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Sequencing the head and neck cancer genome: implications for therapy

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Abstract

Head and neck squamous cell carcinoma (HNSCC) is a disease with significant morbidity and mortality. The advancement of next-generation sequencing technologies now enables the landscape of genetic alterations in HNSCCs to be deciphered. In this review, we describe the mutation spectrum discovered in HNSCCs, especially human papilloma virus (HPV)- and/or tobacco smoke exposure-associated HNSCCs. We also describe related research from two independent investigators and from the Cancer Genome Atlas (TCGA). Emphasis is placed on the therapeutic implications of genes frequently altered in HNSCCs (i.e., *TP53*, *PIK3CA*, and *NOTCH1*) and their corresponding pathways, with a particular focus on recent findings of *NOTCH* pathway activation in HNSCC. We also discuss the application of integrated genomic pathway-based analysis for precision cancer therapy in HNSCC.

Keywords

head and neck cancer; next-generation sequencing; mutation; *NOTCH1*

Introduction

Arising in the oral cavity, oropharynx, larynx, or hypopharynx, head and neck squamous cell carcinoma (HNSCC) is a disease with significant morbidity and mortality.¹ More than 50,000 new cases of HNSCC are diagnosed in the United States yearly, with a mortality rate of 12,000 annually.² This malignancy is highly related to habitual factors, such as smoking, alcohol consumption, and infection with human papilloma virus (HPV), which has been associated with the majority of oropharynx cancers.^{3, 4} The survival rate of HNSCC is only 50% with 5 years after diagnosis, with locoregional relapse being the primary cause of death. Despite significant progress in therapeutic interventions, including surgery,

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radiotherapy, and chemotherapy, there have been only modest improvements in the survival of patients with HNSCC in the past 30 years. Therefore a deeper understanding of the molecular biology of HNSCC is needed to enhance the development of therapeutic approaches, which will improve upon current strategies to combat HNSCC cancers. This review will highlight the importance advances in the next-generation sequencing of the HNSCC genome and emphasize ways in which these advances are likely to affect the development of future therapies of HNSCC cancer.

Recent advances in sequencing the head and neck cancer genome

To gain a comprehensive view of the genetic alterations underlying head neck cancer, our research group and investigators from the University of Pittsburgh and the Broad Institute first analyzed the HNSCC genome using high-throughput next-generation sequencing techniques. Each investigative group sequenced the exons of all known human genes in tumor DNA and compared the sequence to that of the corresponding normal DNA from the identical patients.

We sequenced approximately 18,000 protein-coding genes in 32 primary HNSCC tumors.⁵ Of note, the neoplastic cellularity of the tumors was more than 60%, and among these 32 patients 30 had not been treated with chemotherapy or radiation before their tumor biopsy. DNA was sequenced using an Illumina GAIIX/HiSeq or SOLiD instrument; the average coverage of each base in the targeted regions were 77- and 44-fold for the Illumina and SOLiD instruments, and 92.6% and 90% of targeted bases were represented by at least 10 reads in these platforms. We identified 911 candidate somatic mutations in 725 genes among the 32 tumors; 609 out of 911 candidate somatic mutations were further confirmed by Sanger sequencing or by 454 sequencing. The range of confirmed mutations per tumor was 2 to 78, with a mean and SD of 19 ± 16.5 mutations per tumor. We validated the top 6 genes in additional 88 HNSCC tumors, including *TP53*, *NOTCH1*, *CDKN2A*, *PIK3CA*, *FBXW7*, and *HRAS*, with *TP53* and *NOTCH1* being the most frequently mutated genes found in the combined discovery and prevalence set. Of note, *NOTCH1*, with alterations present in 15% of patients in the combined discovery and prevalence set, has not been reported to be mutated at a significant frequency in other solid tumor types. Moreover, we reported that tumors from patients with a history of tobacco use had more mutations than did tumors from patients who did not use tobacco (21.6 ± 17.8 versus 9.5 ± 6.5 , $P < 0.05$, Welch two sample *t* test), whereas tumors that were negative for human papillomavirus (HPV) had more mutations than did HPV-positive tumors (20.6 ± 16.7 versus 4.8 ± 3 , $P < 0.05$, Welch two sample *t* test).

Paralleled with our study, Stransky *et al.* analyzed whole-exome sequencing data from 74 tumor-normal pairs.⁶ In their study the DNAs were sequenced on Illumina GII or HiSeq platforms, achieving 150-fold mean sequence coverage of targeted exonic regions, with 87% of loci covered at >20-fold, and, on average, identifying 130 coding mutations per tumor, 25% of which were synonymous. They queried 321 of these mutations by mass spectrometric genotyping and validated 288 (89.7%). Using the MutSig algorithm to identify genes harboring more mutations than expected by chance, they identified 39 significant mutations, whereas the majority of mutated genes did not reach statistical significance,

which may indicate that many were passenger events. In the 39 most significantly mutated genes were some that had previously been implicated in HNSCC, e.g., *TP53*, *CDKN2A*, *HRAS*, *PTEN*, and *PIK3CA*. In concordance with our study, particularly noteworthy in the findings of Stransky *et al.* is that point mutations affecting *NOTCH1* occurred in 11% of the HNSCC tumors. In addition to *NOTCH1*, they found non-synonymous point mutations in *NOTCH2* or *NOTCH3* in 11% of the samples. They also reported that the mutation rate of HPV-positive tumors was approximately half that of HPV-negative tumors (mean = 2.28 mutations per megabase compared with 4.83 mutations per megabase); among patients who reported a smoking history, tumors with the highest fraction of G → T transversion showed a tendency toward increased overall mutation rates, which are indicative of smoking-induced mutations, and may represent a robust readout of functional tobacco exposure.

More recently, investigators at the Cancer Genome Atlas (TCGA) research network completed a large-scale analysis of the genetic makeup of HNSCC and uncovered numerous genomic aberrations involved in HNSCC-related tumors.⁷ The study examined tumor and healthy tissue from 279 patients with HNSCC, 80% of tumors were associated with tobacco use and 13% were HPV-positive. All 279 tumor samples showed 15 significantly mutated genes that included *CDKN2A*, *TP53*, *PIK3CA*, *NOTCH1*, *HRAS*, and *NEE2L2*. Among these, *PIK3CA* was mutated in approximately 21% of all samples. Of note, the study revealed 40% to 50% of HPV positive samples had alterations in *PIK3CA* that were linked with very low rates of *EGFR* alterations. Additionally, roughly 5% of samples exhibited *HRAS* mutations and *NOTCH1* was mutated in 18.6 percent.

Table 1 summarizes the representative genes found mutated in studies among investigators from our group, the University of Pittsburgh and the Broad Institute, as well as the TCGA consortium.

Given the extensive heterogeneity and complexity of HNSCC, several research groups performed additional studies in order to better understand the comprehensive genetic alterations in HNSCC. Pickering *et al.* reported analysis of whole exome-sequencing of a panel of 38 oral squamous cell carcinoma (SCC) tumors.⁸ They consistently found mutations of *TP53*, *NOTCH1*, *CDKN2A*, *PIK3CA*, *HRAS*, *FAT1*, and *CASP8*.

To understand the overall HNSCC mutation context, we also performed high depth targeted exon sequencing of 51 highly actionable cancer-related genes with a high frequency of mutation across many types, including head and neck.⁹ These genes were selected from the catalogue of somatic mutations in cancer (COSMIC)¹ based upon the spectrum of mutations in individual cancer types with either an established or potential role in HNSCC development.¹⁰ We detected mutations in the previously reported genes, such as *TP53*, *NOTCH1*, *PIK3CA*, *CDKN2A*, and *FGFR3*, and in two genes, *CEBPA* and *FES*, which have not been previously reported in HNSCC. We also found that HNSCC patients with tobacco smoking had 3.2-fold more mutations than HNSCC patients without tobacco smoking, whereas HPV-negative patients had 4-fold more *TP53* mutations than HPV-positive patients.

¹<http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/>

The relation between tobacco smoke exposure and somatic mutation has been an important topic in the area of HNSCC research. Pickering *et al.* recently compared the genomic alterations in SCC of the tongue between young (<45 years, non-smoker) and older (>45 years) patients using whole-exome sequencing.¹¹ They found that the gene-specific mutation frequencies (e.g., in *FAT1*, *TP53*, and *PIK3CA*) were similar between young and old patients in two independent cohorts (both by Pickering *et al.* and in TCGA cohort). Moreover, the type of base changes observed in the young cohort was similar to those in the old cohort, even though they differed in smoking history. Pickering *et al.* concluded that smoking has only a minor effect on the types of mutations observed in SCC of the tongue.

In contrast, in a clinical and genomic and metagenomic characterization of oral tongue SCC in patients, we found that non-smokers were younger than smokers, were more likely female, and that non-smokers had fewer *TP53* mutations than smokers.¹² We reported that the mutational spectra in non-smokers and smokers with oral tongue SCC were significantly different. Compared to cancers from non-smokers, a greater proportion of mutations in cancers from smokers were either A:T → G:C or A:T → T:A substitutions. Conversely, compared to cancers from smokers, a greater proportion of mutations in cancers from non-smokers were C:G → G:C transversions. In addition, we failed to find the presence of potential causative viral pathogens in patients with oral tongue SCC who do not smoke.

Recent studies have provided evidence that high-risk human HPV-related HNSCC incidence is rapidly increasing.¹³ To understand the genetic alterations in HPV-positive versus HPV-negative HNSCC, Lechner and colleagues performed targeted next-generation sequencing on 182 genes often mutated in oropharyngeal HNSCC tumors.¹⁴ They found that HPV-positive and -negative oropharyngeal carcinomas cluster into two distinct subgroups, with few overlapping genetic alterations. *TP53* mutations were detected in all the HPV-negative samples, whereas *PIK3CA* mutations or amplification and *PTEN* inactivation by gene copy number loss or mutations were seen more frequently in HPV-positive oropharyngeal tumors. In concordance, Braakhuis and colleagues showed that *TP53* mutations are common in oral SCC of young adult patients in which infection with biologically active HPV is rare.¹⁵ With regard to *PIK3CA* mutations, Nocols *et al.* reported a high frequency of activating *PIK3CA* mutations in HPV-positive oropharyngeal cancer.¹⁶ Sewell *et al.* also found that *PIK3CA* mutations were more frequent in HPV-positive oropharyngeal SCCs.¹⁷

Therapeutic implications from sequencing the head and neck cancer genome

As noted above, the application of deep sequencing approaches provides an unprecedented knowledge of the multiplicity and diversity of somatic and/or genetic mutations underlying HNSCC genomes. This knowledge is now contributing to the elucidation of aberrant signaling pathways driving tumor progression and, potentially, to novel opportunities for therapeutic intervention to prevent and treat HNSCC tumors. As revealed by massive parallel sequencing, the genetic alterations in HNSCC are mainly in a handful of molecular pathways and/or biological processes including *TP53* pathways (*TP53*); mitogenic pathways (*RAS/PI3K/mTOR* pathway, *PIK3CA*, *HRAS*); cell cycle (*CDKN2A*); *NOTCH* pathways (*NOTCH1*, *NOTCH2*, *NOTCH3*); and cell communication and death (*FAT1*, *CASP8*).^{18,19}

Below, we will draw attention to therapeutic implications related to the mutations in *TP53*, *PIK3CA*, and *NOTCH1*, and their corresponding pathways.

TP53

TP53 is the most commonly mutated gene, with loss-of-function mutations frequently detected in HNSCC and in many other tumors.^{20,21} More than half of all HNSCCs have mutations in *TP53*, and nearly all of these cancers show defects in the p53 pathway. Alterations have been found in virtually every region of the p53 protein. P53 is a tumor-suppressor protein that has been referred to as the “guardian of the genome” because of its ability to induce senescence, cell-cycle arrest, or apoptosis when cells are exposed to various forms of stress, including DNA damage. The transcriptional activity of p53 leads to the activation of downstream target genes, e.g., *CDKN1A*, *PCNA*, *GADD45*, *BAX*, *NOXA*, *MDM2*, and *miR-34a*.²²

We have provided evidence that the presence and type of *TP53* mutation is of prognostic relevance. In a prospective, multicenter trial with 560 patients registered, we demonstrated that *TP53* mutations generally, and disruptive mutations of *TP53* (resulting in amino acid substitution predicted to disrupt DNA binding) particularly, are significantly associated with short survival in HNSCC.²³ Further, Skinner found that disruptive *TP53* was associated with increased loco-regional recurrence.²⁴ Studies from TCGA also reported the correlation of *TP53* mutations with generally unfavorable indicators, for example in tumor stage and elapsed time to death in HNSCC.²¹ In terms of therapy, Cabelguenne *et al.* reported that *TP53* mutation increased the risk of not responding to cisplatin and 5-FU by 2.7 times, implying that p53 status may be a useful indicator of response to neo-adjuvant chemotherapy in HNSCC.²⁵ More recently, Perrone *et al.* reported a significant association between nonfunctional *TP53* mutations and a low probability of pathological complete response in patients with oral cavity SCC and receiving nonadjuvant cisplatin and fluorouracil chemotherapy in a prospective randomized trial.²⁶ They found that few oral cavity tumors lacking functional p53 responded fully to cisplatin/fluorouracil chemotherapy, while 40% of patients whose tumor contained functional p53 has a complete response by histopathology, suggesting functional p53 status as a biomarker for chemotherapy response in oral cavity cancer.²⁷ The reasons why p53 mutation may lead to refractoriness to chemotherapy remain to be investigated. As proposed by Perrone and colleagues,²⁶ the observed relationship between nonfunctional *TP53* mutations and a low response rate is in line with the current paradigm suggesting that DNA-damaging agents operate mainly by triggering cells to undergo apoptosis, which is impaired by an inefficient p53-dependent apoptotic pathway in oral SCCs. As a consequence, drugs that function by means of p53-independent apoptosis, such as spindle-damaging agents (taxanes), may be effective in oral SCCs carrying nonfunctional *TP53* mutations. Another possibility may be related to increased MAP4 expression, which occurs when p53 is mutated and transcriptionally inactive and enhance microtubule polymerization, taxane binding, and drug sensibility.

Perturbations in p53 signaling pathways are believed to be required for the development of most cancers. There is evidence to suggest that restoration or reactivation of p53 function will have significant therapeutic benefit. Studies from Roh *et al.* showed that the p53-

reactivating small molecule RITA (reaction of the p53 and induction of tumor cell apoptosis) induced prominent accumulation and reactivation of p53 in a *TP53* wild-type HNSCC cell line and promoted apoptosis in association with up-regulation of p21, BAX, and cleaved caspase-3.²⁸ They also showed that in a combined therapy, RITA enhanced cisplatin-induced growth inhibition and apoptosis of HNSCC cells in vitro and in vivo.

In a recent phase II trial of surgery with perioperative INGN 201 (Ad5CMV-p53) gene therapy followed by chemoradiation therapy for advanced, resectable SCC of the oral cavity, oropharynx, hypopharynx, and larynx, Yoo *et al.* demonstrated the feasibility of handling and delivery a very complex gene vector safely in multiple cooperative group institutions without significant incident. Intraoperative INGN 201 gene therapy is technically feasible, but it has many logistical problems, including regulatory requirements when performed in a multi-institutional setting.²⁹ Of interest, in a phase I trial of ALT-801, an interleukin-2/T cell receptor fusion protein (aa264-272 / HLA-A* complex) targeting p53 in patients with advanced malignancies, Fishman *et al.* demonstrated that the ALT-801 regimen could be administered safely and is associated with immunologic changes of potential antitumor relevance.³⁰ In addition, there a clinical trial is in progress focusing on gene therapy in preventing cancer in patients with premalignant carcinoma of the oral cavity or pharynx (NCT00064103) by wild-type p53 gene induction via an adenoviral vector (INGN 201).

***PIK3CA* and *PI3K* pathway**

PIK3CA, a critical gene in the PI3K signaling pathway, is one of the most commonly mutated oncogenes in HNSCC and other solid tumors. The PI3K signaling axis directly influences cancer cell growth, survival, motility, and metabolism.³¹ In HNSCCs, *PIK3CA* is mutated at the frequencies of 6-21% and ~90% of *PIK3CA* mutations found in the helical/kinase domains. In addition to *PIK3CA* mutations, *PIK3CA* copy number increases were found in ~9% of HNSCC tumors.⁵⁸ In an effort to evaluate the mutational events of genes comprising the three major mitogenic pathways that have been previously implicated in HNSCC pathophysiology, namely MAPK, JAK/STAT, and PI3K pathway, Lui *et al.* performed studies indicating the PI3K pathway to be the most frequently mutated oncogenic pathway (30.5%, 46/151), whereas only 9.3% and 8.0% of HNSCC tumors contained mutations in the JAK/STAT or the MAPK pathways, respectively.³² In addition to *PIK3CA* mutation, they found mutations in *PIK3AP1*, *PIK3C2A*, *PIK3C2B*, *PIK3CB*, *PIK3CD*, *PIK3CG*, *PIK3IP1*, *PIK3R1*, *PIK3R2*, *PIK3R3*, *PIK3R4*, *PIK3R5*, *PIK3R6*, *AKT1*, *AKT2*, *AKT3*, *mTOR*, *PTEN*, *PDK1*, *TSC1/2*, and *RICTOR*, all in the PI3K pathway. Lui *et al.* reported that *PI3K* pathway-mutated HNSCC tumors showed an increased rate of cancer gene mutations; and, of interest, all tumors with concurrent mutations of multiple *PI3K* pathway genes were advanced (Stage IV). With respect to therapeutic implications, expression of the *PIK3CA* mutants was found to confer growth advantage compared with overexpression of wild-type *PIK3CA*. Furthermore, patient-derived tumor grafts with *PIK3CA* mutations were exquisitely sensitive to an *mTOR/PI3K* inhibitor (bez-235), in contrast to *PIK3CA*-wildtype tumor grafts, suggesting that activating mutations of the *PI3K* pathway have the potential to serve as biomarkers for treatment selection in HNSCC. In support of this, xenografts developed from an HNSCC cell line with a *PIK3CA* mutation (H1047R) were more sensitive to the combination of bez-235 plus cetuximab compared with

cetuximab alone. Moreover, pharmacologic profiling of antitumor agents in HNSCC cell lines suggested that *PIK3CA* mutation may serve as a predictive biomarker for the drugs targeting the *EGFR/PI3K* pathway.³³ In Sewall's study investigating the significance of *PIK3CA* mutations in HPV-associated oropharyngeal SCCs, the authors reported that mutant *PIK3CA* tumors had a distinct protein expression profile within HPV-positive oropharyngeal SCCs, and *PIK3CA* mutations in HPV-positive cells were associated with activation of the mTOR, but not AKT, signaling pathway; and HPV-positive cells expressing mutant *PIK3CA* were more sensitive to *PIK3CA*/mTOR inhibition versus AKT inhibition, implying that inhibitors for mTOR may have activity against HPV-positive *PIK3CA* mutant oropharyngeal cancers.¹⁷

With regard to the clinical trials with *PIK3CA* and mTOR inhibitors, in a phase I study to evaluate the addition of oral everolimus (mTOR inhibitor) to cisplatin and docetaxel as induction chemotherapy for patients with locally and/or regionally advanced head and neck cancer, Fury *et al.* defined an induction chemotherapy regimen in which patients receive everolimus (7.5 mg daily) plus docetaxel and cisplatin (both 75 mg/m² on day 1 of a 21-day cycle) with pegfilgrastim support; as a result, a larger phase II study of this induction chemotherapy regimen followed by definitive locoregional therapy to assess efficacy and explore candidate biomarkers is warranted.³⁴ In a phase II study of temsirolimus (mTOR inhibitor) and erlotinib (EGFR inhibitor) in patients with recurrent and/or metastatic platinum-refractory HNSCC, Bauman *et al.*³⁵ reported that the combination of erlotinib and temsirolimus was poorly tolerated, and low prevalence of PTEN expression and 8% incidence of *PIK3CA* mutations indicate biological relevance of this pathway in recurrent and/or metastatic HNSCC. A phase II trial (NCT01256385) evaluating temsirolimus with or without cetuximab is successfully enrolling without prohibitive toxicity.³⁵ A variety trials (NCT01602315) of mTOR inhibitors in HNSCC are ongoing, among them a phase Ib/II study of BYL719 (PI3K inhibitor) and cetuximab in recurrent or metastatic HNSCC.

NOTCH1 and the NOTCH pathway

The *NOTCH* signaling pathway has been linked to multiple biologic functions, including regulation of self-renewal capacity, cell-cycle exit, and survival.^{36,37} The pathway is initiated when one cell expressing the appropriate ligand (Jagged or Delta) interacts with another cell expressing a NOTCH receptor (NOTCH1–4). Upon ligand binding, the transmembrane NOTCH receptor is subsequently cleaved by ADAM metalloprotease and γ -secretase complex. The cleaved product, intracellular fragment of NOTCH (NICD), translocates into the nucleus where it interacts with nuclear coactivators (MAML proteins) to turn on transcription factors of target genes. The most prominent targets of the NOTCH pathway include a set of basic-helix-loop factors of the Hes and Hey families.

NOTCH1 mutations have found in 10–15% of the HNSCC tumors, making *NOTCH1* one of the most frequently mutated genes in this tumor type.^{5,6} Among the 28 *NOTCH1* mutations identified in our sequencing study,⁵ ~25% of nonsense mutations and ~15% of indel mutations were detected in HNSCC; inactivation of both alleles probably occurred in at least 9 of the 21 patients with *NOTCH1* mutations. The inactivating/truncating mutations tended to alter the protein N-terminal to the transmembrane domain.⁵ This suggests that *NOTCH1*

is a tumor suppressor in HNSCC. Several studies support this notion: (1) in cutaneous and lung SCC, loss-of-function *NOTCH1* mutations lie within the terminal region;³⁸ (2) ablation of *NOTCH1* results in epidermal and corneal hyperplasia followed by the development of skin tumors and facilitates chemical-induced skin carcinogenesis;³⁹ and (3) impaired Notch signaling promotes de novo cutaneous squamous cell carcinoma in mice.⁴⁰ In addition, in oral squamous cell carcinoma cell lines that have missense or truncating *NOTCH1* mutations, expression of the cleaved/activated form of *NOTCH1* or full-length wild-type *NOTCH1* inhibits cell proliferation both in vitro and in vivo.⁸

However, studies also suggest that *NOTCH* mutation exerts an oncogenic effect. In T cell acute lymphoblastic leukemia/lymphoma, *NOTCH* signaling has previously been implicated as pro-tumorigenic owing to activating mutations and translocations in the genes for *NOTCH* receptors or their regulators.^{41,42}

Given that *NOTCH1* plays the role of oncogene or tumor suppressor gene in different types of tumors and that not every mutation is inactivating, a high priority in our study was placed on a more comprehensive understanding of the complex molecular alterations of *NOTCH* signaling pathway in HNSCC. We explored the comprehensive alterations of *NOTCH* signaling (including 38 of the 44 *NOTCH* pathway members defined by KEGG, whose data were available) in a cohort of 44 HNSCC tumors and 25 normal mucosal samples through a set of expression, copy number, methylation, and mutation analyses.^{9,43} Using an outlier statistical approach, we observed the copy number levels of ligands *JAG1* and *JAG2* and receptor *NOTCH3* were significantly increased in HNSCC tumors, and that none of the *NOTCH* pathway gene promoters are significantly hypomethylated in HNSCC tumors compared with normal mucosa. Our gene set analysis defined differential expression of the *NOTCH* signaling pathway in HNSCC relative to normal tissues.

To assess overall *NOTCH* pathway activation, we analyzed downstream effectors *HES1* and *HEY1* and found that ~32% of HNSCC tumors showed expression of *HES1* and/or *HEY1* consistent with NOTCH activation. Notably, we performed exomic sequencing on 37 HNSCC tumors from the cohort and a total of 5 novel inactivating *NOTCH1* mutations were identified in 4 of the 37 tumors analyzed, none of the mutation was associated with *HES1* or *HEY1* activation. In the meantime, we characterized a subset of wild-type *NOTCH1* HNSCC tumors with increased *HES1/HEY1* expression, a subset validated in TCGA HNSCC cohort. Our data demonstrate a bimodal pattern of NOTCH pathway alterations in HNSCC, with a smaller subset exhibiting inactivating *NOTCH1*-specific mutations, and a larger subset exhibiting other *NOTCH* pathway alterations, including increased expression or gene copy number of either the receptor or ligands, as well as downstream pathway activation. As an additional support to this, our initial functional results showed that in the *NOTCH1* wild-type HNSCC cells, siRNA inhibition of *NOTCH1* or *HEY1* significantly decreased cell growth, and application of GSI-XXI, a *NOTCH* pathway inhibitor, to HNSCC cells also inhibited cell growth.⁴³

Consistent with our study, Pickering and colleagues have discovered changes in gene copy number and expression for other *NOTCH* pathway genes, including increased copy number changes in *NOTCH* receptor ligands *JAG1* and *JAG2* and in *MUMB* that were associated

with elevated mRNA levels.⁸ More recently, Song *et al.* analyzed the *NOTCH1* mutations in Chinese oral squamous cell carcinoma (OSCC); they detected 42 somatic *NOTCH1* mutations, including 7 nonsense mutations and 11 mutations with the heterodimerization domain commonly harboring potential activating mutations in acute lymphoblastic leukemia in 22 of the 51 Chinese OSCC tumors. Patients whose tumors have *NOTCH1* mutation had significantly shorter overall and disease-free survival compared with those patients whose tumors had no *NOTCH1* mutations.⁴⁴

To date, the available potential therapeutic interventions in *NOTCH* signaling pathway include MK-0752 and RO4929097, two potent inhibitors of γ -secretase, an enzyme required for *NOTCH* pathway activation. In a phase I pharmacologic and pharmacodynamic study of MK-0752 in adult patients with advanced solid tumors, MK-0752 demonstrated good tolerability and evidence of *NOTCH* pathway inhibition using a once-per-week dosing schedule. The clinical activity of MK-0752 was demonstrated, predominantly in patients with gliomas.⁴⁵ In phase I study of RO4929097 in patients with refractory metastatic or locally advanced solid tumors, RO4929097 was well tolerated when administered by using both intermittent and continuous dosing schedules.⁴⁶ Currently clinical trials with RO4929097 and MK-0752 are ongoing in a variety of tumor types, including breast cancer, sarcoma, melanoma, pancreatic cancer, non-small cell lung cancer, prostate cancer, and T cell acute lymphoblastic leukemia/lymphoma and results are awaited with interest. Yet, a clinical trial targeting HNSCC tumors was not available yet. Based on our study and as an additional point, in principle there are *NOTCH* inhibiting antibodies and or small molecules available that blocks *NOTCH* signaling pathway, however this would be targeted to patients with ligand or receptor amplification or overexpression, would not likely work on downstream alterations.

Conclusions

The mutation spectrum revealed by high throughput sequencing suggests that the genetic alterations in HNSCC are diverse and complex. The HNSCC tumors associated with HPV infection and/or tobacco smoke exposure exhibit distinctive genetic landscapes. It is essential to identify and characterize the molecular events underlying the pathogenesis of HPV-associated HNSCC, especially those with high translational relevance. In a recent characterization of HPV and host genome interaction in primary head and neck cancers, Parfenov *et al.* found that HPV integration affects the host genome by amplification of oncogenes and disruption of tumor suppressors, as well as by driving inter- and intra-chromosomal rearrangements.⁴⁷ Comparing cancers with integrated versus nonintegrated HPV shows different patterns of DNA methylation and both host and viral gene expression. As an additional point, understanding how the mutational landscape of HNSCC is similar and different from that of other tumors, likely to be greatly facilitated by release of mutation data across over 30 tumor types collected by TCGA, will no doubt be informative.

In HNSCC, inactivating mutations in tumor suppressor genes were identified in a majority of tumors, whereas the activating mutations in oncogenes were mutated at a relatively low rate. It is difficult to target tumor suppressor genes and/or their associated pathways to either restore or reactivate their functions. While an oncogenic target may be targeted with specific

inhibitors, there is a relative lack of readily targeted oncogenes in HNSCC, likely because of their low mutation frequency.

As mutations generated from genomic sequencing represent only one aspect of HNSCC tumorigenesis, a comprehensive molecular characterization of HNSCC, with integration of genetic, epigenetic, transcriptional data, will yield more precise insight into HNSCC tumor biology. We propose that such integrated pathway-based analysis will delineate alternative opportunities for therapeutic treatment toward the goal of targeted precision therapy. As an example, a recent analysis of the five tiers of TCGA data from 378 HNSCC tumors, including somatic mutations, chromosomal aberrations, mRNA expression, microRNA expression and clinical variables, Gross and colleagues reported that *TP53* mutation is frequently accompanied by loss of chromosome 3p, and that the combination of these events is associated with a surprising decrease in survival time (1.9 years versus >5 years for *TP53* alone).⁴⁸ They showed that in HPV-positive tumors, in which HPV inactivates *TP53*, 3p deletion is also common and associated with poor outcomes. The *TP53*–3p event is further influenced by mir-548k expression, which decreases survival further and is mutually exclusive with mutations affecting *RAS* signaling. These results may contribute to molecular stratification alongside clinical variables that is important for patient-tailored treatment programs.

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Table 1

Genes frequently mutated in head and neck cancer

Symbol	Gene description	Chromosome location	Mutation type	Mutation rate, % in Refs 5, 6, 7, respectively
<i>TP53</i>	Tumor protein P53	17p13.1	Missense; nonsense; FS del; FS ins; splice	47, 62, 73
<i>NOTCH1</i>	Notch 1	9q34.3	Misense; nonsense; FS del; splice	15, 14, 19
<i>CDKN2A</i>	Cyclin-dependent kinase inhibitor 2A	9p21	Missense; nonsense; FS del; splice	9, 12, 23
<i>PIK3CA</i>	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit	3q26.1	Missense	6, 8, 21
<i>FBXW7</i>	F-Box and WD repeat domain containing 7, E3 ubiquitin protein ligase	4q31.3	Missense; nonsense	5, 5, 5
<i>HRAS</i>	Harvey rat sarcoma viral oncogene homolog	11p15.5	Missense	4, 5, 4
<i>PTEN</i>	Phosphatase and tensin homolog	10q23.3	Missense; nonsense	NA, 7, 2
<i>TP63</i>	Tumor protein P63	3q28	Missense; nonsense	3, 7, 2
<i>FAT1</i>	FAT atypical cadherin 1	4q35	Missense; nonsense; FS del;	NA, 12, 23
<i>CASP8</i>	Caspase 8, apoptosis-related cysteine peptidase	2q33-34	Missense; nonsense; splice	3, 8, 9
<i>SYNE1</i>	Spectrin repeat containing, nuclear envelope 1	6q25	Missense; nonsense; Indel	13, 20, 18
<i>NOTCH2</i>	Notch 2	1p13-p11	Missense; nonsense	NA, 5, 4
<i>NOTCH3</i>	Notch 3	19p13.2-p13.1	Missense; nonsense	NA, 4, 4
<i>TGFBR2</i>	Transforming growth factor, beta receptor II	3p22	Missense	NA, 3, 4