NF-kappa B activation defines a distinct subset of HPV-associated head and neck cancer

Michael Hajek, MD,[1] Travis Schrank, MD, PhD,[2] Wendell Yarbrough, MD,[2] Natalia Issaeva, PhD [2]
[1]Surgery (Otolaryngology), Yale School of Medicine, New Haven, CT
[2]Otolaryngology, University of North Carolina School of Medicine, Chapel Hill, NC

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Abstract:

Background:

Despite the improved prognosis in HPV-associated head and neck squamous cell carcinoma (HNSCC) as compared to HPV-negative, 20-30% of patients with HPV+ cancer will suffer from recurrent disease.[1] Current treatment, including chemotherapy and radiation therapy, is associated with lifelong morbidity, and there are limited treatments and no curative options for patients who develop recurrent metastatic disease.[2] Therapeutic de-escalation (decreased radiation dose) is being tested through clinical trials; however, those studies select patients based solely on tumor and patient smoking characteristics.[3] Mechanisms of HPV-driven carcinogenesis in HNSCC are not well understood, which limits new therapeutic strategies and hinders the appropriate selection of patients for de-escalation therapy. Tumor necrosis factor 3 (TRAF3), as well as cylindromatosis deubiquitinase (CYLD), are genes involved with negatively regulating the canonical and non-canonical NF-kB pathways. We previously identified a subset of HPV-associated HNSCCs defined by TRAF3/CYLD mutations, which comprise roughly 30% of HPV-associated HNSCCs in the Cancer Genome Atlas (TCGA).[4] This subset is associated with improved patient survival, activation of NF-kB pathways, and maintenance of episomal (as opposed to integrated) HPV.[5] Utilizing an expanded databse of tumors, we propose an updated way to define this distinct subset of HPV+ HNSCC. We also look further into this subset of HPV-associated HNSCCs and propose an alternative mechanism to HPV carcinogenesis, distinct from the canonical viral integration and viral oncogene E6 and E7 expression paradigm.

Methods:

To analyze TRAF3, CYLD, and phosphatidylinositol-4,5-bisphosphae 3-kinase catalytic subunit α (PIK3CA) (as well as the other genes tested in the study) genetic alterations in HNSCC, we obtained data using the cBioPortal for Cancer Genomics for tumors in TCGA, and additionally from a separate UNC tumor database. Raw TCGA data was used for these analyses, downloaded from NCBI dbGaP.
Gene Set Enrichment Analyses were run through the Broad Institute (http://software.broadinstitute.org/gsea/index.jsp).

Results:

1. Creation of a gene expression signature to identify tumors with highly active NF-kB

Given that EBV-associated nasopharyngeal carcinoma is the only other solid tumor (along with HPV+ HNSCC) associated with TRAF3 and CYLD mutations, and that EBV carcinogenesis occurs largely through constituitively active NF-kB allowing for maintenance of a viral episome, we sought to determine if a portion of HPV+ tumors behave in a similar manner.

Results:

- In contrast to canonical HPV carcinogenesis paradigms, we found that 85% of tumors with TRAF3/CLYD mutations have episomal HPV DNA, and only 24% of HPV+ tumors with wild type TRAF3 and CYLD contained episomal HPV.
- To detect which tumors had active NF-κB, we used those with known defects in NF-κB regulators, TRAF3 and CYLD to identify the top 100 differentially expressed genes and used them to create an NF-kB activity classifier (Figure 1).

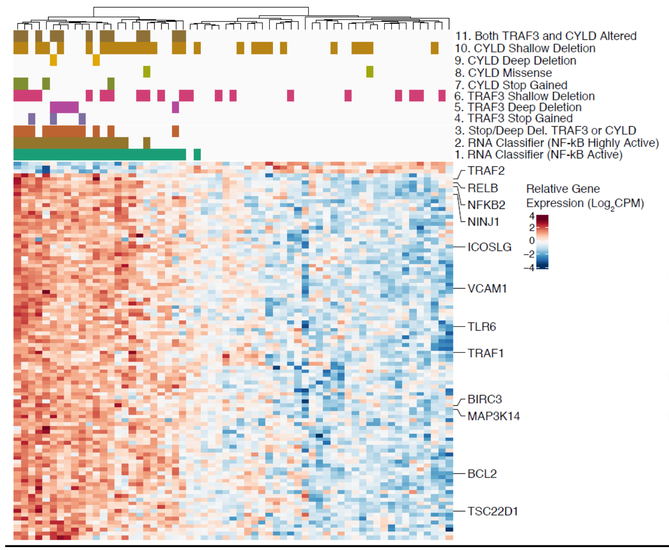

Figure 1. RNA Expression Changes Associated with TRAF3/CYLD alterations in HPV+ HNSCC. Rows show color scale of normalized log2 expression for the top 100 differentially expressed genes between tumors with and without TRAF3/CYLD defects. Columns represent unsupervised clustering of individual tumors. Row annotation – well known NF-κB target genes. Column annotation details: Any TRAF3 and CYLD alteration –missense, nonsense, frameshift, shallow deletion, deep deletion in TRAF3 and/or CYLD – Shallow Deletion Gistic copy-number score = -1, Deep Deletion – Gistic copy-number score = -2, Stop Gained – frameshift or nonsense mutation. Missense – missense or in frame indel. Stop/Deep Del. TRAF3 or CYLD – Any one of nonsense, frameshift, deep deletion inTRAF3 and/or CYLD

Results:

2. NF-κB activation is associated with distinct tumor behavior as indicated by improved survival

- This new way to define this subset of HPV+ HNSCC includes all the tumors with inactivating TRAF3/CYLD mutations, as well as several tumors lacking TRAF3 or CYLD defects that attained high NF-kB activity through other mutations.

Results:

- Interestingly, the subtypes of HPV+ HNSCC identified by the NF-κB activity classifier correlated with survival with a larger HR and lower p value compared to subtypes identified by TRAF3 or CYLD mutation status (Figure 2).

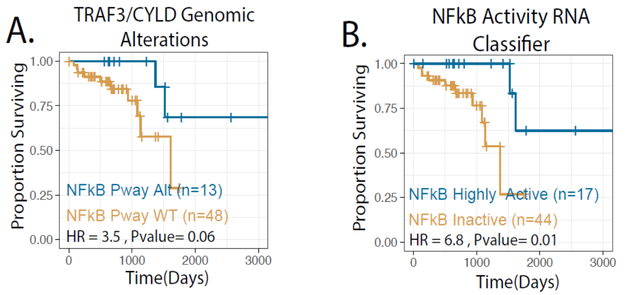

Figure 2. Active NF-κB RNA signature is a significant predictor of improved survival in HPV+ HNSCC patients. Kaplan–Meier survival curves of HPV+ HNSCC patients (TCGA, n=61, p-values = log rank test). HR – Hazard Ratio. NF-kB High Active – Highly NF-kB active tumors by RNA expression as defined according to the gene expression classifier, these were compared to all other tumors (NF-kB Inactive) in the study cohort. NF-kB Pway Alt – Any missense, nonsense, frameshift, shallow deletion, deep deletion in TRAF3 and/or CYLD, these were compared to all other tumors (NF-kB Pway WT) in the study cohort.

- These data suggest that mechanisms in addition to TRAF3 or CYLD gene defects drive NF-κB activation in HPV+ HNSCC, and that NF-κB activation is associated with distinct tumor behavior as indicated by improved survival.

Results:

3. This distinct subset, as defined by NF-kB activity, is validated through its presence in an independent cohort

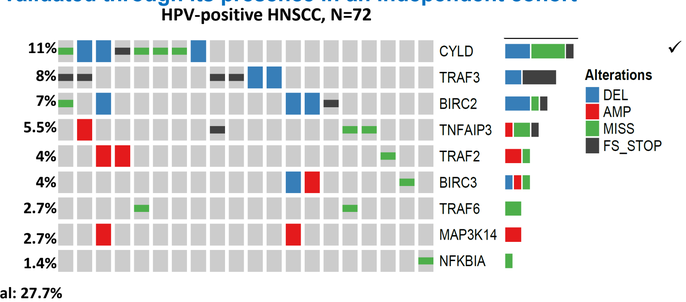

Figure 3. Alterations in the NF-κB regulators in HPV+ HNSCC. Defects in 10 NF-kB regulatory genes in HPV+ HNSCC from the UNC cohort.

Results:

- Since all prior work relied on TCGA, we expanded this work to an independent tumor set from UNC. We utilized tumors with mutations in known regulators of NF-kB signaling, such as TRAF2, MYD88, NF-kBIA, TNFAIP3, TRAF6, BIRC2, BIRC3, and MAP3K14.
- About 28% of tumors contained alterations in NF-κB regulators (Figure 3).
- This correlates with the previously identified 30% of tumors with TRAF3/CYLD defects in TCGA.
- Moreover, As with the TCGA cohort, defects in these genes significantly correlated with improved overall and recurrence-free survival (Figure 4).

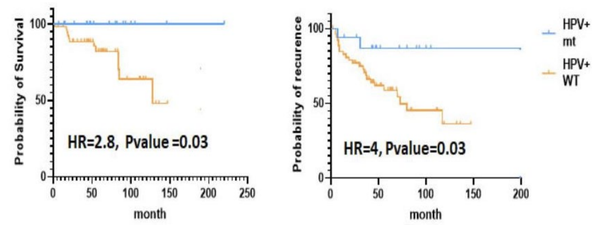

Figure 4. Figure 5. Alterations in NF-κB regulators correlate with improved overall and recurrence-free survival in HPV+ HNSCC. Kaplan–Meier survival curves of 72 HPV+ HNSCC patients from the UNC cohort with (mt) or without (WT) alterations in TRAF3, CYLD, TRAF2, NF-kBIA, TNFAIP3, TRAF6, BIRC2, BIRC3, MAP3K14 genes.

Conclusions:

- ✓ There exists a distinct subset of HPV+ HNSCC, characterized by high NF-kB activity, which confers a significant survival advantage.
- ✓ This subset of HPV+ HNSCCs contains the HPV genome in its episomal form, rather than through viral integration, and implies a distinct mechanism of carcinogenesis via NF-kB activity.
- ✓ The improved survival of HPV+ HNSCC, as compared to HPV-, is skewed due to the presence of this distinct subset of HPV+ tumors.
- ✓ NF-kB active HPV+ HNSCC provides an attractive population for therapeutic de-escalation, both mechanistically and from a survival standpoint.

References:

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