ROLE OF TGF-BETA SIGNALING IN PIK3CA-DRIVEN HEAD AND NECK CANCER INVASION AND METASTASIS

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Introduction

• PI3K pathway alterations (PIK3CA, PTEN, AKT) are common in solid tumors and are highly linked to oncogenesis in multiple tumor types including HNSCC.
  -Gene amplification of PIK3CA ~40%, gain-of-function mutation~10%

• PI3K pathway inhibitors are currently being investigated pre-clinically as targeted therapies for HNSCC.

• While the PI3K pathway has been linked to proliferation and survival, the role of this pathway in invasion and metastasis is unclear.

• We aimed to investigate the in vivo role of PI3K pathway alterations in HNSCC initiation and progression to more specifically guide targeted therapy approaches.
Overexpression of PIK3CA increases susceptibility to 4NQO-induced HNSCC carcinogenesis

Incidence of HNSCC

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<tr>
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<th>control</th>
<th>PIK3CA</th>
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<tbody>
<tr>
<td>5-6 m (visual)</td>
<td>2/25 (8%)</td>
<td>9/23 (39%)*</td>
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<td>10-12 m (patho)</td>
<td>21/25 (84%)</td>
<td>20/23 (87%)</td>
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*p<0.05
High incidence of poorly differentiated tumors and metastasis in PIK3CA tumors

A. Control

B. 100%

Control (n=21)  
Pronly  Mod  Well  CIS

PIK3CA (n=20)  
Pronly  Mod  Well  CIS

C. PET scan

Metastasis at 10-12m:  
PIK3CA: 8/20 (40%)  
control: 0/18
Potential molecular mechanisms mediating PIK3CA-induced HNSCC progression
PDK1 (PHOSPHOINOSITIDE-DEPENDENT KINASE)

- PDK1 is amplified in breast cancer and down-regulation of PDK1 inhibits migration and metastasis in breast cancers
- Controls migration but not cell growth and proliferation in lymphocytes
- Role of PDK1 in HNSCC is unclear, and further studies are ongoing.
PIK3CA, PDK1 and pAKT in human HNSCC

% of samples with ≥2+ staining
Poor/mets vs. well/mod SCC: p<0.01
How does PDK1 contribute to HNSCC invasion and metastasis?

A.

![Bar chart showing TGFβ1 ELISA results for control and PIK3CA in tumor tissues and tumor cells.](chart.png)

B.

![Western blot images showing pSmad3 and Actin levels for control and PIK3CA samples.](image.png)
Potential mechanism of PIK3CA in HNSCC progression

Epithelia

Stroma

Proliferation
Growth
Apoptosis

Initiation

Invasion & Metastasis

PI3K

PIPK2

PIPK3

pAKT

PDK1

Smad3

TGFβ1

EMT, Inflammation, Angiogenesis
Conclusions

- PI3K pathway alterations are common and promote HNSCC progression \textit{in vivo}.

- Overexpression of PIK3CA, the catalytic subunit of PI3K, is associated with poor differentiation and metastasis in our mouse model and in human HNSCC.

- PIK3CA mediated HNSCC appears to be mediated by AKT-independent signaling, particularly through activation/overexpression of PDK1.

- Further characterization of alterations in AKT-independent PI3K targets including PDK1 may lead to novel targeting of HNSCC progression.

  - Personalized and stage specific pathway targeting

  - Radiosensitization
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• **PIK3CA** encodes for p110α, the catalytic subunit of PI3K, which is the most commonly mutated oncogene in solid tumors after RAS.

• **PIK3CA** mutations are correlated with tumor initiation, progression, and metastasis in lung SCC, esophageal SCC, and breast cancer; and poor prognosis and recurrence in colon cancer.

**In HNSCC:**
• Gene amplification of **PIK3CA** ~40%, gain-of-function mutation~10%

• **PIK3CA** overexpression has been correlated with advanced stage, vascular invasion, and lymph node metastasis.
Generation and Characterization of an inducible, head-and-neck specific PIK3CA genetically engineered mouse model (PIK3CA-GEMM)

Keratin 5

K5.GLp65

transactivator

GAL4 ΔPR-LBD p65

target

tata

PIK3CA

X

RU486

+PIK3CA-GEMM

PIK3CA overexpression in oral epithelium
Overexpression of PIK3CA resulted in head and neck epithelial hyperplasia/carcinoma in situ

1 year observation
Epithelial Mesenchymal Transition (EMT) and overexpression of Twist in PIK3CA tumors

Twist downregulates E-Cadherin and promotes EMT
Ex Vivo Cells from the *PIK3CA* tumors are more fibroblast-like and invasive.

*PIK3CA* control

**BD matrigel invasion chamber at 48 hours**
Smad4 loss in mice causes spontaneous head and neck cancer with increased genomic instability and inflammation

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Smad4 is a central mediator of TGF-β signaling, and its expression is downregulated or lost at the malignant stage in several cancer types. In this study, we found that Smad4 was frequently downregulated not only in human head and neck squamous cell carcinoma (HNSCC) malignant lesions, but also in grossly normal adjacent buccal mucosa. To gain insight into the importance of this observation, we generated mice in which Smad4 was deleted in head and neck epithelia (referred to herein as HN-Smad4−/− mice) and found that they developed spontaneous HNSCC. Interestingly, both normal head and neck tissue and HNSCC from HN-Smad4−/− mice exhibited increased genomic instability, which correlated with downregulated expression and function of genes encoding proteins in the Fanconi anemia/Brca (Fanc/Brca) DNA repair pathway linked to HNSCC susceptibility in humans. Consistent with this, further analysis revealed a correlation between downregulation of Smad4 protein and downregulation of the Brca1 and Rad51 proteins in human HNSCC. In addition to the above changes in tumor epithelia, both normal head and neck tissue and HNSCC from HN-Smad4−/− mice exhibited severe inflammation, which was associated with increased expression of TGF-β1 and activated Smad3. We present what we believe to be the first single gene-knockout model for HNSCC, in which both HNSCC formation and invasion occurred as a result of Smad4 deletion. Our results reveal an intriguing connection between Smad4 and the Fanc/Brca pathway and highlight the impact of epithelial Smad4 loss on inflammation.