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Targeting an Immortalization Mutation to Control Glioblastoma

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Abstract

Glioblastoma multiforme (GBM), the most aggressive primary brain tumor, is a very heterogeneous tumor, but its standard treatment of temozolomide and radiation therapy has not changed for the past thirty years. However, ***over 80% of GBM*** tumors have a telomerase reverse transcriptase (TERT) promoter mutation, or TPM. This makes the mutation an attractive target in glioma cells. TERT is the catalytic subunit of telomerase, an enzyme residing in the nucleus that regulates the length of telomeres, a specific sequence of nucleotides located at the end of chromosomes. TERT is often overexpressed in malignant cancers to prevent cells from senescence and to promote rapid and unchecked cellular division, and it is rarely expressed in somatic cells with the exception of stem cells. Targeting the TPM with the pharmacological chaperone RG1534, a small molecule inhibitor, stabilizes the G4 structure in the promoter and the resultant transcription of the protein. Glioblastoma cells were plated, lysed, treated, and their RNA was extracted, reverse transcribed, and amplified in RT-qPCR to determine the effect of TPM inhibition. Biological replicates of TPM inhibition were also plated for western blots to validate the fold gene expression change calculated in qPCR. Both a decrease in TERT mRNA and protein expression was determined, coinciding with high levels of apoptosis. Additionally, western blots were conducted on sub-cellular fractions of different glioblastoma cell lines to show TERT and its expression outside the nucleus. In the preliminary investigation of the functional effects of a normalized TPM, TERT was revealed to be present in the cytosol and mitochondria, prompting that it has non-canonical functions outside of the nucleus. Stabilizing the TPM therefore warrants further study of the functional effects of this mutation's inhibition. Since the standard of care for this cancer has not changed, the TPM is a specific, viable therapeutic target in GBM patients.

Introduction

Glioblastoma multiforme is a grade IV tumor.

- GBM is a cancer of astrocytes; it grows quickly and invasively, often mutated.
- This makes it hard to treat along with reports of incessant recurrence.
- Prognosis: median survival is 12-16 months after diagnosis.

Telomerase reverse transcriptase (TERT) is the catalytic component of telomerase.

- TERT is involved in the elongation of telomeres through the addition of a specific nucleotide sequence (TTAGGG)
- TERT is a mobile protein; it shuttles between the mitochondria and nucleus – its non-canonical functions involve protection against DNA damage and cellular stress via reduction of ROS production in the mitochondria.
- Upregulation of TERT prevents cell senescence, which contributes to GBM's resistance to standard therapies.

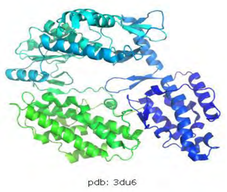


Figure 1. 3-D structure of TERT.

A mutated TERT promoter region (TPM) leads to high levels of the protein's expression in GBM cells.

- It is associated with a higher level of TERT mRNA expression.
- The TPM is present in over 80% of GBM tumors.

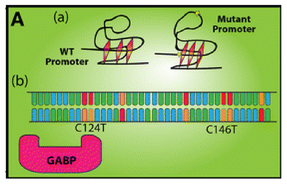
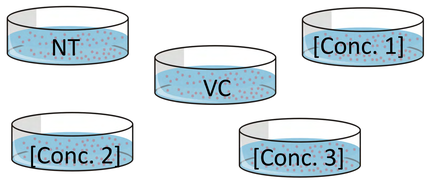


Figure 2. TERT promoter region.

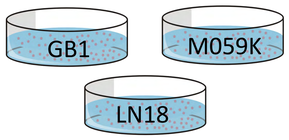
Methods and Workflow

Hypothesis: Stabilizing the TPM inhibits TERT production in GBM, and in doing so affects both the canonical and non-canonical functional pathways of this protein.



T98G and A172 cells are cultured in five 100 mm dishes and treated with RG1534 for 24 hours.

Cells are lysed and RNA is extracted to perform RT-qPCR



Glioma cells are fractionated and TERT expression in sub-cellular fractions were investigated

Results

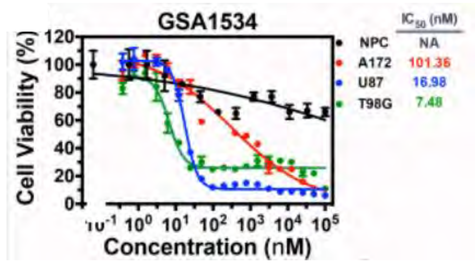


Figure 3. RG1534 potent in glioma cell lines. Cells were treated for 24 hours and drug dose response was measured via Cell Titer Glo.

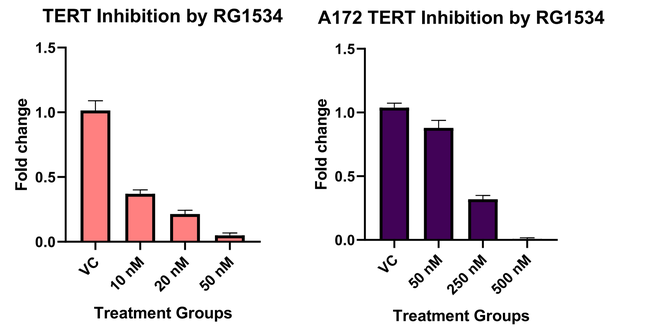


Figure 4. T98G and A172 cells pose a dose dependent response to RG1534. Concentrations were determined by IC50. Fold change in TERT gene expression in cells after treated with drug for 24 hours.

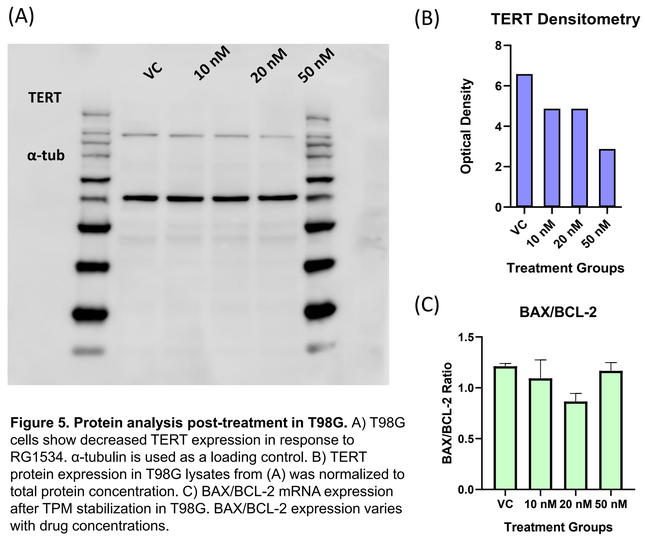


Figure 5. Protein analysis post-treatment in T98G. A) T98G cells show decreased TERT expression in response to RG1534. α-tubulin is used as a loading control. B) TERT protein expression in T98G lysates from (A) was normalized to total protein concentration. C) BAX/BCL-2 mRNA expression after TPM stabilization in T98G. BAX/BCL-2 expression varies with drug concentrations.

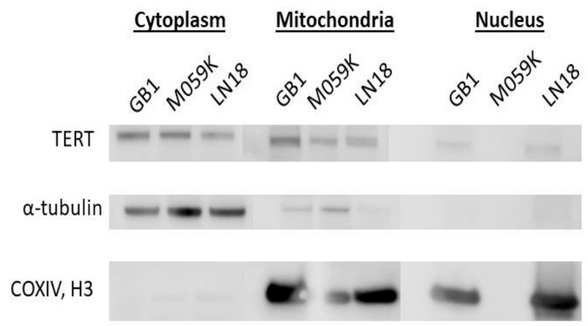


Figure 6. TERT is expressed in non-nuclear locations. Nuclear, cytoplasmic, and mitochondrial markers are shown along with hTERT in different glioblastoma cell lines.

Discussion

- **TERT inhibition via RG1534 is shown to be a feasible strategy to limit tumor cell growth by stabilizing the mutant TERT promoter.**
- A dose dependent response occurs in glioma cells when the mutated TERT promoter region is stabilized, leading to:
  - Decline in mRNA production.
  - Reduction in protein levels of TERT.
- **TERT is localized to the mitochondria.**
  - o TERT's non-canonical functions could reveal more about GBM's tumorigenesis and progression.
- Future steps:
  - o Determining if changes in TERT expression affect GBM vulnerability to standard of care.
  - o Showing how a stabilized TPM affects BAX and BCL-2 expression in various GBM cell lines at different time points.
  - o Further investigating the functional effects of a stabilized TPM:
    - Mitochondrial ROS production (non-canonical).
    - Telomere length (canonical).
  - o Transfecting *TERT*$^{-/-}$ glioma cells to induce TERT expression in subcellular locations.
    - Isolate TERT and its protein-protein interactions to further study its non-canonical functions.

Acknowledgments

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References

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