J Med Biosci Suppl 2025;7: S63 - S68 DOI: 10.58837/CHULA.JMBS.7.S1.11

**Original article**

# Roles of mTOR complex 2 in lipid metabolism of brain cancer cell lines

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## Abstract

***Background**:* Glioblastoma (GBM) is the most aggressive brain cancer, classified as a high-grade glioma found in the central nervous system (CNS). Metabolic reprogramming influences GBM, especially lipid metabolism. Increased lipid synthesis provides energy for cancer cells. Furthermore, activation of different pathways in lipid metabolism supports cell proliferation. mTORC1/2, a protein kinase, plays a role in lipid metabolism. Previous studies show that mTORC1 is involved in lipid regulation, while the functions of mTORC2 remain less well-defined.

***Objective**:* To investigate the roles of mTORC2 in regulating lipid metabolism in GBM.

***Methods**:* The U-87MG cells were treated with 0.1 μM rapamycin and 2 μM AZD8055 as mTORC1 and mTORC1/2 inhibitors, respectively. The expression of phosphorylated proteins targeted downstream of mTORC2 was measured by western blot. Lipid droplets in cells were stained with oil red o, and the amount of lipids was investigated using lipid extraction. The relative gene expressions were identified using RT-qPCR, and cell proliferation in GBM was measured by MTS assay.

***Results**:* The expression of protein p-AKT-Ser473, a downstream target of mTORC2, was decreased with AZD8055 treatment. The outcomes related to lipids showed a reduction in lipid droplets and lower lipid contents in U-87MG cells treated with AZD8055, along with a decrease in gene expressions involved in lipid metabolism. These findings are consistent with significantly reduced cell proliferation, indicating that mTORC2 may regulate lipid processes in GBM, leading to brain tumor progression.

***Conclusion**:* Both mTORC1 and mTORC2 are involved in lipid metabolism, leading to brain cancer progression.

***Keywords**:* Glioblastoma, lipid metabolism, metabolic reprogramming, mTORC2.

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Glioblastoma (GBM) is a rapidly progressing brain tumor that the World Health Organization (WHO) categorizes as a grade IV glioma, the highest classification. It originates from glial cells in the central nervous system (CNS).[1] GBM exhibits a significantly high rate of proliferation and the ability to infiltrate adjacent tissues. Additionally, it has the capacity to survive in stressful environments. These factors contribute to GBM being the most aggressive type of brain tumor [2] and lead to a reduction in the effectiveness of therapeutic approaches such as surgical intervention, radiation therapy, and chemotherapy. GBM can evade these treatments. These factors result in recurrent tumors after treatment. This leads to a poor prognosis for the patient and a low survival rate within five years. [3, 4]

Most brain cancers change various metabolic pathways. Lipid molecules are crucial for supplying energy and serving as building blocks for cell membranes. Cholesterol, a key component of brain lipids, is mainly produced by astrocytes and subsequently delivered to neurons. [5] In GBM, lipid metabolism is altered, leading to an increase in lipid synthesis and upregulation of enzymes such as fatty acid synthase (FASN), which are the rate-limiting step involved in lipid metabolism.[6] Furthermore, the abundance of unsaturated fatty acids present in high-grade intracranial tumors indicates that gliomas have adapted their fatty acid metabolism. Due to the increased presence of polyunsaturated fatty acids in gliomas, it is essential to examine the potential of converting saturated fatty acids to unsaturated ones as a strategy for influencing tumor invasion.[7, 8] Reprogramming of lipids is the key to metabolic disorders that sustain oncogenic signaling in malignant cancer.

The mammalian target of rapamycin (mTOR) serves as a kinase protein that assumes a critical role in the regulation of metabolic processes within cells. The mTOR complex is comprised of two distinct multiprotein complexes: mTORC1, which contains the protein subunit known as regulatory-associated protein of mTOR (RAPTOR), and is responsible for biosynthesis and autophagy to facilitate cellular growth; and mTORC2, which integrates a rapamycin-insensitive companion of mTOR (RICTOR) along with mammalian stress-activated protein kinase-interacting protein 1 (mSIN1), and regulates lipid and glucose metabolism to ensure survival cell proliferation. [9,10] mTORC2 activation by growth factors initiates mTOR signaling, leading to the phosphorylation of downstream effectors belonging to the AGC subfamily of kinases, which in turn regulate the metabolic processes pathway. [11] When mTORC2 is activated, it initiates the activation of transcription factors that play a key role in enhancing lipid metabolism. This shift in lipid processing affects the overall lipid balance within the cells. As a result, these metabolic changes contribute to the improvement of cell survival in GBM. [12] Meanwhile, previous studies mention the mTORC1 functions involved in various processes in lipid metabolism, such as regulation of lipogenesis.[13] However, the functions of mTORC2 signaling involved in lipid metabolism are still not well understood. Therefore, this research aims to examine the roles of mTORC2 signaling involved in lipid metabolism in brain cancer cell lines.

## Materials and methods

### *Drug treatments*

The U-87MG cell lines representing GBM were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% FBS. Then, cells were deprived of the FBS-free medium for 24 hours before the experiment day. After that, the cells were exposed to 0.1 μM of rapamycin, a mTORC1 inhibitor, and 2 μM of AZD8055, a mTORC1/2 inhibitor, for 24 hours before the subsequent experiments.

### *Western blot*

Cells from the drug treatment experiment were extracted to prepare the protein samples using the bicinchoninic acid method (BCA) (Thermo Fisher Scientific). Subsequently, we utilized an 8% separating gel in SDS-PAGE to separate proteins according to size. Afterward, proteins were transferred to a nitrocellulose membrane using the wet transfer system. Protein expression was observed by probing specific primary antibodies, including p-S6-Ser235/236, total S6, p-AKT-Ser473, total AKT, and normalizing with GAPDH as an endogenous control. Next, the membrane was washed with TBST before probing with secondary antibody for 1 hour. Lastly, protein bands were visualized using Odyssey imaging system.

### *Oil Red O Staining*

Firstly, cells from the previous experiment were washed with PBS and fixed in 10% formalin for 2 hours, then rinsed with water and 60% isopropanol for 5 minutes. Secondly, cells were stained with the oil red o working solution for 10 minutes. The oil red o solution was removed from the cells and washed with water. Lastly, lipid droplets were visualized using a microscope (EVLOS, Invitrogen).

### *Quantitative unsaturated fatty acids*

After 24 hours of drug treatment, lipids were extracted from cells to measure lipid contents using the lipid assay kit for unsaturated fatty acids (Abcam). Initially, lipid samples were extracted and dissolved in DMSO. Then, lipids were mixed with 18M sulfuric acid and incubated at 90°C for 10 minutes. After that, samples were cooled to 4°C for 5 minutes. Lipid samples were transferred to a 96-well plate alongside standard lipids to measure OD at 540 nm for background assessment. Finally, vanillin reagent was added to samples and incubated at 37°C for 15 minutes before measuring OD again to assess the signal and subtract the background from it.

### *Real-time quantitative polymerase chain reaction (RT-qPCR)*

Firstly, RNA was extracted from cells using RNeasy mini kit (Qiagen, USA) and subsequently converted to cDNA with iScript™ cDNA Synthesis Kit (Bio-Rad, USA). Secondly, primers for RT-qPCR reaction were targeted at genes in lipid metabolism, such as *SREBP1*, *FASN*, and *ACC1*, including *UGCG*, which regulates glucosylceramide synthase in sphingolipid processes. Then, reactions followed the process of thermal cycling. Finally, data were analyzed by the —”Ct method to observe gene expression compared to the endogenous control.

### *Cell proliferation (MTS assay)*

The cells were cultured in a 96-well plate using 10% FBS medium, following the procedure outlined in the drug treatment experiment. The following day after treatment, for 24 hours, MTS reagent from the MTS assay kit (Promega, Madison, WI, USA) was added to each well plate. This reagent induced reactions between the NADPH-dependent dehydrogenase enzymes in cells and was incubated for 1 hour before measuring OD at 490 nm using a microplate reader. Finally, the data were collected at 24, 48, and 72 hours.

### *Statistical analysis*

Experimental data were analyzed using one-way ANOVA to compare differences among treatment groups. A $P$-value of $< 0.05$ was considered statistically significant.

## Results

### *Western blot*

To investigate the roles of mTORC2, the expression of the downstream target of mTORC2 was examined. The findings demonstrated that treatment with AZD8055 reduced p-AKT-Ser473, while the activated condition and rapamycin treatment maintained phosphorylation in U-87MG cells. The result confirmed that AKT was activated by mTORC2 signaling in GBM (**Figure 1**).

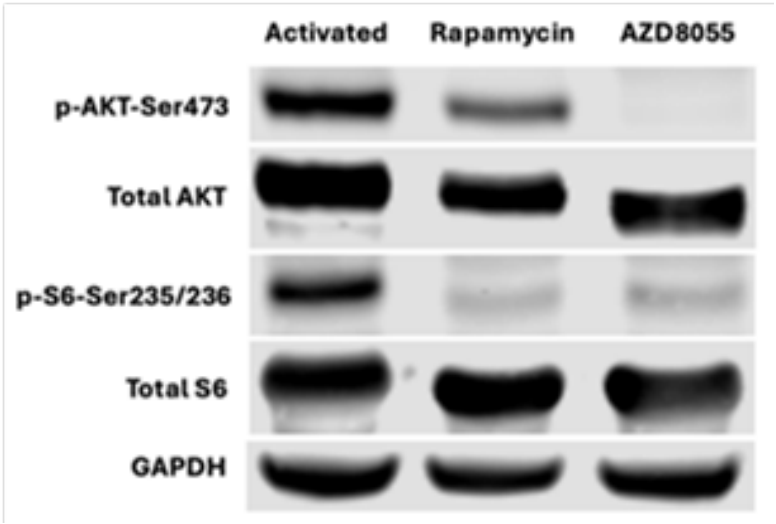


**Figure 1.** Expression of proteins associated with mTOR signaling following treatment with 0.1 μM rapamycin, a mTORC1 inhibitor, and 2 μM AZD8055, a mTORC1/2 inhibitor, for 24 hours.

### *Oil red O staining*

To observe lipid droplet accumulation in GBM, Oil red o staining was performed following treatment. The results revealed that U-87MG cells exhibited reduced lipid content in both treatments, particularly the 2 μM AZD8055 treatment, compared to the activated group (**Figure 2**). The results suggested that drug inhibitors affect lipid content, indicating that mTORC1/2 regulates lipid processes in GBM.

### *Quantitative unsaturated fatty acids*

To investigate the effect of inhibitors on lipid content, total lipid levels in U-87MG cells were measured. The results showed that total lipids in U-87MG cells were significantly reduced in both treatments (rapamycin and AZD8055) as shown in **Figure 3**, which is consistent with the oil red o results. The data indicate that the mTORC1/2 play a role in lipid metabolism, particularly affecting the amount of total unsaturated fatty acids, which serve as substrates for lipid synthesis in lipid metabolism.

### *Real-time quantitative polymerase chain reaction (RT-qPCR)*

To study the expression of a gene involved in lipid metabolism, the expression level of several key genes was analysed. The results showed that *SREBP1*, which is a transcriptional gene-regulated enzyme in lipid synthesis, was decreased in AZD8055 treatment. This led to a decrease in *FASN*, which is a rate-limiting enzyme in fatty acid synthesis, while *ACC1* showed no significant expression. Interestingly, *UGCG*, which regulates glucosylceramide synthase in the production of sphingolipids known as glucosylceramide, was significantly decreased **(Figure 4)**. The data indicated that mTORC2 can regulate lipid synthesis and may influence sphingolipid synthesis in lipid metabolism.

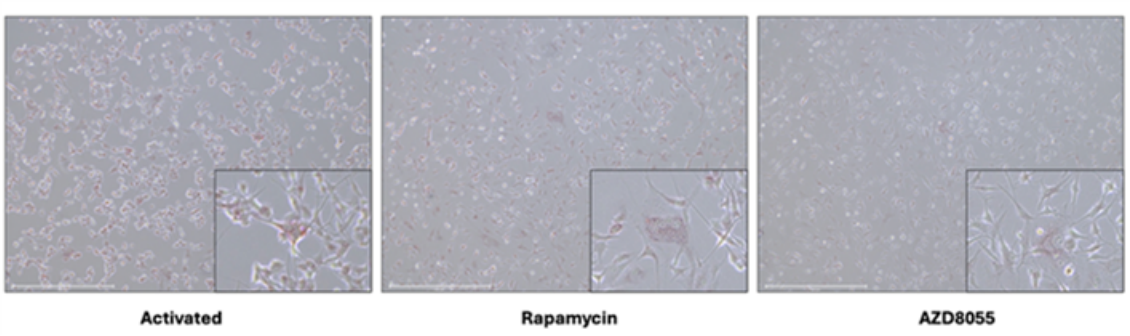


**Figure 2.** Lipid droplets in U-87MG cells through oil red o staining after 0.1 μM rapamycin and 2 μM AZD8055 treatment for 24 hours.

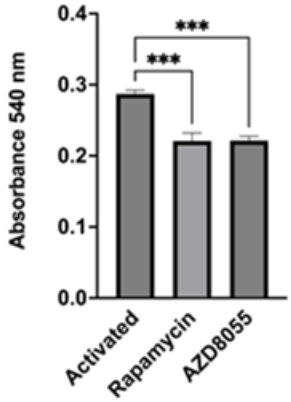


**Figure 3.** The levels of unsaturated fatty acids at an absorbance of 540 nm in the following treatment with 0.1 μM rapamycin and 2 μM AZD8055, in comparison with the activated group after 24 hours (***$P$ <0.001).

### *Cell proliferation (MTS assay)*

To measure cell proliferation in GBM after administering inhibitors, MTS assay was performed. The results showed that cell growth was decreased in both 0.1 μM rapamycin and 2 μM AZD8055 treatment compared to the activated treatment at 24, 48 and 72 h of cell viability (**Figure 5**). This suggested that inhibition of both mTORC1 and mTORC2 effectively suppressed cell proliferation with high statistical significance ($P$ <0.0001).

## Discussion

The alteration of lipid metabolism represents a key factor that influences the progression of brain cancer. The study of proteins related to lipid metabolism may prove beneficial in regulating lipids in GBM. Particularly, mTORC1/2, which is a kinase protein involved in various metabolic processes. [14] Several previous studies have highlighted the role of mTORC1 in lipid metabolism [15], noting that mTORC1 regulates the expression of genes that control functions of enzymes involved in lipid metabolism.[16] Meanwhile, the significance of mTORC2 in lipid metabolism remains relatively under-recognized despite its equally critical role alongside mTORC1. This study investigates the roles of mTORC2 in lipid metabolism associated with GBM by inhibiting mTORC2 activity, thereby influencing the expression of downstream effectors, which was decreased phosphorylation, as illustrated in **Figure 1.** As a result, this impact on lipid metabolism reduces lipid contents in GBM when treated with inhibitors, as shown in **Figures 2 and 3**, respectively. Furthermore, genes related to lipid metabolism resulted in decreased expression. Moreover, mTORC2 inhibition led to a decrease *UGCG* expression, which regulates glucosylceramide synthase to produce glucosylcera-

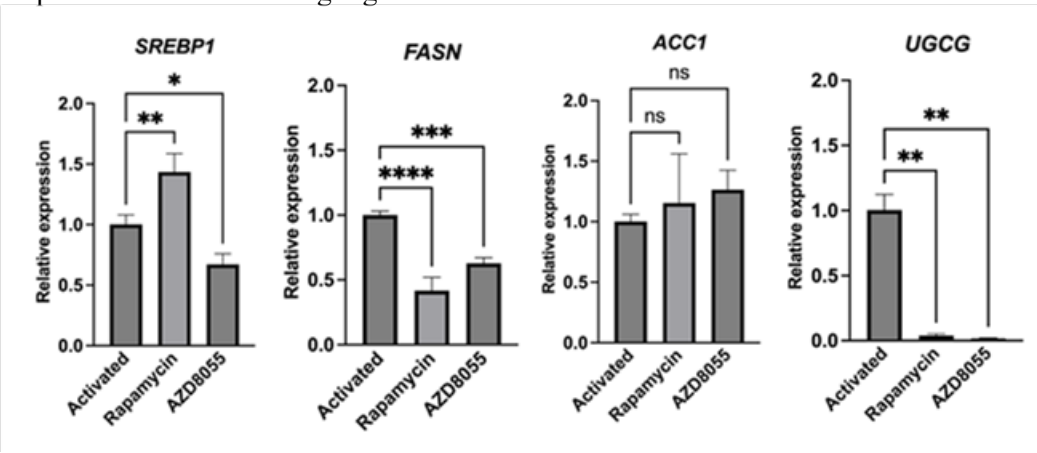


**Figure 4.** The relative expression of genes involved in lipid synthesis and regulated enzyme of sphingolipid in lipid metabolism.
*$P$ <0.05, **$P$ <0.01,***$P$ <0.001, ****$P$ <0.0001

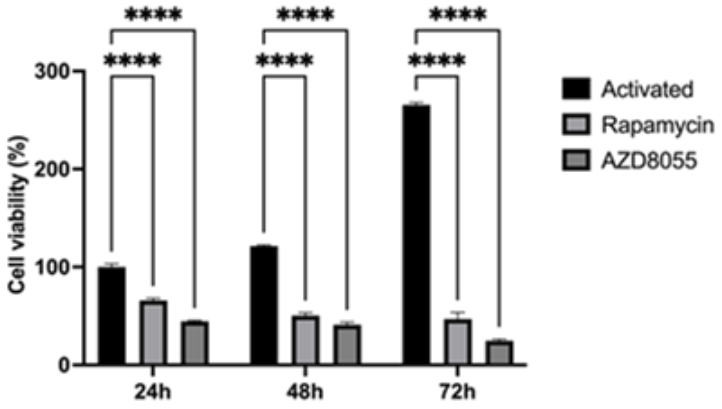


**Figure 5.** The percentage of cell growth after receiving inhibitors, 0.1 μM rapamycin and 2 μM AZD8055, compared to activated treatment for 72 hours (****$P$ <0.0001).

mide as a sphingolipid, as shown in **Figure 4**. These findings are consistent with a previous study reporting that silencing glucosylceramide synthase reduces cell viability in GBM cells. [17] These results indicate that AZD8055 treatment, a mTORC1/2 inhibitor, potentially reduced lipid content similarly to rapamycin, a mTORC1 inhibitor. These effects lead to the inhibition of cell proliferation, as shown in **Figure 5**.

## Conclusion

In conclusion, this research indicated that lipid metabolism is regulated by the involvement of both mTORC1 and mTORC2. While mTORC1 is a well-established regulator, our findings suggest that mTORC2 also contributes to lipid regulation, potentially affecting pathways such as sphingolipid metabolism, leading to brain cancer progression.

## Acknowledgements

This research has been supported by Dr.Naphat Chantaravisoot, and the Department of Biochemistry, Faculty of Medicine, Chulalongkorn University and Ratchadapisek Research Funds (GA68/051) for supporting this research.

## Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Data sharing statement

Data sharing statement. All data generated or analyzed during the present study are included in this published article. Further details are available for noncommercial purposes from the corresponding author on reasonable request.

## References

1. Rasia-Filho AA, Calcagnotto ME, von Bohlen Und Halbach O. Glial cell modulation of dendritic spine structure and synaptic function. Adv Neurobiol 2023; 34:255-310.
2. Ghiaseddin AP, Shin D, Melnick K, Tran DD. Tumor treating fields in the management of patients with malignant gliomas. Curr Treat Options Oncol 2020; 21:76.
3. Taal W, Bromberg JE, van den Bent MJ. Chemotherapy in glioma. CNS Oncol 2015;4:179-92.
4. Taylor OG, Brzozowski JS, Skelding KA. Glioblastoma multiforme: An overview of emerging therapeutic targets. Front Oncol 2019;9:963.
5. Bi J, Chowdhry S, Wu S, Zhang W, Masui K, Mischel PS. Altered cellular metabolism in gliomas - an emerging landscape of actionable co-dependency targets. Nat Rev Cancer 2020;20:57-70.
6. Garcia JH, Jain S, Aghi MK. Metabolic drivers of invasion in glioblastoma. Front Cell Dev Biol 2021; 9:683276.
7. Guo D, Bell EH, Chakravarti A. Lipid metabolism emerges as a promising target for malignant glioma therapy. CNS Oncol 2013;2:289-99.
8. Schlaepfer IR, Nambiar DK, Ramteke A, Kumar R, Dhar D, Agarwal C, et al. Hypoxia induces triglycerides accumulation in prostate cancer cells and extracellular vesicles supporting growth and invasiveness following reoxygenation. Oncotarget 2015;6:22836-56.
9. Luo Y, Xu W, Li G, Cui W. Weighing in on mTOR complex 2 Signaling: The expanding role in cell metabolism. Oxid Med Cell Longev 2018;2018:7838647.
10. Szwed A, Kim E, Jacinto E. Regulation and metabolic functions of mTORC1 and mTORC2. Physiol Rev 2021;101:1371-426.
11. Liu P, Gan W, Chin YR, Ogura K, Guo J, Zhang J, et al. PtdIns(3,4,5)P3-dependent activation of the mTORC2 kinase complex. Cancer Discov 2015;5:1194-209.
12. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell 2017;168:960-76.
13. Li S, Brown MS, Goldstein JL. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. Proc Natl Acad Sci U S A 2010;107: 3441-6.
14. Simcox J, Lamming DW. The central moTOR of metabolism. Dev Cell 2022;57:691-706.
15. Ricoult SJ, Manning BD. The multifaceted role of mTORC1 in the control of lipid metabolism. EMBO Rep 2013;14:242-51.
16. Bakan I, Laplante M. Connecting mTORC1 signaling to SREBP-1 activation. Curr Opin Lipidol 2012;23: 226-34.
17. Morais CM, Cunha PP, Melo T, Cardoso AM, Domingues P, Domingues MR, et al. Glucosylceramide synthase silencing combined with the receptor tyrosine kinase inhibitor axitinib as a new multimodal strategy for glioblastoma. Hum Mol Genet 2019;28: 3664-79.