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A Bimodal Model of Temozolomide-mediated LSD1 Activity in Glioma Cells

Hafkenscheid K.[2,#], Jacuinde B.[2,#], Mari A.[2,#], Gomez A.[2], Philip C.[2], Jay P.[1], Diaz AJ.[1], Wang T.[1,2,*]

[1]Department of Chemistry and Biochemistry
[2]Department of Clinical Science California State University Dominguez Hills, Carson, CA, USA
*Corresponding author: twang@csudh.edu

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[#] The authors contribute equally to the experiment and manuscript writing

Abstract Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor in adults, with a median survival of approximately 14–16 months despite standard treatment. Temozolomide (TMZ) is the frontline chemotherapeutic agent for GBM and is well established as a DNA alkylating agent. However, the potential for TMZ to exert antitumor effects through direct protein-level methylation - particularly through modulation of epigenetic regulatory enzymes - has not been fully characterized. Our previous study has shown that TMZ can methylate recombinant histone H3 and alter histone methylation levels in glioma cells. Building on those findings, the present study examined whether the histone methylation changes observed after TMZ treatment in glioma cells are mediated by lysine-specific demethylase 1 (LSD1/KDM1A), an epigenetic eraser enzyme known to be overexpressed in glioma. Our results showed that TMZ suppresses LSD1 demethylase activity in a concentration-dependent fashion, consistent with a bimodal model of inhibition that becomes operative only at higher drug concentrations (≥150 μM). At lower TMZ concentrations (0–100 μM), LSD1 demethylase activity remained largely unaffected, whereas significant suppression occurred at higher concentrations (150–200 μM). Co-treatment with tranylcypromine (TCP) confirmed effective LSD1 inhibition at lower TMZ concentrations; however, TCP's inhibitory effectiveness diminished at higher TMZ concentrations, suggesting TMZ-induced conformational changes in LSD1 that may reduce the enzyme's accessibility to TCP binding. These findings establish that TMZ functions as a direct histone methylating regulator and suppresses LSD1 demethylase activity in a concentration-dependent manner through both indirect and direct enzyme-level mechanisms. This dual epigenetic activity expands the known pharmacological profile of TMZ and carries meaningful implications for understanding how the drug exerts its downstream effects on chromatin structure and gene expression. These results also inform the growing effort to harness epigenetic mechanisms as therapeutic targets in glioma.

Keywords: *Temozolomide, glioma, LSD1/KDM1A, histone methylation, histone lysine demethylase, therapeutic benefit, chromatin remodeling, epigenetic regulation, tranylcypromine*

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1. Introduction

Gliomas represent the most prevalent primary brain tumors in adults, accounting for approximately 80% of all malignant brain tumors. Among gliomas, glioblastoma multiforme (GBM) is the most aggressive subtype, characterized by rapid proliferation, diffuse infiltration, and resistance to conventional therapies. Despite decades of clinical effort, the prognosis for GBM patients remains poor, with a median survival of 14 to 16 months following the current standard of care. [1,2,3,4,5,6,7] The anatomical constraints imposed by the blood-brain barrier and the complex biology of the central nervous system severely limit therapeutic options, underscoring the urgent need for a deeper mechanistic understanding of existing drugs and the rational development of novel therapeutic strategies.

Temozolomide (TMZ) is an oral alkylating agent and the cornerstone of glioma therapy. Its clinical utility arises from several pharmacological advantages: high oral bioavailability, effective penetration of the blood-brain barrier, and a demonstrated improvement in median patient survival. At physiological pH, TMZ spontaneously decomposes to the reactive intermediate MTIC (5-(3-mehtyltriazen-1yl) imidazole-4-carboxamide), which generates a methyldiazonium ion – the ultimate active methylating species. This ion primarily alkylates guanine at the O_6 position, inducing DNA strand breaks, replication fork collapse, and apoptotic cell death in rapidly dividing tumor cells. [8,9,10,11,12]

Structure-activity relationships have historically driven drug discovery. However, the field has progressively shifted toward a biologically driven paradigm, leveraging

advances in genomics and proteomics to uncover novel molecular targets and unrecognized mechanisms of drug action. [13,14] This paradigm shift has been particularly consequential in oncology, where epigenetic dysregulation, including aberrant DNA methylation, histone modification, and chromatin remodeling, has been established as a hallmark of cancer. Histone methylation plays a central role in regulating gene expression by controlling chromatin accessibility, and the dynamic equilibrium between histone methyltransferases (Writers) and histone demethylases (Erasers) governs the transcriptional state of oncogenes and tumor suppressor genes. Disruption of this balance is increasingly recognized as a key driver of tumor progression. [15,16,17,18,19]

Although TMZ is well established as a DNA methylating agent, its potential to exert antitumor effects through direct protein-level methylation, particularly at histones and their associated proteins, has remained undercharacterized because the methyldiazonium active metabolite generated by TMZ is a potent, chemically non-selective methylating agent. We hypothesize that TMZ may also modify histone proteins, thereby altering the epigenetic landscape of glioma cells independently of its DNA-targeted mechanism. Our previous work demonstrated that TMZ directly methylates recombinant H3 protein in vitro and alters histone methylation levels in glioma cells, as characterized by proteomic mass spectrometry and Western blot analysis. [20,21] However, this trend was not evident in triple-negative breast cancer cells (MDA-MB-231), indicating cell-type-specific effects.[22] We also examined histone lysine demethylase activity following TMZ treatment in MDA-MB-231 breast cancer cells. We observed increased histone methylase activity in MDA-MB-231 cells. [23] Building on these findings, the present study investigates whether TMZ-induced histone modification occurs through the altered activity of lysine-specific demethylase 1 (LSD1), a critical epigenetic eraser enzyme whose overexpression is associated with poor prognosis in glioma and multiple other cancers.

LSD1 (KDM1A) is the flavin adenine dinucleotide (FAD) dependent amine oxidase that catalyzes the removal of mono- and dimethyl marks from lysine residues on histone H3, primarily H3K4 and H3K9 methylations. [24,25,26,27] [28,29,30] By erasing these activating methyl marks, LSD1 regulates chromatin condensation and the transcriptional activation and silencing of tumor suppressor genes, thereby contributing to tumor maintenance and progression. The established LSD1 inhibitor, tranylcypromine (TCP), a monoamine oxidase inhibitor, forms a covalent adduct with the FAD cofactor within the LSD 1 active site and has been widely used as a pharmacological tool to dissect LSD 1 function. Elucidating how TMZ interacts with LSD1 and affects its activity through direct or indirect enzyme modification, substrate-level interference, or both may reveal a clinically significant epigenetic dimension of TMZ's mechanism of action.

In the present study, we employed a histone lysine demethylase assay at increasing TMZ concentrations (0 to 200 mM), both in the presence and absence of TCP inhibitor, to characterize the concentration-dependent effects of TMZ on LSD1 enzymatic activity. The results reveal a bimodal suppression model: at lower TMZ concentration (≤ 100 mM), LSD1 activity is largely preserved. At high concentration (150 to 200 mM), LSD1 activity is significantly reduced. The reduced LSD1 activity by TMZ may be due to both direct and indirect enzyme modification. Notably, high concentration TMZ also decreases the inhibitory effectiveness of TCP, consistent with TMZ-induced structural changes to the LSD1 active site. Together, these findings provide the first evidence that TMZ exerts concentration-dependent suppression of LSD1 activity and suggest that epigenetic modulation of chromatin dynamics represents an additional mechanism underlying TMZ's anti-tumor effects in glioma.

2. Materials and Methods

Chemicals and general procedures

TMZ (>98%) was purchased from Sigma (St. Louis, MO) and used without further purification. All buffers were prepared using analytical-grade reagents without further purification and ultrapure water from a Millipore Synergy UV water filtration system (EMD Millipore, Billerica, MA) with a resistivity of 18 Ω · cm. The pH measurements were made using a calibrated Fisher Scientific AccuMet basic AB15 pH MV benchtop pH meter (Fisher Scientific, Pittsburgh, PA).

Preparation of the anticancer drug TMZ for the LSD1 assay

A Stock solution of 0.2 mM TMZ was prepared in DMSO. Cell culture medium was added to the TMZ stock solution to prepare a 200 µM TMZ solution, which was serially diluted to obtain 50 µM, 100 µM, and 150 µM TMZ concentrations. These TMZ media solutions were then introduced to U87 glioma cells growing in 75 cm2 cell culture flasks. Following TMZ drug treatment, at 70 – 80% confluency, the cells were pelleted and frozen at -80^{o}C. The study included the following treatment groups:

- Control group: Glioma U87 cells treated with diluent (no temozolomide).
- Temozolomide groups: Cells treated with 50µM, 100 µM, 150 µM and 200µM of temozolomide.

Culturing Cancer Cells

U87 MG human glioblastoma cells (ATCC, Manassas, VA) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin and maintained in a 5% CO_2 humidified atmosphere at 37 ^{o}C. 2×10^6 U87 MG cells were seeded into a 75 cm2 culture flask and allowed to attach overnight. After attachment, the culture medium was removed and replaced with a medium containing 0 µM, 50 µM, 100 µM, 150 µM, or 200 µM TMZ, and the cells were incubated for 48 hr. The glioma cells were collected via centrifugation at 70-80% confluency. The cell pellets were then frozen at -80 °C for subsequent assays.

Nuclear protein extraction assay

U87 Glioma cell nuclear protein was extracted using the nuclear protein extraction kit from ABCAM (Catalog # AB113474). 100 µL of pre-extraction buffer was added to each pellet. The sample was left on ice for 15 minutes, with vigorous vortexing for 10 seconds every 3 minutes.

The solution was transferred to an Eppendorf tube and centrifuged for 10 min at 12000rpm at 4°C. The pellet was treated with 2 volumes of extraction buffer and kept on ice for 15min (vortex every 3 min). The solution was then centrifuged for 10 minutes at 14000rpm at 4 °C. The nuclear protein in the supernatant was quantified by BCA assay (Thermo Fisher Scientific).

Protein concentration assay

The bicinchoninic acid (BCA) assay was used to determine nuclear protein concentration. The working solution was prepared by mixing reagent A and reagent B in a 50:1 ratio. 100 µl working solution was added to each well, followed by 10 µl standard or sample in a 96-well plate. The standard samples were prepared at concentrations of 0, 2, 4, 12, 16, and 20 µg/µL. Both standards and samples were performed in duplicate. The plate was incubated at 37°C for 1 hour, during which a violet color developed. Absorbance was measured at 562nm after incubation using a BioTek ELx808 plate reader. The linear regression equation was generated by plotting standard concentration (x-axis)) against absorbance (y-axis). The sample concentrations were then calculated using the linear equation. 15 µg of nuclear protein was used for the demethylase activity assay.

Lysine-Specific Histone Demethylase (LSD1) activity assays in the presence and absence of the inhibitor tranylcypromine (TCP)

The assay is performed using a commercially available assay kit from ABCAM (Catalog # AB113459). Nucleus protein extracted from the U87 glioma cells treated with different concentrations of TMZ was incubated with dimethylated histone (H3K4me2) and assay buffer in the presence and absence of TCP inhibitor. The assay kit provides all the essential reagents for a successful LSD1 activity/inhibition experiment. After an incubation period of 2-3 hours, the demethylated histone H3K4 was recognized with a high-affinity antibody. The amount of demethylated histone is directly proportional to LSD1 enzyme activity. The OD(optical density) measurements were taken using the BioTek ELx 808 plate reader. Each assay was conducted in duplicate. Only the repeated results were used for the data analysis. The average OD values were converted to the relative LSD1 activity, normalized to absorbance per minute per milligram (equation 1). Histone concentration was calculated by comparing it to the LSD activity of a standard sample (equation 2). The TCP percentage inhibition was calculated using equation 3.

$$LSD1Activity\left(OD/\min/mg\right)=\frac{Sample\ OD-Blank\ OD}{Protein\left(ug\right)x\ Incubation\ time\left(\min\right)} \quad (1)$$

$$\left[histone\ produced\right]=\frac{\left[Histone\ Standard\right]xsample\ OD}{standard\ OD} \quad (2)$$

$$\%\ Inhibition = \frac{LSD1activity\left(No\ inhibitor\right)-LSD1activity\left(Withinhibitor\right)}{LSD1activity\left(no\ inhibitor\right)} \quad (3)$$

3. Results

TMZ suppresses LSD 1 activity in a dose - dependent manner

To determine whether TMZ modulates LSD1 demethylase activity, a histone lysine demethylase assay was performed using nuclear protein extracted from glioma cells treated with TMZ at increasing concentrations (0, 50, 100, 150, and 200 µM). The assays were conducted in the presence and absence of the TCP inhibitor of LSD1. Our results show that LSD1relative activity remained largely unchanged at lower TMZ concentration (0 to 100 uM; activity range: 0.039 to 0.041 OD/min/mg) without TCP inhibitor. However, at 150 and 200 mM TMZ concentration, LSD1 activity declined to 0.038 and 0.025 OD/min/mg respectively, representing an approximately 36% reduction at the highest TMZ concentration tested (Table 1).

Table 1. LSD1 relative activity and histone concentration in the absence of TCP inhibitor

TMZ (uM)	Average OD	SD	LSD1 Activity (OD/min/mg)	Histone Product (ng)
0	0.20	0.00262	0.039	0.56
50	0.20	0.00392	0.041	0.57
100	0.20	0.00262	0.041	0.57
150	0.19	0.00288	0.038	0.55
200	0.19	0.00314	0.025	0.45

OD (optical density) readings, SD (standard deviation) and calculated LSD1 relative activity (OD/min/mg) at each TMZ concentration. Histone product (ng) represents the amount of demethylated histone after incubation.

Correspondingly, histone product concentrations remained stable at lower TMZ levels (0.56 – 0.57 ng). But decreased to 0.55 and 0.45 ng. At 150 and 200 uM TMZ concentration, consistent with reduced TMZ-mediated histone demethylase activity.

TMZ Alters the Effectiveness of the LSD1 Inhibitor TCP in a Concentration-Dependent Manner

Table 2. LSD1 relative activity and histone concentration in the presence of TCP inhibitor

TMZ (uM)	Average OD	SD	LSD1 Activity (OD/min/mg)	Histone Product (ng)
0	0.087	0.00071	0.037	0.40
50	0.089	0.00141	0.0044	0.41
100	0.088	0.00071	0.0041	0.41
150	0.097	0.00000	0.0074	0.45
200	0.096	0.00000	0.0070	0.44

OD (optical density) readings, SD (standard deviation) and calculated LSD1 relative activity (OD/min/mg) at each TMZ concentration. Histone product (ng) represents the amount of demethylated histone after incubation.

In the presence of TCP, baseline LSD1 activity was substantially reduced compared to the TCP-absent condition (0.0037 vs. 0.039 OD/min/mg at 0 µM TMZ), confirming effective LSD1 inhibition by TCP under control conditions (Table 2). Interestingly, at higher TMZ concentrations (150 and 200 µM), LSD1 relative activity

in the presence of TCP increased approximately 1.9 - 2 fold in OD/min/mg, respectively, compared to lower TMZ conditions. This paradoxical increase suggests that elevated TMZ concentrations may induce conformational changes in the LSD1 active site that reduce TCP binding pocket accessibility, thereby diminishing TCP's inhibitory effectiveness. Consistent with this interpretation, the difference in histone production between TCP-treated and untreated conditions narrowed at higher TMZ concentrations, indicating reduced TCP inhibitory efficacy with increasing TMZ concentration.

Comparison analysis of LSD1 activity in the presence and absence of TCP LSD1 inhibitors

Figure 1 illustrates the relationship between LSD1 activity and TMZ concentration. The blue color represents LSD1 activity without TCP, while the orange color represents LSD1 activity in the presence of TCP. LSD1 relative activity in the absence of TCP remains consistently high at TMZ concentrations below 100 µM. This indicates that TMZ at these doses has a negligible effect on LSD1 activity. At higher TMZ concentration (≥ 150 µM), LSD1 activity decreased by 36%. When TCP is added, the LSD1 activity drops noticeably, demonstrating that TCP effectively inhibits LSD1. The inhibition efficiency decreases by approximately 1.89 to 2-fold at higher TMZ concentrations. The difference between the LSD1 activity without an inhibitor and with an inhibitor is clear and consistent across this range. From these results, we can conclude that TMZ has little to no effect on LSD1 activity at lower concentrations (below 100 µM). However, at higher concentrations (above 150 µM), TMZ decreases LSD1 enzyme activity and the efficiency of TCP inhibition.

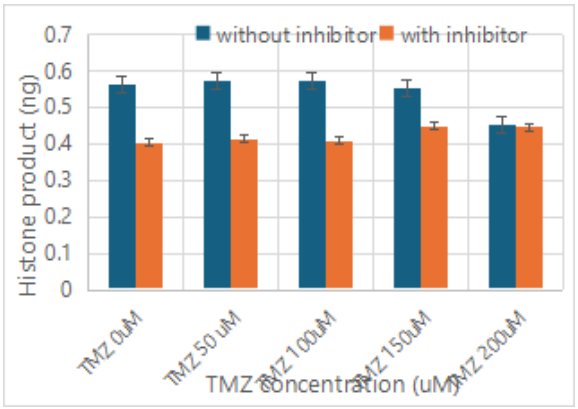

Figure 1. LSD1 relative activity at increasing TMZ concentrations in the presence and absence of the TCP LSD1 inhibitor

Comparison analysis of histone product in the presence and absence of TCP LSD1 inhibitors

Figure 2 displays histone production levels from the LSD1-catalyzed reaction as the concentration of TMZ increases. The data is shown in two colors: blue represents results without TCP, and orange represents results with TCP. At these concentrations, TMZ does not interfere with LSD1 activity, so the observed reduction in histone production can be attributed solely to TCP's inhibition of LSD1. This allows the low TMZ range to serve as a useful baseline for isolating and confirming TCP's effectiveness as an LSD1 inhibitor without the confounding effects of TMZ on LSD1. In summary, at low doses of TMZ, TCP clearly suppresses LSD1 activity. At higher TMZ concentrations, histone production levels were nearly the same in the presence and absence of the TCP inhibitor. Figure 3 is a line graph comparing the LSD1 activity in the presence and absence of the TCP LSD1 inhibitor. Figure 3. Dose-response line graph showing the dose-dependent effect of TMZ on LSD1 relative activity as a function of TMZ concentration with and without TCP.

The decline in activity without TCP at ≥150 µM TMZ, concurrent with the increase in the TCP presence condition, is consistent with TMZ-induced alterations to the LSD1 active site. The results indicate that TCP binding is less effective, and TMZ suppresses LSD1 activity. The results from LSD1 activity and histone product measurements indicate that TMZ may induce conformational changes in LSD1 that alter the TCP-FAD binding pocket.

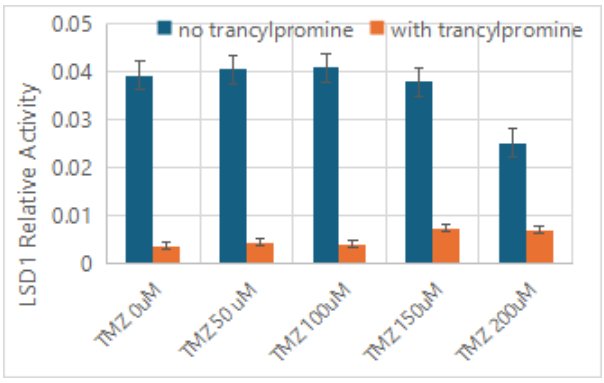

Figure 2. Histone product following the LSD1 demethylation reaction at increasing TMZ concentrations with and without TCP

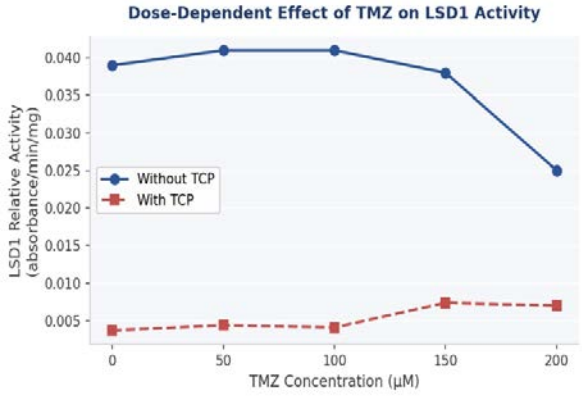

Figure 3. Dose-response line graph showing Dose-Dependent Effect of TMZ on LSD1 relative activity as a function of TMZ concentration with and without TCP

4. Discussion

TMZ suppresses LSD1 activity through both direct and indirect mechanisms at high concentrations

The combined findings of the histone lysine demethylase assay, assessed both with and without the competitive LSD1 inhibitor TCP, support a bimodal model of TMZ-mediated LSD 1 suppression that becomes operative specifically at high drug concentration (≥150 µM). The results are discussed below in the context of the two proposed suppression mechanisms, their downstream

consequences for chromatin structure and gene expression, and the broader implications for epigenetic therapy in glioma.

Direct mechanism: TMZ-mediated LSD1 activity

The first proposed mechanism involves direct chemical modification of the LSD1 enzyme by the reactive methyldiazonium active metabolite generated from TMZ at physiological pH. LSD1(KDM1A) is an FAD - dependent amine oxidase whose catalytic activity depends on the precise geometry and electrostatic environment of its active site, which contains critical lysine and arginine residues involved in substrate recognition and FAD cofactor positioning. Given that TMZ is a potent non-selective methylating agent capable of modifying both lysine and arginine residues, as demonstrated by the Mass spectrometric data in the previous study, it is plausible that at a sufficiently high concentration, TMZ directly methylates residues within the vicinity of the LSD1 catalytic pocket, as shown in Figure 4.

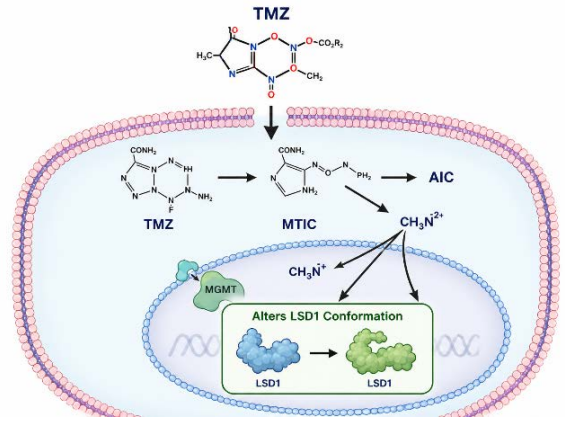

Figure 4. Direct mechanism of TMZ-mediated methylation of LSD1

Direct methylation of active site residues could impair LSD1 function through several non-mutually exclusive mechanisms. (1) Steric occlusion of histone H3 substrate access to the catalytic site. (2) Disruption of the electrostatic interactions required for productive substrate binding or (3) covalent modification of lysine or arginine residues that normally coordinate FAD binding, thereby impairing the oxidative demethylation cycle. The observed decrease in LSD1 relative activity between 0 and 200 uM TMZ concentration in the absence of TCP is consistent with a direct inhibitory interaction between TMZ and the LSD1 enzyme, rather than a purely substrate-level effect.

Importantly, this direct-modification hypothesis is also supported by the behavior of the TCP inhibitor at high TMZ concentrations. TCP inhibits LSD1 by forming a covalent adduct with the FAD LSD1 enzyme cofactor within the active site. The observed partial loss of TCP inhibitory effectiveness at 150 to 200 mM TMZ concentration, evidenced by the increase in LSD1 relative activity in TCP-present samples by 1.9- to 2-fold in OD/min/mg, suggests that TMZ-induced structural alterations to the LSD1 active site affect the accessibility or binding activity of the FAD-TCP adduct. This competitive or allosteric interference provides indirect biochemical evidence that TMZ interacts physically with the LSD1 enzyme at the catalytic domain level.

Indirect mechanism: TMZ-mediated LSD1 activity

The second mechanism is indirect suppression, where TMZ decreases LSD1 activity through various factors in cellular pathways (Figure 5). As demonstrated by both Mass spectrometry and Western blot analysis in the previous study, TMZ directly methylates H3 at multiple lysine and arginine residues, including H3K4. TMZ-mediated methylation of these residues is non-specific to its substrates. Under this model, the apparent suppression of LSD1 activity at higher TMZ concentrations would reflect not a direct inhibition of enzyme function, but rather a reduction due to other factors in cellular pathways or the environment that indirectly alter LSD1 activity, thereby preventing the methylated histone substrate from accessing LSD1 efficiently. This interpretation is consistent with the observed decrease in histone production. In the LSD1 demethylase assay at 150 to 200 mM TMZ concentration without TCP inhibition (0.55 and 0.45 ng, respectively, vs 0.56 and 0.57 ng at lower concentrations).

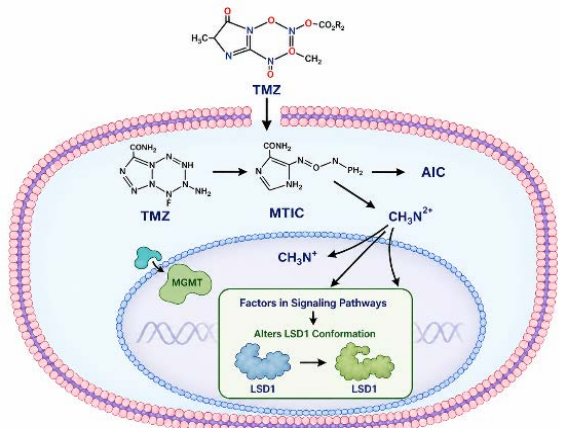

Figure 5. Indirect mechanism of TMZ-mediated LSD1 activity

Effects of LSD1 inhibition by higher concentration TMZ on chromatin structure and gene expression

The suppression of LSD1 demethylase activity by higher concentrations of TMZ has direct structural and transcriptional consequences for chromatin organization in glioma cells. Under normal conditions, active LSD1 continuously removes mono- and dimethyl marks from H3K4. Maintaining a state of relative hypomethylation at this size. This demethylation activity promotes chromatin compaction: H3K4 hypomethylation is associated with nuclear stability and reduced access of RNA polymerase II (Pol II). [31,32,33,34] [35,36,37,38,39] When LSD1 is active and chromatin is in a more compacted conformation, LSD1-dependent target genes, including a subset of oncogenes and proliferation-associated genes that rely on LSD1-mediated chromatin remodeling for their expression, are actively transcribed, as shown in Figure 6.

At higher TMZ concentrations (≥150 uM), inhibition of LSD1 via both direct and indirect mechanisms disrupts this steady-state demethylation activity. The resulting accumulation of methyl marks on H3K4 shifts the chromatin landscape toward a more open, euchromatic configuration at loci normally maintained in a compacted state by LSD1. Paradoxically, this more open chromatin state at LSD1 target loci corresponds to repression rather than activation of LSD1-dependent genes. This occurs

because LSD1 in glioma cells frequently functions as part of transcriptional corepressor complexes: its demethylation of H3K4me1/2 at enhancers and promoters is a prerequisite for the recruitment of downstream repressor machinery, and loss of this activity decommissions LSD1-dependent enhancers, silencing the associated gene programs.

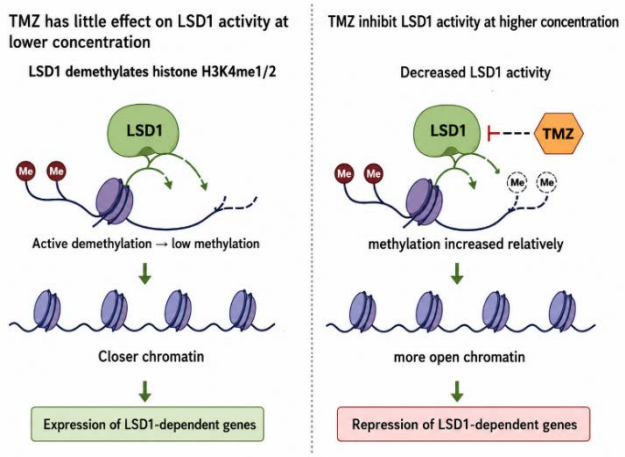

Figure 6. Schematic model of LSD1 activity and TMZ concentration effects on chromatin structure and gene expression

Consequently, higher concentrations of TMZ result in the downregulation of LSD1-dependent oncogenic programs, including genes involved in cell proliferation, self-renewal, and resistance to apoptosis. In contrast, tumor suppressor genes whose silencing is maintained through LSD1-dependent H3K4 demethylation may be depressed. This bidirectional transcriptional reprogramming represents a mechanistically coherent explanation for part of the anti-tumor effect observed at high TMZ concentrations.

Taken together, the suppression of LSD1 by high-concentration TMZ initiates a cascade of epigenetic events, from the direct accumulation of methyl marks to altered chromatin remodeler activity, to larger-scale changes in nucleosome positioning and transcription factor binding, and ultimately to the reprogramming of gene expression networks that underlie glioma cell survival and proliferation. This positions high-dose TMZ not merely as a genotoxic agent, but as a broader epigenetic modulator capable of reshaping the chromatin and transcriptional landscape of glioma cells in ways that may complement and enhance its anti-tumor efficacy. [40,41,42,43,44]

Several Limitations of the current study should be acknowledged. The demethylase assay was conducted using nuclear protein extracted from the glioma cells after treatment with varying concentrations of TMZ. Cellular biology is a dynamic system in which protein expression and functional roles can change over time. There are also notable differences between glioma stem cells (GSCs) and the U87 glioma cell line, as GSCs often exhibit a more robust and complex DNA repair capacity, largely attributable to their stem-like properties and adaptive plasticity. [46,47,48,49,50] While both systems demonstrate that LSD1 inhibition sensitizes cells to TMZ, the prioritized pathways may differ. Our previous study in breast cancer cells demonstrated that TMZ increased LSD1 activity and did not show a significant change in histone methylation levels, as we observed in U87 glioma cells. These differences emphasize that cellular responses are not static within the same system and that the specific cellular environment and the developmental state of the tumor cells heavily influence interactions between LSD1 and TMZ.

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