Integrative and comparative genomic analysis of HPV-positive and HPV-negative head and neck squamous cell carcinomas

Tanguy Y Seiwert[1,2,3,†], **Zhixiang Zuo**[1,†], **Michaela K Keck**[1,†], **Arun Khattri**[1,†], **Chandra S. Pedamallu**[4,5], **Thomas Stricker**[6], **Christopher Brown**[7], **Trevor J. Pugh**[4,5], **Petar Stojanov**[4], **Juok Cho**[4], **Michael S. Lawrence**[4], **Gad Getz**[4], **Johannes Brägelmann**[1], **Rebecca DeBoer**[1], **Ralph R Weichselbaum**[2,8], **Alexander Langerman**[2,9], **Louis Portugal**[2,9], **Elizabeth Blair**[2,9], **Kerstin Stenson**[2,9], **Mark W. Lingen**[2,10], **Ezra EW Cohen**[1,2], **Everett E. Vokes**[1,2,*], **Kevin P. White**[2,3,*], and **Peter S. Hammerman**[4,5,*]

[1]Department of Medicine, Section of Hematology/Oncology, The University of Chicago, Chicago IL60637, USA

[2]The University of Chicago Comprehensive Cancer Center, Chicago, IL60637, USA

[3]Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL60637, USA

[4]Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA

[5]Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA 02215, USA

[6]Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, TN37232, USA

[7]Department of Genetics, University of Pennsylvania, Philadelphia, PA19104, USA

[8]Department of Radiation Oncology, The University of Chicago, Chicago, IL 60637, USA

[9]Department of Otolaryngology/Head and Neck Surgery, The University of Chicago, Chicago, IL60637, USA

[10]Department of Pathology, University of Chicago, Chicago, IL60637, USA

Abstract

Purpose—The genetic differences between Human papilloma Virus (HPV)-positive and negative head and neck squamous cell carcinomas (HNSCC) remain largely unknown. In order to identify differential biology and novel therapeutic targets for both entities we determined mutations and copy number aberrations in a large cohort of locoregionally-advanced HNSCC.

Experimental Design—We performed massively parallel sequencing of 617 cancer-associated genes in 120 matched tumor/normal samples (42.5% HPV-positive). Mutations and copy number aberrations were determined and results validated with a secondary method.

Correspondence: Tanguy Seiwert, Section of Hematology/Oncology, Department of Medicine, The University of Chicago, 5841 S. Maryland Ave, MC2115, Chicago, IL60637, USA, tseiwert@medicine.bsd.uchicago.edu, Tel: +1-773-702-2452, Fax: +1-773-702-3002.
†These authors contributed equally
*These authors contributed equally

Results—The overall mutational burden in HPV-negative and HPV-positive HNSCC was similar with an average of 15.2 versus 14.4 somatic exonic mutations in the targeted cancer-associated genes. HPV-negative tumors showed a mutational spectrum concordant with published lung squamous cell carcinoma analyses with enrichment for mutations in *TP53, CDKN2A, MLL2, CUL3, NSD1, PIK3CA* and *NOTCH* genes. HPV-positive tumors showed unique mutations in *DDX3X, FGFR2/3* and aberrations in *PIK3CA, KRAS, MLL2/3* and *NOTCH1* were enriched in HPV-positive tumors. Currently targetable genomic alterations were identified in *FGFR1, DDR2, EGFR, FGFR2/3, EPHA2* and *PIK3CA. EGFR, CCND1,* and *FGFR1* amplifications occurred in HPV-negative tumors, while 17.6% of HPV-positive tumors harbored mutations in Fibroblast Growth Factor Receptor genes (*FGFR2/3)* including six recurrent *FGFR3* S249C mutations. HPV-positive tumors showed a 5.8% incidence of KRAS mutations, and DNA repair gene aberrations including 7.8% BRCA1/2 mutations were identified.

Conclusions—The mutational makeup of HPV-positive and HPV-negative HNSCC differs significantly, including targetable genes. HNSCC harbors multiple therapeutically important genetic aberrations, including frequent aberrations in the FGFR and PI3K pathway genes.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the fifth most common non-skin cancer worldwide with an annual incidence of 600,000 cases and a mortality rate of 40–50% despite aggressive treatment (1,2). The major known risk factors are environmental exposure to tobacco products, alcohol, and infection with high-risk Human Papilloma Viruses (HPV). The incidence of HPV-positive tumors is rising rapidly in Western countries and HPV-status is the strongest clinically-applicable prognostic marker, portending a favorable prognosis(3, 4).

While HNSCC is widely viewed as comprised of two distinct clinical entities, HPV-positive and HPV-negative tumors, a comprehensive list of differential molecular abnormalities, in particular therapeutically-relevant genetic aberrations has not been reported. A particular problem is the lack of study of HPV-positive HNSCC: Currently no large series of HPV-positive tumors exist and the upcoming cancer genome atlas (TCGA) cohort is compromised of 85% HPV-negative tumors(5). This bias is likely related to selection of surgically resected, earlier stage oral cavity and laryngeal tumors. This may not be representative for clinically more complex Stage IV tumors requiring multimodality or palliative treatments(6–8).

Unlike lung or breast adenocarcinomas, there are currently no defined targetable genetic aberrations for HNSCC, and no approved therapies are tied to genetic alterations as predictive biomarkers. All HNSCC patients are treated with a largely uniform approach based on stage and anatomic location, typically using surgery, radiation, and chemotherapy alone or in combination (9). Cetuximab, an anti-EGFR antibody, is the only approved targeted therapy for HNSCC with a single agent response rate of 10–13%. Despite the modest response rate there are no validated predictive biomarkers for benefit from cetuximab (10,11).

Previous studies have demonstrated frequent mutations of several genes in cohorts of largely HPV-negative HNSCC, most notably *TP53, PIK3CA, CDKN2A,* the *TERT* promoter, and *NOTCH* pathway gene alterations(12–16). However, the genetic makeup of HPV-positive HNSCC remains unclear (15).

In the current study, we investigated a fully annotated patient cohort of 120 locoregionally advanced HNSCC (including 42.5% HPV-positive tumors) treated uniformly with organ-preserving chemoradiotherapy using massively parallel sequencing, copy number profiling, and validation.

We discover distinct mutational and copy number profiles in HPV-positive and HPV-negative tumors and identify for the first time potentially targetable mutations and copy number aberrations that are of high translational relevance.

Materials and Methods

Chicago Head and Neck Cancer Genomics Cohort (CHGC)

Pre-treatment tumor tissues (n=120) and matched normal DNA for patients with locoregionally advanced HNSCC treated at the University of Chicago were obtained from the HNSCC tissue bank (UCCCC#8980).

Sample Preparation

An overview of the tissue-processing is provided in Supplementary Figure S1 and described in detail in the Supplemental Methods.

HPV consensus testing

HPV16/18 status was determined by E6/E7-specific qRT-PCR. Results were corroborated by additional tests to increase accuracy including an E6/E7 DNA based multiplex PCR for five high-risk HPV types (17) as well as p16/CDKN2A expression, and TP53 mutations (18).

Sequencing data generation and analysis

DNA sequencing libraries were prepared following published protocols (19) and enriched using custom capture reagents (Agilent, Nimblegen(validation)). 2×100bp paired-end sequencing occurred using Illumina HiSeq 2000/2500 sequencers.617 cancer-associated genes (Supplementary Table S1A) were targeted and sequenced at high-depth in tumor/normal pairs. Median coverage at targeted bases was 231X in the tumors and 254X in the matched normal. Mutation and indel calling was done using bioinformatic pipelines as described previously employing MuTect (20–22). Furthermore we used VarWalker to determine potentially relevant genetic aberrations (mutations and copy number aberrations) using prioritization via a protein-protein interaction network focusing on frequently mutated and cancer gene census genes (23)

We used an established machine learning based approach – Cancer-Specific High-Throughput Annotation of Somatic Mutations (CHASM) – to predict and prioritize missense

mutations leading to functional changes and thus likely driving tumorigenesis (24). This approach was previously validated to show high specificity, and to a lesser degree sensitivity to identify "drivers" of oncogenesis(24).

Copy number (CN) analysis was performed using sequencing data and the CONTRA algorithm (25). Results were validated on the Nanostring nCounter using predefined/custom cancer gene panels (Nanostring, Seattle, WA).

Detailed methods are available in the Supplementary Methods.

Results

Patient Cohort Description and Sequencing Metrics

The median age of patients was 56 years with a median follow-up of 48 months (Table 1). 51 patients (42.5%) were HPV-positive. 45% of patients had no or light smoking histories while 55% had significant smoking-histories including 35% of HPV-positive tumors (N=18). The cohort consisted of locoregionally-advanced tumors with 67 (55.8%) oropharynx and 115(95.8%) stage IV tumors (Table 1).

Detection of somatic aberrations

Analysis of targeted hybrid capture sequencing data identified a total of 5476 point mutations and 4562 insertion/deletion (indel) events across 120 tumor/normal pairs in targeted 617 genes (Supplementary Table S1A). 1536 of the point mutations and 200 of the indel events were in coding regions. Of the 1536 point mutations in coding regions, 1209 resulted in an amino acid changes in the corresponding proteins. Individuals displayed a mean point mutation rate of 2.33 mutations per Megabase (Mb) in the targeted regions and 0.22/Mb in the coding territory alone with a range of 0–96 mutations in the coding regions of the targeted genes. Among non-synonymous substitutions, transitions and transversions at CpG sites, which are typically associated with tobacco exposure (26), were the most commonly observed mutational context with a rate of 21/Mb. Mutations at Tp*Cp(A/C/T) sites, a substitution type commonly associated with virally induced cancers (26), were the second most commonly observed context at 13/Mb and enriched in the HPV-positive patients. We did not detect a significant difference in overall mutation rates by HPV status at all targeted bases (HPV-negative 2.22/Mb, HPV-positive 2.46/Mb, p = 0.64) or in the coding territory (HPV-negative 0.23/Mb, HPV-positive 0.21/Mb, p=0.79). Coding mutations were validated at a rate of 87% (338/390) in a second independent hybrid capture experiment when evaluating statistically enriched mutations (Tables S1B, C).

We applied the MutSig method to identify cancer-relevant genes demonstrating evidence of statistical selection for mutations in our cohort(20–22, 27). MutSig identified11 genes displaying significant enrichment for mutations as defined by a q-value of <0.1 in the overall cohort (Supplementary Figure S2): *TP53, CDKN2A, PIK3CA, MLL2, TPRX1, CUL3, FLG, NSD1, DDX3X, RPIK4* and *HRAS. FGFR3* and *FBXW7* demonstrated q-values between 0.1 and 1. A second MutSig analysis, in which only genes annotated in the COSMIC database were considered, re-discovered *TP53, CDKN2A, PIK3CA, HRAS, FGFR3* and *FBXW7* and additionally nominated *KRAS, MLL3, FGFR2, ZNF217* and *RIMS2* as statistically

significant with q<0.1. We performed additional significance analyses on the separate HPV-negative and HPV-positive cohorts using the same Mutsig algorithm. Analysis of mutated genes displaying enrichment in the HPV-negative cohorts demonstrated statistical enrichment for mutations of *TP53, CDKN2A, MLL2/3, NOTCH1, PIK3CA, NSD1, FBXW7, DDR2 and CUL3,* in the HPV-negative samples (Figure 1A). Genes displaying statistical enrichment in HPV-positive tumors were PIK3CA, *MLL3, DDX3X, FGFR2/3, NOTCH1, NF1, KRAS, and FBXW7* (Figure 1B).

Somatic copy number alterations (SCNAs) were inferred from sequencing data by read-depth analysis using the CONTRA methodology (Supplementary Methods). We applied a modification of GISTIC 2.0 analysis (28) compatible with the focal sequencing data to identify recurrent peaks of amplification and deletion and identified 192 genes with false discovery rate of less than 0.25 (Supplementary Table S1D–G). Copy number alterations were validated at a rate of 83%(45/54) using the Nanostring nCounter, focusing on the genes found to be significantly altered in this dataset (Supplementary Table S1H). Copy number analysis demonstrated many previously demonstrated regions of amplification and deletion including focal gains of *EGFR, REL, BCL6, PIK3CA, TP63, CCDN1* and *MDM2* and losses of *ATM, CDKN2A, RB1, NOTCH1* and *NF1* (Figures 1C, 1D)(12–15, 29). Significantly amplified regions that occurred primarily in HPV-negative tumors were amplifications of 11q13 likely targeting *CCND1*, 7p11 (*EGFR*) (Figure 2A). Amplification of 3q26–28 a region containing *SOX2/TP63/PIK3CA* (Figures 2A, 2B) occurred in both HPV-positive and HPV-negative tumors. 3p loss and *CDKN2A* deletions (Figure 2C) occurred primarily in HPV-negative tumors while *ATM* deletions occurred primarily in HPV-positive tumors (Figure 2D).

Comparison of altered networks for HPV-positive tumors versus HPV-negative tumors

To identify additional biologic differences between HPV-positive and HPV-negative tumors we employed an unbiased objective protein-protein interaction network based analysis of genetic aberrations (VarWalker) (Figure 3). The altered networks for HPV-negative tumors contained 84 proteins connected by 76 interactions (Figure 3A). For HPV-positive tumors, the altered networks contained 88 proteins connected by 83 interactions (Figure 3B). The two altered networks were substantially different: Alteration of p53 signaling and cell cycle pathway genes occurred almost exclusively in HPV-negative tumors. The alteration of oxidative stress pathway genes (*CUL3, NFE2L2* and *KEAP1*) occurred more often in HPV-negative tumors (Figure 3A). The alteration of DNA damage pathway (*BRCA1, BRCA2, FANCG, FANCA, FANCD2, and ATM*), FGF signaling (*FGFR2, FGFR3* and *FGFR4*), JAK/STAT signaling (*STAT1, JAK1* and *JAK2*), and immunology related genes (*HLA-A, HLA-B*) favored HPV-positive tumors (Figure 3B). HPV-positive and HPV-negative tumors shared network alterations such as PI3K signaling, Notch aberrations, and SMAD signaling (Figures 3A, 3B).

Targetable copy number aberrations and mutations

Somatic mutations in potentially targetable kinase genes occurred in *FGFR2* and *FGFR3* uniquely in HPV-positive tumors with an incidence of 17.6% among HPV-positive tumors. *FGFR2* mutations included N569D and N569K (both in HPV-positive), and *FGFR3*

mutations included six S249C mutations, which were validated by Sanger Sequencing (all in HPV-positive), as well as a K413N somatic mutation (Figures 4A, 4B). We used a previously validated machine learning-based approach named CHASM to predict and prioritize missense mutations with a high likelihood of being drivers of oncogenesis(24). Both *FGFR2* N569K and *FGFR3* S249C have been described in several cancer types(30, 31) and showed low CHASM scores suggestive of oncogenic driver character (Figures 4A/B). Importantly the *FGFR3* S249C mutation was identified recurrently in six HPV-positive tumors. S249C has been reported in one HPV-positive tumor in the TCGA HNSCC cohort and in the TCGA lung squamous cell and bladder carcinoma cohorts. A fraction of *DDR2* and *EPHA2* mutations also showed low CHASM scores, but were not recurrent, and did not cluster in any domain (Figures 4A, 4B).

PIK3CA was the most commonly altered oncogene including a number of established canonical mutations (E542K, E545K, H1047R) (Figures 4A, 4B). Other PI3K pathway mutations included somatic events in *PIK3R1*, *PTEN*, *TSC1* and *TSC2*, all of which have been reported to harbor mutations in other tumor types (Figure 4A). *PIK3CA* mutations were more commonly observed in HPV-positive individuals though the difference was not statistically significant in our cohort (p=0.20).

Mutations in the MAPK pathway genes included seven canonical *HRAS* (G13V, Q61L, K117N), *KRAS* (G12D, G13D, L19F) and *NRAS* (Q61R) events (incidence 5.8%), as well as multiple mutations of unclear significance in the tumor suppressor *NF1* (Figure 4A, 4B).

Clinical correlation

We first examined the mutation difference in smokers vs. non-smokers (Supplementary Table S2). In the overall cohort tumors from smokers displayed a higher overall mutation burden as compared to non-smokers 11.5 mut/pt (10 pack years) versus 5.6 mut/pt (non-smokers)(p = 0.0007, t test). We noted a significant association among smoking and mutations in *TP53* (p = 0.0009), *CSMD3* (p = 0.0062), *RB1CC1* (p = 0.0161), *THSD7A* (p = 0.0319). Mutations in *ZFHX4* (p = 0.0167, Fisher's exact test) and *TRRAP* (p = 0.0392, Fisher's exact test) were significantly enriched in smokers compared to non-smokers. Additional subgroup analyses including drinkers vs. non-drinkers, large primary versus small primary and node negative vs. node positive are provided in Supplementary Table 2, including subgroup analyses for oropharyngeal primary tumors.

The association among HPV status and prognosis was evaluated. As expected HPV positive patients had significantly better prognosis that HPV negative patients for both OS (p = 0.015) and PFS (p = 0.013), and this holds true for the overall cohort as well as oropharyngeal cancers (OS: p=4e-05, PFS: p = 0.0001) (Supplementary Figure S3A).

In an exploratory, hypothesis-forming analysis we correlated gene aberrations including mutations and copy number alterations with overall survival and progression-free survival. In the smaller sub-cohorts of HPV-positive and HPV-negative tumors most associations were not significant, though we did identify in HPV-negative tumors a possible correlation of *PIK3CA* mutations (OS: p = 0.017, PFS: p = 0.004)(Supplementary Figure S3B), as well

as *TP53* wildtype status with poor prognosis (OS: p = 0.262, PFS: p = 0.017) (Supplementary Figure S3C). These findings require validation in future studies.

Discussion

HPV-positive and HPV-negative head and neck cancers are distinct clinical entities. We report for the first time on the underlying differential mutational profiles, generated from a unique patient cohort of poor-prognosis, locoregionally-advanced tumors. Unlike prior exome-focused studies, we employed a focused 617-cancer gene-candidate approach, allowing deep coverage of cancer-relevant genes. This approach is similar, albeit significantly broader than current clinical grade next generation sequencing assays(32).

This is the first cohort to include a large number of HPV-positive tumors, including patients with a significant tobacco history (35% of HPV-positive tumors). While HPV-positive status confers a favorable prognosis, patients with >10 pack-year smoking history have a poorer prognosis (3) and we report a higher mutational burden including presence of KRAS mutationsin such HPV-positive tumors. We identify targetable genetic aberrations including both HPV-positive and HPV-negative tumors including frequent FGFR2/3 aberrations in later group.

Genetic aberrations correlate closely with HPV status, and less with anatomic site (Supplementary Table S2), suggesting that HPV status (not anatomic site) is the most important factor determining tumor biology. Furthermore it was recently reported that anatomic site cannot always be accurately determined and misclassification occurs(18).

The mutational spectrum in HPV-negative HNSCC is very similar to lung and esophageal squamous cell carcinomas with enrichment for mutations in *TP53, CDKN2A, MLL2, CUL3, NSD1, PIK3CA* and *NOTCH* genes, and copy number increases in *EGFR*, *CCND1*and *FGFR1*. In the future these genetic similarities may potentially allow biologically-driven cross-tumor drug development and biomarker discovery.

In contrast HPV-positive tumors show a distinct genetic profile with unique mutations in *DDX3X*, *CYLD* and *FGFR* and enrichment for PI3K pathway alterations and rarer *KRAS* mutations. Somatic aberrations in DNA-repair genes (*BRCA1/2*, Fanconi anemia genes, and *ATM*) may contribute to chemo- and/or radiosensitivity of HPV-positive tumors, and interestingly occurred in HPV-positive tumors in non-/light smokers. Both tobacco smoke and defects in DNA repair are known to induce a large number of genetic aberrations, and may be distinct ways to accumulate genetic aberrations required for the emergence of cancer. Of note a recent *RAD51B* was reported as an integration target for HPV16 leading to loss of its DNA repair function (5).

FGFR and PI3K pathway aberrations are potential therapeutic targets in this population. *FGFR2/3* mutations are of particular interest as they occurred in 17.6% of HPV-positive tumors, most commonly the S249C mutation, which has been shown to be an oncogenic driven in bladder cancer.

One HPV-negative tumor harbored amplification of *FGFR1*, which is being explored clinically for lung squamous cell carcinomas (33, 34). Furthermore oncogenic *FGFR3-TACC3* fusions were recently reported in HPV-positive HNC supporting a prominent role for oncogenic FGFR signaling for HPV-positive HNC while other kinases are genetically unaltered (33–37).

In contrast to prior smaller reports the overall mutational burden in HPV-negative and HPV-positive HNSCC was comparable (15.2 versus 14.4 somatic exonic mutations in the targeted cancer-associated genes), a finding corroborated when analyzing publically available data from the cancer genome atlas (TCGA) HNSCC cohort(5).

HPV-positive tumors in patients with significant tobacco use had a significantly higher mutational burden. While the number of KRAS (N=3) and TP53 (N=2) mutations in HPV-positive oropharyngeal tumors was small, they occurred in patients with significant tobacco history, and showed 4–6-fold higher average mutational burden (NS). Interestingly the two TP53 mutations (H179R, G361fs) co-occurred with KRAS (G12D, L19F) and one PIK3CA mutation (F872L) suggesting multiple oncogenic drivers in highly mutational altered HPV-positive tumors. The KRAS L19F mutation is thought to be modestly transforming (38).

Low-frequency canonical *HRAS, KRAS*, and *NRAS* mutations were identified in both HPV-positive and negative tumors. *RAS* mutations have been associated with poor outcome in other cancer types (e.g. lung adenocarcinomas), as well as resistance to cetuximab in colon cancer (39). Further study in HNC will be important, especially regarding the implications of *KRAS* mutations in otherwise good-prognosis HPV-positive tumors, which were present in 5.8% of HPV-positive tumors. One additional case of a canonical *KRAS* mutation in an HPV-positive tumor was previously reported (29).

The specific genetic events in HPV-positive or negative HNSCC likely contribute to the distinct biologic behavior and may impact therapy in the future. The lack of targeted therapies for squamous cell tumors remains challenging: In addition to *FGFRs* we identify multiple potentially targetable genomic alterations including mutations in the *DDR2, EPHA2* kinases, *PIK3CA* and PI3K pathway genes (e.g. *TSC1/2, NF2*) that are predicted to be oncogenic drivers. Therapeutic implications for these particular changes are being investigated clinically and pre-clinically (33, 40, 41).

The lack of *EGFR* aberrations in HPV-positive tumors is consistent with prior reports (5, 42), and clinical data (10, 11). Other potential therapeutic targets include alterations of genes, which regulate the cell cycle (e.g. *CCND1* amplification as a target for cyclin dependent kinase 4/6 (CDK4/6) inhibitors).

We identify a number of additional novel mutated pathways, providing further insight into the molecular pathogenesis of HNSCC: 1) Similar to lung squamous cell carcinoma, *MLL2* and *MLL3*mutation were observed in both HPV-positive and negative tumors. *MLL* genes encode histone lysine methyltransferases that are involved in chromatin remodeling and recurrently mutated in several cancer types (43–46). Other studies have demonstrated an association with poor clinical outcomes (47). 2) *CUL3, NFE2L2* and *KEAP1*mediate cellular responses to oxidative stress and are a frequent target for mutations in lung squamous cell

carcinomas as well as the HPV-negative HNSCC cohort. *KEAP1* binds to *NFE2L2* and targets *NFE2L2* for ubiquitination and subsequent degradation by the *CUL3/RBX1* complex (48). Mutations are typically associated with heavy tobacco exposure, and distributed across the gene, which is suggestive of loss of function mutations. 3) The *DDX3X* gene was exclusively mutated in HPV-positive tumors. *DDX3X* encodes an ATP-dependent RNA helicase and is involved in RNA processing (Supplementary Figure S4). It has been described to be significantly mutated in medulloblastomas, and can potentiate β-catenin activity by promoter transactivation (43, 44).4) In addition, HPV-positive tumors harbor mutations in *CYLD,* a gene that has not been implicated as a hotspot for mutations, but is well known for its' role in HPV-positive cervical cancer: *CYLD* is involved in differentiation and NFκB signaling and in response to hypoxia activates pro-oncogenic processes (Supplementary Figure S4)(49).5) Lastly, *UBR5* mutations are involved in DNA damage and apoptosis signaling, and in addition to our discovery in HPV-positive and negative tumors, *UBR5* is recurrently mutated in mantle cell lymphomas (Supplementary Figure S4). Investigations into its role in radio- or chemosensitivity are promising(50).

In conclusion, the mutational landscape of HPV-positive HNSCC differs from HPV-negative HNSCC and may help explain the distinct clinical behavior and prognosis. Further study of clinical implications of the observed mutations will be vital with respect to targeted therapies, radiation, and chemotherapy. For the first time we identify targetable genetic aberrations in both HPV-positive and HPV-negative HNSCC. In particular, frequent aberrations in the FGFR family are promising as well asPI3K pathway and cell cycle abnormalities. The significant number of potential targets including rare aberrations (*DDR2, TSC1/2, EPHA2*), or candidate biomarkers such as HRAS/KRAS indicates a need for tumor profiling of HNSCC in the future- including on clinical trials, as well as functional validation in pre-clinical studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Statement of Translational Relevance

Head and Neck Squamous Cell Carcinoma (HNSCC) is comprised of two distinct clinical entities, HPV-positive and HPV-negative HNSCC. A comprehensive list of differential molecular abnormalities, in particular *therapeutically-relevant genetic aberrations* has not been reported. Furthermore, while the incidence of HPV-positive tumors is rising rapidly, there is a lack of HPV-positive cohorts. In our current study, we investigate 120 locoregionally advanced HNSCC (including 42.5% HPV-positive tumors). Tumors show a differential genetic profile based on HPV-status. We discover multiple, novel therapeutic targets with predicted driver character that are of high translational relevance: *EGFR, CCND1,* and *FGFR1* amplifications occurred in HPV-negative tumors, while 17.6% of HPV-positive tumors harbored mutations in Fibroblast Growth Factor Receptor genes (*FGFR2/3*). HPV-positive tumors showed a 5.8% incidence of KRAS mutations, and 7.8% incidence of BRCA1/2 mutations. Our study provides strong support for further clinical investigation of novel therapeutic targets and candidate biomarkers in patients with HNSCC.
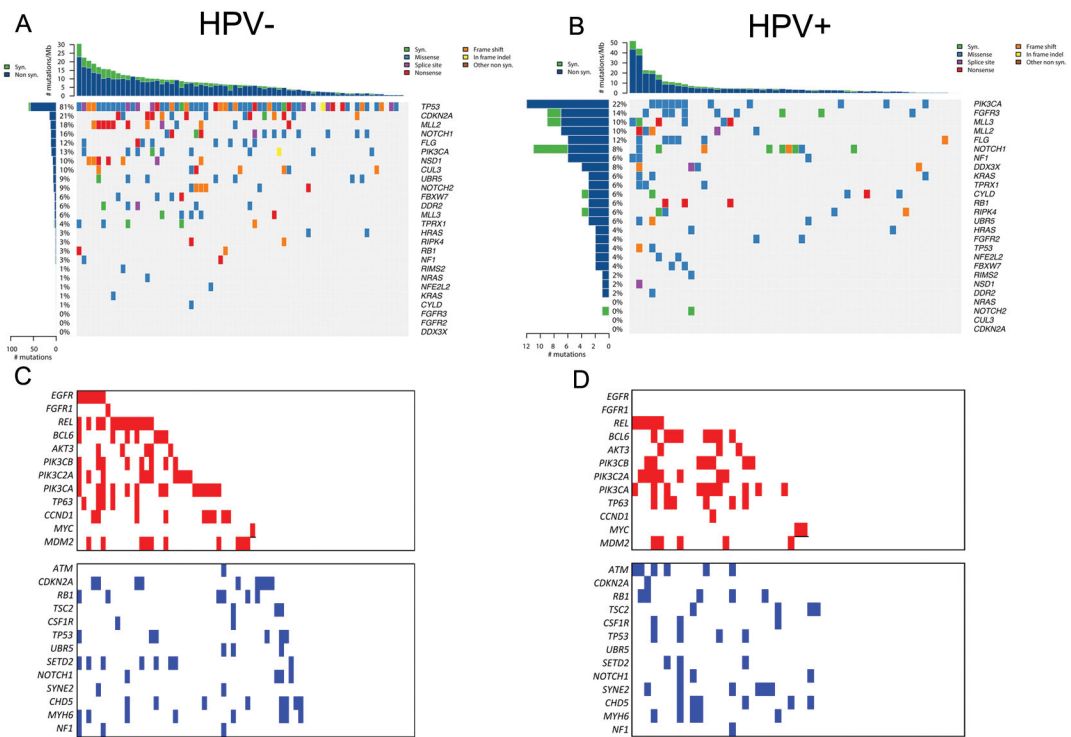
Figure 1. Mutation events sorted by HPV status

(A) Left and right panel respectively: Left histogram: percentage of samples affected by an alteration in the corresponding gene on the right side, top histogram: number of mutations per megabase and sample, heat map:type of alteration events.**(B)** Copy number gains (red) and losses (blue) sorted by HPV status, samples are ordered by copy number events.
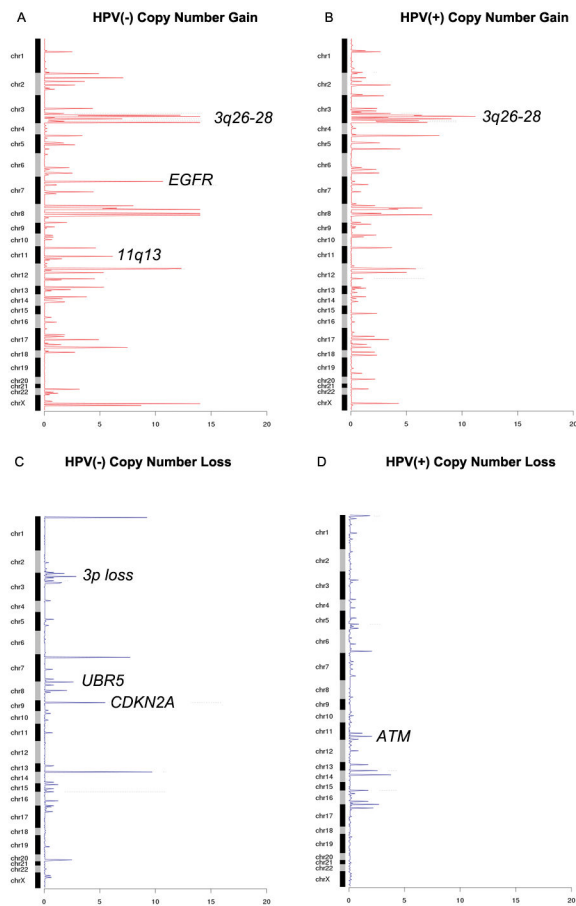
Figure 2. GISTIC-like copy number analysis derived from next generation sequencing data
Shown are the aberrant regions of the chromosome and the respective negative log2 of the q-value of the alteration in each panel, respectively. (**A**) Copy number gains in HPV(−) samples. (**B**) Copy number gains in HPV(+) samples. (**C**) Copy number losses in HPV(−) samples. (**D**) Copy number losses in HPV(+) samples.
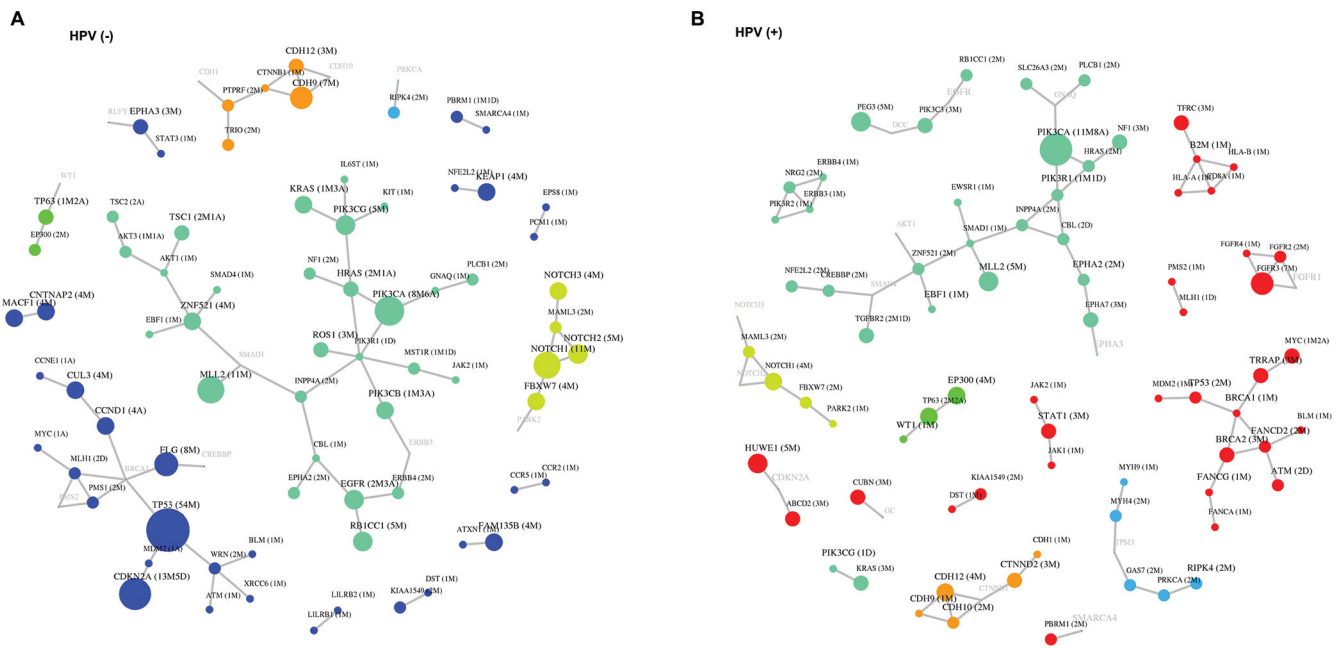
Figure 3. Network based comparison of genetic aberrations in HPV-negative and HPV-positive tumors using protein-protein interaction (PPI) network prioritization (VarWalker) in order to identify significantly altered pathways/networks in each entity

The size of the symbols correlates with frequency of aberrations. M=non-synonymous mutation/s, A=amplification/s, D=deletion/s. Color choices are random, but are intended to highlight similarities and differences between HPV-positive and HPV-negative tumors. PPI connections are shown with grey lines, and connecting genes without genetic aberrations are shown with grey font.
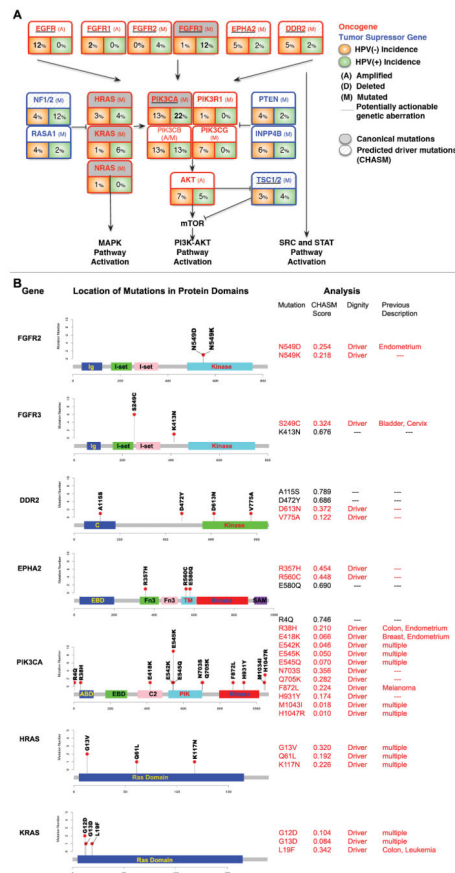
Figure 4. Genomic alterations with translational or clinical relevance by HPV status
(**A**) Shown is the incidence of selected potentially targetable and their association with key pathways in HPV(−) and HPV(+) samples.(B) Gene diagrams for a selection of key mutations in potentially targetable genes, or presumed driver genes. Shown are the genes domains that are affected by somatic mutations, the number of cases that harbor the respective mutation is indicated on the ordinate axis, the CHASM scores and p-values for each mutation (red = <0.5 CHASM score, which indicates predicted oncogenic driver character), as well prior reports of mutations based on occurrence in COSMIC, or prior publications.

Table 1

Overview of clinical and histopathologic characteristics of samples

	Total	HPV Positive	HPV Negative
Pt. (No.)	120 (100%)	51 (42.5%)	69 (57.5%)
Age (Year)	56.72	56.69	56.75
***Gender (No.)**			
Female	24 (20.0%)	4 (3.3%)	20 (16.7%)
Male	96 (80.0%)	47 (39.2%)	49 (40.8%)
****Anatomic Site (No.)**			
OROPHARYNX	67 (55.8%)	47 (39.2%)	20 (16.6%)
ORAL CAVITY	23 (19.2%)	2 (1.7%)	21 (17.5%)
LARYNX	19 (15.8%)	1 (0.8%)	18 (15.0%)
HYPOPHARYNX	8 (6.7%)	0 (0%)	8 (6.7%)
OTHERS	3 (2.5%)	1 (0.8%)	2 (1.7%)
****Tobacco Use (No.)**			
Never/Light Smoker (<10 pack years)	54 (45.0%)	33 (27.5%)	21 (17.5%)
Heavy Smoker (10 pack years)	66 (55.0%)	18 (15.0%)	48 (40.0%)
Alcohol Use (No.)			
Never/Light Drinker	49 (40.8%)	26 (21.7%)	23 (19.1%)
Heavy Drinker	71 (59.2%)	25 (20.8%)	46 (38.4%)
***T Stage (No.)**			
Small primary (T1–2)	39 (32.5%)	23 (19.2%)	16 (13.3%)
Large Primary (T3–4)	81 (67.5%)	28 (23.3%)	53 (44.2%)
N Stage (No.)			
0–1	20 (16.7%)****	5 (4.2%)	15 (12.5%)
2–3	100 (83.3%)	46 (38.3%)	54 (45.0%)
Clinical Stage (No.)			
I–III	5 (4.2%)	3 (2.5%)	2 (1.7%)
IV	115 (95.8%)	48 (40.0%)	67 (55.8%)