ACCSCIENCE PUBLISHING

Advanced Neurology

ORIGINAL RESEARCH ARTICLE

In vitro suppression of glioblastoma cell functions by TG100-115, a transient receptor potential melastatin 7 kinase inhibitor

Yuanyuan Xu[1,2†], Wenliang Chen[1,2†], Rahmah Alanazi[1,2], Xinyang Zhang[1,2], Erin Cross[2], Barbara Gundi[1,2], James T. Rutka[1], F. David Horgen[3], Andrea Fleig[4,5], Zhong-Ping Feng[2], and Hong-Shuo Sun[1,2,6,7*]

[1]Department of Surgery, Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada

[2]Department of Physiology, Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada

[3]Department of Natural Science, College of Natural Sciences and Computational Sciences, Hawaii Pacific University, Honolulu, Hawaii, United States of America

[4]John A. Burns School of Medicine and University of Hawaii Cancer Center at the University of Hawaii at Manoa, Honolulu, Hawaii, United States of America

[5]Center for Biomedical Research at The Queen's Medical Center, Honolulu, Hawaii, United States of America

[6]Department of Pharmacology, Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada

[7]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada

(This article belongs to the *Special Issue: Advanced Neurology 3rd Anniversary Special Issue*)

†*These authors contributed equally to this work.*

***Corresponding author:**
Hong-Shuo Sun
(hss.sun@utoronto.ca)

Citation: Xu Y, Chen W, Alanazi R, *et al*. *In vitro* suppression of glioblastoma cell functions by TG100-115, a transient receptor potential melastatin 7 kinase inhibitor. *Adv Neurol*. 2025; 4(3):88-99.
doi: 10.36922/AN025110023

Received: March 14, 2025

Revised: May 26, 2025

Accepted: May 30, 2025

Published online: July 11, 2025

Abstract

Glioblastomas (GBMs) are highly aggressive and lethal primary brain tumors, known for their rapid proliferation, diffuse infiltration, and resistance to conventional therapies. Recent studies have highlighted the involvement of transient receptor potential melastatin 7 (TRPM7) in regulating GBM progression through its dual function as an ion channel and a serine/threonine protein kinase. TG100-115, initially characterized as a phosphoinositide 3-kinase γ/δ inhibitor, has recently been identified as a novel inhibitor of TRPM7 kinase. However, its potential pharmacological effects in GBM cells have not been fully elucidated. In this study, we investigated the anti-GBM effects of TG100-115 in U251 glioma cells. Cell viability and proliferation were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, whereas cell motility and invasiveness were determined through wound healing and transwell assays, respectively. Western blotting was used to detect the expression of key proteins involved in the apoptotic and molecular signaling pathways. Our findings revealed that TG100-115 significantly diminished the viability of U251 cells by promoting apoptosis while concurrently inhibiting the migratory and invasive activities of GBM cells. Mechanistically, TG100-115 enhanced apoptotic signaling by modulating B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein, and cleaved caspase-3 levels. It also altered the phosphorylation status of protein kinase B and cofilin – both critical for cell survival and cytoskeletal dynamics. In conclusion, these findings suggest that TG100-115, by targeting TRPM7 kinase, exhibits promising therapeutic potential for GBM treatment and provides novel insights into targeting TRPM7-associated pathways in aggressive brain tumors.

Keywords: Transient receptor potential melastatin 7 kinase; TG100-115; Glioblastoma; Proliferation; Migration; Invasion

1. Introduction

Glioblastomas (GBMs) are characterized by aggressive growth and invasiveness, significantly affecting patient survival, neurological function, psychological well-being, and overall quality of life.[1] Despite advancements in therapeutic strategies combining surgery, temozolomide chemotherapy, and radiotherapy, GBMs are associated with a poor prognosis, with a median survival of 14.6 months after diagnosis.[2] The ongoing challenges pertaining to drug resistance, the blood–brain barrier, and tumor heterogeneity highlight the need for new therapeutic approaches. Genomic analyses of gliomas have revealed a wide range of deregulated genes with somatic mutations, including *TERT*, *TP53*, *IDH1*, *ATRX*, and *TTN*, among others, providing important insights into glioma pathogenesis.[3-5] Building on these findings, recent studies have advanced the development of therapies targeting critical signaling pathways, including the epidermal growth factor receptor, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) axis, and vascular endothelial growth factor, along with the exploration of immune checkpoint inhibitors.[6] However, the lack of effective treatment options underscores the urgent need for deeper exploration of the molecular mechanisms of GBM to discover novel therapeutic targets.

Transient receptor potential melastatin 7 (TRPM7) is a non-selective divalent cation channel that facilitates the influx of ions such as calcium, magnesium, and zinc. TRPM7 contains an unusual α-kinase domain within its C-terminal region, enabling its dual function as both an ion channel and a kinase.[7] TRPM7 is widely expressed in various tissues and has been implicated in the progression of multiple malignancies, including pancreatic,[8] breast,[9] gastric,[10] and nasopharyngeal cancers,[11] as well as GBM.[12] Accumulating evidence indicates that TRPM7 plays a pivotal role in the progression of GBM. Inhibition of TRPM7 using compounds, such as carvacrol and waixenicin A, has been shown to suppress GBM cell proliferation, migration, and invasion both *in vitro* and *in vivo* while inducing apoptosis in GBM cells.[12,13] Conversely, pharmacological activation of TRPM7 using naltriben enhances the migratory and invasive capabilities of GBM cells, highlighting its pro-tumorigenic function.[14] Mechanistically, TRPM7 regulates key oncogenic pathways, including the PI3K/Akt and mitogen-activated protein kinase (MAPK) kinase (MEK)/extracellular signal-regulated kinase (ERK)[12,13,15,16] pathways, and modulates the expression of matrix metalloproteinase 2, contributing to tumor cell invasion.[17] Furthermore, TRPM7 has been implicated in the maintenance of glioma stem-like cells through signal transducer and activator of transcription 3 and Notch signaling, and is activated by prostaglandin E2 via the prostaglandin E receptor 3/protein kinase A pathway to promote GBM cell proliferation and motility.[18] These findings collectively underscore TRPM7 as a critical regulator of GBM malignancy and a potential therapeutic target. However, the relative contribution of the kinase domain to GBM regulation, as compared to that of the ion channel, remains insufficiently defined.

The kinase domain of TRPM7 retains functional distinctiveness, with unique implications in human cancer pathogenesis, and mediates tumorigenic processes through the phosphorylation of specific substrates.[19] In contrast to the ion channel domain, which primarily mediates cell proliferation, the kinase domain is specifically required for cytoskeletal regulation and metastatic behavior. Notably, deletion or inactivation of this domain significantly reduces migratory and invasive capacities without affecting proliferation.[20,21] Therefore, selectively inhibiting the TRPM7 kinase domain may offer a therapeutic strategy to suppress cancer metastasis while minimizing effects on normal cell growth.

TG100-115, identified for its potent inhibition of TRPM7 kinase in 2017, demonstrates a potency surpassing the previously known TRPM7 kinase inhibitor, rottlerin, by over 70-fold.[22] In addition, TG100-115 significantly inhibits the migratory and invasive abilities of breast cancer cells.[22] TG100-115 was initially characterized as a potent inhibitor of PI3K, exhibiting selective affinity for the PI3K-γ and PI3K-δ isoforms. In rigorous animal models of myocardial infarction, TG100-115 demonstrated significant cardioprotective effects, reducing infarct size and preserving myocardial function.[23] At present, TG100-115 is the only potent inhibitor known to selectively target the TRPM7 kinase domain, offering valuable insights into the kinase-specific pathophysiological role of TRPM7 in GBM. Such inhibitors serve as important pharmacological tools for investigating the biological functions mediated by TRPM7 kinase activity.

In this study, we investigated the impact of TG100-115 on the proliferation, migration, and invasion of the GBM cell line U251. Furthermore, we explored its effects on apoptosis and potential molecular mechanisms, aiming to provide insights into novel therapeutic strategies for GBM treatment.

2. Materials and methods

2.1. Cell culture

Human GBM cell line U251 was obtained from the American Type Culture Collection (United States of America [USA]). Cell culture procedures followed

protocols previously described in earlier studies.[13,17] Briefly, U251 cells were cultured in 60 mm dishes with Dulbecco's Modified Eagle Medium (Gibco, USA), supplemented with 10% fetal bovine serum (Gibco, USA) and antibiotics (100 U/mL penicillin and streptomycin; Gibco, USA), under standard conditions of 5% carbon dioxide and 95% humidity at 37°C.

2.2. Cell viability and proliferation assay

U251 cells were plated onto 96-well plates at a density of 3,000 cells per well, with 100 μL of culture medium. After 24 h of attachment, the cells were subjected to another 24-h treatment with either dimethyl sulfoxide (DMSO; 0.1%, vehicle control) or TG100-115 at various concentrations (30, 60, 120, 180, and 240 μM). Subsequently, 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (5 mg/mL) was added to each well, and the plates were incubated for 3 h at 37°C with 5% carbon dioxide. Throughout the incubation period, mitochondrial enzymes converted the yellow MTT into insoluble purple formazan. Following this, the medium was aspirated, and the formazan was dissolved in 100 μL of DMSO. Cell viability was assessed using a microplate reader (Synergy H1, Biotek, USA) by measuring absorbance at 490 nm, which corresponds to the formazan content. The cell viability was then calculated as a percentage relative to the control.

2.3. Calculation of half-maximal inhibitory concentration (IC_{50}) values

The half-maximal IC_{50} values of TG100-115 following 24-h incubation in U251 cells were calculated using non-linear regression analysis in GraphPad Prism 10. Log-transformed concentrations of TG100-115 and the corresponding cell viability data were fitted to a sigmoidal dose-response curve (with variable slope) to determine the IC_{50} value.[24]

2.4. Cell migration assay

Cell migration was measured using a wound healing assay, following established protocols.[12,13,17] Briefly, U251 cells were cultured in 24-well plates at a density of 1×10^5/mL per well. A monolayer of cells was scratched with a sterile 200 μL pipette tip to create a wound gap. After rinsing the wells with phosphate-buffered saline to remove detached cells, cultures were treated with either DMSO (0.1%) or TG100-115 at various time points (24, 48, and 72 h) or various concentrations. For each well, images from four different fields were captured using a digital camera connected to an Olympus phase-contrast microscope (×10 objective; CKX41, Olympus, Japan). The wound gap was quantified using the wound healing tool in NIH ImageJ (USA), and wound closure was determined as outlined in prior protocols.[12,13,17]

2.5. Matrigel invasion assay

The transwell assay was performed following the manufacturer's protocol. To evaluate the invasive capacity of U251 cells, BioCoat Matrigel invasion chambers equipped with 8-μm polycarbonate nucleopore filters (Cat. 354480, BD BioSciences, USA) were utilized. After being treated with DMSO (0.1%) or TG100-115 (150 μM) for 24 h, 100 μL of cells (2.5×10^4 cells/mL) in serum-free Dulbecco's Modified Eagle Medium were added to the upper compartment of the chamber. As a chemoattractant, 600 μL of complete medium was added to the lower chamber. Cells that invaded through the Matrigel-coated membrane to the underside of the insert were subsequently fixed with absolute ethanol and stained using 1% toluidine blue. Representative fields were imaged under a phase-contrast microscope (CKX41, Olympus, Japan), and the number of invading cells was quantified using NIH ImageJ (USA) via the cell counting module.

2.6. Western blotting

U251 cells were cultured in 35 mm dishes and grown until reaching approximately 60% confluency. Cells were then treated with either 0.1% DMSO or 150 μM TG100-115 in fresh culture medium and incubated for 24 h. Following treatment, cells were rinsed twice with ice-cold phosphate-buffered saline to remove the residual serum. Western blotting analysis was performed as previously described.[12,13,17] After blocking, the membranes were incubated overnight at 4°C with primary antibodies: Anti-cleaved caspase-3 (1:1,000; 9661S, CST, USA), anti-B-cell lymphoma 2 (Bcl-2; 1:1,000; 3498S, CST, USA), anti-B-cell lymphoma 2-associated X protein (Bax; 1:1,000; 2772S, CST, USA), anti-phosphorylated-Akt (p-Akt; 1:1,000; 9271S, CST, USA), anti-Akt (t-Akt; 1:1,000; 2920S, CST, USA), anti-phosphorylated-ERK1/2 (p-ERK1/2; 1:1,000; 5726S, CST, USA), anti-ERK1/2 (t-ERK1/2; 1:1,000, 4695S, CST, USA), anti-glyceraldehyde-3-phosphate dehydrogenase (1:5,000; 2118S, CST, USA), anti-phosphorylated-cofilin (p-cofilin; 1:1,000; ab12866, Abcam, USA), anti-cofilin (t-cofilin;, 1:1,000; ab134963, Abcam, USA), and anti-TRPM7 (1:1,000; ab85016, Abcam, USA). On the following day, mouse (1:5,000; 7076S, CST, USA) or rabbit (1:10,000; 7074S, CST, USA) secondary antibodies were applied, respectively. Protein bands were detected using a chemiluminescence reagent system (iBright Imaging System FL1500, Thermo Fisher Scientific, USA). Band intensities were quantified using NIH ImageJ (USA) for densitometric analysis.

2.7. Statistical analysis

All data are presented as means ± standard error of the mean. Student's *t*-test was employed to compare the control and treatment groups. One-way analysis of variance, followed by the Newman–Keuls *post hoc* test, was utilized to ascertain statistical significance for multiple comparisons. $p<0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. TG100-115 reduced U251 cell viability and inhibited cell proliferation

We assessed the impact of TG100-115 on U251 cell viability and proliferation through an MTT assay. As shown in Figure 1A and B, our findings show that the 24-h treatment with TG100-115 notably decreased U251 cell viability in a dose-dependent manner ($p<0.001$, $n = 4$), with an IC_{50} of 155.2 μM. The cell proliferation over time is illustrated in Figure 1C. In the control group, cell proliferation increased progressively over the culture period, reaching 108.0 ± 4.4%, 255.9 ± 5.7%, and 383.8 ± 5.4% at 24, 48, and 72 h, respectively. In contrast, treatment with TG100-115 (30 – 240 μM) significantly inhibited U251 cell proliferation at 24, 48, and 72 h, compared to the control group ($p<0.001$, $n = 3$). In Figure S1, numerous U251 cell colonies were observed in the control group 7 days after seeding in six-well plates, as indicated by the crystal violet staining. Treatment with TG100-115 (50 μM) resulted in a significant reduction in U251 cell colony formation to 47.0 ± 4.3%, compared to that observed in controls, 100.0 ± 5.1% ($p<0.01$, $n = 3$). These consistent findings across multiple assays and time points provided strong evidence that TG100-115 effectively inhibited U251 cell viability and proliferation.

3.2. TG100-115 induced apoptosis in U251 cells

We further investigated whether TG100-115 reduces the viability of U251 cells by promoting apoptosis. Cell images were captured 24 h after treatment with TG100-115 (30 –

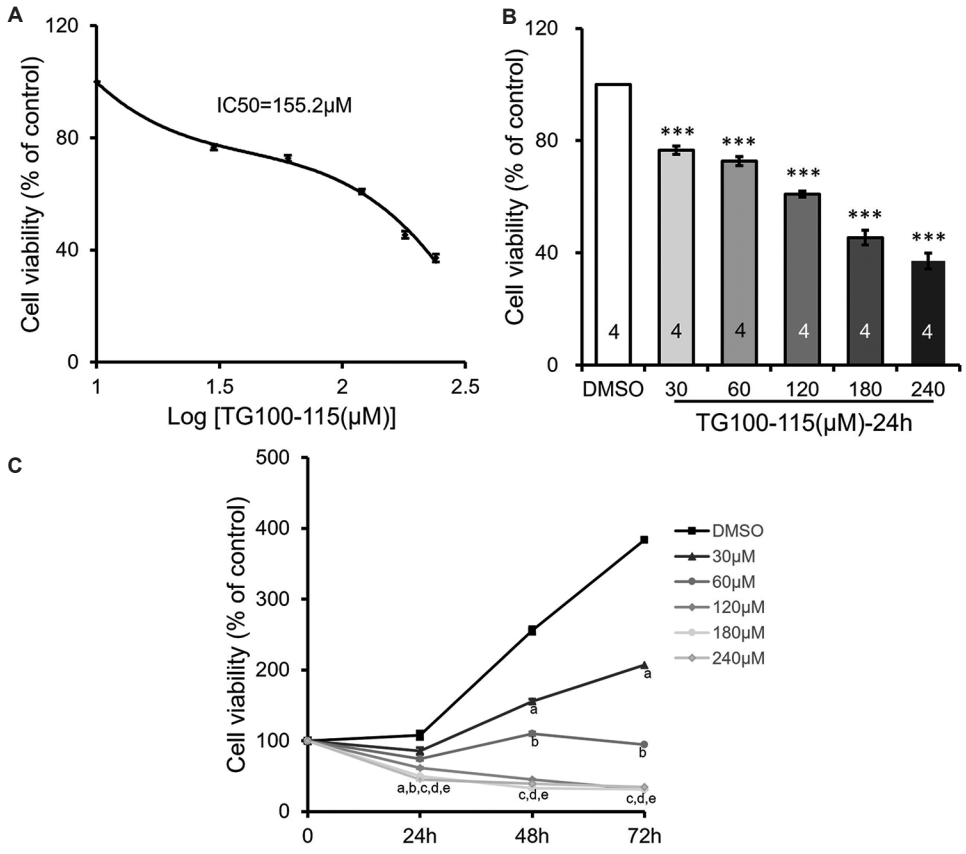

Figure 1. TG100-115 reduced the cell viability and proliferation of U251 cells. (A and B) U251 cells were treated with TG100-115 from 30 μM to 240 μM for 24 h. An MTT assay was used to evaluate the cell viability, and IC_{50} was calculated ($n = 4$). TG100-115 (30 – 240 μM) significantly inhibited U251 cell viability after 24 h (***$p<0.001$ versus DMSO; one-way analysis of variance with subsequent Newman–Keuls test, $n = 4$). (C) TG100-115 inhibited the proliferation of U251 cells. U251 cells were treated with TG100-115 from 30 μM to 240 μM for 24, 48, and 72 h, then an MTT assay was used to measure the proliferation (a, b, c, d, and e represent 30, 60, 120, 180, and 240 μM TG100-115 versus DMSO, respectively, $p<0.001$, $n = 3$).
Abbreviations: DMSO: Dimethyl sulfoxide; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

240 μM), revealing significant damage and the presence of visualized vacuoles when compared to the control group (Figure 2A, $n = 4$). This suggests that TG100-115 treatment resulted in morphological changes in the U251 cells.

To assess apoptosis, we conducted western blot analysis of cleaved caspase-3, Bcl-2, and Bax. The representative images are shown in Figure 2B, the raw blot images are provided in Figures S5–8. Cleaved caspase-3, an active form of the apoptosis-related cysteine peptidase,[25] showed a significantly higher ratio in TG100-115 (150 μM)-treated cells (34.5 ± 2.6%) compared to the control group (6.4 ± 0.7%, $p<0.01$, $n = 4$), as demonstrated in Figure 2C. Bcl-2 and Bax, pivotal regulatory proteins in apoptosis,[26] were also analyzed. Bcl-2, an anti-apoptotic protein, exhibited a significantly lower ratio in TG100-115 (150 μM)-treated cells (14.2 ± 5.3%) compared to the control group (48.5 ± 3.8%, $p<0.05$, $n = 4$), as shown in Figure 2D. Conversely, Figure 2E illustrates a significant increase in Bax level (97.4 ± 13.3%) after treatment with TG100-115 (150 μM) for 24 h, compared to the control group (45.7 ± 9.3%, $p<0.05$, $n = 4$). These findings collectively suggest that TG100-115 induces apoptosis in U251 cells.

3.3. TG100-115 suppressed U251 cell migration

To evaluate the impact of TG100-115 on the migratory behavior of U251 cells, a wound healing assay was performed. In Figure 3, cell images were captured at 0, 4, 8, 16, and 24 h following treatment with either DMSO (0.1%) or TG100-115 (150 μM), and the wound closure rate was analyzed. At 4, 8, 16, and 24 h, the control group exhibited wound closure rates of 14.8 ± 1.4%, 37.4 ± 1.4%, 48.3 ± 1.8%, and 66.1 ± 2.6%, respectively, all of which were notably faster than the TG100-115-treated group at the corresponding time points: 8.6 ± 1.2%, 20.2 ± 2.3%, 28.3 ± 3.4%, and 41.5 ± 2.2% ($p<0.01$, $p<0.001$, $n \geq 6$). This result suggests a significant and time-dependent reduction in the migratory capability of U251 cells treated with TG100-115 when compared to the control group. In Figure 4, we further investigated the migration of U251 cells with various doses of TG100-115 (60, 120, 180 μM) for 24 h, using DMSO (0.1%) as the control. The wound closure rate in the control group was 66.1 ± 2.6%, while in the treatment groups, it was significantly reduced to 48.8 ± 3.7%, 43.2 ± 3.7%, and 37.4 ± 1.4%, respectively ($p<0.01$, $p<0.001$, $n \geq 6$). This result indicates a significant reduction in the migration of U251 cells treated with TG100-115 compared to the control across different doses.

3.4. TG100-115 inhibited U251 cell invasion

The Matrigel transwell assay was utilized to assess U251 cell invasion. As depicted in Figure 5, U251 cell invasion was significantly reduced after 24-h exposure to TG100-115 at 150 μM. The relative invasiveness decreased from 100.0 ± 8.8% to 53.8 ± 3.8% with TG100-115 treatment ($p<0.01$, $n = 6$). This result indicates that, *in vitro*, TG100-115 diminishes the invasion of U251 cells.

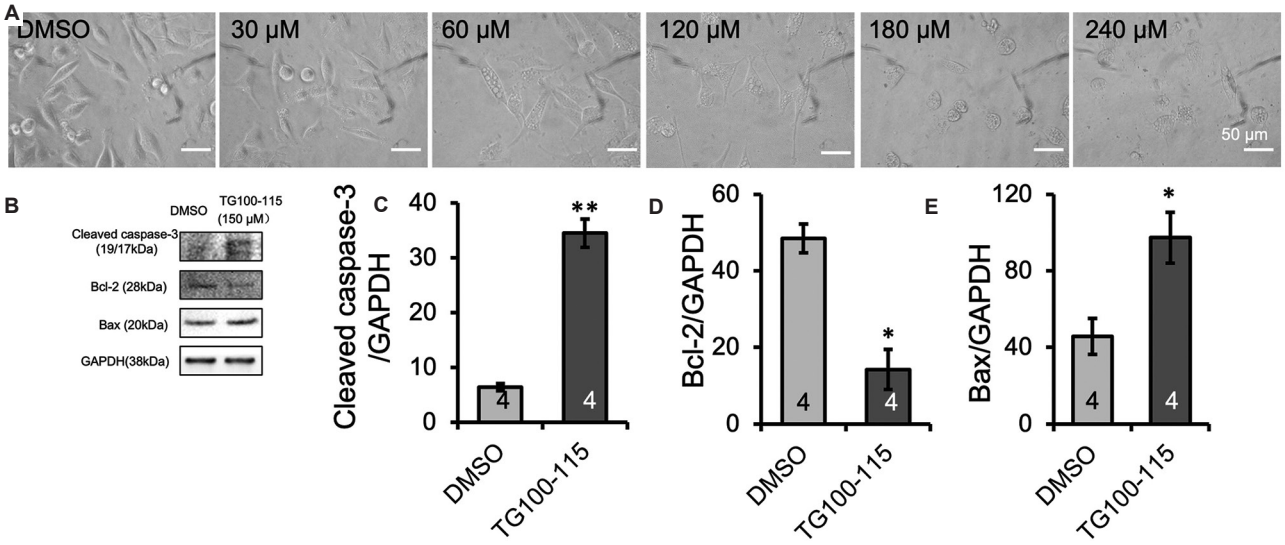

Figure 2. TG100-115 induced apoptosis in U251 cells. (A) Representative images of U251 cells with or without TG100-115 (30 – 240 μM) treatment for 24 h. Scale bar: 50 μm, magnification ×20. U251 cells treated with TG100-115 significantly showed cell damage and the presence of vacuoles when compared to the control group. (B) Representative images of western blotting results. (C) TG100-115 significantly increased the ratio of cleaved caspase-3/GAPDH. (D) TG100-115 significantly reduced the ratio of Bcl-2/GAPDH. (E) TG100-115 significantly increased the ratio of Bax/GAPDH.
Note: Statistical significance determined at *$p<0.05$ and **$p<0.01$ using Student's *t*-test, $n = 4$.
Abbreviations: Bax: B-cell lymphoma 2-associated X protein; Bcl-2: B-cell lymphoma 2; DMSO: Dimethyl sulfoxide; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

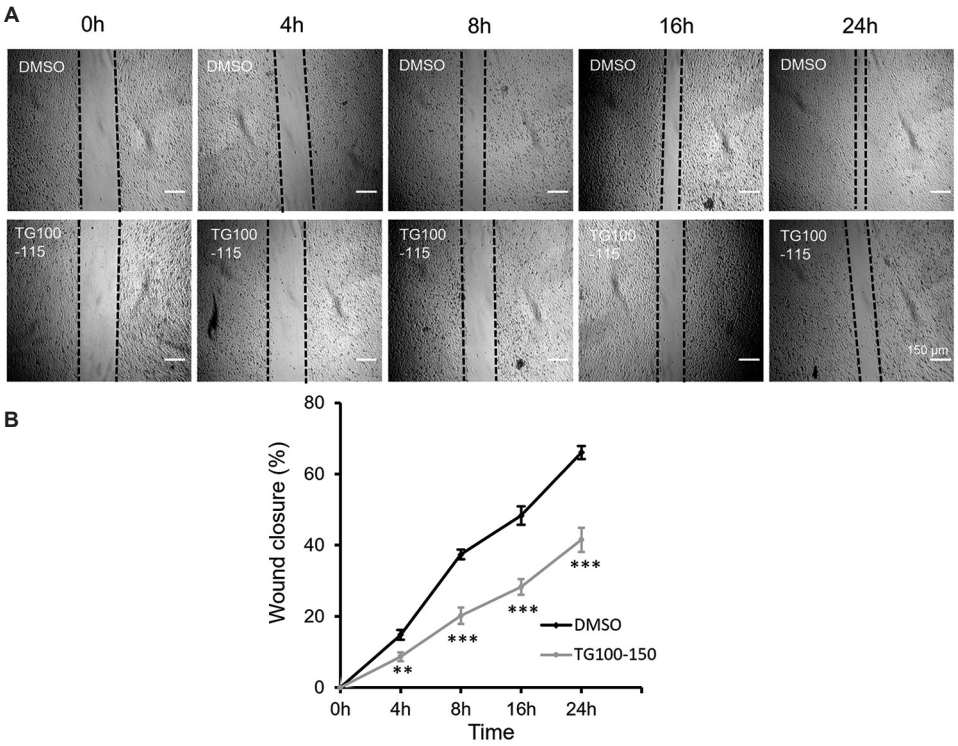

Figure 3. TG100-115 inhibited the migration of U251 in a time-dependent manner. (A) Representative images of wound healing. After being scratched with a 200 μL pipette tip, U251 cells were treated with TG100-115 (150 μM) or 0.1% DMSO. Then, the images were captured at 0, 4, 8, 16, and 24 h, and the gap closure was analyzed. Scale bar: 150 μm, magnification: ×10. (B) The wound closure of the TG100-115 treatment groups was significantly decreased compared to DMSO at the corresponding time points.
Note: Statistical significance determined at $^{**}p<0.01$ and $^{***}p<0.001$ versus DMSO using one-way analysis of variance with subsequent Newman–Keuls test, $n \geq 6$.
Abbreviation: DMSO: Dimethyl sulfoxide.

3.5. TG100-115 downregulated PI3K/Akt signaling

The PI3K/Akt and MAPK/ERK signaling pathways play key roles in regulating the proliferation, migration, and invasion of GBM cells.[16,27,28] Consequently, we explored the phosphorylation status of Akt and ERK1/2. In Figure 6A, TG100-115 (150 μM) markedly reduced p-Akt protein levels in U251 cells while leaving t-Akt protein levels unchanged, the raw blot images are provided in Figures S9-10. In Figure 6B, the p-Akt/t-Akt ratio was significantly reduced in TG100-115-treated cells (4.1 ± 1.1%) compared to the control group (21.1 ± 1.8%, $p<0.001$, $n = 4$). This indicates that TG100-115 treatment led to a notable decrease in the p-Akt/Akt ratio, suggesting a potential impact on the activity of the PI3K/Akt signaling pathway.

Subsequently, the protein levels of p-ERK1/2 and t-ERK1/2 were assessed. In cells treated with TG100-115 (150 μM), there was no significant alteration observed in the protein levels of both p-ERK1/2 and t-ERK1/2 compared to the TG100-115 group. Quantification of the p-ERK1/2/t-ERK1/2 ratio revealed no significant difference in TG100-115-treated cells (120.7 ± 6.7%) when compared to the control group (138.2 ± 10.1%, $p>0.05$, $n = 4$), as illustrated in Figure 6D, the raw blot images are provided in Figures S11-12. These findings suggest that TG100-115 did not affect the MAPK/ERK signaling pathway.

3.6. TG100-115 upregulated phosphorylation of cofilin

Cofilin, an actin-interacting protein capable of breaking down actin filaments, undergoes regulatory control through phosphorylation, leading to its inactivation. This dynamic regulation is essential for the proper functioning of cellular processes involving cytoskeletal restructuring and cellular motility.[29] Here, we investigated the effect of TG100-115 on cofilin regulation. In Figure 6A, the result revealed that TG100-115 treatment (150 μM) for 24 h increased the phosphorylation level of cofilin, with no change in total cofilin (t-cofilin), the raw blot images are provided in Figures S13-14. In Figure 6D, the ratio of p-cofilin/t-cofilin was significantly higher in TG100-115-treated cells (241.5 ± 22.8%) compared to the control group (34.7 ± 18.0%, $p<0.01$, $n = 4$). This indicates that TG100-115 influences the phosphorylation status of cofilin, shedding

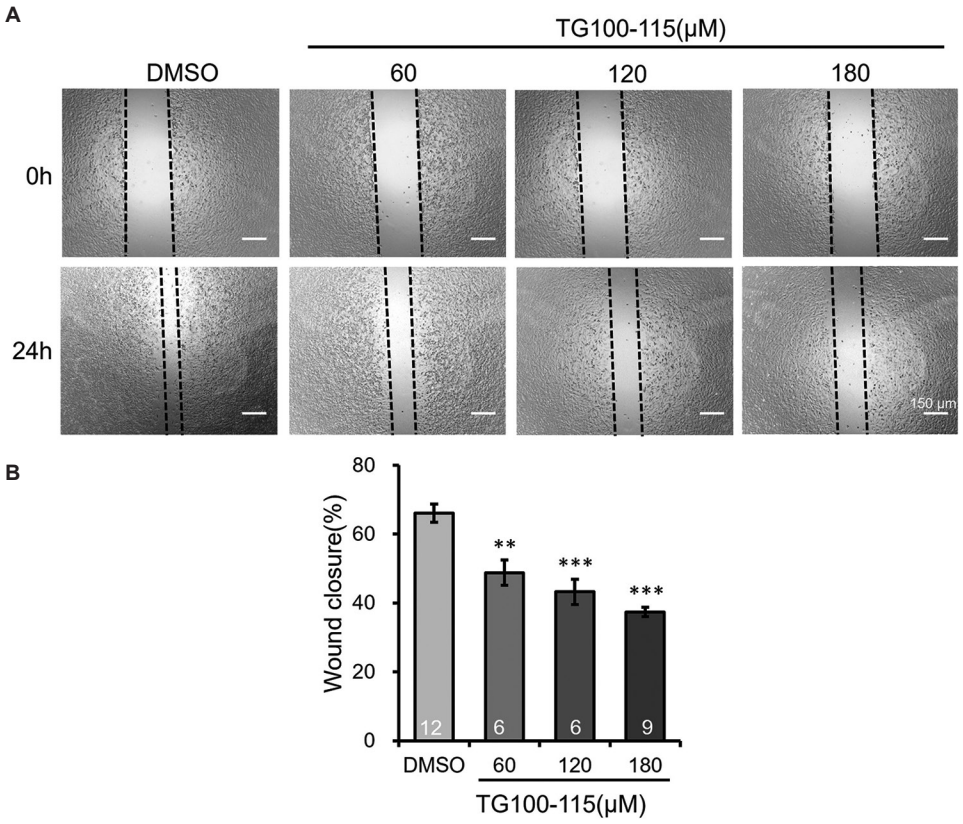

Figure 4. TG100-115 inhibited the migration of U251 cells at different doses for 24 h. (A) Representative images of wound healing. After being scratched with a 200 μL pipette tip, U251 cells were treated with TG100-115 at doses of 60, 120, 160 μM or 0.1% DMSO. Then, the images were captured at 24 h, and the gap closure was analyzed. Scale bar: 150 μm, magnification: 10×. (B) The wound closure of TG100-115 treatment at different doses was significantly decreased compared with DMSO.
Note: Statistical significance determined at $^{**}p<0.01$ and $^{***}p<0.001$ using one-way analysis of variance with subsequent Newman–Keuls test, $n \geq 6$.
Abbreviation: DMSO: Dimethyl sulfoxide.

light on its potential role in cellular processes associated with migration and invasion.

3.7. TG100-115 did not affect the protein expression of TRPM7

Finally, we investigated the impact of TG100-115 treatment on the protein expression of TRPM7 in U251 cells. Following a 24-h exposure to either DMSO (0.1%) or TG100-115 (150 μM), we assessed TRPM7 expression through western immunoblotting. As shown in Figure S2, the raw blot images are provided in Figure S16, TG100-115 exhibited no significant influence on the protein expression of TRPM7 when compared to the control group (19.1 ± 3.8% vs. 20.3 ± 4.6%, respectively, $p>0.05$, $n = 4$). This suggests that TG100-115 did not markedly influence the protein expression of TRPM7 under the conditions examined in U251 cells.

4. Discussion

This study demonstrates the role of TG100-115 in GBM cell biological functions. Our results showed that TG100-115 significantly reduced the viability and proliferation of U251 cells in a dose-dependent manner, with an IC_{50} of 155.2 μM. In addition, TG100-115 exhibited substantial inhibitory effects on U251 cell migration and invasion. Furthermore, we found that the anti-GBM activities of TG100-115 were involved in PI3K/Akt and cofilin-dependent signaling. In this study, experiments were initially performed using both U87 and U251 glioma cell lines, with preliminary data obtained from both models. However, due to variability in experimental conditions and inconsistencies in U87 cell status, only the U251 cell data have been reported here. Nevertheless, the relevant results from U87 cells are included in Figure S3, raw blot images are provided in Figure S17. This data demonstrated that TG100-115 significantly reduced U87 cell viability, migration, colony formation, and Akt phosphorylation, consistent with the findings observed in U251 cells. Thus, the results from both cell lines support the conclusion that TG100-115 exerts anti-GBM effects via similar mechanisms.

Our study showed that TG100-115 attenuated PI3K/Akt signaling. Akt, the central molecule of PI3K/Akt signaling, undergoes activation by phosphorylation, promoting tumor cell survival, proliferation, migration, invasion, and treatment resistance.[30] Recently, Akt was identified as a direct substrate of TG100-115,[31] and kinase-deficient murine neutrophils confirmed the essential role of TRPM7 kinase in neutrophil function via Akt/mTOR signaling.[32] Our findings exhibited that TG100-115 markedly reduced Akt phosphorylation. It should be noted that TG100-115 was previously recognized as a potent PI3K inhibitor, particularly targeting the PI3K-γ and δ isoforms.[22,23,32] We performed MTT and wound-healing assays using PIK-294, a selective blocker of PI3K p110δ. A PI3K p110δ inhibitor was specifically used because GBMs express a low level of PI3K p110γ.[33] In Figure S4A, our data revealed that PIK-294 significantly reduced the viability of U251 cells at higher doses (120 and 180 μM), but not at lower doses (10, 30, and 60 μM), with 24-h incubation. In contrast, TG100-115 significantly reduced cell viability even at a lower dose of 30 μM after 24 h of incubation. This data suggests that the PI3K inhibitory activity of TG100-115 could partially affect the reduction of cell viability.

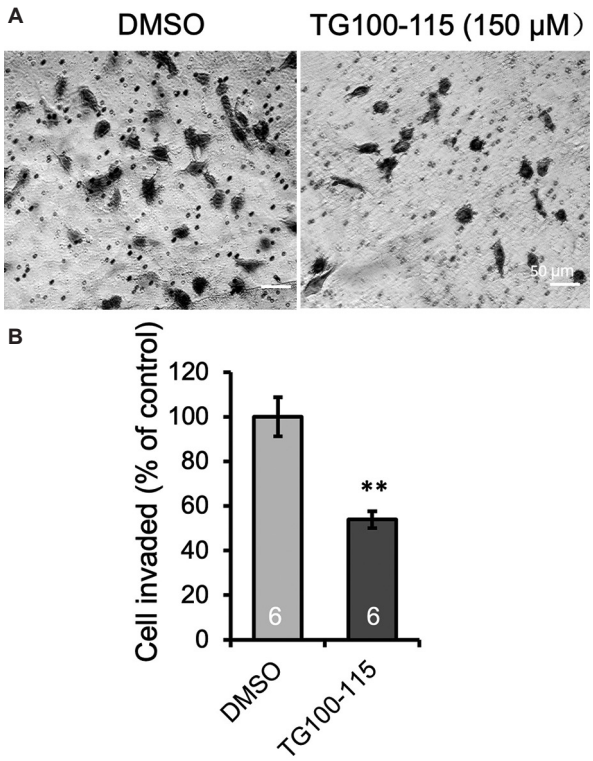

Figure 5. TG100-115 inhibited U251 cell invasion. (A) The representative images are from transwell experiments to detect *in vitro* cell invasion. Scale bar: 50 μm, magnification: ×10. (B) The invasion of TG100-115-treated cells (150 μM) was significantly decreased compared with DMSO (**p<0.01, Student's t-test, n = 6).
Abbreviations: DMSO: Dimethyl sulfoxide.

Previous studies have indicated that PI3K p110-δ is essential for glioma cell migration and invasion. CAL-101, a PI3K p110δ-specific inhibitor, has been shown to moderately reduce GBM cell proliferation and migration without significantly affecting tumor growth in GBM xenograft mouse models.[34] In our experiment, another PI3K p110δ-specific inhibitor, PIK-294, did not significantly influence the migration of U251 cells at concentrations of 60 and 120 μM (Figure S4B), likely due to the lower doses and different cell lines used. In contrast, TG100-115 significantly reduced migration at the same doses (Figure 2). This suggests that the anti-migration effect of TG100-115 on U251 cells may be mediated through its

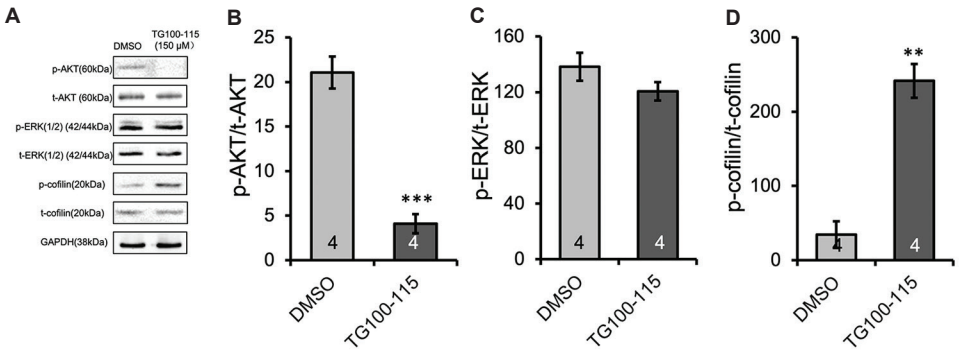

Figure 6. The underlying molecular mechanisms are mediated by TG100-115 in U251 cells. U251 cells were treated with TG100-115 (150 μm) for 24 h, and the protein expression was detected by western blotting. (A) Representative images of western blotting results. (B) TG100-115 significantly reduced the ratio of p-Akt/t-Akt. (C) TG100-115 did not change the ratio of p-ERK/t-ERK (p>0.05). (D) TG100-115 significantly increased the ratio of p-cofilin/t-cofilin.
Note: Statistical significance determined at **p<0.01 and ***p<0.001 using Student's t-test, n = 4.
Abbreviations: Akt: Protein kinase B; DMSO: Dimethyl sulfoxide; ERK: Extracellular signal-regulated kinase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; p: Phosphorylated; t: Total.

inhibition of TRPM7 kinase, rather than PI3K blockade. Further studies using small interfering RNA targeting TRPM7 or kinase-mutated cell lines are needed to clarify the inhibitory activity of TG100-115 on TRPM7 kinase.

The MAPK/ERK pathway contributes to cancer progression and treatment resistance.[35] ERK1/2 is activated by MEK phosphorylation and phosphorylates transcription factors, influences gene expression, and promotes cancer, alongside GBM cell proliferation and invasion.[36] We previously reported that TRPM7 downregulation reduced the phosphorylation of ERK1/2.[12] However, we observed that TG100-115 showed no significant effect on ERK1/2, suggesting that TRPM7 regulates MAPK/ERK signaling potentially through its channel domain, but not its kinase domain. This is worthy of further investigation.

Bcl-2, an anti-apoptotic molecule, is overexpressed in GBM[37] and other types of cancer,[38] enhancing resistance to cytotoxic treatments.[39] Conversely, Bax, a pro-apoptotic Bcl-2 family member, when released from Bcl-2 inhibition through pharmacological means, promotes apoptosis in GBM cells both *in vitro* and *in vivo*.[26] Caspase-3 activation by cleavage promotes apoptosis.[40] Caspase-3 activity is modulated by the Bcl-2/Bax balance, where Bax leads to the release of cytochrome c and a subsequent cascade of caspase activations.[13,17] We found that TG100-115 diminished Bcl-2 expression and augmented Bax expression, while also increasing cleaved caspase-3 expression. Our results demonstrate that TG100-115 promotes GBM cell apoptosis through the Bcl-2/Bax/caspase-3 pathway, likely contributing to its inhibitory effects on GBM cell viability.

Cofilin is crucial for cell motility, particularly in tumor cell migration and invasion.[29,41] Its phosphorylated state suppresses activity, affecting actin filament dynamics and cellular movement, thus underscoring its importance in cancer metastasis.[29,42] Park *et al.*[43] have highlighted cofilin upregulation in human GBM tissues and its potential as a therapeutic target, with its inhibition reducing glioma cell motility.[43] Our laboratory has shown that TRPM7 inhibitors decreased cofilin activity, hindering GBM cell invasion and migration.[12,13] Consistently, this study also showed that TG100-115 increased cofilin phosphorylation. Intriguingly, recent research indicates that the TRPM7 kinase domain interacts with cofilin in neuroblastoma cells and mouse brains.[44,45] Cofilin phosphorylation can also be regulated by the Akt/mTOR pathway, influencing melanoma cell behaviors, which suggests cofilin as a potential downstream effector of Akt signaling.[46] Therefore, TRPM7 kinase may modulate cofilin activity through Akt-dependent signaling, affecting GBM migration and invasion.

TG100-115 was previously identified as a significantly more potent inhibitor of the TRPM7 kinase than other recognized inhibitors (exhibiting an IC_{50} of 1.07 ± 0.14 μM), as demonstrated by utilizing the cyclic adenosine monophosphate response element-binding protein peptide as an *in vitro* substrate and adopting an assay system for kinase inhibitory compounds.[22] Despite the demonstrated capability of TG100-115 to reversibly inhibit TRPM7-like currents, this effect required significantly higher concentrations, specifically 100 μM.[22] Interestingly, our study concluded that TG100-115 does not alter the protein expression of TRPM7, indicating that its inhibitory action is primarily linked to the kinase domain, rather than affecting the overall expression of the TRPM7 channel-kinase. Such findings highlight the inhibitory potency of TG100-115 in targeting the kinase-specific functions of TRPM7, making it a valuable tool for studying the functional implications of TRPM7 kinase in GBMs.

5. Conclusion

In summary, this study provides compelling *in vitro* evidence for the potential therapeutic efficacy of TG100-115 in GBMs. The potential molecular mechanism underlying the inhibitory effects of TG100-115 on cell viability, proliferation, migration, and invasion involves blocking TRPM7 kinase, thus altering multiple signaling pathways, including Bcl-2/Bax/caspase-3, Akt, and cofilin. These findings collectively position TG100-115 as a promising candidate for further exploration in preclinical and clinical studies.

Acknowledgments

We would like to thank the Second Affiliated Hospital of Guangdong Medical University and the Zhuhai Campus of Zunyi Medical University for their valuable support and contributions to this study.

Funding

This work was supported by the following grants: Canadian Institutes of Health Research (CIHR PJT-153155) to ZPF and the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants (RGPIN-2016-04574 and RGPIN-2022-04589) to HSS.

Conflict of interest

Hong-Shuo Sun is an Editorial Board Member, and Zhong-Ping Feng serves as an Associate Editor for this journal, but they were not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions

Conceptualization: Hong-Shuo Sun, Zhong-Ping Feng, Wenliang Chen
Formal analysis: Yuanyuan Xu, Rahmah Alanazi
Investigation: Yuanyuan Xu, Wenliang Chen
Methodology: Yuanyuan Xu, Wenliang Chen
Writing–original draft: Yuanyuan Xu, Xinyang Zhang, Erin Cross, Barbara Gundi
Writing–review & editing: Hong-Shuo Sun, Zhong-Ping Feng, James T. Rutka, F. David Horgen, Andrea Fleig

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The data supporting this study can be accessed by submitting a request to the lead or corresponding author.

References

1. Wasilewski A, Serventi J, Kamalyan L, Wychowski T, Mohile N. Acute care in glioblastoma: The burden and the consequences. *Neurooncol Pract*. 2017;4(4):248-254.

   doi: 10.1093/nop/npw032

2. Stupp R, Hegi ME, Mason WP, *et al*. 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*. 2009;10(5):459-466.

   doi: 10.1016/S1470-2045(09)70025-7

3. Kannan S, Murugan AK, Balasubramanian S, Munirajan AK, Alzahrani AS. Gliomas: Genetic alterations, mechanisms of metastasis, recurrence, drug resistance, and recent trends in molecular therapeutic options. *Biochem Pharmacol*. 2022;201:115090.

   doi: 10.1016/j.bcp.2022.115090

4. Murugan AK, Alzahrani AS. Isocitrate dehydrogenase IDH1 and IDH2 mutations in human cancer: Prognostic implications for gliomas. *Br J Biomed Sci*. 2022;79:10208.

   doi: 10.3389/bjbs.2021.10208

5. Murugan AK, Kannan S, Alzahrani AS. Immune checkpoint expression and therapeutic implications in IDH1-mutant and wild-type glioblastomas. *Curr Probl Cancer*. 2025;55:101182.

   doi: 10.1016/j.currproblcancer.2025.101182

6. Le Rhun E, Preusser M, Roth P, *et al*. Molecular targeted therapy of glioblastoma. *Cancer Treat Rev*. 2019;80:101896.

   doi: 10.1016/j.ctrv.2019.101896

7. Fleig A, Chubanov V. TRPM7. In: Nilius B, Flockerzi V, editors. *Mammalian Transient Receptor Potential (TRP) Cation Channels*. Vol. 1. Berlin, Heidelberg: Springer; 2014. p. 521-546.

8. Yee NS, Kazi AA, Li Q, Yang Z, Berg A, Yee RK. Aberrant over-expression of TRPM7 ion channels in pancreatic cancer: Required for cancer cell invasion and implicated in tumor growth and metastasis. *Biol Open*. 2015;4(4):507-514.

   doi: 10.1242/bio.20137088

9. Guilbert A, Gautier M, Dhennin-Duthille I, Haren N, Sevestre H, Ouadid-Ahidouch H. Evidence that TRPM7 is required for breast cancer cell proliferation. *Am J Physiol Cell Physiol*. 2009;297(3):C493-C502.

   doi: 10.1152/ajpcell.00624.2008

10. Kim BJ, Park EJ, Lee JH, Jeon JH, Kim SJ, So I. Suppression of transient receptor potential melastatin 7 channel induces cell death in gastric cancer. *Cancer Sci*. 2008;99(12):2502-2509.

    doi: 10.1111/j.1349-7006.2008.00982.x

11. Chen JP, Luan Y, You CX, Chen XH, Luo RC, Li R. TRPM7 regulates the migration of human nasopharyngeal carcinoma cell by mediating Ca(2+) influx. *Cell Calcium*. 2010;47(5):425-432.

    doi: 10.1016/j.ceca.2010.03.003

12. Chen WL, Barszczyk A, Turlova E, *et al*. Inhibition of TRPM7 by carvacrol suppresses glioblastoma cell proliferation, migration and invasion. *Oncotarget*. 2015;6(18):16321-16340.

    doi: 10.18632/oncotarget.3872

13. Wong R, Gong H, Alanazi R, *et al*. Inhibition of TRPM7 with waixenicin A reduces glioblastoma cellular functions. *Cell Calcium*. 2020;92:102307.

    doi: 10.1016/j.ceca.2020.102307

14. Wong R, Turlova E, Feng ZP, Rutka JT, Sun HS. Activation of TRPM7 by naltriben enhances migration and invasion of glioblastoma cells. *Oncotarget*. 2017;8(7):11239-11248.

    doi: 10.18632/oncotarget.14496

15. Majewska E, Szeliga M. AKT/GSK3β signaling in glioblastoma. *Neurochem Res*. 2017;42(3):918-924.

    doi: 10.1007/s11064-016-2044-4

16. McCubrey JA, Steelman LS, Chappell WH, *et al*. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. *Biochim Biophys Acta*. 2007;1773(8):1263-1284.

    doi: 10.1016/j.bbamcr.2006.10.001

17. Chen WL, Turlova E, Sun CL, *et al*. Xyloketal B suppresses glioblastoma cell proliferation and migration *in vitro*

through inhibiting TRPM7-regulated PI3K/Akt and MEK/ERK signaling pathways. *Mar Drugs*. 2015;13(4):2505-2525.

doi: 10.3390/md13042505

18. Tian Y, Yang T, Yu S, Liu C, He M, Hu C. Prostaglandin E2 increases migration and proliferation of human glioblastoma cells by activating transient receptor potential melastatin 7 channels. *J Cell Mol Med*. 2018;22(12):6327-6337.

doi: 10.1111/jcmm.13931

19. Meng S, Alanazi R, Ji D, *et al*. Role of TRPM7 kinase in cancer. *Cell Calcium*. 2021;96:102400.

doi: 10.1016/j.ceca.2021.102400

20. Guilbert A, Gautier M, Dhennin-Duthille I, *et al*. Transient receptor potential melastatin 7 is involved in oestrogen receptor-negative metastatic breast cancer cells migration through its kinase domain. *Eur J Cancer*. 2013;49(17):3694-707.

doi: 10.1016/j.ejca.2013.07.008

21. Wan J, Guo AA, Chowdhury I, *et al*. TRPM7 induces mechanistic target of rap1b through the downregulation of miR-28-5p in glioma proliferation and invasion. *Front Oncol*. 2019;9:1413.

doi: 10.3389/fonc.2019.01413

22. Song C, Bae Y, Jun J, *et al.* Identification of TG100-115 as a new and potent TRPM7 kinase inhibitor, which suppresses breast cancer cell migration and invasion. *Biochim Biophys Acta Gen Subj*. Apr 2017;1861(4):947-957.

doi: 10.1016/j.bbagen.2017.01.034

23. Doukas J, Wrasidlo W, Noronha G, *et al*. Phosphoinositide 3-kinase gamma/delta inhibition limits infarct size after myocardial ischemia/reperfusion injury. *Proc Natl Acad Sci U S A*. 2006;103(52):19866-19871.

doi: 10.1073/pnas.0606956103

24. Alandağ C, Kancağı DD, Karakuş Sir G, *et al*. The effects of thymoquinone on pancreatic cancer and immune cells. *Rev Assoc Med Bras (1992)*. 2022;68(8):1023-1026.

doi: 10.1590/1806-9282.20220066

25. Asadi M, Taghizadeh S, Kaviani E, *et al*. Caspase-3: Structure, function, and biotechnological aspects. *Biotechnol Appl Biochem*. 2022;69(4):1633-1645.

doi: 10.1002/bab.2233

26. Fulda S. Cell death-based treatment of glioblastoma. *Cell Death Dis*. 2018;9(2):121.

doi: 10.1038/s41419-017-0021-8

27. Murugan AK. mTOR: Role in cancer, metastasis and drug resistance. *Semin Cancer Biol*. 2019;59:92-111.

doi: 10.1016/j.semcancer.2019.07.003

28. Murugan AK. Special issue: PI3K/Akt signaling in human cancer. *Semin Cancer Biol*. 2019;59:1-2.

doi: 10.1016/j.semcancer.2019.10.022

29. Bravo-Cordero JJ, Magalhaes MA, Eddy RJ, Hodgson L, Condeelis J. Functions of cofilin in cell locomotion and invasion. *Nat Rev Mol Cell Biol*. 2013;14(7):405-415.

doi: 10.1038/nrm3609

30. Manning BD, Cantley LC. AKT/PKB signaling: Navigating downstream. *Cell*. 2007;129(7):1261-1274.

doi: 10.1016/j.cell.2007.06.009

31. Hoeger B, Nadolni W, Hampe S, *et al*. Inactivation of TRPM7 kinase targets AKT signaling and cyclooxygenase-2 expression in human CML cells. *Function (Oxf)*. 2023;4(6):zqad053.

doi: 10.1093/function/zqad053

32. Nadolni W, Immler R, Hoelting K, *et al.* TRPM7 kinase is essential for neutrophil recruitment and function via regulation of Akt/mTOR signaling. *Front Immunol*. 2020;11:606893.

doi: 10.3389/fimmu.2020.606893

33. Pridham KJ, Le L, Guo S, *et al*. PIK3CB/p110beta is a selective survival factor for glioblastoma. *Neuro Oncol*. Mar 27 2018;20(4):494-505.

doi: 10.1093/neuonc/nox181

34. Zhao HF, Wang J, Jiang HR, Chen ZP, To SS. PI3K p110β isoform synergizes with JNK in the regulation of glioblastoma cell proliferation and migration through Akt and FAK inhibition. *J Exp Clin Cancer Res*. 2016;35:78.

doi: 10.1186/s13046-016-0356-5

35. Yuan J, Dong X, Yap J, Hu J. The MAPK and AMPK signalings: Interplay and implication in targeted cancer therapy. *J Hematol Oncol*. 2020;13(1):113.

doi: 10.1186/s13045-020-00949-4

36. Barbosa R, Acevedo LA, Marmorstein R. The MEK/ERK network as a therapeutic target in human cancer. *Mol Cancer Res*. 2021;19(3):361-374.

doi: 10.1158/1541-7786.MCR-20-0687

37. Qiu B, Wang Y, Tao J, Wang Y. Expression and correlation of Bcl-2 with pathological grades in human glioma stem cells. *Oncol Rep.* 2012;28(1):155-160.

doi: 10.3892/or.2012.1800

38. Kirkin V, Joos S, Zornig M. The role of Bcl-2 family members in tumorigenesis. *Biochim Biophys Acta*. 2004;1644(2-3):229-249.

doi: 10.1016/j.bbamcr.2003.08.009

39. Fels C, Schafer C, Huppe B, *et al*. Bcl-2 expression in higher-grade human glioma: A clinical and experimental study. *J Neurooncol*. 2000;48(3):207-216.

doi: 10.1023/a:1006484801654

40. Porter AG, Janicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ*. 1999;6(2):99-104.

doi: 10.1038/sj.cdd.4400476

41. Chang CY, Leu JD, Lee YJ. The actin depolymerizing factor (ADF)/cofilin signaling pathway and DNA damage responses in cancer. *Int J Mol Sci*. 2015;16(2):4095-4120.

doi: 10.3390/ijms16024095

42. Bernstein BW, Bamburg JR. ADF/cofilin: A functional node in cell biology. *Trends Cell Biol*. 2010;20(4):187-195.

doi: 10.1016/j.tcb.2010.01.001

43. Park JB, Agnihotri S, Golbourn B, *et al.* Transcriptional profiling of GBM invasion genes identifies effective inhibitors of the LIM kinase-Cofilin pathway. *Oncotarget*. 2014;5(19):9382-9395.

doi: 10.18632/oncotarget.2412

44. Abumaria N, Li W, Clarkson AN. Role of the chanzyme TRPM7 in the nervous system in health and disease. *Cell Mol Life Sci*. 2019;76(17):3301-3310.

doi: 10.1007/s00018-019-03124-2

45. Liu Y, Chen C, Liu Y, *et al.* TRPM7 is required for normal synapse density, learning, and memory at different developmental stages. *Cell Rep.* 2018;23(12):3480-3491.

doi: 10.1016/j.celrep.2018.05.069

46. Li J, Yang R, Yang H, *et al.* NCAM regulates the proliferation, apoptosis, autophagy, EMT, and migration of human melanoma cells via the Src/Akt/mTOR/cofilin signaling pathway. *J Cell Biochem*. 2020;121(2):1192-1204.

doi: 10.1002/jcb.29353