Association between p53 and Human Papillomavirus in Head and Neck Cancer Survival

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Abstract

Background: High-risk human papillomavirus (HPV-HR) is a significant risk factor for head and neck cancer (HNC), abrogating normal p53 function. In addition, HPV and p53 have been associated with prognosis of these tumors but the findings have been inconsistent. We examined p53 expression and HPV-HR individually and jointly for differences in predicting HNC survival. Methods: HNC patients (n = 294) were evaluated for p53 by immunohistochemical staining. HPV was detected by PCR/dot blot hybridization and sequencing. Results: HNC tumors showed 48% with p53 overexpression and 27% with HPV-HR. Multivariate analyses showed that p53 positivity was significantly associated with higher risk of disease-specific [hazard ratio (HR); 2.0; 95% confidence interval (95% CI), 1.1-3.7] and recurrence-free mortality (HR, 2.8; 95% CI, 1.4-5.3). HPV− cases had significantly worse disease-specific survival (HR, 2.8; 95% CI, 1.3-6.3) compared with HPV-HR cases. When analyzed jointly, with p53 /HPV-HR tumors as the reference group, p53 /HPV− patients had the worst disease-specific (HR, 5.3; 58% versus 15%, P = 0.006) and recurrence-free survival rates (HR, 9.5; 17% versus 89%, P = 0.001), in contrast to the p53 /HPV− and p53 /HPV-HR groups, which had less elevated and different risks for disease-specific survival (HR, 2.5 and 1.7, respectively) and recurrence-free survival (HR, 4.2 and 7.2, respectively). Conclusion: Joint assessment of p53/HPV status provides different HRs for each clinical outcome in the four biomarker groups that are distinct from the individual biomarkers. These findings suggest that joint assessment of p53/HPV provides a better indicator of prognosis and potentially different types of treatments. (Cancer Epidemiol Biomarkers Prev 2008;17(2):421–7)

Introduction

p53 is a critical factor in cell cycle control and apoptosis, and loss of function has been shown to play a pivotal role in cancer development and progression (1, 2). The prevalence of p53 alterations in head and neck cancer (HNC) has been reported to be between 39% and 62% (3-7) and these changes have been associated with two major risk factors for HNC: tobacco and alcohol (6, 8, 9). The presence of high-risk human papillomavirus (HPV-HR) detected in ~25% of all HNC tumors (7, 10-12) is a significant risk factor for HNC that is independent of tobacco and alcohol (10, 11). In cervical cancer, there is a high correlation between HPV and wild-type p53 presence (13, 14). Whether this association is similar in HNC is unclear. The E6 oncoprotein of HPV-HR has been shown to promote p53 degradation and lead to p53 inhibition (15). Because the mechanism of p53 modulation by HPV-HR does not involve p53 mutation, it is possible that the clinical outcomes for HNC associated with HPV-HR may be different from those associated with tobacco and alcohol. In addition, HNC patients show low survival (50%) and high recurrence rates (25%; refs. 7, 12, 16-18). p53 and HPV-HR have been examined in association with HNC prognosis with conflicting results observed (19). Overexpression of p53 was related to worse prognosis in some investigations (3, 5, 20) but not in others (4, 17), whereas HPV-HR has been associated with better prognosis (7, 21). Studies of cervical cancer have not been able to address the p53/HPV relationship with survival because HPV-HR is a necessary cause of these tumors and thus cannot serve as a model for predicting outcomes. Only one large investigation (7) of HNC has reported the independent effects of p53 mutation and HPV in tumors and correlated these with prognosis. After adjusting for p53 and HPV status and other HNC risk factors related to clinical outcomes, they found that neither p53 mutations nor HPV positivity significantly affected disease-specific survival. To date, there have been no investigations of both the independent and the combined effects of p53 and HPV on HNC survival. This study examined the association between p53 expression and HPV status, both as individual and joint risk factors, and those effects on prognosis in HNC patients. The purpose was to determine whether these joint findings would better characterize differences in HNC clinical outcomes over that of the individual marker profiles.

Received 9/17/07; revised 11/16/07; accepted 11/27/07.


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Cancer Epidemiol Biomarkers Prev 2008;17(2). February 2008
using a routinely available pathology lab test to detect p53 alterations.

Materials and Methods

Patients and Tumor Specimens. Participants signed an informed consent form before enrollment. Patients were recruited between 1994 and 2004 from the University of Iowa Hospitals and the local Veterans Affairs Medical Center. Patients (n = 294) completed a self-administered questionnaire about demographics, dose and duration of tobacco and alcohol use, sexual practices, history of HPV-related diseases, and oral lesions. Information about previous HNC history, tumor stage, site, grade, histology, nodal involvement, and treatments was retrieved from medical records and pathologic reports. All cases were histologically confirmed.

H&E-stained slides from formalin-fixed and paraffin-embedded biopsy blocks were reevaluated to verify the presence of malignant tissue, diagnosis, histology, stage, and tumor grade. For each case, serial 4-μm-thick sections were cut from the block with the highest tumor percentage for immunohistochemical staining of p53 and HPV detection. Tumor stage was based on the 1997 American Joint Committee on Cancer criteria (22). Tumor sites were grouped into oral cavity, oropharynx, and larynx/hypopharynx as suggested in the American Joint Committee on Cancer Staging Manual (ref. 22; excluding nasopharynx). Most cases (92%) were squamous cell carcinoma and 24 were of other nonsquamous cell carcinoma histologic types.

Laboratory Methods. For each patient, two slides from the same cut series of the same block were used. Slides for p53 staining were deparaffinized by passage through xylene and then dehydrated in graded alcohol. One was stained with mouse anti-human p53 protein monoclonal antibody D-O7 (DAKO Co.), whereas the other was stained with mouse anti-human IgG for verification of specificity of staining for the monoclonal antibody.

The p53 immunohistochemistry slides were reviewed by one pathologist (T.H.H.). Strong nuclear staining in tumor tissues was considered a positive reaction. The p53 positive distribution was scored on a semiquantitative scale initially as follows: 1, negative; 2, <10% positive; 3, 10% to 80% positive; 4, >80% positive; 5, every tumor cell positive. Tumor cells were counted p53 positive only if they were part of the consistent morphologic feature of the tumor. Before analyses, p53 scales were changed into positive for cases in categories 2 to 5, and negative for cases equal to 1. All those with a score of 2 (n = 4) and 3 (n = 20), plus a random sample of others (n = 26), were rereviewed to verify the initial interpretation.

HPV detection and typing from paraffin-embedded tissue were essentially done as described earlier (10). To confirm that HPV was detected from tumor cells within the tumor tissue, a subset of cases (n = 55) was examined by laser capture microdissection (23). Two case tumors were consistently hβ-globin negative and were excluded from analyses.

Statistical Methods. Risk factors included age, gender, tobacco and alcohol use, number of sexual partners, oral-genital sex, tumor site, stage, grade, nodal involvement, and histology (squamous cell carcinoma versus others). Only 4% identified themselves as non-Caucasian. In preliminary analyses, we compared these two groups. There were no significant (P ≤ 0.05) or near-significant (P ≤ 0.10) associations between these racial groups and demographic characteristics, risk factors, biomarkers, or clinical outcomes. Race could not be included in multivariate logistic or proportional hazards models because of the small sample size. Multivariate unconditional logistic regression was used to examine the association between dependent variables and risk factors. Odds ratios and 95% confidence intervals measured the magnitude of the associations. Fisher’s exact test for small sample sizes was used to test the association of racial groups and other variables of interest, as well as 5-year survival and recurrence, with the biomarker groups.

Survival was measured in years from the date of diagnosis until death or until the patient was last known to be alive. Recurrence was measured from the date of first diagnosis to the date of recurrence. Date of death or date last known to be alive was obtained from the Iowa National Cancer Institute Surveillance Epidemiology and End Results Cancer Registry (14), the university hospital tumor registry, and National Death Index (24). Disease-specific survival and recurrence-free survival were analyzed for p53 and HPV, modeled independently (p53, HPV) and then jointly in groups (p53+/HPV-HR, p53+/HPV−, p53−/HPV+, and p53−/HPV-HR). In disease-specific analyses, patients who died of causes other than HNC were censored. Survival curves were estimated by the Kaplan-Meier method (25), compared by the log-rank test (26), and generated in S-Plus 6.2 (27). Cox proportional hazards models (28) adjusted for factors prognostically significant in HNC survival and recurrence whereas hazard ratios (HR) measured the magnitude of the association (29). No variables violated the proportional hazards assumption (30). Statistical significance was based on two-tailed tests and P ≤ 0.05. Statistical analyses were done using SAS version 9.1 (31).

Results

Patient Characteristics and Risk Factors. The majority (62%) of cases was male and the average age was 60 years. p53 was detected in 48% and HPV-HR in 27% of cases (95% HPV-16, 4% HPV-33, and 1% HPV-18). The distribution of cases by tumor site was 56% in the oral cavity, 30% in the oropharynx, and 14% in the larynx/hypopharynx. The prevalence of HPV-HR by site was 57% in the oropharynx, 16% in the oral cavity, and 10% in the larynx/hypopharynx. When examined by joint p53/HPV status, 11% were p53+/HPV-HR, 35% p53+/HPV−, 37% p53−/HPV+, and 16% p53−/HPV-HR. After adjustment for age, alcohol, tobacco, and HPV status, p53 status was not significantly associated with any demographic or risk factors including HPV status (Table 1), although the risk was slightly elevated in heavy alcohol users. The adjusted odds ratios between p53 and pathologic characteristics (Table 2) also showed no associations with site, stage, grade, nodal involvement, or histology.

Survival. Included in the disease-specific survival analyses were 234 newly diagnosed cases with follow-up status. Thirty-four were excluded from recurrence-free analyses because they were never disease-free (n = 200).
Patients with missing data were compared with those without missing data and there were no statistical differences in risk factors, survival, or recurrence. Figure 1 shows Kaplan-Meier curves for disease-specific (Fig. 1A) and recurrence-free survival (Fig. 1B) for the four biomarker groups. The reference group is p53+/HPV-C0. In Fig. 1A, only p53+/HPV exhibited worse disease-specific survival compared with p53−/HPV-C0. The recurrence-free survival curves in Fig. 1B exhibited significantly worse survival for all three biomarker groups compared with the reference.

Table 3 displays adjusted HRs for disease-specific and recurrence-free survival with p53 expression, HPV status, and the four p53/HPV groups. Alcohol and tobacco were not significantly associated with prognosis but were included in the final models based on their established significance as risk factors for HNC. There were no significant differences in the results of the p53/HPV groups and survival by site or histologic groups; thus, all sites or types were combined in reporting the analyses. The median disease-specific survival times for p53+/HPV-C0 and p53−/HPV-C0 were 1.7 and 1.9 years, respectively, whereas...
Disease-Specific Survival by p53 Status and HPV Status

Recurrence-Free Survival by p53 Status and HPV Status

the median times for HPV-HR and HPV− were 2.3 and 1.6 years, respectively (data not shown). The median disease-specific survival times for the four groups were p53−/HPV-HR, 2.4 years; p53+/HPV-HR, 2.2 years; p53−/HPV−, 1.7 years; and p53+/HPV−, 1.6 years (data not shown). When analyzed individually, both p53+ and HPV− cases were associated with significantly worse disease-specific survival (Table 3). There was no interaction between p53 and HPV for disease-specific survival ($P = 0.75$). Similar to the unadjusted analyses (Fig. 1A), adjusted assessments of the joint biomarkers showed the greatest risk in those who were p53+/HPV−. Disease-specific mortality also was greater with increasing age and for those in stage III or IV. Compared with surgery/radiation treatment, surgery-only patients had a reduced risk of death due to HNC after controlling for stage and other risk factors. Better 5-year disease-specific survival was seen in the p53−/HPV-HR reference group: 58%
compared with 15% for p53+/HPV- (P = 0.006), 14% for p53+/HPV+ (P = 0.04), and 17% for p53+/HPV-HR (P = 0.15; data not shown).

The median times to recurrence for p53+ and p53- were 1.6 and 1.9 years respectively, whereas the median times for HPV-HR and HPV- were 2.5 and 1.5 years (data not shown). When p53 and HPV-HR were examined independently (Table 3), p53 positivity was significantly associated with increased tumor recurrence and the HPV- group had a nonsignificant elevated risk (HR, 1.9). There was no significant interaction between the two biomarkers and risk of recurrence (P = 0.16). The median times to recurrence for the four biomarker groups were p53+/HPV-HR, 2.7 years; p53+/HPV-, 1.9 years; p53-/HPV+, 1.5 years; and p53-/HPV-, 1.4 years (data not shown). Compared with the p53+/HPV-HR group, the other biomarker groups had much higher recurrence risks (Table 3; Fig. 1B). This is in contrast to the patterns shown for disease-specific survival in which only the p53+/HPV- tumors had significantly worse prognosis. Stage was not significantly associated with recurrence. Surgery-only cases had a lower risk of recurrence compared with those who had surgery/X-ray therapy. The p53+/HPV-HR reference group again had better 5-year recurrence-free survival compared with the other groups: 83% versus 11% for p53+/HPV+ (P = 0.001), 13% for p53+/HPV-HR (P = 0.03), and 13% for p53-/HPV- (P = 0.006; data not shown).

Discussion

This is the first study of HNC to evaluate differences in prognosis associated with the joint assessment of p53 and HPV.

Table 3. Adjusted HRs of p53/HPV for survival and recurrence

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HR (95% CI)</th>
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<tbody>
<tr>
<td></td>
<td>Disease-specific survival</td>
</tr>
<tr>
<td>p53</td>
<td>1.0</td>
</tr>
<tr>
<td>p53-</td>
<td>2.0 (1.3-3.7)</td>
</tr>
<tr>
<td>p53+</td>
<td>1.0</td>
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<tr>
<td>HPV-HR</td>
<td>2.8 (1.3-6.3)</td>
</tr>
<tr>
<td>HPV-</td>
<td>1.0</td>
</tr>
<tr>
<td>p53+/HPV-HR</td>
<td>1.0</td>
</tr>
<tr>
<td>p53+/HPV-</td>
<td>1.7 (0.4-6.4)</td>
</tr>
<tr>
<td>p53-/HPV+</td>
<td>2.5 (0.8-7.6)</td>
</tr>
<tr>
<td>p53-/HPV-</td>
<td>5.3 (1.9-14.7)</td>
</tr>
<tr>
<td>Age</td>
<td>1.03 (1.01-1.05)</td>
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<td>Alcohol*</td>
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<tr>
<td>Tobacco*</td>
<td>1.0 (0.98-1.002)</td>
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<td>Stage</td>
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</tr>
<tr>
<td>III</td>
<td>4.1 (1.6-10.8)</td>
</tr>
<tr>
<td>IV</td>
<td>1.0</td>
</tr>
<tr>
<td>Treatment</td>
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<td>Surgery and XRT</td>
<td>1.2 (0.6-2.5)</td>
</tr>
</tbody>
</table>

NOTE: Models were adjusted for age, alcohol, tobacco, stage, and treatment. Abbreviation: XRT, X-ray therapy.
*Drinks per week.
**Pack-years.

protein overexpression, as a readily available marker of many p53 pathway alterations, and HPV status in these tumors, as compared with evaluating p53 or HPV as single tumor markers. Our results indicate that evaluating multiple biomarkers as four distinct groups reveals greater variation in clinical outcomes that are not apparent by assessing the individual biomarkers separately. These multiple marker distinctions are consistent with known differences in the mechanisms leading to p53 loss of function by damage to the p53 gene or by HPV infection. In the joint biomarker assessment, the prognostic results differ from the individual biomarker assessment because the effects in the four p53/HPV patient groups are evaluated in more distinctly similar (homogeneous) groups than are the p53 or HPV groups. The individual p53 or HPV groups examine only the effect of a single marker without consideration for the effect of the other marker on prognosis. Greater precision in predicting distinct outcomes should also lead to administering more effective, targeted treatment regimens.

In our investigation, patients with tumors that were p53+/HPV-HR had the highest survival and lowest recurrence rates whereas those with cancers that were p53+/HPV- had significantly worse outcomes after adjusting for other risk factors and pathologic characteristics. Because studies have shown that HPV-HR in HNC is associated with increased survival (7, 21, 32) whereas p53+ status is associated with worse survival (3, 5), those results were not unexpected. Although those who were p53+ in the individual biomarker models had modestly elevated HRs for each clinical outcome compared with the p53- patients, these HRs were almost always lower than those of the four joint p53/HPV groups. Likewise, compared with the individual HPV- outcomes, the HRs for the combined p53/HPV patient groups usually were higher and distinctly different in each joint group. Neither the HRs associated with p53 nor HPV status alone would have predicted the high HRs and low survival or high recurrence seen for the p53+/HPV- cases. Furthermore, the other two joint biomarker groups (p53+/HPV-HR and p53-/HPV-) had different clinical outcomes compared with the best or worst survival groups, with each other, and with risks based solely on the individual biomarker HRs. These findings again suggest that diverse molecular mechanisms involved in head and neck carcinogenesis influence subsequent survival. Thus, the p53/HPV combined group assessment provides a more complete picture of the association between the two biomarkers and prognosis than do the biomarkers examined individually. The results of our study show that HPV-HR tumors have higher recurrence rates if they also overexpress p53 than do p53+/HPV+ tumors despite the lower risk of recurrence shown in the HPV-HR biomarker group alone (Table 3). This finding suggests that p53 status plays a more significant role in recurrence than does HPV.

Previous studies used either p53 overexpression or TP53 mutations (3, 5, 7, 33, 34). Like other studies (7, 34), we found that HPV-HR HNC were less likely to overexpress p53 than were HPV- tumors (11% versus 37%). Mao et al. (33) found p53 mutations in 10% of HPV+ and 32% of HPV- whereas Gillison et al. (7) reported 18% HPV+ tumors and 28% HPV- tumors harbored a TP53 mutation. The adjusted HR in our study showed that p53+ patients had worse disease-specific survival and
modestly higher tumor recurrence, consistent with some studies (3, 5, 18) but not with others (4, 15, 35) which found no prognostic significance for p53. Geisler et al. (3) found similar increased HRs as in our findings among those with p53 overexpression for disease-specific survival (HR, 3.1) after adjusting for age, stage, and treatment, but they did not examine HPV. Gillison et al. (7) performed multivariate analyses to control for the effect of one marker while examining the independent effect of the other and found that disease-specific survival was not significantly associated with p53 mutation or HPV⁺. Recurrence was not examined. In contrast, we (21) and Cabelguenne et al. (36) found that disease-specific survival was significantly better in HPV-HR HNC despite the fact that most positive cases had advanced-stage disease.

Because of p53 modulation by the HPV-HR E6 oncoprotein, prognostic findings based on p53 alone may be inaccurate if the effects of HPV are not taken into account. Whereas most TP53 gene mutations in cancers also block p53 protein degradation and lead to its accumulation, the E6 oncoprotein of HPV-HR types complexes with p53 and ultimately lead to its degradation (4, 37, 38). Therefore, the p53⁺ rate is expected to be higher in HPV⁺ tumors than in HPV-HR tumors. This association between p53 and HPV status also was seen in our study but it was not statistically significant. This may be due to mutant p53 that is resistant to E6 degradation in some HPV-HR tumors (37, 39). Although immunohistochemistry would also detect wild-type p53, overexpression of wild-type p53 in HNC is unlikely. Elevated levels of p53 would be inconsistent with cancer growth because they would likely trigger at least some of the p53 effector pathways and lead to apoptosis, senescence, differentiation, and/or cell cycle arrest. The overexpression of p53 in the absence of detectable TP53 mutations observed by others (4, 40) may thus reflect limitations in mutational analysis rather than for wild-type TP53 (4).

In contrast, some TP53 mutations such as frame-shifts, defective splice sites, deletions, or nonsense mutations may abolish detectable p53 expression (6). However, even if some of our patients were misclassified as p53⁻ but truly had p53⁺ mutations, the difference in the risk between our reference group (p53⁻/HPV-HR cases) and that of each other group would be reflected in a HR that would underestimate the true difference in risk of death. Yet in comparison with the reference group, each of the other groups has elevated HRs for each clinical outcome.

Strengths of this study include a large number of HNC cases in which to examine the independent and joint effects of p53 and HPV status for clinical outcomes while adjusting for confounders that are likely to have had an effect on disease outcomes. The study size also allowed us to examine the effects of each p53/HPV group on prognosis as stratified analyses, not just controlling one biomarker while evaluating the association of another marker on outcomes, which may be misleading if there are different molecular interactions reflected in the groups. This study also benefited from survival data available through the Iowa National Cancer Institute Surveillance Epidemiology and End Results Cancer Registry, which provides follow-up status on 98% of patients. The remaining survival information was identified through the U.S. National Death Index. The 10-year duration of follow-up is the longest of the published p53/HPV studies.

Our findings suggest that there are multiple, different molecular mechanisms that lead to HNC and have clinical consequences for survival and recurrence. Examining the joint effects of p53 and HPV groups increased information about differences in HNC clinical outcomes over that of the individual assessment of either biomarker. These distinctions should assist clinical practice in applying treatment strategies that need to be more (or less) aggressive, based on the molecular profile of the tumor. p53 activity was identified using a routinely available test in pathology labs and HPV status is increasingly being assessed in medical labs as well. These tests would provide low-cost, quick means for characterizing p53/HPV molecular profiles to assist in treatment protocols of HNC. The best treatment for a given joint biomarker is not yet clear. Before specific treatment protocols can be more firmly established, further assessment will require a retrospective study of large numbers of HNC cases in a variety of tumor-node-metastasis stages, receiving a variety of treatments, and with known tumor marker status.

References


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