**EphB4 and EphrinB2 Play Dichotomous Roles in Head and Neck Squamous Cell Carcinoma Metastasis**

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**Purpose of Study:**

Distant metastasis remains a significant challenge in head and neck squamous cell carcinoma (HNSCC) with a median overall survival of only 10 months. The EphB4-ephrinB2 signaling axis has been shown to modulate progression of numerous cancer types, but its role in HNSCC metastasis is poorly understood. This study characterizes how EphB4 and ephrinB2 impact the development of metastasis to guide rational drug design for HNSCC patients.

**Methods:**

The MOC2 cell line was used as an orthotopic murine model of HNSCC. Previous work with this model has shown that EphB4 is primarily expressed on cancer cells, while ephrinB2 is primarily expressed on blood vessels. Thus, MOC2 EphB4 knockdown and control tumors were compared to elucidate how loss of cancer cell EphB4 mediates HNSCC metastasis. Additionally, ephrinB2$^{fl/fl}$Tie2$^{Cre}$ genetically engineered mice were compared to wild-type controls to investigate how deletion of vascular ephrinB2 impacts HNSCC metastasis. Tumors were implanted in the buccal mucosa of C57BL/6 (wild-type) or ephrinB2$^{fl/fl}$Tie2$^{Cre}$ mice. Three fractions of 8 Gy radiation were administered to the tumor once tumor volumes reached 100-200mm$^3$. Development of lung metastasis was detected by weekly computed tomography (CT) scans to generate metastasis-free survival curves. As a proof-of-concept, tumor-bearing mice were treated with ephrinB2-Fc plasmid by hydrodynamic tail vein injection. This plasmid acts as an EphB4 agonist that avoids simultaneous activation of ephrinB2, allowing specific pharmacological activation of EphB4.

**Summary of Results:**

Knockdown of EphB4 in MOC2 cancer cells coupled with radiation therapy (RT) significantly increased lung metastasis (HR, 2.654; 95% CI, 1.188 to 5.930). In contrast, deletion of vascular ephrinB2 in the context of RT significantly reduced lung metastasis (HR, 0.2972; 95% CI, 0.09223 to 0.9577). Treatment of MOC2 tumor-bearing mice with ephrinB2-Fc significantly improved overall survival (HR, 0.4964; 95% CI, 0.1957 to 1.259) and decreased lung metastasis (HR, 0.07271; 95% CI, 0.01064 to 0.4967).

**Conclusions:**
In the MOC2 model of HNSCC, cancer cell EphB4 inhibits metastasis while vessel ephrinB2 promotes distant spread. These findings support targeted activation of EphB4 and inhibition of ephrinB2 as a promising approach to mitigate HNSCC metastasis and improve patient outcomes.