



Multiple Pathway-Dephosphorylated ASK-1 Confers Temozolomide-Resistance to Human Glioma Cells

Kai GAO¹, Kun SHE¹, Jie FAN², Lianbin SHAO¹, Zhiwu JI¹

¹Affiliated Hospital of Hebei University of Engineering, Department of Neurosurgery I, Handan 056002, Hebei, China

²Affiliated Hospital of Hebei University of Engineering, Clinical Lab, Handan 056002, Hebei, China

Corresponding author: Zhiwu JI ✉ jizhiwu88@126.com

ABSTRACT

AIM: To elucidate the function of ASK-1 and the role of its modulators in the induction of Temozolamide (TMZ) resistance in glioma and the underlying mechanism.

MATERIAL and METHODS: ASK-1 phosphorylation, the IC₅₀ of TMZ, cell viability, and apoptosis were assessed in the U87 and U251 glioma cell lines and the derived TMZ-resistant cell lines U87-TR and U251-TR. We then blocked ASK-1 function, either with an inhibitor or by overexpression of multiple ASK-1 upstream modulators, to further explore the role of ASK-1 in TMZ-resistant glioma.

RESULTS: TMZ-resistant glioma cells showed high IC₅₀ values of TMZ, high survival, and low levels of apoptosis following the TMZ challenge. ASK-1 phosphorylation, but not protein expression, was higher in U87 and U251 cells than in TMZ-resistant glioma cells exposed to TMZ. The addition of the ASK-1 inhibitor selonsertib (SEL) resulted in the dephosphorylation of ASK-1 in U87 and U251 cells after the TMZ challenge. SEL treatment increased the TMZ resistance of U87 and U251 cells, as evidenced by the increased IC₅₀ and cell survival rate and low apoptosis rate. Overexpression of some ASK-1 upstream suppressors [Thioredoxin (Trx), protein phosphatase 5 (PP5), 14-3-3, and cell division cycle 25C (Cdc25C)] led to various degrees of ASK-1 dephosphorylation and a TMZ-resistant phenotype in U87 and U251 cells.

CONCLUSION: Dephosphorylation of ASK-1 induced TMZ resistance in human glioma cells, and several ASK-1 upstream suppressors, including Trx, PP5, 14-3-3, and Cdc25C, are involved in this phenotypic change induced by dephosphorylation of ASK-1.

KEYWORDS: Glioma, TMZ, ASK-1, drug resistance, SEL

ABBREVIATIONS: **TMZ:** Temozolomide, **SEL:** Selonsertib, **Trx:** Thioredoxin, **PP5:** Protein phosphatase 5, **Cdc25C:** Cell division cycle 25C, **GBM:** Glioblastoma, **ER:** Endoplasmic reticulum, **WB:** Western blotting, **PVDF:** Polyvinylidene fluoride, **FCM:** Flow cytometry, **ANOVA:** Analysis of variance, **Redox:** Reduction/oxidation, **TR:** TMZ-resistant

INTRODUCTION

Gliomas are a series of malignant brain tumors that include glioblastoma (GBM), anaplastic astrocytoma, and oligodendroglioma (11). GBM is the most common type, accounting for 54% of gliomas and 16% of all primary

brain tumors (14). It is also the most lethal brain tumor, with a median survival of 15 months (9,14).

Since 2005, the standard treatment for patients diagnosed with GBM consists of surgery followed by daily temozolomide (TMZ) and radiotherapy. TMZ is a second-generation imid-

Kai GAO : 0000-0002-6654-2393
Kun SHE : 0000-0002-6287-7372
Jie FAN : 0000-0001-7238-5200

Lianbin SHAO : 0000-0002-6358-5996
Zhiwu JI : 0000-0002-5461-2702

azotetrazine prodrug with good bioavailability and tolerance that is capable of crossing the blood-brain barrier and displays mild GBM-inhibiting activity (2,18,19). However, many patients with GBM develop TMZ resistance during treatment. Thus, owing to either pre-existing intrinsic tumor resistance or acquired resistance that develops during treatment, TMZ treatment offers only a minimal reduction in median overall survival (6). Many cellular signaling pathways, such as the sonic hedgehog, notch, and Wnt/ β -catenin pathways, are associated with TMZ resistance in GBM (20,21).

ASK-1 is a MAP3K that activates the JNK and p38 pathways (7). Diverse stressors can activate ASK-1, including endoplasmic reticulum (ER) and osmotic stresses, heat shock, lipopolysaccharides, and inflammatory cytokines. ASK-1 plays a key role in H₂O₂-triggered apoptotic cell death (16). ASK-1 can interact with various molecules in a context-dependent manner. However, the role of ASK-1 in the development of TMZ resistance in gliomas is not known. In the present study, we assessed the role of ASK-1 activation (phosphorylation) in TMZ resistance of gliomas. We used a chemical inhibitor of ASK-1, selonsertib (SEL), and various upstream suppressors, including protein phosphatase 5 (PP5), thioredoxin (Trx), 14-3-3, and cell division cycle 25C (Cdc25C), to block ASK-1 activation to probe the mechanism by which ASK-1 regulates TMZ resistance in glioma.

■ MATERIAL and METHODS

Cell Culture and Transfection

Glioma cell lines (U87 and U251) and derived TMZ-resistant (TR) glioma cells (U87-TR and U251-TR) were cultivated in DMEM supplemented with 10% FBS. Cells cultivated to 50–70% confluence were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Fresh medium was added every 4–6 h. After 48 hours, the transfected cells were used for subsequent experiments.

Generation of High-Dose Shock-Triggered TMZ-Resistant Model Glioma Cells

Glioma cells in the logarithmic phase were inoculated into a 250 mL culture flask. At 70%–80% confluence, TMZ was added to a final concentration of 5 μ g/mL. After 2 h of treatment, the medium was replaced, and the surviving cells were inoculated into a new flask. When these cells reached 70%–80% confluence, they were treated with TMZ as described above. This process was repeated until the cell death rate was <5% at a TMZ concentration of 0.4–0.5 μ g/mL. The obtained TR U87 and U251 cell lines were named U87-TR and U251-TR, respectively.

Western Blotting (WB)

Cells were lysed to obtain total proteins, which were then separated by electrophoresis. The separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes. Nonspecific binding was blocked with 5% skim milk for 120 min. The membranes were incubated with primary and secondary antibodies for the indicated times. Bands were detected, and the grey values were analyzed.

Cell Survival Assay

Cell survival was assessed using the CCK-8 assay according to the manufacturer's instructions. Briefly, cells were inoculated into 96-well plates, CCK-8 (10 μ l) was added, and the cells were incubated for 2 h at 37 °C. Then, the optical density at 450 nm (OD₄₅₀) was measured using a microplate reader (Infinite M200, Tecan, Switzerland). The half inhibitory concentration (IC₅₀) of TMZ was determined.

Flow Cytometry

Cell apoptosis was evaluated by flow cytometry (FCM) using an Annexin V-FITC/PI apoptosis kit (BD Pharmingen™). Cells were suspended in a binding buffer (20 μ L) and incubated with annexin V-FITC (10 μ L) and PI (5 μ L). The apoptosis rate was measured using FCM.

Data Analysis

Data are means \pm standard deviation. The t-test and one-way analysis of variance (ANOVA) were used to analyze the differences between two groups and three or more groups, respectively. Statistical significance was set at $p < 0.05$.

■ RESULTS

Levels of Phosphorylated ASK-1 were Reduced in U87-TR and U251-TR Cells

To assess ASK-1 activation in TR U87 and U251 glioma cells, TR glioma model cells were established. The IC₅₀ of TMZ was determined for the parental cell lines and the TR model cells. The TMZ IC₅₀ was markedly higher in the TR glioma cells than in the parental cell lines, which is characteristic of TMZ resistance (Figure 1A). Cell survival was evaluated following the challenge with 500 μ g/mL TMZ. The results showed that TMZ significantly reduced cell survival; however, relative to the parental cells, the numbers of surviving U87-TR and U251-TR cells were higher (Figure 1B). Cell death following TMZ treatment was assessed using FCM. TMZ treatment induced significant apoptosis in U87 and U251 cells, whereas TMZ treatment only alleviated apoptosis in the TR cell lines (Figure 1C). These data show that the TR model cell lines were successfully established.

To elucidate ASK-1 activation in TR cells, WB was used to measure ASK-1 expression and phosphorylation in U87 and U251 cells and the derived TR cell lines. The results showed that ASK-1 expression levels were not different in all four cell lines, with or without TMZ treatment. However, TMZ treatment increased ASK-1 phosphorylation levels in the parental glioma cells. Interestingly, following TMZ treatment, ASK-1 phosphorylation levels were lower in the TR glioma cell lines than in the parental cells (Figure 1D).

Reduction of ASK-1 Phosphorylation Induced by SEL Treatment Increased the TMZ Resistance of Glioma Cells

We hypothesized that dephosphorylation of ASK-1 may bolster TMZ resistance in glioma cells. Thus, we used an ASK-1 inhibitor, SEL (10), to block the phosphorylation of ASK-1 in glioma cells. We found that co-administration of SEL and TMZ ameliorated the phosphorylation of ASK-1 (Figure 2).

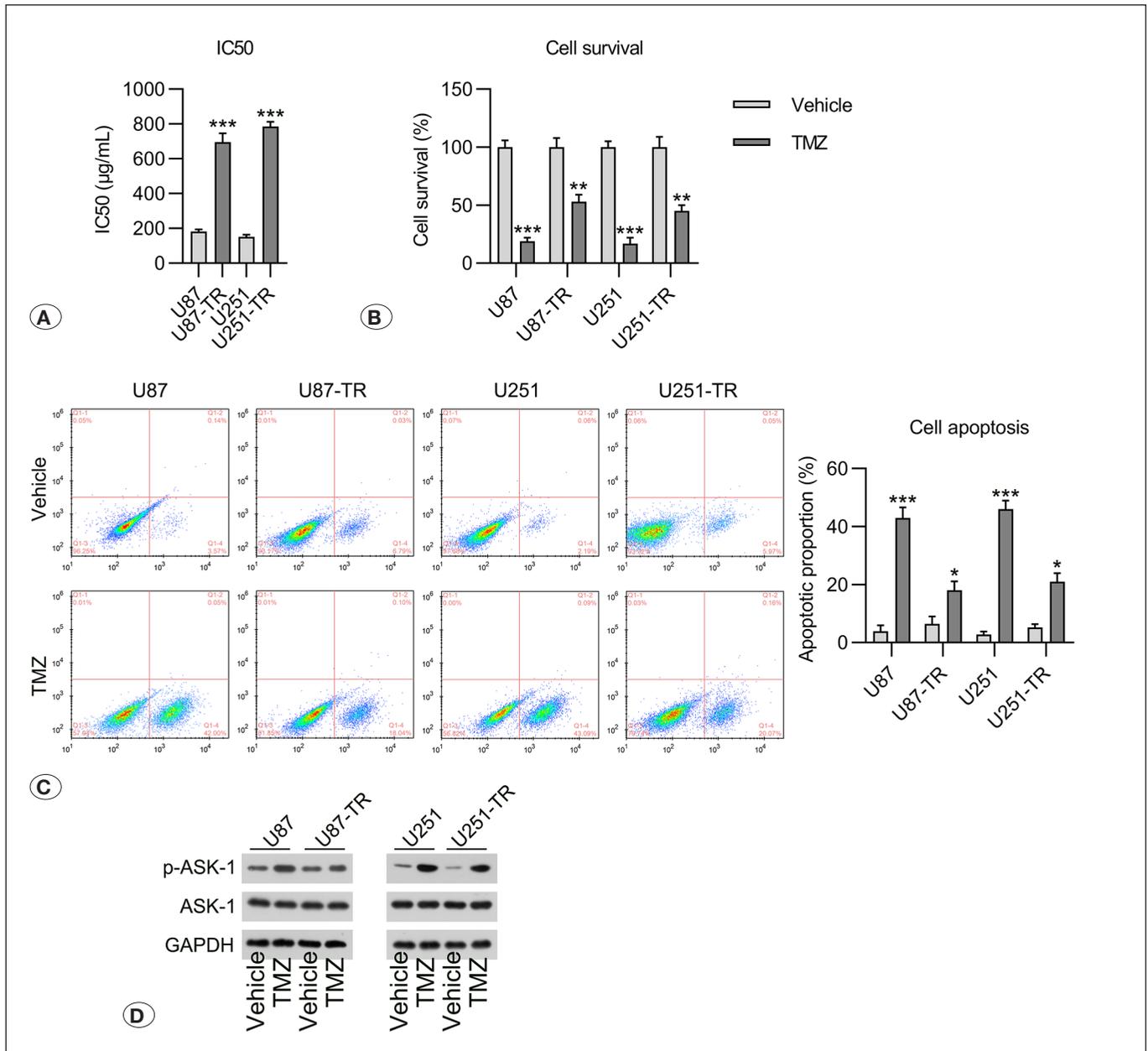


Figure 1: Phosphorylation of ASK-1 was reduced in TMZ-resistant glioma cell lines. **A)** IC₅₀ value of TMZ in U87 and U251 glioma cell lines, and their TMZ-resistant (TR) derivative lines. **B)** The survival rate of glioma cells with or without 500 µg/mL TMZ treatment, as assessed by the CCK-8 assay. **C)** The apoptosis rate of cells with or without TMZ treatment as determined by flow cytometry. **D)** Phosphorylation of ASK-1 in glioma cells as evaluated by western blotting. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

We subsequently determined the IC₅₀ of TMZ and assessed cell survival and apoptosis under high doses of TMZ in cells co-treated with SEL and TMZ. Co-treatment with SEL and TMZ markedly enhanced the IC₅₀ value of TMZ in U87 and U251 cells compared to the value in cells treated with TMZ alone (Figure 3A). Cell viability was also increased in glioma cells co-treated with SEL and TMZ compared to the viability of cells treated with TMZ alone (Figure 3B). In addition, apoptosis of TMZ-exposed glioma cells was reduced by SEL treatment (Figure 3C). These data suggest that the dephosphorylation of ASK-1 induces TMZ resistance in glioma cells.

Multiple Pathway-Dephosphorylated ASK-1 Increased the TMZ Resistance of Glioma Cells

Several studies have demonstrated that ASK-1 is activated via various pathways and several modulators can lead to the dephosphorylation of ASK-1, including Trx (1), PP5 (12), 14-3-3 (23), and Cdc25C (3). We hypothesized that ASK-1 may act as the central protein in the TMZ resistance of glioma cells. To test this hypothesis, we overexpressed Trx, PP5, 14-3-3, and Cdc25C in the glioma cells, which was confirmed by WB (Figure 4).

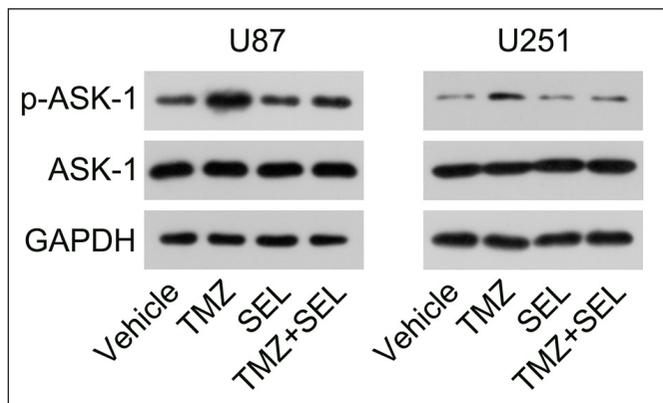


Figure 2: Administration of the ASK-1 inhibitor selonsertib reduced ASK-1 phosphorylation in TMZ-treated glioma cells. Glioma cells were treated with TMZ (500 µg/mL) and/or selonsertib (SEL; 500 µg/mL) for 48 h. Phosphorylation of ASK-1 in glioma cells was examined by western blotting.

We then explored the involvement of Trx, PP5, 14-3-3, and Cdc25C in TMZ resistance in gliomas regulated by ASK-1. Overexpression of Trx, PP5, 14-3-3, and Cdc25C reduced the IC50 of TMZ in glioma cells (Figure 5A). The reduction in the viability of TMZ-treated glioma cells was partially reversed by overexpression of Trx, PP5, 14-3-3, and Cdc25C to different degrees (Figure 5B). Overexpression of Trx, PP5, 14-3-3, and Cdc25C also reduced the proportion of apoptotic TMZ-treated U87 and U251 cells (Figure 5C). Collectively, our data indicate that Trx, PP5, 14-3-3, and Cdc25C increase TMZ resistance in glioma cells by dephosphorylating ASK-1.

DISCUSSION

TMZ, an alkylating agent, induces double-strand breaks in DNA and subsequent apoptosis of glioma cells. Long-term use of TMZ during tumor treatment may lead to acquired drug resistance, which is a major problem (22). However, the mechanism underlying TMZ resistance in GBM is unknown.

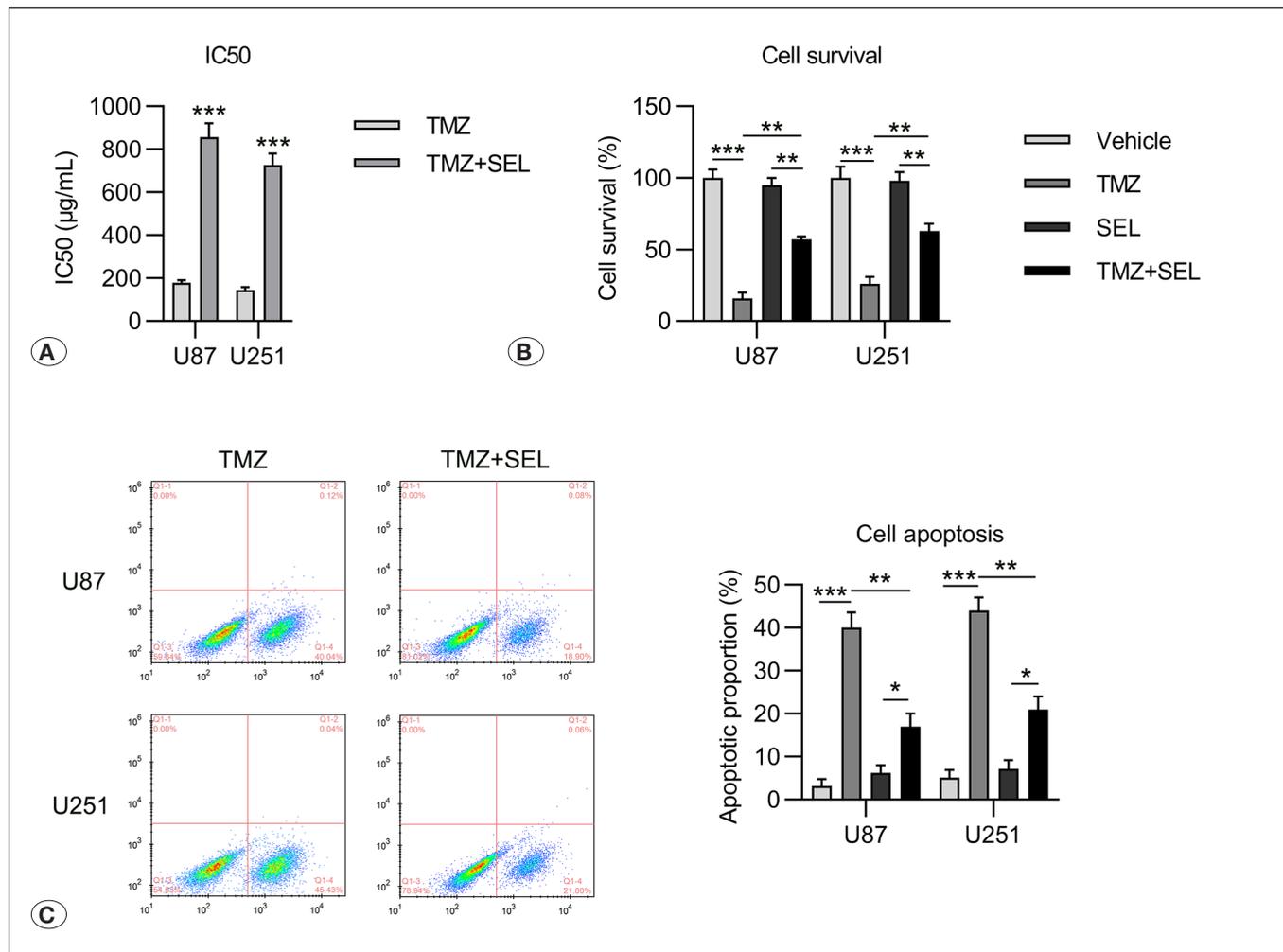


Figure 3: Administration of SEL increased TMZ resistance in glioma cells. Glioma cells were treated with TMZ (500 µg/mL) and/or SEL (100 µg/mL) for 48 h. **A)** IC50 values of TMZ in U87 and U251 glioma cell lines and their derived TMZ-resistant (TR) cell lines. **B)** The survival rate of glioma cells with or without 500 µg/mL TMZ treatment as assessed by the CCK-8 assay. **C)** The apoptosis rate of cells with or without TMZ treatment as determined using flow cytometry. * p<0.05, ** p<0.01, *** p<0.001.

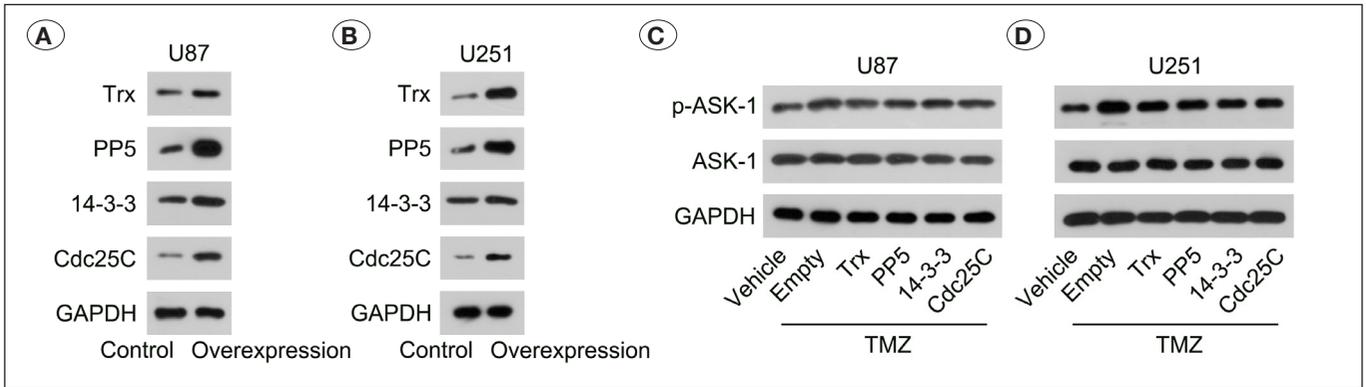


Figure 4: Overexpression of Trx, PP5, 14-3-3, and Cdc25C reduced ASK-1 phosphorylation in TMZ-treated glioma cells. Glioma cells were transfected with Trx, PP5, 14-3-3, and Cdc25C for 24 h and then treated with 500 $\mu\text{g}/\text{mL}$ TMZ for 48 h. Phosphorylation of ASK-1 in the transfected and treated glioma cells was examined by western blotting.

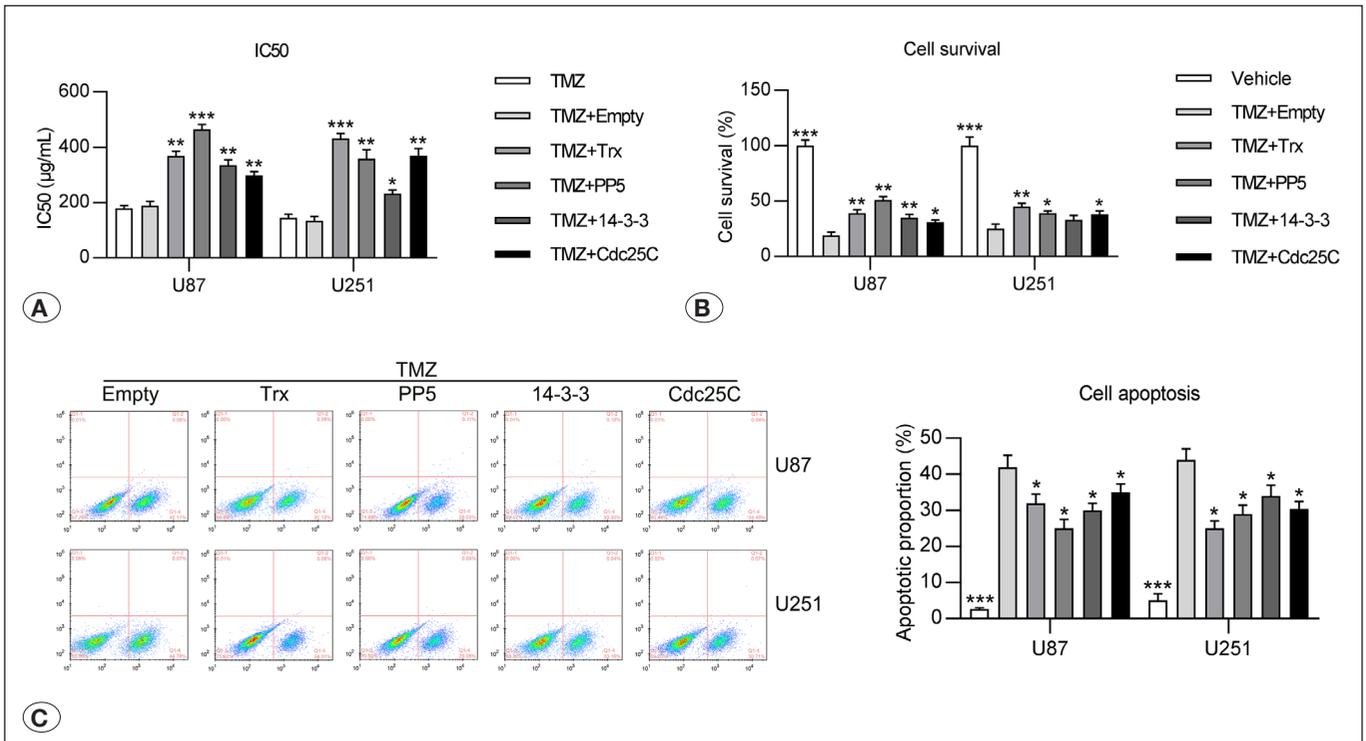


Figure 5: Overexpression of Trx, PP5, 14-3-3, and Cdc25C increased TMZ resistance in glioma cells. Glioma cells were transfected with Trx, PP5, 14-3-3, and Cdc25C for 24 h and then treated with TMZ for 48 h. **A)** TMZ IC₅₀ values in U87 and U251 glioma cells and their derived TMZ-resistant (TR) cell lines. **B)** The survival rate of glioma cells with or without 500 $\mu\text{g}/\text{mL}$ TMZ treatment as assessed by the CCK-8 assay. **C)** The apoptosis rate of cells with or without TMZ treatment as determined using flow cytometry. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. TMZ + Empty group.

In the present study, we found that ASK-1 phosphorylation in U87 and U251 glioma cells was elevated following TMZ treatment. Compared to the levels in parental cells, ASK-1 phosphorylation was markedly reduced in TR glioma cells; thus, ASK-1 might play an important role in gliomas. In our study, blocking phosphorylation of ASK-1 increased the IC₅₀ of TMZ, restored cell viability, and reduced apoptosis in TMZ-exposed U87 and U251 cells.

ASK-1 belongs to the MAP3K family and modulates the JNK and p38 MAPK pathways. Owing to its pleiotropic functions and wide expression, ASK-1 activity is strictly controlled through complex molecular mechanisms, including dimerization and phosphorylation. In response to various cell stresses, including bacterial and viral infections, endoplasmic reticulum stress, reactive oxygen species, calcium influx, and inflammatory signals, ASK-1 is activated via homodimerization and subsequent autophosphorylation. Phosphorylated ASK-

1 activates the JNK and p38 MAPK pathways, which leads to apoptosis (17). Several studies have demonstrated that ASK-1 has various functions in numerous tumors. In skin tumors, ASK-1 facilitated inflammatory cytokine production in macrophages, which caused tumor progression (8). In gastric tumors, excess ASK-1 was detected in transformed tissue, which might decrease cell proliferation through positive feedback signaling (5). In hepatic tumors, the deletion of ASK-1 promoted the occurrence of hepatocellular carcinoma, indicating that ASK-1 functions as an inhibitor of hepatocarcinogenesis (13). However, the function of ASK-1 in gliomas is not well understood, especially its role in TMZ resistance. In the present study, we showed that ASK-1 phosphorylation was reduced in TMZ-treated TR glioma cell lines. Chemical inhibition of ASK-1 increased the IC50 of TMZ and the cell survival rate and inhibited apoptosis in glioma cells following high-dose TMZ treatment. These data indicate that blocking ASK-1 phosphorylation in glioma cells results in TMZ resistance.

Recently, numerous proteins that interact with ASK-1 have been identified, including Trx, PP5, 14-3-3, and Cdc25C. The reduction/oxidation (redox) regulatory protein Trx has two cysteine residues in its active site that are required for activation, and only the inactive, reduced form of Trx can bind to ASK-1 and suppress its kinase activity. When Trx is oxidized, it dissociates from ASK-1, resulting in the activation of ASK-1 by autophosphorylation, which is required for its kinase activity (15). Another negative ASK-1 regulator, PP5, is a serine/threonine phosphatase that specifically interacts with the form of ASK-1 activated by H₂O₂ and dephosphorylates the threonine required for activation, thereby inactivating ASK-1. Thus, PP5 plays an essential role in negative ASK-1 feedback (12). ASK-1 binds to 14-3-3 via Ser966 in its 14-3-3 recognition motif in a phosphorylation-dependent manner; binding to 14-3-3 physically inhibits the phosphorylation of ASK-1 (4). Cdc25C was confirmed to be a binding partner of ASK-1 in an *in vitro* binding assay. During the interphase of the cell cycle, Cdc25C binds to ASK-1 and inversely modulates ASK-1 through the dephosphorylation of Thr838 (3). Our data showed that following overexpression of Trx, PP5, 14-3-3, and Cdc25C, the level of phosphorylated ASK-1 was reduced in TMZ-treated glioma cells. These data implicate these four proteins in the mechanism underlying the TR phenotype of glioma cells through ASK-1 dephosphorylation.

CONCLUSION

This study showed evidence of the TMZ resistance-promoting role of ASK-1 in glioma cells. Reduction of ASK-1 phosphorylation by either chemical inhibition or overexpression of upstream repressors improved TMZ resistance in glioma cells. Our findings provide novel targets for the development of therapeutics for TR gliomas.

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AUTHORSHIP CONTRIBUTION

Study conception and design: KG, ZJ

Data collection: KS, JF, LS

Analysis and interpretation of results: KS, JF, LS

Draft manuscript preparation: KG

Critical revision of the article: ZJ

All authors (KG, KS, JF, LS, ZJ) reviewed the results and approved the final version of the manuscript.

REFERENCES

- Al-Kandari N, Fadel F, Al-Saleh F, Khashab F, Al-Maghrebi M: The thioredoxin system is regulated by the ASK-1/JNK/p38/survivin pathway during germ cell apoptosis. *Molecules* 24(18):3333, 2019
- Baumert BG, Hegi ME, van den Bent MJ, von Deimling A, Gorlia T, Hoang-Xuan K, Brandes AA, Kantor G, Taphoorn MJB, Hassel MB, Hartmann C, Ryan G, Capper D, Kros JM, Kurscheid S, Wick W, Enting R, Reni M, Thiessen B, Dhermain F, Bromberg JE, Feuvret L, Reijneveld JC, Chinot O, Gijtenbeek JMM, Rossiter JP, Dif N, Balana C, Bravo-Marques J, Clement PM, Marosi C, Tzuk-Shina T, Nordal RA, Rees J, Lacombe D, Mason WP, Stupp R: Temozolomide chemotherapy versus radiotherapy in high-risk low-grade glioma (EORTC 22033-26033): A randomised, open-label, phase 3 intergroup study. *Lancet Oncol* 17:1521-1532, 2016
- Cho YC, Park JE, Park BC, Kim JH, Jeong DG, Park SG, Cho S: Cell cycle-dependent Cdc25C phosphatase determines cell survival by regulating apoptosis signal-regulating kinase 1. *Cell Death Differ* 22:1605-1617, 2015
- Goldman EH, Chen L, Fu H: Activation of apoptosis signal-regulating kinase 1 by reactive oxygen species through dephosphorylation at serine 967 and 14-3-3 dissociation. *J Biol Chem* 279:10442-10449, 2004
- Hayakawa Y, Hirata Y, Nakagawa H, Sakamoto K, Hikiba Y, Kinoshita H, Nakata W, Takahashi R, Tateishi K, Tada M, Akanuma M, Yoshida H, Takeda K, Ichijo H, Omata M, Maeda S, Koike K: Apoptosis signal-regulating kinase 1 and cyclin D1 compose a positive feedback loop contributing to tumor growth in gastric cancer. *Proc Natl Acad Sci U S A* 108:780-785, 2011
- Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG: Cancer drug resistance: An evolving paradigm. *Nat Rev Cancer* 13:714-726, 2013
- Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, Gotoh Y: Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 275:90-94, 1997
- Iriyama T, Takeda K, Nakamura H, Morimoto Y, Kuroiwa T, Mizukami J, Umeda T, Noguchi T, Naguro I, Nishitoh H, Saegusa K, Tobiume K, Homma T, Shimada Y, Tsuda H, Aiko S, Imoto I, Inazawa J, Chida K, Kamei Y, Kozuma S, Taketani Y, Matsuzawa A, Ichijo H: ASK1 and ASK2 differentially regulate the counteracting roles of apoptosis and inflammation in tumorigenesis. *EMBO J* 28:843-853, 2009

9. Koshy M, Villano JL, Dolecek TA, Howard A, Mahmood U, Chmura SJ, Weichselbaum RR, McCarthy BJ: Improved survival time trends for glioblastoma using the SEER 17 population-based registries. *J Neurooncol* 107:207-212, 2012
10. Loomba R, Lawitz E, Mantry PS, Jayakumar S, Caldwell SH, Arnold H, Diehl AM, Djedjos CS, Han L, Myers RP, Subramanian GM, McHutchison JG, Goodman ZD, Afdhal NH, Charlton MR: The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: A randomized, phase 2 trial. *Hepatology* 67:549-559, 2018
11. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, Soffietti R, von Deimling A, Ellison DW: The 2021 WHO classification of tumors of the central nervous system: A summary. *Neuro Oncol* 23:1231-1251, 2021
12. Morita K, Saitoh M, Tobiume K, Matsuura H, Enomoto S, Nishitoh H, Ichijo H: Negative feedback regulation of ASK1 by protein phosphatase 5 (PP5) in response to oxidative stress. *EMBO J* 20:6028-6036, 2001
13. Nakagawa H, Hirata Y, Takeda K, Hayakawa Y, Sato T, Kinoshita H, Sakamoto K, Nakata W, Hikiba Y, Omata M, Yoshida H, Koike K, Ichijo H, Maeda S: Apoptosis signal-regulating kinase 1 inhibits hepatocarcinogenesis by controlling the tumor-suppressing function of stress-activated mitogen-activated protein kinase. *Hepatology* 54:185-195, 2011
14. Ostrom QT, Cioffi G, Waite K, Kruchko C, Barnholtz-Sloan JS: CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2014-2018. *Neuro Oncol* 23:iii1-iii105, 2021
15. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H: Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17:2596-2606, 1998
16. Sakauchi C, Wakatsuki H, Ichijo H, Hattori K: Pleiotropic properties of ASK1. *Biochim Biophys Acta Gen Subj* 1861:3030-3038, 2017
17. Schuster S, Feldstein AE: NASH: Novel therapeutic strategies targeting ASK1 in NASH. *Nat Rev Gastroenterol Hepatol* 14:329-330, 2017
18. Shi Z, Chen Q, Li C, Wang L, Qian X, Jiang C, Liu X, Wang X, Li H, Kang C, Jiang T, Liu LZ, You Y, Liu N, Jiang BH: MiR-124 governs glioma growth and angiogenesis and enhances chemosensitivity by targeting R-Ras and N-Ras. *Neuro Oncol* 16:1341-1353, 2014
19. Stupp R, Taillibert S, Kanner AA, Kesari S, Steinberg DM, Toms SA, Taylor LP, Lieberman F, Silvani A, Fink KL, Barnett GH, Zhu JJ, Henson JW, Engelhard HH, Chen TC, Tran DD, Sroubek J, Tran ND, Hottinger AF, Landolfi J, Desai R, Caroli M, Kew Y, Honnorat J, Idbaih A, Kirson ED, Weinberg U, Palti Y, Hegi ME, Ram Z: Maintenance therapy with tumor-treating fields plus temozolomide vs temozolomide alone for glioblastoma: A randomized clinical trial. *JAMA* 314:2535-2543, 2015
20. Tan Z, Song L, Wu W, Zhou Y, Zhu J, Wu G, Cao L, Song J, Li J, Zhang W: TRIM14 promotes chemoresistance in gliomas by activating Wnt/ β -catenin signaling via stabilizing Dvl2. *Oncogene* 37:5403-5415, 2018
21. Ulasov IV, Nandi S, Dey M, Sonabend AM, Lesniak MS: Inhibition of Sonic hedgehog and Notch pathways enhances sensitivity of CD133(+) glioma stem cells to temozolomide therapy. *Mol Med* 17:103-112, 2011
22. Xia J, He Y, Meng B, Chen S, Zhang J, Wu X, Zhu Y, Shen Y, Feng X, Guan Y, Kuang C, Guo J, Lei Q, Wu Y, An G, Li G, Qiu L, Zhan F, Zhou W: NEK2 induces autophagy-mediated bortezomib resistance by stabilizing Beclin-1 in multiple myeloma. *Mol Oncol* 14:763-778, 2020
23. Yan Y, Xu Y, Gao YY, Zong ZH, Zhang Q, Li C, Wang HQ: Implication of 14-3-3 ϵ and 14-3-3 θ / τ in proteasome inhibition-induced apoptosis of glioma cells. *Cancer Sci* 104:55-61, 2013