Changzheng Hospital Affiliated to Naval Military Medical University, and informed consents were provided by participants or their families.

Quantitative Real Time-Polymerase Chain Reaction

Total RNAs of tissue samples and cells were extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacture's instruction. The reverse transcription of Mettl3 was performed using high capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). TaqMan Universal Master MixII (Invitrogen, Carlsbad, CA, USA) was used for quantitative Real Time-PCR and the internal control was GAPDH. The primers used in this study were as follows: Mettl3-forward: 5'-TCCGGTTAGCCTTCGGG-3', Mettl3-reverse: 5'-TGAAATATGTTGACCCAGCTCATCT-3'; GAPDH-forward: 5'-TTTGCTGGTTCCGATGCTGA-3', GAPDH-reverse: 5'-AGTTAAAAGCAGCCCTGGTGA-3'.

Cell Culture

Human glioma cell lines U87 and LN229 were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA), and maintained in high glucose Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA) complemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, CA, USA). All cells were incubated at 37°C and 5% CO_2.

Cell Transfection and Treatments

Sh-NC, sh-Mettl3, vector-NC and vector-Mettl3 were transfected into U87 and LN229 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocols. The inhibitors of PI3K (LY294002, ApexBio, Houston, TX, USA) and Akt (GSK690693, ApexBio, Houston, TX, USA) were added into the culture medium in the concentration of 50 μM for 1 h, then, the culture medium was replaced with normal culture medium.

Proliferation Assays of Cells

Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology, Shanghai, China) was adopted to determine the ability of cell growth. U87 and LN229 were divided into six groups: short hairpin- negative control (sh-NC), short hairpin-Mettl3 (sh-Mettl3), Vector-negative control (Vector-NC), Vector-Mettl3, sh-Mettl3, and LY294002 treated, sh-Mettl3, and GSK690693 treated. All cells were seeded at 96-well plates for 24 or 48 hours after pretreatment. 10 μl CCK-8 solution was added into each well, and the cells were incubated in the 37°C incubator for 2 h. A microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the absorbance at 450 nm.

Western Blot

The cells were collected by phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, MO, USA), and lysed by pre-cooling RIPA buffer on the ice for 0.5 h. The lysis buffer was transferred into new 1.5 ml microfuge tubes and centrifuged in 4°C at 14000 rpm/min for 20 min. The primary antibodies were used at first: mouse anti-PI3K (Abcam, Cambridge, MA, USA, ab140307), Rabbit anti-Akt (Abcam, Cambridge, MA, USA, 8805), Rabbit anti-p-Akt (Abcam, Cambridge, MA, USA, ab38449), Rabbit anti-mTOR (Abcam, Cambridge, MA, USA, ab2723), Rabbit anti-p-mTOR (Abcam, Cambridge, MA, USA, ab109268) and anti-GAPDH HRP Mouse mAb (Multi Sciences Biotechnology, Hangzhou, China, ab011-040). The following secondary antibodies were used: anti-Mouse IgG-HRP (Thermo Fisher Scientific, Waltham, MA, USA, RA230188), anti-Rabbit IgG-HRP (Abcam, Cambridge, MA, USA, ab2761). We visualized the images with Molecular Imager Imaging System (Tanon, Shanghai, China). Analysis of intensity of images was performed with Adobe Photoshop software.

Wound Healing Assay

U87 and LN229 cells were seed into 6-well plates at a number of 1×10^5 cells/well, the wound was scraped with pipette tips. We cultured the cells for 24 hours, and DM2500 bright field microscope (LEICA, Wetzlar, Germany) was used to capture the migration of cells, and the Image J software was adopted to measure the distance.

Transwell Invasion Assay

Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) was used to coat the upper chamber and 1×10^5 cells were seeded on it. 500 µl DMEM supplement with 10% FBS were added into the lower chamber. Cultured cells for 24 hours and fixed the invasion cells with 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA). Cells were stained with crystal violet solution.

Animal Experimental Protocol

All animal experiments were conducted in line with National Institute of Health's Guidelines for the Care and Use of Laboratory Animals. Animal experiments were approved by the Animal Care and Research Committee of Changzheng Hospital Affiliated to Naval Military Medical University. Mice were cultured in specific-pathogen-free (SPF) conditions. U87 and LN229 cells were transfected with empty vector, overexpression of Mettl3 (OE-Mettl3). 5×10^6 transfected cells were injected subcutaneously into the nude mice. After 4 weeks, we euthanized the mice, and calculated the volume of the tumors by the formula as follows: length × width^2 × 0.5.

Apoptosis Analysis

To measure the apoptosis ability of the transfected cells, the cells were treated with Annexin V-APC/7-AAD (BD Biosciences, Franklin Lakes, NJ, USA) in the light of the manufacturer's protocol. We washed the cells with cold PBS three times, and then, suspended them at a density of 1×10^6 cells/ml with binding buffer. 5 µl of APC and 5 µl of AAD were added into the cells. Finally, we incubated the cells for 15 minutes at room temperature in the dark place, and the total volume was made up to 500 µl with 1 x binding buffer. We analyzed and counted the cell samples by flow cytometry (FACScan, BD Biosciences, Franklin Lakes, NJ, USA).

Colony Formation Assay

The transfected cells were seeded into a 10 cm cell culture dish (2200 cells/well) and incubated at 37°C in 5% CO_2 for 14 days. Finally, we fixed the cells with 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MA, USA) and stained the cell with crystal violet for number counting.

Statistical Analysis

Statistical analysis was carried out with GraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA) and data are shown as mean ± standard deviation (SD). The comparison between the two groups were analyzed by Student's *t*-test. The *p*-value less than 0.05 was considered statistically significant.

Results

Mettl3 Is Downregulated in Glioma Tumor Tissues

It was reported that the reduction of m6A mRNA methylation could promote the tumorigenicity of glioblastoma stem cells[7]. To investigate whether the m6A mRNA methylation plays the same role in the human glioma, we detected the expression of its "writer" Mettl3 in glioma tissues by qRT-PCR. The results showed that expression of Mettl3 decreased as the condition of the glioma worsened (Figure 1A). Then, we tested the expression of Mettl3 protein in different grades of glioma by Western blot, showing that the relative protein levels of Mettl3 appeared the same trend as the mRNA level of Mettl3 (Figure 1B).

Mettl3 is Downregulated in Glioma Cell Lines

To confirm the function of Mettl3 in glioma, we detected the expression of Mettl3 in glioma cell lines, including U87 and LN229. The expression of mRNA and protein were detected by qRT-PCR and Western blot. As shown in Figure 2A and B, the expression of Mettl3 in U87 and LN229 was significantly lower than that in the normal human astrocytes HA1800.

Deficiency of Mettl3 Enhances Proliferation while Inhibits the Apoptosis of Glioma Cell Lines

To explore the function of the Mettl3 on the development of glioma, we knocked down Mettl3 in U87 and LN229 cells with Mettl3 shRNA. The results of qRT-PCR showed the efficiency of the downregulation of Mettl3 (Figure 3A). CCK-8 assay was used to detect the proliferation of transfected cells, showing

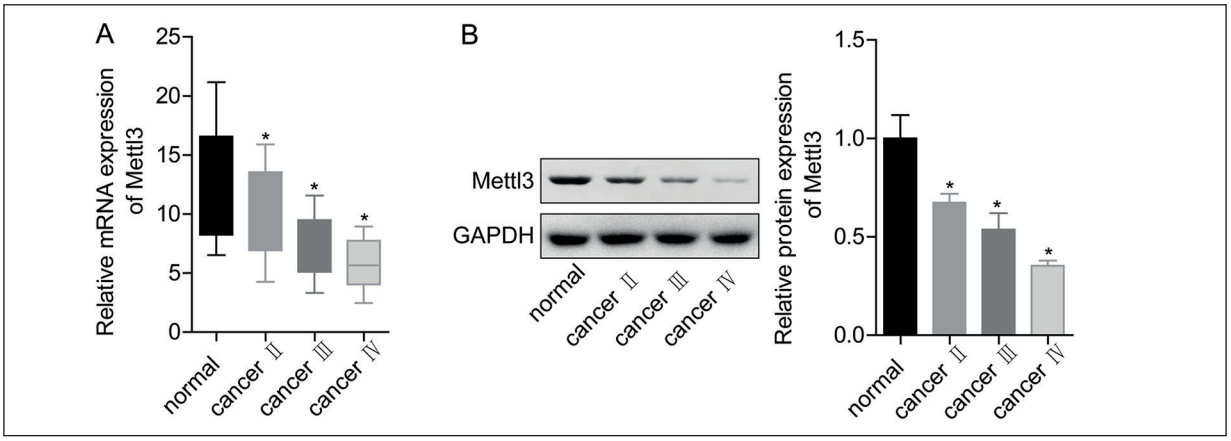

Figure 1. Downregulation of Mettl3 was related to malignancy in glioma (**A**) the expression of Mettl3 mRNA in different grade glioma classified by WHO detected by qRT-PCR. **B,** The expression of Mettl3 protein in glioma of WHO grades II-IV detected by Western blot, $^{*}p<0.05$ tumor tissues *vs.* the adjacent normal tissues.

that the downregulation of Mettl3 promoted the proliferation of U87 and LN229 cells (Figure 3B, C). The analysis of cell apoptosis was performed with flow cytometry. As shown in Figure 3D and E, the downregulation of Mettl3 decreased the apoptosis of U87 and LN229 cells. The results of colony formation assay further confirmed that deficiency of Mettl3 accelerated the colony formation of U87 and LN229 cells (Figure 3F, G).

Deficiency of Mettl3 Promotes the Migration and Invasion of Glioma Cell Lines

To investigate the function of the Mettl3 on the migration and invasion of U87 and LN229 cells, we performed wound healing assay and transwell invasion assay. Downregulation of Mettl3 enhanced the migration (Figure 4A, B) and invasion (Figure 4C, D) of U87 and LN229 cells.

Overexpression of Mettl3 Inhibits the Proliferation and Induced the Apoptosis of Glioma Cell Lines

To further confirm the critical role that Mettl3 plays in U87 and LN229 cells, we transfected the cells with OE-Mettl3. The results of qRT-PCR showed that Mettl3 was overexpressed in U87 and LN229 cells (Figure 5A). In consistent with previous results, the upregulation of Mettl3 inhibited the proliferation (Figure 5B, C), while enhanced the apoptosis of U87 and LN229 cells (Figure 5D, E). Moreover, the colony formation

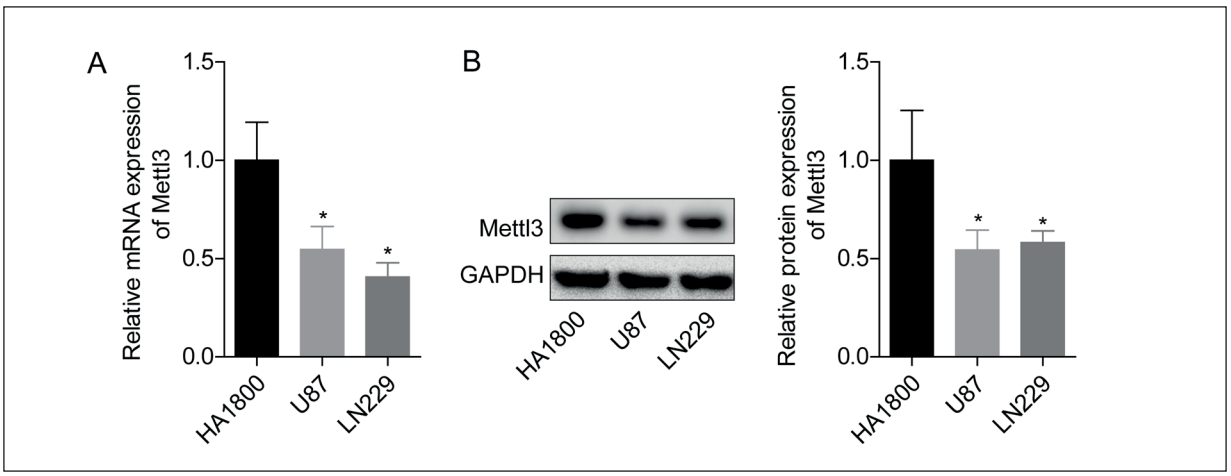

Figure 2. Expression of Mettl3 in glioma cell lines (**A**) Expression of Mettel3 mRNA in human astrocyte cells and glioma cell lines. **B,** Expression of Mettel3 protein in human astrocyte cells and glioma cell lines, $^{*}p<0.05$ glioma cell lines *vs.* HA1800 cells.

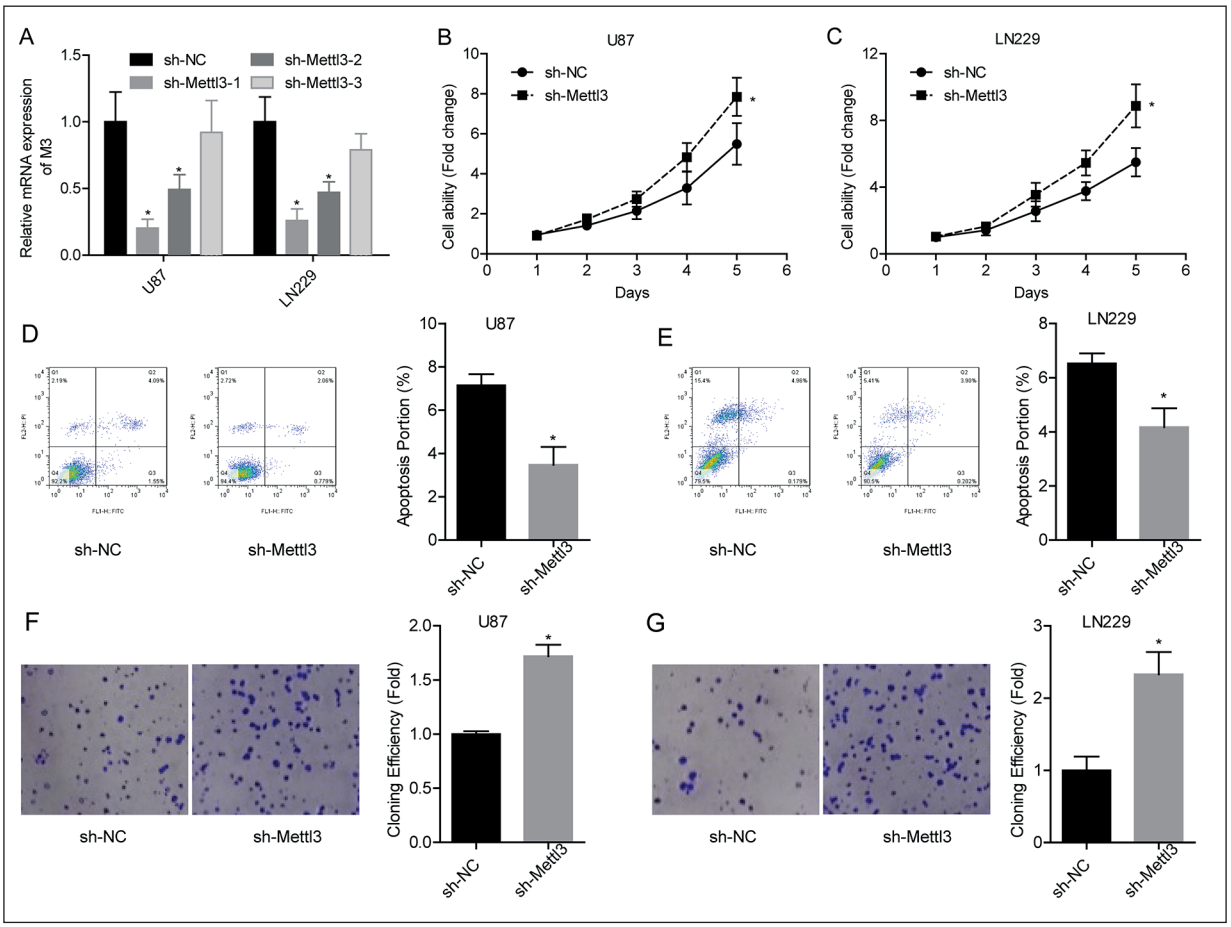

Figure 3. Downregulation of Mettl3 promoted glioma cells growth (**A**) qRT-PCR was adopted to validate the efficiency of Mettl3 shRNA. Effect of Mettl3 deficiency on U87 (**B**) and LN299 (**C**) cells proliferation was detected by CCK-8 assay. Effect of Mettl3 deficiency on U87 (**D**) and LN299 (**E**) cells apoptosis was measured by flow cytometry. Effect of Mettl3 deficiency on U87 (**F**) and LN299 (**G**) cells proliferation was assessed by colony formation assay, magnification times: 200×, $^{*}p<0.05$ sh-Mettl3 group *vs.* sh-NC group.

assay showed that overexpression of Mettl3 attenuated the colony formation of U87 and LN229 cells (Figure 5F, G).

Overexpression of Mettl3 Inhibits the Migration and Invasion of Glioma Cells

To validate the contribution of Mettl3 in the inhibition of the tumor growth of glioma, we overexpressed Mettl3 in U87 and LN229 cells and tested the abilities of migration and invasion by wound healing assay and transwell invasion assay. The results of wound healing assay indicated that the overexpression of Mettl3 inhibited the migration of U87 (Figure 6A) and LN229 (Figure 6B) cells. As expected, the invasion abilities of U87 and LN229 cells were also decreased by the overexpression of Mettl3 (Figure 6C, D).

Mettl3 Regulates the Growth of Glioma Tumors by Targeting PI3K/Akt Pathway

Liu et al[13] indicated that m6A mRNA methylation acted as a regulatory role in PI3K/Akt pathway in endometrial cancer. To investigate whether m6A mRNA methylation has the same function on PI3K/Akt in glioma, we detected the phosphorylation level of Akt and mTOR after downregulating Mettl3 in U87 and LN229 cells. The results of Western blot suggested that the downregulation of Mettl3 could enhance the expression of PI3K and the phosphorylation level of Akt and mTOR in U87 and LN229 cells (Figure 7A, B). To further show whether the PI3K/Akt was the underlying pathway of the Mettl3 on regulating the tumorigenesis of glioma, we treated the

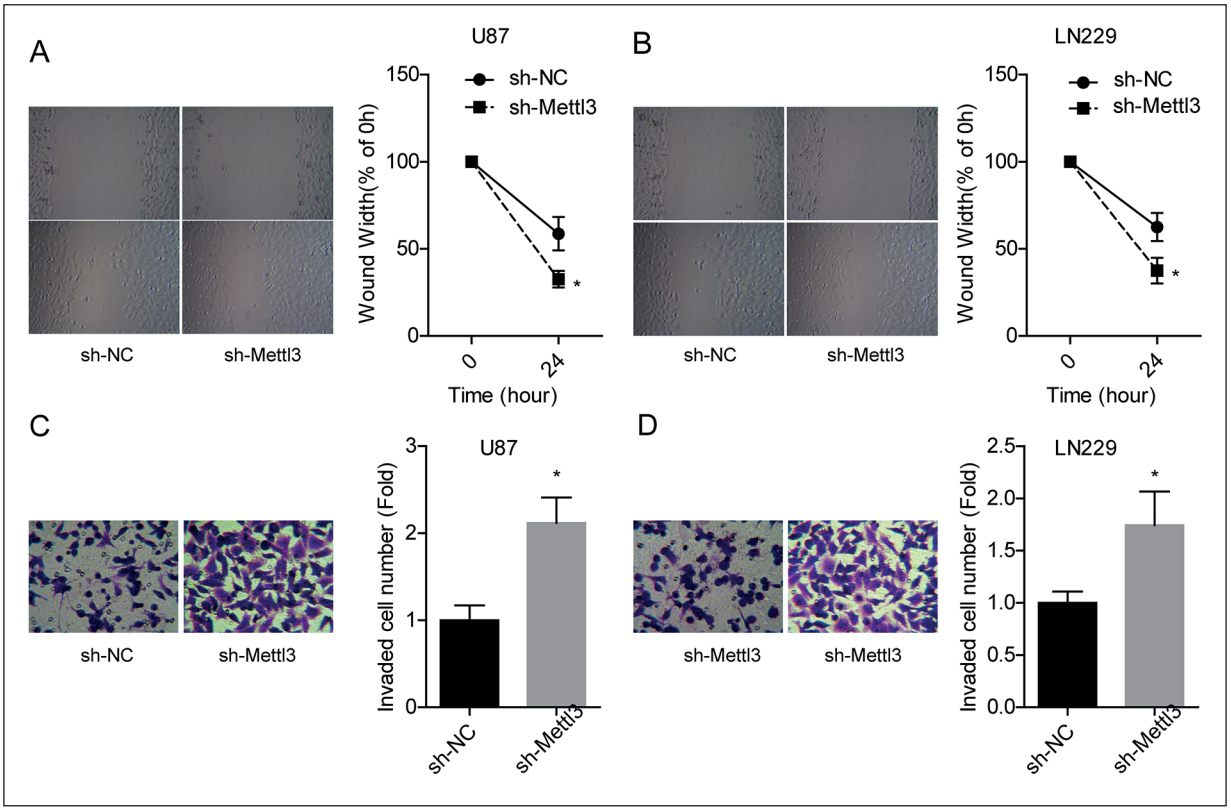

Figure 4. Downregulation of Mettl3 enhanced the migration and invasion of glioma cells U87 and LN229 cells were separated into two groups: sh-NC group, sh-Mettl3 group. Migration of U87 (**A**) and LN229 (**B**) cells was assessed by wound healing assay, magnification times: 200×. Invasion of U87(**C**) and LN229 (**D**) cells was detected by transwell assay, magnification times: 200×, $^{*}p<0.05$ sh-Mettl3 group *vs.* sh-NC group.

cells with vector-Mettl3, LY294002 (the inhibitor of PI3K), GSK690693 (the inhibitor of Akt) and performed CCK-8 assay and wound healing assay. As shown in Figure 7C-H, the treatment of LY2940002 and GSK690693 abolished the promotion of proliferation and migration of U87 and LN229 cells caused by downregulation of Mettl3.

Overexpression of Mettl3 Inhibits U87 and LN229 Cells Growth In Vivo

According to the results of Mettl3 *in vitro* assay, we presumed that Mettl3 played a significant role in the tumorigenesis of glioma. To confirm its function *in vivo*, we transfected the vector -NC and vector-Mettl3 into U87 and LN299 cells and injected the cells subcutaneously into nude mice. As detected in Figure 8A-D, the tumor volumes of vector-Mettl3 group were prominently larger than that in the vector-NC group. The results of Western blot showed that the expression of PI3K and the phosphorylation level of Akt and mTOR were significantly downregulated in the tumor tissues of vector -Mettl3 group (Figure 8E, F).

Discussion

Previous studies demonstrated that m6A mRNA methylation played important roles in a variety of physiological functions. However, the exact role of m6A in biology function was still unknown. There were studies[13,14] showing that reducing m6A level of m6A-tagged transcripts by knocking down Mettl3 could enhance self-renewal and damaged differentiation of mESCs, while others[6] validated that the loss of the Mettl3 or Methyltransferase-like 14 (Mettl14) which was defined as another "writer" of m6A decreased the self-renewal of mECSs. Recently, more attention was paid on the regulatory function of m6A on promoting

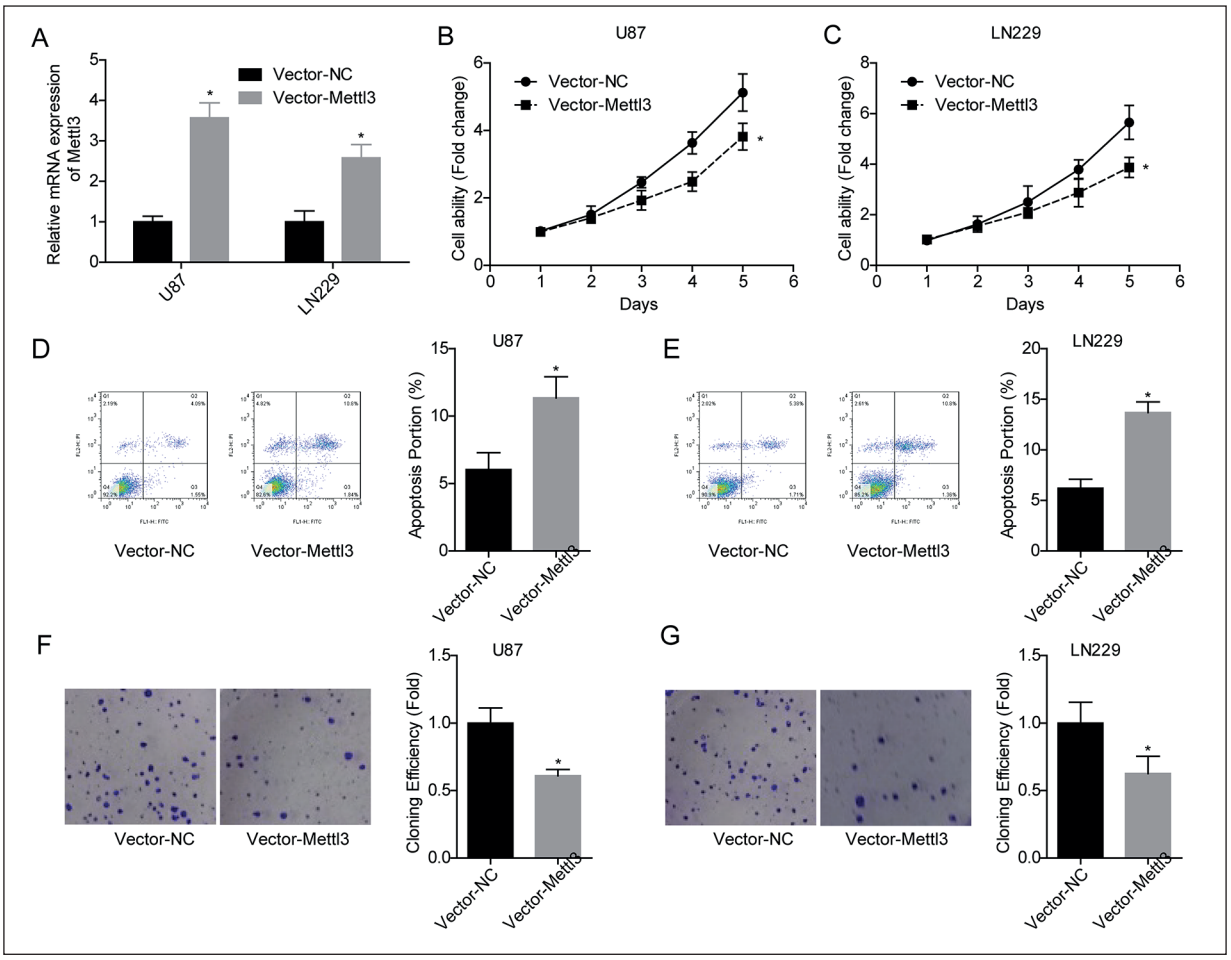

Figure 5. Overexpression of Mettl3 inhibited the glioma cells growth U87 and LN229 cells were divided into two groups: Vector-NC group, Vector -Mettl3 group. **A,** The mRNA level of Mettl3 detected by qRT-PCR. **B, C,** Upregulation of Mettl3 inhibited the proliferation of U87 and LN229 cells. **D, E,** Upregulation of Mettl3 promoted the apoptosis of U87 and LN229 cells. **F, G,** Upregulation of Mettl3 reduced the rate of colony formation of U87 and LN229, magnification times: 200×, $^{*}p<0.05$ Vector -Mettl3 group *vs.* Vector-NC group.

some cancers, such as human acute myeloid leukemia[15] and breast cancer[16]. While, other researches reflected that the reduction of the mRNA m6A level by knocking down Mettl3 enhanced the proliferation of the glioblastoma stem-like cells and the growth of endometrial cancer was promoted either[7,13].

Our study validated that the expression of Mettl3 was significantly downregulated in glioma tissues compared to the adjacent normal tissues. The downregulation of Mettl3 promoted the proliferation, migration, and invasion of glioma cells, while inhibited the apoptosis of glioma cells. Moreover, the upregulation of Mettl3n can also inhibit the cell growth of U87 and LN229 *in vitro.*

The PI3K/Akt pathway has significant functions on various biological processes, and disorder of the PI3K signaling has contribution to a variety of diseases including diabetes, Parkinson disease, cancers, and choroba autoimmunologiczna[17-20]. Brazil et al[21] demonstrated that PI3K signaling pathway played the important role in cell growth, apoptosis, and metabolism, and the expression of PI3K and Akt protein is upregulated in tumor tissues such as gastric cancer, endometrial cancer and breast cancer[22-24]. In this study, we found that PI3K/Akt/mTOR acted as a crucial downstream signaling of Mettl3 in glioma. As shown in our results, the knockdown of Mettl3 could enhance the phosphorylation level of Akt and

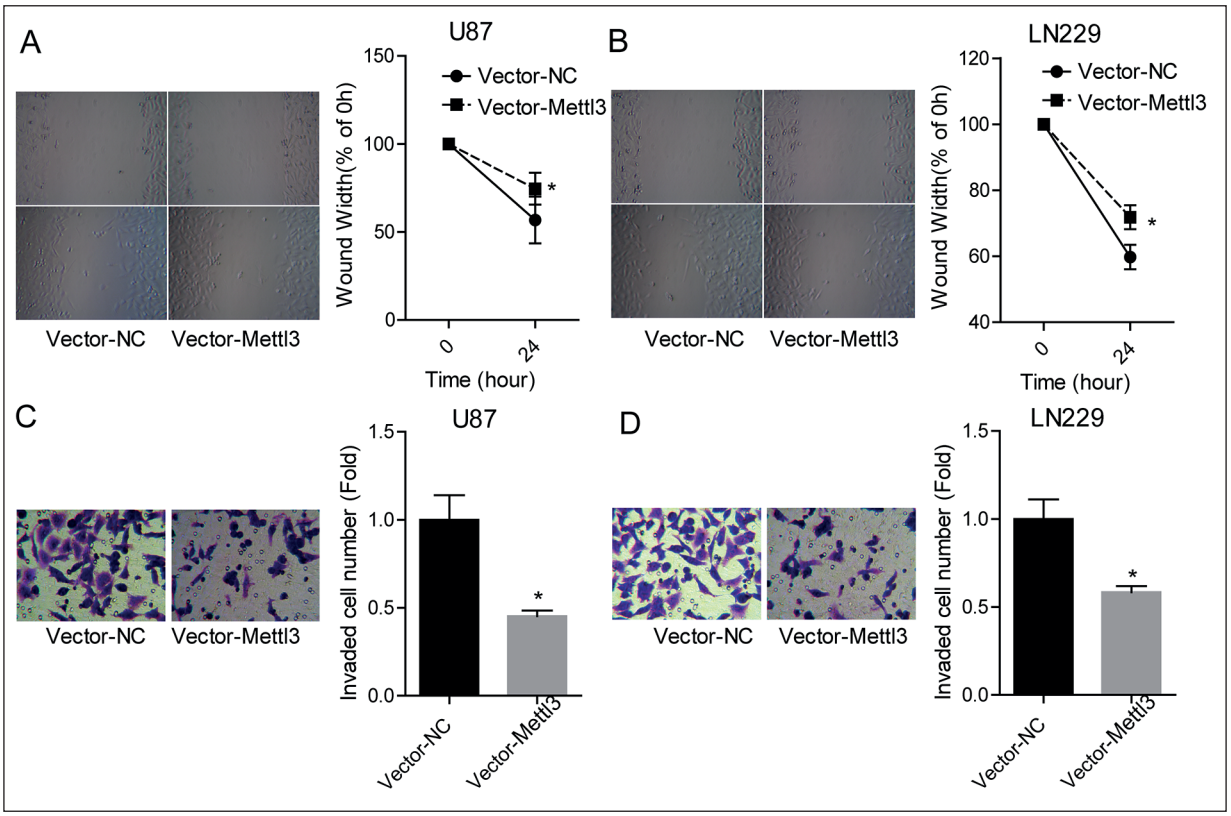

Figure 6. Overexpression of Mettl3 inhibited migration and invasion of glioma cells U87 and LN229 cells were divided into two groups: Vector-NC group, Vector -Mettl3 group. **A, B,** Overexpression of Mettl3 inhibited the migration of U87 and LN229 accessed by wound healing assay, magnification times: 200×. **C, D,** Over expression of Mettl3 inhibited the invasion of U87 and LN 229 cells, magnification times: 200×, $^{*}p<0.05$ Vector -Mettl3 *vs.* Vector-NC.

mTOR, while the inhibition of Akt and mTOR could reverse the increase of cell proliferation and migration caused by downregulation of Mettl3. In addition, the overexpression of Mettl3 in U87 and LN229 could downregulate the relative level of PI3K and phosphorylation level of mTOR and Akt *in vivo*.

According to our study, Mettl3 was reported as a crucial regulator of PI3K signaling in numerous kinds of cancers. Depletion of Mettl3 could promote the tumorigenesis in endometrium by activating Akt signaling[13]. In renal cell carcinoma (RCC), the downregulation of Mettl3 was validated to enhance the proliferation and migration of RCC cell lines by dysregulating of PI3K/Akt pathway[9]. Our work demonstrated that Mettl3 regulated the abilities of proliferation, migration, and invasion in glioma cell lines by targeting PI3K/Akt /mTOR pathway. In summary, our findings prove that Mettl3 may act as an anti-oncogene in progression of glioma.

Conclusions

To sum up, the findings of this study indicate that Mettl3 can function as a tumor suppressor in the development of glioma. The inactivation of PI3K/Akt signaling pathway underlies the molecular mechanism by which Mettl3 inhibits the progression of glioma. However, as the modification of m6A on RNA was dynamic, how Mettl3 inactivates PI3K/Akt signaling pathway is still unclear. Further investigations should be conducted to illustrate the mechanisms through which Mettl3 regulates the activation of PI3K/Akt signaling pathway.

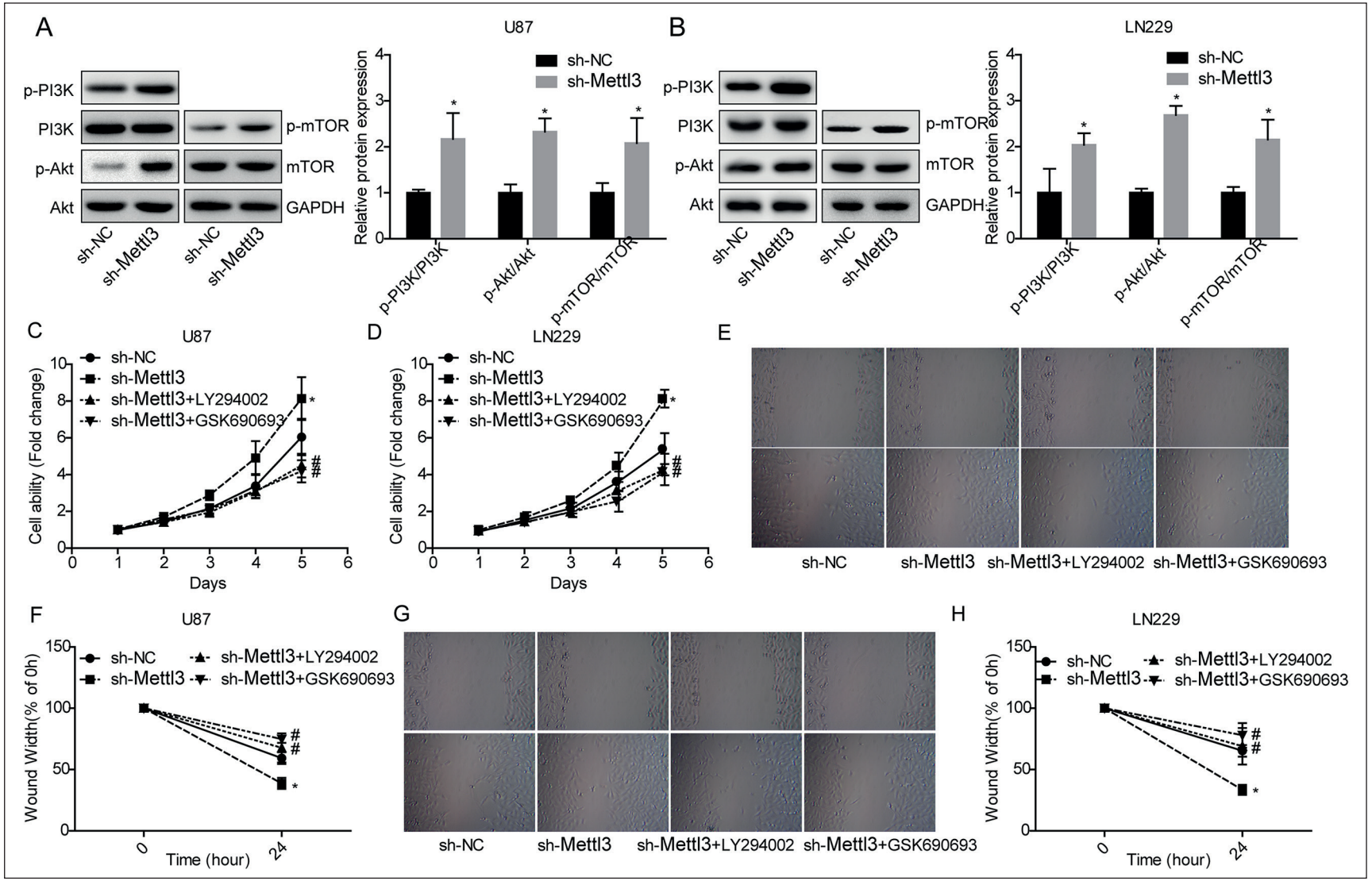

Figure 7. Deficiency of Mettl3 promoted malignancy of glioma by activating PI3K/Akt pathway U87 and LN229 cells were divided into four groups: sh-NC, sh-Mettl3, sh-Mettl3 +LY294002, sh-Mettl3 +GSK690693. **A, B,** Deficiency of Mettl3 induced changes on the markers of PI3K/Akt/mTOR pathway with enhancements of the PI3K, p-Akt, and p-mTOR. **C, D,** Inhibitors of Akt and mTOR decreased the proliferation of transfected U87 and LN229 cells. **E-H,** Inhibitors of Akt and mTOR reduced the migration of transfected U87 and LN229 cells, magnification times: 200×, $^{*}p<0.05$ sh-Mettl3 *vs.* sh-NC, $^{\#}p<0.05$ sh-Mettl3 +LY294002 *vs.* sh-Mettl3, $^{\#}p<0.05$ sh-Mettl3 + GSK690693 *vs.* sh-Mettl3.

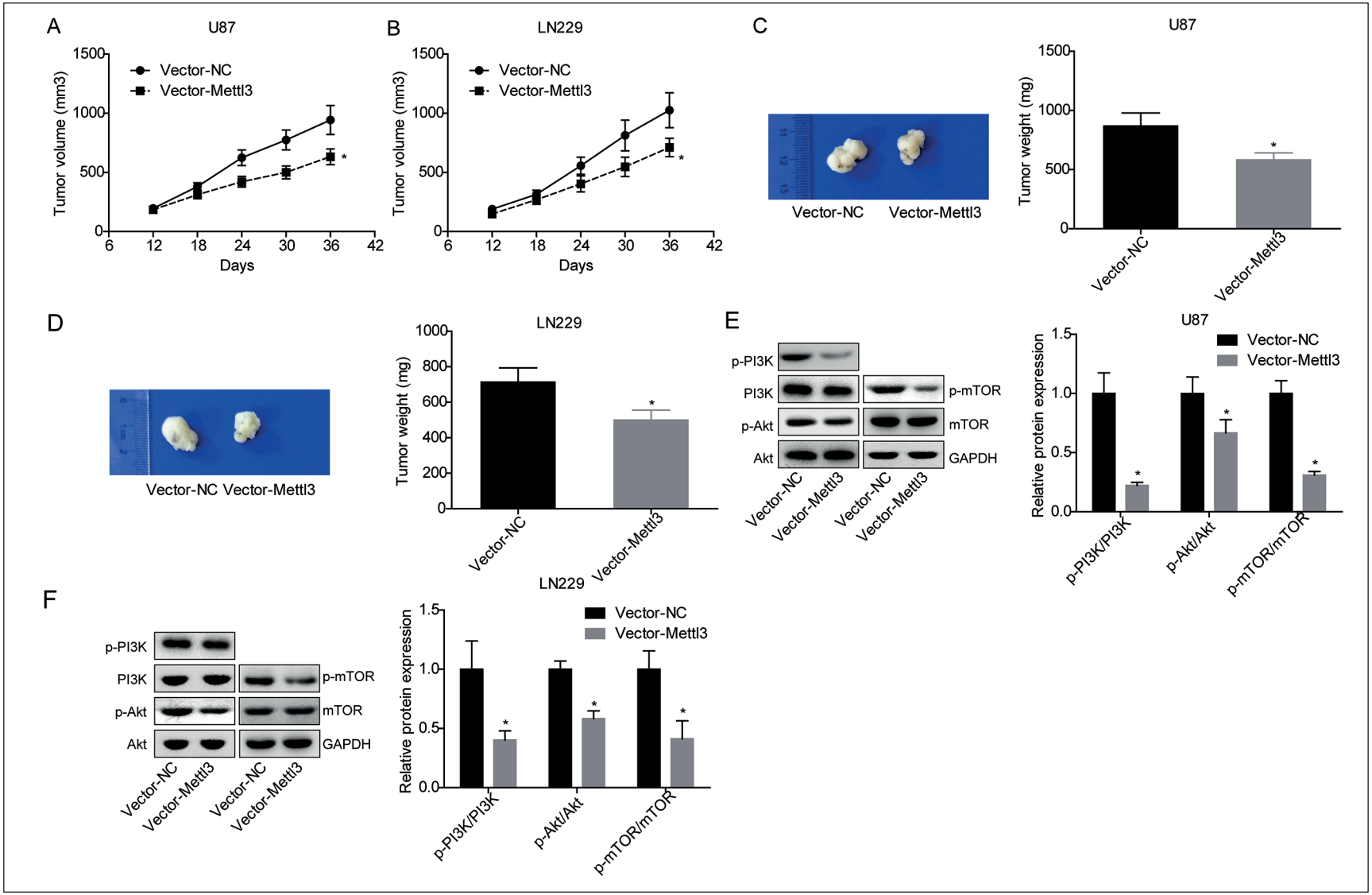

Figure 8. Overexpression of Mettl3 inhibited the tumor growth *in vivo* U87 and LN229 cells were divided into two groups: Vector-NC group and Vector -Mettl3 group. **A, B,** The volume of tumors was detected every 6 day after U87 and LN229 were injected into nude mice. **C, D,** The weight of tumors was detected after sacrificing the nude mice. **E, F,** The phosphorylation levels of PI3K and Akt proteins were detected after sacrificing the nude mice, $^{*}p<0.05$ Vector -Mettl3 *vs.* Vector-NC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Jansen M, Yip S, Louis DN. Molecular pathology in adult gliomas: diagnostic, prognostic, and predictive markers. Lancet Neurol 2010; 9: 717-726.
2) Armstrong TS, Wen PY, Gilbert MR, Schiff D. Management of treatment-associated toxicites of anti-angiogenic therapy in patients with brain tumors. Neuro Oncol 2012; 14: 1203-1214.
3) Gravina GL, Mancini A, Marampon F, Colapietro A, Delle Monache S, Sferra R, Vitale F, Richardson PJ, Patient L, Burbidge S, Festuccia C. The brain-penetrating CXCR4 antagonist, PRX177561, increases the antitumor effects of bevacizumab and sunitinib in preclinical models of human glioblastoma. J Hematol Oncol 2017; 10: 5.
4) Han L, Liu D, Li Z, Tian N, Han Z, Wang G, Fu Y, Guo Z, Zhu Z, Du C, Tian Y. HOXB1 is a tumor suppressor gene regulated by miR-3175 in glioma. PLoS One 2015; 10: e0142387.
5) Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG, He C. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 2011; 7: 885-887.
6) Wang Y, Li Y, Toth JI, Petroski MD, Zhang Z, Zhao JC. N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Nat Cell Biol 2014; 16: 191-198.
7) Cui Q, Shi H, Ye P, Li L, Qu Q, Sun G, Sun G, Lu Z, Huang Y, Yang CG, Riggs AD, He C, Shi Y. m(6)A RNA methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells. Cell Rep 2017; 18: 2622-2634.
8) Deng X, Su R, Weng H, Huang H, Li Z, Chen J. RNA N(6)-methyladenosine modification in cancers: current status and perspectives. Cell Res 2018; 28: 507-517.
9) Li X, Tang J, Huang W, Wang F, Li P, Qin C, Qin Z, Zou Q, Wei J, Hua L, Yang H, Wang Z. The M6A methyltransferase METTL3: acting as a tumor suppressor in renal cell carcinoma. Oncotarget 2017; 8: 96103-96116.
10) Liu N, Pan T. N6-methyladenosine-encoded epitranscriptomics. Nat Struct Mol Biol 2016; 23: 98-102.
11) Zhao BS, Roundtree IA, He C. Post-transcriptional gene regulation by mRNA modifications. Nat Rev Mol Cell Biol 2017; 18: 31-42.
12) Bokar JA, Rath-Shambaugh ME, Ludwiczak R, Narayan P, Rottman F. Characterization and partial purification of mRNA N6-adenosine methyltransferase from HeLa cell nuclei. Internal mRNA methylation requires a multisubunit complex. J Biol Chem 1994; 269: 17697-17704.
13) Liu J, Eckert MA, Harada BT, Liu SM, Lu Z, Yu K, Tienda SM, Chryplewicz A, Zhu AC, Yang Y, Huang JT, Chen SM, Xu ZG, Leng XH, Yu XC, Cao J, Zhang Z, Liu J, Lengyel E, He C. m(6)A mRNA methylation regulates AKT activity to promote the proliferation and tumorigenicity of endometrial cancer. Nat Cell Biol 2018; 20: 1074-1083.
14) Geula S, Moshitch-Moshkovitz S, Dominissini D, Mansour AA, Kol N, Salmon-Divon M, Hershkovitz V, Peer E, Mor N, Manor YS, Ben-Haim MS, Eyal E, Yunger S, Pinto Y, Jaitin DA, Viukov S, Rais Y, Krupalnik V, Chomsky E, Zerbib M, Maza I, Rechavi Y, Massarwa R, Hanna S, Amit I, Levanon EY, Amariglio N, Stern-Ginossar N, Novershtern N, Rechavi G, Hanna JH. Stem cells. m6A mRNA methylation facilitates resolution of naive pluripotency toward differentiation. Science 2015; 347: 1002-1006.
15) Vu LP, Pickering BF, Cheng Y, Zaccara S, Nguyen D, Minuesa G, Chou T, Chow A, Saletore Y, MacKay M, Schulman J, Famulare C, Patel M, Klimek VM, Garrett-Bakelman FE, Melnick A, Carroll M, Mason CE, Jaffrey SR, Kharas MG. The N(6)-methyladenosine (m(6)A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. Nat Med 2017; 23: 1369-1376.
16) Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, Chen I, He X, Semenza GL. Hypoxia induces the breast cancer stem cell phenotype by HIF-dependent and ALKBH5-mediated m(6)A-demethylation of NANOG mRNA. Proc Natl Acad Sci U S A 2016; 113: E2047-E2056.
17) Huang X, Liu G, Guo J, Su Z. The PI3K/AKT pathway in obesity and type 2 diabetes. Int J Biol Sci 2018; 14: 1483-1496.
18) Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2002; 2: 489-501.
19) Jha SK, Jha NK, Kar R, Ambasta RK, Kumar P. p38 MAPK and PI3K/AKT signalling cascades in Parkinson's disease. Int J Mol Cell Med 2015; 4: 67-86.
20) Manning BD, Toker A. AKT/PKB signaling: navigating the network. Cell 2017; 169: 381-405.
21) Brazil DP, Yang ZZ, Hemmings BA. Advances in protein kinase B signalling: AKTion on multiple fronts. Trends Biochem Sci 2004; 29: 233-242.
22) Fang WL, Huang KH, Lan YT, Lin CH, Chang SC, Chen MH, Chao Y, Lin WC, Lo SS, Li AF, Wu CW, Chiou SH, Shyr YM. Mutations in PI3K/AKT pathway genes and amplifications of PIK3CA are associated with patterns of recurrence in gastric cancers. Oncotarget 2016; 7: 6201-6220.
23) Dong P, Konno Y, Watari H, Hosaka M, Noguchi M, Sakuragi N. The impact of microRNA-mediated PI3K/AKT signaling on epithelial-mesenchymal transition and cancer stemness in endometrial cancer. J Transl Med 2014; 12: 231.
24) Yang W, Schwartz GN, Marotti JD, Chen V, Traphagen NA, Gui J, Miller TW. Estrogen receptor alpha drives mTORC1 inhibitor-induced feedback activation of PI3K/AKT in ER+ breast cancer. Oncotarget 2018; 9: 8810-8822.