Frequent Mutation of the PI3K Pathway in Head and Neck Cancer Defines Predictive Biomarkers


ABSTRACT

Genomic findings underscore the heterogeneity of head and neck squamous cell carcinoma (HNSCC). Identification of mutations that predict therapeutic response would be a major advance. We determined the mutationally altered, targetable mitogenic pathways in a large HNSCC cohort. Analysis of whole-exome sequencing data from 151 tumors revealed the phosphoinositide 3-kinase (PI3K) pathway to be the most frequently mutated oncogenic pathway (30.5%). PI3K pathway–mutated HNSCC tumors harbored a significantly higher rate of mutations in known cancer genes. In a subset of human papillomavirus-positive tumors, PIK3CA or PIK3R1 was the only mutated cancer gene. Strikingly, all tumors with concurrent mutation of multiple PI3K pathway genes were advanced (stage IV), implicating concerted PI3K pathway aberrations in HNSCC progression. Patient-derived tumorgrafts with canonical and noncanonical PIK3CA mutations were sensitive to an mTOR/PI3K inhibitor (BEZ-235), in contrast to PIK3CA–wild-type tumorgrafts. These results suggest that PI3K pathway mutations may serve as predictive biomarkers for treatment selection.

SIGNIFICANCE: Treatment options for HNSCC are limited, in part, because of an incomplete understanding of the targetable mutations that “drive” tumor growth. Here, we define a subgroup of HNSCC harboring activating mutations of genes in the PI3K pathway where targeting the pathway shows anti-tumor efficacy. These results suggest that PI3K pathway mutation assessment may be used to guide HNSCC therapy.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is a frequently lethal cancer with few effective therapeutic options. Recent genomic findings in head and neck cancer revealed a wide spectrum of unexpected genetic aberrations (1, 2). This genomic heterogeneity of HNSCC tumors underscores an obstacle to the identification of effective molecular targeting agents likely to benefit the majority of patients with HNSCC. To date, there is a translational gap between genomics and...
treatment selection for patients with HNSCC. TP53 mutation is the single most common mutational event. Yet the loss-of-function of this tumor suppressor gene has remained challenging to exploit therapeutically. Mitogenic pathways are crucial for cancer development and progression. In other malignancies, mutations of growth pathway genes have been shown to result in pathway activation, enhanced tumor growth, and increased sensitivity to agents targeting the mutated pathway. However, the potential of genomics-based therapy selection has not been widely investigated in HNSCC.

We and others recently reported genomic mutational profiles of more than 100 HNSSC tumors (1, 2). Here, we analyzed an additional 45 HNSSC tumors by whole-exome sequencing using the Illumina platform. In an effort to identify mutationally altered, targetable mitogenic pathways in HNSSC, we combined all currently available mutational data (from whole-exome sequencing) of 151 HNSSC primary tumors and evaluated the mutational events of genes in three major mitogenic pathways that have been previously implicated in HNSSC pathophysiology, namely the mitogen-activated protein kinase (MAPK; ref. 3), Janus-activated kinase (JAK)/signal transducer and activator of transcription (STAT) (4), and the phosphoinositide 3-kinase (PI3K) pathways (3). These key mitogenic pathways are targetable in human cancers with a variety of agents currently in various stages of clinical development.

RESULTS

Nearly One Third of HNSSC Tumors Harbor PI3K Pathway Mutations

To date, whole-exome sequencing data of 106 HNSSC tumors are available. Here, we reported the whole-exome sequencing data of an additional 45 HNSSC tumors collected at the University of Pittsburgh (Pittsburgh, PA; Supplementary Table S1). Our results, similar to previous reports, showed a high degree of intertumor mutation heterogeneity, further confirming the complexity of HNSSC biology and the associated challenges in defining subsets of patients likely to respond to specific targeted therapies. With the aim of identifying mutationally altered, targetable mitogenic pathways in HNSSC, we assessed the mutational events of genes comprising three major mitogenic and targetable pathways in HNSSC: the JAK/STAT, MAPK, and PI3K pathways. Pathway component genes were defined as follows: JAK/STAT pathway (JAK1, JAK2, JAK3, STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6, SOCS3, SHP2, IL6ST, IL6R, and IL6), MAPK pathway (ERK1, ERK2, MEK1, MEK2, RAF1, ARAF, BRAF, HRAS, KRAS, NRAS, SHC1, SHC2, SHC3, and GRB2), and PI3K pathway (PIK3CA, PIK3AP1, PIK3CA, PIK3CB, PIK3CG, PIK3CD, PIK3CG, PIK3CP1, PIK3R1/2/3/4/5/6, AKT1/2/3, MTOR, PTEN, PDK1, TSC1/2, RICTOR, and RPTOR). Strikingly, almost one third of all HNSSC tumors analyzed in our cohort (30.5%; 46 of 151 tumors) harbored PI3K pathway mutations, whereas only 9.3% (14 of 151) and 8.0% (12 of 151) of tumors contained mutations in the JAK/STAT or the MAPK pathways, respectively (Fig. 1A and Supplementary Table S2A–S2E). In contrast to other smoking-related aerodigestive tract cancers where KRAS is frequently mutated (5), the HNSSC MAPK mutational profile is characterized primarily by HRAS mutations, which accounted for seven of 12 pathway mutations identified. PIK3CA is the most commonly mutated gene in the HNSSC PI3K mutational profile (Fig. 1B). These results show that despite the genomic heterogeneity of HNSSC tumors, the PI3K pathway is the most frequently somatically mutated mitogenic pathway in HNSSC tumors, found in 30.5% of cases, providing a potential approach to treat a substantial subset of patients.

A detailed analysis of the PI3K pathway mutational events showed that 19 of 151 tumors (12.6%) harbor a PIK3CA mutation (Fig. 1B). This mutation rate is similar to that detected in prior reports of HNSSC tumors (7.4% and 10.8% rate (6, 7)). Furthermore, we found PIK3CG and PTEN mutations in 4.0% (six of 151) of HNSSC tumors, whereas PIK3R1 (also known as p85), PIK3R5, and PIK3AP1 were mutated in 2.7% tumors (four of 151). Other components of the PI3K pathway were mutated in less than 2% of cases (Fig. 1B). Major downstream effectors of the PI3K pathway, including PDK1 and AKT1, were not mutated, whereas AKT2 and MTOR were only mutated in 1.3% (two mutations) of HNSSC tumors. Although PIK3CA gene amplification data were not available for the previously sequenced tumors, in the newly added cohort, PIK3CA was amplified in 24.4% (11 of 45) of the cases.

Previous reports noted loss of heterozygosity of the tumor suppressor PTEN in HNSSC by PCR-based microsatellite analysis primarily using D10S215 and/or D10S541 probes in relatively small HNSSC cohorts (e.g., 17 and 39 tumors, respectively; refs. 8, 9). Although comprehensive analysis of PTEN copy number was not available in the published genomic HNSSC studies (1, 2), PTEN gene copy number change was analyzed using a highly sensitive Affymetrix Genome-Wide Human SNP Array 6.0 platform in our 45 newly sequenced HNSSC tumors. Our results showed that PTEN gene copy loss (≥1 copy loss) was found in only 8.6% of cases (four of 45), indicating that PTEN loss is not likely to be the primary mediator of PI3K pathway alteration in this cohort of 45 HNSSC tumors (unlike other cancers such as glioblastoma, where PTEN loss can be as high as 20–60%; refs. 10, 11). However, all four tumors with PTEN gene copy loss expressed relatively low levels of PTEN protein when compared with HNSSC tumors without PTEN gene copy alteration (P < 0.001; Supplementary Fig. S1).

PI3K Pathway–Mutated HNSSC Tumors Show an Increased Rate of Cancer Gene Mutations

To determine whether HNSSC tumors harboring mutations in PI3K pathway genes contained a higher number of mutations in other cancer-associated genes, we compared the mutation rates of PI3K pathway–mutated tumors with non-PI3K pathway–mutated tumors. We found that tumors harboring PI3K pathway mutations have higher rates of mutation than non-PI3K–mutated HNSSC tumors. As shown in Fig. 1C, PI3K pathway–mutated HNSSC tumors harbored 2.3 times more nonsynonymous mutations (165.5 ± 24.1 vs. 72.1 ± 6.6 mutations; P < 0.0001) than tumors without PI3K mutations, indicating increased genomic instability in tumors harboring PI3K pathway mutations. Furthermore, cancer gene filtering analysis showed that these PI3K pathway–mutated HNSSC tumors harbored twice as many cancer gene mutations than those without PI3K pathway mutations [Fig. 1D; 7.2 ± 0.8 vs. 3.6 ± 0.3; P < 0.0001: defined by the Cancer Gene Census, Catalogue
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RESEARCH BRIEF

A

B

C

D

E

Figure 1. Mutations in oncogenic signaling pathways in HNSCC. A, mutation rates of the major mitogenic pathways (the PI3K pathway, the MAPK pathway, and the JAK/STAT pathway) in 151 HNSCC patient tumors determined by whole-exome sequencing. Components of each pathway examined are displayed under each pie chart. B, bar graph detailing the number of mutations (dark bars) of each particular component of the PI3K pathway as well as the percentage of HNSCC tumors harboring these mutations (gray bars). C, PI3K pathway–mutated HNSCC tumors have higher rates of nonsynonymous (non-syn.) mutations and (D) cancer gene mutations when compared with HNSCC tumors without any PI3K pathway mutations. Bar graph representing the average number of nonsynonymous mutations per tumor (C) and the average number of cancer gene mutations per tumor (D) in 151 HNSCC tumors. Statistical significance was calculated by Fisher’s exact test; \( P < 0.0001 \) (N = 151). E, graphical representation of the number of HNSCC tumors with mutation of multiple components of the PI3K, MAPK, and the JAK/STAT pathways, respectively.

of Somatic Mutations in Cancer (COSMIC) Database; ref. 12). These results suggest that PI3K pathway mutations in HNSCC may facilitate the expansion or selection of tumor cells that are already genetically unstable, and thus harbor more genomic aberrations, including aberrations in known cancer genes. This contention is supported by further analysis showing that DNA damage/repair genes [based on the DNA damage gene list in the cBio portal database (13), which includes ATM, ATR, CHEK1/2, BRC1/2, FANC, MLH1, MSH2, MDC1, PARP1, and RAD51] were found to be mutated at a significantly higher frequency in the PI3K-mutated tumors (average mutation rate of 37.0%; 17 mutations in 46 tumors) compared with tumors without PI3K pathway mutations (average mutation rate of 15.2%; 16 mutations in 105 tumors; \( P = 0.033 \)).

The association between PI3K pathway mutations and genomic instability is observed in HNSCC derived from all anatomic sites in our cohort (e.g., oral cavity, pharynx, and larynx; data not shown). Mutation rates in laryngeal tumors (186.3 ± 27.1; \( n = 32 \); data not shown) are significantly higher than the rates of mutation in tumors from the other anatomic locations (78.6 ± 8.3; \( n = 116 \); \( P = 0.0005 \); data not shown). In addition, the prevalence of PI3K pathway mutations is higher in laryngeal tumors (53.1% ± 9.0%; \( n = 32 \); data not shown) as compared with tumors from the other anatomic locations (25.0% ± 4.0%; \( n = 116 \); \( P = 0.0005 \); data not shown).

Comutation analysis showed that tumors with PI3K pathway mutation(s) are also associated with mutations of several known tumor suppressor genes including ARID1A, MLL, and MLL3 (\( P < 0.05 \); Supplementary Table S3), which contribute to chromatin remodeling and transcriptional regulation in cancers (14–17). Intriguingly, ARID1A has been shown to influence signaling through the PI3K pathway, suggesting that ARID1A may regulate the PI3K pathway and expand the number of tumors susceptible to targeting the PI3K pathway (18). Of note, PI3K pathway–mutated tumors are not associated with TP53 (\( P = 1.0 \)) or NOTCH1 mutations (\( P = 0.34 \); data not shown).
Interestingly, we also identified three tumors where PIK3CA or PIK3R1 was the only known mutated cancer gene [HN_00361, HN_63027, and HN414PT with the respective PI3K mutations of PIK3R1 (453_454insN), PIK3CA(E542K), and PIK3CA(H1047L)]. Strikingly, all three tumors were associated with infection by the human papillomavirus (HPV), suggesting that, although the number of HPV-positive HNSCC tumors in this cohort is relatively small (15 cases, five of which (33%) harbored PI3K pathway mutations; see Supplementary Table S4), a subset of HPV-positive HNSCC tumors (20%; three of 15 cases) may be driven by PI3K pathway mutation(s) alone, without an associated increase in underlying genomic instability.

Only Advanced-Stage HNSCC Tumors Harbor Multiple PI3K Pathway Mutations

In HNSCC tumors containing PI3K pathway mutations, 21.7% (10 of 46) harbored mutations in more than one PI3K pathway member gene, indicating that genetic alterations at multiple levels in the PI3K pathway are relatively common in HNSCC (Table 1). In contrast, HNSCC tumors rarely, if ever, harbored multiple mutations in the MAPK pathway (0 tumors), or the JAK/STAT pathway (only one contained both JAK3 and STAT1 mutations; HN_63080; Fig. 1E). Strikingly, all of these HNSCC tumors (100%; 10 of 10 cases) with multiple PI3K pathway mutations were advanced (stage IV; Table 1). None of these tumors was associated with HPV infection. These findings suggest that concerted PI3K pathway aberrations may contribute to HNSCC progression. This finding seems to be unique to HNSCC. Examination of recently published tumor datasets including breast, colon, and lung squamous cell carcinoma (SCC) showed that only one of 25 breast tumors, one of 27 colon carcinomas, and 0 of 31 lung SCC tumors that harbored multiple PI3K pathway mutations were stage IV [data not shown; eBio portal (13)]. Although all 10 tumors with multiple PI3K pathway mutations were advanced (stage IV), there is no significant association between advanced disease and individual PI3K pathway mutations (data not shown). In addition, mutation rates do not vary significantly between stage IV and earlier stage (I–II) HNSCC (data not shown). In the absence of models assessing the specific contribution of each mutation to cell growth or survival, it is not possible to determine the precise biologic effect(s) of individual mutations in tumors that harbor more than one mutation in the PI3K pathway.

PIK3CA Canonical and Novel Mutations Increase Survival and Pathway Activation in HNSCC Tumors

PIK3CA is a critical gene in the PI3K signaling pathway. In HNSCC tumors, the most common sites of PIK3CA mutations included H1047R/L (eight mutations total), E545K/G (four mutations), and E542K (three mutations; Fig. 2A), all of which represent previously reported canonical (“hotspot”) mutation sites. This HNSCC PIK3CA mutation pattern (~90% of mutations found in the helical/kinase domains) is similar to that observed in cervical and breast cancers, as well as lung SCC, but is distinct from other tumors such as endometrial cancer, lung adenocarcinoma, glioblastoma multiforme, and prostate carcinoma (Supplementary Table S5). In addition, we detected four previously unreported, novel PIK3CA mutations (R115L, G363A, C971R, and R975S). To determine the functional consequences of these mutations, we stably expressed, by retroviral infection, each of the novel mutations and a hotspot mutation (H1047R) in a representative HNSCC cell line that is wild-type (WT) for all PI3K pathway components. Overexpression of WT PIK3CA (mimicking PIK3CA gene amplification), and expression of all the engineered PIK3CA mutants individually, resulted in enhanced growth compared with infection by enhanced GFP (EGFP) control. Furthermore, the canonical hotspot mutation showed significantly enhanced growth compared with overexpression of WT PIK3CA (P = 0.0001). The novel mutations were found to confer moderate growth advantage compared with simulated WT amplification (R115L, P = 0.1174; G363A, P = 0.9637; C971R, P = 0.6503; R975S, P = 0.0958). Immunoblotting of cell lysates revealed that enhanced HNSCC growth, conferred by the introduction of the novel mutations, was associated with increased PI3K pathway activation as reflected by elevated expression of phosphorylated AKT (Fig. 2B and C). In the absence of complete functional characterization of these novel mutations, these findings should be considered supportive but not definitive evidence of oncogenic function.

HNSCC Patient Tumorgrafts with PIK3CA Mutations Are Exquisitely Sensitive to BEZ-235

Reports in other cancers suggest that tumors with PI3K pathway activation may be more sensitive to PI3K pathway inhibitors (19). To determine the predictive value of PIK3CA mutational status in HNSCC, we examined the sensitivity of HNSCC cell lines that did and did not harbor intrinsic activating driver PIK3CA(H1047R) hotspot mutations to PI3K pathway inhibitors. As shown in Fig. 3A, HNSCC cell lines containing endogenous PIK3CA(H1047R) mutations (CAL-33 and Detroit 562; ref. 20) showed increased sensitivity to PI3K pathway inhibition by the mTOR/PI3K inhibitor BEZ-235 compared with representative HNSCC cells with WT PIK3CA [SCC-9 and PE/CA-PJ34(clone C12)]. Next, mice bearing CAL-33 xenografts were found to be sensitive to BEZ-235 treatment in vivo when compared with vehicle control (Fig. 3B). Because of the lack of HPV-positive HNSCC cell line models that contain PIK3CA mutations, we developed an HPV-positive PIK3CA-mutated HNSCC patient tumorgraft model (E542K; Fig. 3C) to determine the sensitivity of HPV-positive PIK3CA-mutated HNSCC tumors to PI3K pathway targeting. As shown in Fig. 3D, BEZ-235 treatment (at 25 mg/kg/day by oral gavage) significantly inhibited the growth of an HPV-positive PIK3CA-mutated HNSCC patient tumorgraft in vivo (P < 0.0001). Inhibition of tumor growth was accompanied by decreased PI3K signaling as evidenced by downregulation of p-AKT(S473) (P = 0.0124), and p-S6(S235/236) (P < 0.0001) in the BEZ-235-treated tumors (Fig. 3E). Another HNSCC patient-derived tumorgraft model (HPV-negative) harboring a PIK3CA mutation (E110K) was also found to be sensitive to BEZ-235 treatment (Supplementary Fig. S2). In contrast, patient tumorgrafts with WT PIK3CA and low baseline p-AKT levels were not sensitive to the growth-inhibitory effects of BEZ-235 (Fig. 3F and Supplementary Fig. S3). These results indicate that activating mutations of the PI3K pathway have the potential to serve as biomarkers for treatment selection in HNSCC. Xenografts developed from an HNSCC cell line harboring a PIK3CA mutation (H1047R) were more sensitive to the combination of BEZ-235
plus cetuximab [the only U.S. Food and Drug Administration (FDA)-approved molecular targeting agent in HNSCC] compared with cetuximab alone (Supplemental Fig. S4), suggesting that targeting PI3K in the setting of PIK3CA-mutant tumors can enhance treatment responses to cetuximab.

**DISCUSSION**

The increasing number of targeted agents for cancer treatment results in an unprecedented opportunity for personalized cancer medicine. Selection of therapies based on mutation status of molecular targets has transformed clinical management and survival of several human malignancies. The EGF receptor (EGFR) monoclonal antibody cetuximab is the only targeted therapy that is FDA-approved to date for HNSCC treatment, yet there are no biomarkers that can be assessed in the primary tumor to predict clinical responses to this agent. The recent elucidation of HNSCC genomics offers an opportunity to identify genetic subgroups of HNSCC tumors to guide treatment decisions.

In this report, we used a bioinformatic approach to identify mutationally altered, targetable mitogenic pathways in HNSCC. Analyses of all currently available HNSCC whole-exome sequencing data (a total of 151 primary HNSCC tumors) revealed several key findings with important implications for HNSCC pathobiology and treatment. The PI3K pathway is the most frequently mutated oncogenic pathway in HNSCC, with the relative number of PI3K-mutated tumors compared with RAS/MAPK and JAK/STAT–mutated tumors being approximately threefold greater. Similar ratios of PI3K pathway mutations (relative to RAS/MAPK or JAK/STAT) are seen in SCC of the lung and in cervical cancer, both of which share common risk factors with HNSCC, including tobacco and HPV infection, respectively. In contrast, the RAS/MAPK pathway is more frequently mutated than the PI3K pathway in colon and thyroid cancers, and both the PI3K and RAS/MAPK pathways are mutated at comparable rates in lung adenocarcinomas (13). The percentage of HNSCC tumors harboring multiple mutations in the PI3K pathway is similar to that observed in breast cancers (4.9%; 25 of 507 tumors) and glioblastomas (9.1%; 25 of 276 tumors), higher than in thyroid cancer (0.3%; one of 323 tumors), and much lower than in most other cancers, including uterine carcinoma (65.7%; 163 of 248 tumors), melanoma (24.9%; 63 of 253 tumors), and, interestingly, lung SCC (17.4%; 31 of 178 tumors), which otherwise shares...
common risk factors and similar relative rates of pathway
mutation with HNSCC (13).

Using novel patient-derived tumorgraft models with an
oncogenic PIK3CA(E542K) mutation, we showed that these
tumors are exquisitely sensitive to a PI3K pathway inhibitor
(BEZ-235). Similar results were shown in another HNSCC
patient-derived tumorgraft model with a PIK3CA(R115L)
mutation, previously reported in breast cancer (21). In con-
trast, treatment of human-derived heterotopic tumorgrafts
with WT PIK3CA and low basal expression levels of phospho-
AKT with a PI3K pathway inhibitor was ineffective. These
findings suggest that (i) PI3K pathway inhibitors can be
effective for treating HNSCC tumors with PI3K mutations;
and (ii) mutation-guided treatment responses can be evaluated/
monitored using patient-derived HNSCC tumorgraft models
in vivo. In fact, early-phase clinical trial results showed that
patients with solid tumors harboring a PIK3CA hotspot
mutation (H1047R) were found to be responsive to PI3K
pathway inhibitors (22). However, the effects of other PIK3CA
mutations on mediating drug sensitivity in HNSCC preclin-
ical models or clinical trials have not been previously reported.
Findings from our study indicate that PIK3CA(E542K) muta-
tion, as well as other non-hotspot mutations (such as E110K),
may also identify an HNSCC subgroup potentially responsive
to PI3K pathway inhibitors. In particular, our results using
HNSCC patient-derived tumorgrafts suggest that HNSCC
tumors with activating PIK3CA mutations may be more
sensitive to a dual PI3K/mTOR inhibitor (such as BEZ-235)
compared with tumors with WT PIK3CA (Fig. 3E and Sup-
plementary Fig. S3), as indicated by significant inhibition of
p-S6 expression in the PIK3CA mutated, but not in the WT
tumorgrafts. In fact, a recent report of five HNSCC cases
found that mTOR-based targeted therapy may be more effec-
tive in HNSCC tumors harboring PIK3CA mutation and/or
PTEN loss (23).

PI3K pathway–mutated HNSCC tumors were found to
have a higher rate of nonsynonymous mutations, including
an increased number of defined cancer gene mutations, com-
pared with tumors without PI3K pathway mutations. This
observation implies that the PI3K pathway–mutated HNSCC
tumors have an “oncogenic” advantage even with genomic
instability, and/or that PI3K-mutated HNSCC tumors
intrinsically display a “mutator” phenotype rendering them
more prone to mutation.

**Figure 2.** PIK3CA mutations in HNSCC tumors. A, schematic of all PIK3CA mutations found in 151 HNSCC tumors by whole-exome sequencing. The
amino acid (a.a) positions of each domain are shown in gray below each domain. The number of mutational events at each site is indicated by a filled trian-
gle (▲) in the graph above. Blue triangles indicate mutations found in HPV-positive HNSCC tumors. ABD, p85-binding domain; RBD, Ras-binding domain;
C2, superfamily; Helical, PIK domain. Kinase, kinase domain of PIK3CA. B, effects of PIK3CA mutations on PI3K signaling in HNSCC cells. WT PIK3CA,
hotspot mutant H1047R, and novel mutants R115L, G363A, C971R, and R975S were stably expressed in an HNSCC cell line harboring no endogenous
mutations in the PI3K pathway. PE/CA-PJ34 (clone C12) cells, by retroviral infection. Shown here is a representative Western blot analysis with densit-
ometry values normalized to β-tubulin loading controls for each engineered cell line. Increased phosphorylation of AKT at the T308 and/or S473 residue
was generally observed in HNSCC cells stably expressing WT or mutant PIK3CA constructs relative to the EGFP-expressing HNSCC cells, indicating
enhanced activation of the PI3K signaling pathway. C, effects of PIK3CA mutations on HNSCC cell growth. HNSCC cells stably expressing WT or mutant
PIK3CA constructs showed enhanced growth at 72 hours in media with 2% FBS by MTT assay compared with cells expressing EGFP vector control
(***, P < 0.0001). PIK3CA(H1047R)-expressing cells further showed enhanced growth when compared with WT PIK3CA amplification (P = 0.001).
Data shown here represent growth studies from three sets of independent replicate cell lines (separate infections, n = 18 for each group).
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Figure 3. PIK3CA mutation enhances sensitivity to PI3K pathway inhibition. A, HNSCC cells containing endogenous PIK3CA mutation (H1047R, CAL-33, and Detroit 562) and cells containing WT PIK3CA (SCC-9, PE/CA-PJ34 (clone C12)) were treated with a PI3K/mTOR inhibitor, BEZ-235, followed by growth measurements at 48 hours (n = 4). Experiments were repeated three times with similar results. DMPS, dimethyl sulfoxide B, BEZ-235 inhibited growth of CAL-33 xenografts (with endogenous PIK3CA(H1047R) mutation). CAL-33 cells (0.5 x 10^6 cells) were inoculated into the flanks of nude mice. Treatment was started when the tumors became palpable 8 days after tumor cell inoculation. BEZ-235 (25 mg/kg/day; n = 9) or vehicle (Veh; n = 10) was given by oral gavage daily. Treatment with BEZ-235 significantly reduced tumor size when compared with vehicle control (*, P = 0.01; **, P < 0.05; ***, P = 0.0002; ****, P < 0.0001). C, Sanger sequencing results showing PIK3CA(E542K) mutation in the HPV-positive HNSCC patient tumors that were implanted into the flanks of NOD/SCID mice. BEZ-235 (25 mg/kg) or vehicle control was given daily by oral gavage. Mice were given vehicle (n = 7) or BEZ-235 (n = 5) when tumors became palpable. Treatment with BEZ-235 significantly reduced the tumor size when compared with vehicle control (P < 0.0001). D, Western blot analyses showing the effects of BEZ-235 vs. vehicle control on expression of PI3K signaling components in the PIK3CA-mutated patient tumors. Tumors were harvested for Western blotting at the end of the experiment on day 22. Densitometry values of band intensity are shown below each band (normalized to total β-tubulin level). Phospho-AKT(547) (S473) and phospho-S6 (S235/236) levels were significantly reduced upon BEZ-235 treatment when compared with the vehicle-treated tumors (P = 0.0124 and P < 0.0001, respectively). E, HNSCC patient tumors from a WT PIK3CA tumor expressing low levels of pAKT were implanted into the flanks of NOD/SCID mice, and treatment was started when tumors became palpable. BEZ-235 (25 mg/kg; n = 6) or vehicle control (n = 6) was given daily by oral gavage. Treatment with BEZ-235 failed to significantly reduce the tumor size when compared with vehicle control (P = 0.300).

Pathway-mutated tumors can be partly explained by PIK3CA “driver” mutations’ growth-promoting activity (ref. 24; Fig. 2C), whereas the “mutator” phenotype of these tumors is supported by our finding that PI3K pathway-mutated tumors are associated with higher rates of mutation in DNA damage/repair genes, and with ARID1A and MLL3 mutations, which are important tumor suppressor genes (15, 16, 25). It is possible that both the “oncogenic/growth” advantage and “mutator” phenotype associated with PI3K pathway-mutated HNSCC tumors are necessary for HNSCC progression; especially because PI3K pathway mutations in these tumors are not associated with TP53 mutation, a previously recognized tumor suppressor alteration that contributes to HNSCC carcinogenesis. Although the relationship of PI3K pathway mutations and TP53 mutation has not been carefully examined in most cancers, a recent study in bladder cancer showed that PIK3CA mutations were significantly more common in TP53-WT tumors (26). Hence, PI3K pathway mutations may mediate tumor progression in the absence of TP53 genetic alteration.

Our finding that all 10 HNSCC tumors with concurrent mutations of multiple PI3K pathway genes were advanced-stage cancers (stage IV) suggests the potential involvement of concurrent alterations of multiple nodes of the PI3K pathway in HNSCC progression. This agrees with the recent report that in addition to PIK3CA mutation, other pathway components such as PIK3R1 and PIK3R2, when mutated, can also serve to drive cell growth/survival (27). Although the effects of multiple PI3K pathway mutations on cancer cell growth or progression have not been previously investigated, our results support the possibility that genetic alterations at multiple nodes in this oncogenic pathway, a common feature
of many solid tumors, may identify a subgroup of patients with cancer most likely to respond to PI3K pathway inhibitors. These cumulative findings identify the PI3K pathway as the most frequently mutated mitogenic pathway in HNSCC tumors. Prospective identification of patients whose tumors harbor these mutations is likely to identify a subgroup of individuals who may benefit from treatment with PI3K pathway inhibitors.

METHODS

Additional methods are detailed in the Supplementary Data.

Cell Cultures

The HNSCC cell lines Detroit 562 and SCC-9 were obtained from the American Type Culture Collection, and the PE/CA-PJ34 (clone C12) cells were obtained from Sigma-Aldrich. CAL-33 was a kind gift from Dr. Gerard Milano (University of Nice, Nice, France). All cell lines were genotypically verified. The HNSCC cell lines were cultured in the respective culture medium containing 10% fetal calf serum, 1× penicillin/streptomycin solution (Invitrogen); CAL-33 and Detroit 562 in Dulbecco’s Modified Eagle Medium (DMEM), SCC-9 in DMEM/F12 with 0.4 μM hydrocortisone, and the PE/CA-PJ34 (clone C12) cells in Iscove’s Modified Dulbecco Minimum Essential Medium with 2 μmol/L glutamine (Mediatech, Inc.). All cell lines were maintained in a humidified cell incubator at 37°C, 5% CO₂.

Cancer Gene Census Comparison and Comutation Analysis

A mutation comparison program was written in Visual Basic for Microsoft Excel to compare the existence of HNSCC mutations versus a reference list of mutations of interest (in this case, cancer genes). The program allows side-by-side comparison between multiple groups (two or more) to discover common mutational events, as well as the number of common events in multiple groups. A cancer gene list was generated in each subgroup of tumors by comparing the Cancer Gene Census list (COSMIC Database) with a nonsynonymous mutation gene list of each tumor subgroup (the PI3K-mutated tumors, tumors without PI3K mutation, PIK3CA-mutated tumors, and PIK3CA-WT tumors) using this comparison program. This analysis allows us to discover the number of cancer genes mutated in each subgroup.

Mutation Validation by Sanger Sequencing

Sanger sequencing was conducted on patient tumors that were grafted for tumorgen studies. About 25 to 50 mg of tumor tissue (pathologically confirmed HNSCC with >70% tumor cell content) was used for extraction of DNA by Qiaamp DNA Mini Kit (Qagen, Inc.). Sequencing primers for HNSCC-associated PIK3CA hotspot mutations were synthesized (Sigma-Aldrich) and used for Sanger sequencing. The primer sequences for E542 site mutation are: 5’-ctcagagtcctcttcctattaactcagagaggg-3’ (forward) and 5’-ctcagagttttgcagagactc-3’ (reverse). Sanger sequencing was conducted at the Genomics and Proteomics Core Laboratories at the University of Pittsburgh.

HNSCC Tumorgraft Model and Drug Treatment

BEZ-235 was obtained as a kind gift from Novartis. HPV-positive HNSCC patient tumorigrafs were derived under the auspices of an Institutional Review Board–approved protocol, with WT PIK3CA or PIK3CA(E542K) mutation implanted into the flanks of nonobese diabetic/severe combined immunodeficient γ (NOD/SCIDγ) mice, and treatment was started when tumors became palpable. BEZ-235 (25 mg/kg) or vehicle control was given daily by oral gavage. Tumor volumes were measured every 2 days.

Disclosure of Potential Conflicts of Interest

L.A. Garraway has received a commercial research grant from Novartis; has ownership interest (including patents) in Foundation Medicine; and is a consultant/advisory board member of Foundation Medicine, Novartis, Millennium, and Boehringer Ingelheim. G.B. Mills has received commercial research grants from AstraZeneca, Celgene, CeMines, Exelixis/Sanoﬁ l, GlaxoSmithKline, Roche, Wyeth, and Pfizer/Puma; has ownership interest (including patents) in Catena Pharmaceuticals, PTV Ventures, and Spindle Top Ventures; and is a consultant/advisory board member of AstraZeneca, Catena Pharmaceuticals, Critical Outcome Technologies, Daiichi Pharmaceuticals, Targeted Molecular, Foundation Medicine, Han AllBio Korea, Komen Foundation, Novartis, Symphogen, and Tumor Therapeutics. J.R. Grandis receives research support from Bristol-Myers Squibb, Novartis, and Atellas (previously OSI Pharmaceuticals).

Authors’ Contributions


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