The effects of ephrinB2 signaling on proliferation and invasion in glioblastoma multiforme

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Abstract

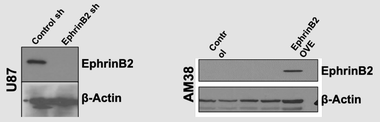
- Glioblastoma Multiforme (GBM) modulates invasive and proliferative pathways to modulate tumorigenesis.
- The Eph family of receptor tyrosine kinases are implicated in several malignancies.
- EphrinB2, a member of this family, has recently emerged as a critical therapeutic target responsible for regulating those pathways, but is heavily embedded in controversy.
- Is EphrinB2 a tumor-suppressor or oncogene?
- To reconcile the contrasting results, we analyzed the effects of manipulating ephrinB2's function on expression levels of its cognate receptor, EphB4.
- Our data show that the activation of EphB4 by its cognate ligand produces a dichotomously anti-proliferative and pro-invasive effect, based on the activation of either forward receptor or reverse ligand signaling, in GBM tumors.
- In order to understand GBM cell behavior, it's important to examine the interaction between Eph-ephrin binding between cells rather than ephrinB2 signaling alone.

Introduction

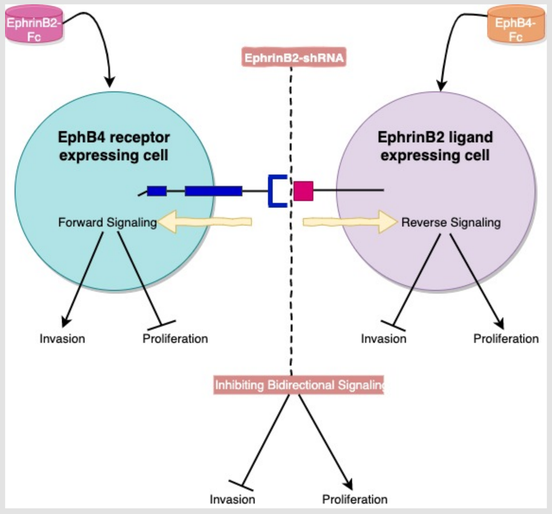
- GBM is the most aggressive type of CNS malignancy.
- Current treatment is surgery followed by concurrent radiation and chemotherapy. Survival is still abysmal in these patients.
- GBM tumors have been shown to utilize signaling networks involved in embryogenesis to become aggressively invasive and proliferative.
- Such a network is the Eph-Ephrin system. The Eph family of membrane bound receptor tyrosine kinases are instrumental in modulating early developmental functions of migration, adhesion, repulsion.
- In GBM specifically, dysregulation of various receptors and ligands of the Eph family have been shown to contribute to the invasion, migration, and proliferation seen abundantly in GBM.
- EphrinB2 and EphB4 are two such members of this family that have been heavily implicated in GBM, as both are overexpressed in GBM and are unique binding partners.
- Although ephrinB2 is promiscuous in the sense that it can bind to a wide variety of Eph receptors, it binds to EphB4 with the highest binding affinity. In contrast, EphB4 exclusively binds to ephrinB2.
- Upon cell-to-cell contact, the EphB4 receptor and ephrinB2 ligand can either transduce a forward signal into the EphB4-expressing cell or a reverse signal into the ephrinB2-expressing cell. Thus, the signaling axis is said to be bidirectional.
- Past studies illustrated that the Eph family is heavily implicated in the established "go or grow" model in GBM, where actively proliferating cells are less invasive while actively invading cells are less proliferative.
- In our model, we sought to determine the role of the EphB4 and ephrinB2 signaling axis on the proliferation and invasion pathways involved in GBM tumorigenesis.

Materials and Methods

- Cell Lines: Human GBM cell lines U87 and AM38. We generated ephrinB2-shRNA clones in the U87 line and ephrinB2-overexpression clones in the AM38s.



- TCGA: Gene expression data was obtained from The Cancer Genome Atlas (TCGA) for 580 GBM patients and 530 Low-Grade Glioma patients. Overall survival and disease-free survival were calculated using the Kaplan-Meier method using log-rank tests for comparisons. DFS was defined as time from the date of diagnosis to the date of the last known occasion that the patient was disease-free.



- Orthotopic *n vivo* model: Female athymic nude mice were used and 200,000 cell/mouse were orthotopically implanted into the right putamen using stereotactic surgical techniques.
- Bioluminescence and CBCT imaging: For the AM38 mice, 1.5 mg D-luciferin was intraperitoneally injected 10 minutes prior to imaging in the Xenogen IVIS 200. For the U87 mice, 200 µl of iodinated CT contrast (IV) was injected 3-15 mins prior to CBCT imaging.
- IHC: Whole brains were harvested from orthotopic experiments in which either the AM38 gain of function of U87 loss of function cells were xenotransplanted intracranially into nude mice. The sections were stained with an anti-PCNA antibody at a 1:200 dilution in 2% milk.
- Boyden Chamber invasion Assay: U87/AM38 cells were serum starved for 4h prior to seeding in serum-free media in the upper chamber of a 24 well-plate insert with 8µM pores. Matrigel was used as the ECM, through which cells invaded. Invading cells were stained with 0.1% crystal violet.

Results

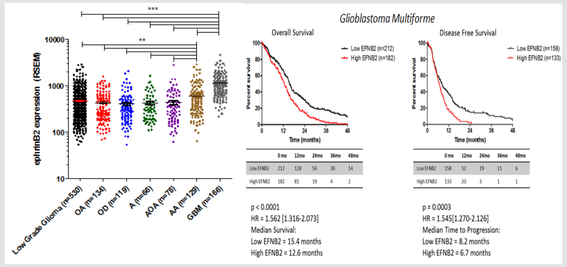


Fig 1. EphrinB2 is overexpressed in GBM and high expression leads to worse overall and disease-free survival.

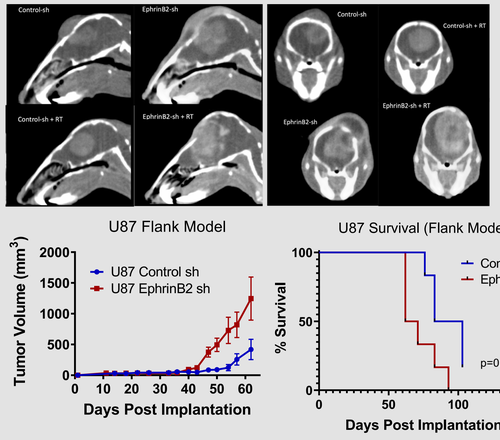


Fig 2. Knockdown of ephrinB2 increases tumor volumes and decreases survival *in vivo*.

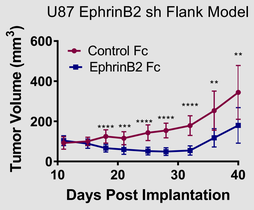


Fig 3. Stimulating forward signaling of EphB4 using an ephrinB2-Fc protein in the U87 ephrinB2-knockdown tumors significantly decreased tumor volumes.

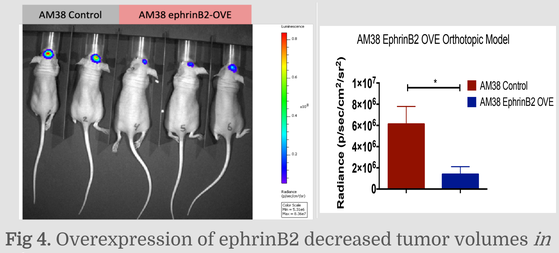


Fig 4. Overexpression of ephrinB2 decreased tumor volumes *in vivo*.

Results (continued)

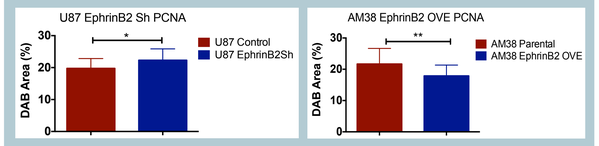


Fig 4. Levels of a proliferation marker (PCNA) increase upon knockdown of ephrinB2 and decrease upon overexpression of ephrinB2 by IHC.

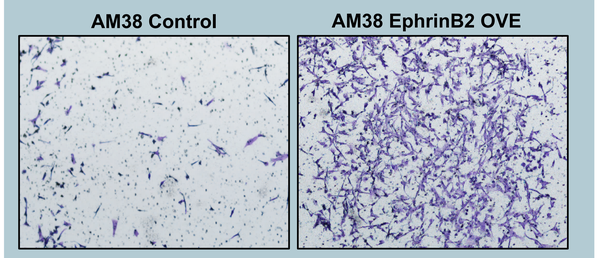


Fig 5. Overexpression of ephrinB2 lead to increased invasion *in vitro*.

Conclusions

- Past studies have shown that ephrinB2 is a key player in modulating GBM tumorigenesis, but the role it plays has been unclear thus far.
- Our data suggest that it may be the signaling axis of ephrinB2 that determines the activation or inhibition of downstream signaling pathways, and consequentially the full effect on tumor growth.
- Our data suggest that activation of EphB4 by its cognate ligand, ephrinB2, is anti-proliferative and pro-invasive in GBM tumors.
- It may be possible that signaling between EphB4 and ephrinB2 may be beneficial in targeting the proliferation aspect of these tumors.

Acknowledgements

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