2019

# KDM1A Inhibition Enhances Chemotherapy Response in Glioblastoma

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# KDM1A inhibition enhances chemotherapy response in glioblastoma

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## Introduction

Glioblastomas (GBM) have dismal survival rates of 1 year – 34.6% and 5 years – 4.75% which affects 13,000 patients yearly. As of now, standard of care treatment consists of surgical resection, external beam radiation therapy, adjuvant chemotherapy with temozolomide (TMZ), and tumor treating fields. Regardless of the extensive therapy, patients will eventually fall victim to this deadliest disease. Deregulated oncogenic and epigenetic signaling mechanisms contribute to chemo and radiation therapy resistance. The lysine-specific demethylase-1 (KDM1A/LSD1) demethylates both mono- and dimethylated lysine residue-4 and -9 specifically on histone H3. KDM1A regulates gene expression programs by changing the epigenetic histone marks at the gene prompters. Emerging studies have shown that KDM1A is overexpressed in glioblastoma and other cancers. However, the role of KDM1A in therapy resistance of GBM remains unknown. In this study, we tested the hypothesis that KDM1A inhibition sensitizes GBM to TMZ therapy.

## Objective

The objective of this study is to test the efficacy of KDM1A inhibition on chemotherapy sensitization of glioblastoma cells.

## Methods

To study the role of KDM1A in GBM cells, we have generated KDM1A knockout (KDM1A-KO) cells and KDM1A-shRNA transfected GBM cells (KDM1A-KD). Confirmation of these knockdowns and knockouts was done using western blotting. The effect of KDM1A-KO and –KD on cell viability and survival of U251 and T98G cells was studied using MTT cell viability assays and clonogenic cell survival assays respectively. A mechanistic study was conducted using RT-qPCR which examined the expression of DNA repair genes in U251 and U251 KDM1A-KO GBM cells.

### Fig. 1. Confirmation of KDM1A-KO and KD in GBM cells

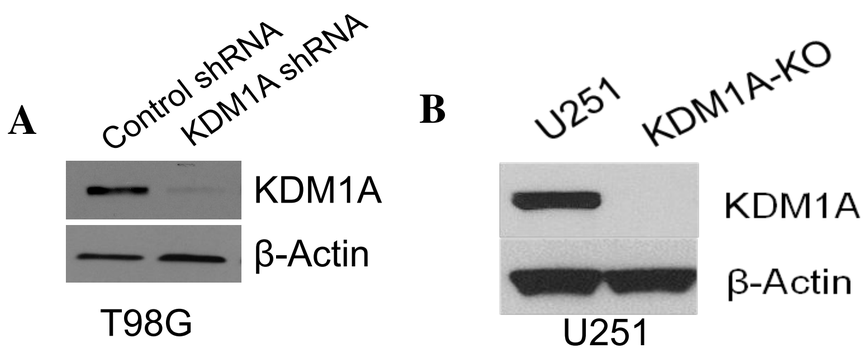


**Fig 1 A,** Knockdown of KDM1A in T98G was tested using western blotting. **B,** Knockout of KDM1A in U251 was tested using western blotting.

### Fig. 2. KDM1A-KO and –KD sensitize TMZ-sensitive and TMZ-resistant GBM cells to TMZ treatment

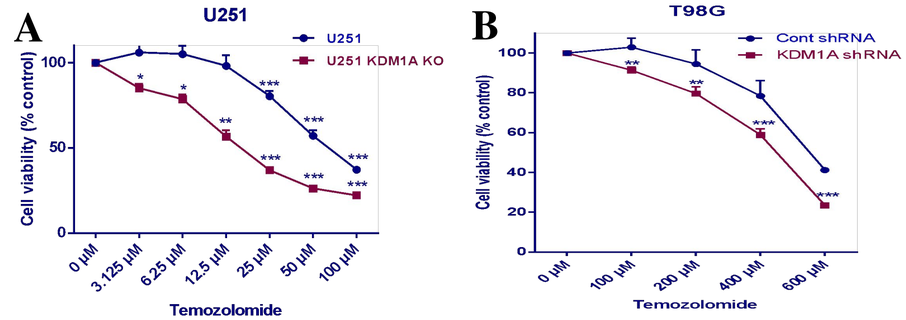


**Fig. 2 A,** U251 control and KDM1A-KO cells were treated with various amounts of TMZ for 7 days, and the cell viability was determined using MTT assays. **B,** T98G cells expressing control shRNA or KDM1A-shRNA were treated with varying amounts of TMZ for 7 days and the cell viability was determined using MTT assays.

### Fig. 3. KDM1A-KO and –KD enhanced the TMZ efficacy in reducing the cell survival

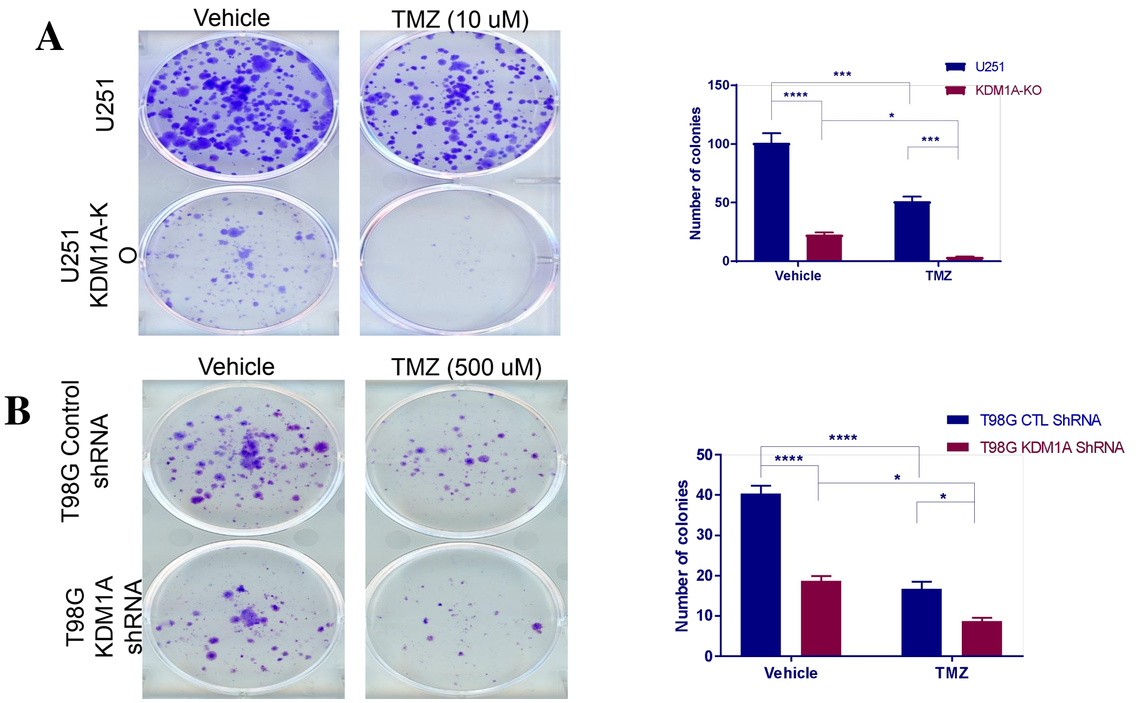


**Fig. 3 A,** U251 or KDM1A-KO cells were treated with TMZ for 5 days and cell survival was determined after 14 days using colony formation assays. **B,** T98G control shRNA or KDM1A-shRNA cells were treated with TMZ for 5 days and cell survival was determined after 14 days using colony formation assays.

### Fig. 4. Knockout of KDM1A reduced the expression of DNA repair genes in GBM cells

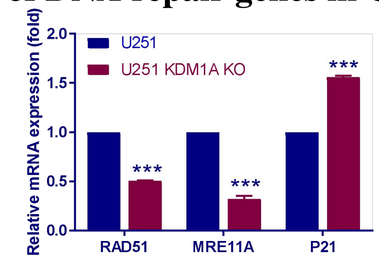


**Fig. 4 A,** Expression of DNA repair and apoptosis genes in U251 or KDM1A KO was analyzed using RT-qPCR

## Results

Western blot analysis confirmed the knockout and knockdown of KDM1A in GBM cells. Cell viability assays showed that KDM1A knockout or knockdown potentiated the cytotoxic effect of TMZ in TMZ sensitive and TMZ resistant GBM cells. Further, clonogenic survival assays demonstrated that KDM1A knockout or knockdown enhanced the TMZ efficacy in reducing the cell survival of GBM cells. Importantly, qRT-PCR assays showed that KDM1A knock out reduced the expression of DNA repair genes and induced the apoptotic gene p21.

## Conclusion

Our results provided the evidence that knockout or knockdown of KDM1A sensitizes GBM cells to chemotherapy and may lead to the development of KDM1A inhibitor therapy in conjunction with TMZ.

Moraima Rodriguez

# Bibliography

1. Burg, Jonathan M., et al. “Lysine-Specific Demethylase 1A (KDM1A/LSD1): Product Recognition and Kinetic Analysis of Full-Length Histones.” *Biochemistry*, no. 11, 2016, p. 1652. *EBSCOhost*, doi:10.1021/acs.biochem.5b01135.
2. Chooi Yeng Lee. “Strategies of Temozolomide in Future Glioblastoma Treatment.” *OncoTargets & Therapy*, vol. 10, Jan. 2017, pp. 265–270. *EBSCOhost*, doi:10.2147/OTT.S120662.
3. Mosammaparast, Nima, et al. “The Histone Demethylase LSD1/KDM1A Promotes the DNA Damage Response.” *Journal of Cell Biology*, no. 3, 2013, p. 457. *EBSCOhost*, doi:10.1083/jcb.201302092.
4. Sareddy, G. R., et al. “Novel KDM1A Inhibitors Induce Differentiation and Apoptosis of Glioma Stem Cells via Unfolded Protein Response Pathway.” *Oncogene*, no. 17, 2017, p. 2423. *EBSCOhost*, doi:10.1038/onc.2016.395.

Research Narrative for “Research Thing” Contest

By Moraima Rodriguez

This research was conducted at The University of Texas Health Science Center in San Antonio, TX over the course of 9 months under the supervision of Dr. Gangadhara Sareddy. This study is based on the hypothesis that the protein KDM1A could increase chemotherapy response in glioblastoma (brain cancer) when inhibited. When conducting this research, we had the previous knowledge that a protein known as KDM1A was related to chemotherapy response in glioblastoma due to previous research done by Dr. Sareddy in 2017 which indicated that KDM1A inhibition induced cell death in glioblastoma stem cells via unfolded protein response pathway (as seen in article 4 in the bibliography). This along with other studies that connected the inhibition of KDM1A and cell death in glioblastoma cells, allowed us to develop the hypothesis that when exposed to different levels of chemotherapy and KDM1A inhibition, there would be an increase in the response level of the glioblastoma cells, meaning more cell death would be seen. The way we went about testing this was first identifying certain glioblastoma cell lines we wanted to work with, which ended up being U251 and T96G. U251 is considered a very weak cell line and easy to kill while T98G is a rather difficult cell line to kill. We wanted to see two different ends of the spectrum and decided that these two commercial cell lines would be appropriate. Once we identified the cell lines we wanted to work with, we inhibited KDM1A using a virus and then tested the cell lines using western blot in order to ensure that the protein was inhibited. As seen in figure 1 both cell lines had the KDM1A “knockout” or “knockdown” which is just another way of saying the protein is no longer active. Western blot confirmed this by showing a lack of bands present in the KDM1A knockdown/knockout cell lines. After

confirming this knockdown/knockout we began by exposing the cell lines to different levels of a chemotherapy called temozolomide (TMZ) and comparing those results to the regular cell lines. As seen in figure 2, there was a decrease in the amount of cell viability in the knocked out/down cell lines. This was done by conducting a MTT assay which is way to expose a cell line to multiple levels of something in one trial. After repeating the experiment multiple times, we determined that the next step in our journey was to have a visible representation of cell survival rates. So in order to do that we conducted colony formations, which showed us how many cell colonies formed over a week when exposed to TMZ. We did this with both the regular cell lines and the knockdown/out cell lines. As seen in figure 3, there was a significant decrease in the amount of cell colonies formed when compared to the vehicle plates. With all this evidence after multiple trials we could then say that there was a definite increase in response to chemotherapy when KDM1A was inhibited. Even then we wanted to test the function of the cell when KDM1A was inhibited, so we used a test called RT-qPCR in order to determine how well DNA repair genes and cell death genes were being used in the cell lines. As seen in figure 4, there was a significant decrease in the function of both RAD51 and MRE11A which are both DNA repair genes. As well as a significant increase in the P21 genes which is a cell death gene, which means that more cells were dying and less were being repaired by the genes. With all of this evidence, we were able to conclude that knockout or knockdowns of KDM1A sensitized glioblastoma cells to chemotherapy and may lead to the development of KDM1A inhibitor therapy in conjunction with chemotherapy.