INTRODUCTION

Glioblastoma multiforme (GBM) is one of the most malignant human intracranial tumors. Temozolomide (TMZ) is the primary alkylating agent for GBM patients. However, many GBM patients are resistant to TMZ. Therefore, patients with GBM urgently need more effective therapeutic options. 20(S)-ginsenoside-Rg3 (20(S)-Rg3) is a natural chemical with anti-tumor effects, but at present there is little understanding of its functional mechanism. Several research reports have demonstrated that O6-methylguanine DNA-methyltransferase (MGMT) repairs damaged DNA and contributes to TMZ resistance in gliomas. In addition, recent studies have shown that MGMT gene expression could be regulated by the Wnt/β-catenin pathway. However, whether 20(S)-Rg3 inhibits MGMT expression and augments chemosensitivity to Temozolomide (TMZ) in glioma cells remains unclear. In this study, we explored the modulating effects of 20(S)-Rg3 on MGMT. We used glioma cell lines, primary cell strain (including T98G, U118 and GBM-XX; all of them are MGMT-positive glioma cell lines) and xenograft glioma models to examine whether 20(S)-Rg3 increased the sensitivity to TMZ and to reveal the underlying mechanisms. We found that the MGMT expression was effectively downregulated by 20(S)-Rg3 via the Wnt/β-catenin pathway in glioma cell lines, and TMZ resistance was significantly reversed by 20(S)-Rg3. Meanwhile, 20(S)-Rg3 shows no obvious cytotoxicity at its effective dose and is well tolerated in vivo. In addition, we found that 20(S)-Rg3 significantly restrains the epithelial-mesenchymal transition (EMT) progression of glioma cells. Taken together, these results indicate that 20(S)-Rg3 may be a novel agent to use in treatment of GBM, especially in TMZ-resistant GBM with high MGMT expression.

KEYWORDS

20(S)-ginsenoside-Rg3, glioblastoma multiforme, O6-methylguanine DNA-methyltransferase, temozolomide, Wnt/β-catenin pathway
a decade. Although current multimodal therapies have improved, the prognosis of GBM is still very poor, with a median survival of only 14.6 months. The rapid recurrence and TMZ resistance of glioma are the main difficulties in treatment. Therefore, it is critical to find new therapies for GBM treatment and to better understand the molecular mechanism of TMZ resistance. TMZ exerts antitumor effects by methylating the O6 position of guanine (O6-G) in DNA and causes base mispairing by continuously inducing O6-methylguanine adduct. It then induces cell cycle arrest and, ultimately, apoptosis. However, the DNA repair protein MGMT (O6-methylguanine-DNA-methyltransferase) reverses the mutagenic and lethal effects of TMZ by removing the methyl adducts from the O6 position of guanine; therefore, MGMT mediates the resistance of glioma cells to TMZ. Furthermore, clinical studies have demonstrated that patients with low expression of MGMT are more sensitive to TMZ chemotherapy, proving that the expression of MGMT remains a main cause of GBM’s chemotherapy resistance. Thus, reduction of MGMT expression in glioma cells may contribute to overcoming the resistance to TMZ.

20(S)-ginsenoside-Rg3 is a stereoisomer of 20(R)-ginsenoside-Rg3; they are the 2 stereoisomeric pairs of ginsenoside-Rg3 which is a tetracyclic triterpenoids saponins monomer, extracted and purified from Panax ginseng C. A. Meyer. Studies report that 20(S)-ginsenoside-Rg3 displays more effective clinical effects than 20(R), especially in the antitumor area. Several studies have shown that drug 20(S)-Rg3 is nontoxic and well-tolerated in organisms, including in mice, rats, dogs and humans. Referring to the pharmacokinetic characteristics of 20(S)-Rg3, Zhang et al. report that the drug concentration of 20(S)-Rg3 in the serum of mice with 40 mg/kg 20(S)-Rg3 administered by i.p. injection daily for 1 week was maintained at approximately 200 µM. Taken together, these reports indicate that 20(S)-Rg3 has great potential for clinical application. In our laboratory, we initially discovered that 20(S)-Rg3 can effectively down-regulate MGMT expression in the glioma cell line T98G. Therefore, our study mainly explored whether 20(S)-Rg3 plays a synergistic role in improving the effect of TMZ treatment in GBM. Herein, we show that 20(S)-Rg3 inhibits MGMT expression by modulating Wnt/β-catenin pathways and remarkably potentiates the sensitivity of glioma to TMZ chemotherapy. Meanwhile, we found that 20(S)-Rg3 significantly inhibits the process of epithelial mesenchymal transition in glioma cells.

2 | MATERIALS AND METHODS

2.1 | Reagents and chemicals

20(S)-ginsenoside-Rg3, molecular formula C42H66O13 and molecular weight 785.01 g/mol, was purchased from Shanghai Tauto Biotech (>98% purity; Shanghai, China). TMZ, molecular formula C16H12N2O2 and molecular weight 194.15 g/mol, was purchased from Sigma-Aldrich.

2.2 | GBM cell culture

The T98G and U118 GBM cell line were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). GBM-XX is a primary cell strain derived from the surgical specimen of a patient with World Health Organization grade IV GBM undergoing resection in accordance with a protocol approved by the Ethics Committee of our hospital and with prior informed consent from the patient. The culture medium was composed of DMEM (Life Technologies/Gibco, Carlsbad, CA, USA) and 10% FBS (Life Technologies/Gibco, Carlsbad, CA, USA), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco, Grand Island, NY, USA). The cells were cultured at a density of 1 × 105 cells/mL. These cells were incubated at 37°C with 5% CO2 and 100% humidity.

2.3 | Total RNA extraction, reverse transcription and qPCR

Total RNA was extracted from cell lines using TRIzol (TaKaRa, Dalian, China) according to the manufacturer’s protocol. For mRNA analysis, the Primer-Script one step RT-PCR kit (TaKaRa) was used to reverse transcribe RNA into cDNA. The cDNA templates were amplified by RT-PCR using the SYBR Premix Dimmer Eraser kit (TaKaRa); GAPDH was used as an internal control. The real-time PCR performed were performed in triplicate. The primer sequences used were as follows: for GAPDH, 5′-CTCAACGGATTTGGTCTGTATT-3′ (forward) and 5′-AGTCTCTGGGTGGGACAGTCT-3′ (reverse); for MGMT, 5′-GGGAATGAAGTAATG-3′ (reverse). The relative mRNA expression change was calculated using the 2−ΔΔCt method.

2.4 | Western blot

Western blot assay was carried out as previously described. Cells were washed with PBS and then lysed with RIPA lysis buffer (Solarbio, China) and protease inhibitors (Roche Applied Science, Indianapolis, Switzerland). The protein concentration was measured using the bicinchoninic acid (BCA) protein assay kit (Beyotime Biotechnology, Shanghai, China). Equal amounts of protein were subjected to 10% SDS-polyacrylamide gel electrophoresis and transferred onto PVDF membranes. The membranes were sequentially blocked with 5% non-fat milk for 2 hours and incubated with primary antibodies overnight at 4°C. The primary antibodies used were anti-MGMT (1:500, Abcam, Cambridge, UK) and anti-Survivin (1:1000, Abcam), anti-β-catenin, anti-CD44, anti-C-Jun, anti-C-Myc, anti-cyclinD1, anti-LEF1, anti-TCF1/TCF7, anti-MMP7, anti-Axin2, anti-Met, anti- PARP, anti-caspase-3, anti-BAX, anti-Bcl2, anti-cleaved-caspase-3, anti-E-cadherin, anti-N-cadherin and anti-Vimentin (these primary antibodies are all: 1:1000, Cell Signaling Technology, Boston, MA, USA), as well as anti-GAPDH, and secondary antibodies were HRP-conjugated goat anti-mouse or goat anti-rabbit IgG antibody (1:1000, Beyotime). All experiments were performed in triplicate.
2.5 | Cell viability assay

Cell viability and proliferation was, respectively, evaluated using the cell counting kit-8 solution (CCK-8) assay (Dojindo, Japan). Cells were collected and seeded at a density of 5 × 10^3 cells/well in 96-well plates for CCK-8 assay. After various treatments, cells were incubated with 10 μL CCK-8 solution for 1 hour following the manufacturer’s specifications. The data was assessed by measuring the optical density (OD) at 450 nm using a microplate reader (BioTek, Winooski, VT, USA).

2.6 | Wound healing assay

Approximately 1 × 10⁶ cells were seeded into 6-well plates and incubated at 37°C until cells reached a confluence of at least 90%. Wounds were created by scratching cell monolayers with a 200-μL plastic pipette tip and then incubated in fresh medium containing 1% FBS and different concentrations of 20(S)-Rg3 for 24 hours. Photographs were taken to estimate the mean number of migrating cells per field.

2.7 | Transwell invasion assay

Cell invasion assays were performed using 24-well Transwell plates (Corning, NY, USA) pre-coated with Matrigel (BD, USA). Approximately 1 × 10^5 cells were seeded in the upper chamber with serum-free medium in triplicate. Medium containing 10% FBS (300 μL) and different concentrations of 20(S)-Rg3 was added to the lower chamber as a chemo-attractant. After incubation for 24 hours, the cells above the Matrigel layer were removed by cotton swab, and the cells below the membrane were fixed by methanol, stained with 1% crystal violet for 10 minutes, and counted from 5 randomly chosen fields for each well.

2.8 | Immunofluorescence analysis

Cells were cultured on glass coverslips in 6-well plates, and 20(S)-Rg3 was added to the culture media for 72 hours and then fixed in a solution of 4% paraformaldehyde in PBS for 30 minutes. The cells were permeabilized with 1% Triton X-100 in PBS for 10 minutes, and blocked in 5% goat serum in PBS for 1 hour. Coverslips were then incubated in primary antibodies overnight at 4°C in the dark with the following dilutions: E-cadherin, N-cadherin and Vimentin (1:400, Cell Signaling Technology). Cells were washed and incubated in secondary antibody (Abcam) for 1.5 hour at 37°C. The slips were immediately examined using fluorescence microscopy (Olympus BX51).

2.9 | Annexin V-FITC/PI apoptosis assay

Cell apoptosis was measured using an annexin V-FACS apoptosis detection kit (Becton Dickinson, Lake Franklin, NJ, USA) according to the manufacturer’s instructions. To be brief, after treatments (20(S)-Rg3 alone, TMZ alone and 20(S)-Rg3 combined with TMZ for 72 hours), cells were washed twice with PBS and incubated in 300 μL 1× binding buffer containing 5-μL annexin V-FITC for 10 minutes and then resuspended with propidium iodide in the dark for 5 minutes at 25°C. The stained cells (containing 200 000 cells/sample) were analyzed using an FC 500 flow cytometer (Beckman Coulter, Brea, CA, USA) within 1 hour according to the manufacturer’s protocol.

2.10 | Tumor xenograft experiment and tumor peritoneal metastasis experiment

For the tumor xenograft experiment, each 4-week-old male nude mouse (6 mice per group) was subcutaneously injected with U118 cells (100 μL, 2 × 10^6). Tumor volumes were calculated as .5 × length × width² on a weekly basis. Therapeutic experiments were started when the tumors reached approximately 100 mm³ after 7 days and mice were randomly divided into 2 groups (6 mice/group). Because the drug concentration in the serum of mice with 40 mg/kg 20(S)-Rg3 administered i.p. daily for 1 week reached a peak at approximately 200 μmol/L, 23 and 30 mg/kg TMZ alone was unable to significantly inhibit glioma growth in vivo xenograft models. 25 20 mg/kg of 20(S)-Rg3 and 20 mg/kg TMZ were selected for in vivo experiments. One of the groups received an i.p. injection of TMZ 20 mg/kg alone every day for a 28-day continuum, and the other group received TMZ 20 mg/kg and 20(S)-Rg3 20 mg/kg in combination every day for a 28-day continuum. After 4 weeks, mice were killed and tumors were excised and weighed; total tissue protein was then extracted from tumor tissue for western blot of MGMT expression level.

For the tumor peritoneal metastasis experiment, GBM-XX cells (100 μL, 2 × 10^6) were i.p. injected into each 4-week-old male nude mouse, and then mice were randomly divided into 2 groups (4 mice for each group); 2 groups received PBS or 20 mg/kg of 20(S)-Rg3 every day, respectively. Mice were killed to count the peritoneal metastatic nodules after 20 days. All animal experiments were performed in the animal laboratory center of Xinhua Hospital (Shanghai JiaoTong University School of Medicine, Shanghai, China). The study protocol was approved by the Animal Care and Use committee of Xinhua Hospital.

3.1 | 20(S)-Rg3 significantly downregulated the expression of O³-methylguanine DNA-methyltransferase in glioma cell lines

To evaluate the toxic effect of 20(S)-Rg3 and TMZ on GBM, three human GBM cell lines, T98G, U118 and GBM-XX, were treated...
with 20(S)-Rg3 at different concentrations (0, 60, 120, 240, 360 and 480 μmol/L) and TMZ at different concentrations (0, 75, 150, 300, 450 and 600 μmol/L) for 72 hours, respectively. MTT assay showed that 20(S)-Rg3 and TMZ exhibited a concentration-dependent killing of diverse GBM cell lines, with an IC50 (half maximal inhibitory concentration) value of around 200 and 250 μmol/L, respectively (Figure 1A,B). To avoid cytotoxicity using a single reagent, we used a 100 μmol/L concentration of 20(S)-Rg3 and a 100 μmol/L concentration of TMZ for the following study.

To explore whether 20(S)-Rg3 will exert an effect on the expression of MGMT, we first detected MGMT expression levels before and after treatment with 20(S)-Rg3 (100 μmol/L for 72 hours) in glioma cell lines T98G, U118 and GBM-XX by qRT-PCR. As show in Figure 1C, MGMT levels in GBM cell lines were significantly depressed by 20(S)-Rg3. Western blotting showed that all cells, U118

![Figure 1](image_url)

**Figure 1** 20(S)-Rg3 inhibits the expression of O6-methylguanine DNA-methyltransferase (MGMT) in glioma cell lines. T98G, U118 and GBM-XX cells were seeded in 96-well flat-bottom plates at 5000 cells/well, cultured in DMEM supplemented with 10% FBS, and then treated with increasing concentrations of 20(S)-Rg3 or temozolomide (TMZ), or DMSO as a control; 72 h later, 10 μL of cell counting kit-8 mix reagent was added to 100 μL of media per well, and the cells were incubated at 37°C for 2 h. The optical density (OD) was measured at 450 nm with a spectrophotometer. A,B, The half maximal inhibitory concentration of 20(S)-Rg3 and TMZ on glioma cells is approximately 200 and 250 μmol/L. C, T98G, U118 and GBM-XX were treated with 20(S)-Rg3 (100 μmol/L) for 72 h, and the total RNA was extracted with TRizol; then the expression of MGMT mRNA level was determined by quantitative real-time PCR (n = 4). ***P < .001 compared with the control group. D,E, T98G, U118 and GBM-XX were treated with 20(S)-Rg3 (100 μmol/L) for 72 h, and 20(S)-Rg3 (100 μmol/L) was added to the culture media for 72 h. Then expression of MGMT in glioma cells was determined by immunocytochemistry assay (n = 4). *P < .05, **P < .01 compared with control group.
and GBM-XX were MGMT-positive, while with the treatment of 20(S)-Rg3 (100 μmol/L for 72 hours), MGMT expression was significantly suppressed in all glioma cell lines (Figure 1D,E). In addition, the immunocytochemistry results are similar to those of quantitative RT-PCR and western blot (Figure 1F,G).

### 3.2 | GinsenosideRg3 inhibits O6-methylguanine DNA-methyltransferase expression in glioblastoma by modulating Wnt/β-catenin pathways

To determine whether the 20(S)-Rg3 inhibits MGMT expression is related to Wnt/β-catenin signaling, a series of western blot experiments were carried out. These results suggested that expression of the key downstream effector β-catenin of the Wnt signaling pathway is obviously suppressed by 20(S)-Rg3 (100 μmol/L for 3 days). As shown in Figure 2, the related nuclear transcription factors LEF1 and TCF1/TCF7 were distinctly decreased. In addition, the target genes CD44, C-Jun, C-Myc, cyclinD1, MMP7 and survivin were decreased, and the changes were similar to those of MGMT as well, but there was no obvious effect on MET and Axin-2.

### 3.3 | 20(S)-Rg3 augments temozolomide-mediated chemotherapy

To explore whether the 20(S)-Rg3 could augment TMZ-mediated chemotherapy by downregulating the expression of MGMT, we performed a cell viability assay. The assay demonstrated that 20(S)-Rg3 (100 μmol/L for 3 days) had no obvious cytotoxicity by itself, and TMZ (100 μmol/L for 3 days) alone did not cause significant cytotoxicity in cell lines T98G, U118 and GBM-XX. However, as shown in Figure 3A,B,C, 20(S)-Rg3 significantly enhanced the cytotoxicity of TMZ (100 μmol/L for 3 days) in all three glioma cell lines. In addition, to investigate whether compared with the treatment of 20(S)-Rg3+ TMZ on glioblastoma cells simultaneously, pretreatment of 20(S)-Rg3 on glioblastoma cells may be more effective, we pretreated cells with 20(S)-Rg3 (100 μmol/L) and then performed CCK8 assays. As shown in Figure S1, the results showed that pretreatment of 20(S)-Rg3 was more effective in the first 24 hours on T98G and U118 cells, but for 72 hours, there was no significant difference in the results on all three glioblastoma cell lines. Taken together, the results suggested that MGMT expression after 20(S)-Rg3 pretreatment for 72 hours was lower than 20(S)-Rg3 treatment for 24 hours, and sensitivity of TMZ to glioblastoma cells is negatively correlated with the expression of MGMT in glioblastoma cells.

Similar results were shown in the analysis of flow cytometry. From Figure 3D,E, compared with the control, when 20(S)-Rg3 (100 μmol/L for 3 days) or TMZ (100 μmol/L for 3 days) alone was used on glioma cell lines, neither of them could induce an increase in cell apoptosis obviously; however, the combination of 20(S)-Rg3 and TMZ can significantly increase the apoptotic effect of glioma cells. For T98G cells, the early and the late apoptosis percentage was 5.4% and 2.2% in the control group, 5.4% and 2.2% in the 20(S)-Rg3 group, and 6.7% and 3.0% in the TMZ group, while in the 20(S)-Rg3+ TMZ group the early apoptosis percentage was up to 15.6% and the late apoptosis percentage was up to 44.8% (P < .05). The results in U118 and GBM-XX cell lines were consistent with T98G.

We carried out western blotting experiments to detect changes in the expression of apoptosis-related proteins in T98G cells, including BAX, Bcl-2, caspase-3, cleaved-caspase-3, PARP and cleaved-PARP. As shown in Figure 3F, 20(S)-Rg3+ TMZ increased the expression of BAX, cleaved-caspase-3, cleaved-PARP and conversely decreased Bcl-2, caspase-3 and PARP expression in glioma cell line T98G. These results illustrated that 20(S)-Rg3(100 μmol/L for 3 days) + TMZ (100 μmol/L for 3 days) could induce apoptosis in glioma cells through mitochondrial signaling pathways.
3.4 | 20(S)-Rg3 displays synergistic activity with temozolomide in in vivo xenograft models

To further examine the effect of 20(S)-Rg3 synergistic activity with TMZ in vivo, U118 cells were injected into nude mice subcutaneously. According to some references, we selected 20 mg/kg 20(S)-Rg3 and 20 mg/kg TMZ for the in vivo experiments. When the subcutaneous tumors reached a mean group size of around 100 mm³, mice were treated every day for 4 weeks with 20 mg/kg TMZ alone or 20 mg/kg 20(S)-Rg3 + 20 mg/kg TMZ. 20(S)-Rg3 showed superior synergistic activity with TMZ. Compared to TMZ alone, 20(S)-Rg3 plus TMZ treatment showed dramatically suppressed subcutaneous tumor growth (Figure 4A,B).
Western blot for the subcutaneous tumors indicated that the MGMT level in tumor tissue was significantly suppressed (Figure 4C). In addition, the combination of 20(S)-Rg3 and TMZ did not display adverse consequences or obvious toxicity; it was well-tolerated in nude mice.

3.5 | 20(S)-Rg3 restrains the epithelial-mesenchymal transition progression of glioma cells in vitro and in vivo

In addition, we found that 20(S)-Rg3 significantly inhibited the migration and invasion of glioma cells. As shown in Figure 5, the results of wound healing (Fig. 5a, b) and transwell invasion assays (Figure 5C, D) indicated that 20(S)-Rg3 dramatically inhibited cell migration and invasion in cell lines T98G, U118 and GBM-XX in a dose-dependent manner.

To further explore the mechanism of inhibition of glioma cell migration and invasion by 20(S)-Rg3, we performed western blot assays for epithelial-mesenchymal transformation-related proteins, including E-cadherin, N-cadherin and vimentin. Western blot analyses showed that the expression levels of an epithelial marker, E-cadherin, were increased by 20(S)-Rg3 (120 μmol/L for 3 days) in T98G, U118 and GBM-XX cell lines, and of mesenchymal markers, N-cadherin and Vimentin, were the opposite to E-cadherin in all three glioma cell lines (Figure 6A). In addition, the results of
immunofluorescence analyses in T98G and GBM-XX were similar to those of western blot assays (Figure 6B). These results suggested that 20(S)-Rg3 inhibits the epithelial cells to transdifferentiate into mesenchymal cells; in other words, 20(S)-Rg3 could restrain the EMT progression of glioma cells.

Finally, to investigate the effect of 20(S)-Rg3 on glioma cell peritoneal spreading in vivo, we injected GBM-XX cells into nude mice intraperitoneally. Seven days later, 20 mg/kg of 20(S)-Rg3 was injected in one group of mice every day by i.p. injection for 20 days in a row, while the control group mice were injected with equal amount of PBS. Twenty days later, the number of intraperitoneal metastatic nodules in the 20(S)-Rg3 (20 mg/kg/day) treatment group was significantly less than in the control group (Figure 6C,D). Thus, 20(S)-Rg3 can prevent glioma cell peritoneal spreading and metastasis in vivo.

4 | DISCUSSION

As the most common malignant intracranial tumor, glioblastoma has a very fast course and extremely poor prognosis. At present, the treatment options for glioma patients are still very limited. The Stupp's regimen has been widely used in clinical practice for over a decade, and increases the life expectancy of GBM patients. Postoperative adjuvant chemotherapy plays an important role in Stupp's regimen, and TMZ is the first choice for chemotherapeutic agents. The addition of TMZ can bring additional benefits to patients with gliomas for an additional average 2.5 months to overall survival (OS). However, the emergence of TMZ resistance in the majority of GBM patients brings another great challenge to the treatment of patients.

The alkylating agent TMZ can spontaneously dissolve into the reactive intermediate 5-(3-methyl-1-triazeno)imidazole-4-carboxamide in the human body, and then it methylates the N7/O6 positions of guanine and the N3 position of adenine. The most important mismatch in these 3 methylation positions is the O6 position of guanine (O6-G), which causes DNA base mispairing by continuously inducing O6-methylguanine adduct, finally leading to cell cycle arrest and cellular apoptosis. This is how TMZ exerts its chemotherapeutic effects.

Unfortunately, MGMT is frequently expressed in tumor cells of glioma patients. MGMT mediates the chemoresistance of glioma cells to TMZ by directly removing the methyl group from O6-methylguanine. After each MGMT molecule transfers the methyl group from O6-methylguanine, it will deactivate itself. Therefore, the cytotoxicity of TMZ is theoretically determined by the expression level of MGMT. Because of these factors, much effort has been directed towards increasing the TMZ chemosensitivity by downregulating the expression of MGMT of glioma cells. Shirai et al. reported that TMZ itself was shown to partially deplete MGMT protein in tumors, and MGMT would be depleted by a high dose of TMZ by suicide inhibition. O6-BG, sulforaphane and STAT3 inhibitor have been shown to reverse resistance to TMZ by targeting downregulation of MGMT expression.

20(S)-Rg3 is a small molecule agent with a molecular weight of 785.01 g/mol, and it is reported that 20(S)-Rg3 can be maintained in plasma with a high drug concentration, and is nontoxic and well-tolerated. 20(S)-Rg3 has been reported to have remarkable anti-tumor effects; however, its mechanism remains poorly understood. In this study, we used 2 MGMT-positive glioma cell lines and an MGMT-positive primary cell strain and xenograft glioma models to examine whether 20(S)-Rg3 potentiates the sensitivity to TMZ and to reveal the underlying mechanisms.

Wnt/β-catenin signaling plays a pivotal role in the occurrence and development of many types of malignant tumors, including GBM. Inhibition of the β-catenin expression level and the transcription factor TCF/LEF1 expression level for Wnt/β-catenin signaling lead to downregulation of Wnt/β-catenin signaling activity. Targeting Wnt/β-catenin pathways provide a new therapeutic strategy for GBM. Recent studies have shown that the Wnt/β-catenin pathway could be involved in regulating the expression level of MGMT in cancers. In the present study, we revealed that 20(S)-Rg3 (100 μmol/L for 3 days) significantly inhibits the expression level of MGMT protein in glioma cells by modulating Wnt/β-catenin signalling in vitro. The key downstream effector β-catenin and 2 important nuclear transcription factors for Wnt/β-catenin pathways were all depressed by 20(S)-Rg3, leading to several target genes being decreased. He et al. reported that ginsenoside Rg3 inhibits colorectal tumor growth by downregulating the Wnt/β-catenin pathway, which partly consist with our result.

Meanwhile, 20(S)-Rg3 by itself showed no significant cytotoxicity at its effective dose (100 μmol/L for 3 days), but when 20(S)-Rg3 was given concomitantly with TMZ, it strongly enhanced the pro-apoptotic effect of TMZ. 20(S)-Rg3 inhibited the viability of glioma cells and induced apoptosis by decreasing the expression of caspase-3, PARP and anti-apoptosis protein Bcl-2, and increasing the expression of the pro-apoptosis proteins Bax, cleaved-PARP and cleaved caspase-3. Accumulating studies have reported that increased MGMT gene expression is closely related to the occurrence of TMZ resistance. To our knowledge, we have revealed for the first time that 20(S)-Rg3 inhibits MGMT gene expression by modulating Wnt/β-catenin/MGMT pathways and potentiates chemosensitivity to TMZ in glioma cells in vitro and in vivo.
Moreover, it is generally recognized that epithelial-mesenchymal transition (EMT) causes dissemination of tumor cells from primary sites by inducing polarized epithelial tumor cells to acquire mesenchymal cell phenotype. Thus, it is considered as an important factor in promoting the cell migration and invasion ability of tumor cells. Therefore, we examined the effect of 20(S)-Rg3 on migration and invasiveness of glioma cells, and checked the expression of critical EMT markers, E-cadherin, N-cadherin and vimentin, and found that 20(S)-Rg3 significantly restraints the epithelial-mesenchymal transition progression of glioma cells in vitro and in vivo. However, the underlying mechanism of 20(S)-Rg3 that inhibits the EMT process on glioma cells is uncertain at present, and in future we will carry out further related exploration.

In sum, we revealed that 20(S)-Rg3 demonstrates good reversal performance of TMZ resistance in MGMT-positive glioma treatment; meanwhile, 20(S)-Rg3 effectively restrains the epithelial-mesenchymal transition progression of glioma cells. Our study suggests that 20(S)-Rg3 could potentially be used to counteract TMZ resistance in MGMT-positive glioblastomas.

**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.
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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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