Chemical Activation of Piezo1 to Enhance TRAIL-induced Apoptosis in Glioblastoma Cells

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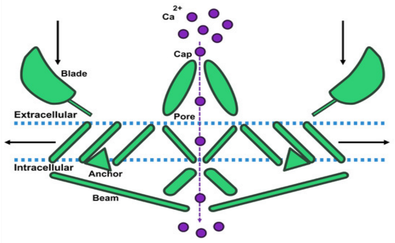
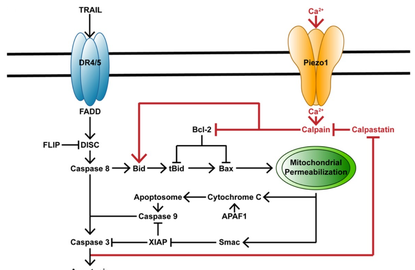
Introduction

- Mean survival post-treatment for glioblastoma multiforme (GBM) is 14-15 months
- Tumor Necrosis Factor-related apoptosis-inducing ligand (TRAIL) triggers apoptosis in multiple tumor types
- Activating mechanoreceptor Piezo1 via Yoda1 has been shown to induce TRAIL sensitization in other cancer cell lines
- Immortalized LN18 cells are resistant to Temozolomide (TMZ), the current standard chemotherapy, while immortalized U87 cells are TMZ sensitive



Schematic of Piezo1 and pore channel opening
Dombroski, et al, *Cells*, 2021.



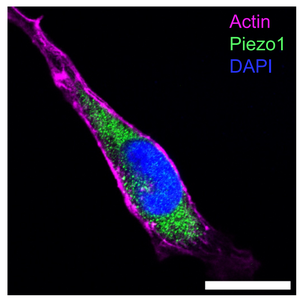
TRAIL-induced apoptosis pathway
Hope, et al, *Cell Death and Disease*, 2019.

Materials and Methods

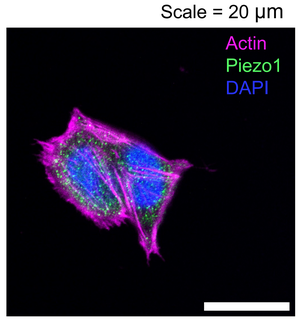
Treatment with TRAIL + Yoda1

- Cells were treated with 25 ng/mL TRAIL and 10μM Yoda1
  - U87 cells were treated for 24 h and LN18 for 4 h

Scale = 20 μm



U87- more metastatic, TMZ sensitive



LN18- less metastatic, TMZ resistant

Flow cytometry assays

- Annexin V and Propidium Iodide (AV-PI) assays were used to measure cell viability, apoptosis, and necrosis
  - Cells were treated with DMSO (10μM), Yoda1 (10μM), TRAIL (25 ng/mL), low dose TMZ (50μM) and high dose TMZ (200 μM)
- JC-1 assays were used to quantify mitochondrial depolarization of LN18 cells
  - The same protocol was followed as the AV-PI assay, with cells stained with 1 mL of 40 μM JC-1.
- Calcium flux assays were performed to quantify calcium influx
  - 1mM Fluo-4 and 2mM Fura Red fluorescent dyes were used
  - Cells treated with 1 μM, 5 μm, 10 μM, and 50 μM of DMSO and Yoda1
- Piezo1 antibody was used to quantify ion channel expression

Data analysis

- FlowJo software used for gating and analysis
- GraphPad Prism used for generating graphs and statistics

Results

Cell viability decreases in GBM cells following TRAIL and Yoda1 treatments

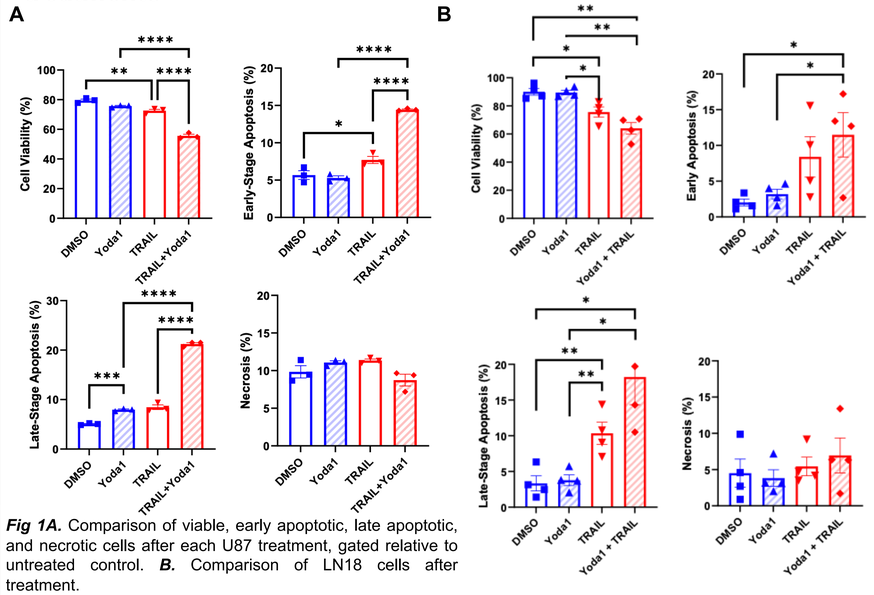


Fig 1A. Comparison of viable, early apoptotic, late apoptotic, and necrotic cells after each U87 treatment, gated relative to untreated control. ***B.*** Comparison of LN18 cells after treatment.

TRAIL and Yoda1 treatments result in decreased cell viability relative to the standard of care

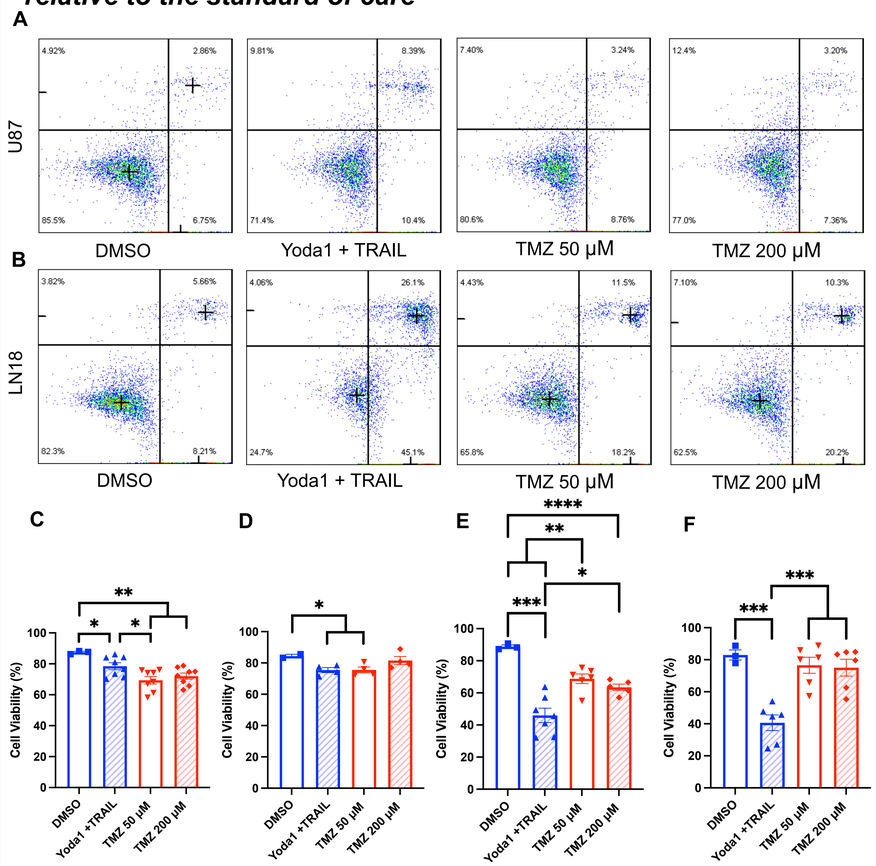


Fig 2A. Representative flow plots of U87 cells 72 h post-treatment, gated relative to DMSO (vehicle control). ***B.*** Representative flow plots of LN18 cells 8h post-treatment. ***C.*** U87 cells 24 h after treatment, ***D.*** U87 cells 72 h after treatment, ***E.*** LN18 cells 4h after treatment, and ***F.*** LN18 cells 8h after treatment.

Results

Treatment with TRAIL and Yoda1 increases mitochondrial depolarization in GBM cells

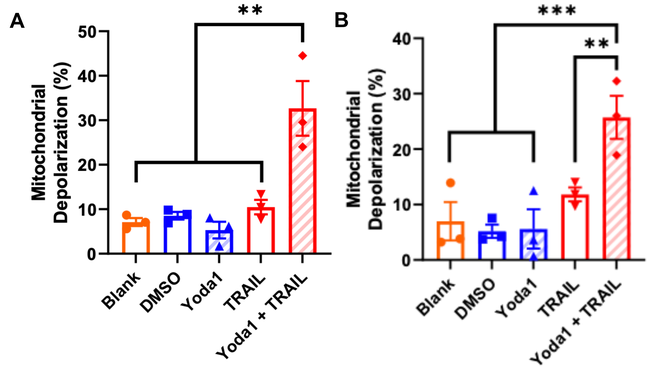


Fig 3. Comparison of mitochondrial depolarization for ***A.*** U87 cell and ***B.*** LN18 cell treatments.

U87 and LN18 cells exhibit different levels of Piezo1 expression and activation

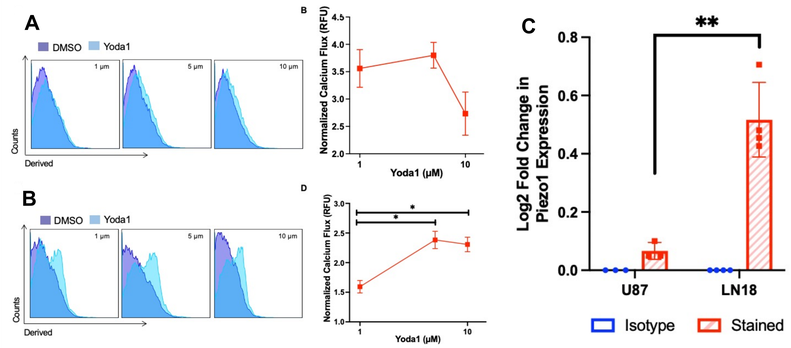


Fig. 4A. Comparison of Yoda1 induced calcium influx in U87 cells measured in ratiometric flux units (RFU) normalized to DMSO control. ***B.*** Comparison of Yoda1 induced calcium influx in LN18 cells measured in ratiometric flux units (RFU) normalized to DMSO control. ***C.*** Analysis of Piezo1 expression in U87 and LN18 cells.

Conclusions

- TRAIL + Yoda1 treatment results in a significant increase in apoptosis relative to controls and TMZ, demonstrating therapeutic effectiveness
- The TRAIL apoptotic pathway, activated by Yoda1 and Piezo1, executes intrinsic apoptosis, since the combined treatment caused significant mitochondrial depolarization
- The differing sensitivity of U87 and LN18 cells to TRAIL + Yoda1 may be related to Piezo1 expression

References and Acknowledgements

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