TTK Protein Kinase promotes temozolomide resistance through inducing autophagy in glioblastoma

Jian Yu, Ge Gao, Xiangpin Wei and Yang Wang*

Abstract

Background: Temozolomide (TMZ) resistance remains the main therapy challenge in patients with glioblastoma multiforme (GBM). TTK Protein Kinase (TTK) contributes to the radioresistance and chemoresistance in many malignancies. However, the role of TTK in the TMZ resistance of GBM cells remains unknown.

Methods: The expression of TTK was measured by western blot. The proliferation of GBM cells was assessed through MTT assay and clonogenic assay. Cell apoptosis was evaluated using western blot. LC3B puncta were detected using immunohistochemistry staining. The mouse xenograft model was used to investigate the role of TTK in vivo.

Results: Knockdown of TTK increased the sensitivity of GBM cells to TMZ treatment, while overexpression of TTK induced TMZ resistance. Two specific TTK inhibitors, BAY-1217389 and CFI-402257, significantly inhibited GBM cell proliferation and improved the growth-suppressive effect of TMZ. In addition, the knockdown of TTK decreased the autophagy levels of GBM cells. Inhibition of TTK using specific inhibitors could also suppress the autophagy process. Blocking autophagy using chloroquine (CQ) abolished the TMZ resistance function of TTK in GBM cells and in the mouse model.

Conclusions: We demonstrated that TTK promotes the TMZ resistance of GBM cells by inducing autophagy in vitro and in vivo. The use of a TTK inhibitor in combination with TMZ might help to overcome TMZ resistance and improve therapy efficiency in GBM.

Keywords: TTK, Temozolomide, Glioblastoma, Autophagy, Resistance
that TTK participates in cell proliferation and division, and is essential for chromosome alignment at the centromere during mitosis and centrosome duplication [7]. In addition, accumulating evidence indicates that TTK is related to poor prognosis and malignant progression in gastric cancer [8], colon cancer [9], clear cell renal cell carcinoma [10], prostate cancer [11], breast cancer [12, 13], non-small-cell lung cancer [14], and medulloblastoma [15]. Recent research has shown that high TTK mRNA level correlates with earlier development of clinical symptoms, increased tumor aggressiveness, and poor outcome in patients with glioma [16]. The up-regulated TTK promotes proliferation and clonogenicity of glioma stem-like cells (GSCs) in vitro and in vivo [17].

TTK has also been implicated in mediating the radiosensitivity of glioma cells. Chen et al. demonstrate that hepatic leukemia factor (HLF)-mediated miR-132 directly suppresses TTK expression. TTK acts as an oncogene and contributes to the radioresistance of glioma cells [18]. Inhibition of TTK using NMS-P715 enhances radiosensitivity of human GBM cells through impairing DNA repair ability [19]. Additionally, TTK selective inhibitor MPS1-IN-3 has been proved to sensitize glioblastoma cells to antimitotic drugs [20]. However, the role of TTK in the TMZ resistance of GBM remains unclear.

In the current study, we demonstrated that knockdown of TTK sensitizes GBM cells to TMZ treatment, while overexpression of TTK promotes TMZ resistance of GBM. TTK specific inhibitors increased the sensitivity of GBM cells to TMZ. Moreover, TTK facilitated TMZ resistance through inducing autophagy in vitro and in vivo. Our study is beneficial to overcoming TMZ resistance and develops a novel therapeutic method for GBM.

**Methods**

**Cell lines and cell culture**

U87 MG cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). U251 and HEK293T cell was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). U251, U87, and HEK293T were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS, Gibco, Waltham, MA, USA) and 1% penicillin/streptomycin (B540732, Sangon Biotech, Shanghai, China) at 37 °C with 5% CO₂.

**Antibodies and reagents**

Antibodies for TTK (10381–1-AP), β-actin (20536–1-AP) were purchased from Proteintech (Wuhan, China); antibodies for poly [ADP-ribose] polymerase 1 (PARP1, 9532), cleaved caspase 3 (9664), and caspase 3 (9662) were obtained from Cell Signaling Technology (Danvers, MA, USA); antibodies for p62 (ab109012), Ki-67 (ab15580), and LC3B (ab192890) were obtained from Abcam (Cambridge, UK); BAY-1217389 (S8215), Chloroquine (CQ, S6999), Temozolomide (TMZ, S1237), and Thiazolyol Blue (MTT, S6821) were acquired from Selleck Chemicals (Houston, TX, USA). CFI-402257 (A12037) was acquired from Adooq Bioscience (Irvine, CA, USA).

**Western blot**

U251 and U87 cells were lysed using the RIPA Lysis Buffer (AR0105–100, Boster, Pleasanton, CA, USA) containing 1 mM phenylmethylsulfonyl fluoride (PMSF). After being centrifuged, the protein concentration was measured with a bicinchoninic acid (BCA) protein assay kit (C503021, Sangon Biotech). The proteins were electrophoresed on 12–15% SDS–PAGE gels and then transferred onto polyvinylidene fluoride (PVDF) membranes. After that, the membranes were incubated with 5% skimmed milk for 1 h at room temperature (RT), indicated primary antibodies overnight at 4 °C, and then with the appropriate secondary antibodies for 1 h at RT in turn. The blots were imaged with an enhanced chemiluminescence detection system and GE Amersham Imager Al680 (GE, Chicago, IL, USA). β-actin was used as a loading control. Data were analyzed by ImageJ software (US National Institutes of Health).

**Colony formation assay**

Cells (800 cells per well) were seeded in the 6-well plate and incubated for the indicated time. Then, the colonies were stained with 0.1% crystal violet which dissolved in methanol. Colonies with > 50 cells per colony were counted with ImageJ software.

**MTT assay**

Cells were seeded into a 96-well plate at a density of 3000 cells per well. After treatment, the culture medium was replaced by MTT solution and incubated for 4 h at 37 °C. After that, the medium was gently removed, and 200 μL dimethyl sulfoxide (DMSO) was added into each well. Next, the plate was put on a shaker and gently vortexed for 10 min at RT in the dark to fully dissolve the crystals. The absorbance values were measured at 490 nm using a microplate reader (ThermoFisher Scientific, Waltham, MA, USA).

**Plasmid construction**

The PLKO.1 TTK shRNA1 (TRCN0000006358) and PLKO.1 TTK shRNA2 (TRCN0000011012) were purchased from Sigma-Aldrich (St. Louis, MO, USA). PCMV TTK plasmids were generated by Genechem (Shanghai, China). PLKO.1 and PCMV empty plasmids were used as
or not were subcutaneously injected into the nude mouse. The size of the tumor was measured by vernier calipers and calculated as the length × width² × 0.5. When the tumor volumes reached approximately 100 mm³, the animals were randomly divided into 3 groups with 6 animals per group and the treatments were initiated by intraperitoneal injection of temozolomide and/or CQ. Two weeks post injection, the mice were sacrificed to determine the tumor volumes and photographed.

Statistical analysis
All experiments were repeated at least three times. The results were expressed as mean±standard error of the mean (SEM) and were analyzed using SPSS 26.0 software (IBM, Chicago, IL, USA). The comparison between the two groups was conducted with Student’s t-test. For comparisons among multiple groups, one-way analysis of variance (ANOVA) with Fisher’s least significant difference (LSD) test was used. Images were processed using GraphPad Prism 9.00 (GraphPad Software, La Jolla, CA, USA) and Adobe Photoshop CC 22.0 (Adobe, San Jose, CA, USA). p<0.05 was considered as statistically significant.

Results

Knockdown of TTK sensitizes GBM cells to TMZ
To determine the role of TTK in the TMZ sensitivity of GBM cells, two independent TTK shRNAs (shRNA1 and shRNA2) were stably transfected into U251 and U87 cells. The knockdown efficiency of TTK was confirmed using western blot (Fig. 1A). Then, U251 and U87 cells with TTK knockdown were incubated with 100 μM temozolomide (TMZ) for 48 or 72 h. The MTT assay showed that knockdown of TTK significantly decreased the cell viability of U251 and U87 cells treated with TMZ compared with control groups (Fig. 1B). Next, western blot was performed to investigate the apoptosis in U251 and U87 cells with TTK knockdown in response to TMZ. The results demonstrated that cells with TTK knockdown showed an increased level of apoptotic protein cleaved caspase 3 and cleaved PARP1 (Fig. 1C and D). Moreover, the clonogenic assay also showed that knockdown of TTK impaired the colony forming ability of GBM cells upon TMZ treatment (Fig. 1E and F). Therefore, these results suggested that knockdown of TTK increases the TMZ sensitivity of GBM cells.

Overexpression of TTK promotes TMZ resistance in glioma cells
To better clarify the function of TTK in the TMZ resistance of GBM cells, we overexpressed TTK by transfecting the PCMV TTK plasmid into U251 and U87 cells. Western blot showed that the protein level of TTK was dramatically increased in cells transfected with PCMV
TTK (Fig. 2A). Correspondingly, overexpression of TTK increased the cell viability in U251 and U87 cells with TMZ treatment (Fig. 2B). Additionally, overexpression of TTK reduced the protein level of cleaved caspase 3 and cleaved PARP1 induced by TMZ treatment (Fig. 2C and D). The clonogenic assay also confirmed that U251 and U87 cells with TTK overexpression possessed stronger colony forming ability (Fig. 2E and F). Taken together, our results indicated that overexpression of TTK conferred TMZ resistance in GBM cells.
Fig. 2  Overexpression of TTK promoted TMZ resistance in GBM cells. U251 and U87 cells were stably transfected with PCMV (Ctr) or PCMV TTK (TTK), then treated with 100 μM temozolomide (TMZ) for 48 and 72 h. A Western blot was used to determine the protein levels of TTK and β-actin. B MTT assay was conducted to analyze the cell viability. C U251 and U87 cells transfected with PCMV (Ctr) or PCMV TTK (TTK) were treated with 100 μM TMZ for 72 h, western blot was performed to determine the protein levels of cleaved PARP1, cleaved caspase 3, and β-actin. D Quantification of relative protein levels in (C). E Clonogenic assay was performed to assess the colony formation efficiency of U251 and U87 cells stably transfected with PCMV (Ctr) or PCMV TTK (TTK) with or without 20 μM TMZ treatment. F Quantification of the number of clones in (E). The uncropped blots are displayed in Additional file 1. (Data are mean ± SEM, #p > 0.05, *p < 0.05, **p < 0.01, n = 3)
TTK inhibitors significantly decrease glioma cell viability

To examine the effect of TTK inhibitors on the proliferation of GBM cells, U251 and U87 cells were treated with a series of concentrations of BAY-1217389 (BAY) or CFI-402257 (CFI) for 48h. Results from the MTT assay demonstrated that different concentrations of BAY and CFI exhibited a strong growth suppressive effect on U251 and U87 cells in a dose dependent manner (Fig. 3A and B). BAY and CFI have been reported to induce cell apoptosis in a variety of cancer cells. Next, we turned to explore whether BAY and CFI could increase the apoptosis in GBM cells. Western blot results showed that the protein levels of cleaved caspase 3 and cleaved PARP1 were dramatically increased under BAY and CFI treatment in U251 and U87 cells (Fig. 3C and D). Moreover, compared with the control group, BAY and CFI stimulation significantly inhibited the colony forming abilities of GBM cells (Fig. 3E and F). Thus, TTK specific inhibitors significantly inhibited GBM cell proliferation.

TTK inhibitors increase TMZ sensitivity in GBM cells

Next, we tried to investigate whether TTK inhibitors could enhance TMZ sensitivity in glioma cells. U251 and U87 cells treated with different concentrations of TMZ were incubated with BAY and CFI for 48h, and MTT assay was performed to detect cell viability. As shown in Fig. 4A and B, compared with TMZ alone treated group, BAY and CFI in combination with TMZ treatment showed a dramatic decrease of cell viability in U251 and U87 cells. Moreover, the clonogenic assay showed that the colony forming suppressive effect of TMZ and BAY/CFI on GBM cancer cells was significantly better than that of TMZ alone (Fig. 4C-D). Collectively, these findings indicated that TTK inhibitors enhances the lethal effect of TMZ in GBM cells. Combined TTK inhibitors with TMZ might possess a bright future in the clinical treatment of GBM.

TTK promotes TMZ resistance through inducing autophagy

The cellular autophagy process has been shown to play a vital role in the chemoresistance of several cancers [21, 22]. Next, western blot was performed to explore the autophagy levels in U251 and U87 cells with TTK knockdown and overexpression. The results showed that knockdown of TTK significantly decreases the protein level of autophagy marker LC3-II, and increased the expression of autophagy substrate p62 compared with those in the control group. Correspondingly, overexpression of TTK increased the LC3-II level and decreased p62 level in U251 and U87 cells (Fig. 5A and B). TTK inhibitors could also significantly inhibit autophagy level, as indicated by the downregulated LC3-II level and upregulated p62 level in GBM cells (Fig. 5C and D). Meanwhile, immunofluorescence staining of LC3B showed an increased number of LC3B puncta in U251 cells transfected with PCMV TTK (Fig. 5E and F). To clarify the effect of autophagy on TTK-induced drug resistance, CQ was added to inhibit the autophagy process. The MTT assay showed that the TMZ resistant effect of TTK was reversed in U251 and U87 cells treated with CQ (Fig. 5G).

Therefore, these results suggested that overexpression of TTK enhances TMZ resistance via inducing autophagy in GBM cells.

TTK promotes TMZ resistance through autophagy in vivo

Given the in vitro findings, we attempted to investigate the effect of TTK-induced autophagy on TMZ sensitivity in the mouse xenograft model. U251 cells with TTK overexpression or not were subcutaneously injected into the nude mice. Two weeks later, the tumor-bearing mice received intraperitoneal injection of TMZ or and CQ for 15 days. The tumor volumes in cells transfected with PCMV TTK were significantly larger than those in cells transfected with PCMV control, indicating the TMZ resistant function of TTK in vivo. However, the administration of CQ reversed the effect of TTK overexpression (Fig. 6A-C). IHC staining showed that overexpression of TTK increases the protein level of Ki-67 and decreased the level of cleaved caspase 3, while inhibition of autophagy using CQ exhibited the opposite effect (Fig. 6D and E). Thus, our results indicated that TTK confers TMZ resistance through autophagy in the mouse xenograft model.

Discussion

As a dual-specificity protein kinase, TTK has been first proven to play a key role in spindle assembly checkpoint (SAC), and subsequent studies find that TTK is also involved in other processes, such as centrosome duplication, DNA damage response, organ development, tumor progression, chemoresistance, and radioresistance [23]. The expression of TTK is almost undetectable in normal tissues except the testis and placenta. However, TTK is commonly expressed in a variety of tumor tissues, including glioma, making it a potential target for cancer therapies [24, 25]. Inhibition of TTK activity sensitizes basal-like breast cancer to radiation therapy by destroying DNA repair efficiency [26]. TTK is overexpressed in cisplatin-resistant ovarian cancer cells and ovarian cancer patients with acquired resistance to cisplatin. TTK knockdown increases the sensitivity of cisplatin-resistant ovarian cancer cells to cisplatin via the PI3K/AKT signaling pathway [27]. Inhibition of TTK alters cell-cycle progression and exacerbates centrosome abnormalities, thus promoting the radiosensitivity of liver cancer cells in a p21-mediated manner [28]. In this study, we
Fig. 3. TTK inhibitors inhibited cell proliferation of GBM cells. A U251 and U87 cells were treated with different concentrations (0, 0.1, 1, 5, 10, 20, 50, 100 nM) of BAY-1217389 (BAY) for 48 h. MTT assay was used to detect the cell viability. B U251 and U87 cells were treated with different concentrations (0, 0.01, 0.1, 0.5, 1, 2, 5, 10 μM) of CFI-402257 (CFI) for 48 h. MTT assay was used to detect the cell viability. C U251 and U87 cells were treated with BAY (U251, 10 and 20 nM; U87, 20 and 50 nM) or CFI (U251, 1 and 2 μM; U87, 2 and 5 μM) for 48 h, western blot was performed to determine the protein levels of cleaved PARP1, cleaved caspase 3, and β-actin. D Quantification of relative protein levels in (C). E Clonogenic assay was performed to assess the colony formation efficiency of U251 and U87 cells with BAY (U251, 5 nM; U87, 10 nM) or CFI (U251, 0.5 μM; U87, 1 μM) treatment. F Quantification of the number of clones in (E). The uncropped blots are displayed in Additional file 1. (Data are mean ± SEM, *p < 0.05, **p < 0.01, n = 3)
demonstrated that knockdown of TTK decreases the cell viability and increases the apoptosis in GBM cells with TMZ treatment. However, overexpression of TTK reached the opposite conclusions. Overexpression of TTK also decreased the sensitivity of GBM cells to TMZ in the mouse model. Therefore, TTK is a promising therapeutic target and molecular biomarker for effective GBM treatment. Targeted inhibition of TTK may have a bright clinical prospect in the near future.

A variety of TTK inhibitors have been developed to improve the effectiveness of tumor therapy or overcome drug resistance. Up to now, several studies have yielded encouraging results about the anti-proliferative activity of TTK inhibitors in combination with chemotherapy or radiotherapy [29]. CFI-402257, a selective orally bioavailable inhibitor of TTK, displays strong anti-tumor efficiency in combination with cisplatin/pemetrexed in malignant mesothelioma (MM) [30]. Another TTK inhibitor, NTRC0066–0, inhibits the proliferation via inducing chromosome missegregation in cell lines and in mice. NTRC0066–0 combined with a therapeutic dose of docetaxel results in extended tumor remission and doubled survival time in triple negative breast cancer (TNBC) [31]. TTK inhibitors BAY 1161909 and BAY 1217389 in combination with antimitotic cancer drugs achieve obvious enhancement effects over paclitaxel or TTK inhibitor monotherapy through abrogating SAC in a variety of xenograft models [32]. NMS-P715 has been shown to sensitize GBM cells to radiation therapy through impairing DNA double-strand breaks (DSB) and induction of postradiation mitotic catastrophe [19]. Here, we reported that TTK inhibitors BAY 1217389 and CFI-402257 induces cell apoptosis and significantly decreases the proliferation of GBM cells dose-dependently. Additionally, these two inhibitors effectively increased the TMZ sensitivity in GBM. Combined TTK inhibitors and TMZ induced greater cell apoptosis and showed a stronger anti-proliferative effect than the control group. Thus, these results highlight the potential value of TTK inhibitors as therapeutic options to overcome TMZ resistance in GBM. The combination of TMZ with TTK inhibitors may have an attractive therapeutic effect in clinical treatment.

Autophagy is a conserved molecular pathway that eliminates damaged and defective cellular materials, including nucleic acids, proteins, and organelles, via lysosome-mediated degradation [33]. Autophagy plays vital roles in several cellular functions, including tumorigenesis, tumor-stroma interactions, and tumor microenvironment [34]. It is believed that autophagy...
protects the cancer cells from various chemotherapeutics by providing recycled nutrients and energy to cells [35]. The previous study has proved that TMZ induces autophagy in malignant glioma cells and suppression of autophagy using CQ or Bafilomycin A1 (Baf A1) can increase the therapeutic efficacy of TMZ [36]. The relationship between TTK and autophagy in GBM cells remains obscure. In the current study, we demonstrated that TTK knockdown impaires the autophagy and TTK overexpression promoted autophagy in GBM cells. Inhibition of TTK using specific inhibitors also markedly decreased cellular autophagy level. Suppression of autophagy using CQ could counteract the TMZ resistance induced by TTK overexpression in vitro and in a xenograft model. Our results suggest that blocking autophagy could be a promising approach to overcome TMZ resistance in GBM cells with high TTK expression.

Fig. 5 TTK prompted TMZ resistance through inducing autophagy in GBM cells. A Western blot was used to determine the protein levels of LC3-I, LC3-II, p62, and β-actin in U251 and U87 cells transfected with PLKO.1 (Ctr), TTK shRNA1 (sh-1), TTK shRNA2 (sh-2), PCMV (Ctr), or PCMV TTK (TTK). B Quantification of relative protein levels in (A). C Western blot was used to determine the protein levels of LC3-I, LC3-II, p62, and β-actin in U251 and U87 treated with BAY (U251, 10 nM; U87, 20 nM), and CFI (U251, 1 μM; U87, 2 μM) for 12 h. D Quantification of relative protein levels in (C). E Immunofluorescence staining was used to detect LC3B puncta in U251 cells transfected with PCMV (Ctr) or PCMV TTK (TTK). Scale bar: 10 μm. F Quantification of the number of LC3 puncta/cell. G U251 and U87 cells transfected with PCMV or PCMV TTK were treated with TMZ (100 μM) or/and CQ (50 μM) for 48 h or 72 h. The MTT assay was used to detect cell viability. The uncropped blots are displayed in Additional file 1. (Data are mean ± SEM, *p < 0.05, **p < 0.01, n = 3)
Conclusions
In this study, we demonstrated that knockdown of TTK sensitizes GBM cells to TMZ, while overexpression of TTK promotes TMZ resistance in GBM. Two specific TTK inhibitors dramatically decreased GBM cell viability and enhanced the growth suppressive effect of TMZ. Mechanistically, TTK contributed to TMZ resistance through inducing autophagy in vitro and in vivo. Our findings help to reveal the mechanism of TMZ resistance and provide potential solutions for overcoming TMZ resistance in clinical practice.

Fig. 6 Overexpression of TTK induced TMZ resistance through autophagy in vivo. About 5 × 10⁶ U251 cells stably transfected with PCMV (Ctr) or PCMV TTK (TTK) were subcutaneously injected into the nude mice. When the tumor volume reached about 100 mm³, the mice then received intraperitoneal injection of TMZ (50 mg/kg, every day) or/and CQ (30 mg/kg, every day) for 2 weeks. A Representative image of gross tumors from the mice. B Tumor volumes of each group were measured. C The mouse body weight of each group. D Representative images of IHC staining of TTK, Ki-67, and cleaved caspase 3 in tumor tissues. Scale bar: 100 μm. E Quantification of the positive ratio of TTK, Ki-67, and cleaved caspase 3 in (D). (Data are mean ± SEM, *p < 0.05, **p < 0.01, n = 6)

Abbreviations
ATCC: American Type Culture Collection; BCA: Bicinchoninic acid; CQ: Chloroquine; DAB: 3,3′-diaminobenzidine; DMSO: Dimethyl sulfoxide; EDTA: Ethylenediaminetetraacetic acid; FBS: Fetal bovine serum; GBM: Glioblastoma multiforme; HLF: Hepatic leukemia factor; IHC: Immunohistochemistry; Mps1: Monopolar spindle 1; PARP1: Poly [ADP-ribose] polymerase 1; PMSF: Phenylmethylsulfonyl fluoride; PVDF: Polyvinylidene fluoride; RT: Room temperature; SAC: Spindle assembly checkpoint; SEM: Standard error of the mean; TMZ: Temozolomide; TTK: TTK Protein Kinase.
Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12885-022-09899-1.

Additional file 1.

Acknowledgments
Not Applicable.

Authors’ contributions
JY and YW lead the cellular experiments and were major contributors in writing the manuscript. GG lead the animal experiments. XPW assisted in data analysis and manuscript writing. JY and YW designed and arranged the study. All authors read and approved the final manuscript.

Funding
This work was financially supported by the Anhui Provincial Natural Science Foundation of China (No. 1708085QH174).

Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate
Animal experiments were approved by the Animal Research Committee of The First Affiliated Hospital of the University of Science and Technology of China and in accordance with the ARRIVE guidelines. All methods used in the current study were approved by the Ethics Committee of The First Affiliated Hospital of the University of Science and Technology of China and were performed according to the Declaration of Helsinki.

Consent for publication
Not Applicable.

Competing interests
The authors have no conflicts of interest to declare.

Received: 15 February 2022   Accepted: 13 July 2022
Published online: 18 July 2022

References
1. Lyne SB, Yamini B. An alternative pipeline for glioblastoma therapeutics: a systematic review of drug repurposing in glioblastoma. Cancers (Basel). 2021;3(8):1953.
2. Ostrom QT, Cioffi G, Gittleman H, Patil N, Waite K, Kruchko C, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2012-2016. Neuro-Oncology. 2019;21(Suppl 5):v1–v100.
3. Wang M, Zhang C, Wang X, Yu H, Zhang H, Xu J, et al. Tumor treating fields (TTFields)-based cocktail therapy: a novel blueprint for glioblastoma treatment. Am J Cancer Res. 2021;11(4):1069–86.
4. Zhang Z, Yin J, Lu C, Wei Y, Zeng A, You Y. Exosomal transfer of long non-coding RNA SBF2-AS1 enhances chemoresistance to temozolomide in glioblastoma. J Exp Clin Cancer Res. 2019;38(1):166.
5. Combes G, Barysz H, Garand C, Gama Braga L, Alharbi I, Thebault P, et al. Mps1 Phosphorylates Its N-Terminal extension to relieve autoinhibition and activate the spindle assembly checkpoint. Curr Biol. 2018;28(6):872–83.e875.
6. Lan W, Cleveland DW. A chemical tool box defines mitotic and interphase roles for Mps1 kinase. J Cell Biol. 2010;190(1):21–4.
7. Kuijt TEF, Lambers MLA, Weterings S, Ponsioen B, Bolhaqueiro ACF, Staijen DHM, et al. A biosensor for the mitotic kinase MPS1 reveals spatiotemporal activity dynamics and regulation. Curr Biol. 2020;30(19):3862–3870.e3866.
8. Huang H, Yang Y, Zhang W, Liu X, Yang G. TTK regulates proliferation and apoptosis of gastric cancer cells through the Akt-mTOR pathway. FEBS Open Bio. 2020;10(8):1542–9.
9. Zhang L, Jiang B, Zhu N, Tao M, Jun Y, Chen X, et al. Mitotic checkpoint kinase Mps1/TTK predicts prognosis of colon cancer patients and regulates tumor proliferation and differentiation via PKChalpa/ERK1/2 and PI3K/Akt pathway. Med Oncol. 2019;37(1):5.
10. Liu XD, Yao DW, Xin F. TTK contributes to tumor growth and metastasis of clear cell renal cell carcinoma by inducing cell proliferation and invasion. Neoplasma. 2019;66(6):946–53.
11. Chen S, Wang J, Wang L, Peng H, Xiao L, Li C, et al. Silencing TTK expression inhibits the proliferation and progression of prostate cancer. Exp Cell Res. 2019;385(1):111669.
12. Tang J, Lu M, Cui Q, Zhang D, Kong D, Liao X, et al. Overexpression of ASPM, CDC20, and TTK confer a poorer prognosis in breast Cancer identified by gene co-expression network analysis. Front Oncol. 2019;9:310.
13. King JL, Zhang B, Li Y, Li KP, Ni JJ, Saavedra H, et al. TTK promotes mesenchymal signaling via multiple mechanisms in triple negative breast cancer. Oncogenesis. 2018;7(96).
14. Chen X, Yu C, Gao J, Zhu H, Cui B, Zhang T, et al. A novel USP9X substrate TTK contributes to tumorigenesis in non-small-cell lung cancer. Theranostics. 2018;8(9):2348–60.
15. Ailinova I, Ng J, Harris P, Birks D, Donson A, Taylor MD, et al. MPS1 kinase as a potential therapeutic target in medulloblastoma. Oncol Rep. 2016;36(5):2633–40.
16. Kessler AF, Feldheim J, Schmitt D, Feldheim JJ, Monoranu CM, Ernestus RI, et al. Monopolar spindle 1 kinase (MPS1/TTK) mRNA expression is associated with earlier development of clinical symptoms, tumor aggressiveness and survival of glioma patients. Biommedicines. 2020;8(7):192.
17. Wang J, Xie Y, Bai X, Wang N, Yu H, Deng Z, et al. Targeting dual specificity protein kinase TTK attenuates tumorigenesis of glioblastoma. Onco. target. 2019;8(9):3081–8.
18. Chen S, Wang Y, Ni C, Meng G, Sheng X. HLF/mir-132/TTK axis regulates cell proliferation, metastasis and radiosensitivity of glioblastoma cells. Biomed Pharmacother. 2016;83:898–904.
19. Maachani UB, Kramp T, Hanson R, Zhao S, Celiku Q, Shankavaram U, et al. Targeting MPS1 enhances Radiosensitization of human glioblastoma by modulating DNA repair proteins. Mol Cancer Res. 2015;13(5):852–62.
20. Tannous BA, Karami M, Van der Stoop PM, Kwatowski N, Wang J, Zhou W, et al. Effects of the selective MPS1 inhibitor MPS1-IN-3 on glioblastoma sensitivity to antimitotic drugs. J Natl Cancer Inst. 2013;105(7):1322–31.
21. Chang H, Zou T. Targeting autophagy to overcome drug resistance: further developments. J Hematol Oncol. 2020;13(1):159.
22. Mele L, Del Vecchio V, Liccardo D, Prisco C, Schwedtfeger M, Robinson N, et al. The role of autophagy in resistance to targeted therapies. Cancer Treat Rev. 2020;88:102043.
23. Liu X, Winery M. The MPS1 family of protein kinases. Annu Rev Biochem. 2012;81:561–85.
24. Saijo-Hisamimoto A, Katagiri T, Kakuchi S, Nakamura T, Tsunoda T, Nakamura Y. Genome-wide profiling of gene expression in 29 normal human tissues with a cDNA microarray. DNA Res. 2002;9(2):35–45.
25. Yamabuki T, Daigo Y, Kato T, Hayama S, Tsunoda T, Miyamoto M, et al. Genome-wide gene expression profile analysis of esophageal squamous cell carcinomas. Int J Oncol. 2006;28(5):1375–84.
26. Chandler BC, Moubaddel L, Ritter CL, Liu M, Cameron M, Wilder-Romans K, et al. TTK inhibition radiosensitizes basal-like breast cancer through impaired homologous recombination. J Clin Invest. 2020;130(2):958–73.
27. Liu Y, Zhu K, Guan X, Xie S, Wang Y, Tong Y, et al. TTK is a potential therapeutic target for cisplatin-resistant ovarian cancer. J Ovarian Res. 2021;14(1):128.
28. Zhang H, Yao W, Zhang M, Lu Y, Tang J, Jiang M, et al. TTK inhibitor promotes radiosensitivity of liver cancer cells through p21. Biochem Biophys Res Commun. 2021,550:84–91.
29. Xie Y, Wang A, Lin J, Wu L, Zhang H, Yang X, et al. MPS1/TTK: a novel target and biomarker for cancer. J Drug Target. 2017;25(2):112–8.
30. Szymiczek A, Carbone M, Pastorino S, Napolitano A, Tanji M, Minaai M, et al. Inhibition of the spindle assembly checkpoint kinase Mps1/TTK enhances the efficacy of triple-targeted kinase inhibitors on glioblastoma xenografts. Oncogene. 2020;39(1):158–70.
31. Maia AR, de Man J, Boon U, Janssen A, Song JY, Omerzu M, et al. Inhibition of the spindle assembly checkpoint kinase TTK enhances the efficacy of...
of docetaxel in a triple-negative breast cancer model. Ann Oncol. 2015;26(10):2180–92.

32. Wengner AM, Siemeister G, Koppitz M, Schulze V, Kosemund D, Klar U, et al. Novel Mps1 kinase inhibitors with potent antitumor activity. Mol Cancer Ther. 2016;15(4):583–92.

33. Klionsky DJ, Petroni G, Amaravadi RK, Baehrecke EH, Ballabio A, Boya P, et al. Autophagy in major human diseases. EMBO J. 2021;40(19)e108863.

34. Li X, Zhou Y, Li Y, Yang L, Ma Y, Peng X, et al. Autophagy: a novel mechanism of chemoresistance in cancers. Biomed Pharmacother. 2019;119:109415.

35. Lim SM, Mohamad Hanif EA, Chin SF. Is targeting autophagy mechanism in cancer a good approach? The possible double-edge sword effect. Cell Biosci. 2021;11(1):56.

36. Kanzawa T, Germano IM, Komata T, Ito H, Kondo Y, Kondo S. Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells. Cell Death Differ. 2004;11(4):448–57.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.