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

Ángel A. García1, Lawrence Bronk1, Sharmishtha Chakraborty1, Krishna P.L. Bhat2, David R. Grosshans1
1. Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX.
2. Department of Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX.

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.

Methods

- **U87 Clonogenic Assay**
  - Seeding Density: 200, 400 cells/well
  - Irradiation LETs: 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

ATRX Loss Promotes PRT Sensitivity

IDH1R132H Promotes PRT Sensitivity

- **ATRX Loss Promotes PRT Sensitivity**
- **IDH1R132H Promotes PRT Sensitivity**

Discussion

Our results clearly demonstrate the heightened efficacy of PRT over XRT in both U87-IDH1WT and U87-IDH1R132H glioblastoma cells. Additionally, we provide clear evidence for the role of ATRX loss in promoting PRT sensitivity in TSS43-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 IDH1R132H inhibitor Ivosidenib in isogenic Doxycycline activated IDH1R132H GSCs.
- Quantification of HR and NHEJ specific DNA damage foci in PRT irradiated U87s and GSCs.

Acknowledgements

This research was funded by the National Institute of Health (NIH) R01 grant entitled “Synaptic basis of deficits in attention and executive function following cranial radiation.” A. García acknowledges Dr. Grosshans, Bhat, Bronk, and his advisory committee for their continued mentorship and support throughout this project. GSCs were generously donated to A. García by Dr. Bhat and Dr. Cahill.