Biochemical and Biophysical Research Communications 463 (2015) 638–643

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

TAZ promotes temozolomide resistance by upregulating MCL-1 in human glioma cells

Tian Tian [a, c, *, 1], Aimin Li [b, 1], Hong Lu [a, c], Ran Luo [d], Mingzhi Zhang [b], Zhaoming Li [b, c, **]

[a] Department of Neurology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, PR China
[b] Department of Oncology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, PR China
[c] Institute of Clinical Medicine, The First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, PR China
[d] Department of Neurosurgery, Yichang Central People's Hospital and the First Affiliated Hospital of China Three Gorges University, 443002, PR China

ARTICLE INFO

Article history:
Received 22 May 2015
Accepted 30 May 2015
Available online 1 June 2015

Keywords:
TAZ
Temozolomide resistance
Glioma
Apoptosis

ABSTRACT

Temozolomide is a novel cytotoxic agent currently used as first-line chemotherapy for glioblastoma multiforme (GBM). However, intrinsic or acquired chemoresistance to temozolomide remains the greatest obstacle to the successful treatment of human GBM. The principal mechanism responsible for this resistance is largely unknown. In the present study, we showed that expression of transcriptional co-activator with PDZ-binding motif (TAZ) in glioma cells correlated with temozolomide chemoresistance in human glioma cells. Overexpression of TAZ promoted temozolomide resistance in U-87MG cells, whereas knockdown of TAZ expression sensitized temozolomide-resistant U-251MG cells to temozolomide. Further, TAZ inhibits temozolomide induced apoptosis via upregulation of MCL-1 (myeloid cell leukemia 1) and high expression of TAZ predicts a poor prognosis for GBM patients. In conclusion, our results suggest that TAZ had a critical role in the resistance to temozolomide in glioma cells, and it may provide a promising target for improving the therapeutic outcome of temozolomide-resistant gliomas.

1. Introduction

Glioblastoma multiforme (GBM) is the most aggressive malignant primary brain tumor in adults [1,2]. Despite surgical operation combined with radiotherapy and chemotherapy, the prognosis for GBM patients remains poor, with a median survival of ~1 year [1,2]. Temozolomide is one of the most commonly used chemotherapy drugs against GBM that act by inhibiting the proliferation of glioma cells and inducing apoptosis [3,4]. Although it is currently the most promising chemotherapy for GBM, intrinsic or acquired resistance to temozolomide is a major cause of treatment failure in patients with malignant gliomas [5]. Thus, identifying the diverse mechanisms underlying its highly malignant nature and poor response to therapy will be instrumental for developing efficacious therapeutic regimens.

The Hippo pathway plays an important role in cell proliferation, organ size control, and tumorigenesis [6]. The transcriptional co-activator with PDZ-binding motif (TAZ) and Yes-associated protein (YAP) are the primary downstream effectors of the Hippo signaling pathway, and these two effectors can be phosphorylated and inhibited by the Hippo pathway [7]. TAZ plays a key role in the tumor initiation and progression of diverse cancers, including breast, lung, colorectal, ovarian and brain cancer [8–10]. The aberrant overexpression of TAZ has been observed in GBM and its overexpression significantly correlated with poor differentiation in glioma [11]. Studies reported that glioblastoma cell proliferation can be inhibited and cell differentiation promoted by targeting TAZ [12]. Moreover, TAZ has been identified as a core player in driving the mesenchymal differentiation of malignant glioma, which exhibits poor overall survival and treatment resistance [13]. We therefore speculate that TAZ might be involved in the mechanism of acquired chemoresistance to temozolomide in GBM.

In this study, we found that overexpression of TAZ promoted the chemoresistance of human glioma cells to temozolomide by upregulating MCL-1. Furthermore, high levels of TAZ expression were correlated to shorter overall survival in GBM patients.

* Corresponding author. Department of Neurology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, PR China.
** Corresponding author. Department of Oncology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, PR China.
E-mail addresses: tiantian_2016@126.com (T. Tian), zhaomingli2013@163.com (Z. Li).
[1] Contribute equally.

http://dx.doi.org/10.1016/j.bbrc.2015.05.115
0006-291X/

2. Materials and methods

2.1. Cell culture

Human glioblastoma cell lines (T98G, U-138MG, A-172 and U-87MG) were obtained from American Type Culture Collection ATCC (Rockville, MD, USA). Other glioblastoma cell lines (LN382, AM-38, U-251MG and KMG4) were obtained from Biofavor company (Wuhan, China). All cell lines were cultured in Dulbecco's modified Eagle's medium (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), 100 units/ml penicillin, and 0.1 mg/ml streptomycin (Invitrogen, California, USA) in 5% CO_2 atmosphere at 37 °C.

2.2. Cell transfection

The TAZ cDNA was subcloned into plasmid pcDNA4/TO (Invitrogen, California, USA) and the plasmid for overexpression of TAZ were designated as pcDNA4/TO–TAZ. U-87 MG cells were seeded in six-well plate and transfected with vector control pcDNA4/TO or pcDNA4/TO-TAZ by using Lipofectamine 2000 reagent as recommended by the manufacturer (Invitrogen, California, USA). Twenty-four hours after transfections the cells were passaged and selected using 100 μg/ml Zeocin for 2 weeks, and then got the U-87MG/TAZ stable cell lines. Two distinct, non-overlapping small interfering RNA (siRNA) oligonucleotides targeting TAZ (siTAZ#1: 5′-GGAUACAGGAGAAAACGCA-3'; siTAZ#2: 5′-AAACACCCAUGAACAUCAA-3′), MCL-1 (siMCL-1#1: 5′-GGACUUUUAUACCUGUUAU-3′; siMCL-1#2: 5′-GGAGGCCUCGGCCCGGCGATT-3′) and negative control siRNA were synthesized by RiboBio (Guangzhou, China) and cells were transfected with Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA).

2.3. Cell viability assay

Cells were seeded into 96-well culture plates and incubated at 37 °C. Temozolomide was purchased from Sigma (St. Louis, MO) and dissolved in DMSO. After treatment with different concentrations of temozolomide for 72 h, the 20 μL of tetrazolium bromide (5 mg/mL, GE Healthcare) was added to each well and incubated for 4 h at 37 °C. The culture medium was removed and 150 μL of DMSO was added to solubilize the crystals for 20 min at room temperature and the absorbance at 570 nm was read by an ELISA plate reader (Model 680, Bio-Rad, CA). Each temozolomide concentration was tested in triplicate in 96-well plates, and experiments were repeated independently at least three times. The 50% inhibitory concentration (IC50) was calculated with GraphPad Prism software using the sigmoidal dose–response function.

2.4. Establishment of temozolomide-resistant U-251MG cell line

To establish the temozolomide-resistant cell line, U-251MG cells were exposed to a low dose of temozolomide in culture media for 6 months and established temozolomide-resistant cells designated as U-251 MG/TR. IC50 for the growth inhibition of temozolomide to U-251MG and U-251 MG/TR are 18.4 μM and 141.6 μM, respectively.

2.5. RNA isolation and real-time PCR

Total RNA was isolated using the RNeasy mini kit (Qiagen, Germany) and the cDNA was prepared using the SuperScript® III First-Strand Synthesis System (Invitrogen, California, USA). Quantitative PCR was performed using SYBR Green dye on an Applied Biosystems 7300 Real-time PCR system (Applied Biosystems, Foster City, CA).

2.6. Western blot analysis

Western blot analysis was performed as we previously described [14]. Briefly, cells were lysed in cold lysis buffer containing protease inhibitor mixture. Proteins were resolved on SDS-PAGE, and then transferred onto polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences, Piscataway, NJ, USA). The membrane was blocked in TBS-T buffer containing 5% (w/v) non-fat milk at room temperature for 1 h and then probed with antibodies for TAZ (sc-48805, Santa Cruz Biotech), MCL-1 (sc-819, Santa Cruz Biotech) and GAPDH (sc-32233, Santa Cruz Biotech) at 4 °C overnight. Detection was performed with the SuperSignal West Femto Maximum Sensitivity Substrate Trial Kit (Pierce, Rockford, IL, USA). The band images were digitally captured and quantified with a FluorChem FC2 imaging system (Alpha Innotech, San Leandro, CA, USA).

2.7. Apoptosis assays

Cells were plated in six-well plates and treated with 100 μM temozolomide for 6 h, and then cells were washed twice and placed in temozolomide free medium to grow for 48 h. Cell apoptosis was determined by Annexin V/PI fow cytometry assays (BD Biosciences, San Diego, CA) as described previously [15].

2.8. Kaplan–Meier analysis of survival probability using The Cancer Genome Atlas (TCGA) network

Publically available microarray and clinical data of patients with glioma were acquired from The Cancer Genome Atlas (TCGA) [16]. Survival analysis within the glioblastoma data set of the TCGA database (n = 504) was performed using the Kaplan–Meier analysis module of the R2 microarray analysis and visualization platform (http://r2.amc.nl). Clinical data of these patients is available at the Data Portal:https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm?mode=ApplyFilter&showMatrix=true&diseaseType=GBM&tumorNormal=TN&tumorNormal=T&tumorNormal=NT. Gene expression data were collected using Affymetrix® gene chips. The Affymetrix probe-set for TAZ was 202132_at. Kaplan–Meier analysis was conducted online, and the resulting survival curves and P values (log-rank test) were downloaded from the Internet. The cutoff value of 177.3 was used for separating the high and low expression groups of TAZ, which is determined using the online R2 microarray platform algorithm.

2.9. Statistical analysis

All data were expressed as mean ± SEM. Between groups and among groups comparisons were conducted with Student t test and ANOVA, respectively. Mann–Whitney U test is used for nonparametric variables. The Spearman rank correlation test was assessed to verify the association between expression levels of TAZ and their resistance to temozolomide (IC50) in glioma cells. Statistical analysis was performed using GraphPad Prism software version 4.0 (PRISM4) (GraphPad Software Inc, LaJolla, CA), and $P < 0.05$ was considered significant.

3. Results

3.1. Expression of TAZ in glioma cells correlates with their resistance to temozolomide

To determine whether TAZ expression is associated with chemoresistance of glioma cells, we examined the mRNA expression of TAZ and temozolomide sensitivity in eight glioma cell lines. First, the IC50 values of temozolomide, which stand for the

concentration of temozolomide needed for preventing cell proliferation by 50%, were determined in these cell lines. Three of them LN382, T98G, and U138MG, exhibited a higher IC50 than the other five cell lines A-172, AM-38, U-87MG, U-251MG and KMG4 (Fig. 1A). Subsequently, we examined the mRNA expression of TAZ in these cell lines. The correlation between the IC50 values and the relative mRNA expression of TAZ was analyzed. The IC50 values of temozolomide significantly correlated with the expression level of TAZ in these glioma cells (Fig. 1B, Spearman $r = 0.833$, $p = 0.015$). Furthermore, we established temozolomide resistant human glioma cell line (U-251 MG/TR) by exposure of U-251MG cells to a low dose of temozolomide in culture media for 6 months. IC50 for the growth inhibition of temozolomide to U-251MG and U-251 MG/TR are 18.4 μM and 141.6 μM, respectively (Fig. 1C). The expression of TAZ increased significantly in U-251 MG/TR cells compared with parental U-251MG cells (Fig. 1D). These results suggest that TAZ expression correlates with temozolomide resistance in glioma cells.

3.2. TAZ confers resistance to temozolomide in glioma cells

To confirm that overexpression of TAZ is directly responsible for temozolomide resistance in glioma cells, we carried out the following experiments. First, TAZ was overexpressed in a temozolomide sensitive cell line, U-87MG. Results showed that overexpression of TAZ rendered U-87MG cells more resistant to temozolomide. U-87 MG cells transfected with vector control had an IC50 value of 15.2 μM; whereas the IC50 value of U-87MG cells overexpressed with TAZ was 92.4 μM (Fig. 2A). These results indicate that overexpression of TAZ enhances resistance of glioma cells to temozolomide. Second, we knocked down the expression of TAZ in temozolomide-resistant U-251 MG/TR cells by using two distinct, non-overlapping siRNAs targeting TAZ. As shown in Fig. 2B, knockdown of TAZ in U-251 MG/TR cells sensitizes their response to temozolomide treatments ($IC50_{siControl}$: 163.9 μM; $IC50_{siTAZ\#1}$: 18.45 μM; $IC50_{siTAZ\#2}$: 21.01 μM; $P < 0.05$). In summary, these findings suggest that TAZ overexpression confers resistance to temozolomide in glioma cells.

3.3. TAZ inhibits temozolomide induced apoptosis via upregulation of MCL-1

To determine the mechanisms by which TAZ enhances resistance of glioma cells to temozolomide, we analyzed the effect of TAZ overexpression on temozolomide induced apoptosis. U-87 MG cells stably transduced with TAZ were treated with 100 μM temozolomide for 6 h, and then cells were washed twice and placed in temozolomide free medium to grow for 48 h. Cell apoptosis was determined by Annexin V/PI fow cytometry assays. The apoptosis rate of vector control group and TAZ overexpression group were (37.5 ± 6.2)% and (13.1 ± 2.7)%, respectively (*, $P < 0.05$, Fig. 3A). It suggests that overexpression of TAZ inhibits temozolomide induced apoptosis. To study underlying molecular mechanisms by which TAZ inhibits temozolomide induced apoptosis, we analyzed several apoptosis related proteins and found MCL-1, an anti-apoptotic BCL-2 family member, was significantly upregulated in U-87MG cells stably transduced with TAZ (Fig. 3B), whereas the cells with TAZ knockdown showed a reduced MCL-1 expression (Fig. 3C). To determine whether TAZ inhibits temozolomide induced apoptosis via upregulation of MCL-1, we knocked down MCL-1 expression by using two distinct, non-overlapping siRNAs targeting MCL-1 in U-87MG cells stably transduced with TAZ. Cell apoptosis assay showed that knockdown of MCL-1 attenuated the anti-apoptotic advantage conferred by TAZ significantly (*, $P < 0.05$, Fig. 3D). Taken together, these data suggest that TAZ inhibits temozolomide-induced apoptosis via upregulation of MCL-1 in glioma cells.

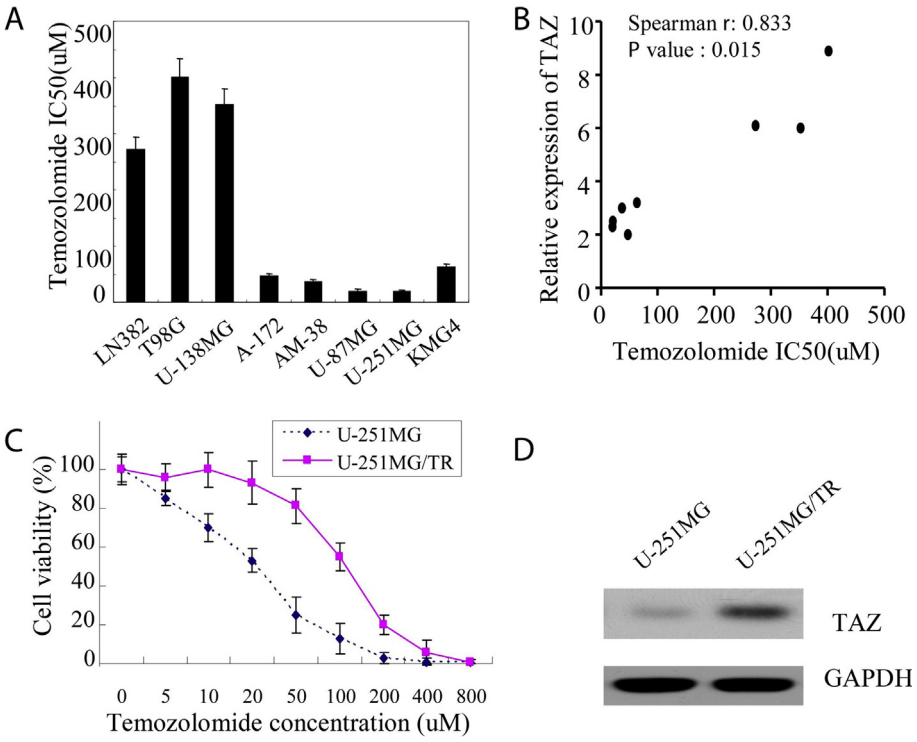

Fig. 1. Correlation between TAZ expression and temozolomide resistance in glioma cells. A, the IC50 values of temozolomide in different glioma cell lines. B, The correlation between the relative TAZ expression and the IC50 values in glioma cells was quantified by Spearman's rank correlation. C, Cell viability was assessed by MTT assay. D, Western blot analysis showed the upregulated expression of TAZ in U-251 MG/TR cells. All experiments were performed in triplicate.

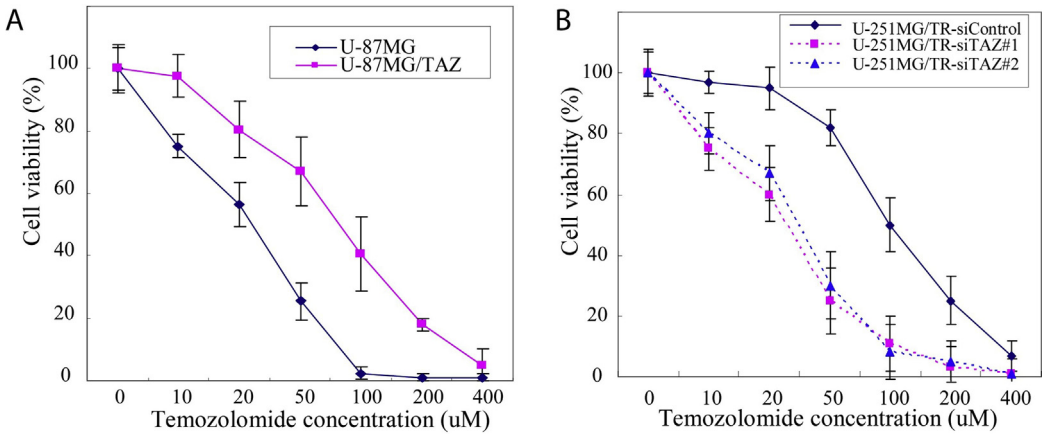

Fig. 2. TAZ confers resistance of glioma cells to temozolomide. Overexpression of TAZ in U-87MG cells (A) or knockdown of the endogenous TAZ expression in U-251 MG/TR cells (B), and then these cells were treated with gradient concentrations of temozolomide for 72 h. The percentage of cell survival as a function of drug concentration was plotted. All data represent the means ± SEM of three replications.

3.4. High TAZ expression indicates a poor prognosis for glioblastoma patients

We next analyzed the clinical outcome data in glioblastoma patients in TCGA database (n = 504). Survival analysis within the TCGA database was performed using the Kaplan–Meier analysis module of the R2 microarray analysis and visualization platform (http://r2.amc.nl). These glioblastoma patients were divided into two groups: high expression (TAZ high; n = 252) and low expression (TAZ low; n = 252). Kaplan–Meier analysis revealed that glioblastoma patients with high expression of TAZ had both a poor progress-free survival (Fig. 4A; P = 0.006) and a worse overall survival (Fig. 4B; P = 0.023). Taken together, these results suggest that high TAZ expression predicts a poor prognosis for glioblastoma patients.

4. Discussion

Temozolomide is one of the most commonly used alkylating agents in the treatment of malignant gliomas; however, its survival benefit remains unsatisfactory because of the intrinsic or acquired chemoresistance of glioma cells [17,18]. In the present study, we report that TAZ promotes temozolomide resistance in glioma cells. Moreover, elevated expression of TAZ indicates a poor prognosis for glioblastoma patients.

TAZ is an important mediator of the Hippo pathway and plays important roles in tumor initiation and progression [19]. It has been revealed that aberrant expression of TAZ is associated with chemoresistance in breast cancer and oral cancer [20,21]. To explore the relationship between TAZ and the temozolomide

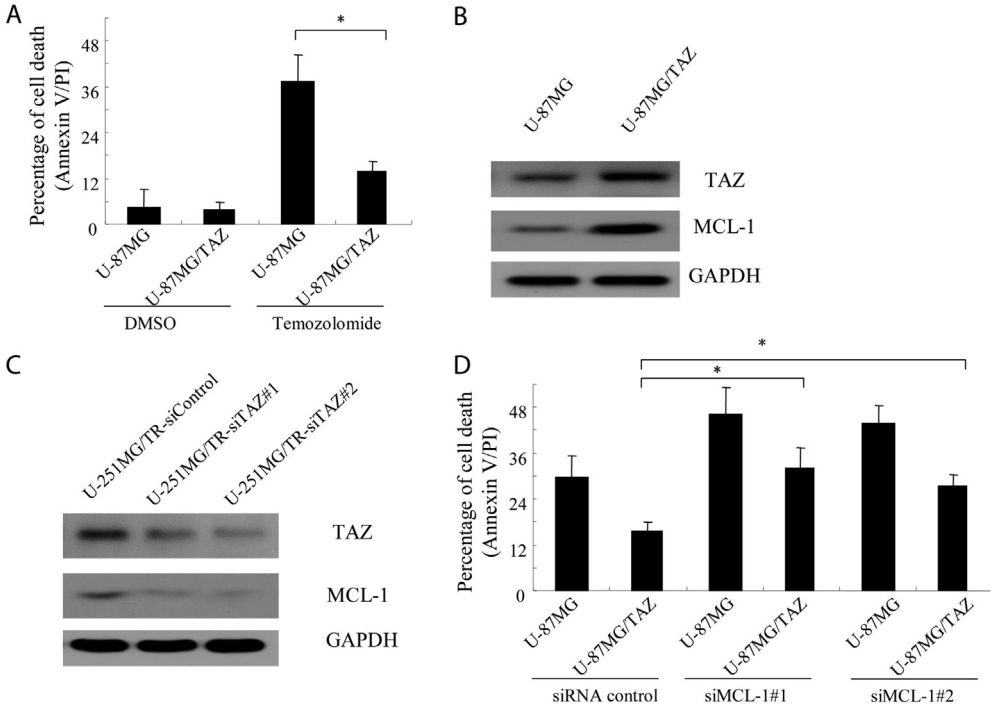

Fig. 3. TAZ inhibits temozolomide induced apoptosis via upregulation of MCL-1. A, U-87MG cells stably transfected with vector control and TAZ were treated with temozolomide and then cell apoptosis was determined by Annexin V/PI assay. B and C, Western blot analysis showed that TAZ upregulated MCL-1 expression in glioma cells. GAPDH was used as an internal control. D, Cell apoptosis assay showed that knockdown of MCL-1 attenuated the anti-apoptotic advantage conferred by TAZ. All data represent the means ± SEM of three replications.

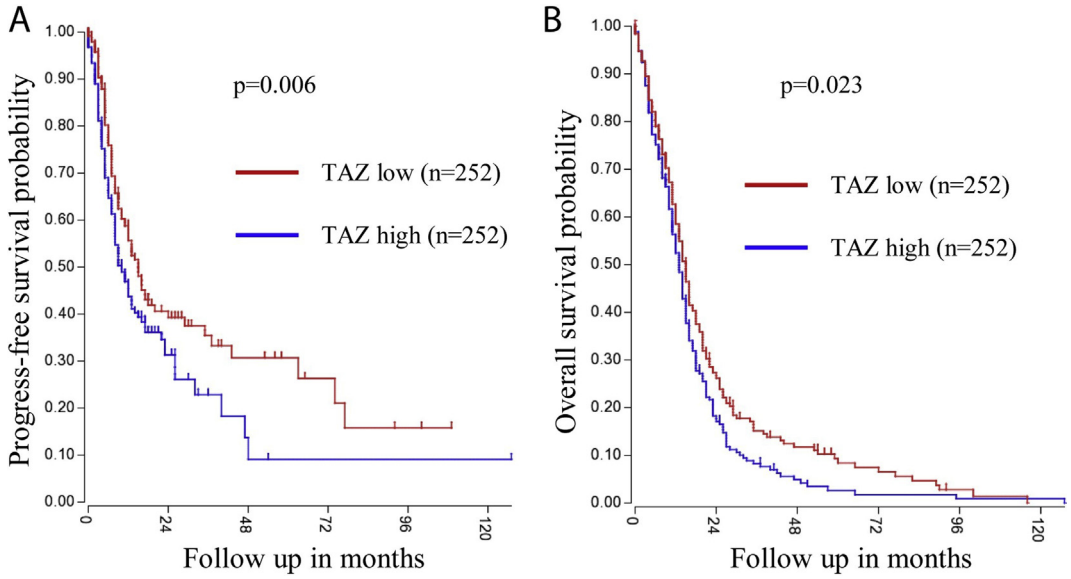

Fig. 4. High TAZ expression indicates a poor prognosis for glioblastoma patients. Kaplan–Meier analysis of glioblastoma patients in TCGA database (n = 504) revealed that patients with high expression of TAZ had a poor progress-free survival (A; P = 0.006) and a worse overall survival (B; P = 0.023).

chemoresistance in glioma cells, we first examined the level of TAZ expression in glioma cell lines and their response to temozolomide. We found that the expression level of TAZ correlated with the chemoresistance to temozolomide in glioma cells. Forced overexpression of TAZ could enhance resistance of glioma cells to temozolomide. By contraries, knockdown of endogenous TAZ sensitized glioma cells to temozolomide.

Temozolomide is known to block the cell cycle and lead to cell apoptosis by targeting nuclear DNA [17]. Dysregulation of apoptosis-regulating genes and proteins is one of the most common mechanisms of temozolomide resistance [5,17]. Therefore, we further explored the possible mechanisms involved in the TAZ-mediated resistance to temozolomide by evaluating the effect of forced TAZ overexpression on temozolomide-induced apoptosis. In this study we showed that overexpression of TAZ rendered U-87MG cells more resistant to temozolomide, and we also found that an anti-apoptotic BCL-2 family member, MCL-1, could be significantly upregulated by TAZ. Previous studies reported that MCL-1 is essential for temozolomide induced cell death in human glioma, and thus may be a target to overcome therapeutic resistance toward temozolomide [22,23]. Consistent with previous studies, our results also showed that TAZ inhibited temozolomide induced apoptosis via upregulation of MCL-1. In addition, interrogation of the TCGA glioblastoma cohort revealed that high TAZ expression predicted a poor prognosis for glioblastoma patients.

In conclusion, our findings demonstrate that the mechanism responsible for resistance of glioma cells to temozolomide is associated with TAZ-mediated upregulation of MCL-1. TAZ may function as an important modifier of the response of glioma cells to temozolomide. A new strategy combining current regimens with compounds targeting TAZ may significantly improve the therapeutic outcome of temozolomide-resistant GBM.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This study was supported by funds from the National Natural Science Foundation of China (81172118, 81301087 and 81402380), China Postdoctoral Science Foundation (2013M540574 and 2014T70689) and Youth Innovation Fund Project of the First Affiliated Hospital, Zhengzhou University (to Tian Tian).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.05.115.

References

[1] M.M. Mrugala, Advances and challenges in the treatment of glioblastoma: a clinician's perspective, Discov. Med. 15 (2013) 221–230.
[2] D.A. Reardon, P.Y. Wen, Therapeutic advances in the treatment of glioblastoma: rationale and potential role of targeted agents, Oncologist 11 (2006) 152–164.
[3] R. Stupp, M.E. Hegi, W.P. Mason, M.J. van den Bent, M.J. Taphoorn, R.C. Janzer, S.K. Ludwin, A. Allgeier, B. Fisher, K. Belanger, P. Hau, A.A. Brandes, J. Gijtenbeek, C. Marosi, C.J. Vecht, K. Mokhtari, P. Wesseling, S. Villa, E. Eisenhauer, T. Gorlia, M. Weller, D. Lacombe, J.G. Cairncross, R.O. Mirimanoff, E.O.f.R.a.T.o.C.B.T.a.R.O. Groups, N.C.I.o.C.C.T. Group, Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial, Lancet Oncol. 10 (2009) 459–466.
[4] R. Stupp, W.P. Mason, M.J. van den Bent, M. Weller, B. Fisher, M.J. Taphoorn, K. Belanger, A.A. Brandes, C. Marosi, U. Bogdahn, J. Curschmann, R.C. Janzer, S.K. Ludwin, T. Gorlia, A. Allgeier, D. Lacombe, J.G. Cairncross, E. Eisenhauer, R.O. Mirimanoff, E.O.f.R.a.T.o.C.B.T.a.R.O. Groups, N.C.I.o.C.C.T. Group, Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma, N. Engl. J. Med. 352 (2005) 987–996.
[5] K. Messaoudi, A. Clavreul, F. Lagarce, Toward an effective strategy in glioblastoma treatment. Part I: resistance mechanisms and strategies to overcome resistance of glioblastoma to temozolomide, Drug Discov. Today (2015 Mar 2) pii: S1359-6446(15)00080-X. http://dx.doi.org/10.1016/j.drudis.2015.02.011. [Epub ahead of print].
[6] A. Errico, Targeted therapies: hippo effector YAP1 inhibition-towards a new therapeutic option to overcome drug resistance, Nat. Rev. Clin. Oncol. 12 (2015) 190.
[7] M. Santucci, T. Vignudelli, S. Ferrari, M. Mor, L. Scalvini, M.L. Bolognesi, E. Uliassi, M.P. Costi, The Hippo Pathway and YAP/TAZ-TEAD Protein-Protein Interaction as Targets for Regenerative Medicine and Cancer Treatment, J. Med. Chem. (2015 Mar 11) [Epub ahead of print].
[8] S. Piccolo, S. Dupont, M. Cordenonsi, The biology of YAP/TAZ: hippo signaling and beyond, Physiol. Rev. 94 (2014) 1287–1312.
[9] M. Kodaka, Y. Hata, The mammalian Hippo pathway: regulation and function of YAP1 and TAZ, Cell. Mol. Life Sci. 72 (2015) 285–306.
[10] X. Varelas, The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease, Development 141 (2014) 1614–1626.

[11] P.D. Li, X.J. Wang, Q. Shan, Y.H. Wu, Z. Wang, Evaluation of TAZ expression and its effect on tumor invasion and metastasis in human glioma, Asian Pac. J. Trop. Med. 7 (2014) 757–760.
[12] J. Yuan, G. Xiao, G. Peng, D. Liu, Z. Wang, Y. Liao, Q. Liu, M. Wu, X. Yuan, MiRNA-125a-5p inhibits glioblastoma cell proliferation and promotes cell differentiation by targeting TAZ, Biochem. Biophys. Res. Commun. 457 (2015) 171–176.
[13] K.P. Bhat, K.L. Salazar, V. Balasubramaniyan, K. Wani, L. Heathcock, F. Hollingsworth, J.D. James, J. Gumin, K.L. Diefes, S.H. Kim, A. Turski, Y. Azodi, Y. Yang, T. Doucette, H. Colman, E.P. Sulman, F.F. Lang, G. Rao, S. Copray, B.D. Vaillant, K.D. Aldape, The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma, Genes Dev. 25 (2011) 2594–2609.
[14] Z. Li, T. Tian, X. Hu, X. Zhang, L. Li, F. Nan, Y. Chang, X. Wang, Z. Sun, F. Lv, M. Zhang, Targeting Six1 by lentivirus-mediated RNA interference inhibits colorectal cancer cell growth and invasion, Int. J. Clin. Exp. Pathol. 7 (2014) 631–639.
[15] Z. Li, T. Tian, X. Hu, X. Zhang, F. Nan, Y. Chang, F. Lv, M. Zhang, Six1 mediates resistance to paclitaxel in breast cancer cells, Biochem. Biophys. Res. Commun. 441 (2013) 538–543.
[16] C.G.A.R. Network, Comprehensive genomic characterization defines human glioblastoma genes and core pathways, Nature 455 (2008) 1061–1068.
[17] T.C. Johannessen, R. Bjerkvig, Molecular mechanisms of temozolomide resistance in glioblastoma multiforme, Expert Rev. Anticancer Ther. 12 (2012) 635–642.
[18] J.R. Silber, M.S. Bobola, A. Blank, M.C. Chamberlain, O(6)-methylguanine-DNA methyltransferase in glioma therapy: promise and problems, Biochim. Biophys. Acta 1826 (2012) 71–82.
[19] M. Kim, T. Kim, R.L. Johnson, D.S. Lim, Transcriptional Co-repressor Function of the Hippo Pathway Transducers YAP and TAZ, Cell Rep. 11 (2015) 270–282.
[20] Z. Li, Y. Wang, Y. Zhu, C. Yuan, D. Wang, W. Zhang, B. Qi, J. Qiu, X. Song, J. Ye, H. Wu, H. Jiang, L. Liu, Y. Zhang, L.N. Song, J. Yang, J. Cheng, The hippo transducer TAZ promotes epithelial to mesenchymal transition and cancer stem cell maintenance in oral cancer, Mol. Oncol. 9 (2015) 1091–1105.
[21] M. Bartucci, R. Dattilo, C. Moriconi, A. Pagliuca, M. Mottolese, G. Federici, A.D. Benedetto, M. Todaro, G. Stassi, F. Sperati, M.I. Amabile, E. Pilozzi, M. Patrizii, M. Biffoni, M. Maugeri-Saccà, S. Piccolo, R. De Maria, TAZ is required for metastatic activity and chemoresistance of breast cancer stem cells, Oncogene 34 (2015) 681–690.
[22] C. Gratas, Q. Séry, M. Rabé, L. Oliver, F.M. Vallette, Bak and Mcl-1 are essential for temozolomide induced cell death in human glioma, Oncotarget 5 (2014) 2428–2435.
[23] R.Y. Li, L.C. Chen, H.Y. Zhang, W.Z. Du, Y. Feng, H.B. Wang, J.Q. Wen, X. Liu, X.F. Li, Y. Sun, D.B. Yang, T. Jiang, Y.L. Li, C.L. Jiang, MiR-139 inhibits Mcl-1 expression and potentiates TMZ-induced apoptosis in glioma, CNS Neurosci. Ther. 19 (2013) 477–483.