



Inhibition of cholinergic signaling enhances radiation treatment efficacy in glioblastoma



REDISCOVERING RADIATION MEDICINE AND EXPLORING NEW INDICATIONS

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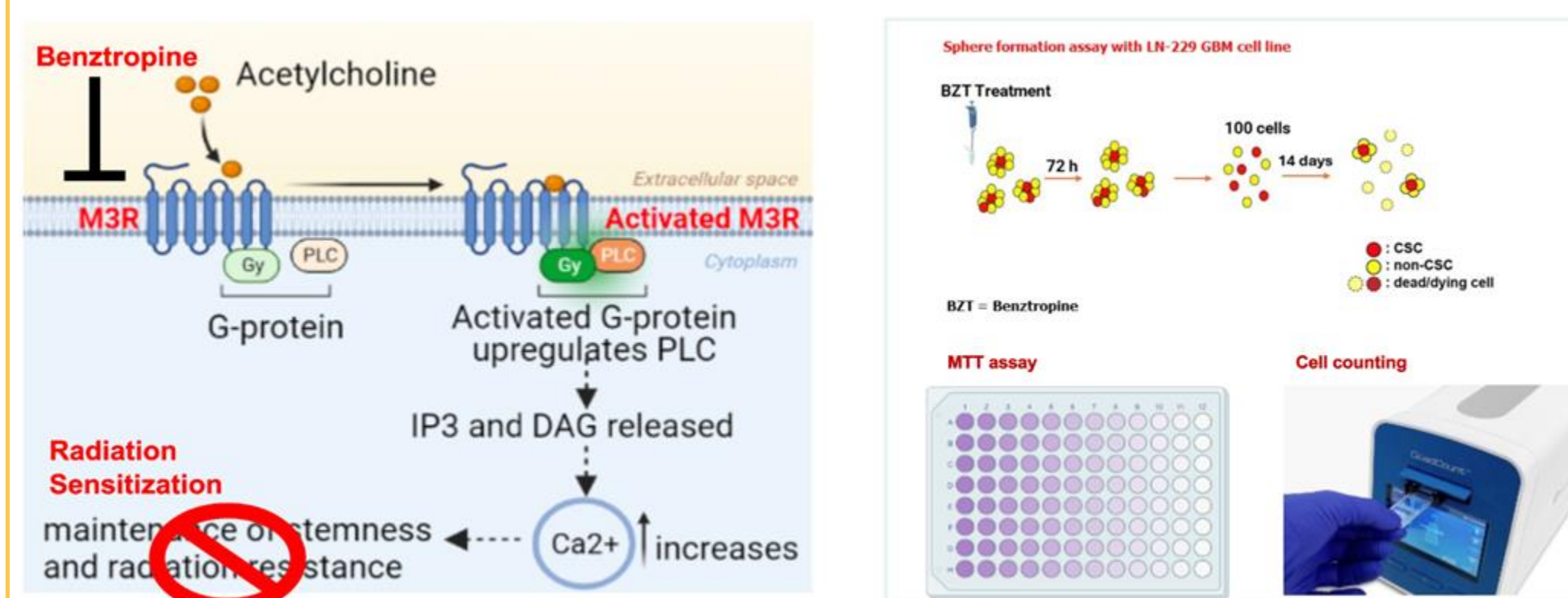
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INTRODUCTION

- Glioblastoma (GBM) is the most aggressive malignant primary brain tumor in adults with a median survival of <2 yrs
- Glioblastoma stem cells (GSCs) are one of the drivers of tumor recurrence and treatment resistance
- Understanding mechanisms of GSC resistance is vital for developing more targeted GBM therapies
- In the brain, acetylcholine (ACh) promotes normal cell proliferation and normal stem cell maintenance, and can signal through the muscarinic receptor (M3 mAChR) encoded by CHRM3

Hypothesis: Targeting Ach signaling via M3 mAChR using benztrapine, a muscarinic antagonist, may lead to radiation sensitization of GBM

METHODS



- Human GBM cell line LN-229 were treated with benztrapine at 1µM and 3µM, then given a radiation with viability and sphere formation assays being conducted.

RESULTS

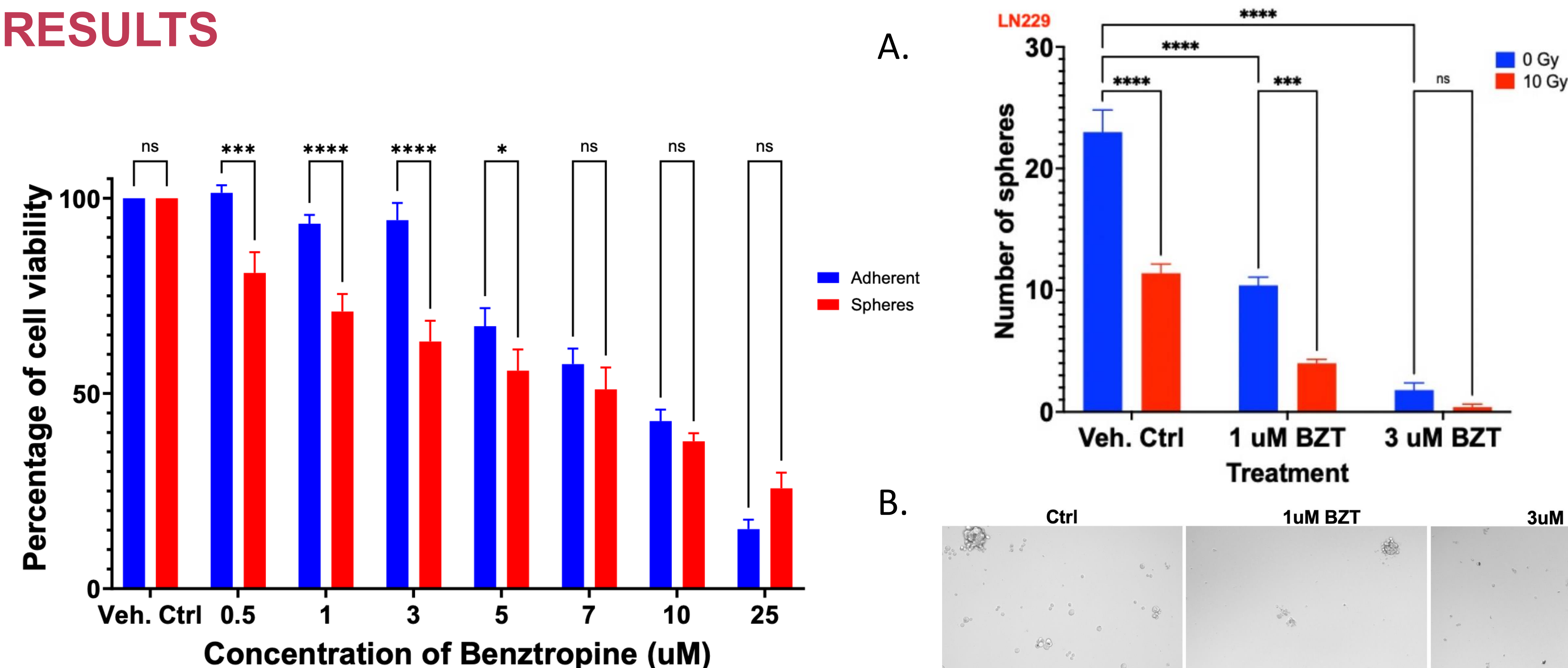


Figure 1. Benztrapine (BZT) decreases the cell viability of LN-229 spheres greater than more differentiated cells A) Dose-response assay demonstrates sensitivity of LN-229 cells grown under adherent versus sphere-forming conditions to BZT. LN-229 cells were seeded at 100,000 cells/well with cell viability assessed after 72 hours of drug treatment (n=6). Values are mean + SEM of 6 biological and 3 technical replicates. *P<0.05, ****P<0.001, ****P<0.0001

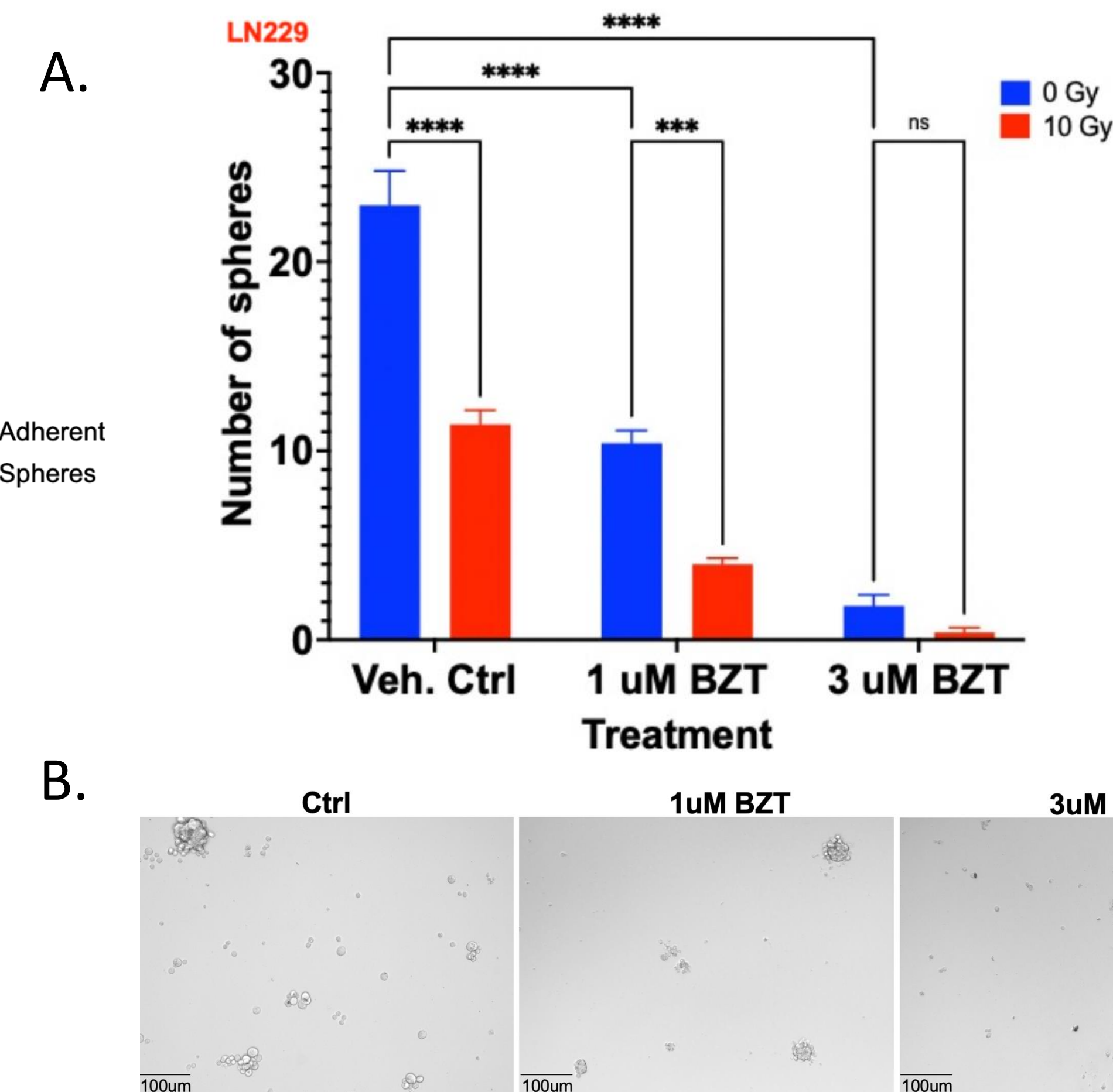


Figure 2. BZT+ Radiation decreases GBM stemness. LN-229 spheres were treated for 72hours with BZT or vehicle control (DMSO) followed by radiation and 100 cells/well were immediately seeded in ultra-low attachment plates with continued drug treatment for 7 days. Spheres > 50 µm were then counted. Values are mean + SEM of 6 technical replicates and 3 biological replicates. ***P<0.001, ****P<0.0001.

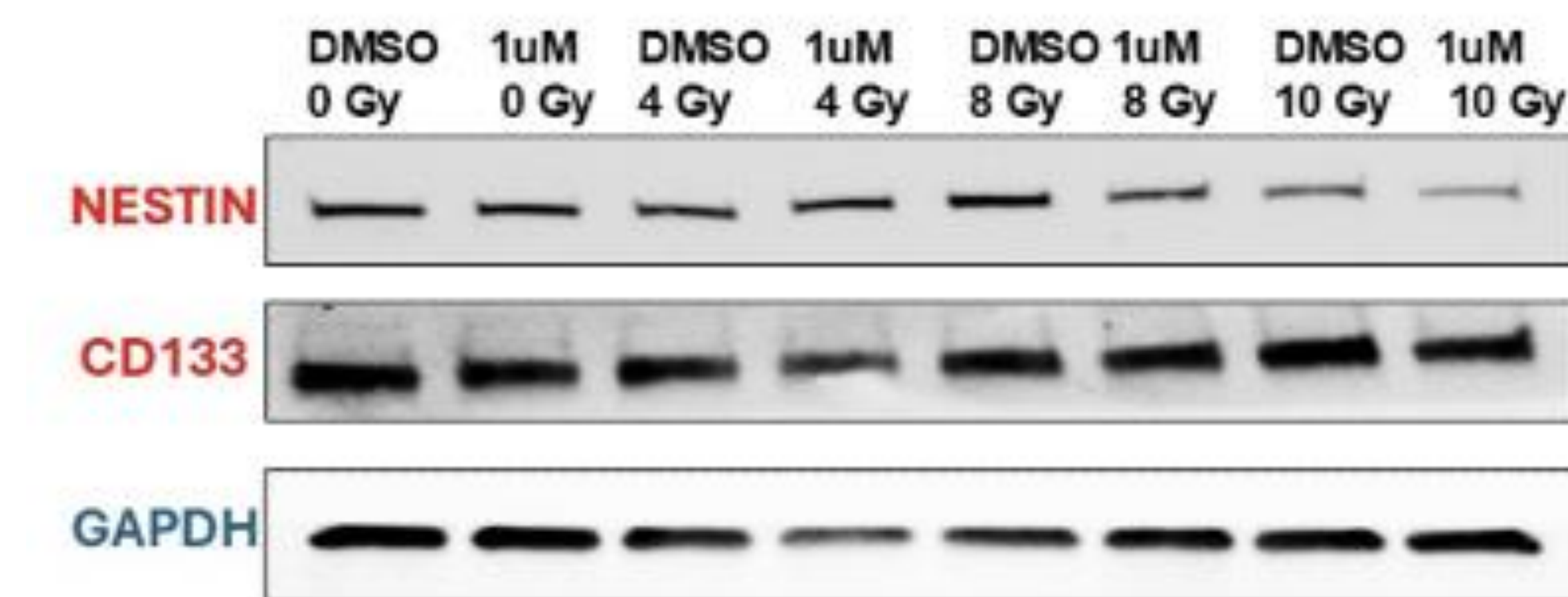


Figure 3. BZT+ Radiation decreases GBM stemness. LN-229 spheres were treated for 72hours with BZT or vehicle control (DMSO) followed by radiation. Lysates were collected 48 hours after radiation treatment. Immunoblotting was performed for nestin and CD133. GAPDH served as loading control.

CONCLUSIONS AND FUTURE DIRECTIONS

- Targeting ACh signaling via M3R with muscarinic antagonists such as BZT decreases stemness in GBM and enhances cell death
- Application: Pharmacological inhibition of cholinergic signaling using BZT may provide a promising strategy to target GSCs when coupled with radiotherapy
- Next step: Expansion to additional cell lines and animal models to determine effects in vivo

REFERENCES

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