Assessing the Role of Extracellular Matrix-Integrins in Metastatic Squamous Cell Carcinoma

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Abstract

Purpose: Patients with head and neck squamous cell carcinoma (HNSCC) have a dismal survival rate. The effects of the extracellular matrix (ECM) on cancer progression have been long studied, but the roles of specific integrins in the process of HNSCC metastasis remains to be dissected. This study aims to determine how HNSCC cells affect the production of laminin-binding integrins, and how the integrins participate in the ECM interactions necessary for a metastatic phenotype.

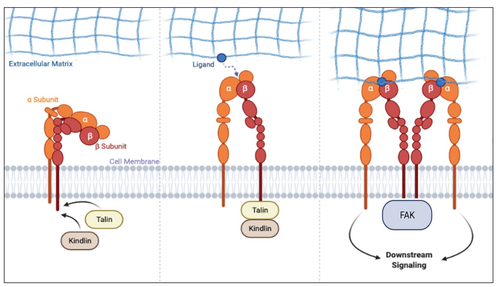
Experimental design: Our laboratory has produced syngeneic mouse HNSCC cell lines derived from Keratin15+ stem cells with Smad4 loss and Kras^{G12D} mutation. In syngeneic recipients, HNSCCs derived from one tumor transplanted to the flank mouse skin produced spontaneous metastases to the lung while HNSCCs derived from another did not form metastases. Having the same genotype, these cell lines serve as models to examine cancer cell interactions with the ECM and resulting effects on invasion and metastasis. We performed bulk RNA sequencing (RNAseq) to compare cultured metastatic HNSCC cells versus non-metastatic counterparts and identify differentially expressed genes that regulate HNSCC cells and ECM interactions. We performed immunoassays and functional invasion assays to evaluate ECM-cancer cell signaling and influence on cancer cell invasion in these two cell lines.

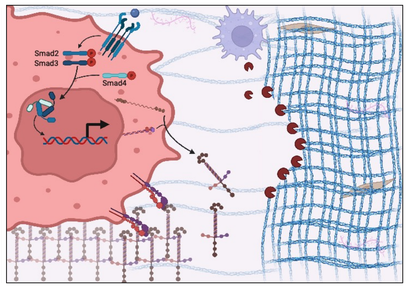
Results: By RNAseq analysis we found that, relative to non-metastatic HNSCC cells, metastatic cells have increased levels of integrins, the main mechanoreceptors for numerous ECM ligands and matrix proteins. TCGA data shows that laminin-binding integrins, including integrins α4 and β6, were greater at the RNA and protein levels. Additionally, metastatic cells expressed higher levels of laminin coding genes compared to non-metastatic HNSCC cells. Correlatively, high expression of these matrix proteins is associated with worse patient survival. Additionally, metastatic HNSCC cells displayed differential genetic expression of apical junctions, TGFβ signaling components, and regulators of ECM remodeling. Furthermore, we demonstrated by ELISA and western blot analysis that metastatic HNSCC cells have aberrant TGFβ-Smad signaling as indicated by elevated release of TGFβ-1 protein and higher levels of phosphorylated Smad2 and Smad3 relative to non-metastatic HNSCC counterparts. Treating metastatic cells with TGFβ-1 significantly increased their motility and invasion. Conversely, migration and motility of metastatic HNSCC cells were radically reduced by the TGFβ inhibitor galunisertib. Additionally, we leveraged a mouse cytokine to reveal that plasma from mice bearing metastatic tumors had greater circulation of CXCL16, MMP-9, proliferin and serpin E1. These proteins are associated with ECM remodeling and metastasis in HNSCC.

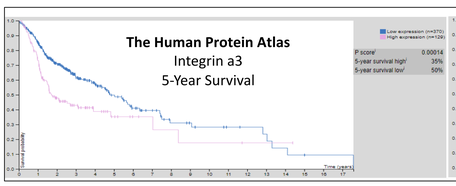
Conclusions: HNSCC cells with metastatic properties upregulate integrins which bind to laminin matrix proteins and have elevated invasive and migratory capacity contributed from activated TGFβ signaling.

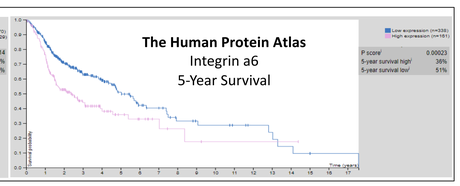
ECM-Integrin Interactions in Cancer

- Squamous Cell Carcinomas (SCC) has a high global incidence with distant **metastasis being the primary cause of patient mortality**
- The standard of care can eradicate the primary tumor, but treatment options for metastatic disease is severely limited









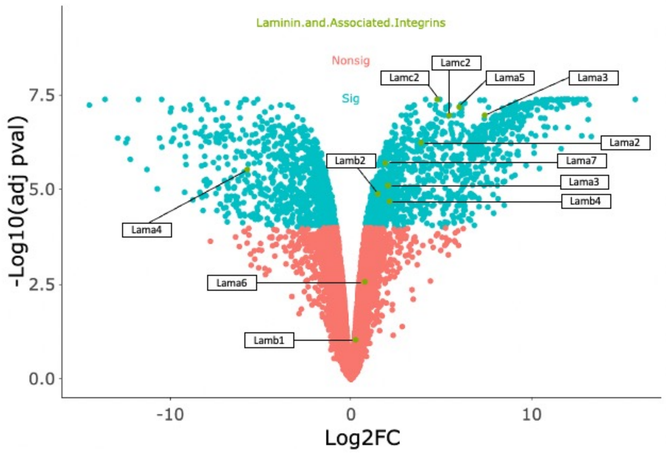
Hypothesis

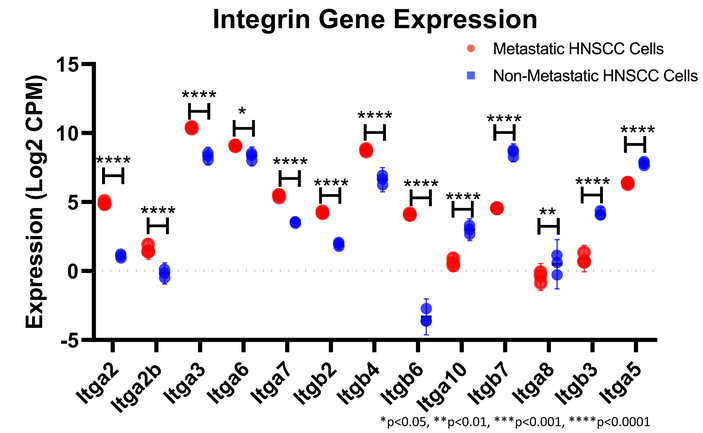
SCC cells upregulate laminins and integrin expression to facilitate metastasis.

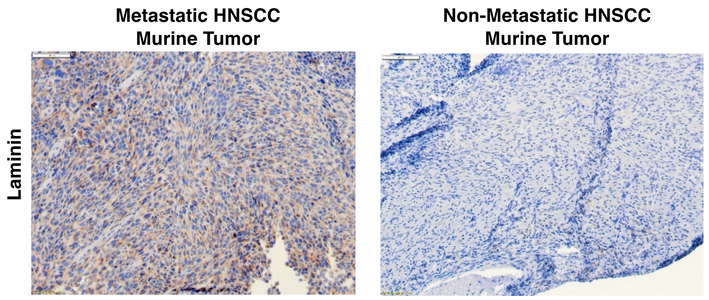
Methodology

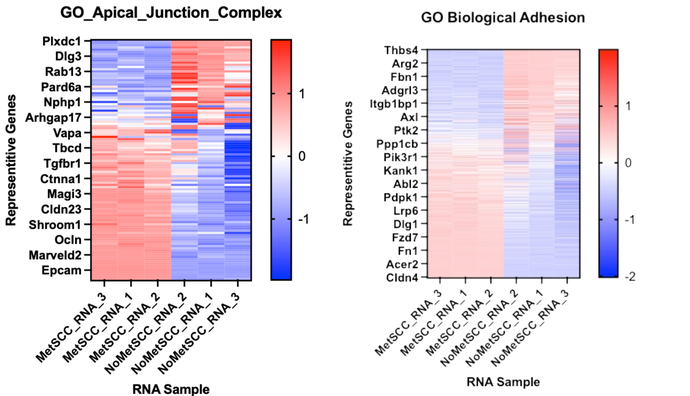
- Syngeneic lines derived from both metastatic and non-metastatic tumors to study both cell autonomous activity and tumor-stromal interactions
- Transwell and scratch assays to assess invasion and migration *in vitro*
- Evaluate metastatic burden *in vivo* by subcutaneous and orthotopic tumor transplants
- Transcriptome analysis with RNAseq
- Assess protein expression of PI3K, TGFb-Smad, and ECM-Integrin signaling pathway members by immunoblotting, IF, and IHC

Elevated Integrin and Laminin Expression in Metastatic SCC Cells

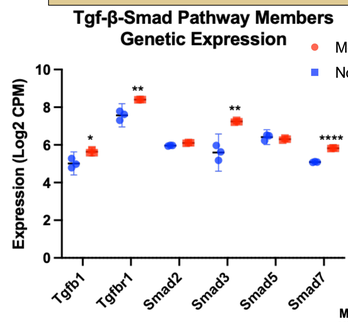


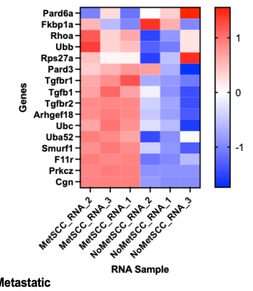


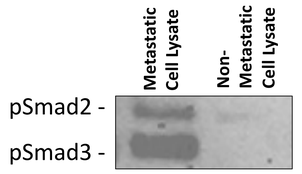


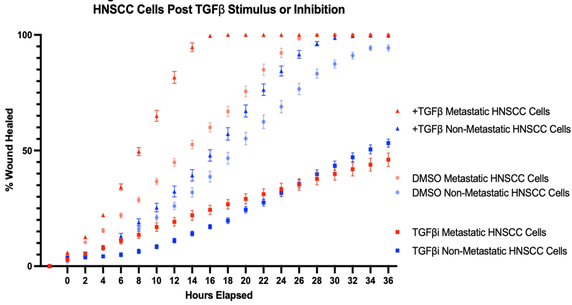


TGFβ-Dependent Motility in Metastatic SCC

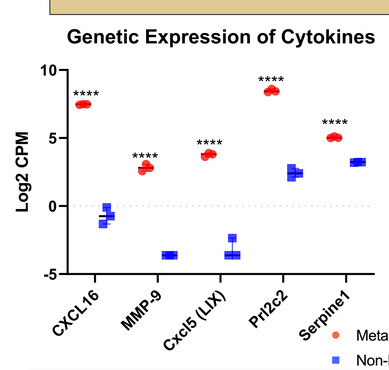


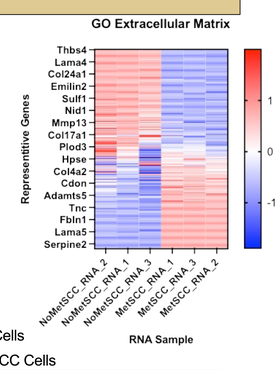






Elevated ECM Remodeling in Metastatic SCC





Summary

- Metastatic HNSCC cells have elevated levels of laminins, laminin-binding integrins, and ECM remodeling proteins
- Metastatic HNSCC cells have heightened TGFβ-Smad pathway activity
- Metastatic HNSCC cells have heightened TGFβ-dependent motility

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