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Original Articles

Cell surface vimentin-targeted monoclonal antibody 86C increases sensitivity to temozolomide in glioma stem cells

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ABSTRACT

Glioblastoma multiforme (GBM) is the most prevalent and aggressive brain tumor. The current standard therapy, which includes radiation and chemotherapy, is frequently ineffective partially because of drug resistance and poor penetration of the blood-brain barrier. Reducing resistance and increasing sensitivity to chemotherapy may improve outcomes. Glioma stem cells (GSCs) are a source of relapse and chemoresistance in GBM; sensitization of GSCs to temozoliomide (TMZ), the primary chemotherapeutic agent used to treat GBM, is therefore integral for therapeutic efficacy. We previously discovered a unique tumor-specific target, cell surface vimentin (CSV), on patient-derived GSCs. In this study, we found that the anti-CSV monoclonal antibody 86C efficiently increased GSC sensitivity to TMZ. The combination TMZ + 86C induced significantly greater antitumor effects than TMZ alone in eight of 12 GSC lines. TMZ + 86C–sensitive GSCs had higher CSV expression overall and faster CSV resurfacing among CSV^- GSCs compared with TMZ + 86C–resistant GSCs. Finally, TMZ + 86C increased apoptosis of tumor cells and prolonged survival compared with either drug alone in GBM mouse models. The combination of TMZ + 86C represents a promising strategy to reverse GSC chemoresistance.

1. Introduction

Glioblastoma multiforme (GBM) is the most prevalent and aggressive malignant brain tumor and has a median survival duration of approximately 15 months from diagnosis [1]. The current standard of care for GBM patients is surgical resection followed by radiotherapy and chemotherapy. This therapy is not effective in most patients because of factors including drug resistance and poor penetration of the blood-brain barrier. The primary chemotherapeutic agent used to treat GBM is temozolomide (TMZ), a methylating agent that does cross the blood-brain barrier [2,3] but whose efficacy is constrained by frequent development of resistance [4–6]. Median survival duration is increased only 2.5 months by adjuvant combined radiation and TMZ treatment. The efficacy of TMZ is further constrained by toxic effects outside of the central nervous system and by the biologic limits to achieving a sustained tumoricidal concentration in the tissue, as with most other systemic therapies [7]. Intensification of TMZ, in the form of dose-dense adjuvant TMZ, was associated with significantly greater high-grade toxicity without any survival benefit compared with the standard regimen for GBM [8]. There is a great need for more efficacious therapeutic strategies to improve clinical management and survival outcomes in GBM patients.

Glioma stem cells (GSCs) exhibit resistance to radiation and to anticancer drugs such as TMZ [9–12], and elimination of GSCs is considered key to ensuring the long-term survival of GBM patients [13]. Since GSCs are also responsible for tumor initiation and recurrence, they are attractive candidate targets for anticancer therapy. Identifying new drugs that can specifically target and kill GSCs is a critical step in improving GBM patient outcomes.

A potential approach to targeting GSCs is through cell surface vimentin (CSV). Unlike intracellular vimentin, which is found in both cancer cells and normal mesenchymal cells, CSV is tumor-specific. It has been found primarily on cancer cells, including circulating tumor cells, GBM cells, and GSCs [14–16], and can serve as a therapeutic target for such cells. Our previous study showed that the novel monoclonal antibody 86C, which binds CSV on cancer stem cells and is internalized,

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induces apoptosis of the target cells, suggesting that targeting GSCs using 86C is a promising approach for the treatment of GBM [16]. On the basis of these findings, we hypothesized that targeting CSV with 86C would help overcome TMZ resistance in GSCs, increasing cell killing. Our findings show that 86C efficiently targets GSCs expressing CSV and that this intervention increases GSC sensitivity to TMZ. The addition of 86C reduced the dose of TMZ required to eliminate GSCs, which will ultimately reduce its toxic effects. Treatment with 86C combined with TMZ decreased resistance to chemotherapy and resulted in a striking recovery of GSC sensitivity to TMZ.

2. Materials and methods

2.1. Ethics statement

The mice used in this study were maintained under the guidelines of the National Institutes of Health and euthanized according to procedures approved by the Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center (MD Anderson). Tumor sample collection from patients with GBM at MD Anderson was conducted under protocol #LAB03-0687, which was approved by the institutional review board, after informed consent was obtained from the patients.

2.2. Cell lines and cell culture

Twelve GSC cell lines (GSC6-27, GSC7-2, GSC8-11, GSC11, GSC17, GSC20, GSC23, GSC28, GSC272, GSC280, GSC295, and GSC300) were provided by Dr. Frederick Lang (MD Anderson) and were cultured in serum-free Dulbecco's modified Eagle medium supplemented with epidermal growth factor (20 ng/mL), basic fibroblast growth factor (20 ng/mL), and 2% B27 (Life Technologies, Carlsbad, CA). GL261 murine glioma cells were cultured in 10% fetal bovine serum and Dulbecco's modified Eagle medium. Cells were dissociated using Accutane (Invitrogen, Carlsbad, CA) and then used for experiments. No further authentication of cell lines was conducted in our laboratory.

2.3. Drugs

The stock solution of TMZ (50 mM; Sigma-Aldrich, St. Louis, MO) was prepared by dissolving the drug in dimethyl sulfoxide (DMSO). The final concentration of DMSO was kept to less than 0.5% (v/v), and it did not contribute to toxicity.

2.4. TMZ treatment and analysis of cell viability in vitro

GSCs were plated at a density of 5×10^3/100 mL of culture medium and treated with TMZ alone or in combination with 86C (TMZ + 86C) at various concentrations in triplicate for 3 days. Cell viability was determined by normalizing the absolute values of absorbance for cells that received each treatment with that of controls and is expressed as a percentage.

2.5. Isolation of CSV^- and CSV^+ cells by flow cytometry

Single-cell suspensions were blocked for 10 min at room temperature with FcR blocker (Miltenyi Biotic, Bergisch Gladbach, Germany) in a 1:1000 dilution and then incubated with 2 μg of the CSV-binding antibody 84–1, produced in our laboratory, for 15 min at room temperature. After two washings, the cells were incubated with 2 μg of goat anti-mouse Alexa Fluor 405–conjugated secondary antibody in phosphate-buffered saline solution (PBS) plus 2% serum for 15 min in the dark at room temperature. CSV^- and CSV^+ cells were isolated using a FACSAria Fusion cell sorter (BD Biosciences, San Jose, CA). CSV^- cells were cultured for 30 min, 4 h, 1 day, or 2 days, and CSV expression was analyzed.

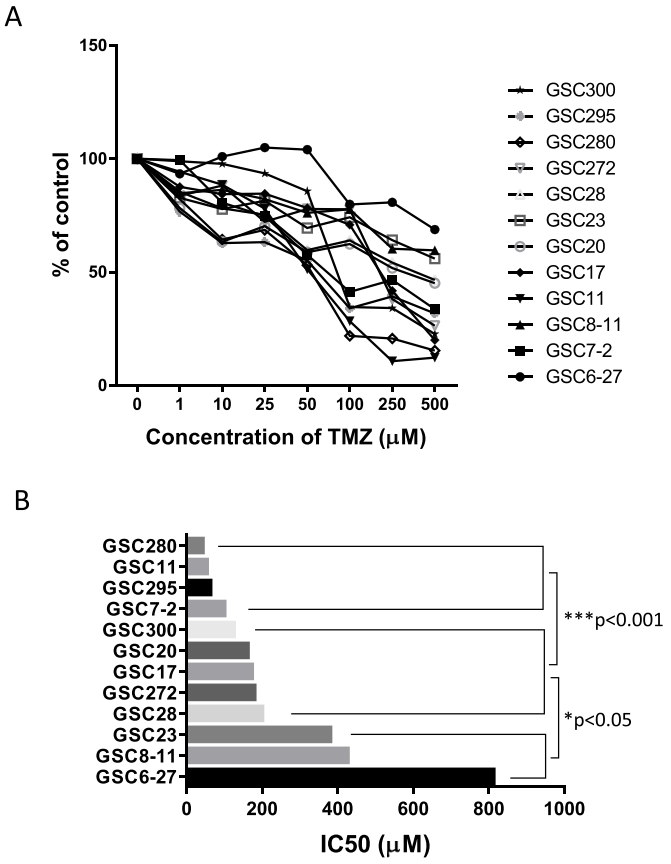

Fig. 1. Median inhibitory concentrations (IC_{50}) of temozolomide (TMZ) for 12 GSC cell lines. (A) Effects of TMZ on cell viability were determined. At 72 h after addition of TMZ (final concentration, 0–500 μM) to the culture medium, the viable cells were counted and the numbers expressed as percentages of the untreated control cells. (B) IC_{50} represents the TMZ concentration required for a 50% decrease in cell growth compared with the control.

2.6. Detection of CSV expression by flow cytometry

Single-cell suspensions were blocked for 10 min at room temperature with FcR blocker in a 1:1000 dilution and then incubated with 0.4 μg of 84–1 for 15 min at room temperature. After two washings, cells were incubated with 0.5 μg of goat anti-mouse Alexa Fluor 405–conjugated secondary antibody in PBS plus 2% serum for 15 min in the dark at room temperature. Cells were analyzed on an Attune flow cytometer (Life Technologies), and the results were evaluated using FlowJo 10.0 software (Tree Star, Inc., Ashland, OR).

2.7. Western blotting and immunoprecipitation

Vimentin-null T47D cells, LN18-shCtrl cells, and LN18-shVim cells were lysed in RIPA buffer on ice for 30 min. Lysates were centrifuged at 15,000 × g for 5 min at 4 °C. The supernatant was collected for subsequent procedures. Immunoblotting was performed as previously described [17]. Briefly, 86C (1:1000), β-actin antibody (1:1000, Biolegend) and secondary antibodies were diluted in 5% nonfat milk/TBST, incubated overnight at 4 °C, and blots were developed with Enhanced Chemiluminescence Plus (GE Biosciences). Immunoprecipitation was performed using 2 mg of total protein lysate and 1 μg (30 μl) of agarose beads were added to the mixture of protein lysate and 86C and incubated at 4 °C overnight. The lysate mixture was then centrifuged at 3000 rpm for 30 s at 4 °C. After discarding the supernatant, the pellet