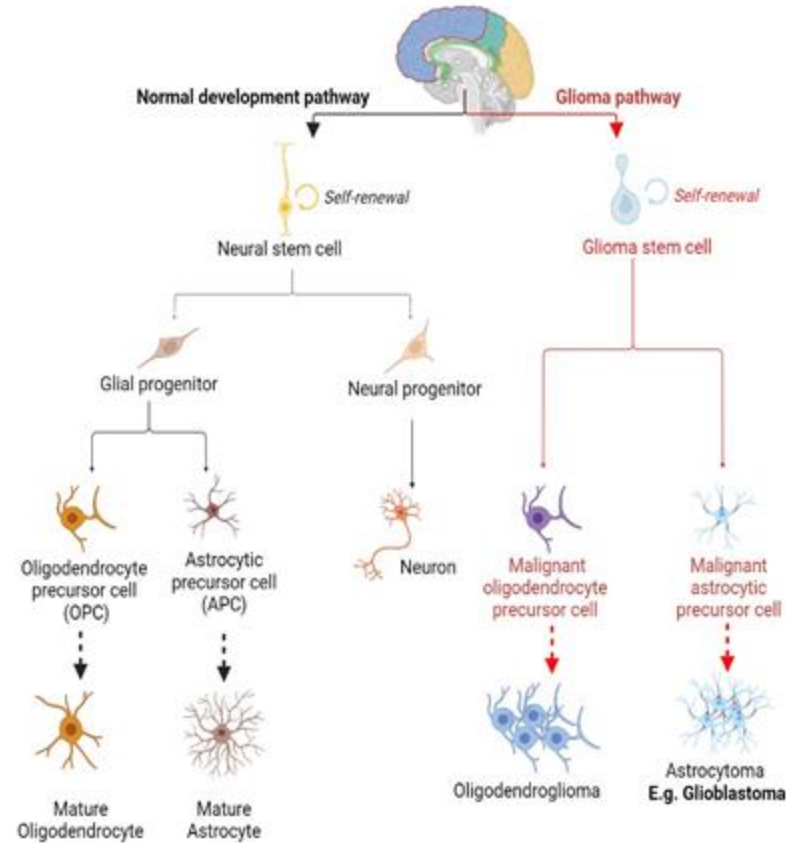


Inhibition of Cholinergic Signaling Enhances Radiation Treatment Efficacy in Glioblastoma

Ayanfeoluwa Kolawole, ASTRO MSF Fellow
Dartmouth Hitchcock Medical Center
Mentor: Dr. Kelli B. Pointer
09/28/2025

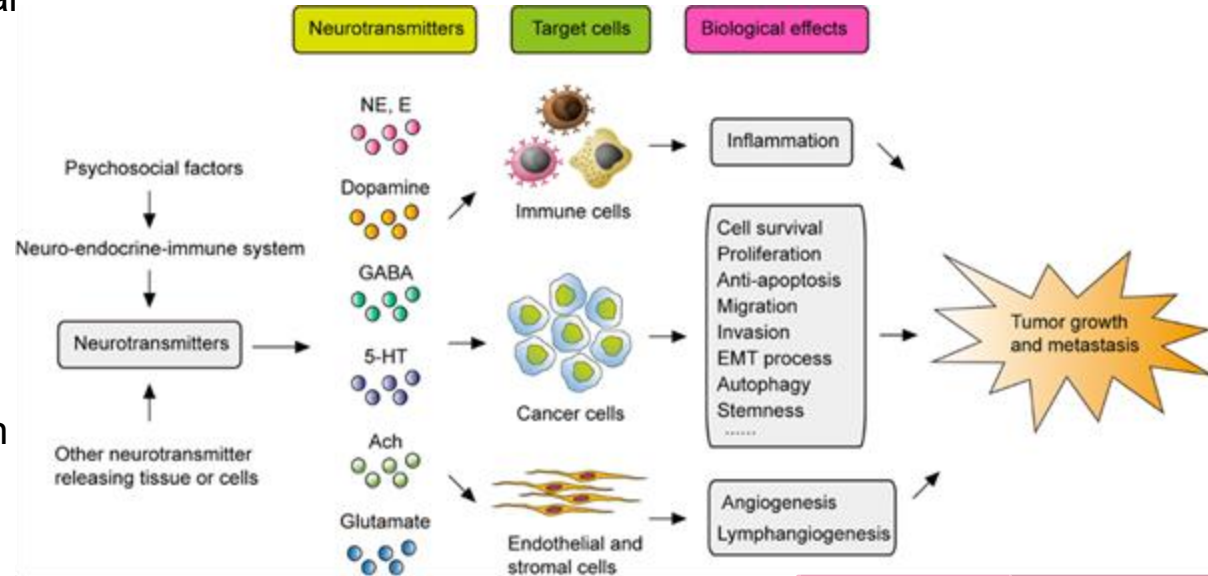
Glioblastoma are aggressive tumors resistant to standard therapies

- Glioblastoma (GBM) is the most aggressive malignant primary brain tumor with a median survival of < 2 years
- Standard treatment includes surgery, chemotherapy, and radiation therapy
- Cancer stem cells in GBM resistant standard treatment and contribute to poor outcomes



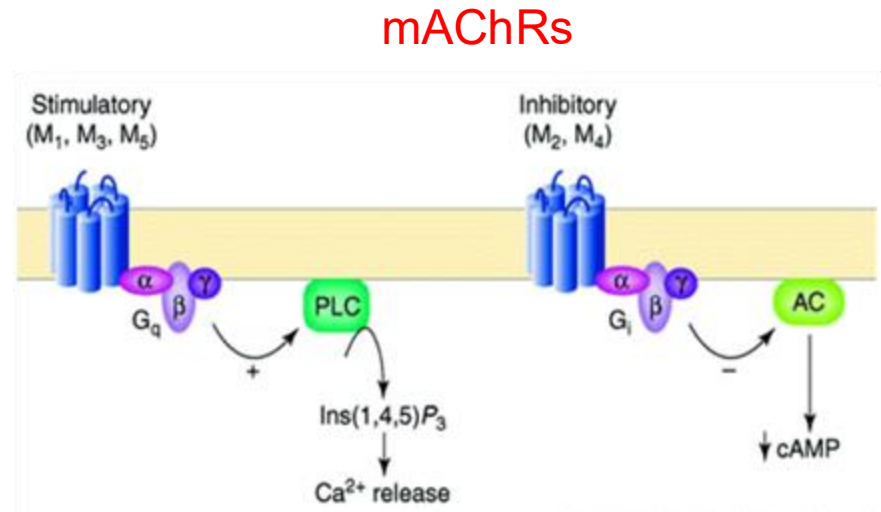
Neurotransmitters and Ach in cancer

- Neurotransmitters are chemical messengers that facilitate information transfer between neurons and target cells
- Neurotransmitters such as glutamate, dopamine and **acetylcholine (ACh)** have been implicated in tumorigenesis of GBM
- Most GBMs occur in ACh-brain lobes



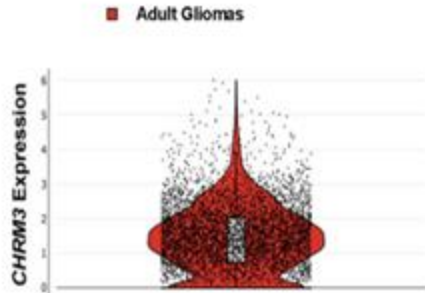
Acetylcholine signals through muscarinic receptors

- Acetylcholine (ACh):
 - Normally aids in memory, learning, attention, arousal, etc.
 - In the brain, ACh signals mainly in a paracrine manner and binds cholinergic receptors
 - maintains **stemness** in normal stem cell populations in the brain
- ACh can signal through G-protein coupled receptors called **muscarinic ACh receptors (mAChRs)**

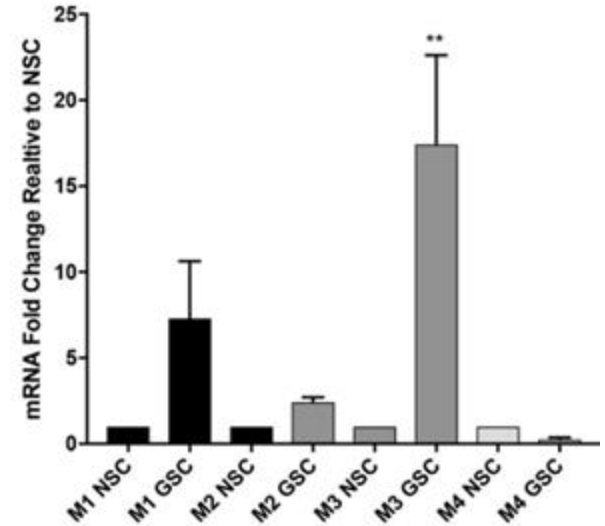
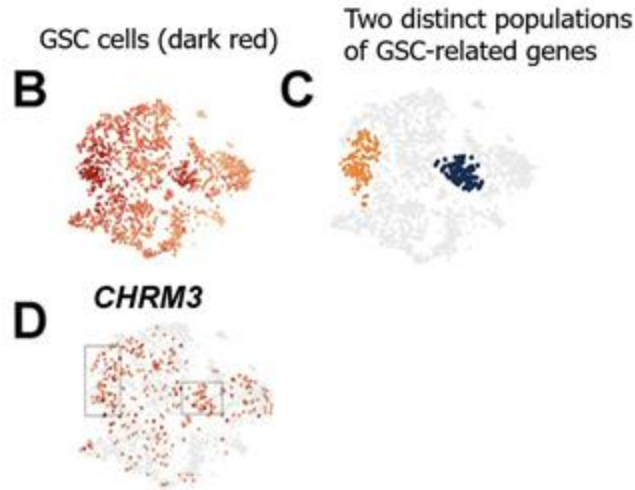


M3 mAChR (CHRM3) expression is upregulated in GBMs

Public scRNA-seq data shows high *CHRM3* expression in adult gliomas

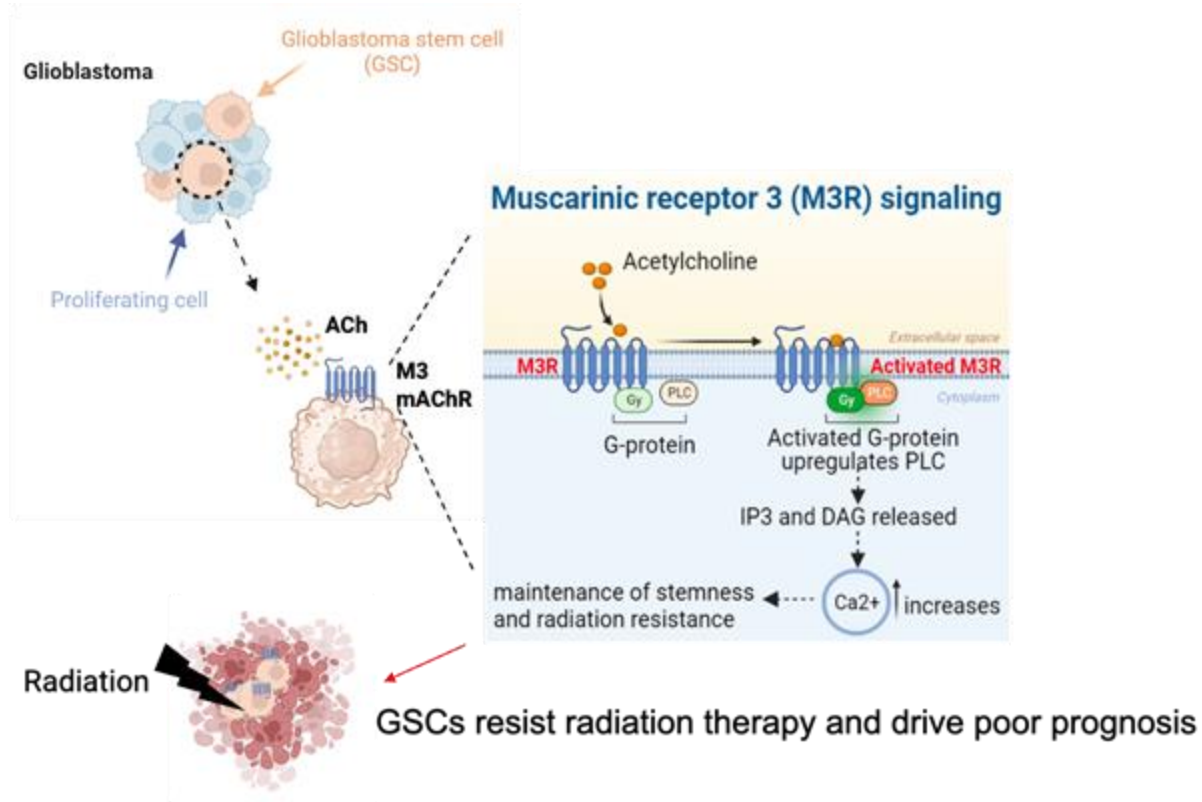


CHRM3 expression is upregulated in murine GSCs

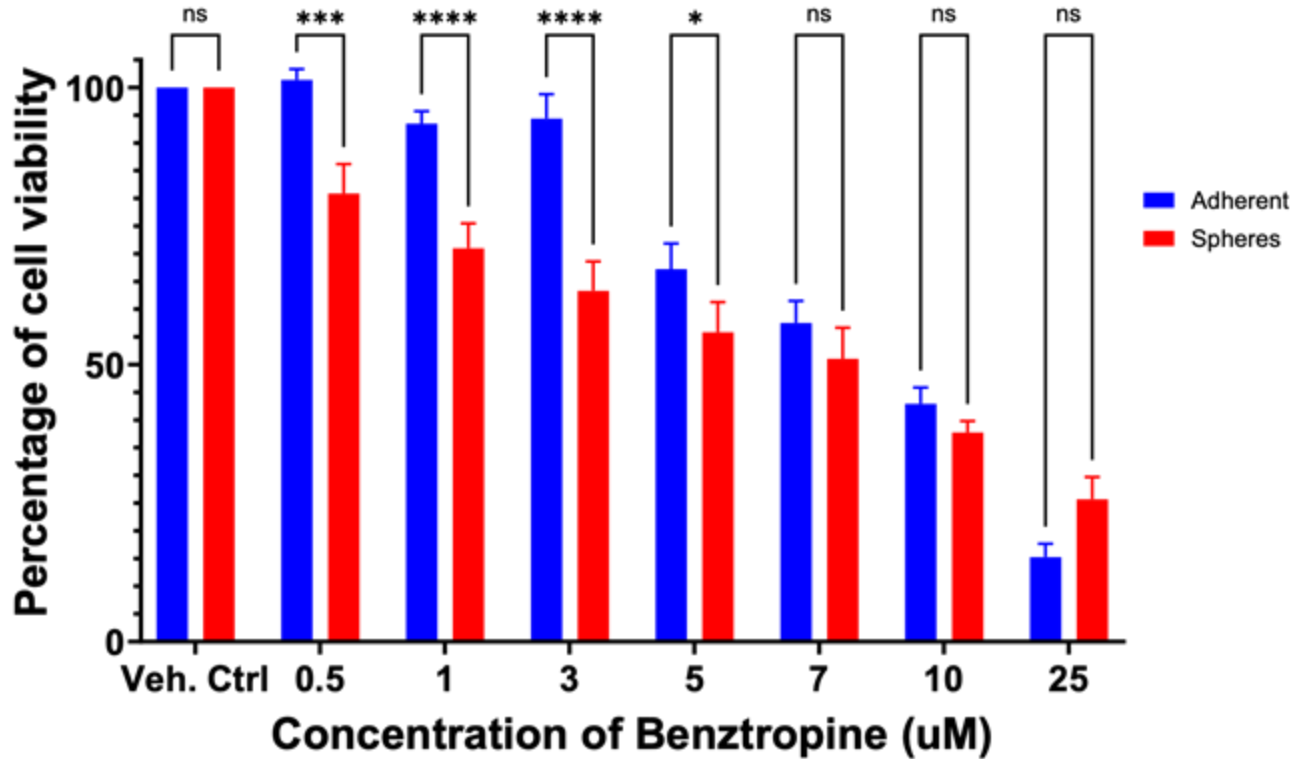


n=3, $P = 0.0060$

Targeting Ach signaling via M3R using Benztropine may lead to radiation sensitization of GSCs



BZT decreases the cell viability of LN-229 spheres

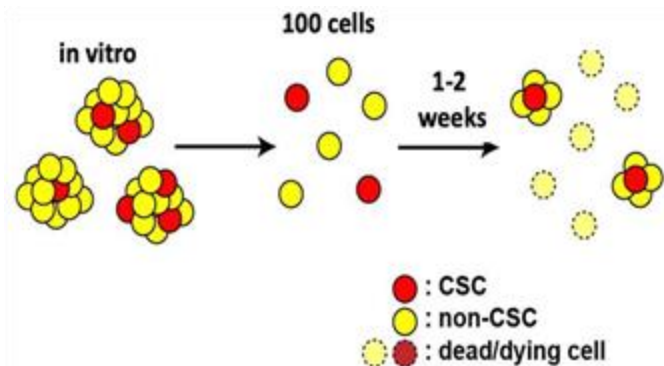


n = 6.

* $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

Values are mean + SEM of 3 technical and 6 biological replicates. Two-way ANOVA.

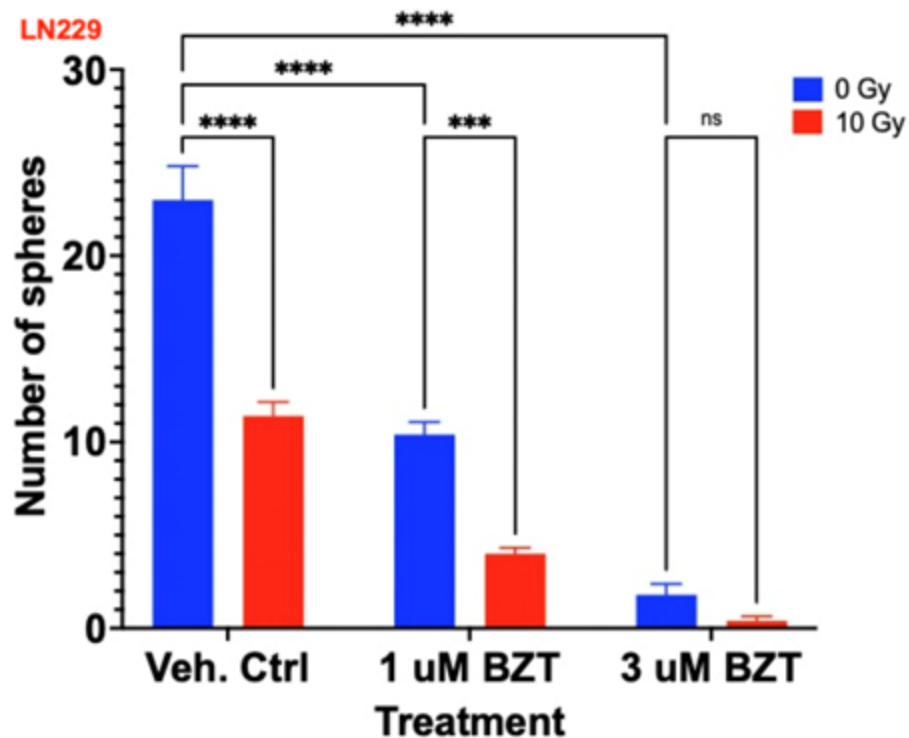
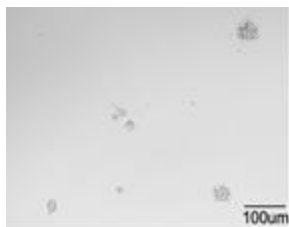
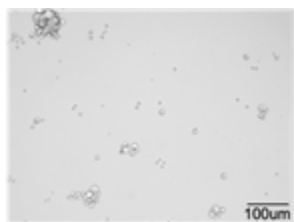
BZT + radiation decreases stemness in GBM



Control

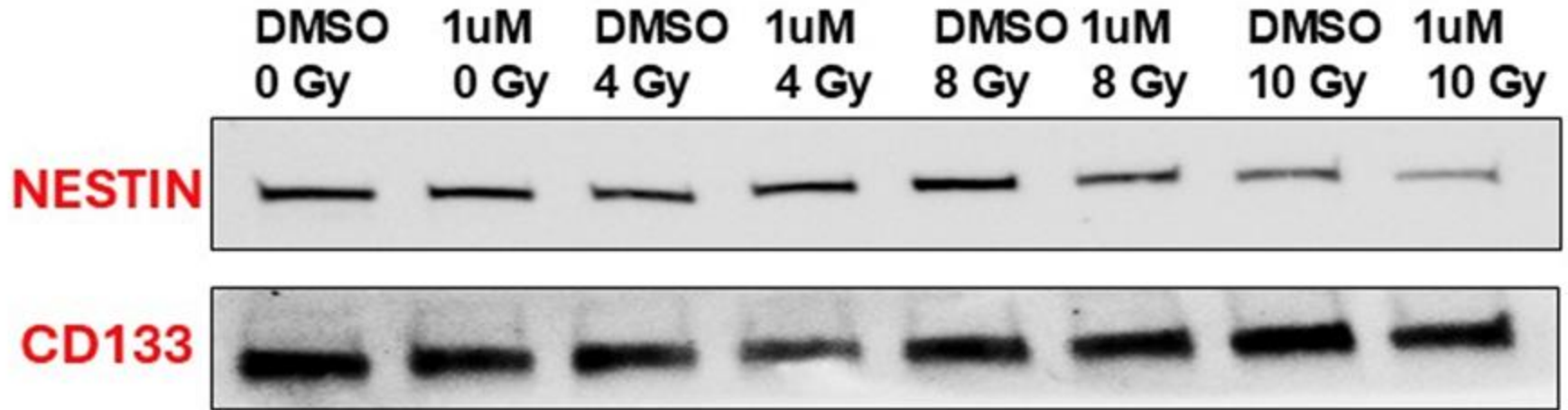
1 μ M

3 μ M

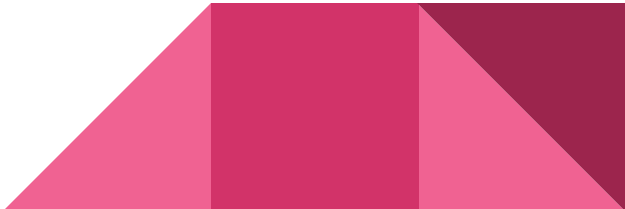


*** P <0.001, **** P <0.0001.
Values are mean \pm SEM of 3 technical and 6 biological replicates. Two-way ANOVA

BZT + radiation decreases stemness in GBM



Conclusion

- Benztropine decreases viability and stemness of GSCs
 - Benztropine increases radiation sensitivity in GBM in an additive manner
 - Application: Pharmacological inhibition of cholinergic signaling using BZT may provide a promising strategy to target GSCs when coupled with radiotherapy
 - Next step: Currently conducting in-vivo analysis, expansion to other cell lines and looking at gene expression changes
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5P30 CA023108, Couch Family