

Berberine Inhibits Glioma Cell Migration and Invasion by Suppressing TGF- β 1/COL11A1 Pathway and Enhances Chemosensitivity

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Research Article

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Abstract

Background: The expression of collagen type XI alpha 1 chain (COL11A1) is up-regulated in many cancers, affecting the risk of metastasis, drug resistance, and poor prognosis. Berberine is an isoquinoline alkaloid present in many traditional Chinese medicines and it has been shown to reduce collagen accumulation in pulmonary fibrosis, diabetic nephropathy and arthritis. However, its effect on COL11A1 in glioma needs to be further elucidated.

Methods: Western blot was performed to detect the expression level of COL11A1 in several glioma cells, and siCOL11A1 was performed to investigate the effect of COL11A1 on the migration and invasion ability of glioma cells. CCK-8, wound healing experiment and transwell experiment were performed to detect the effect of berberine on the proliferation, migration and invasion of glioma cells. The xenografts experiment in nude mice was performed to test the effect of berberine on inhibiting glioma *in vivo*.

Results: Our results showed that COL11A1 is highly expressed in glioma cell lines and associated with migration, invasion, and chemoresistance of glioma cells. Knocking down COL11A1 caused decreased expression of MMPs, Snail, and MGMT. Berberine could inhibit the migration and invasion of glioma cells by suppressing the TGF- β 1/COL11A1 pathway and changes actin cytoskeleton arrangement. In addition, berberine increased the chemosensitivity of glioma cells to temozolomide, which may be related to the down-regulation of Snail and MGMT proteins caused by berberine. High-throughput sequencing of xenografts in nude mice also showed that berberine inhibited the expression of COL11A1 *in vivo*.

Conclusions: Our results suggest that berberine that targets COL11A1 to inhibit glioma migration, invasion and chemoresistance, may serve as a promising candidate for the development of anti-glioma drugs in the future.

1. Introduction

Glioma is the most common type of primary tumor in the central nervous system, accounting for approximately 80% of primary brain malignancies[1]. The World Health Organization classifies gliomas into I-IV grades; grade IV glioblastoma (GBM) has the highest malignancy and accounts for more than 50% of all glioma cases[1, 2]. Currently, treatment for glioma includes surgery, radiotherapy, and chemotherapy; temozolomide (TMZ) is the most commonly used chemotherapy drug for GBM. TMZ penetrates the blood-brain barrier and induces glioma cell apoptosis. However, glioma cells may become resistant to TMZ; preventing glioma chemotherapy resistance is paramount to patient outcomes[3]. Studies have shown that O-6-methylguanine-DNA methyltransferase (MGMT) expression is closely related to TMZ resistance[4]; however, clinical trials aimed at reducing TMZ resistance have yet to achieve satisfactory results[5].

Collagen is the most abundant component of the extracellular matrix (ECM). Collagen type XI alpha 1 chain (COL11A1) is a member of the collagen family[6]. COL11A1 represents a minor fibrillar collagen type, which is essential for skeletal development and collagen fiber assembly[7]. Additionally, many

studies have shown that COL11A1 is highly expressed in many cancers, including ovarian, pancreatic, colon, breast, and lung cancers, where it is associated with the risk of cancer metastasis and drug resistance[8–13]. However, the relationship between COL11A1 expression and glioma remains unclear. At present, it has been found that Solanum incanum extract (SR-T100), LY2157299, SC66 and AZD5653 target COL11A1 to inhibit the proliferation and chemoresistance of cancer cells[14]. COL11A1 is produced by cancer cells or cancer-related fibroblasts and secreted into the ECM[15]. COL11A1 participates in activating intracellular signaling pathways through discoidin domain receptor 2 and $\alpha 1\beta 1$ integrin on the cell surface and induces drug resistance of cancer cells[16]. Therefore, the development of drugs targeting COL11A1 may help reduce tumor resistance and inhibit tumor progression.

Berberine is an isoquinoline alkaloid present in many traditional Chinese medicines. Berberine has been used in China for many years as an over-the-counter drug for the treatment of bacterial diarrhea[17]. In addition, berberine is considered a candidate treatment for diabetes[18]. Several recent studies have shown that berberine has anti-tumor properties[19]. Our previous studies have shown that berberine may induce programmed cell death of glioma cells through ERK1/2-mediated mitochondrial damage and that it may inhibit the proliferation of glioma cells by activating wild-type p53 or inhibiting mutant p53 activity[20, 21]. Finally, berberine may reduce collagen accumulation in pulmonary fibrosis, diabetic nephropathy, and arthritis[22–24]. However, the effect of berberine on collagen in cancers remains unclear.

In this study, we demonstrated that COL11A1 promoted migration, invasion and chemoresistance of gliomas. On the other hand, we demonstrated that berberine could inhibit glioma cell migration, invasion and reduce chemotherapy resistance by inhibiting COL11A1. These findings suggest that berberine may be a candidate drug targeting COL11A1 in the treatment of glioma.

2. Materials And Methods

2.1 Cell Culture

Human glioma cells U87 MG, U251, U118 MG, A172, T98G, LN18, LN229, and human umbilical vein endothelial cell (HUVEC) were purchased from the American type culture collection (ATCC). All cell lines were cultured in DMEM (Gibco, CA, USA) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, MO, USA) and 1% penicillin/streptomycin (Gibco, CA, USA), under 5% CO₂ atmosphere at 37°C.

2.2 Cell Proliferation Assay

Cell proliferation was determined by a cell counting kit-8 (CCK8; Beyotime, Shanghai, China). Cells were seeded in 96-well plates at a density of 5×10^3 cells per well overnight before the application of different treatments. After 48 h, 10 μ l CCK8 reagent was added to each well, and the plates were incubated for 2 h at 37°C. The optical density (OD) of each well was measured at 450 nm.

2.3 Wound Healing Assay

U87 MG and T98G cells were seeded in 6-well plates. The cells were scratched with a 1-mL pipette tip when the cells reached 90% confluence, and floating cells were washed twice with phosphate saline (PBS). The cells were then incubated in DMEM containing 3% FBS. The wound width was measured at different times by a microscope and the rate of wound closure was calculated.

2.4 Cell Invasion Assays

The invasive capacity of cells was tested by 24-well Matrigel invasion chambers (Corning, NY, USA) with an 8- μ m pore membrane. U87 MG and T98G cells were seeded in the upper chamber of a transwell device with 0.2 mg/ml matrigel (Corning, NY, USA) at a density of 2×10^4 cells per well in 200 μ l serum-free medium, and 700 μ l complete medium was added to the lower chamber. After 24 h, the medium was removed from the upper and lower chambers, the cells were fixed with 4% paraformaldehyde for 20 min and then stained with 0.1% crystal violet for 15 min. Cells that failed to penetrate the surface of the upper chamber were carefully wiped off with a cotton swab, and the penetrated cells were counted under a microscope. Five fields per chamber were randomly counted for statistical analysis in triplicate.

2.5 siRNA Synthesis and Transfection

Small interfering RNA against COL11A1 and the negative control siRNA were chemically synthesized by Transheep (Shanghai, China). The sequences of the COL11A1 siRNA were as follows: 5'-GCAUGGUAUUCAGCAAUdTdT-3' (sense) and 5'-AAUUUGCUGAAUACCAUGCdTdT-3' (antisense). Lipofectamine 3000 transfection reagent (Invitrogen, Carlsbad, CA, USA) was used for siRNA transient transfection, according to the manufacturer's instructions. The cells were collected and subjected to subsequent analysis 72 h after transfection.

2.6 Western Blot

After treatment with berberine (Chroma, Chengdu, China) or TGF- β 1 (Peprotech, NJ, USA), the cells were lysed with an RIPA lysis buffer (Beyotime, Shanghai, China) with added protease inhibitor (TargetMol, Shanghai, China), and an equal amount of protein was separated by the SDS-PAGE gel. Primary antibodies anti-COL11A1, anti-MGMT, anti-MMP2, anti-MMP3, anti-MMP9, and anti-TGF- β 1 were purchased from Abcam (Cambridge, UK); anti-GAPDH antibodies were purchased from Engibody (DE, USA).

2.7 Enzyme Linked Immunosorbent Assay

Human TGF- β 1 enzyme linked immunosorbent assay kit was purchased from Cusabio (Wuhan, China). U87MG and T98G cells were treated with berberine for 48 h and then centrifuged at 4°C 1000 \times g for 15 min to obtain the supernatant. According to the manufacturer's instructions, samples were acidified with 1 N HCl for 10 min and neutralized with 1.2 N NaOH to activate TGF- β 1 in the samples. Biotin-labeled antibody working solution and horseradish peroxidase-labeled avidin working solution were sequentially added into an enzyme-labeled plate; finally, a substrate solution and a termination solution were added, and the OD values of each well were measured by a microplate reader at a wavelength of 450 nm within 5 min.

2.8 Xenograft Experiments

All animal experimental operations were performed in accordance with the procedures for experimental animals, approved by the Animal Ethics Committee of the First Hospital of Jilin University (No. 20210547). BALB/c-nu male mice aged 5-6 weeks were purchased from Charles River Laboratories (Beijing, China), and 2×10^6 U87 MG cells were implanted subcutaneously. Five mice were used in each group. The mice were intraperitoneally injected with 5 mg/kg berberine and the same volume of solvent, respectively. Tumor length and width were measured with vernier caliper every 3 days. Tumor volume was calculated according to the following formula: $\text{volume} = 1 / 2 \times \text{length} \times \text{width}^2$. Two weeks after the berberine treatment, the mice were sacrificed by cervical dislocation after intraperitoneal injection of pentobarbital sodium (100mg/kg) and the tumor tissue was excised.

2.9 RNA-Sequencing Analysis

According to the manufacturer's protocol, the isolated nude mice xenografts were placed on ice and the total RNA was extracted with RNAeasy Animal RNA Isolation Kit (Beyotime, Shanghai, China). For high-throughput sequencing, the libraries were prepared, following the manufacturer's instructions, and applied to Illumina HiSeq X Ten system for 150 nt paired-end sequencing. We used edgeR software, which is dedicated to performing differential gene expression analysis. The mathematical model used was a negative binomial distribution model. EdgeR allows analysis of the differential expression between two or more samples, and the analysis results used fold change (FC) and false discovery rate (FDR) values to determine whether a gene was differentially expressed. Significant expression difference was defined as FC of ≥ 2 or ≤ 0.5 and FDR of < 0.05 .

2.10 Immunofluorescence

Paraffin sections were dewaxed, dehydrated with gradient alcohol, subjected to antigen repair, and then rinsed with 0.01 M PBST for 5 min \times 3 times. In addition, 3% BSA was blocked in a wet box for 30 min. Anti-COL11A1 (abcam, Cambridge, UK) was incubated overnight at 4°C and rinsed with 0.01 M PBST for 5 min \times 3 times. The sections were incubated with CY 3-labeled goat anti-rabbit antibody at room temperature for 2 h. After PBST washing, the slices were sealed with glycerol and photographed under a fluorescence microscope.

2.11 Statistical Analysis

Statistical analyses were performed using GraphPad Prism software version 8.0 (GraphPad Software, La Jolla, CA, USA). All measurement data were reported as means \pm standard deviations. One-way ANOVA was used to compare the mean difference of two or more groups. P-values of < 0.05 were considered statistically significant.

3. Results

3.1 COL11A1 is up-regulated in glioma cells and associated with migration, invasion and chemoresistance

We compared the expression of COL11A1 protein in seven glioma cells with that in HUVEC cells. Western blot analysis of seven glioma cells showed that COL11A1 expression in six glioma cells was higher than that in the HUVEC (Figure 1A, B). To investigate the mechanism of COL11A1 resistance to chemotherapy in glioma cells, TMZ-sensitive U87 MG cells and TMZ-resistant T98G cells were selected for subsequent experiments. To examine whether COL11A1 expression affected the rates of invasion and chemotherapy resistance of glioma cells, we performed COL11A1 interfering sequences for gene knockdown in U87 MG and T98G cells. Our results showed that siRNA effectively reduced the expression of COL11A1 in two glioma cells. The knock-down of COL11A1 significantly inhibited the expression of MMP2, MMP3, and MMP9, whose high expression may be related to the risk of tumor migration, invasion, and metastasis. In addition, the expression of Snail decreased with the knock-down of COL11A1. Since U87 MG cells do not express MGMT, knocking down COL11A1 did not cause a change in the content of MGMT in U87 MG cells. In contrast, T98G cells highly express MGMT, and knocking down COL11A1 may effectively inhibit the expression of MGMT (Figure 1C, D). The results of a wound healing experiment showed that the migration ability of glioma cells decreased significantly after knocking down COL11A1 (Figure 1E). Similarly, transwell invasion assay findings indicated that the invasion ability was significantly reduced in knock-down COL11A1 glioma cells (Figure 1F). In addition, CCK8 results showed that TMZ had lower IC50 values for two glioma cells after than before the knock-down of COL11A1 (Figure 1G, H). These results indicate that COL11A1 expression is associated with cell migration, invasion ability and chemotherapy resistance of glioma cells.

3.2 Berberine suppresses proliferation, migration and invasion of glioma cells

Currently, drugs targeting COL11A1 are rarely studied. As a multi-target antitumor drug, whether berberine (Figure 2A) can inhibit COL11A1 is still unclear. Firstly, we examined the effect of berberine on proliferation, migration and invasion of glioma cells. After glioma cells were treated with different concentrations of berberine for 48h, we used the CCK8 kit to detect glioma cell viability. The results of CCK8 assay showed that berberine effectively inhibited the viability of U87 MG and T98G cells (Figure 2B, C). Next, we analyzed the influence of berberine on the rates of migration and invasion of glioma cells. The results of a wound healing experiment showed that berberine inhibited the migration ability of glioma cells in a concentration-dependent manner (Figure 2D-F). Transwell invasion assay confirmed that berberine inhibited the invasion ability of U87 MG and T98G cells with the increase of concentration (Figure 2G).

3.3 Berberine inhibits plasma membrane ruffles formation and changes actin cytoskeleton arrangement in glioma cells

Cancer cells rely on their actin cytoskeleton, and coordinate the movement of cancer cells by generating protrusions on the cell membrane, thus promoting the migration of cancer cells[25]. After the glioma cells were treated with berberine, the cells were observed under a light microscope. The glioma cells in the control group had many plasma membrane ruffles that resembled lamellipodia and/or filopodia-like structures. After berberine treatment, the number of plasma membrane ruffles of glioma cells was significantly reduced, and intracellular vacuoles appeared (Figure 3A). The actin cytoskeleton in U87 MG and T98G cells was stained with F-actin probe; actin in the glioma cells of the control group was arranged in a parallel manner, indicating that it was more invasive. Actin in the berberine-treated glioma cells was distributed sporadically or arranged in a circular pattern (Figure 3B). These findings suggest that berberine may effectively affect plasma membrane ruffles formation and changes actin cytoskeleton arrangement to reduce the migration and invasion ability of glioma cells.

3.4 Berberine inhibits TGF- β 1/COL11A1/MMP axis in glioma cells

To further investigate the potential molecular mechanism of berberine in inhibiting the migration and invasion of glioma cells, western blot assay was performed to examine protein changes in berberine-treated glioma cells. The results showed that berberine effectively inhibited the expression of COL11A1 in U87 MG and T98G cells (Figure 4A-D). Previous studies have shown that TGF- β 1 could activate COL11A1 to promote the invasion of ovarian cancer cells[26]. Meanwhile, Berberine inhibited the expression of TGF- β 1. In addition, berberine inhibited the expression of a variety of matrix metalloproteinases (MMPs)–downstream of COL11A1, including MMP2, MMP3, and MMP9, thereby reducing the invasion of glioma cells (Figure 4A, B). However, the analysis of TGF- β 1 content in the supernatant of cell cultures with an enzyme linked immunosorbent assay kit revealed that berberine had no significant effect on TGF- β 1 content in U87 MG cell culture medium; however, berberine decreased the TGF- β 1 content in T98G cell culture medium (Figure 4E, F). U87 MG cells have been shown to produce considerable TGF- β 1 RNA, but to secrete relatively small amounts of TGF- β 1 into the medium. Increased expression of endogenous TGF- β 1 inhibits U87 MG cell growth in vivo, that is, endogenous TGF- β 1 inhibits U87 MG growth in an autocrine manner[27]. Our results showed that berberine did not reduce TGF- β 1 secretion in U87 MG cell culture medium, and maintained a relatively certain level of endogenous TGF- β 1 inhibiting the growth of U87 MG cells.

3.5 Exogenous TGF- β 1 reversed the effect of berberine on inhibiting the migration and invasion of glioma cells

To verify the effect of exogenous TGF- β 1 on migration and invasion of glioma cells treated with berberine, we treated glioma cells with TGF- β 1 and berberine simultaneously. After adding exogenous TGF- β 1, we found that exogenous TGF- β 1 could enhance the migration ability of U87 MG and T98G cells and reverse the effect of berberine on inhibiting the migration ability of glioma cells (Figure 5A-C). TGF- β 1 also reversed the effect of berberine on inhibiting the invasion of glioma cells (Figure 5D). In addition, western blot assay showed that TGF- β 1 could promote the expression of COL11A1. After treatment of

glioma cells with berberine and TGF- β 1 at the same time, the inhibitory effect of berberine on COL11A1 expression was weakened. Exogenous TGF- β 1 also promoted the expression of Snail, MMP2, MMP3, and MMP9 (Figure 5E, F). These results indicate that TGF- β 1 could reverse the inhibition of migration and invasion caused by berberine-induced suppression of COL11A1 in glioma cells.

3.6 Berberine increases the sensitivity of glioma cells to TMZ

To verify whether berberine could increase the therapeutic effect of TMZ on glioma cells, we used berberine and TMZ to treat glioma cells simultaneously. After treating glioma cells with 10 mg/L and 25 mg/L berberine in combination with TMZ, we found that berberine enhanced the sensitivity of U87 MG and T98G cells to TMZ (Figure 6A, B). Cell counting under a fluorescence microscope revealed that berberine may reduce chemoresistance of glioma cells (Figure 6C). Western blot assay showed that berberine inhibited the expression of MGMT and Snail in T98G cells, thereby increasing the sensitivity of T98G cells to TMZ. Since U87 MG cells did not express MGMT, the mechanism of berberine increasing the sensitivity of U87 MG cells to TMZ may be associated with berberine inhibiting Snail expression (Figure 6D, E).

3.7 Berberine inhibits glioma growth and reduces COL11A1 level in mouse xenografts

To examine the effect of berberine in inhibiting glioma *in vivo*, U87MG cells were used for subcutaneous xenografts in nude mice. The total RNA was extracted from the tissues 2 weeks after the intraperitoneal injection of berberine, which was used for RNA sequencing to observe changes to the tumor-related genes. Berberine (5 mg/kg) effectively inhibited the growth of glioma *in vivo*, compared with that observed in the control group (Figure 7A). After berberine treatment, the weight of tumor was significantly reduced (Figure 7B); however, berberine had no significant effect on the weight of mice (Figure 7C). The effect of berberine on reducing the tumor volume increased with the duration of berberine administration (Figure 7D). Similarly, in the berberine-treated gliomas, expression levels of 42 and 31 genes were up and down-regulated, respectively, and the levels of ECM-related proteins, including COL11A1, were significantly decreased, compared to those observed in the control group (Figure 7E). Hematoxylin and eosin staining showed that the arrangement of cells in berberine-treated tumors was looser, and the vacuoles in cells and intercellular spaces were larger than those observed in the control group. Immunohistochemistry findings revealed that berberine effectively inhibited the expression of Ki-67 in glioma cells (Figure 7F). Immunofluorescence findings showed that the content of COL11A1 in the xenografts was significantly reduced after berberine treatment (Figure 7G).

4. Discussion

In this study, we demonstrated that COL11A1 is highly expressed in gliomas and associated with migration, invasion and chemoresistance of glioma cells. Knocking-down COL11A1 could effectively reduce the migration and invasion ability of glioma cells and increase the sensitivity of glioma cells to TMZ. In the present study, we also proved that knocking-down COL11A1 significantly inhibited the expression of MGMT, Snail, MMP2, MMP3, and MMP9. Moreover, we found that berberine inhibited the migration and invasion of U87 MG and T98G cells *in vitro*, reducing the apophysis of the plasma membrane ruffles and changing the arrangement of actin cytoskeleton. Western blot assay findings revealed that berberine inhibited the migration and invasion of glioma cells by inhibiting the TGF- β 1/COL11A1/Snail/MMP axis. The addition of exogenous TGF- β 1 could reverse the antitumor effect of berberine. In addition, berberine increased the chemosensitivity of glioma cells to TMZ by inhibiting Snail and MGMT expression. Finally, berberine inhibits glioma growth *in vivo*. RNA sequencing of the tumor tissue showed that berberine inhibited the expression of COL11A1 in glioma (Figure 8).

The ECM interacts with cancer cells, and regulates many biological behaviors of cancer cells, including their proliferation, metabolism, invasion, chemoresistance, and angiogenesis[28]. As a minor fibrillar collagen, COL11A1 is highly expressed in a variety of cancers, where it is associated with poor prognosis[14, 15]. COL11A1 may promote the proliferation of pancreatic cancer cells and inhibit their apoptosis by promoting the phosphorylation of AKT^{ser437}. The COL11A1/AKT axis changed the balance between BCL-2 and BAX, induced the destruction of mitochondrial transmembrane function, and inhibited the release of cytochrome C, thereby promoting apoptosis escape and chemotherapy resistance of pancreatic cancer cells[13]. In ovarian cancer, COL11A1 promoted fatty acid β -oxidation by activating Src-Akt-AMPK signaling pathway, up-regulated fatty acid metabolism, and promoted cisplatin resistance in ovarian cancer[9]. In another study, the c/EBP β binding site in the COL11A1 promoter was identified as the primary factor for cisplatin-and paclitaxel-induced overexpression of COL11A1[29]. In addition, COL11A1 was found to up-regulate MMP3 and to promote tumor progression[8]. COL11A1 also promoted Twist1 activation by activating NF- κ B, which in turn promoted chemoresistance in ovarian cancer cells[30]. AKT and TGF- β 1 have been shown to be upstream molecules of COL11A1 that promote tumor progression by promoting COL11A1 expression[26, 29]. Some AKT inhibitors and TGF- β 1 inhibitors may reverse COL11A1-induced resistance to chemotherapy[31, 32].

Several recent studies have examined the role of TGF- β 1 signaling pathway in cancer. TGF- β 1 is a powerful cytokine produced by cancer cells that affects the expression of genes involved in ECM remodeling and adhesion[33]. However, TGF- β 1 shows a dual effect in cancers. TGF- β 1 inhibits tumor progression in the early stage of cancer and exhibits pro-tumor effects in the late stage of cancer, promoting cancer migration, invasion, and metastasis[34]. TGF- β 1 may be involved in the progression of renal and ovarian cancer by promoting the expression of COL11A1[26, 33]. Our study has found that TGF- β 1 promoted the expression of COL11A1 in gliomas and thus promoted the migration and invasion of glioma cells. Meanwhile, another study has shown that TGF- β 1 secreted by U87 MG cells can inhibit cell growth *in vivo* in an autocrine manner[27]. This also explains that berberine had no effect on TGF- β 1 secreted by U87 MG, but reduced TGF- β 1 secretion by T98G cells. Notably, berberine inhibited the TGF- β 1

protein levels in U87 MG and T98G cells, while the addition of exogenous TGF- β 1 partially reversed the inhibition of berberine on COL11A1 and its downstream proteins.

At present, TMZ is a commonly used chemotherapeutic drug for the treatment of glioblastoma; however, chemotherapy resistance limits its efficacy. Increasing the sensitivity of glioma to TMZ may improve patient prognosis[1]. MGMT may contribute to cancer TMZ chemotherapy resistance. The methylation level of the MGMT promoter may be a biomarker of glioma recurrence risk[35]. A recent study has shown that the expression of Snail gene inhibited TMZ-induced apoptosis and increased the migration and invasion ability of glioma cells[36]. This evidence suggests that Snail is one of the key molecules for TMZ chemotherapy resistance in glioma cells. The present study has shown that COL11A1 promoted the expression of Snail and thus promoted invasion and chemotherapy resistance of glioma cells. Berberine may inhibit the migration and invasion ability of glioma cells by inhibiting the TGF- β 1/COL11A1/Snail/MMP axis. In U87 MG cells, berberine's inhibition of snail expression may increase the sensitivity of U87 MG cells to TMZ chemotherapy due to the lack of MGMT expression. In T98G cells, berberine inhibited the expression of both MGMT and Snail, thereby reducing chemotherapy resistance.

5. Conclusion

In summary, the present study has demonstrated that high expression of COL11A1 is a risk factor for poor prognosis of gliomas. COL11A1 is an upstream molecule of Snail, MMP2, MMP3, and MMP9. Berberine may reduce the migration and invasion ability of glioma cells by inhibiting TGF- β 1/COL11A1 pathway; it may also improve the sensitivity of glioma cells to TMZ chemotherapy by inhibiting MGMT and Snail. Collectively, berberine may be a candidate drug for the treatment of COL11A1 high-expression glioma.

List Of Abbreviations

BBR	Berberine
COL11A1	Collagen XI alpha1 chain
GBM	Glioblastoma
TMZ	Temozolomide
MGMT	O-6-methylguanine-DNA methyltransferase
ECM	Extracellular matrix
HUVEC	Human umbilical vein endothelial cell
ATCC	American type culture collection
FBS	Fetal bovine serum
CCK8	Cell counting kit-8
OD	Optical density
PBS	Phosphate saline
ELISA	Enzyme linked immunosorbent assay
FC	Fold change
FDR	False discovery rate
MMPs	Matrix metalloproteinases

Declarations

Ethics approval and consent to participate: The animal experimental was approved by the Animal Ethics Committee of the First Hospital of Jilin University (No. 20210547).

Consent for publication: Not applicable.

Availability of data and materials: Not applicable.

Competing interests: The authors declare that they have no conflict of interest.

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Authors' contributions: Yuxue Sun, Yong Chen and Ziqiang Liu designed the research; Yuxue Sun, Haiyan Huang, Zhixin Zhan, Haijun Gao, Chaochao Zhang, Jiacheng Lai, Junguo Cao and Ziqiang Liu performed

the research; Yuxue Sun, Chaoyue Li analyzed the data; Yuxue Sun and Haiyan Huang wrote the manuscript; Yong Chen and Ziqiang Liu revised the manuscript.

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References

1. Zhang L, He A, Chen B, Bi J, Chen J, Guo D, *et al.* A HOTAIR regulatory element modulates glioma cell sensitivity to temozolomide through long-range regulation of multiple target genes. *Genome Res.* 2020; 30: 155–163.
2. Chen R, Smith-Cohn M, Cohen AL, Colman H. Glioma Subclassifications and Their Clinical Significance. *Neurotherapeutics.* 2017; 14: 284–297.
3. Nejo T, Yamamichi A, Almeida ND, Goretsky YE, Okada H. Tumor antigens in glioma. *Semin Immunol.* 2020; 47: 101385.
4. Wick W, Weller M, van den Bent M, Sanson M, Weiler M, von Deimling A, *et al.* MGMT testing—the challenges for biomarker-based glioma treatment. *Nat Rev Neurol.* 2014; 10: 372–385.
5. Warren KE, Gururangan S, Geyer JR, McLendon RE, Poussaint TY, Wallace D, *et al.* A phase II study of O6-benzylguanine and temozolomide in pediatric patients with recurrent or progressive high-grade gliomas and brainstem gliomas: a Pediatric Brain Tumor Consortium study. *J Neurooncol.* 2012; 106: 643–649.
6. Xu S, Xu H, Wang W, Li S, Li H, Li T, *et al.* The role of collagen in cancer: from bench to bedside. *J Transl Med.* 2019; 17: 309.
7. Vazquez-Villa F, Garcia-Ocana M, Galvan JA, Garcia-Martinez J, Garcia-Pravia C, Menendez-Rodriguez P, *et al.* COL11A1/(pro)collagen 11A1 expression is a remarkable biomarker of human invasive carcinoma-associated stromal cells and carcinoma progression. *Tumour Biol.* 2015; 36: 2213–2222.
8. Wu YH, Chang TH, Huang YF, Huang HD, Chou CY. COL11A1 promotes tumor progression and predicts poor clinical outcome in ovarian cancer. *Oncogene.* 2014; 33: 3432–3440.
9. Nallanthighal S, Rada M, Heiserman JP, Cha J, Sage J, Zhou B, *et al.* Inhibition of collagen XI alpha 1-induced fatty acid oxidation triggers apoptotic cell death in cisplatin-resistant ovarian cancer. *Cell Death Dis.* 2020; 11: 258.
10. Toss MS, Miligy IM, Gorringer KL, Aleskandarany MA, Alkawaz A, Mittal K, *et al.* Collagen (XI) alpha-1 chain is an independent prognostic factor in breast ductal carcinoma in situ. *Mod Pathol.* 2019; 32: 1460–1472.
11. Galvan JA, Garcia-Martinez J, Vazquez-Villa F, Garcia-Ocana M, Garcia-Pravia C, Menendez-Rodriguez P, *et al.* Validation of COL11A1/procollagen 11A1 expression in TGF-beta1-activated immortalised human mesenchymal cells and in stromal cells of human colon adenocarcinoma. *BMC Cancer.* 2014; 14: 867.

12. Shen L, Yang M, Lin Q, Zhang Z, Zhu B, Miao C. COL11A1 is overexpressed in recurrent non-small cell lung cancer and promotes cell proliferation, migration, invasion and drug resistance. *Oncol Rep.* 2016; 36: 877–885.
13. Wang H, Ren R, Yang Z, Cai J, Du S, Shen X. The COL11A1/Akt/CREB signaling axis enables mitochondrial-mediated apoptotic evasion to promote chemoresistance in pancreatic cancer cells through modulating BAX/BCL-2 function. *J Cancer.* 2021; 12: 1406–1420.
14. Liu Z, Lai J, Jiang H, Ma C, Huang H. Collagen XI alpha 1 chain, a potential therapeutic target for cancer. *FASEB J.* 2021; 35: e21603.
15. Raglow Z, Thomas SM. Tumor matrix protein collagen XIalpha1 in cancer. *Cancer Lett.* 2015; 357: 448–453.
16. Rada M, Nallanthighal S, Cha J, Ryan K, Sage J, Eldred C, *et al.* Inhibitor of apoptosis proteins (IAPs) mediate collagen type XI alpha 1-driven cisplatin resistance in ovarian cancer. *Oncogene.* 2018; 37: 4809–4820.
17. Kumar A, Ekavali, Chopra K, Mukherjee M, Pottabathini R, Dhull DK. Current knowledge and pharmacological profile of berberine: An update. *Eur J Pharmacol.* 2015; 761: 288–297.
18. Cicero AF, Baggioni A. Berberine and Its Role in Chronic Disease. *Adv Exp Med Biol.* 2016; 928: 27–45.
19. Yun D, Yoon SY, Park SJ, Park YJ. The Anticancer Effect of Natural Plant Alkaloid Isoquinolines. *Int J Mol Sci.* 2021; 22.
20. Sun Y, Yu J, Liu X, Zhang C, Cao J, Li G, *et al.* Oncosis-like cell death is induced by berberine through ERK1/2-mediated impairment of mitochondrial aerobic respiration in gliomas. *Biomed Pharmacother.* 2018; 102: 699–710.
21. Liu Z, Chen Y, Gao H, Xu W, Zhang C, Lai J, *et al.* Berberine Inhibits Cell Proliferation by Interfering with Wild-Type and Mutant P53 in Human Glioma Cells. *Onco Targets Ther.* 2020; 13: 12151–12162.
22. Ni WJ, Ding HH, Zhou H, Qiu YY, Tang LQ. Renoprotective effects of berberine through regulation of the MMPs/TIMPs system in streptozocin-induced diabetic nephropathy in rats. *Eur J Pharmacol.* 2015; 764: 448–456.
23. Yue M, Tao Y, Fang Y, Lian X, Zhang Q, Xia Y, *et al.* The gut microbiota modulator berberine ameliorates collagen-induced arthritis in rats by facilitating the generation of butyrate and adjusting the intestinal hypoxia and nitrate supply. *FASEB J.* 2019; 33: 12311–12323.
24. Chitra P, Saiprasad G, Manikandan R, Sudhandiran G. Berberine inhibits Smad and non-Smad signaling cascades and enhances autophagy against pulmonary fibrosis. *J Mol Med (Berl).* 2015; 93: 1015–1031.
25. Machesky LM. Lamellipodia and filopodia in metastasis and invasion. *FEBS Lett.* 2008; 582: 2102–2111.
26. Cheon DJ, Tong Y, Sim MS, Dering J, Berel D, Cui X, *et al.* A collagen-remodeling gene signature regulated by TGF-beta signaling is associated with metastasis and poor survival in serous ovarian cancer. *Clin Cancer Res.* 2014; 20: 711–723.

27. Pan JJ, Chang WJ, Barone TA, Plunkett RJ, Ostrow PT, Greenberg SJ. Increased expression of TGF-beta1 reduces tumor growth of human U-87 Glioblastoma Cells in vivo. *Cancer Immunol Immunother.* 2006; 55: 918–927.
28. Nissen NI, Karsdal M, Willumsen N. Collagens and Cancer associated fibroblasts in the reactive stroma and its relation to Cancer biology. *J Exp Clin Cancer Res.* 2019; 38: 115.
29. Wu YH, Chang TH, Huang YF, Chen CC, Chou CY. COL11A1 confers chemoresistance on ovarian cancer cells through the activation of Akt/c/EBPbeta pathway and PDK1 stabilization. *Oncotarget.* 2015; 6: 23748–23763.
30. Wu YH, Huang YF, Chang TH, Chou CY. Activation of TWIST1 by COL11A1 promotes chemoresistance and inhibits apoptosis in ovarian cancer cells by modulating NF-kappaB-mediated IKKbeta expression. *Int J Cancer.* 2017; 141: 2305–2317.
31. Wu YH, Huang YF, Chen CC, Chou CY. Akt inhibitor SC66 promotes cell sensitivity to cisplatin in chemoresistant ovarian cancer cells through inhibition of COL11A1 expression. *Cell Death Dis.* 2019; 10: 322.
32. Zhang Q, Hou X, Evans BJ, VanBlaricom JL, Weroha SJ, Cliby WA. LY2157299 Monohydrate, a TGF-betaR1 Inhibitor, Suppresses Tumor Growth and Ascites Development in Ovarian Cancer. *Cancers (Basel).* 2018; 10.
33. Boguslawska J, Rodzik K, Poplawski P, Kedzierska H, Rybicka B, Sokol E, *et al.* TGF-beta1 targets a microRNA network that regulates cellular adhesion and migration in renal cancer. *Cancer Lett.* 2018; 412: 155–169.
34. Colak S, Ten Dijke P. Targeting TGF-beta Signaling in Cancer. *Trends Cancer.* 2017; 3: 56–71.
35. Mathur R, Zhang Y, Grimmer MR, Hong C, Zhang M, Bollam S, *et al.* MGMT promoter methylation level in newly diagnosed low-grade glioma is a predictor of hypermutation at recurrence. *Neuro Oncol.* 2020; 22: 1580–1590.
36. Liang H, Chen G, Li J, Yang F. Snail expression contributes to temozolomide resistance in glioblastoma. *Am J Transl Res.* 2019; 11: 4277–4289.

Figures

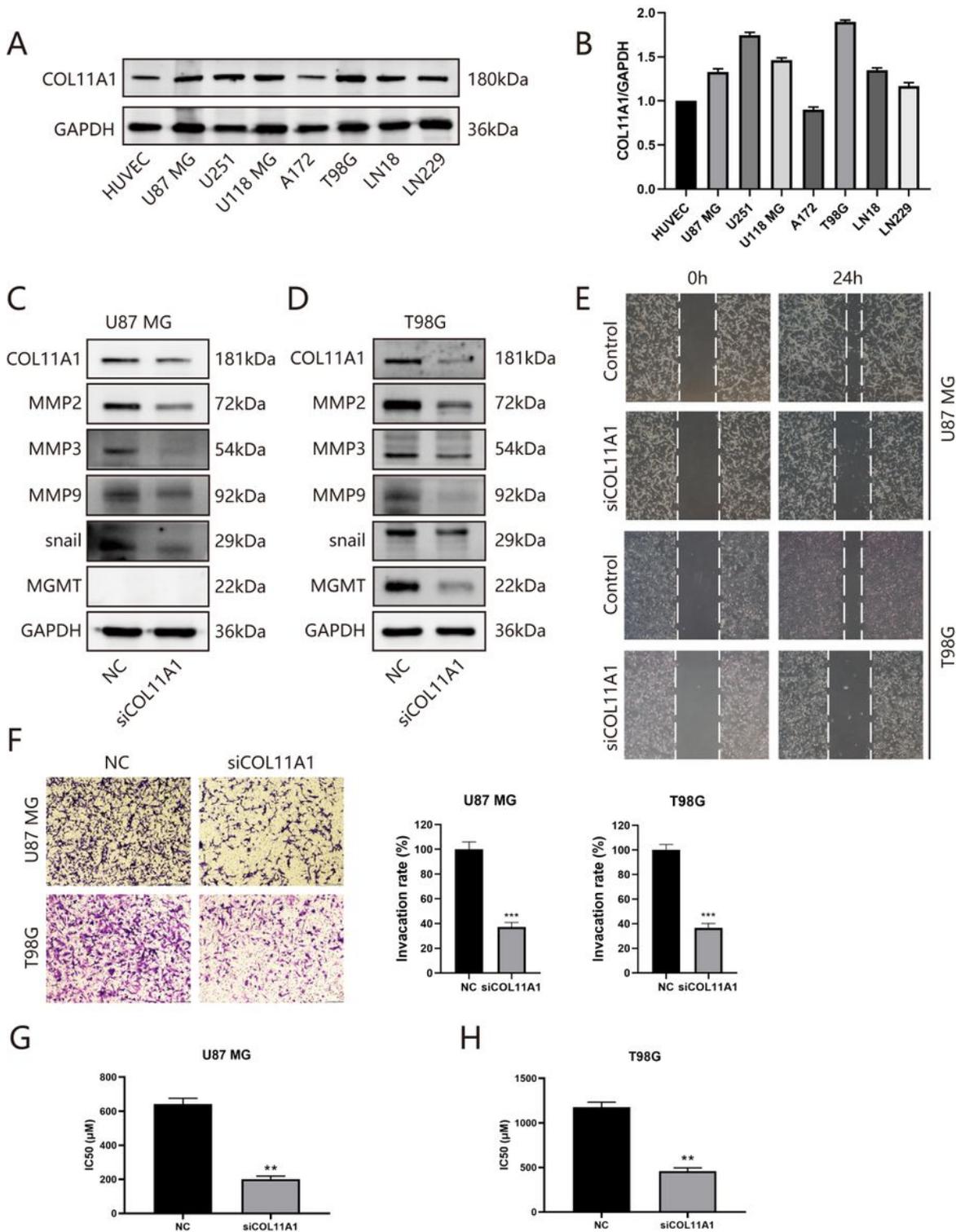


Figure 1

COL11A1 is associated with migration, invasion and chemoresistance of glioma cells A,B. Expression levels of COL11A1 in several glioma cells and HUVEC. C,D. Western blot showed that after the knock-down of COL11A1, the expression levels of MMP2, MMP3, MMP9 and snail in U87 MG and T98G cells were decreased, while the expression of MGMT in T98G cells was decreased, with GAPDH was used as a loading control. E,F. Wound Healing Assay and transwell experiments showed that knocking down

COL11A1 decreased the migration and invasion ability of U87 MG and T98G cells. G,H. Knocking down COL11A1 reduced the IC50 value for TMZ. **p < 0.01, ***p < 0.001.

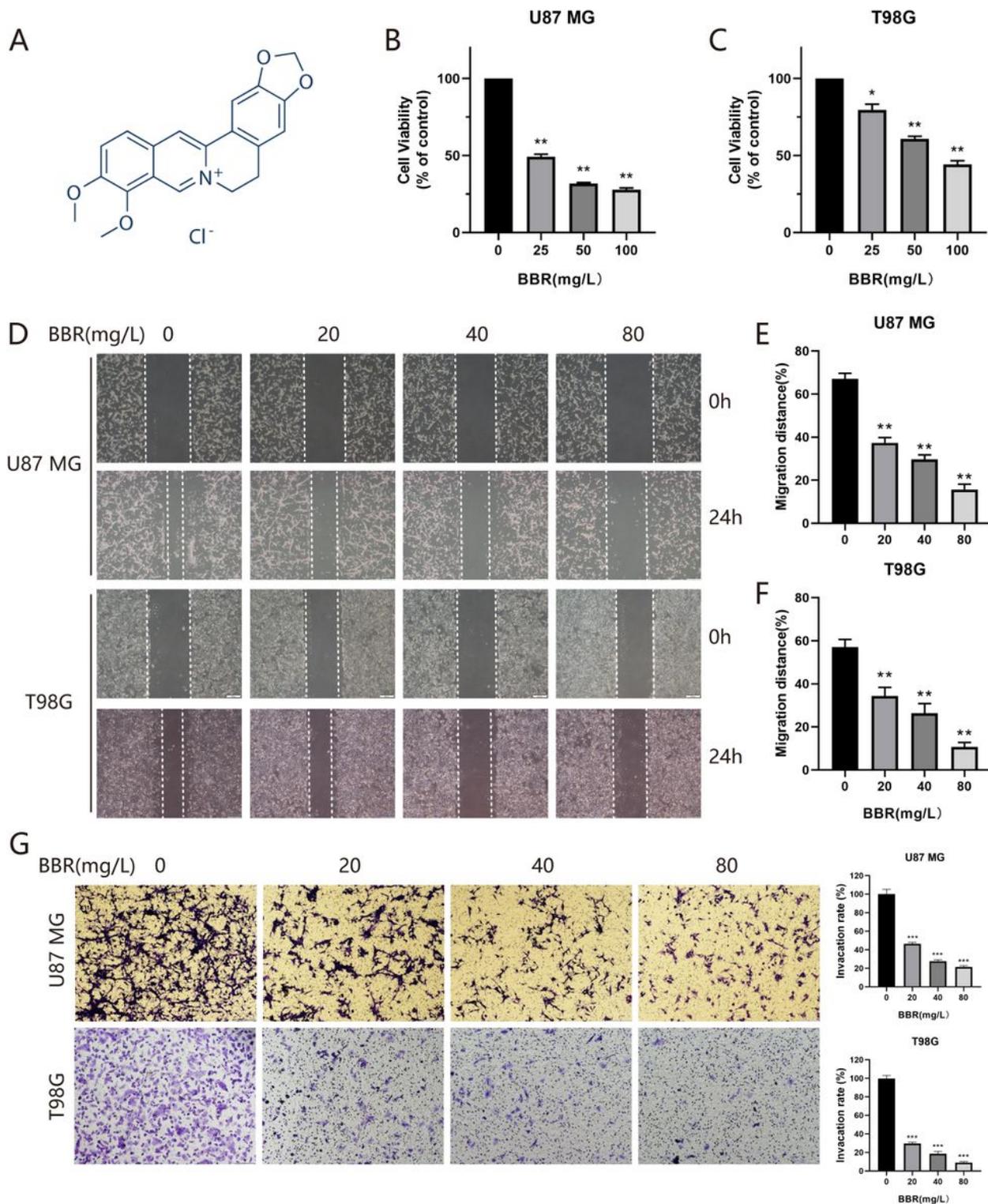


Figure 2

Berberine inhibited migration and invasion of glioma cells. A. Chemical structural formula of berberine. B,C. CCK8 experiment showed that berberine inhibited the proliferation of glioma cells. D-F. Berberine

inhibit that migration of glioma cells. G. Berberine reduced the invasion ability of glioma cells. ** $p < 0.01$, *** $p < 0.001$.

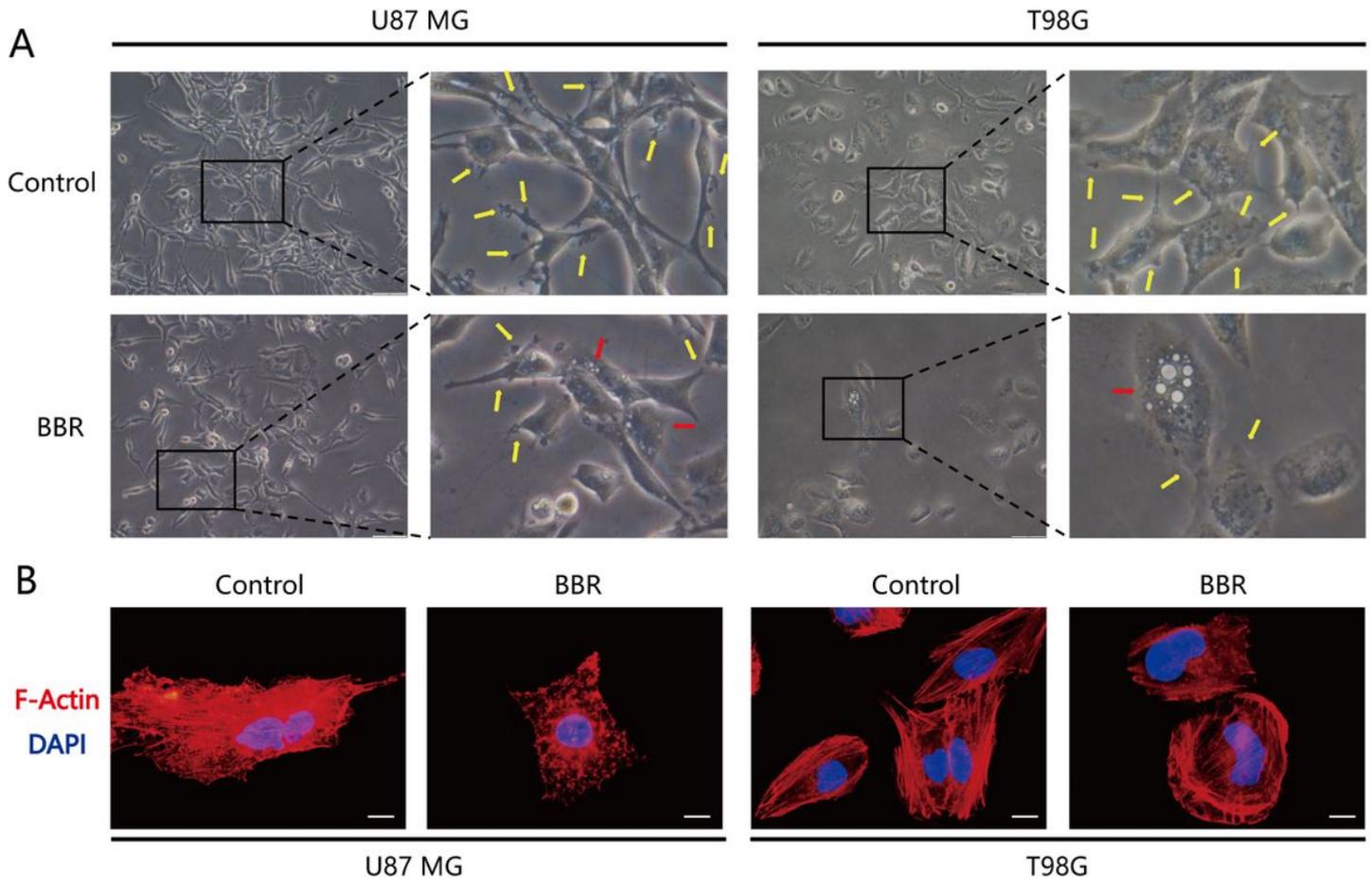


Figure 3

Berberine inhibits plasma membrane ruffles formation and changes actin cytoskeleton arrangement in glioma cells

A. Morphological changes of glioma cells treated with berberine under the microscope. B. The F-actin probe was used to stain the actin cytoskeleton of U87 MG and T98G cells, and berberine was shown to affect the cytoskeleton of glioma cells.

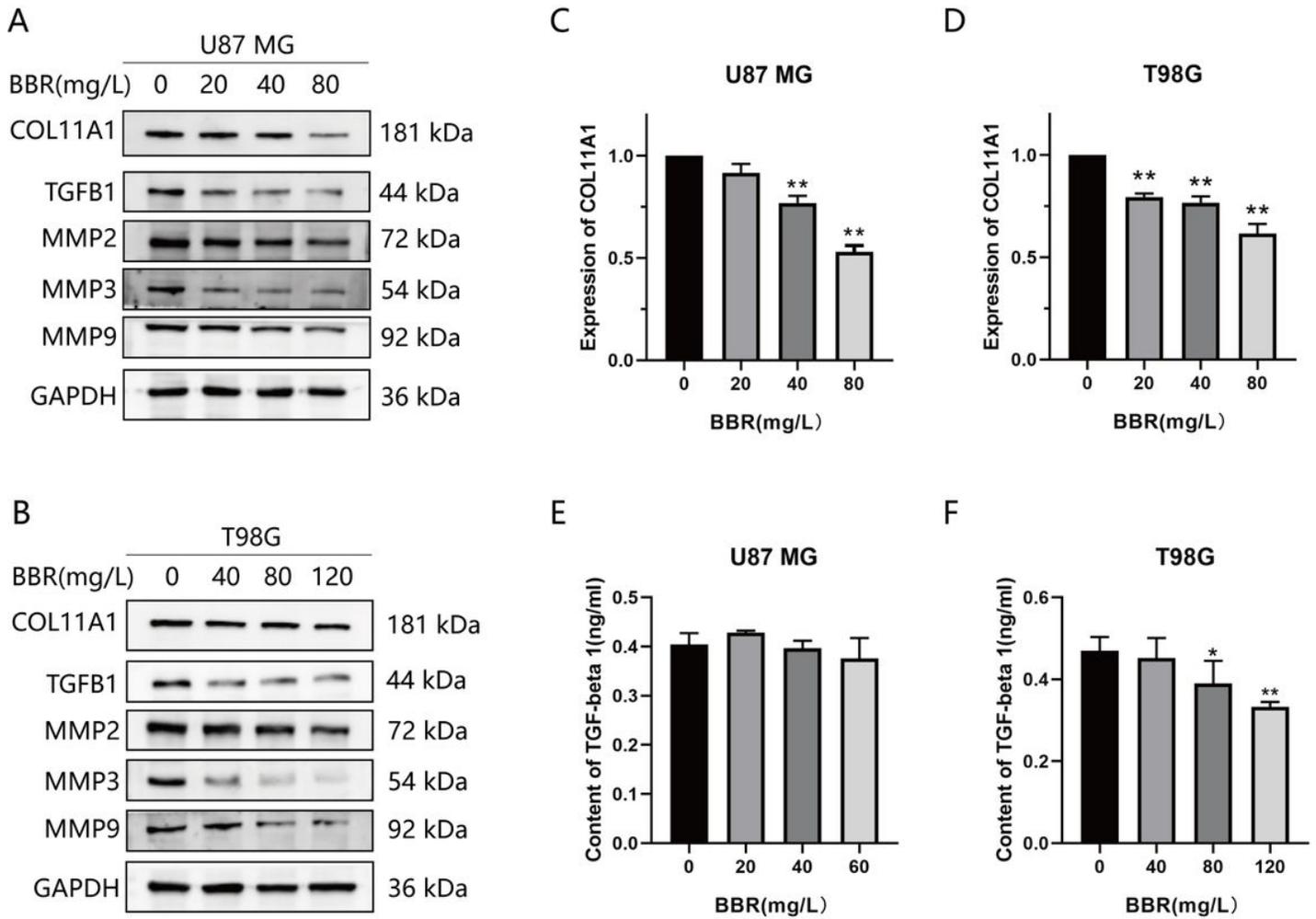


Figure 4

Berberine inhibited TGF- β 1/COL11A1/MMPs axis in glioma cells. A,B. Western blot analysis showed that berberine inhibited the expression of TGF- β 1, COL11A1, MMP2, MMP3, and MMP9 proteins in glioma cells. GAPDH was used as a loading control. C,D. Gray scale analysis of COL11A1 protein content after berberine treatment of U87 MG and T98G cells. E,F. After berberine treatment for 48h, the content of TGF- β 1 in the culture medium was detected by ELISA kit. * $p < 0.05$, ** $p < 0.01$.

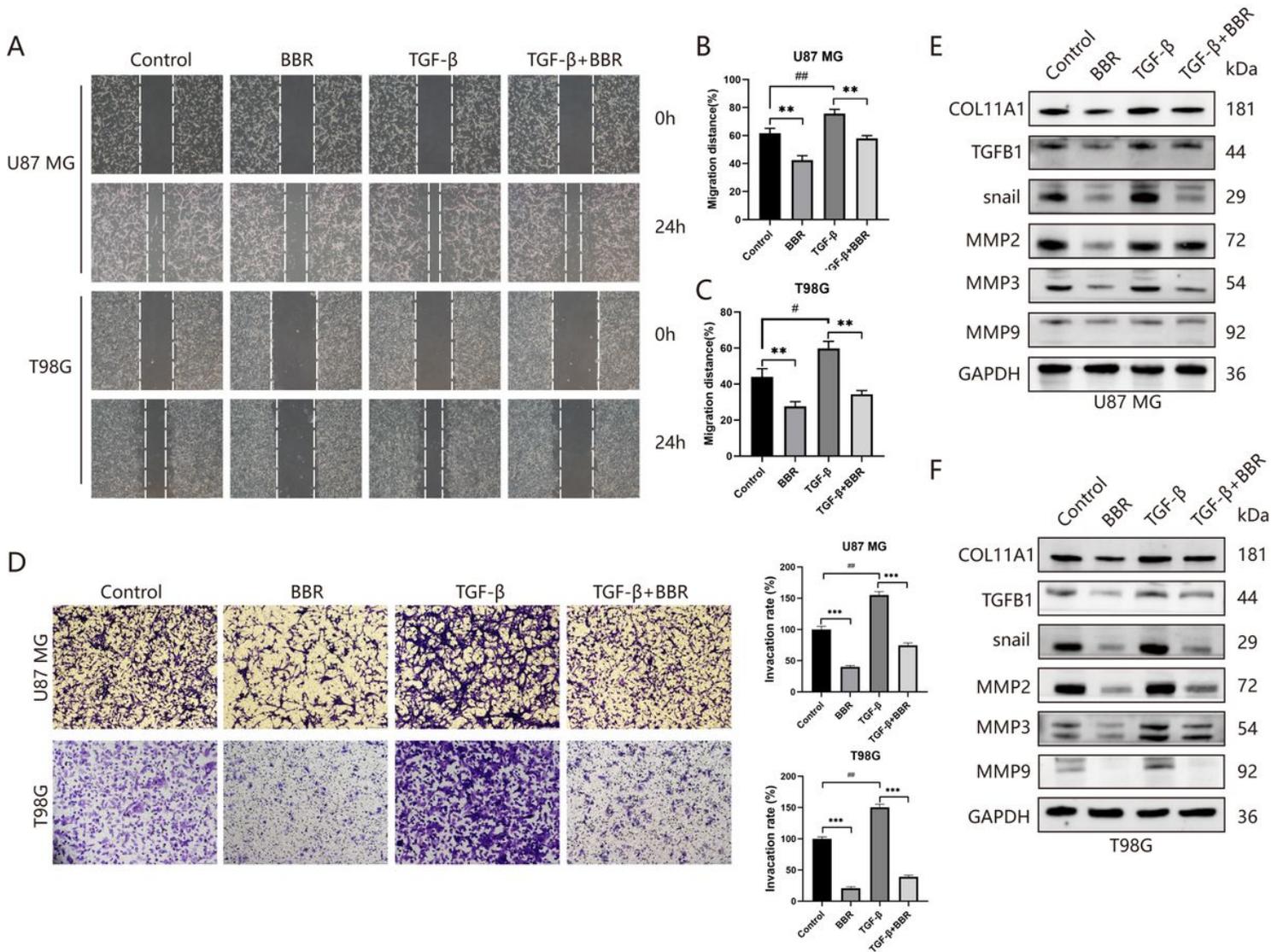


Figure 5

TGF- β 1 reversed the effect of berberine on inhibiting the migration and invasion of glioma cells. A-C, Migration of glioma cells after treatment with TGF- β 1 and berberine alone and treatment with TGF- β 1 and berberine together for 24 h. TGF- β 1 promoted the migration of glioma cells, while berberine inhibited the migration of glioma cells. After TGF- β 1 and berberine were added together, the ability of berberine to inhibit the migration of glioma was decreased. #p < 0.05, ##p < 0.01, **p < 0.01. D. Transwell experiments showed that TGF- β 1 reduced the ability of berberine to inhibit the invasion of glioma cells. ##p < 0.01, ***p < 0.001. E, F. Western blot showed that TGF- β 1 promoted the expression of COL11A1 and downstream proteins. GAPDH was used as a loading control.

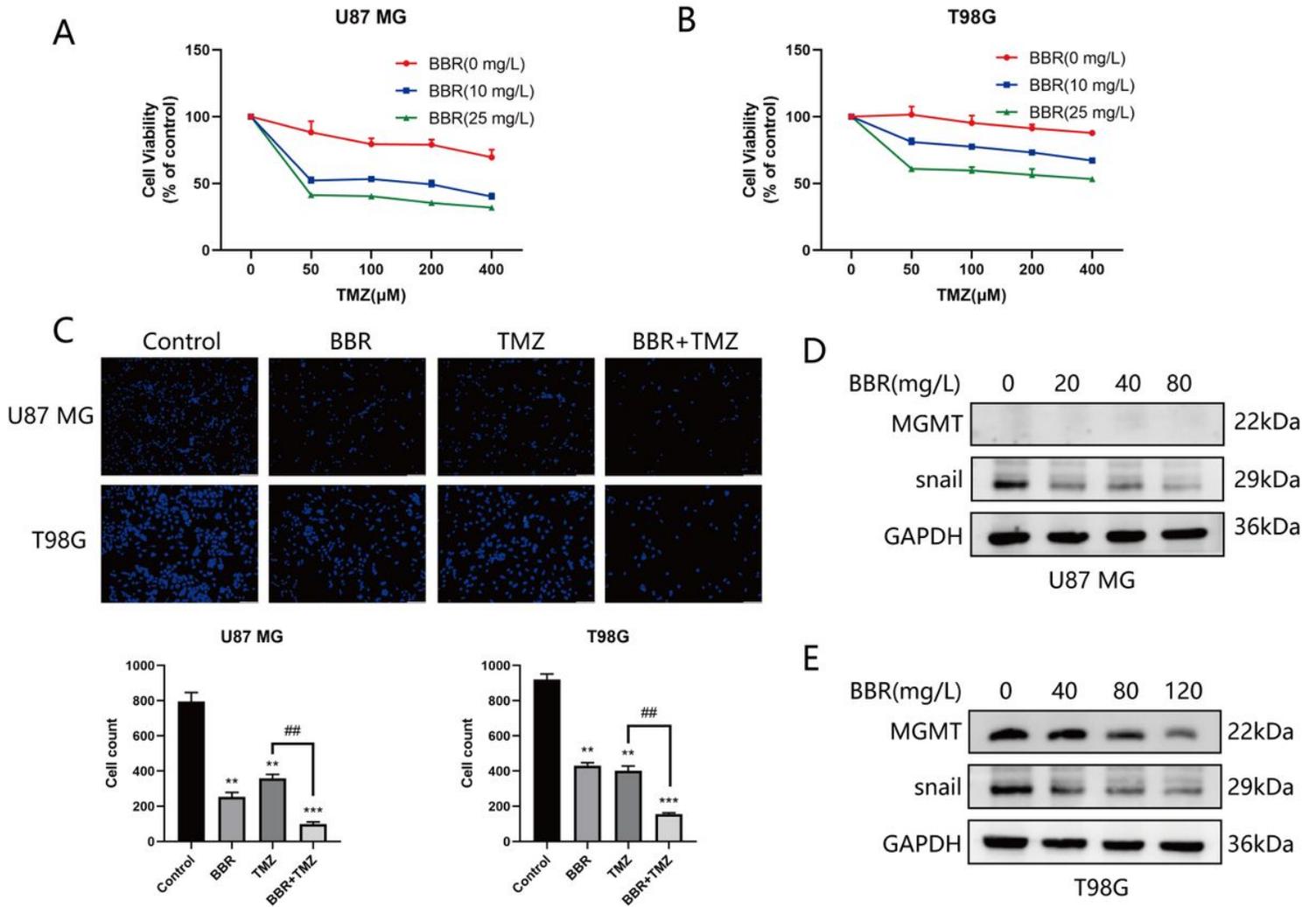


Figure 6

Berberine increased the sensitivity of glioma cells to TMZ. A,B. After the combination of berberine and TMZ, CCK8 kit was used to detect the cell viability of glioma cells. C. Glioma cells were counted under fluorescence microscope after DAPI staining, and the number of glioma cells was significantly reduced after the combination of berberine and TMZ. D. Berberine inhibited the expression of snail in TMZ-sensitive U87 MG cells and had no effect on MGMT protein. GAPDH was used as a loading control. E. Berberine inhibits the expression of snail and MGMT proteins in TMZ-resistant T98G cells, with GAPDH was used as a loading control. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ## $p < 0.01$.

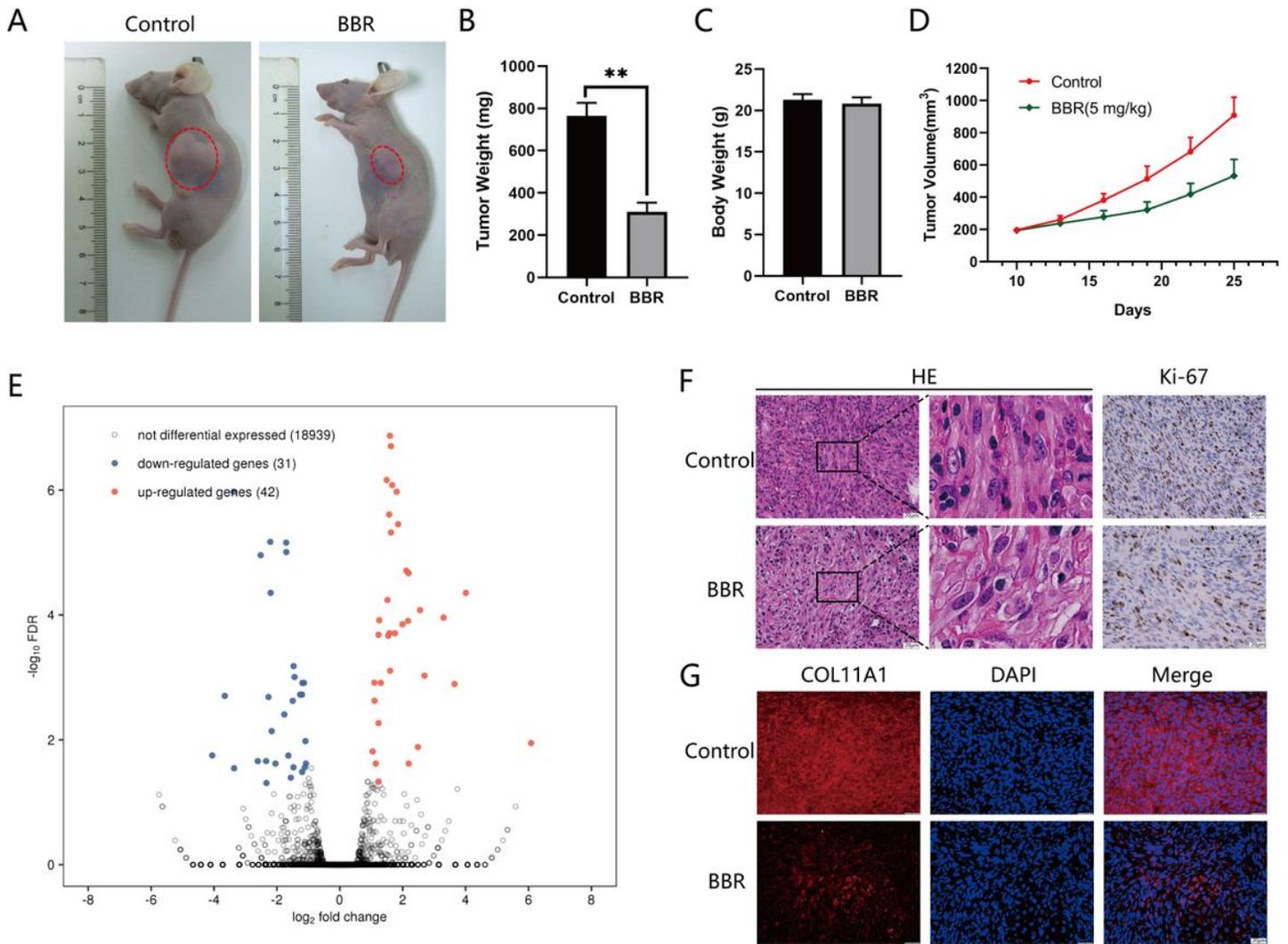


Figure 7

Berberine inhibited glioma growth in vivo. A,B. Berberine inhibited the growth of xenografts and reduced tumor weight in nude mice. C. Body weights of nude mice in the control group and berberine treatment group. D. Tumor volume changes of nude mice in control group and berberine treatment group. E. RNA sequencing showed the difference of gene expression in tumor after berberine treatment. The abscissa indicated the multiple of expression change (log value based on 2), and the ordinate indicated the statistical significance of expression change difference (log value based on 10). F. HE staining showed that berberine changed the arrangement of tumor cells, and immunohistochemistry showed that berberine reduced the expression of Ki-67 in tumors. H. Immunofluorescence showed that berberine inhibited the expression of COL11A1 in tumors. ** $p < 0.01$.

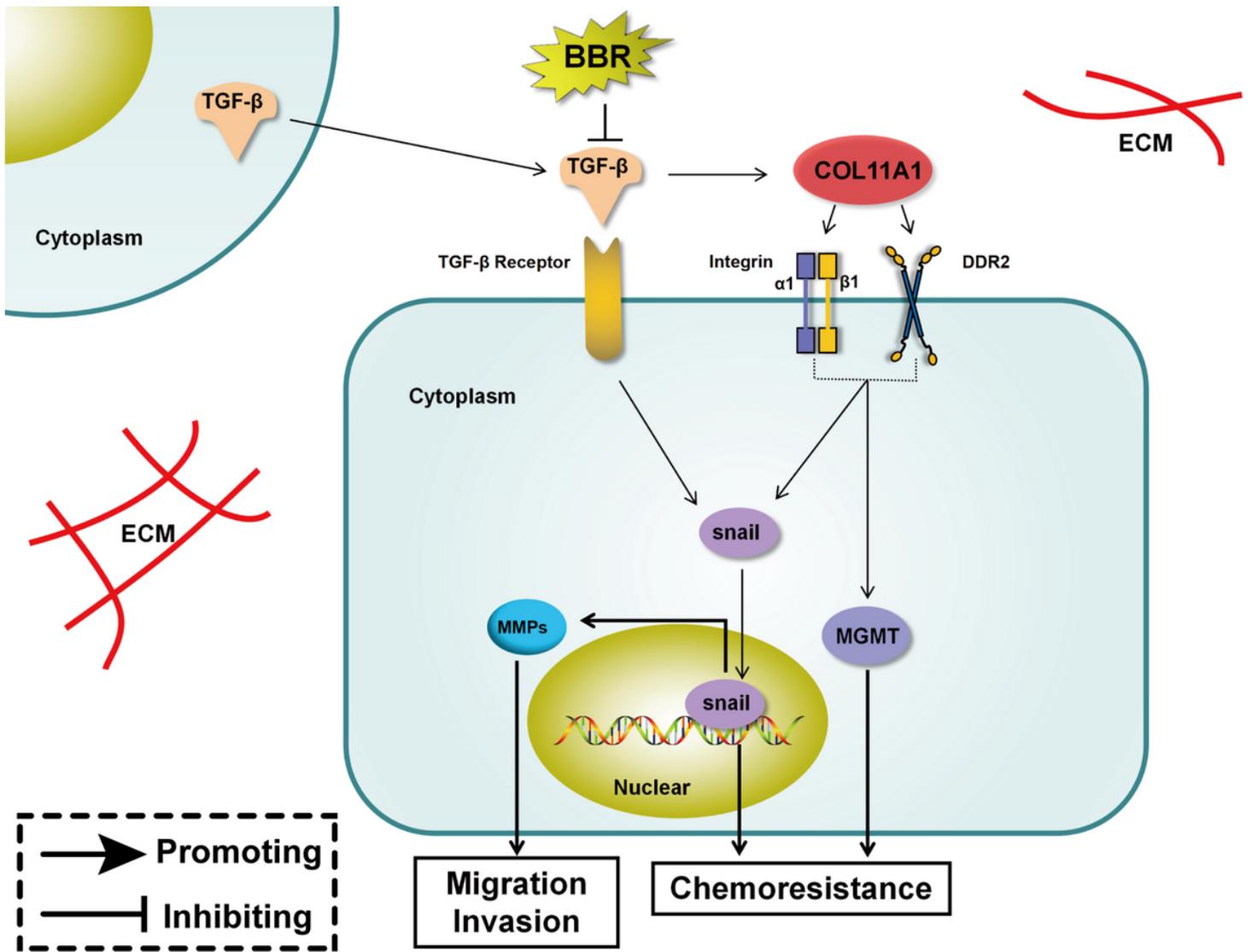


Figure 8

A model to explain the hypothetical effect of berberine on inhibiting the migration, invasion and chemotherapy resistance of glioma cells.