Targeting Androgen Receptors to Treat Glioblastoma (GBM), A Translational Research Using Human GBM Cell Lines and Animal Models

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Introduction

Background:

Glioblastoma (GBM) is most common type of brain gliomas, accounting for up to 40-60% of all malignant primary brain tumors in adults, occurring in 2–3 cases per 100,000 persons in Europe and North America. The standard treatment for this cancer comprises of surgery, radiation and chemotherapy that usually takes more than half a year to complete. However, the average survival time for patients with GBM is only fifteen months including the treatment time. GBM usually is very difficult to be completely resected due to its large volume in the brain at diagnosis. It has high recurrence rate even after complete resection. Radiation has played a major role in treating this cancer but the prognosis of the patients are dismal. It is important to identify some novel drugs that can make radiation work better. However, most drugs cannot penetrate into brain which make it challenging to find suitable medications to treat brain tumor. Recently, enzalutamide, a drug that can penetrate to brain, has been approved for use in prostate cancer patients. This drug targets a protein called androgen receptor (AR), which has been shown to be involved in cancer stem cells and resistance to radiation therapy in prostate cancer. There are very limited information about AR expressions in GBM but a few reports showing that AR is overexpressed in GBM. AR might be a novel target for treating GBM. Our preliminary data confirmed that enzalutamide does suppress GBM cells *in vitro*. We thus hypothesize that direct AR inhibition plays a role in inhibiting GBM growth.

Objective(s):

To confirm the inhibitory effect of AR inhibitor both *in vitro* and *vivo*. To find out the mechanism of inhibitory effect of AR inhibitor. Furthermore, we will explore the effect of AR inhibitor on cancer stem cell.

Significance:

This is the first translational research proposed in GBM using a FDA-approved medication of a second generation AR antagonist, enzalutamide; based on the results from this grant, future studies in human patients will be immediately carried out to test enzalutamide in GBM patients concurrently and/or adjuvantly with radiation therapy *via* clinical trials.

Results

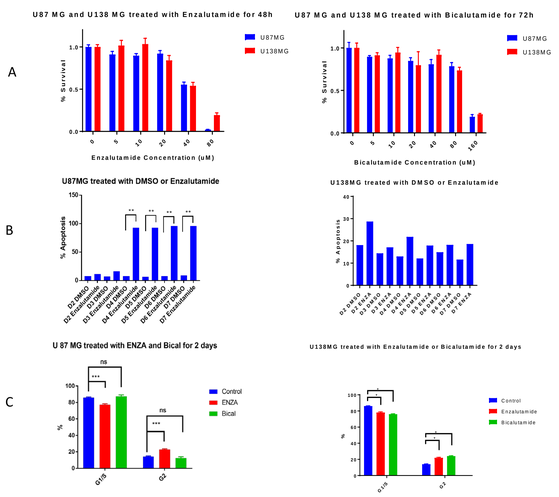
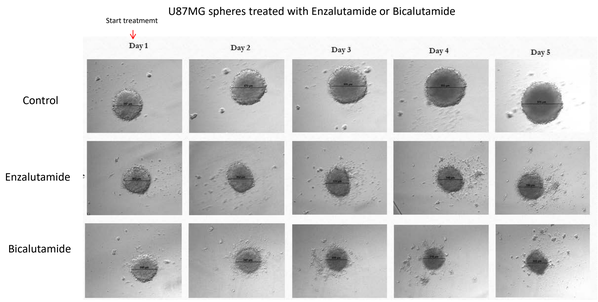
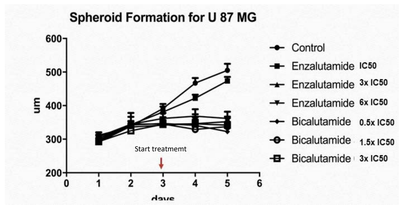


Figure 1. A. U87MG and U138G human GBM cell lines showed dose-dependent response in cell proliferation to enzalutamide or bicalutamide treatment in *vitro* with cell titer blue assays. B. U87MG and U138MG human GBM cell lines were treated with DMSO (control) or ENZA for 2 d, 3 d, 4 d, 5 d, 6 d, and7 d, respectively and then analyzed with the apoptotic markers Annexin V and PI using flow cytometry. Enzalutamide induces apoptosis of U87MG after 3 days' treatment. However, we did not see a significant apoptosis-inducing effect of Enzalutamide on U138MG cell line which carries p53 mutation. C. U87MG and U138MG were arrested in G2 phase after 2 days' treatment of Bicalutamide or Enzalutamide which predict that these drugs could be potentially used as radiosensitizers.

Results





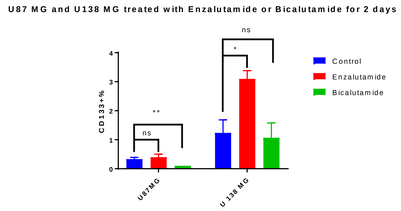


Figure 2. U138MG and U87MG were cultured in ultralow attachment 6-well plates or 96-well plates using low serum (0.5% FBS) media. The diameters of the tumor spheres were measured every day (left bottom panel) showing significant growth suppression with either drug treatment. After dissociating the tumor spheres, the cells were sorted with CD133 cancer stem cell marker using flow cytometry.

Methods

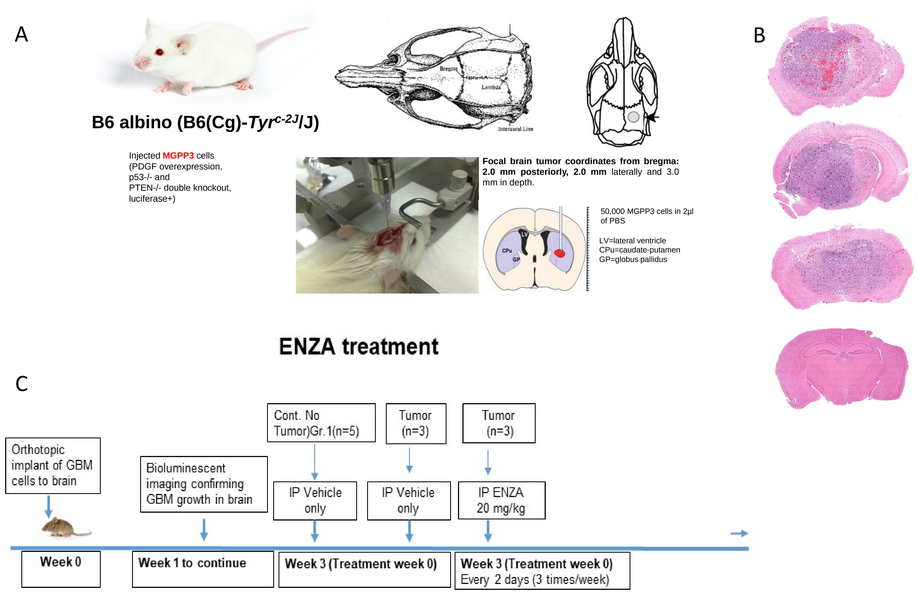


Figure 3. A. Syngeneic orthotopic GBM mouse model using stereotactic microinjection of mouse GBM cells to B6 albino mouse brain. Development of tumor in the injection site is shown via brain MRI (not shown) and by H&E staining (B). C. Scheme of experimental planning for treating orthotopic GBM mice with enzalutamide.

Results

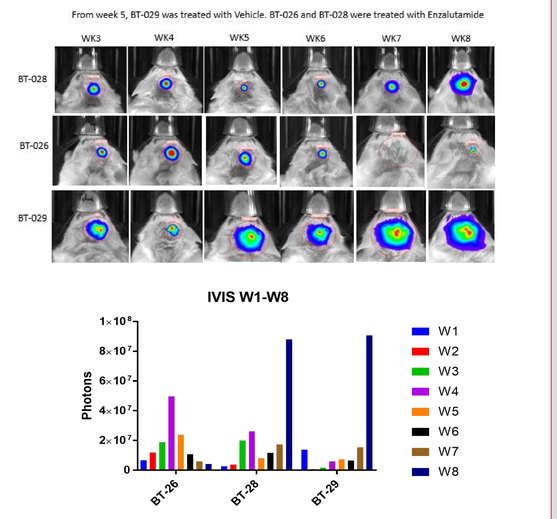


Figure 4. After starting enzalutamide IP treatment at week 5, bioluminescent signal from the tumor decreased in BT-026 mouse. For BT-028 mouse, the tumor signal decreased until week 7 and regressed at week 8. The tumor kept growing in BT-029 mouse which was treated with saline only as negative control.

Conclusions

The AR inhibitors, both enzalutamide and bicalutamide, could inhibit the proliferation of GBM both *in vitro* and *in vivo*. The mechanism could be partly through arresting the cell cycle at G2 phase and induction of apoptosis in tumor cells. The role of AR in promoting cancer stem cells in GBM needs to be further studied. AR inhibitor could inhibit the tumor proliferation *in vivo* but drug resistance may happen after prolonged treatment.

References

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Acknowledgements

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