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 these pathways, but is heavily embodied in controversy.
- Is EphrinB2 a tumor-suppressor or oncoprotein?
- 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.

Materials and Methods

- Cell Lines: Human GBM cell lines U87 and AM38. We generated ephrinB2-shRNA clones in the U87 line and ephrinB2-overexpressing 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-specific 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 follow-up condition that the patient was disease-free.

Results

- Orthotopic in vivo model: Female athymic nude mice were used 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.
- So 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.

Results (continued)

- Bioluminescence and CBCT imaging: For the U87 mice, 200 μCi of iodinated CT contrast (IV) was injected 3 days post-implantation, the tumor was imaged in the Xenogen IVIS 200. For the AM38 mice, 1.5 mg of bioluminescent marker (IV) was injected 3 days post-implantation. The tumor was imaged using the IVIS 200.

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 ephrinB2 may be beneficial in targeting the proliferation and invasion aspect of these tumors.
- It may be possible that signaling between EphB4 and ephrinB2 may be beneficial in targeting the proliferation aspect of these tumors.

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