Molecular Biology Research Communications 2026;15(1):11-20 DOI:10.22099/mbrc.2025.52011.2079 MBRC

Original Article Open Access

# CPUK02 sensitizes U87 glioblastoma cell lines to TMZ treatment via autophagy flux inhibition

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## ABSTRACT

Adjuvant chemotherapy with TMZ (Temozolomide) does not improve the survival of patients suffering from GBM (Glioblastoma). Given the importance of autophagy and UPR (Unfolding Protein Response) in chemotherapy resistance, as well as the role of *Beclin-1*, *LC3IIβ*, and *P62* in the regulation of autophagy, we evaluated the effect of TMZ along with CPUK02 on U87 cells as a model of Glioblastoma cancer in this study. To achieve this goal, we treated the U87 cells with different doses of TMZ (50, 100, 200, 400, and 800 µM) and CPUK02 (1, 0.5, 0.25, 0.125, 0.06, 0.03, 0.01, and 0.007 µM); then, cell viability was assessed by MTT assay. The gene expression of *Beclin1, P62, LC3IIβ,* and *XBP-1s* was analyzed using quantitative real-time polymerase chain reaction. The comparison of the control group with the groups treated with the TMZ drug showed that, in 48 and 72 hours, doses of TMZ more than $IC_{50}$ (100 µM) ($p$<0.001) significantly led to cell death. CPUK02 doses more than 0.125 ($p$<0.0001) significantly led to cell death. TMZ and CPUK02 combination therapy (100 and 0.03 µM, respectively) increased the expression of *Beclin-1, LC3IIβ*, and *P62* and activated the IRE-1 arm of UPR by increasing the expression of XBP-1s. TMZ and CPUK02 treatment inhibits the autophagic flux (*p62, LC3IIβ*). Increased *XBP-1s* expression might contribute to the enhanced TMZ sensitivity. This combination therapy is promising for TMZ-resistant cancers, but it needs further investigation.

**Keywords:** CPUK02; TMZ; Autophagy; Glioblastoma; UPR

## INTRODUCTION

Glioblastoma multiforme (GBM) is a type of glioma, a primary brain tumor originating from glial cells, and accounts for 80% of malignant CNS (Central nervous system) tumors [1]. GBM development is linked to factors like ionizing radiation, vinyl chloride, pesticides, smoking, and manufacturing-related hazards [2]. TMZ (Temozolomide) resistance complicates GBM treatment. Efforts focus on enhancing TMZ efficacy and overcoming resistance [3].

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more accurately evaluate the specific effects of CPUK02. To strengthen the conclusion—that one of the pathways affected by this compound is autophagy—it is essential to examine other glioblastoma cell lines in future studies. While qRT-PCR was used to measure mRNA levels of autophagy markers in this study, future research should include Western blot analysis to validate these findings. Furthermore, quantifying protein levels in each signaling pathway would provide a more complete picture and strengthen the conclusions.

Combined TMZ and CPUK02 significantly enhanced the sensitivity of U87 glioma cells to chemotherapy compared to TMZ alone. This study provides the first evidence that CPUK02 can potentiate the anti-tumor effects of TMZ. Mechanistically, while CPUK02 increased autophagy initiation, impaired autophagy flux was observed. Additionally, upregulated *XBP-1s* suggests a potential role for ER stress in the observed effects. Targeting CPUK02 emerges as a potential therapeutic avenue for glioblastoma, with the possibility of synergistic effects when combined with the current standard treatment, TMZ

**Acknowledgements:** The authors would like to thank Shiraz University of Medical Sciences, Shiraz, Iran and also Center for Development of Clinical Research of Nemazee Hospital and Dr. Nasrin Shokrpour for editorial assistance.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Ethics approval and consent to participate:** This study was approved by the vice-chancellor of research and technology, and ethics committee of Zanjan University of Medical Sciences [ethical code: IR.ZUMS.BLC.1402.015]. Ethical considerations were observed in all steps of the research.

**Authors' Contribution:** HR, MS did all experiments, figures, and table preparation, and prepared the first draft of the manuscript. SD set up all experiments and prepared the second draft of the manuscript. PM co-correspond to the project, made the initial plan of the project, supervised the direction of the project, and did a final proof of the manuscript. MH co-correspond to the project, made the final plan for the project, supervised the direction of the project, and did a final proof of the manuscript. All authors have read and agreed to the published version of the manuscript.

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