Investigating the effects of IDH and ATRX mutations on post-radiation survival and DNA damage repair mechanisms in glioma stem cells

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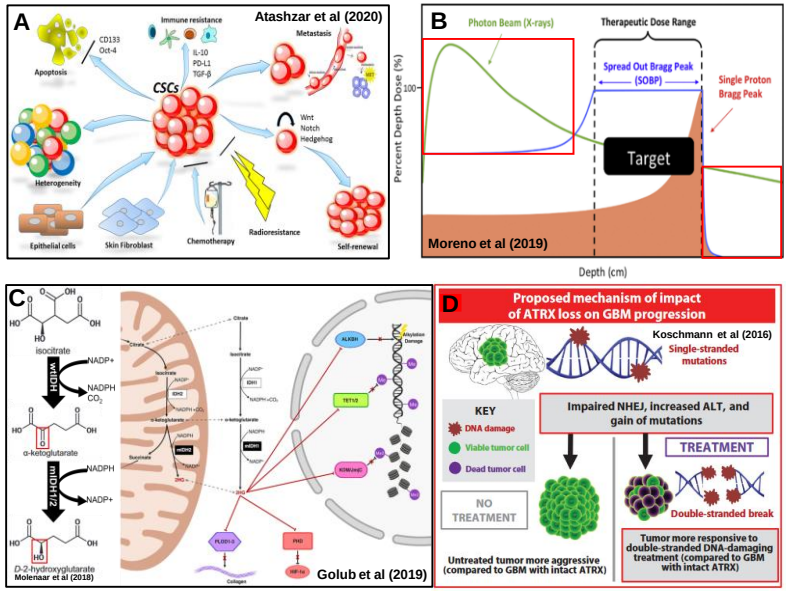
Introduction

- **Glioblastoma Multiforme (GBM):** 15 month median survival time.
- **Glioma Stem Cells (GSCs):** Self-renewable cells in GBM tumors, promote chemoradioresistance, tumorigenesis, and metastasis.
- **Isocitrate Dehydrogenase Mutations (mIDH)** present in 70-80% of gliomas, IDH1^{R132H} correlated with improved patient survival.
- **ATRX loss** often co-presents with mIDH in oligodendrogliomas.
- **Proton radiation therapy (PRT):** Highly tumor specific, cytotoxicity via DNA damage, more effective than X-ray therapy (XRT).
- Mechanisms by which mIDH and ATRX loss affect PRT response in GSCs not well understood.
- **Hypothesis: ATRX loss and mIDH promote higher radiosensitivity in GSCs irradiated with PRT vs. XRT.**



(A) Cancer Stem Cells (CSCs) contribute to poor patient outcomes by promoting therapeutic resistance and tumor growth. **(B)** PRT selectively irradiates the tumor while sparing surrounding critical brain structures required for normal cognition more effectively than XRT. **(C)** Mutant IDH promotes conversion of α-KG to 2-HG, which inhibits cytoplasmic and nuclear demethylases leading to DNA and histone hypermethylation. **(D)** ATRX loss inhibits GBM progression when treated with radiation therapy (DNA damaging treatment) by promoting ALT and genetic instability while also impairing NHEJ capabilities.

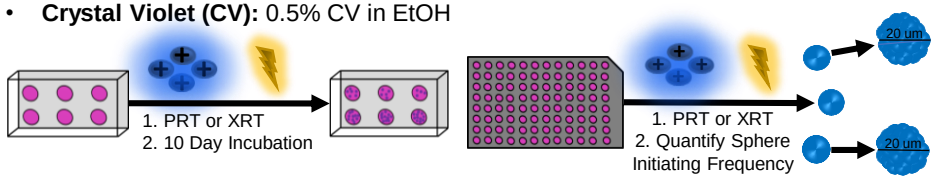
Methods

U87 Clonogenic Assay

- **Seeding Density:** 200, 400 cells/well
- **Irradiation Doses:** 2, 4, 6, 8 Gy
- **Proton LETs:** 3.3, 11.1 KeV/µm
- **Crystal Violet (CV):** 0.5% CV in EtOH

GSC Limiting Dilution Analysis

- Adapted from **Hu and Smyth (2009)**
- **Seeding Density:** 2, 5, 10, 20 cells/well
- **Irradiation Doses:** 1, 2, 3, 4 Gy



ATRX Loss Promotes PRT Sensitivity

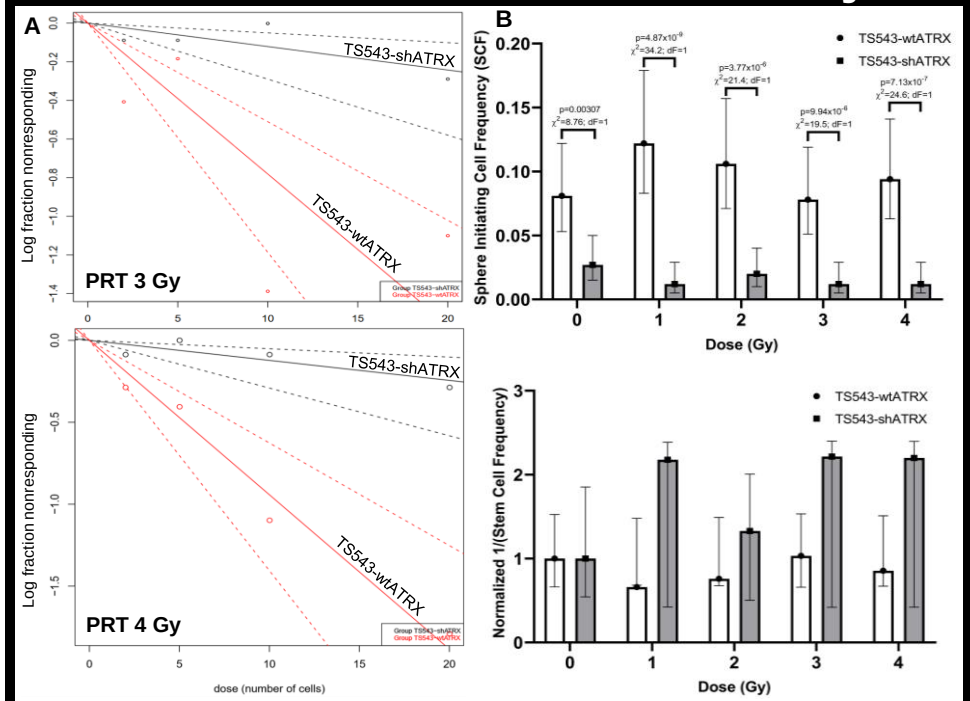


Figure 1: ATRX loss improves response to PRT in isogenic GSCs
(A) ATRX loss increased the fraction of non-spheroid forming cultures at 1-4 Gy. Data for 3 and 4 Gy shown. **(B)** TS543-shATRX exhibited lower SCFs (plotted with 95% CI) than TS543-wtATRX four days after PRT.

IDH1^{R132H} Promotes PRT Sensitivity

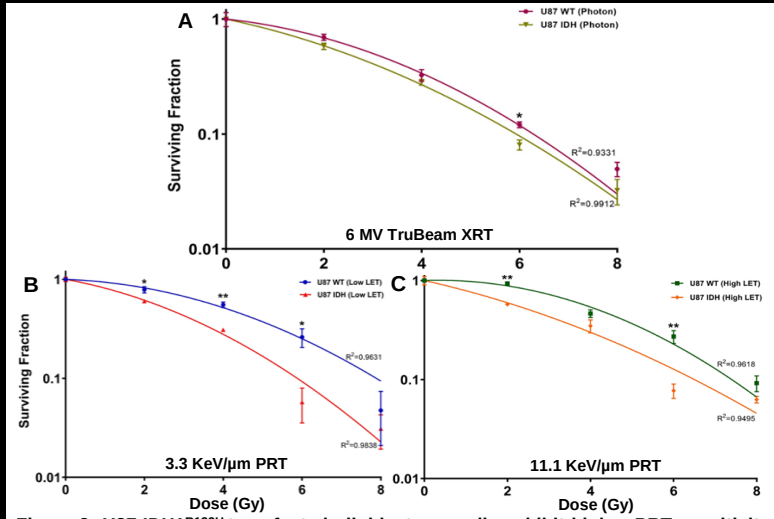


Figure 2: U87-IDH1^{R132H} transfected glioblastoma cells exhibit higher PRT sensitivity than U87-IDH1^{WT}. Survival plots after treatment with (A) Photons, (B) Protons at Low LET (3.3 KeV/µm), and (C) Protons at High LET (11.1 KeV/µm). *p<0.05 **p<0.01.

Discussion

Our results clearly demonstrate the heightened efficacy of PRT over XRT in both U87-IDH1^{WT} and U87-IDH1^{R132H} glioblastoma cells. Additionally, we provide clear evidence for the role of ATRX loss in promoting PRT sensitivity in TS543-shATRX isogenic GSCs. These results show the potential benefits of mIDH and ATRX loss as targets for therapeutic intervention in treating chemoradioresistant GSCs.

Future Directions

- Investigate the combination of **PRT/XRT and IDH1^{R132H} inhibitor Ivosidenib** in isogenic Doxycycline activated IDH1^{R132H} GSCs.
- Quantification of **HR and NHEJ specific DNA damage foci** in PRT irradiated U87s and GSCs.

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