

**Title:** Targeting treatment-refractory glioma stem cells via activation of the integrated stress response

**Authors:** Saad Sheikh, Xiaobing Tian, Deeksha Saxena, Gary D. Kao, Jay F. Dorsey

The high incidence of glioblastoma recurrence necessitates additional therapeutic strategies. Heterogeneous populations of cells, including glioma stem cells (GSC) have been implicated in disease recurrence. GSC are able to survive gamma irradiation and temozolomide (TMZ) treatment due to upregulation of DNA damage pathways. One potential strategy to target treatment resistant tumor populations may be via the integrated stress response (ISR). Modulation of the ISR pathway also allows for sensitization of treatment resistant cells to TNF related apoptosis inducing ligand (TRAIL). We generated a novel cell-based death receptor assay to identify potent inducers of ISR dependent DR5 expression. We used this assay to screen compounds from three commercially available libraries, and identified 1-Benzyl-3-cetyl-2-methylimidazolium iodide (NH125) as a potent inducer of DR5 expression. NH125 engages the EIF2alpha-ATF4-CHOP axis culminating in DR5 expression at high nanomolar to low micromolar doses. CHOP was found to be essential for NH125-mediated TRAIL synergy. NH125 also produced a significant decrease in GSC viability when compared to TMZ and radiotherapy. GSC demonstrate an increased sensitivity to NH125 treatment when compared to other cancer cells, and synergizes with TRAIL at lower doses than all other cell lines tested. Furthermore, NH125 treatment leads to a unique transcriptional profile in GSC that involves activation of ISR and GADD45 pathways. Taken together, these data suggest that engaging the ISR pathway may represent a promising strategy to target treatment refractory GSC. Based upon this data, we are now conducting a high throughput screen of approximately 3000 bioactive molecules to uncover additional therapeutic lead agents.