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

Diemmy Nguyen, Khalid N.M. Abdelazeem, Sophia Corbo, Laurel B. Darragh, Shilpa Bhatia, Mike W. Matsumoto, Elena B. Pasquale, Sana D. Karam

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most prevalent cancer type with a 5-year survival rate below 50%. Despite advances in treatment modalities, the prognosis remains notably poor, especially for patients with advanced disease. Approximately 10% of HNSCC patients present with distant metastases, and up to 30% additional patients develop distant metastases as their disease progresses. Patients with distant metastases have a median overall survival of only 10 months. These grim statistics underscore the urgent need for innovative therapeutic strategies to reduce distant metastases in the context of HNSCC.

EphB4 and its main ligand ephrinB2 have been shown to modulate progression of numerous cancer types, but their role in metastasis is poorly understood. Our lab has shown that EphB4 is primarily expressed on cancer cells, while ephrinB2 is expressed on blood vessels in a mouse model of HNSCC. We have also shown that inhibition of cancer cell EphB4 accelerates tumor growth, while deletion of vascular ephrinB2 yields the opposite effect. This study expands upon our previous findings to characterize the role of EphB4 and ephrinB2 in metastasis and guide rational drug design for HNSCC patients.

Methods

Cell Lines

The MOC2 cell line was used as an orthotopic murine model of HNSCC. MOC2 EphB4 knockdown and control cell lines were used to examine how loss of cancer cell EphB4 mediates HNSCC metastasis.

Animal Models

Genetically engineered (ephrinB2$^{fl/fl}$Tie2^{Cre}) mice were used to knock out vascular ephrinB2. The incidence of metastases was compared to wild-type controls to investigate how deletion of vascular ephrinB2 impacts HNSCC metastasis. All animal protocols have been approved by IACUC (protocol #250).

Pharmacological Intervention

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.

Experimental Design

- MOC2 EphB4 knockdown or control 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.

Results

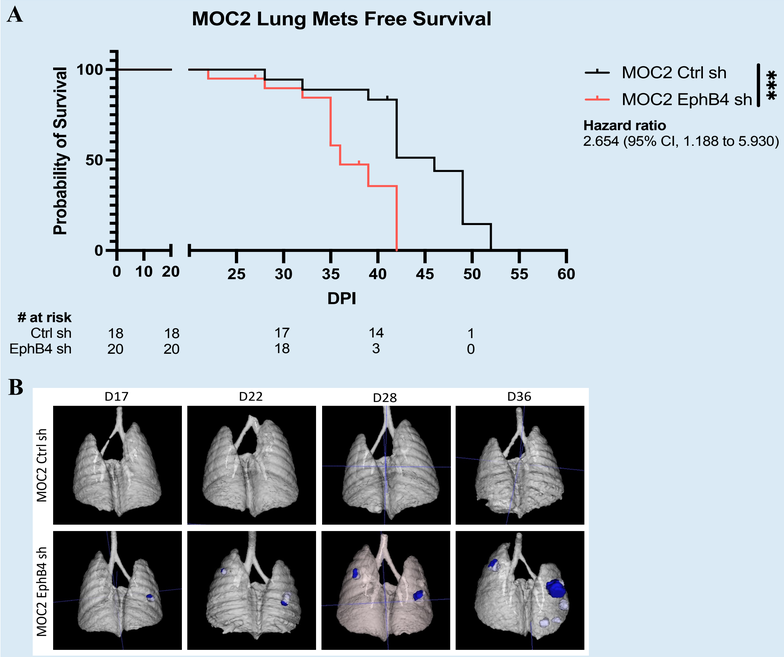

Figure 1: Knockdown of cancer cell EphB4 significantly increases metastasis

A) Kaplan-Meier curves showing metastasis-free survival of MOC2 control (Ctrl) shRNA versus EphB4 shRNA tumors implanted in C57BL/6J (wild-type) mice. Numbers at risk indicate mice that were alive without metastases at specified days post-implantation (DPI). Significance was determined by a log-rank Mantel-Cox test. ***p = 0.0009. B) 3D lung contouring of representative mice implanted with MOC2 control or EphB4 shRNA knockdown tumors at different timepoints.

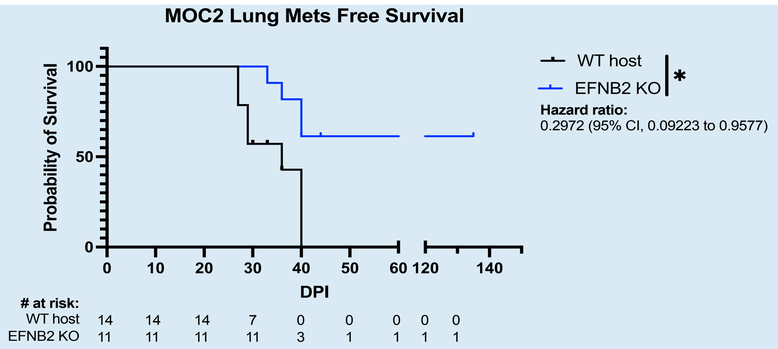

Figure 2: Deletion of vascular ephrinB2 significantly reduces metastasis

Kaplan-Meier curves showing metastasis-free survival of wild-type (WT) and ephrinB2$^{fl/fl}$Tie2^{Cre} (EFNB2 KO) mice. Significance was determined by a log-rank Mantel-Cox test. *p = 0.0172.

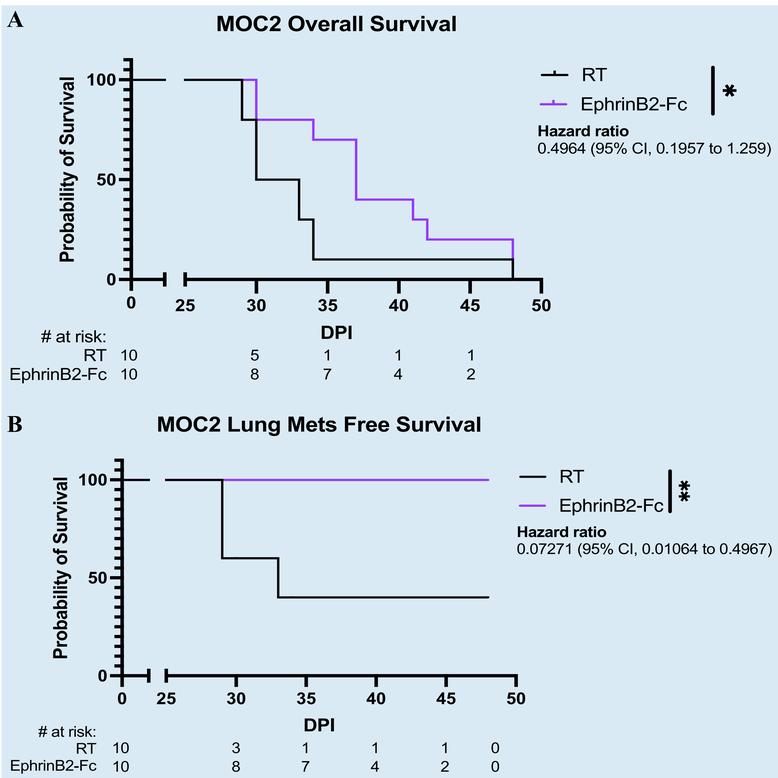

Figure 3: Deletion of vascular ephrinB2 significantly reduces metastasis

Kaplan-Meier curves showing therapeutic effects of ephrinB2-Fc plasmid on overall survival (A) and metastasis-free survival (B) in MOC2 implanted C57BL/6 mice. For metastasis-free survival curves, numbers at risk indicate mice that were alive without metastases at specified timepoints. Significance was determined by a log-rank Mantel-Cox test. p-values are indicated for the figures A, *p = 0.0485; B, **p = 0.0075.

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.

Acknowledgments

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University of Colorado Anschutz Medical Campus