Murine Glioma Cells That Develop Resistance To Topoisomerase Inhibitors Remain Sensitive To RSL3 But Not IKE

Peter B. Wu, B.S., Athanassios Dovas, Ph.D., Peter D. Canoll, M.D., Ph.D., Jeffrey N. Bruce, M.D.

Poster 2580
No Disclosures
Introduction

- Etoposide is a topoisomerase inhibitor targeting the cell cycle that has been shown to improve survival in animal malignant glioma models.
- Ferroptosis is lipid peroxide mediated cell death, and a metabolic target that could overcome resistance to cell cycle directed therapy.
- RSL3 inhibits GPX4, while IKE inhibits SLC7a11. Both are investigational drugs that induce ferroptosis.
- To investigate the potential for enhanced glioma therapy, we sought to generate an etoposide resistant glioma cell line to test its susceptibility to ferroptosis induction.
Methods

• The “MGPP3” murine glioma cell line was grown in increasing concentrations of etoposide, up to 200nM, which yielded the etoposide resistant “ME200” line.

• Dose-response curves were generated by incubating cells in drug, and viability assessed via CellTiter-Glo Assay.

• Flow cytometry was conducted by incubating cells in drug before staining the cells with BODIPY, which detects lipid peroxidation.

• Glutathione assay was conducted by incubating cells in drug before using the commercially available Ab1388801 kit.
ME200 is resistant to etoposide and topotecan.

Dose response curves are shown for etoposide (left) and topotecan (right). MGPP3 cells (circles) show significantly greater sensitivity to both drugs ($p<0.001$) compared to ME200 cells (squares).
ME200 Is Resistant to IKE

Dose response curve showing that MGPP3 (black line) cells are susceptible to IKE, while ME200 (blue line) cells are not. IKE’s effects on MGPP3 cells (red line) are rescued by ferroptosis inhibitor Ferrostatin. No difference is seen in the ME200 cells (green line).

Glutathione assay shows a significant decrease in GSH in response to IKE in the MGPP3 cells (black, left) (p<.0001) but not the ME200 cells (grey, right).

Flow cytometry shows an increase in lipid peroxidation in response to IKE in the MGPP3 cells (left) but not the ME200 cells (right).
ME200 Is Susceptible to RSL3

Flow cytometry shows an increase in lipid peroxidation in response to RSL3 in both the MGPP3 and ME200 cell lines.

Dose response curve showing that MGPP3 (red line) and ME200 (black line) cells are susceptible to RSL3, RSL3’s effects on MGPP3 (blue line) and ME200 (green line) cells are rescued by ferroptosis inhibitor Ferrostatin.
qPCR shows that compared with the MGPP3 cells, the ME200 cells have decreased expression of SLC7a11, which is IKE’s target (p=.002). It also shows that CHAC1 (p=.02) and ATF4 (p=.008) have less expression in the ME200 cells. GPX4, which is RSL3’s target, showed similar expression between the two cells.
Discussion

• Current glioma therapies are largely directed at the cell cycle. However, recurrence with eventual resistance to these drugs is a virtual inevitability. This study suggests that ferroptosis, a cell cycle independent target, is a promising avenue for addressing this issue.

• The development of etoposide resistance was associated with altered metabolism and downregulation of ferroptosis related genes.

• Metabolic therapies may be a means of treating glioma cells that have become resistant to cell cycle directed therapy.
Summary Points

• We were able to successfully derive an etoposide resistant cell line from the MGPP3 line, which was named “ME200”

• The ME200 line was also resistant to topotecan, in spite of never having been exposed to the drug

• Despite resistance to etoposide, the ME200 cells remained sensitive to the ferroptosis inducing drug RSL3

• The ME200 cells were resistant to IKE, suggesting that mechanisms of resistance can affect some metabolic sensitivities but not others.

• Ferroptosis provides a possible mechanism for targeting glioma cells that have become resistant to cell cycle directed therapy