IGSF4 Methylation as an Independent Marker of Human Papillomavirus–Positive Oropharyngeal Squamous Cell Carcinoma

Kang Mei Chen, MD; Josena K. Stephen, MD; Shaleta Havard, AuD; Meredith Mahan, MS; George Divine, PhD; Maria J. Worsham, PhD

IMPORTANCE Human papillomavirus (HPV) is a known causative agent for oropharyngeal squamous cell carcinoma (OPSCC). Whereas it is becoming more firmly established that HPV-positive head and neck squamous cell carcinoma is associated with better survival outcomes, believed to be because of better response to chemoradiation therapy, the specific mechanisms for these improved survival outcomes remain underexplored.

OBJECTIVE To examine the relationship between HPV status and promoter methylation in an OPSCC cohort.

DESIGN, SETTING, AND PARTICIPANTS Real-time quantitative polymerase chain reaction was used to examine oncogenic HPV type 16 in a retrospective cohort of 121 patients with primary OPSCC. Aberrant promoter methylation of IGSF4, DAPK1, and ESR1 genes, known to be methylated in head and neck squamous cell carcinoma, including OPSCC, was examined by means of quantitative methylation-specific polymerase chain reaction.

INTERVENTIONS Patients received standard therapy.

MAIN OUTCOMES AND MEASURES Univariate associations between HPV and methylation were analyzed using Fisher exact tests followed by multivariable logistic regression. Cox proportional-hazards regression was used to model the risk of death given age, race, sex, HPV status, methylation, stage, smoking, and treatment.

RESULTS In univariate logistic regression analyses, HPV-positive status was significantly associated with Caucasian race (P = .02), treatment (radiotherapy only, P = .01; chemoradiotherapy, P = .007), and IGSF4 methylation (P = .005). The final multivariate logistic model, after controlling for patient characteristics (sex, age, smoking, race, and treatment) with backward variable selection among genes, retained IGSF4 methylation (OR, 4.5 [95% CI, 1.6-12.8]; P = .005), Caucasian race (OR, 2.9 [95% CI, 1.0-8.3]; P = .053), treatment (radiotherapy only vs neither: OR, 11.62 [95% CI, 2.02-66.82]; P = .02; chemoradiotherapy vs neither: OR, 11.15 [95% CI, 1.92-64.65]; P = .01), male sex (OR, 4.7 [95% CI, 1.3-17.0]; P = .02), and younger age (OR, 0.9 [95% CI, 0.90-1.0]; P = .008) as independent predictors of HPV-positive status. Cox regression modeling indicated HPV-negative status, age, male sex, smoking, and radiation treatment as independent predictors of mortality.

CONCLUSIONS AND RELEVANCE Methylation of IGSF4 is an independent predictor of HPV-positive status. DNA methylation in conjunction with HPV infection appears to play a role in OPSCC.
Multiple risk factors are associated with the development of head and neck squamous cell carcinoma (HNSCC), the most common ones being tobacco and alcohol use. In addition to these, infection with the human papillomavirus (HPV) is also a causative agent for some HNSC
cancers.3,2,3 and an independent risk factor for oropharyngeal squamous cell carcinoma.3,5 (OPSCC). A recent meta-analysis of 5681 patients with HNSC
cancers revealed that the prevalence of HPV infection was significantly higher among patients with OPSCC (35.6%) than among those with oral (23.5%) or laryngeal squamous cell carcinoma.6 Approximately 95% of these HNSCC subgroups contain high-risk HPV type 16 (HPV16) genomic DNA sequences.8

Human papillomavirus status underlies HNSCC pathogenesis and is of clinical significance. Patients with HPV-positive tumors, particularly those with OPSCC, have improved prognosis.3,9 Whereas it is becoming more firmly established that HPV-positive HNSCC is associated with better survival outcomes, believed to be because of better response to chemoradiotherapy,9 the specific mechanisms for these improved survival outcomes remain underexplored. Molecular alterations are known to occur early in HNSCC,10 and epigenetic events of promoter hypermethylation represent important tumor-specific markers occurring early in tumor progression. Our group has demonstrated, using high-throughput methods, the contribution of both genetic11,12 and epigenetic events,13 often working together,14 in the development and progression of HNSCC.15 The goal of our study was to examine the status of HPV16 infection and methylation status of IGSF4, DAPK1, and ESR1, candidate genes associated with HNSCC pathogenesis,14,15 in an OPSCC cohort.16

Methods

Cohort

The retrospective study cohort comprised 121 patients with primary OPSCC.16 To examine the relationship between HPV status and promoter methylation, variables included age, sex, race as self-reported, smoking, treatment (radiotherapy, chemotherapy), HPV16 status, and methylation status of IG SF4, DAPK1, and ESR1. This study was approved by the Henry Ford Health System institutional review board. Informed consent was waived due to the retrospective study design.

DNA Extraction

Whole 5-μm tissue sections or microdissected OPSCC lesions and adjacent normal tissue, when present, were processed for DNA extraction as previously described.17

HPV16 Detection by Means of Quantitative Polymerase Chain Reaction

Tumor HPV DNA concentrations were measured using a real-time quantitative polymerase chain reaction (PCR) system as previously described.16 The cutoff value for HPV16-positive status was ≥0.03 (≥3 HPV genome copies/100 cells).16,18,19

Bisulfite Modification and Quantitative Methylation-Specific Polymerase Chain Reaction (QMSP) Assay

Genomic DNA (100 ng) from formalin-fixed paraffin-embedded OPSCC tissue and control universal methylated DNA (Chamicon International Inc) were modified using the EZ DNA methylation gold kit (Zymo Research) during which methylated DNA is protected and unmethylated cytosine is converted to uracil.17 Quantitative methylation-specific PCR detects the presence of neoplastic DNA with a sensitivity of 1 cell in 1000.20 This PCR approach is more sensitive than conventional PCR and more specific due to the use of an internally binding, fluorogenic hybridization probe.21,22 An advantage of QMSP is that it can measure the amount of methylation in a sample. Primers and probes were specifically designed to amplify IG SF4, DAPK1, and ESR1 genes.21,22 Primers and probes to an internal reference gene (ACTB) in run in parallel to standardize the input DNA.

Polymerase chain reaction was carried out using the EpiTect MethyLight PCR Kit (Qiagen) according to the manufacturer’s protocol in 96-well plates using the 7900HT Sequence detector (Applied Biosystems). Each plate included multiple water blanks and serial dilutions of a universal methylated positive control DNA (100 ng/μL, Chemicon), which was bisulfite converted in the laboratory for constructing the standard curve. These were run with the samples on each plate in duplicate. To determine the relative levels of methylated promoter DNA in each sample, the values of the gene of interest were compared with the values of the internal reference gene (ACTB) to obtain a ratio that is then multiplied by 100 to give a percentage value (gene of interest/reference gene × 100). The methylation results obtained by QMSP in our analysis were classified to give a binary status, in which any quantity of methylation in a sample was considered positive.25

Data Analysis

Univariate associations of HPV and methylation were analyzed using Fisher exact tests and by logistic regression for crude odds ratios, followed by multivariable logistic regression. Backward variable selection among genes was used to arrive at a final model. Univariate survival analyses were performed using log-rank tests. A multivariable Cox proportional hazards model was built to identify possible independent predictors of survival. Race, sex, HPV status, smoking, stage, treatment (radiotherapy and chemotherapy), and methylation were analyzed as categorical variables, whereas age was examined as a continuous variable. Statistical significance was set at P < .05, and all analyses were performed using SAS, version 9.2.

Results

Of the 121 primary OPSCCs, 67 were HPV negative and 51 HPV positive.16 In 3 cases, HPV status was not evaluable, yielding a final cohort of 118 cases of OPSCC with definitive HPV status. This cohort comprised 68 Caucasians, 49 African Americans (AAs) (42%), and 1 patient of unknown race; 92 were men, and 26, women. Of the 118 cases, methylation status for ESR1, IG SF4, and DAPK1 were obtained for 93, 104, and 105 cases,
respectively. Demographic characteristics by HPV status are presented in Table 1. Age of the cohort ranged from 37.5 to 88.2 years. Of the 118 cases, 25 were early stage (1 carcinoma in situ [stage 0], 24 stage 1 and 2), 90 were late stage (stage 3 and 4), and in 3 cases stage was missing. Analysis of the cohort characteristics of age, race, sex, HPV status, stage, marital status, smoking, treatment, and date of diagnosis has been previously reported.16

Caucasian race was associated with HPV positivity (71% vs 29% for AA race; \( P = .02 \)). Comparison of methylation status of IGSF4, DAPK1, and ESR1 by HPV status demonstrated that IGSF4 was more frequently methylated in HPV-positive than HPV-negative patients (\( P = .004 \)) (Table 2). In univariate logistic regression analyses (Table 3), Caucasians with OPSCC were more likely to be HPV positive as compared with AAs with OPSCC (OR, 2.5 [95% CI, 1.2-5.5]; \( P = .02 \)). Methylation of IGSF4 was also associated with HPV-positive status (OR, 3.2 [95% CI, 1.4-7.3]; \( P = .005 \)). Treatment (radiotherapy only vs neither or chemoradiotherapy vs neither) was associated with being HPV positive (OR, 4.64 [95% CI, 1.40-15.40]; \( P = .01 \); and OR, 5.53 [95% CI, 1.60-19.09]; \( P = .007 \), respectively). Correlation of smoking with HPV-positive status in never vs current and past vs current smokers was not statistically significant (\( P = .06 \) for both).

The final multivariable logistic regression model (Table 3), after controlling for patient characteristics (sex, age, smoking, race, and treatment) with backward gene selection, indicated IGSF4 methylation as an independent predictor of HPV-positive status (OR, 4.5 [95% CI, 1.6-12.8]; \( P = .005 \)). In this analysis, age as a continuous variable (OR, 0.9 [95% CI, 0.90-1.0]; \( P = .008 \)), sex as male (OR, 4.7 [95% CI, 1.3-17.0]; \( P = .02 \)), race as Caucasian (OR, 2.9 [95% CI, 1.0-8.3]; \( P = .053 \)), and treatment (radiotherapy only vs neither: OR, 11.62 [95% CI, 2.02-66.82]; \( P = .02 \); chemoradiotherapy vs neither: OR, 11.15 [95% CI, 1.92-64.65]; \( P = .01 \)) were also independent predictors of HPV status.

Univariate overall survival (Table 4) was significantly associated with HPV status (\( P = .003 \)), age (\( P = .04 \)), smoking

### Table 1. Demographic Characteristics by Human Papillomavirus (HPV) Status

<table>
<thead>
<tr>
<th>Variable</th>
<th>HPV Status</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (( n = 67 ))</td>
<td>Positive (( n = 51 ))</td>
</tr>
<tr>
<td><strong>Age, mean (SD) [range], y</strong></td>
<td>62.4 (9.8) [37.5-88.2]</td>
<td>60.5 (11.6) [41.9-87.7]</td>
</tr>
<tr>
<td><strong>Race, No. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>32 (48)</td>
<td>36 (71)</td>
</tr>
<tr>
<td>African American</td>
<td>34 (52)</td>
<td>15 (29)</td>
</tr>
<tr>
<td><strong>Sex, No. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>49 (73)</td>
<td>43 (84)</td>
</tr>
<tr>
<td>Female</td>
<td>18 (27)</td>
<td>8 (16)</td>
</tr>
<tr>
<td><strong>Smoking, No. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>42 (63)</td>
<td>21 (41)</td>
</tr>
<tr>
<td>Past</td>
<td>19 (28)</td>
<td>21 (41)</td>
</tr>
<tr>
<td>Never</td>
<td>6 (9)</td>
<td>9 (18)</td>
</tr>
<tr>
<td><strong>Stage, No. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early (0-2)</td>
<td>18 (27)</td>
<td>7 (14)</td>
</tr>
<tr>
<td>Late (3-4)</td>
<td>47 (70)</td>
<td>43 (86)</td>
</tr>
<tr>
<td>Missing</td>
<td>2 (3)</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Treatment, No. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neither radiotherapy nor chemotherapy</td>
<td>20 (30)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Radiotherapy only</td>
<td>28 (42)</td>
<td>26 (51)</td>
</tr>
<tr>
<td>Both radiotherapy and chemotherapy</td>
<td>19 (28)</td>
<td>21 (41)</td>
</tr>
</tbody>
</table>

### Table 2. Methylation and Human Papillomavirus (HPV) Status for 118 Patients

<table>
<thead>
<tr>
<th>Methylation</th>
<th>HPV Status, No. (%)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DAPK1 (( n = 105 ))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>28 (46)</td>
<td>17 (39)</td>
</tr>
<tr>
<td>Yes</td>
<td>33 (54)</td>
<td>27 (61)</td>
</tr>
<tr>
<td><strong>ESR1 (( n = 93 ))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>34 (64)</td>
<td>23 (58)</td>
</tr>
<tr>
<td>Yes</td>
<td>19 (36)</td>
<td>17 (43)</td>
</tr>
<tr>
<td><strong>IGSF4 (( n = 104 ))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>34 (59)</td>
<td>14 (30)</td>
</tr>
<tr>
<td>Yes</td>
<td>24 (41)</td>
<td>32 (70)</td>
</tr>
</tbody>
</table>
incidence of HPV-related OPSCC has been increasing. Sanders et al determined that there would be a 4.7% reduction in this incidence if oncogenic HPV infection could be prevented. The association between HPV and HNSCC (for both incidence and prognosis) is strongest for OPSCC. Human papillomavirus-positive OPSCC has been noted as a distinct variant of HNSCC characterized by high prevalence of HPV infection, better patient outcome, nonkeratinizing histologic subtype, and overexpression of p16, with worse outcomes in OPSCC for AAs compared with Caucasians partially explained by fewer HPV-positive cases in AAs.

Several recent studies have shown that an HPV-positive status confers certain advantages to a subset of HNSCCs. Patients with HPV-positive HNSCCs have better survival outcomes than those with HPV-negative HNSCCs, believed to be because of better response to chemoradiation therapy. However, the mechanism for this improved prognosis remains underexplored.

Promoter hypermethylation is widely recognized as a mechanism in the progression of HNSCC. There is growing evidence that the DNA methylation regulatory pathways are targets for E6 and E7 oncogenes. Genome-wide methylation differences between HPV-positive and HPV-negative tumors have been noted in squamous cell carcinoma cell lines and primary HNSCC tumors. In particular, promoter methylation of CCNA1 in HPV-positive cases with lower expression has been reported. In addition, epigenetic downregulation of this gene has been shown to be previously common in oral squamous cell carcinomas by means of pyrosequencing methylation assay. In this study, IGSF4 was more frequently methylated in HPV-positive than HPV-negative patients with OPSCC (70% vs 41%, respectively; $P = .004$) and was an independent predictor of HPV-positive status (OR, 4.5; $P = .005$). Although the methylation rates of HPV-positive tumors are similar for DAPK1 and IGSF4 (61% and 70%, respectively), the $P$ values reflect the difference in methylation rates relative to those seen in HPV-negative tumors. For DAPK1, there is only a 7% absolute difference (61% vs 54%), but for IGSF4 there is a 29% absolute difference (70% vs 41%). This explains the large difference between the respective $P$ values. IGSF4 is frequently methylated in saliva from HNSCC tumors and in matched HPV16-

### Table 3. Crude and Adjusted Odds of Being Human Papillomavirus (HPV) Positive

<table>
<thead>
<tr>
<th>Variable</th>
<th>Crude OR (95% CI)</th>
<th>$P$ Value</th>
<th>Adjusted OR (95% CI)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race, Caucasian vs African American</td>
<td>2.5 (1.2-5.5)</td>
<td>.02</td>
<td>2.9 (1.0-8.3)</td>
<td>.053</td>
</tr>
<tr>
<td>Sex, male vs female</td>
<td>2.0 (0.7-4.9)</td>
<td>.15</td>
<td>4.7 (1.3-17.0)</td>
<td>.02</td>
</tr>
<tr>
<td>Age</td>
<td>0.98 (0.95-1.02)</td>
<td>.34</td>
<td>0.9 (0.9-1.0)</td>
<td>.008</td>
</tr>
<tr>
<td>Smoking, vs current</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>3.0 (0.9-9.6)</td>
<td>.06</td>
<td>2.5 (0.8-7.9)</td>
<td>.21</td>
</tr>
<tr>
<td>Past</td>
<td>2.2 (1.0-5.0)</td>
<td>.06</td>
<td>2.6 (0.6-12.4)</td>
<td>.13</td>
</tr>
<tr>
<td>Treatment, vs neither</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiotherapy only</td>
<td>4.64 (1.40-15.40)</td>
<td>.01</td>
<td>11.62 (2.02-66.82)</td>
<td>.02</td>
</tr>
<tr>
<td>Chemoradiotherapy</td>
<td>5.53 (1.60-19.09)</td>
<td>.007</td>
<td>11.15 (1.92-64.65)</td>
<td>.01</td>
</tr>
<tr>
<td>Methylation, yes vs no</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAPK1</td>
<td>1.3 (0.6-3.0)</td>
<td>.46</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>ESR1</td>
<td>1.3 (0.6-3.1)</td>
<td>.52</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>IGSF4</td>
<td>3.2 (1.4-7.3)</td>
<td>.005</td>
<td>4.5 (1.6-12.8)</td>
<td>.005</td>
</tr>
</tbody>
</table>

Values reflect the differences between HPV-positive and HPV-negative tumor groups. The $P$ values reflect the statistical significance of these differences. The adjusted odds ratios and $P$ values are adjusted for multiple variables, including smoking status, race, and age. The adjusted odds ratios are derived from a multivariate logistic regression analysis with age, smoking, and race as independent variables. The $P$ values reflect the statistical significance of these differences.
positive saliva and HNSCC tumor pairs.37 IGSF4 is a novel immunoglobulin-like intercellular adhesion molecule first characterized as a tumor suppressor of non-small-cell lung cancer and termed TSLC1,38,39 in which silencing was primarily achieved by allelic loss and promoter methylation. IGSF4 is located in the long arm of chromosome 11 at 11q23.2 and spans more than 300 kilobases.38 In HNSCC, promoter hypermethylation of IGSF4 is a primary as well as a disease progression event.14,15 IGSF4 hypermethylation is also a highly frequent event in cervical cancers, where its epigenetic silencing has been implicated in the progression from high-risk HPV-containing, high-grade cervical intraepithelial neoplasia lesions to invasive cervical cancer.40 Furthermore, IGSF4 silencing was accompanied by complete loss or significant decrease of IGSF4 mRNA expression in these cell lines. In esophageal squamous cell carcinoma (ESCC), loss of IGSF4 protein expression as a consequence of promoter hypermethylation, a late-stage event in ESCC carcinogenesis, has been implicated in invasion, metastasis, and aggressive tumor behavior through the disruption of cell-cell interactions.41 A hypermethylated IGSF4 suggests a promising new therapeutic target in ESCC for restoration of gene expression by demethylating agents.42 In this study, the significant association of IGSF4 with HPV-positive HNSCC suggests that HPV-positive tumors are to a greater extent driven by promoter hypermethylation in this tumor suppressor gene.

From a clinical significance standpoint, recent studies are beginning to establish a mechanistic role for promoter methylation with improved survival outcomes in HPV-positive HNSCC. Gubanova et al42 showed that promoter hypermethylation and concordant low SMG-I expression not only was correlated with HPV-positive status and improved patient survival but also enhanced response to radiotherapy in HPV-positive HNSCC cell lines. A more recent study identified an HPV-related promoter methylation signature of 5 genes (derived from a more global discovery approach) with strong correlation to and strong predictive power for clinical outcome of patients with OPSCC.43 Our study found no association of methylation and overall survival. This may be largely due to the candidate gene approach with only a limited number of genes. Even though IGSF4 methylation was associated with HPV positivity, it did not share HPV's association with survival.

At the univariate level, AAs had poorer survival outcomes when compared with Caucasians (P = .008). In AAs with OPSCC, survival disparities are attributed to racial differences in the prevalence of HPV-positive tumors.16,29,44-45 African Americans had a significantly lower prevalence of HPV-positive tumors as compared with Caucasians, with corresponding worse survival for AAs. A recent study from our group16 reported that HPV status has a substantial impact on overall survival in AAs with OPSCC. Among AAs, HPV-positive patients had better survival than HPV-negative patients (HR, 3.44; P = .001), a finding not previously reported, presumably due to a paucity of multietnic cohorts and limited numbers of AA patients. The HPV-negative AA patients also did worse than both HPV-positive Caucasians and HPV-negative Caucasians. In this study, univariate survival analysis also showed that current smokers were more likely to die than never and past smokers (P = .003), with no difference in survival between never and past smokers. Tobacco use is one of the most common risk factors associated with the development of HNSCC. Most patients in our cohort had a history of smoking (87%). Recent studies have found that the risk of OPSCC progression and death increases as a direct result of tobacco exposure.3,46

Cox multivariate proportional hazards modeling retained HPV status, age, and treatment as independent predictors of overall survival in both Cox regression models. The HPV-negative patients had more than 2 times the risk of death as compared with HPV-positive patients. For every 1-year increase in age, the risk of death increased by a factor of 1.1, in keeping with studies showing increasing rate of cancer with age. Patients who received both radiotherapy and chemotherapy did worse than those with radiotherapy only, in both the univariate and multivariate models. The late-stage proportion (95%) among patients with chemoradiation treat-
ments was higher than that for radiotherapy only (74%, adjusted \( P = .03 \)), suggesting that higher-stage tumors are associated with combined chemoradiation treatment. Stage at diagnosis was not retained after multivariable survival model variable selection; however, even when it is included, it does not explain the survival differences seen for treatment group (Table 5).

Conclusions

Identification of epigenetically affected genes has become an important tool for understanding aberrant gene expression and mechanisms of tumorigenesis. Our group\(^4\) and others\(^3\) have shown that HPV can modulate the HNSCC epigenome. In our study, \( IGSF4 \) methylation was an independent predictor of HPV-positive HNSCC.

Further confirmatory studies should include gene expression and protein assays for correlation of methylation status. Subsequent assessment in independent retrospective and prospective studies in which HPV status and methylation of \( IGSF4 \) are determined concomitantly to assess association with survival outcomes would support \( IGSF4 \) as a potential therapeutic target, as well as a surrogate marker for HPV-positive OPSCC. Further exploration of \( DAPK1 \) or other potential promoters should await additional evidence of stronger associations with HPV status. Independent validation would strengthen the evidence for \( IGSF4 \) as a molecular classifier of this disease and as a potential demethylating therapeutic target in HNSCC.

Table 5. Multivariable Cox Proportional Hazards Models

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Predictors(^a)</th>
<th>( P ) Value</th>
<th>( P &lt; .20 ) Predictors Only(^b)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male vs female</td>
<td>2.2 (0.8-6.3)</td>
<td>.14</td>
<td>1.9 (0.9-3.9)</td>
<td>.07</td>
</tr>
<tr>
<td>Age</td>
<td>1.1 (1.0-1.1)</td>
<td>.004</td>
<td>1.0 (1.0-1.1)</td>
<td>.004</td>
</tr>
<tr>
<td>Smoking vs current</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>0.4 (0.1-1.0)</td>
<td>.12</td>
<td>0.5 (0.3-0.9)</td>
<td>.02</td>
</tr>
<tr>
<td>Never</td>
<td>0.5 (0.2-1.5)</td>
<td>.06</td>
<td>0.4 (0.1-1.2)</td>
<td>.12</td>
</tr>
<tr>
<td>Race, Caucasian vs African American</td>
<td>0.8 (0.3-1.7)</td>
<td>.52</td>
<td>0.7 (0.4-1.1)</td>
<td>.12</td>
</tr>
<tr>
<td>Treatment vs radiotherapy only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both chemotherapy + radiotherapy</td>
<td>3.1 (1.3-7.4)</td>
<td>.03</td>
<td>2.5 (1.3-4.5)</td>
<td>.01</td>
</tr>
<tr>
<td>Neither chemotherapy nor radiotherapy</td>
<td>2.7 (0.9-7.9)</td>
<td>.08</td>
<td>1.9 (1.0-3.7)</td>
<td>.05</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late vs early</td>
<td>1.7 (0.6-4.7)</td>
<td>.48</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Missing vs early</td>
<td>3.5 (0.3-37.1)</td>
<td>.30</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>HPV status, negative vs positive</td>
<td>2.7 (1.1-6.7)</td>
<td>.04</td>
<td>2.1 (1.1-3.7)</td>
<td>.02</td>
</tr>
<tr>
<td>Methylation, yes vs no</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( IGSF4 )</td>
<td>1.2 (0.4-3.8)</td>
<td>.74</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>( DAPK1 )</td>
<td>0.7 (0.3-2.0)</td>
<td>.54</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>( ESR1 )</td>
<td>1.2 (0.4-3.2)</td>
<td>.77</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: HPV, human papillomavirus; HR, hazard ratio.

\(^a\) Model with all possible predictors (\( n = 75 \): dead = 38, alive = 37).

\(^b\) Model with only \( P < .20 \) predictors (\( n = 116 \): dead = 63, alive = 53).
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