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Genomic alterations in head and neck squamous cell carcinoma determined by cancer gene-targeted sequencing

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Background: To determine genomic alterations in head and neck squamous cell carcinoma (HNSCC) using formalin-fixed, paraffin-embedded (FFPE) tumors obtained through routine clinical practice, selected cancer-related genes were evaluated and compared with alterations seen in frozen tumors obtained through research studies.

Patients and methods: DNA samples obtained from 252 FFPE HNSCC were analyzed using next-generation sequencing-based (NGS) clinical assay to determine sequence and copy number variations in 236 cancer-related genes plus 47 introns from 19 genes frequently rearranged in cancer. Human papillomavirus (HPV) status was determined by presence of the HPV DNA sequence in all samples and corroborated with high-risk HPV *in situ* hybridization (ISH) and p16 immunohistochemical (IHC) staining in a subset of tumors. Sequencing data from 399 frozen tumors in The Cancer Genome Atlas and University of Chicago public datasets were analyzed for comparison.

Results: Among 252 FFPE HNSCC, 84 (33%) were HPV positive and 168 (67%) were HPV negative by sequencing. A subset of 40 tumors with HPV ISH and p16 IHC results showed complete concordance with NGS-derived HPV status. The most common genes with genomic alterations were *PIK3CA* and *PTEN* in HPV-positive tumors and *TP53* and *CDKN2A/B* in HPV-negative tumors. In the pathway analysis, the PI3K pathway in HPV-positive tumors and DNA repair-p53 and cell cycle pathways in HPV-negative tumors were frequently altered. The HPV-positive oropharynx and HPV-positive nasal cavity/paranasal sinus carcinoma shared similar mutational profiles.

Conclusion: The genomic profile of FFPE HNSCC tumors obtained through routine clinical practice is comparable with frozen tumors studied in research setting, demonstrating the feasibility of comprehensive genomic profiling in a clinical setting. However, the clinical significance of these genomic alterations requires further investigation through application of these genomic profiles as integral biomarkers in clinical trials.

Key words: DNA mutation, copy number variation, human papillomavirus, head and neck squamous cell carcinoma

introduction

Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous disease arising from the mucosal lining of the upper aerodigestive tract including the nasal cavity, paranasal sinuses, oral cavity, pharynx, and larynx. Common causes of HNSCC are tobacco and alcohol use [1]. High-risk human papillomavirus (HPV) is an established cause of HNSCC arising primarily in the oropharynx [2]. Recent data suggest that a subset of nasal cavity and paranasal sinus HNSCCs is also associated with HPV [3]. Clinically, patients with HPV-positive HNSCC, especially nonsmokers, have significantly better prognosis compared with patients with HPV-negative HNSCC after treatments for newly diagnosed as well as recurrent/metastatic diseases [4–7].

To improve understanding of the underlying cancer biology and leverage the findings to optimize the HNSCC management, there have been concerted efforts to genomically characterize HNSCC. To date, four large studies have reported the genomic alterations in HNSCC; three studies evaluated the genomic alterations using whole exome sequencing (WES) and whole

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genome sequencing (WGS) [8–10], and one study applied a targeted approach of evaluating 617 selected cancer-associated genes [11]. However, these earlier studies were conducted using frozen primary tumors, which are not common tissue collection or storage method in clinical practice. In this study, we evaluated genomic alterations of HNSCC using formalin-fixed, paraffin-embedded (FFPE) tumors obtained through routine clinical practice and compared with publically available sequencing data generated from frozen tumors. In addition, we have evaluated carcinomas arising in the sinonasal tract where ~20% of the tumors are high-risk HPV positive [3] and established similarities in their HPV-specific profiles compared with HPV-positive oropharynx SCC.

materials and methods

patient characteristics and next-generation sequencing-based genomic profiling assay

DNA extracted from FFPE tissue samples for 252 HNSCC patients was analyzed by Clinical Laboratory Improvement Amendments (CLIA)-certified, next-generation sequencing (NGS)-based assay, ordered by clinicians as a routine clinical practice, designated as the Foundation Medicine (FM) cohort (FoundationOne, Foundation Medicine, Cambridge, MA). Methods for the NGS-based clinical cancer gene assay used in this project have been previously published and assay performance has been rigorously validated [12]. We also provide a summary of the sequencing methods in supplementary File, available at *Annals of Oncology* online. The version of the FoundationOne assay used in this study was in use between December 2012 and August 2014 and evaluated exons of 236 cancer-related genes and introns of 19 genes frequently re-arranged in cancer (supplementary Table S1, available at *Annals of Oncology* online).

Copy number and mutation data from 279 HNSCC samples (TCGA cohort) with known HPV status were downloaded from cBioPortal (http://www.cbioportal.org/public-portal/); TCGA copy number data were GISTIC transformed before analysis [10, 13]. The genomic information of 236 genes contained in the FoundationOne assay was extracted. Similarly, the genomic information of overlapping 122 genes contained in the FoundationOne assay was extracted from the University of Chicago dataset (Chicago cohort) [11]. The genomic features selected from TCGA and Chicago data were tabulated with the genomic profiles from the FoundationOne assay. Because gene-rearrangement data were not available in the Chicago cohort [11], we considered only short variants and copy number alterations in 236 cancer-related genes.

determination of the HPV tumor status by sequencing, immunohistochemistry, *in situ* hybridization and the multivariate organization of combinatorial alterations algorithm in HNSCC

To assess viral content of specimens, sequencing reads are aligned to a multitude of clinically relevant viral genomes, including all common isoforms of HPV as previously published [14]. Immunohistochemistry was carried out to determine p16 expression using a p16 mouse monoclonal antibody (predilute, mtm-CINtech, E6H4) and high-risk HPV status was determined by *in situ* hybridization (ISH) using a cocktail probe (GenPoint HPV Probe Cocktail, Dako) as previously described [4]. The HPV-specific genomic profile was determined using the multivariate organization of combinatorial alterations (MOCA) algorithm [15]. Again the summary of the methods is provided in supplementary File, available at *Annals of Oncology* online.

ranking of gene-specific alterations

For HPV-positive and HPV-negative samples, we ranked genes by the percent of samples they were altered in, across the three cohorts. If a gene was not characterized in a particular cohort (denoted by *NA*), that cohort was removed from the average for the corresponding gene (e.g. *SOX2* from the Chicago cohort).

estimation of alteration-per-sample counts for aggregate data

The FM cohort ($N = 252$) was consisted of 40 samples from Johns Hopkins University (FM-JHU) with a mutation profile per tumor and 212 samples with only aggregate data (FM-non-JHU). For the aggregate data, we knew the HPV tumor status associated with each alteration, but we were unable to determine which, if any, genes were altered more than once in a single sample. We estimated an alteration-per-sample count for each gene using the TCGA and the FM-JHU cohorts for which sample-specific genomic data were available; this estimation was done separately for HPV-positive and HPV-negative samples. For example there were 170 *TP53* mutations observed in 160 HPV-negative samples in the aggregate data, indicating that

Table 1. Clinical characteristics of head and neck squamous cell carcinoma in the Foundation Medicine cohort

	HPV+ ($N = 84$)	HPV− ($N = 168$)
Mean age (min, max)	56.2 years (30 years, 84 years)	60.7 years (18 years, 85 years)
Gender		
Male	65 (77.4%)	120 (71.4%)
Female	19 (22.6%)	48 (28.6%)
Tissue of origin		
Primary		
Paranasal sinus	6 (7.1%)	7 (4.2%)
Nasal cavity	8 (9.5%)	5 (3.0%)
Mouth	1 (1.2%)	8 (4.8%)
Tongue	10 (11.9%)	34 (20.2%)
Tonsil	10 (11.9%)	2 (1.2%)
Larynx	0 (0%)	7 (4.2%)
Regional		
Lymph node	9 (10.7%)	17 (10.1%)
Distant		
Lung	12 (14.3%)	9 (5.4%)
Bone	2 (2.4%)	6 (3.6%)
Liver	2 (2.4%)	2 (1.2%)
Chest wall	0 (0%)	3 (1.8%)
Skin	1 (1.2%)	5 (3.0%)
Trachea	1 (1.2%)	1 (0.6%)
Adrenal gland	0 (0%)	2 (1.2%)
Pleura	1 (1.2%)	2 (1.2%)
Pleural fluid	2 (2.4%)	0 (0%)
Brain	1 (1.2%)	0 (0%)
Parotid gland	0 (0%)	2 (1.2%)
Salivary gland	0 (0%)	1 (0.6%)
Miscellaneous		
Head and neck	8 (9.5%)	38 (22.6%)
Soft tissue	6 (7.1%)	7 (4.2%)
Ear	0 (0%)	1 (0.6%)
Not provided	4 (4.8%)	9 (5.4%)

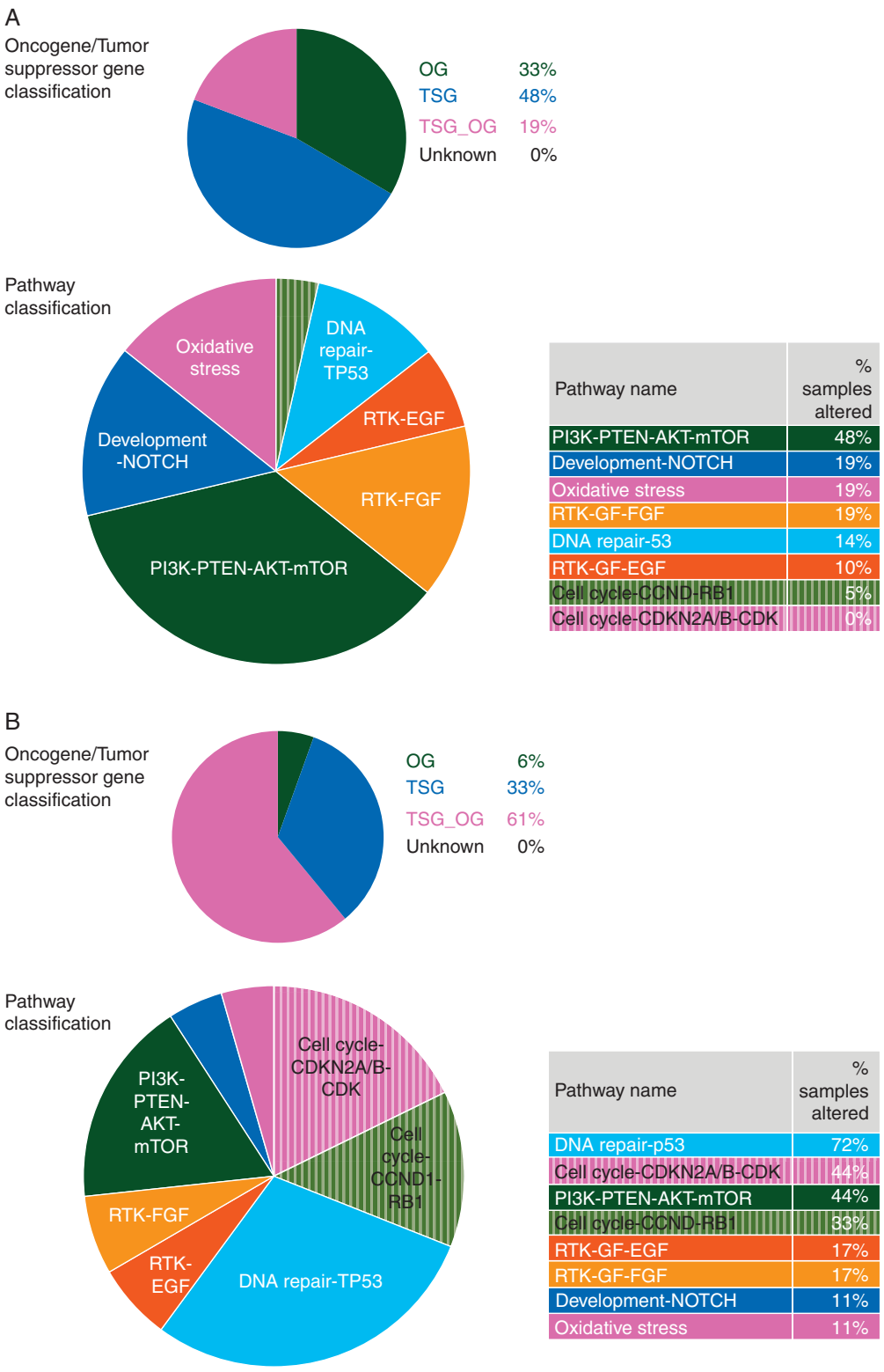

Figure 1. (A) Tumor suppressor gene (TSG) and oncogene (OG) classification, and pathway evaluation of altered genes in human papillomavirus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) in the Foundation Medicine–Johns Hopkins University (FM-JHU) cohort. (B) TSG and oncogene classification, and pathway evaluation of altered genes in HPV-negative HNSCC in the FM-JHU cohort. Only pathways altered in ≥8 of 40 FM-JHU samples are included in the pie charts and tables.

some samples had more than one *TP53* mutation. Because *TP53*-mutated samples had an average of 1.19 *TP53* mutations in the HPV-negative sample-specific data, we estimated that 143 of the 160 aggregate samples had at least one *TP53* mutation (e.g. 170/1.19 = 143).

pathway assignments

We mapped the 236 altered genes from the three studies to the set of curated pathways available from the National Cancer Institute (NCI) Pathway Interaction Database (PID) [16]. NCI PID contains signaling interactions associated with major biomolecular and cellular processes. For genes that could be assigned to multiple pathways, we chose the pathways with the highest fraction of mapped genes from the 236 altered gene set. Additionally, knowledge of the functional classification of the major signaling molecules in the pathway was applied. As an example, the 'ErbB receptor signaling network' is a subpathway in the 'Epidermal growth factor/neuregulin signaling pathways', which are 'Growth factor signaling pathways'. Since this signaling is mediated by receptor tyrosine (*RTK*) signaling kinases, this and other pathways were grouped into *RTK–GF* pathway names.

determination of HPV-specific tumor suppressor and oncogene alteration fractions

Genes altered in the HPV-positive and HPV-negative HNSCC cohorts were categorized as 'tumor suppressor gene' (TSG), 'oncogene' (OG) or 'unknown' when no classification was available [17, 18]. Cohort samples were then uniquely labeled as TSG (a sample has alterations in TSGs only), OG (a sample has alterations in OGs only), TSG + OG (a sample has alterations in both tumor suppressors and OGs), or unknown (a sample has alterations in genes with unknown TSG/OG classification only) among the 236 selected genes. We normalized the categories' sample counts by the total number of altered samples in the cohort.

results

patient characteristics and genomic profiles

Genomic alteration data from 252 FFPE HNSCC tumors were available in the FM cohort. The HPV status of 252 HNSCC patients was determined by NGS; 84 (33%) were HPV-positive and 168 (67%) were HPV-negative. The high-risk HPV ISH and p16 immunohistochemistry were available for 40 tumors in the FM-JHU cohort and showed 100% concordance when compared with HPV tumor status determined by NGS. Twenty-two of 84 (26.2%) HPV-positive tumors and 37 of 168 (22%) HPV-negative tumors were obtained from distant metastatic sites. Clinical characteristics are summarized in Table 1.

Table 2. Top 30 of 236 most commonly altered genes in HPV-positive head and neck squamous cell carcinoma

Gene name	Alteration	HPV+ FM ($N = 84$) (%)	HPV+ Chicago ($N = 51$) (%)	HPV+ TCGA ($N = 36$) (%)	Pathway	Average (%)
PIK3CA	Mut/Ampl	30	35	56	PI3K–PTEN–AKT–mTOR	37
SOX2	Ampl	11	NA	28	Transcription-Sox2	16
MLL2 (KMT2D)	Mut	13	20	17	NA	16
RB1	Mut/Loss	7	24	6	Cell cycle-CCND-RB1	12
BCL6	Ampl	1	18	25	RTK–JAK–STAT	11
EP300	Mut	10	12	14	Cell cycle-CCND-RB1	11
NOTCH1	Mut	6	18	11	Development-NOTCH	11
PTEN	Mut/Loss	15	8	3	PI3K–PTEN–AKT–mTOR	11
FGFR3	Mut	1	24	11	RTK–GF–FGF	10
ASXL1	Mut	5	10	19	NA	9
KLHL6	Ampl	1	NA	25	NA	8
FBXW7	Mut/Loss	12	6	3	Development-NOTCH	8
TP53	Mut	5	16	3	DNA repair	8
ATM	Mut	1	16	8	DNA repair	7
BRCA2	Mut	6	12	3	DNA repair	7
BRIP1 (BACH1)	NA	0	16	8	DNA repair	7
LRP1B	Mut	2	12	8	RTK	7
ATRX	NA	0	18	3	NA	6
KDM6A	Mut/Loss	7	NA	3	NA	6
BRCA1	Mut	2	14	3	DNA repair	6
BLM	NA	0	18	0	DNA repair	5
JAK2	NA	0	14	6	RTK–JAK–STAT	5
NF1	Mut	2	14	0	Ras	5
HRAS	Mut	1	12	3	Ras	5
MYC	Ampl	5	6	3	TGF-β—SMAD	5
ATR	Mut	2	NA	8	DNA repair	4
FGF19	Ampl	4	NA	6	RTK–GF–FGF	4
FGF3	Ampl	4	NA	6	RTK–GF–FGF	4
FGF4	Ampl	4	NA	6	RTK–GF–FGF	4
RICTOR	Ampl	4	NA	6	PI3K–PTEN–AKT–mTOR	4

HPV-positive HNSCC has a distinct HPV-specific genomic profile

We determined the differential genomic alterations in HPV-positive and HPV-negative HNSCC in the FM-JHU cohort with available sample-specific genomic data. First, we evaluated general distribution of the TSGs and OGs [17, 18]. In both HPV-positive and -negative HNSCCs, the mutations/copy number variations were more common in TSGs compared with OGs; however, HPV-positive tumors had a significantly higher frequency of having only OG alterations compared with HPV-negative tumors among the 236 evaluated genes (33% versus 6%, respectively; Figure 1A and B).

The most common alteration by mutation and/or copy number variation in HPV-positive tumors was *PIK3CA* (30%) in the FM dataset, which is consistent with the TCGA and Chicago datasets (35% and 56%, respectively; Table 2). The PI3K pathway was also the most commonly altered pathway seen in HPV-positive tumors including alterations of *PTEN* (15%), *AKT1* (5%), *RICTOR* (4%), *mTOR* (2%), *AKT2* (2%), and *PIK3R1* (2%) in addition to *PIK3CA* (Figure 1A, Table 2, and supplementary Table S2, available at *Annals of Oncology* online). The most common alteration in HPV-negative tumors was *TP53* (87%) in the FM dataset, which is again consistent with TCGA and Chicago cohorts (84% and 80%, respectively; Table 3). The most commonly altered pathways excluding *TP53* are the cell cycle and PI3K pathways in HPV-negative tumors (Figure 1B, Table 3 and supplementary Table S3, available at *Annals of Oncology* online). This suggests that the selected cancer gene analysis using FFPE tumors obtained through routine clinical practice yield comparable assessment of genomic alterations to frozen tumors.

genomic profiles of HPV-positive SCC of oropharynx and nasal cavity/paranasal sinus

We further evaluated a HPV-specific genomic profile using the MOCA algorithm in the TCGA cohort in order to assess the molecular similarities between HPV-positive oropharynx carcinoma and HPV-positive nasal cavity/paranasal sinus carcinoma. The top two most discriminating genes of HPV tumor status were *TP53* and *CDKN2A/B.* The MOCA-derived composite marker combining *TP53* mutation and *CDKN2A/B* loss discriminated HPV tumor status in TCGA cohort with 97% sensitivity and 91% specificity (P value = 4×10^{-11}). We also compared the genomic alterations in oropharynx and nasal cavity/paranasal sinus carcinomas (Figure 2). As seen in the oropharynx, absence of *TP53* mutation and intact *CDKN2A/B* are also

Table 3. Top 30 of 236 most commonly altered genes in HPV-negative head and neck squamous cell carcinoma

Gene name	Alteration	HPV− FM (N = 168) (%)	HPV− Chicago (N = 69) (%)	HPV− TCGA (N = 243) (%)	Pathway	Average (%)
TP53	Mut	87	80	84	DNA repair	84
CDKN2A/B	Mut/Loss	54	32	57	Cell cycle-CDKN2A/B-CDK	53
FGF19	Ampl	23	NA	32	RTK–GF–FGF	28
FGF3	Ampl	22	NA	31	RTK–GF–FGF	27
FGF4	Ampl	22	NA	31	RTK–GF–FGF	27
PIK3CA	Mut/Ampl	16	29	34	PI3K–PTEN–AKT–mTOR	27
CCND1	Ampl	24	13	32	Cell cycle-CCND-RB1	26
NOTCH1	Mut/Loss	16	26	21	Development-NOTCH	20
LRP1B	Mut/Loss	6	30	22	RTK	18
SOX2	Ampl	8	NA	21	Transcription-Sox2	16
MLL2 (KMT2D)	Mut	10	20	18	NA	15
EGFR	Mut/Ampl	13	12	16	RTK–GF–EGF	14
KLHL6	NA	0	NA	21	NA	13
BCL6	Mut	1	12	18	RTK–JAK–STAT	11
ATR	Mut	2	NA	16	DNA repair	10
NFE2L2	Mut	7	1	14	Oxidative stress	10
NOTCH2	Mut	7	17	9	Development-NOTCH	9
MYC	Ampl	5	1	15	TGF-β–SMAD	9
FGFR1	Mut/Ampl	5	10	12	RTK–GF–FGF	9
ATRX	NA	0	30	7	NA	8
JAK2	Ampl	4	17	7	RTK–JAK–STAT	8
SMAD4	Mut/Loss	7	7	8	TGF-β–SMAD	7
RICTOR	Ampl	5	NA	9	PI3K–PTEN–AKT–mTOR	7
ZNF703	Mut/Ampl	4	NA	9	NA	7
BRCA2	Mut	7	16	4	DNA repair	7
FOXL2	NA	0	NA	11	NA	7
PRKDC	NA	0	NA	11	PI3K–PTEN–AKT–mTOR	7
GPR124	NA	0	NA	11	Cell adhesion-integrin	6
KDM6A	Mut	2	NA	9	NA	6
APC	Mut	3	14	6	Ras	6

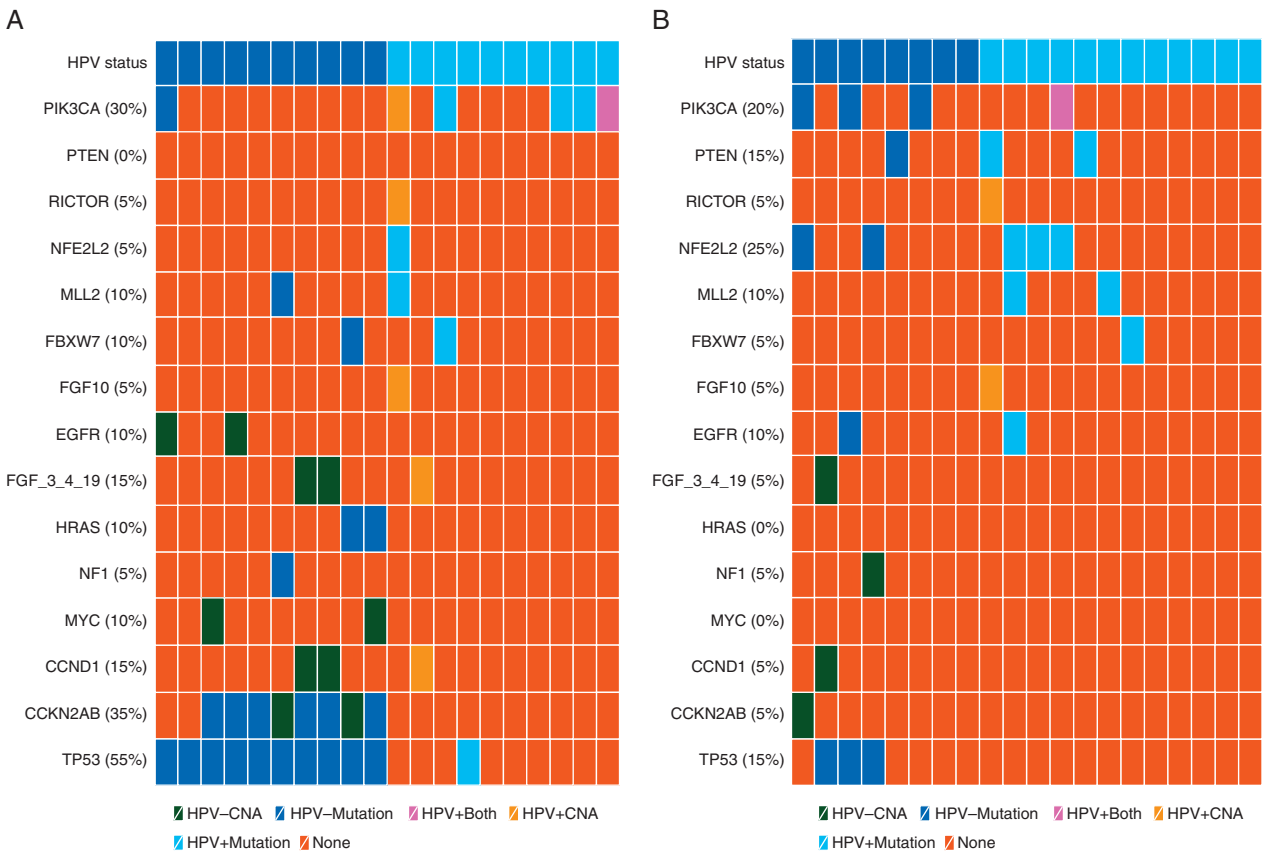

Figure 2. Genomic alteration profiles of (A) head and neck squamous cell carcinoma (HNSCC) including oral cavity, oropharynx, larynx, and hypopharynx primary sites, and (B) HNSCC including nasal cavity and paranasal sinus primary sites.

significantly associated with HPV-positive status in sinonasal carcinomas; however, mutations in *NFE2L2* were more frequent in nasal cavity/paranasal sinus carcinoma than conventional HNSCC sites.

discussion

To realize the goal of personalized medicine, understanding the molecular underpinnings of HNSCC and the use of clinically applicable biomarker assays are key factors. Molecular characterization of HNSCC has been extensively published [8–11]; however, the WES or WGS data were generated in research settings using frozen tumors. While these datasets provide comprehensive evaluation of the molecular landscape, there are downsides to directly implementing the research methods to clinical application including; (i) FFPE tumors have fragmented and damage DNA such that robust DNA extraction and construction of sequencing library could be a challenge, (ii) diagnostic clinical samples are small tissue fragments, fine-needle aspirations or cell blocks which may be sufficient for histological testing, but frequently insufficient for comprehensive WES or WGS due to limited DNA yield, (iii) many clinical samples do not have high tumor cell contents in the tumor specimen; therefore, high sequence coverage across the entire tested regions is not always possible, and (iv) the analysis of WES or WGS data is labor intensive, time consuming, and costly, therefore, limiting the clinical applicability. Application of the targeted gene sequencing from FFPE tumors obtained from a routine clinical setting makes a practical sense and it is a first step toward clinical implementation.

In our study, we obtained aggregate genomic profile data from Foundation Medicine, Inc., for comparison with TCGA and Chicago datasets [10, 11]. Analysis of aggregate data included an estimated count of gene-specific alterations per patient based on the alteration frequency in the non-aggregate, patient-specific data (see Materials and Methods); this approach could be problematic if the distribution of gene-specific alterations varied significantly among the cohorts (e.g. a gene was, on average, altered ≥2 times per sample in the aggregate data). However, despite the heterogeneity of the FM cohort obtained from multiple tumor sites and varying medical practice settings with non-standardized tissue procurement methods, our data reveal remarkable similarities to the TCGA and Chicago cohorts supporting the feasibility of routine clinical testing to determine genomic alterations using FFPE tumors.

Corroborating a similar finding from the TCGA Network [10], our multicohort study revealed that HPV-positive and HPV-negative HNSCC have distinct mutation profiles and most of the discriminating power is in the lack of *TP53* mutations in HPV-positive HNSCCs and in the loss of *CDKN2A/B* in HPV-negative HNSCC. Indeed, loss of p53 function in HPV-positive HNSCCs through expression of the viral oncoprotein, E6, would

negate selective pressure of gaining *TP53* mutations. *CDKN2A* which encodes p16 protein is the most widely used surrogate marker of HPV-positive cancers and it is clearly established that up to 90% of HPV-negative HNSCC tumors lack p16 expression due to mutations, loss of heterozygosity, and/or promoter hypermethylation [10].

We found that *PIK3CA* was the most frequently mutated OG among HPV-positive patients in this multicohort study; a similar finding was recently reported by the TCGA Network [10]. In addition to confirming the PI3K pathway to be the most commonly altered pathway and potentially a very promising therapeutic target in HPV-positive HNSCC, we report the fibroblast growth factor (FGF) pathway may be a relevant therapeutic target in HPV-positive HNSCC. Novel agents targeting the FGF pathway are in active development [19–21]. With emerging data showing that there are phenotypical differences among the *TP53* mutations associating with a high or low risk of poor survival and some mutants displaying oncogenic properties by a 'gain-of-function' [22, 23], relative lack of common TSG mutations in HPV-positive HNSCC may also suggest favorable response to targeted agents such as PI3K or FGFR inhibitors which warrant clinical evaluation. For HPV-negative patients with mutations predominantly in TSGs, innovative trial designs to evaluate synthetic lethality through combination regimens are necessary. Recent advances in understanding of excessive reliance on the G2-M checkpoint in tumors lacking p53 function have led to evaluation of Wee1 and Chk1 inhibitors, which suggest a significant potential for clinical development in HNSCC [24, 25].

In addition to the oropharynx site within HNSCC, the presence of high-risk HPV is also found in nasal cavity/paranasal sinus carcinoma suggesting HPV as an important etiologic agent of carcinomas arising in the sinonasal tract. Unlike in HPV-positive oropharynx SCC, there is very limited molecular and clinical data for nasal cavity/paranasal sinus carcinoma, likely due to its rarity with annual incidence of only 0.5–1.0 patients per 100 000 [26, 27]. In the absence of the sinonasal tract cancer-specific data, clinicians have a tendency to extrapolate the data from the oropharynx SCC. Our genomic data suggest that further studies to evaluate the role of HPV and *NFE2L2* (Nrf2) pathway in nasal cavity/paranasal sinus carcinoma are warranted.

Our study expands the current genomic data available surrounding HNSCC; however, further investigation is required to determine the clinical significance of these genomic alterations before use in routine clinical decision-making. Our data indicate that specimens obtained through routine clinical practice exhibit similar genomic profiles compared with those obtained in an academic setting, and demonstrate the feasibility of comprehensive genomic profiling using a CLIA-certified assay in a clinical setting. This is the first step toward development of future clinical trials using these genomic profiles as integral biomarkers.

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disclosure

GMF, ZRC, RY, PJS, SMA and VAM are employed by and have equity interest in Foundation Medicine, Inc. All remaining authors have declared no conflict of interest.

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Phase I trial of everolimus in combination with thoracic radiotherapy in non-small-cell lung cancer

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Background: This phase I study evaluated the safety and efficacy of the oral mTOR inhibitor everolimus in combination with thoracic radiotherapy followed by consolidation chemotherapy in locally advanced or oligometastatic untreated non-small-cell lung cancer (NSCLC).

Patients and methods: Everolimus dose was escalated in incremental steps [sequential cohorts of three patients until the occurrence of dose-limiting toxicity (DLT)] and administered orally weekly (weekly group: dose of 10, 20 or 50 mg) or daily (daily group: 2.5, 5 or 10 mg), 1 week before, and during radiotherapy until 3.5 weeks after the end of radiotherapy. Two cycles of chemotherapy (cisplatin–navelbine) were administrated 4.5 weeks after the end of radiotherapy.

Results: Twenty-six patients were included in two centers, 56% had adenocarcinoma and 84% had stage III disease. In the weekly group (12 assessable patients), everolimus could be administered safely up to the maximum planned weekly dose of 50 mg; however, one patient experienced a DLT of interstitial pneumonitis at the weekly dose level of 20 mg. In the daily group (9 assessable patients): one DLT of interstitial pneumonitis with a fatal outcome was observed at the daily dose level of 2.5 mg; two other DLTs (one grade 3 esophagitis and one bilateral interstitial pneumonitis) were found at the daily dose level of 5 mg. Overall there were five patients with G3–4 interstitial pneumonitis related to treatment. Among 22 assessable patients for response, there were 9 (41%) partial response and 7 (32%) stable disease. At a median follow-up of 29 months, the 2-year overall survival and progression-free survival actuarial rates were 31% and 12%, respectively.

Conclusion: In previously untreated and unselected NSCLC patients, the recommended phase II dose of everolimus in combination with thoracic radiotherapy is 50 mg/week. Pulmonary toxicity is of concern and should be carefully monitored to establish the potential role of mTOR inhibitor with concomitant radiotherapy.

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Key words: everolimus, thoracic radiotherapy, concomitant combination, non-small-cell lung cancer, phase I

introduction

Chemoradiotherapy is the treatment of choice for locally advanced unresectable non-small-cell lung cancer (NSCLC) but the optimal chemotherapy regimen to use with concurrent thoracic radiotherapy is unknown [1–3] Also, as the prognosis of

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