Priority Report

Cancer Research

Genomic Profiling of Penile Squamous Cell Carcinoma Reveals New Opportunities for Targeted Therapy

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Abstract

Penile squamous cell carcinoma (PeSCCA) is a rare malignancy for which there are limited treatment options due to a poor understanding of the molecular alterations underlying disease development and progression. Therefore, we performed comprehensive, targeted next-generation sequencing to identify relevant somatic genomic alterations in a retrospective cohort of 60 fixed tumor samples from 43 PeSCCA cases (including 14 matched primary/metastasis pairs). We identified a median of two relevant somatic mutations and one high-level copy-number alteration per sample (range, 0–5 and 0–6, respectively). Expression of HPV and p16 was detectable in 12% and 28% of patients, respectively. Furthermore, advanced clinical stage, lack of p16 expression, and *MYC* and *CCND1* amplifications were significantly associated with shorter time to progression or PeSCCA-specific survival. Notably, four cases harbored *EGFR* amplifications and one demonstrated *CDK4* amplification, genes for which approved and investigational targeted therapies are available. Importantly, although paired primary tumors and lymph node metastases were largely homogeneous for relevant somatic mutations, we identified heterogeneous *EGFR* amplification in primary tumor/lymph node metastases in 4 of 14 cases, despite uniform EGFR protein overexpression. Likewise, activating *HRAS* mutations occurred in 8 of 43 cases. Taken together, we provide the first comprehensive molecular PeSCCA analysis, which offers new insight into potential precision medicine approaches for this disease, including strategies targeting EGFR. *Cancer Res; 75(24); 5219–27. ©2015 AACR.*

Introduction

Penile squamous cell carcinoma (PeSCCA) accounts for over 95% of penile malignancies. Although rare in Western nations (incidence of 0.3–1/100,000; refs. 1, 2), PeSCCA can constitute up to 17% of malignant disease in men in the developing world (1). PeSCCA primarily affects older men (ages 50–70) and is rare in men less than 20 years of age (3). Risk factors for PeSCCA include high-risk HPV infection, phimosis, lichen sclerosis, and tobacco use (2). PeSCCA has a multitude of histologic subtypes that have distinct clinical and prognostic associations (3). The presentation of PeSCCA can be either ulcerated or exophytic, and the disease shows a tendency toward lymphatic dissemination toward the inguinal nodes, with 30% to 60% of patients having palpable lymphadenopathy at presentation (1). Although surgery alone can cure approximately 80% of patients with limited lymph node involvement (4), advanced PeSCCA requires incorporation of systemic therapies in the neoadjuvant, consolidative, or adjuvant setting to improve outcomes over single modality approaches (5), although the evidence level for treatment guidelines is relatively low (6).

The key molecular alterations driving PeSCCA development and potential therapeutic targets are incompletely understood. Loss of heterozygosity (LOH) at *CDKN2A* has been reported to correlate with aggressive PeSCCA behavior (7) and frequent alterations of *TP53* have also been reported (8). In addition, reports demonstrating high levels of EGFR overexpression via immunohistochemistry in upwards of 88% of cases of PeSCCA suggest this signaling pathway may have an important role in PeSCCA carcinogenesis (9), although only limited reports of anti-EGFR–based therapies in PeSCCA have been reported (10) As comprehensive profiling of somatic genomic alterations in PeSCCA has not been reported, we performed next-generation sequencing (NGS) on a cohort of PeSCCA cases representing the spectrum of pathologic grades, stages, and morphologic subtypes to identify driving alterations and potential precision medicine approaches.

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Materials and Methods

Tissue samples

Sixty formalin-fixed, paraffin-embedded (FFPE) tumor samples from 43 cases of PeSCCA (diagnosed between 2005 and 2013) were selected from the University of Michigan Department of Pathology Tissue Archive with Institutional Review Board (IRB) approval, including matched primary/metastatic tissue in 14 cases. Diagnostic slides were reviewed by board certified Anatomic Pathologists with subspecialty genitourinary pathology training (A.S. McDaniel and S.A. Tomlins) using the 2004 WHO diagnostic criteria to determine diagnosis, subtype, grade, and tumor content (by H&E estimation). Clinicopathologic data was obtained from medical records. DNA isolation was performed as described (11).

Targeted NGS

Targeted NGS of FFPE tumor tissue was performed with IRB approval. Targeted, multiplexed PCR-based NGS was performed essentially as described on each isolated tumor component using the DNA component of the Oncomine Comprehensive Panel (OCP), a custom panel comprised of 2,462 amplicons targeting 126 genes (11). Barcoded libraries were generated from 20 ng of DNA per sample and sequencing of multiplexed templates was performed using the Ion Torrent Personal Genome Machine (PGM) or Proton sequencer (details of sequencing and data analysis are described in Supplementary Materials and Methods). Data analysis, including variant prioritization using Oncomine, is described in Supplementary Materials and Methods. Sanger sequencing was performed to validate representative NGS calls, with details presented in Supplementary Materials and Methods.

Human papillomavirus analysis and typing

HPV detection and typing was performed via PCR of genomic DNA, followed by direct Sanger sequencing as described in Supplementary Materials and Methods.

p16 and EGFR immunohistochemistry

Immunohistochemistry for p16 and EGFR was performed using mouse monoclonal anti-p16 (clone E6H4) and rabbit monoclonal anti-EGFR (clone 5B7) antibodies as described in Supplementary Materials and Methods.

Statistical analysis

Statistical analyses were performed using R or MedCalc as described in Supplementary Methods and Methods. Two-tailed tests were used for all comparisons.

Results

NGS of PeSCCA

Our PeSCCA cohort comprised 60 tumor samples (from 43 patients) with an average patient age of 63 years (range 39–92). Circumcision status was available for 34 patients, with seven reported as circumcised and 27 as uncircumcised. Matched primary and metastatic tumor samples were available for 14 patients. Tumor samples spanned histologic grade: 13 low grade (30%), 2 low to moderate grade (5%), 19 moderate grade (43%), 6 moderate to poor grade (14%), and 4 poor grade (9%) tumors. Twelve patients (29%), 13 (31%), 3 (7%), and 14 (33%) tumors were clinical stage I, II, III and IV, respectively (considering nodal status at concurrent or subsequent resection if dissected). The histologic subclassification of the 42 primary tumors included 29 (67.4%) with usual type histology, 5 warty (11.6%), 4 papillary (9.3%), 2 basaloid (4.6%), 2 verrucous (4.6%), and 1 warty-basaloid (2.3%), representative of the usual distribution seen at our institution and similar to other published cohorts (3). One case consisted of a metastasis only, with no primary tumor available. With a median follow-up of 1.28 years (range 0.1–7.96), 3 had documented local recurrences, 6 patients showed distant progression, and 8 patients died of disease. Detailed clinicopathologic data are provided in Supplementary Table S1.

Targeted NGS was performed on 20 ng of genomic DNA extracted from FFPE tissues, using the DNA component of the OCP, with sequencing performed using IonTorrent PGM or Proton sequencers. The OCP, which will be used in the NCI MATCH trial (a sequencing informed umbrella protocol), is comprised of 2,462 amplicons targeting 126 genes selected on the basis of pan-cancer analysis that prioritized recurrently altered oncogenes, tumors suppressors, and genes subject to high-level copy-number alterations (CNA), combined with a comprehensive analysis of known/investigational therapeutic targets (11). Sequencing resulted in an average targeted base coverage depth of 535× and 309 total variant calls per sample (detailed coverage statistics are provided in Supplementary Table S2).

Identification of prioritized somatic variants in PeSCCA

After sequencing and data analysis, somatic variants were filtered using predefined Oncomine criteria to nominate relevant alterations (driving or potentially targetable), resulting in a total of 94 nonsynonymous point mutations, stopgains/nonsense mutations, or short insertion/deletions (indels) present in the 60 samples (median 2, range 0–5). *TP53*, *CDKN2A*, *PIK3CA*, and *HRAS* were the most frequently mutated genes, with variants in 29, 20, 9, and 8 samples, respectively. Detailed information describing all prioritized variants is provided in Supplementary Table S3. Representative somatic variant calls (7/7 tested, 100%) were validated by Sanger sequencing of genomic DNA, with representative IGV and Sanger sequencing of a prioritized variant shown in Supplementary Fig. S1.

Identification of prioritized CNAs in PeSCCA

Copy-number analysis demonstrated 72 high-level, prioritized CNAs (median 1, range 0–6), including 54 CNA gains and 18 CNA losses. High-level gains were most frequently identified in *MYC* (11 samples), *CCND1* (8 samples), *SOX2* (8 samples), *ATP11B* (5 samples), *EGFR* (6 samples), and *TERT* (4 samples). Of the 18 high-level losses, 13 (72%) involved *CDKN2A*. The loss of *CDKN2A* in sample 3B resulted in loss of heterozygosity of a germline I49T variant (based on variant allele frequency), consistent with complete *CDKN2A* inactivation (data not shown). Prioritized likely gain- or loss-of-function somatic mutations in oncogenes and tumor suppressors (see below) and high-level CNAs for each case are shown in an integrative heatmap (Fig. 1 and Supplementary Fig. S2), and complete copy-number profiles are shown in Fig. 2.

Heterogeneity of prioritized alterations in paired primary tumors and lymph node metastases

Matched primary tumor and metastasis samples showed 82.6% (19/23) and 85% (17/20) SNV/indel concordance considering total and prioritized alterations, respectively. Matched tumor and metastasis samples showed decreased CNA concordance, with only 14 of 34 (41.6%) prioritized CNAs shared between matched

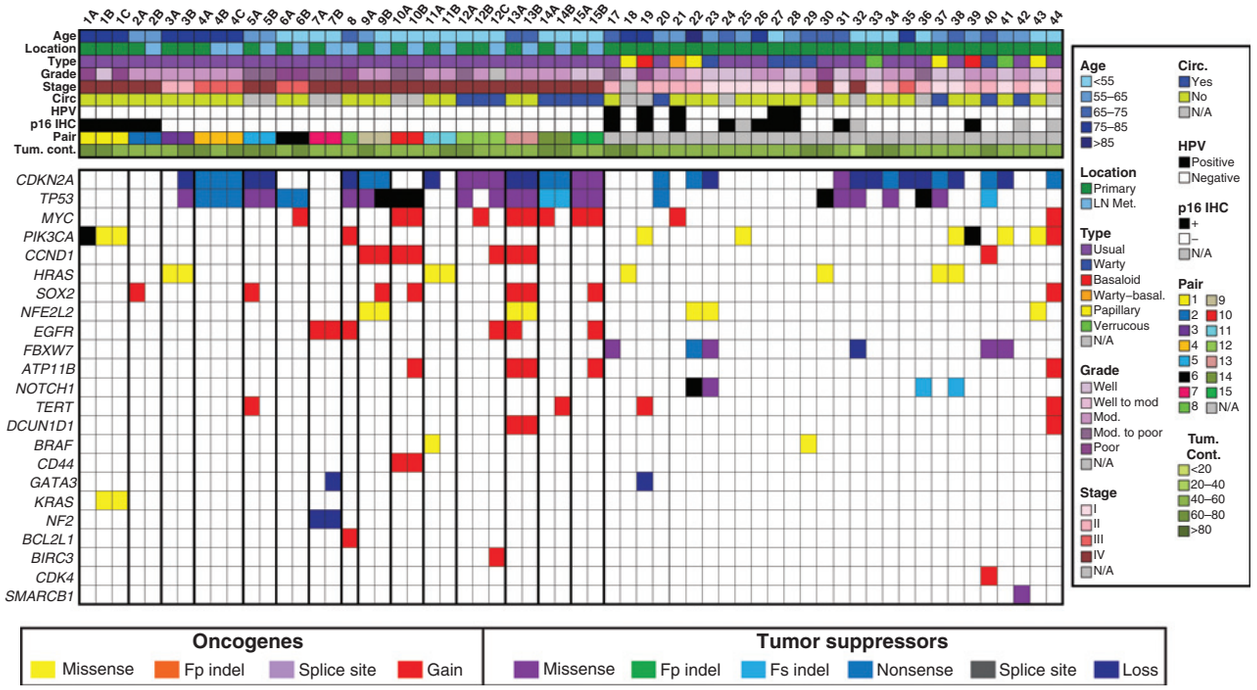

Figure 1.

Integrative molecular profiling of somatic genomic alterations in PeSCCA. Heatmap of nonsynonymous mutations and high-level CNAs from 60 PeSCCA samples (from 43 patients), including 14 matched tumor/lymph node metastases pairs. Samples were assessed by targeted NGS using the DNA component of the OCP, a custom panel comprised of 2,462 amplicons targeting 126 genes selected on the basis of pan-cancer somatic alteration analysis coupled with potential therapeutic prioritization. Rows represent genes and are ordered in decreasing variant frequency and columns represent individual samples in numerical order with paired tumor samples on the left. Clinicopathologic features are indicated in the header according to the legend (right); paired samples are indicated by color in the header. Prioritized alteration type in oncogenes and tumor suppressors are indicated according to the legend (bottom). Circ, circumcision status; Tum. Cont, tumor content; LN Met, lymph node metastasis; basal, basaloid; Mod, moderate; Fp, frame-preserving; FS, frame-shift.

pairs. Estimated tumor content varied by a maximum of only 20% in matched samples and absence of called variants in samples lacking alterations were confirmed by visual inspection of IGV and copy-number profiles, respectively. Together, these results support substantial intertumoral heterogeneity between prioritized alterations in matched primary/metastasis pairs, particularly for CNAs.

HPV/p16 (CDKN2A) status and association with somatic alterations in PeSCCA

Given the importance of HPV in the pathogenesis of certain PeSCCA subtypes, we next determined the HPV status of all patients in our cohort. We assessed for HPV DNA via PCR of FFPE-isolated genomic DNA with two distinct consensus primer sets (GP5/GP6 and My09/My11) followed by direct Sanger sequencing of the PCR products. As shown in Supplementary Table S4, HPV DNA was detected in 5 of the 43 patients (12%), with HPV 16 present in four samples (samples 19, 21, 27, and 28) and HPV 33 present in one sample (sample 17). HeLa cells and primary cervical cancer tissue samples were used as positive controls and were positive using both primer sets (data not shown). Of note, HPV-positive PeSCCA samples showed a median of 1 genomic alteration (including somatic variants and CNAs) per sample (range, 0–3), which was significantly lower than HPV negative samples (median = 2 alterations, range 0–10, two-sided Student unpaired *t* test $P = 0.04$).

As p16 expression (encoded by *CDKN2A*) has also been used as an HPV infection surrogate, we evaluated p16 by immunohistochemistry (Supplementary Table S4) for all samples with available tissue (54/60 samples). Diffuse positive p16 expression was noted in 11 patients (28%), with representative photomicrographs shown in Supplementary Fig. S3. All cases found to harbor HPV DNA by PCR also expressed p16, and all matched primary and metastases showed concordant p16 expression status.

HPV status and p16 expression were both significantly associated with histologic cancer type (two-sided Fisher exact test $P = 0.003$ and $P = 0.04$, respectively), with basaloid, warty, and warty-basaloid types showing more frequent positivity than usual, papillary and verrucous tumors, as expected (12). We also assessed associations between HPV status/p16 expression and alteration status in genes harboring ≥ 5 prioritized alterations. p16 expression was significantly associated with lack of *CDKN2A* and *TP53* alterations (two-sided Fisher exact test $P = 7.0\text{E}{-5}$ and $P = 0.0004$, respectively; both significant after multiple hypothesis testing correction). Likewise, HPV positivity was also associated with lack of prioritized *CDKN2A* alterations ($P = 0.01$) and was more frequent in samples lacking *TP53* alterations ($P = 0.05$).

Association of prioritized somatic alterations and clinicopathologic parameters

No significant associations between the number of prioritized alterations (single nucleotide variant/indel only, CNA only, or

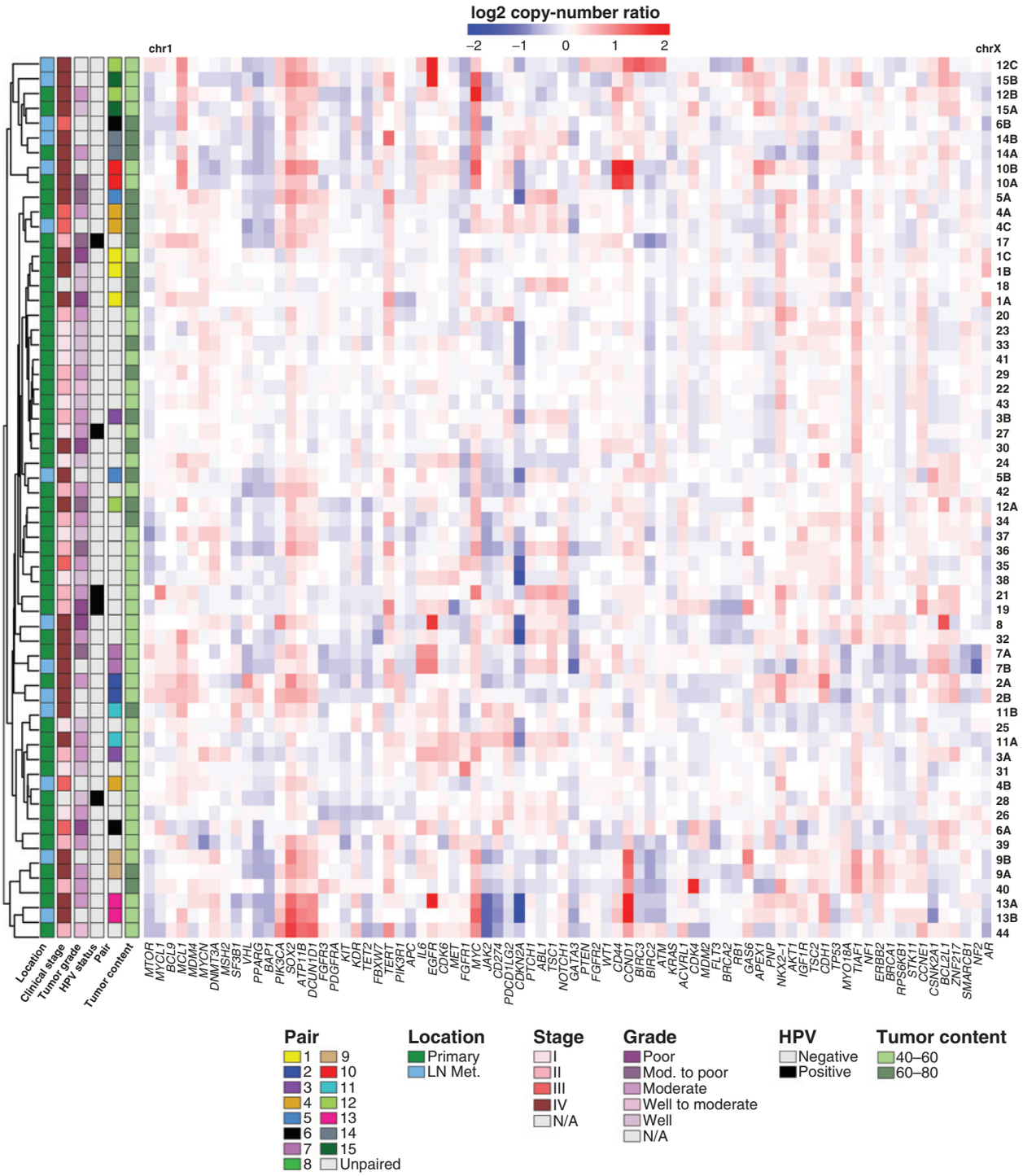

Figure 2.
PeSCCA somatic copy-number heatmap. GC content corrected, normalized read counts per NGS amplicon were divided by those from composite normal tissue, yielding a copy-number ratio for each gene (cancer/composite normal), with red and blue indicating gain and loss, respectively, according to the $\log_2$ color scale (above). Columns represent individual targeted genes in genome order (from chromosome 1 to X). Clinicopathologic features are indicated below the heatmap as in Fig. 1.

combined) and tumor grade and/or clinical stage were present. Furthermore, no significant relationship existed between overall alteration of an individual gene and tumor grade and/or stage. However, after controlling for grade, age, and tumor content, a one-unit (one alteration) increase in total number of alterations in the most frequently mutated genes (*CDKN2A/EGFR/MYC/HRAS/TP53*) was associated with 2.9 times the odds of being in a higher clinical stage (ordinal logistic regression, OR 95% confidence interval, 1.5–6.6; $P = 0.005$).

Likewise, the Kaplan–Meier analysis demonstrated that increasing clinical stage was significantly associated with shorter event-free survival (combined progression or PeSCCA specific death, log-rank test $P = 0.0005$, log-rank test for trend $P = 0.0001$; Fig. 3A). Among other clinicopathologic parameters, positive p16 expression was significantly associated with longer event-free survival (log-rank test $P = 0.03$, Fig. 3A). Although all events occurred in HPV-negative patients, HPV status was not significantly associated with event-free survival ($P = 0.23$; data not shown). Likewise, although Kaplan–Meier analysis did not demonstrate significant differences in event-free survival by histologic subtype (Supplementary Fig. S4), we had a very limited number of high-risk tumor subtypes (basaloid and mixed basaloid tumors) with short follow-up. Of note, while 28% of combined high and intermediate risk (usual) tumor subtypes had events, none of the low-risk subtype PeSCC (papillary, verrucous, or warty) in our cohort had events.

We also assessed the association of each of the genes with $\geq$5 prioritized alterations with event-free survival. As shown in Fig. 3B, alterations in *CCND1* (log-rank test $P < 0.0001$), *MYC* ($P < 0.0001$), *TP53* ($P = 0.01$), *EGFR* ($P = 0.03$), and *ATP11B* ($P = 0.045$) were significantly associated with shorter event-free survival, with *CCND1* and *MYC* remaining significant after multiple hypothesis testing correction. Given the small number of alterations in some genes and the relatively few numbers of events, these results should be considered exploratory.

Potential precision medicine approaches for PeSCCA

Given the lack of available targeted therapies for locally advanced or metastatic disease, we were particularly interested in potentially actionable alterations identified through our comprehensive profiling. Our prioritized variant list was evaluated for potential actionability using the Oncomine database. Briefly, for each sample the "most actionable" alteration was identified by giving preference to (i) variants referenced in FDA drug labels, (ii) variants referenced in NCCN treatment guidelines for PeSCCA or other squamous cell carcinomas (SCC), (iii) variants referenced in NCCN treatment guidelines in other cancer types, and (iv) variants referenced as inclusion criteria for a clinical trial. This approach prioritized potential treatment strategies directed against KRAS (in one patient), CDK4 (in one patient), and EGFR (in 5 patients).

Intertumoral EGFR amplification heterogeneity in paired primary tumors and lymph node metastases and discordance with EGFR protein expression

EGFR overexpression has been reported in a large percentage of PeSCCA, and investigational use of anti-EGFR–targeted therapies has been reported in advanced PeSCCA (10). We performed EGFR IHC on 26 tissue blocks from 5 patients with matched primary and metastatic foci, with multiple sections tested from each (see Supplementary Materials and Methods for details). EGFR showed strong diffuse expression in all tested samples. Furthermore, as shown in Fig. 4, despite identical histology and uniform EGFR overexpression by IHC, *EGFR* copy-number status showed significant heterogeneity between paired samples from the same patient (i.e., primary and metastatic foci). For example, while patient 7 showed concordant one copy *EGFR* gains in both the primary and lymph node samples, patient 13 displayed a high-level *EGFR* gain in the primary tumor that was not present in the metastatic site (while other CNAs were present in both samples). Conversely, for patient 15, *EGFR* showed no significant CNA in the primary tumor but the profiled metastasis showed a high-level gain. In each case, tumor content estimation by histology and variant allele frequency of clonal alterations were sufficient for identification of high-level CNAs in each component. Interestingly, EGFR protein expression did not correlate with *EGFR* amplification status, as samples with and without *EGFR* high-level gains showed similar expression by IHC (Fig. 4). Taken together, our results support *EGFR* gains/amplifications in approximately 10% of PeSCCA cases, with significant heterogeneity between paired primary tumors and lymph node metastases. Similarly, EGFR expression by IHC does not appear to be correlated with *EGFR* copy number.

Comparison of PeSCCA with other SCCAs

We compared the prioritized somatic alteration spectrum in our PeSCCA cohort to integrated molecular profiling data for lung (Lu), head and neck (HN), and cervical (Ce) SCCA using The Cancer Genome Atlas (TCGA) studies in cBioPortal (13). As shown in Supplementary Fig. S5, across of a set of the 9 most frequently altered genes (and *KRAS*) in our PeSCCA cohort (at least one gene altered in 87% of our PeSCCA samples), at least one of these genes was altered in 98%, 92%, and 52% of LuSCC, HNSCC, and CeSCC samples, respectively, (two-sided Fisher exact test $P < 0.0001$ for each type vs. CeSCC). These differences were driven largely by more frequent *TP53* and/or *CDKN2A* alterations in PeSCCA (63% samples altered), LuSCC (90% samples altered), and HNSCC (79% samples altered) compared with CeSCC (4%, two-sided Fisher exact test $P < 0.0001$ for each type vs. CeSCC), consistent with the much greater rate of HPV infection in CeSCC.

Discussion

The genomic landscape of PeSCCA is only partially appreciated, with a limited number of single gene studies (focusing on *TP53*, *CDKN2A*, and *EGFR*), and a single report assessing genome-wide CNAs via array comparative genomic hybridization (14). Here, via targeted NGS on routine FFPE archival tissues, we report the first comprehensive assessment of putative driving somatic genomic alterations with near term potential actionability in PeSCCA utilizing a representative cohort of PeSCCA tumors (including primary tumor and lymph node metastasis pairs). *TP53*, *CDKN2A*, *PIK3CA*, *MYC*, *HRAS*, and *SOX2* were among the most frequently altered genes. No significant associations were present between mutation status for an individual gene and tumor grade, stage, or histology. Recent pan-cancer integrated genomic analyses have demonstrated a molecular convergence among SCCs from various anatomic sites with frequent alterations in *TP53*, *CDKN2A*, *PIK3CA*, *MYC*, and *SOX2* noted (15). Of note, SCCA from organ sites with high HPV infection rates [e.g., cervix (Ce)] show much lower *TP53* and *CDKN2A* alteration

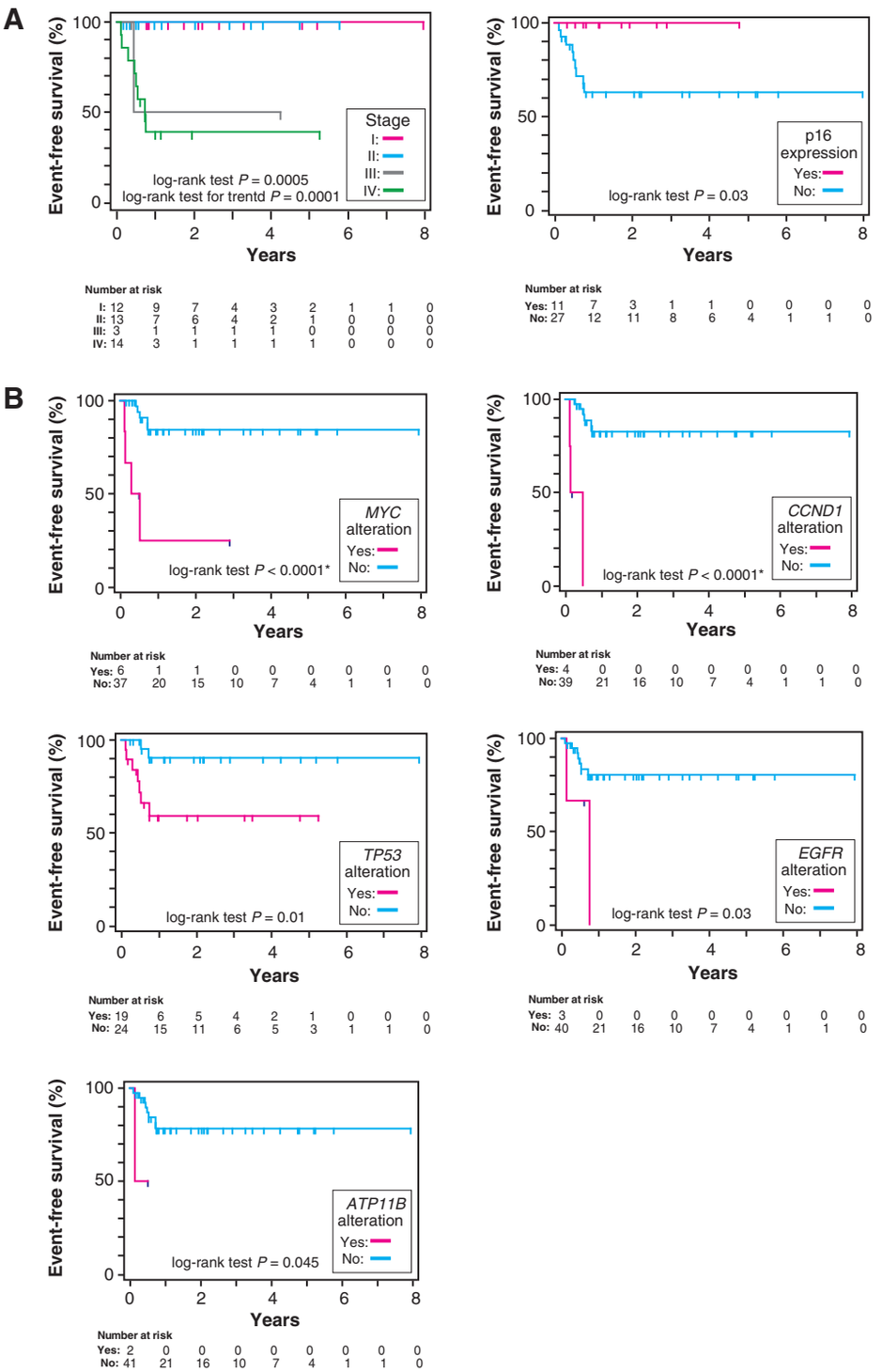

Figure 3.
Kaplan–Meier analysis of clinicopathologic and genomic alterations significantly associated with event-free survival. Outcome analysis was performed for all profiled patients (considering primary specimens if matched primary tumors/lymph node metastases were profiled) using combined distant progression and PeSCCA-specific death as a composite endpoint. A, clinicopathologic parameters significantly associated with event-free survival. Log-rank test *P* values and numbers at risk are shown. B, as in A, but assessing prioritized alteration status in frequently altered genes in our cohort. *, Log-rank *P* values remaining statistically significant after Bonferroni multiple comparison correction based on the number of genes assessed.

rates (see Supplementary Fig. S5), consistent with *TP53* and *RB1* inactivation by the HPV E6 and E7 oncoproteins (16). Taken together, our data support environmentally exposed epithelia (e.g., the penile surface) as sharing a common set of genomic alterations driving SCCA development.

We detected high-risk HPV infection in 12% of samples, which is lower than previously reported for most PeSCCA cohorts (22%–72% infection frequency; reviewed in ref. 2) but comparable with rates recently reported in another North American cohort (17). The reasons for the lower rate of HPV

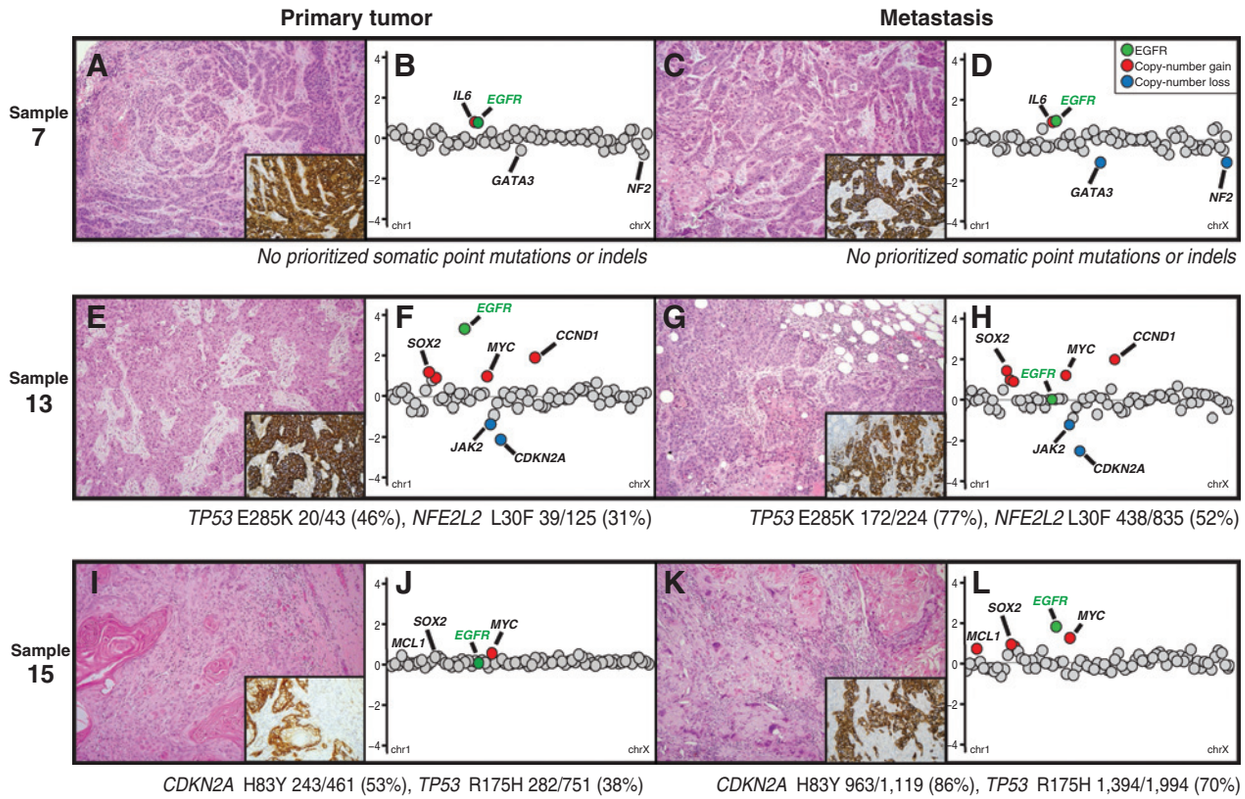

Figure 4.
Heterogeneity of *EGFR* amplifications in paired PeSCCA primary tumor/lymph node metastases. Histology, EGFR expression by IHC, and copy-number profiles for three matched tumor/lymph node metastasis pairs are shown. Histology from paired primary tumor and metastatic foci were highly concordant (H&E stains at ×100 are shown in A, C, E, G, I, and K). EGFR IHC shows strong intense membranous staining in all samples (insets of A, C, E, G, I, and K). Corresponding copy-number plots and prioritized somatic mutations for these samples are shown in B, D, F, H, J, and L. Each point represents the $\log_2$ copy-number ratio for a targeted gene (shown in genome order). For sample 7, *EGFR* is amplified in both the tumor and metastases (green points). For sample 13, *EGFR* is highly amplified in the primary tumor (F) but no CNA is present in the paired metastasis (H). Conversely, in sample 15, *EGFR* shows no CNA in the primary tumor (J) but is amplified in the metastatic focus (L). Additional CNAs (high-level gains and losses shown in red and blue, respectively) and prioritized somatic mutations are highly concordant across paired samples.

positivity reported here and by Bezerra and colleagues compared with previously published international cohorts is not entirely clear; however, differences in patient demographics, barriers to health care access, and clinical practice pattern variations may all contribute (3). The mutational burden was significantly less in HPV positive versus negative PeSCCA in our cohort, and no HPV-positive PeSCCA harbored *TP53* alterations nor *EGFR* amplifications, consistent with SCCs of other sites (18–20). In our cohort, p16 overexpression was found in 28% of patients, including all HPV-positive cases. Like SCCs of other organs, our results support HPV-driven PeSCCA as having distinct biologic and epidemiologic characteristics.

Of note, p16 positivity was significantly associated with longer event-free survival (combined progression or PeSCCA-specific death) and no HPV-positive patients had events (although results were not statistically significant). Previous studies have generally shown HPV and p16 positivity to associate with favorable prognosis (17, 21–23). Likewise, although exploratory due to the limited number of samples with alterations and events, *MYC* and *CCND1* amplifications were both significantly associated with decreased event-free survival.

While surgery is curative for many patients with PeSCCA, there is a decided lack of therapeutic options, particularly targeted therapies, with aggressive disease, although both radiotherapy- and chemotherapy-based approaches can be effective in selected clinical scenarios (24–26). Rational approaches targeting the EGFR signaling axis have been employed on the basis of descriptions of high EGFR expression in most PeSCCA. While only a small number of PeSCCA patients have been treated with anti-EGFR therapies, results from the largest series so far only showed a partial response rate of 23.5% (10).

We show discordance between EGFR expression via IHC and *EGFR* copy number, with only 10% (6/60) samples showing *EGFR* amplification near uniform EGFR overexpression in all PeSCCA. Our *EGFR* amplification rate is comparable with that reported in SCCs from other sites (e.g., ∼15% in head and neck, ∼7% in lung, and 9%–12% in vulvar SCCA; refs. 18–20; Supplementary Fig. S5). Given the limited success of anti-EGFR therapies in PeSCCA despite EGFR protein overexpression, we hypothesize that tumors with *EGFR* amplification may be more sensitive to EGFR targeting. A planned trial evaluating cetuximab in metastatic PeSCCA stipulates wild-type *KRAS* as an inclusion

criterion (NCT02014831). Importantly, our cohort showed a higher frequency of activating *HRAS* (5 patients with G12S/D mutations, one with Q61K) versus *KRAS* alterations (one patient with G12S). Although the clinical impact of activating *HRAS* mutations and EGFR therapy has not been investigated, extended *KRAS* and *NRAS*-activating mutations predict resistance to EGFR-based therapy in colorectal cancer (27, 28). Together, our data suggest that activating *HRAS* mutations and *EGFR* CNA heterogeneity (between paired primary tumors and metastases) may complicate EGFR-targeted therapy efforts in PeSCCA.

In summary, we have performed the first systematic exploration of clinically relevant somatic genomic alterations in PeSCCA, finding opportunities for potential therapeutic targets as well as similarities to SCCs from other sites. While a targeted genomic analysis as described has limited capabilities for detecting complex structural rearrangements, novel mutations, and germline variants, the panel utilized herein is specifically curated for detecting alterations associated with approved, guideline-referenced, or current clinical trial therapeutic agents to maximize clinical relevance. This approach may have potential applications in characterizing routine pathologic material for rationally driven clinical trials in PeSCCA to develop novel precision medicine approaches for a disease with few therapeutic options.

Disclosure of Potential Conflicts of Interest

S. Sadis is a Director, Oncology at Thermo Fisher Scientific. S.A. Tomlins reports receiving a commercial research grant and has received travel support from ThermoFisher Scientific. D.R. Rhodes and S.A. Tomlins are co-founders and equity holders in Strata Oncology, Inc. No potential conflicts of interest were disclosed by the other authors..

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