Vulvar Squamous Cell Carcinoma (VSCC) as Two Diseases: HPV Status Identifies Distinct Mutational Profiles Including Oncogenic Fibroblast Growth Factor Receptor 3

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Abstract

Purpose: Patients with advanced or recurrent invasive vulvar squamous cell carcinoma (VSCC) have limited treatment options and a grave prognosis. Understanding the genomic landscape may facilitate the identification of new therapies and improve clinical outcomes.

Experimental Design: A retrospective chart review and molecular analysis of patients with VSCC from 2000 to 2016 was performed at the Ottawa Hospital Research Institute. The presence of oncogenic human papillomavirus (HPV) was determined by nested PCR and amplified DNA was sequenced using the Ion AmpliSeq Cancer Hotspot v2 Panel. The patients were divided into two groups according to HPV status (HPV-positive versus HPV-negative) and clinical outcome correlated with mutation status using descriptive statistics.

Results: In 43 VSCC patients, there was a high mutation rate in both HPV-positive (73%) and HPV-negative (90%) disease with the two subgroups expressing distinct genetic profiles. HPV-positive tumors were characterized by oncogenic mutations in PIK3CA (27%), FGFR3 (14%), and PTEN (9%), whereas HPV-negative tumors were found to have mutations in TP53 (57%), HRAS (24%), PI3KCA (19%), and CDKN2A (14%). Mutation S249C in FGFR3 occurred in 14% of HPV-positive tumors. While there were notable differences in the occurrence of TP53, HRAS, PTEN, and FGFR3 mutations according to HPV status, only the rate of TP53 mutations was statistically significant (P = 0.0004). No significant difference in prognosis was found between patients with HPV-positive and HPV-negative VSCC.

Conclusions: HPV-positive VSCC is characterized by oncogenic FGFR3 mutations that helps classify this subtype as a separate disease. Inhibitors of FGFR3 merit consideration as a therapeutic strategy in this neglected cancer in women. Clin Cancer Res; 23(15): 4501–10. ©2017 AACR.

Introduction

Vulvar squamous cell carcinoma (VSCC) is a relatively rare gynecologic cancer with an age-standardized incidence of 4/100,000 (1). As detailed in our recent review of targeted therapies in VSCC, this “forgotten cancer in women” has suffered from a paucity of basic and clinical research and has seen minimal improvement in treatment for decades (2). Although early-stage disease is frequently amenable to surgery, radical excision is associated with significant morbidity and a high rate of recurrence. Approximately, a third of patients will recur (3) and undergo standard treatment with radiotherapy and platinum-based chemotherapy. Patients with distant metastases have a median survival of approximately 5 months (4). Therefore, there is an urgent need for us to increase our understanding of the molecular pathogenesis of VSCC and improve patient outcomes.

As in other human papillomavirus (HPV)-driven cancers, VSCC is linked to the viral oncoproteins E6 and E7, which dysregulate apoptosis and cell-cycle activity. The pathogenesis of VSCC appears to arise from two distinct mechanisms leading to two different precursor lesions (5). HPV-positive and HPV-negative VSCCs are characterized by the well-defined precursor lesions, usual vulvar intraepithelial neoplasia (uVIN) and differentiated vulvar intraepithelial neoplasia (dVIN), respectively. Despite the introduction of prophylactic HPV vaccines for young women (age 9–23 years), the rates of HPV-positive cancers are not anticipated to decline for several decades given the vaccine’s lack of therapeutic efficacy in those already infected with HPV and the long lag time for carcinogenesis in infected individuals (6). Notably, there has been an increase in the incidence of VSCC in North America, largely as a result of HPV-positive VSCC, which now accounts for 50% of all cases (7). In some tumor types, including head and neck squamous cell carcinoma (HNSCC), a better prognosis has
Translational Relevance

In this study using next-generation sequencing, we provide supporting evidence that human papillomavirus (HPV)-positive and HPV-negative VSCC should be regarded as two distinct clinical diseases, each characterized by a relatively high mutational burden with unique molecular profiles. The FGFR3 S249C mutation occurred in 14% of HPV-positive tumors, a finding that has not been previously described in VSCC. Given the molecular diversity of VSCC, biomarker identification and screening will be critical to advancing the treatment of this disease with molecular therapies. Overall, a number of therapeutically relevant mutations, including FGFR3, merit interrogation with novel molecular targeted agents in future clinical trials in VSCC.

been demonstrated in patients with HPV-positive neoplasms when compared with HPV-negative disease (8). However, the prognostic relevance of HPV infection in VSCC is unclear and there are no predictive biomarkers that guide treatment decisions.

In recent years, the identification of driver mutations in a number of cancers has enabled personalized therapies to be tailored to the unique genomic characteristics of the tumor, resulting in improved survival outcomes. In VSCC, genomic investigations have focused on establishing the prevalence of single gene mutations (9, 10) or a limited set of predetermined genes thought to be therapeutically relevant (11). Previous reports have suggested an adverse prognosis associated with specific gene mutations (12, 13) with TP53, PTEN, and CDKN2A mutations identified as the most common molecular aberrations in VSCC (14). However, given the tumor suppressor function of these genes, the development of targeted therapies has remained a challenge. In recent years, there has been only one phase II clinical trial in VSCC with a targeted agent namely, the anti-EGFR tyrosine kinase inhibitor erlotinib. Erlotinib demonstrated significant response (partial response = 27.5%; stable disease = 40%) but had a limited sustained response rate (PFS = 3 months; ref. 15). Furthermore, in this study, no activating EGFR mutations were identified that predicted response, as in non–small cell lung cancer (16). Consequently, systemic therapies in VSCCs are currently restricted to cytotoxic chemotherapy and there is no approved targeted agent in the first-line or recurrent setting (17).

Effective therapeutic alternatives in advanced and recurrent VSCC remain an urgent unmet clinical need.

Next-generation sequencing (NGS) can detect mutations in multiple genes and allows for comprehensive analysis of variant mutations. Newer NGS technologies have identified novel genomic targets and activated molecular pathways in a number of tumor types and are used to guide treatment with targeted therapies. In VSCC, the genomic landscape remains poorly defined and is hampered by the inconsistent reporting of HPV status and the limited application of NGS technologies (14, 18). Advances in our understanding of the molecular biology of VSCC may provide an understanding of HPV-positive and HPV-negative disease and help establish these conditions as separate entities that merit distinct therapies. Given the limitations of current treatments, the identification of novel actionable mutations has important therapeutic implications in the management of VSCC.

In this study, we undertook a retrospective review of patient clinical data and performed NGS mutation analysis on archival tumor to define the genomic profiles of HPV-positive and HPV-negative VSCC.

Methods

Patient tumors

A retrospective chart review of clinical data and molecular analysis of archival tissue from patients diagnosed with VSCC from 2000 to 2016 at The Ottawa Hospital Cancer Centre (TOHCC), Ottawa, Canada was undertaken. The study was approved by the Ottawa Hospital Research Institute’s Research Ethics Board (#20120078-01H). Patients were included in the study if they had adequate tumor for molecular analysis from a pretreatment biopsy or tumor resection, as determined by the study pathologist (H.S. Sekhon). For eligible patients, clinicopathologic data were collected from the electronic medical record including age at diagnosis, stage, smoking history, lymph node involvement, tumor size, depth of invasion, treatment, time to progression (TTP), and overall survival (OS).

DNA was isolated from the sections of formalin-fixed, paraffin embedded (FFPE) tumor blocks from biopsies or tumor resection specimens of VSCC to determine HPV status and for molecular analysis. Unstained 10-μm thick sections on slides were prepared from FFPE blocks. Using pathologist-annotated H&E slides as a reference, tumor-containing regions (tumor cellularity >80%) from the unstained sections were then microdissected into mineral oil with the Avenio Millisect (an automated tissue dissection instrument currently in development that allows for more precise isolation of tumor compared with manual methods; Fig. 1) Samples were transferred to a 1.5-μL microcentrifuge tube, deparaffinized in 300 μL of mineral oil, and heated to 80°C for 30 seconds. Genomic DNA was isolated using the QIAamp FFPE Tissue Kit (Qiagen). The tissue was digested by adding 180 μL of buffer ATL and 30 μL of proteinase K followed by an overnight incubation at 56°C or until the specimen was completely lysed. Proteinase K (Qiagen) was inactivated by incubation at 90°C for 1 hour. The aqueous phase was carefully transferred to a QIAamp mini elute column where isolation was continued as per manufacturer’s protocol. DNA was quantified via Qubit 3.0 (Life Technologies).

HPV status

Each tumor was evaluated for the presence of high-risk HPV. Before testing HPV status, DNA integrity was verified by PCR for human β-globin using primers GH20 (GAA GAG CCA AGG ACA GGT AC) and PC04 (CAA CIT CAT CCA GTG TCA CC) from Invitrogen. HPV status was tested by nested PCR of the L1 region using two primer sets from Invitrogen, My09/11 PGMY and GP5+/GP6+. HPV-positive PCR products were gel purified for HPV genotyping. The purified samples underwent cycle sequencing using BigDye Terminator Chemistry v3.1 (Applied Biosystems) according to the manufacturer’s recommended protocol for PCR fragments. Purified products were sequenced on a 3730 DNA Analyzer using the 50-cm capillary array and base calls were automatically generated using Sequencing Analysis Software (Applied Biosystems).

Genomic profiling using next-generation sequencing

Library preparation. Barcoded libraries were generated using the Ion AmpliSeq Cancer Hotspot v2 Panel and the Ion AmpliSeq...
Library Kit (Thermo Fisher) as per the manufacturer’s recommendations and as described previously (19). This panel included 207 amplicons that cover over 2,800 hotspots and 20,000 bases in 50 genes of oncogenic significance. A summary of the genes included in this panel is provided in Supplementary Table S1. Multiplexed barcoded libraries were then loaded onto either the Ion 316 or 318 chip for sequencing. On average, the number of mapped reads was 360,000 per library. The mean depth of coverage was 1,600 per amplicon and the uniformity of coverage was 97.5%.

Molecular analysis. The Ion Torrent Suite v5.0 was used to analyze the raw signal data. Detected sequence variations (single nucleotide variants and insertions or deletions) were identified using the Variant Caller v5.0. Annotated variants were prioritized using IonReporter with publicly available databases COSMIC v68 (COSMIC) and dbSNP build 137 (dbSNP). Only variants with a minimum of 300 depth of coverage and a >5% allelic frequency were included.

Statistical analysis. Descriptive statistics using χ², Fisher exact test, and t tests were used for categorical and continuous data, respectively, and were analyzed separately by HPV status. TTP and OS were estimated using the Kaplan–Meier approach and compared between groups using the log-rank test. Cox proportional hazards models were used to adjust for covariates.

Digital droplet PCR. The digital droplet PCR (ddPCR) technique was used to confirm and quantify novel mutations. Water–oil emulsion droplet technology is used to fractionate samples into 10,000 to 20,000 droplets, with PCR amplification of the template molecules occurring in each individual droplet. Positive and negative fluorescent droplets are then analyzed by a droplet reader to determine the target concentration. ddPCR was performed using the Bio-Rad QX100 system according to manufacturer’s guidelines. Labeled (FAM or HEX) fluorescent probes designed for FGFR3 c.746C>G, p.S249C (catalog no. 10031246) together with the respective wild-type (WT; catalog no. 10031249) locus, were provided by Bio-Rad. Droplets were generated on the QX100 Droplet Generator and read on the QX100 Droplet Reader (Bio-Rad). The data were analyzed using the QuantaSoft software from Bio-Rad.

FISH. Each sample was stained with commercially available antibody FGF receptor 3 (C51F2) rabbit mAb (Cell Signaling Technology) diluted 1:100 in 1% Tween, 1% Triton X-100 PBS. Antigen retrieval was performed at 125°C for 10 minutes in a pressure cooker while submerged in citrate buffer, pH 9 (Agilent Technologies). Vectastain elite ABC HRP kit was used for secondary antibody staining and streptavidin (Vector Laboratories). FGFR3 expression was visualized using Vector Red chromogen (Vector Laboratories).

The p16 expression was determined using ready-to-use primary mouse mAb (clone E6H4) directed to human p16INK4a protein (CINtec Histology Kit, Roche mtm Laboratories AG). Polymer reagent conjugated with horseradish peroxidase and secondary goat anti-mouse Fab antibody was used to visualize signal with DAB chromogen on Leica Bond Max stainer (Leica Biosystems) with hematoxylin counterstain.

Results. Molecular analysis was attempted on a total of 52 patient tumors. Specimen degradation prevented molecular analysis in 9 samples yielding molecular data on a total of 43 patient tumors. Specimen degradation prevented molecular analysis in 9 samples yielding molecular data on a total of 43 patient tumors, which formed the study cohort. Patient clinical and demographic data is detailed in Table 1, with data represented separately for HPV-positive and HPV-negative cohorts. The mean age of diagnosis in the HPV-positive and HPV-negative subgroups was not significantly different at 66 and 64 years, respectively (P = 0.711). Significant differences were observed between subgroups in terms of stage at presentation (P = 0.008) and depth of tumor infiltration with a mean depth invasion of 9.84 mm (range 2.5–22 mm) and 5.49 mm (range 0.5–17 mm) in the HPV-negative and HPV-positive groups, respectively (P = 0.007). Patients in the HPV-positive group were more likely to have a positive tumor margin at primary surgical resection than patients in the HPV-negative group (P = 0.02).
HPV status was established on all 43 patient samples and HPV-positive and HPV-negative tumors were equally represented between the two groups (Table 2). The HPV genotype was not determined in 3 tumors due to sample fragmentation. HPV sequences included HPV 16, 18, 31, and 33 with HPV 16 being the most commonly detected oncogenic HPV subtype.

The distribution of genomic aberrations by HPV subgroup is presented in Fig. 2. The overall prevalence of one or more oncogenic mutations in the full study cohort was 83%, with a relatively high rate of mutations noted in both HPV-positive (73%) and HPV-negative disease (90%). The molecular profiles were found to differ by HPV status, with HPV-positive cancers characterized by oncogenic mutations in PIK3CA 6/22 (27%), FGFR3 3/22 (14%), PTEN 2/22 (9%), and TP53 2/22 (9%), while HPV-negative cancers were found to have mutations in TP53 12/21 (57%), HRAS 5/21 (24%), PIK3CA 4/21 (19%), and CDKN2A 2/21 (10%). Low frequency mutations (e.g., APC, KRAS, ERBB4, ATM, SMARCB1) were more common in HPV-positive disease. Notably, 13 (30%) tumors harbored more than one mutation of oncogenic significance. Germline mutations in KIT at M541L, as reported in the COSMIC database, were found in both HPV-negative and HPV-positive subgroups, with M541L being more common in the latter. The S249C FGFR3 mutation occurred only in HPV-positive tumors, a finding that has not been previously described in VSCC. All three FGFR3 mutations in HPV-positive tumors result in the conversion of serine at 249 to cysteine, which is predicted to cause receptor activation. FGFR3 mutations were established as somatic by mutational analysis of the adjacent normal tissue. There was one mutation in the final position of FGFR3, T806M, and to our knowledge there is no data to suggest that this is an activating mutation. The mutation allele frequency and COSMIC reference for each sample are detailed in Supplementary Fig. S1.

Morphologic assessment of the three FGFR3-mutation positive tumors exhibited a broad-based invasive pattern (Fig. 3A).
rather than infiltrative cellular invasion and marked intratumoral inflammatory infiltrate. Two cases had predominantly lymphocytic infiltrate (Fig. 3B) while the third case had mainly neutrophilic infiltrate. Interestingly, FGFR3 expression was more intense at the periphery of tumor nests where tumor cells come in contact with the mesenchyme (Fig. 3C). These tumors were also strongly and diffusely positive for p16 expression (Fig. 3D).
Within the limitations of the NGS panel, the rate of oncogenic mutations in patient tumors was compared between HPV-negative and HPV-positive VSCC (Table 3). In the study sample, only the rate of mutations in \( \text{TP53} \) was significantly different between the two subgroups \( (P = 0.0004) \). Although there were distinct differences in the incidence of \( \text{HRAS}, \text{PTEN}, \text{FGFR3}, \) and \( \text{KIT} \) mutations between HPV-negative and HPV-positive VSCC, the limited sample size in the study cohort precluded statistically significant results.

Patient prognosis was stratified by HPV status taking into account clinical and pathologic data in both univariate and multivariate analysis (Supplementary data). In all patients, prognosis was not found to differ by HPV status, with no statistically significant differences in TTP and OS in both univariate (Supplementary Fig. S2A and S2B) and multivariate analysis (Supplementary Fig. S3A and S3B). Trends in prognosis appear to correlate with mutation status and the lack of statistical significance may be due to the relatively small sample size (Supplementary Figs. S4 and S5A–S5G). As expected, in the HPV-negative group, patients with mutations in \( \text{TP53} \) trended toward a poorer
prognosis (Supplementary Fig. S4B and S4C). In the HPV-positive group, patients with mutations in FGFR3 trended toward a better prognosis (Supplementary Fig. S4F and S4G). In all patients, the number of molecular aberrations did not correlate with TTP and OS (Supplementary Fig. S6A–S6D). In the dataset, having one mutation compared with more than one mutation did not have a significant impact on prognosis. Furthermore, an increasing number of mutations did not associate with a poorer prognosis in this limited study cohort (data not shown).

Discussion

Genetic mutations by HPV status

In this study, NGS technology was applied to an HPV-characterized cohort of VSCC patients to better define the genomic landscape of VSCC using a 50-gene panel of established oncogenic significance. In the overall sample population, there was a relatively high rate of mutations and a previously unreported S249C FGFR3 mutation was observed in HPV-positive VSCC.

Over 83% of the tumors included in the molecular analysis harbored one or more oncogenic mutation. The high mutation rate in HPV-positive VSCC (73%) was unexpected and has not been previously described. Studies in HNSCC have supported that the increased genomic complexity of HPV-positive patients may be associated with a high prevalence of smoking among all patients with VSCC and not just those with HPV-negative disease (20). In support of this finding, the current study did not find a significant difference in the smoking incidence between HPV-positive and HPV-negative patients. A previous genotyping study reported by Spaans and colleagues of over 500 gynecologic positive and HPV-negative patients. A previous genotyping study reported by Spaans and colleagues of over 500 gynecologic specimens (n = 25; ref. 21). In their study, hotspot mutational analysis with mass spectrometry was applied using a 13-gene panel assay purposefully developed for gynecologic cancers. The results of the current study highlight the greater sensitivity of NGS technology for mutational profiling and the identification of novel mutations. Although the oncogenes surveyed in this study represent a relatively broad molecular profile, the Ion Amplicon Panel is limited by the ability to survey a panel of only 50 genes. Furthermore, the ability to detect molecular aberrations such as gene fusions or copy number variation is beyond the scope of the technique. However, a primary advantage of the methods used for this analysis is the suitability for sequencing from FFPE with low DNA yield.

Notwithstanding, mutational profiles help to characterize HPV-positive and HPV-negative VSCC as separate diseases by reflecting distinct mutational patterns. HPV-negative disease was defined by somatic mutations in TP53, HRAS, PI3K, and CDKN2A and this is consistent with a previous report in VSCC (14). Conversely, the current analysis revealed that FGFR3 and PTEN mutations are features of HPV-positive VSCC, while PI3K mutations were observed in both subgroups. Although our sample size is relatively small, we documented an incidence of FGFR3 mutations in HPV-positive tumors of 14%. This new finding in HPV-positive VSCC has also been observed in other HPV-linked malignancies. In particular, a comprehensive study of a broad panel of cancer-associated genes in 120 matched samples in HNSCC revealed a similar mutational spectrum with an incidence of FGFR2/3 mutations of 17.6% (20). The study by Seiwert’s group performed a network-based analysis of genetic aberrations, which demonstrated altered networks associated with HPV-negative disease in TP53, cell-cycle, and oxidative stress pathways, whereas HPV-positive disease demonstrated alterations in the DNA damage pathway, fibroblast growth factor (FGF) signaling, and immunology-related genes.

FGFR3 S249C is a novel mutation that has not been previously reported in molecular studies in VSCC (14). This may be due, in part, to the limited numbers of HPV-positive patients in previous VSCC reports that screened specifically for FGFR3 mutations (11). FGF signaling is receptor-mediated by transmembrane tyrosine kinase receptors belonging to the immunoglobulin superfamily (22). Although elevated levels of FGFR expression have been described in a number of cancers, such as prostate, breast, lung, multiple myeloma, and HNSCC, it is uncertain as to whether aberrant expression is an underlying driver of carcinogenesis or a consequence of disease. Mutations in FGFR3, which are believed to be activating mutations, modulate cell growth and division by upregulation of the FGFR3 protein (23). FGFR3 is one of the most commonly mutated genes in low-grade human urothelial cell carcinoma (UCC). In UCC, most of the mutations are FGFR3 S249C, corresponding to the ligand-binding domain, with an additional cysteine leading to constitutive dimerization and receptor activation (24). Other somatic FGFR3 mutations found in UCC are identical to the germline-activating mutations that cause achondroplasia, a nonlethal skeletal dysplasia (25).

While the clinical relevance of this novel mutation in VSCC has not yet been explored, FGFR3 maybe a novel therapeutic target in the future management of HPV-driven VSCC. In the current study, all three FGFR3 mutation–positive cases are also strongly positive for p16 protein expression which is known to be upregulated in HPV-positive VSCC. All three cases are also positive for FGFR3 expression that appears to be more intense at the periphery of the tumor nests. Whether this plays any role in mesenchyme-to-tumor interaction remains to be determined. The other notable feature in FGFR3 mutation–positive tumors is marked intratumoral inflammatory infiltrate and the significance of this pathologic finding is the focus of future study.

Inconsistent reporting of HPV status in VSCC has made genetic analyses and patient prognosis difficult to interpret. The current analysis on HPV-characterized tumors found a considerable number of genomic aberrations in both HPV-negative and HPV-positive VSCC. Although a high rate of TP53 mutations in HPV-negative tumors was observed in this study, the significance of this is not clear. In HPV-positive malignancies, high-risk E6 and E7 independently induce genomic instability in normal cells (26) and contribute to the degradation of the tumor suppressors TP53 and RB, which drive cellular proliferation (27, 28). Other mechanisms that play a role in establishing a suitable environment for viral replication include altered DNA repair pathways and epigenetic changes, such as promoter methylation. Enhanced DNA damage is a feature of HPV-positive cancers as E6 and E7 increase the frequency of foreign DNA into the host genome, activate the ATM pathway, and abrogate cell-cycle checkpoints (28). In addition, HPV infection downmodulates the cellular immune response by repressing the IFN antiviral response. To better understand the genetic influence on VSCC and its relevance to the development of targeted therapies, future studies should include data on oncogenic HPV status.
In this study, HPV status was correlated with patient prognosis and no significant impact on TTF and OS was detected. While these findings may be dismissed on account of a limited sample size, they are consistent with previous reports that have shown that HPV is not an independent prognostic factor in VSCC (29, 30). This is in contrast to the improved prognosis reported in HPV-positive HNSCC patients (20) which has led to the justification of HPV and/or p16\(^{\text{INK4a}}\) as prognostic tumor biomarkers. The lack of prognostic value of HPV status in the current study may be explained by the relatively high mutational burden in the subgroup of HPV-positive patients. Furthermore, there were significantly more patients in the HPV-positive cohort with positive resection margins which may account for a poorer outcome. Similarly, the correlation with specific genetic mutations and patient outcomes did not lead to statistically significant results. However, trends observed in patient tumors with TP53 and FGFR3 mutations warrant further investigation in larger studies.

In addition to somatic mutations in both HPV-positive and HPV-negative tumors, this analysis also revealed the presence of genetic variants. In particular, the alteration at M54IL in KIT was found in both subgroups with a preponderance of this mutation seen in HPV-positive tumors. This finding supports the suggestion of an inherited component to VSCC that has previously been described in VSCC (18) as well as in other HPV-linked cancers (31). Although analysis of peripheral blood samples is beyond the scope of this study, a number of reports have focused on inherited polymorphisms to investigate immunodeficient populations who are at risk of persistent HPV infection and malignant transformation to VSCC. In particular, within the tumor necrosis family, a single-nucleotide polymorphism located in the lymphotxin alpha (LTA) gene, was significantly associated with a 51% increased risk of vulvar cancer and a 31% increased risk of cervical cancer (32). Indeed, genetic studies of VSCC on patient tumor must take into consideration the inherited genetic predisposition among patients as well as the effect of epigenetic modifications which will likely have a collective influence on response to systemic therapies.

Targeted therapeutic strategies in VSCC

Our results confirm that VSCC is molecularly diverse, with aberrations identified in no less than 15 oncogenes. The identification of a broad array of mutations of oncogenic significance in both HPV-positive and HPV-negative disease has important implications in the future management of these distinct subtypes of VSCC with targeted agents. As a rare gynecologic cancer, given the overlapping genomic profile of VSCC with other gynecologic cancers and HPV-positive cancers, the inclusion of VSCC patients in clinical trials with targeted agents in biomarker-selected patients will be essential to advancing the management of VSCC. In this respect, potential avenues of further research in VSCC are discussed below.

Dysregulation of the PI3K pathway is a common feature of many gynecologic cancers (33) and HPV oncogenic proteins have been shown to activate the PI3K pathway (34). In our series, there was a high prevalence of PI3KCA mutations in both HPV-positive and HPV-negative disease, making PI3K pathway inhibition an attractive treatment strategy in the management of patients with VSCC. Preclinical data in PI3K-mutant cell lines support the PI3K pathway as a target in gynecologic malignancies (35). However, the results of early clinical trials of single-agent PI3K/AKT/mTOR inhibitors demonstrate a limited duration of response (36, 37) and suggest that combination regimens will be required. Combination strategies which abrogate the feedback mechanisms and activate alternate signaling pathways, such as MAPK, may overcome therapeutic resistance to PI3K inhibition and are under active investigation (38).

Our study is the first to report FGFR3 mutations in VSCC although mutations in the FGFR family of receptor genes have been previously reported in other gynecologic cancers (39) as well as HPV-positive malignancies (20). FGFR inhibitors are under active investigation in these and other cancers known to harbor FGFR mutations, including NSCLC (40) and UCC [ref. 41; NCT 01004224, NCT02154490, NCT01752920]. Strategies for the clinical development of targeting FGFRs have included both nonselective agents such as tyrosine kinase inhibitors (TKI) as well as more potent selective inhibition of FGFR. There have been encouraging signs of clinical activity with cediranib, a VEGF/PDG/FGFR multi-TKI in a phase II trial in recurrent endometrial cancer (42). The newer generation compounds have shown greater tolerability and specifically, the pan-FGFR receptor TKI JNJ-42756493, has demonstrated responses in patients with glioblastoma, uterine, and endometrial cancer, all in tumors having FGFR2 or FGFR3 translocations (43). Therefore, logically targeting FGFR is important and FGFR inhibition should be actively pursued as a therapeutic strategy particularly in HPV-positive patients harboring FGFR mutations. However, despite promising biologic activity, the challenges in screening limited patient populations for which uniformity in molecular diagnostics is lacking is not to be underestimated (44).

In addition, other cellular pathways may be considered in the clinical development of targeted therapeutics in VSCC. Although direct targeting of HRAS and CTNNB1 regulators of cell division have been unsuccessful as a therapeutic strategy across many tumor types (45), targeting their downstream effectors may be a viable therapeutic alternative. In the case of HRAS-mutant disease, a number of MEK inhibitors are approved in the management of melanoma (Abramson, R. 2017. Overview of Targeted Therapies for Cancer. https://www.mycancergenome.org), and may have clinical application in VSCC. As an essential component of the WNT signaling pathway, modulation of CTNNB1 activity with WNT inhibitors in VSCC should also be considered as part of the strategy in the clinical development of these agents (46).

Conclusion

In this study, we provide supporting evidence that HPV-positive and HPV-negative VSCC should be regarded as two distinct clinical diseases, each characterized by a relatively high mutation burden with unique molecular profiles. Given the molecular diversity of VSCC, biomarker identification and screening will be critical to advancing the treatment of this disease with molecular therapies. VSCC harbors a number of therapeutically relevant mutations, including FGFR3, which should encourage the inclusion of this rare malignancy in future clinical trials with targeted agents.

Disclosure of Potential Conflicts of Interest

B. Lo reports receiving speakers bureau honoraria from Pfizer Inc. G.D. Goss is a consultant/advisory board member for AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly and Company, and Pfizer Inc. No potential conflicts of interest were disclosed by the other authors.
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**Oncogenic Mutations in HPV-characterized VSCC**

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