Propranolol Anti-tumor Effect In GBM is Mediated By Broad Changes In Gene Expression Networks

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Disclosure Statement

- The lead author has no conflict of interest that need to be disclosed
Repurposing Propranolol as an Anti-Tumor Agent for Glioblastoma

- Glioblastoma multiforme (GBM) is the most lethal primary brain tumor and there is an urgent need for more effective treatments.
- Propranolol, a pan-beta receptor antagonist, has demonstrated potent anti-tumor properties against VHL-associated hemangioblastomas and other solid tumors, including breast cancer and melanoma.
- Preliminary *in-vitro* studies have demonstrated that propranolol exerts an anti-tumor effect on multiple GBM cell lines.
- The exact mechanisms of Propranolol’s anti-tumor action remain unknown and was the focus of this investigation.
Study Objective Methods & Study Overview

- **Study Objective:** To identify the mechanistic underpinnings of propranolol’s anti-tumor effect on GBM cells

- **Methods:**
  - Six GBM cell lines (U251, GL261, 9L, S635, LN229 and A172) were treated with varying concentrations of propranolol (0-400µM) *in vitro*
  - Therapeutic response in propranolol-treated GBM cells was assessed with the following *in-vitro* assays:
    - Cell viability and migration assays
    - Immunoblots
    - Quantitative RT-PCR
    - Flow Cytometry
    - RNA-sequencing analyses
Propranolol Decreases the Viability of GBM Cell Lines

- Propranolol-treated GBM cells demonstrated reduced viability with an IC$_{50}$ of 100µM-200µM 24-hours post-exposure (A)
- Propranolol-treated U251 and S635 cells exhibited a 3-fold increase in the rate of apoptosis as determined by flow cytometry. Representative data shown (n=3, p<0.0001) (B)
Propranolol Decreases GBM Cell Migration and VEGF-A Expression

- Despite QT-PCR demonstrating increased expression of VEGF-A in propranolol treated GBM cells (A), a notable decrease in VEGF protein expression in propranolol treated GBM cells was appreciated in immunoblots (B) and immunofluorescence (C), suggesting translational downregulation of VEGF by propranolol.

- GBM cells (S635 and U251) exposed to 50µM and 100µM of propranolol for 48 hours exhibited a 6-fold reduction in migratory capacity via transwell assay (Figure A, p<0.001) (D).
Propranolol Alters Functional Gene Clusters Involving Cell Cycle and Cholesterol Synthesis

- RNA-seq analysis in U251 glioma cells treated with 100µM of propranolol for 24-hours revealed a statistically significant decrease in genes that control chromosomal replication and a substantially increase in the expression of genes involved in cholesterol biosynthesis.
Propranolol Modulated the Expression Genes Associated with Cellular Proliferation

- Analysis of individual genes that were differentially expressed at significant levels revealed that propranolol modulated the expression of several genes that have been previously implicated previously in cellular proliferation and/or GBM progression

- Propranolol downregulated the expression of the following genes:
  - KRT6B (86 fold, p<0.0001)
  - ALOX5 (8.5 fold, p<0.0001)
  - CXCR4 (7.5 fold, p<0.0001)

- Propranolol upregulated the expression of the following genes:
  - KRT6B (86 fold, p<0.0001)
  - DDIT4L (34.4 fold, p<0.0001)
  - CCN3 (25.4 fold, p<0.0001)
Conclusion

- Propranolol, a well-tolerated, FDA-approved beta antagonist decreases glioma proliferation, decreases its migratory potential, downregulates VEGF protein expression and leads to numerous gene expression changes that decrease GBMs proliferative potential.

- Given a long track record of safety and tolerability, clinical efforts may be pursued to probe propranolol’s anti-tumor potential in patients with GBM.
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