Role of DNA Ligase I in Modulating PARP1 Inhibitor-Mediated Chemosensitization in Glioblastoma

ABSTRACT #5676

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ABSTRACT

BACKGROUND + OBJECTIVE

- Glioblastoma (GBM) is the most aggressive primary malignant brain tumor and remains associated with limited therapeutic options and poor patient survival.
- Poly(ADP-ribose) polymerase inhibitors (PARPi), which impair DNA repair and exacerbate replication stress, are being explored as chemosensitizers in GBM. However, variable PARPi-mediated temozolomide (TMZ) sensitization underscores the need to define molecular determinants of response.
- Here, we investigate whether DNA ligase I (LIG1), a key DNA nick-sealing enzyme, regulates PARPi–TMZ sensitivity in GBM.

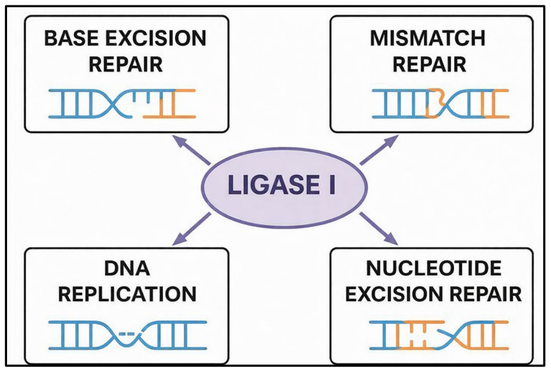

FIGURE 1. Schematic representation of LIG1 function across diverse DNA repair pathways. The diagram was generated with assistance from artificial intelligence.

METHODS

- Human GBM cell lines (U251, LN229, and T98G) were transfected with non-targeting or LIG1-specific siRNA. Knockdown efficiency was confirmed by immunoblotting.
- Cell survival was assessed using clonogenic and CyQUANT assays, while DNA damage signaling was evaluated by immunoblotting.

RESULTS

- LIG1 knockdown sensitized U251 GBM cells to the PARP inhibitor AZD9574 (30 nM), whereas siCtrl had no appreciable effect.
- Compared with controls, LIG1 silencing further increased TMZ sensitivity in TMZ-responsive U251 and LN229 cells but did not alter TMZ response in a resistant line.
- The greatest cytotoxicity was observed with combined LIG1 knockdown and PARPi–TMZ treatment. This effect extended to the MGMT-unmethylated T98G line, which is modestly sensitized to TMZ by PARPi; LIG1 silencing further enhanced sensitivity to the combination.

CONCLUSION

- LIG1 suppression enhances GBM sensitivity to PARPi/TMZ co-treatment, with increased single-agent activity in select models.
- Targeting LIG1 may improve the therapeutic efficacy of DNA-damaging regimens, warranting further evaluation of LIG1 inhibitors for efficacy and safety.

RESULTS

EFFECTS OF LIG1 KNOCKDOWN ON U251 SENSITIVITY TO AZD9574/TMZ

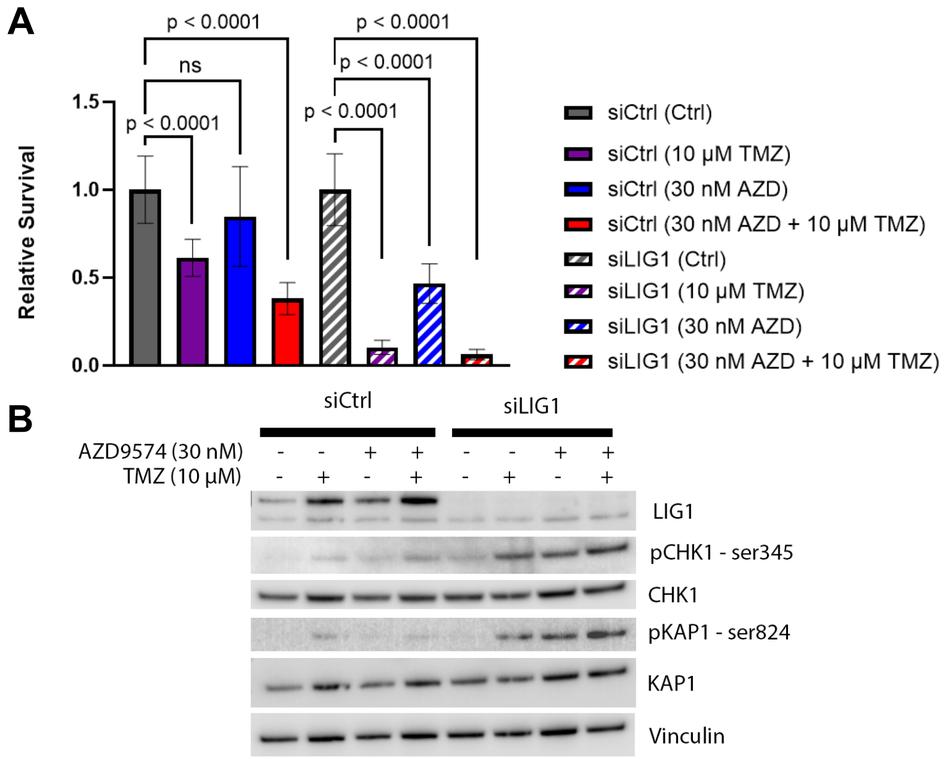

Figure 2. LIG1 knockdown sensitizes U251 GBM cells to AZD9574 ± TMZ. (A) Clonogenic survival of U251 cells treated for 24h with TMZ (10 μM) and/or AZD9574 (30 nM). Cells were plated 48h after transfection with siCtrl or siLIG1, and colonies were quantified after 14 days. (B) Western blot analysis of LIG1 expression and DNA damage signaling under the same conditions.

EFFECTS OF LIG1 KNOCKDOWN ON LN229 SENSITIVITY TO AZD9574/TMZ

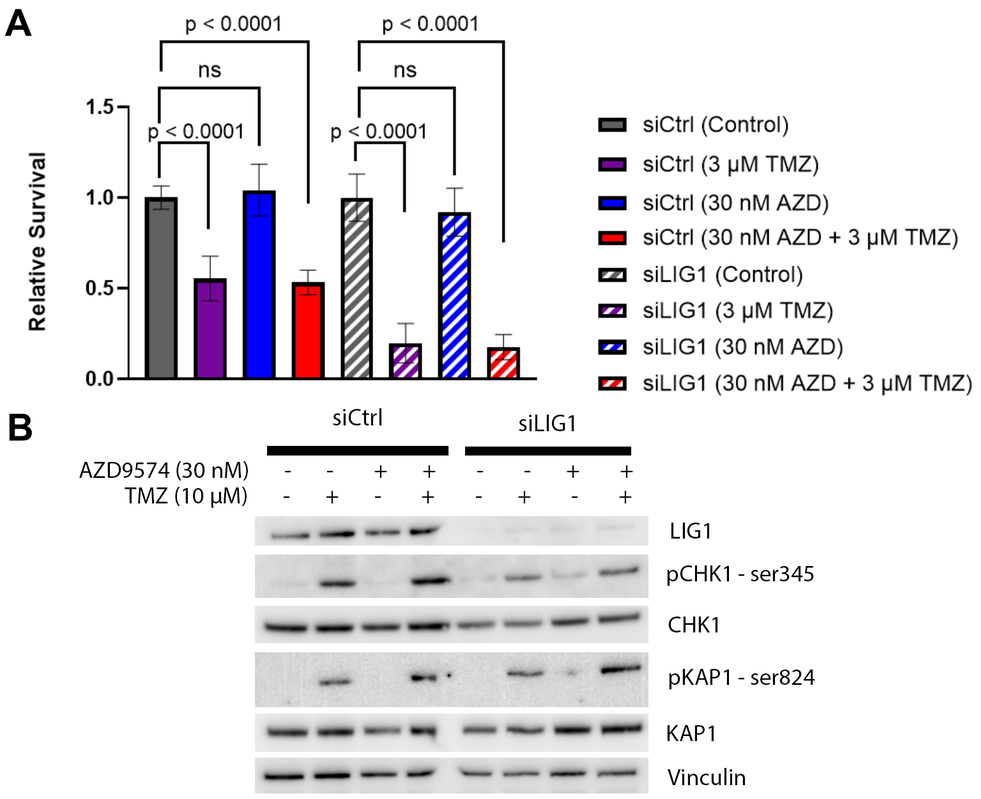

Figure 3. LIG1 knockdown sensitizes LN229 cells to AZD9574 ± TMZ. (A) Clonogenic survival of U251 cells treated for 24h with TMZ (3 μM) and/or AZD9574 (30 nM). Cells were plated 48h after transfection with siCtrl or siLIG1, and colonies were quantified after 14 days. (B) Western blot analysis of LIG1 expression and DNA damage signaling under the same conditions.

EFFECTS OF LIG1 KNOCKDOWN ON T98G SENSITIVITY TO AZD9574/TMZ

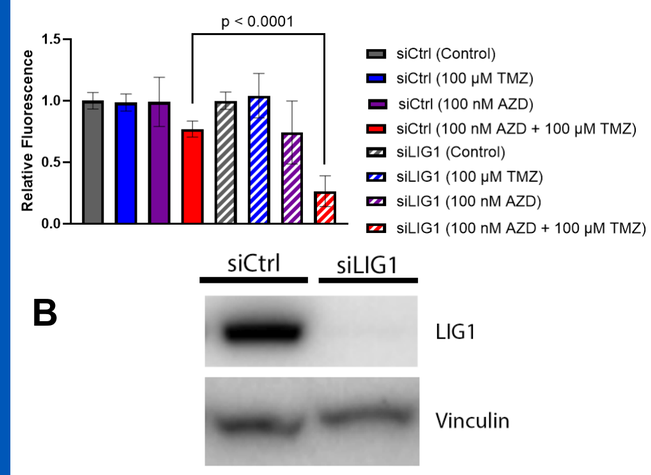

Figure 4. LIG1 knockdown enhances AZD9574-mediated TMZ sensitization in T98G cells. (A) CyQUANT proliferation assay of T98G cells treated with TMZ (100 μM) and/or AZD9574 (100 nM). Cells were plated 48h after transfection with siCtrl or siLIG1, treated next day and analyzed after 6 days. **(B)** Western blot confirming LIG1 knockdown.

DISCUSSION

- LIG1 inhibition may sensitize a subset of GBM to PARP inhibitor therapy.
- While LIG1 knockdown enhances TMZ sensitivity in TMZ-responsive cells but not in resistant models, suggesting a potential role in O6MeG processing, it also increases sensitivity to the AZD9574/TMZ combination regardless of MGMT methylation status.
- Further studies evaluating the efficacy and toxicity of LIG1 inhibition are needed to support clinical translation.

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