Use of In Situ Hybridization to Detect Human Papillomavirus in Head and Neck Squamous Cell Carcinoma Patients Without a History of Alcohol or Tobacco Use

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Context.—Head and neck squamous cell carcinoma is commonly associated with tobacco and alcohol use. There are, however, a group of patients without a significant history of tobacco or alcohol use, and the etiology of these tumors is incompletely understood.

Objective.—To examine tumors in this subpopulation for association with human papillomavirus (HPV) using newly available in situ hybridization probes.

Design.—Between October 2004 and October 2005, 22 patients who did not use alcohol or tobacco were included. Formalin-fixed, paraffin-embedded tissue sections were used to perform in situ hybridization using newly available probe sets (Ventana Medical Systems, Tucson, Ariz). The slides were examined for the presence of integrated HPV using light microscopy. Positive and negative xenograft controls were run with the assay.

Results.—The mean age of the patients was 64 years. There were 14 men and 8 women. The most common anatomic sites included tongue (n = 8), tonsil (n = 7), and larynx (n = 7). All cases and controls were successfully stained. Only 2 cases were positive for high-risk HPV, and both demonstrated an integrated pattern. Both cases were tumors of the tonsil. No cases were positive for low-risk HPV.

Conclusions.—These results demonstrate that the new probe sets for HPV can be used very efficiently in clinical pathology material of head and neck squamous cell carcinoma. Our data show that high-risk HPV is an uncommon finding in head and neck squamous cell carcinoma from patients who do not have a history of tobacco or alcohol use; low-risk HPV was not seen in any case.

Tobacco and alcohol use are well known and established risk factors for head and neck squamous cell carcinoma (HNSCC). However, there is a subset of HNSCC patients who do not report a history of tobacco or alcohol use. These patients comprise a unique population, and other etiologies for their HNSCC have been investigated. One such area of research is the role of human papillomavirus (HPV).

It has been reported that HPV may be associated with HNSCC in patients without a history of tobacco or alcohol consumption.1-6 These studies found a significantly higher incidence of HPV compared with other cohorts. Furthermore, the most common site of infection was the oropharynx.1,4 There appear to be clinical implications from knowing the HPV status in HNSCC. Numerous reports have demonstrated a survival outcome advantage in patients with HPV-positive tumors.5-7 Furthermore, with the recent approval of the HPV vaccine for cervical cancer, research is ongoing as to the utility of such a vaccine against HNSCC in this patient population.4-8

Human papillomavirus serotypes 16 and 18 have been associated with cancer development.7-9 The incidence of these patients with HPV varies widely. Across published reports, HPV positivity varies from 20% to 90%.7 This variation may be due to the variety and high sensitivity of the methods used to identify HPV. One of the most common methods is polymerase chain reaction (PCR).10 However, this method is known to be sensitive to contamination and may be prone to false negatives.11 If accurate investigation of HPV-associated tumors is to be done, then it will be necessary to provide an accurate and sensitive method of determining HPV involvement.

We present a series of patients without a history of alcohol or tobacco use who underwent detection of HPV using novel and newly available, well-validated, low- and high-risk probe sets (Ventana Medical Systems, Tucson, Ariz). Both low- and high-risk serotypes were screened in pathologic specimens. This technique allows for accurate and rapid detection of HPV infectivity in pathologic samples. Furthermore, it allows for direct visualization of HPV in HNSCC cells.
MATERIALS AND METHODS
All patients with HNSCC who did not have a history of alcohol or tobacco use were screened from October 2004 through October 2005 after Cleveland Clinic Institutional Review Board approval. Patients were surveyed by the physician on initial presentation and diagnosis. Nonsmokers constituted patients who replied that they had never smoked currently or in the past. Nondrinkers constituted patients who claimed no regular or routine use of alcoholic beverages currently or in the past.

Histologic sections were reviewed, and the diagnosis was confirmed. Blocks were selected to contain both tumor and normal adjacent epithelium as an internal control. Fresh formalin-fixed, paraffin-embedded tissue sections were cut from the tissue block and mounted on coated slides. These slides were used to perform chromogenic in situ hybridization (ISH), according to standard protocols. The probe sets used for the assay included are well validated to identify low- or high-risk HPV (Ventana Medical Systems). The high-risk family 16 probe cocktail has an affinity to high-risk HPV genotypes 16, 18, 31, 33, 35, 39, 51, 52, 56, 58, and 66. The low-risk family 6 probe cocktail has an affinity to HPV genotypes 6 and 11. The slides were examined for the presence of integrated HPV using light microscopy. Positive signal is indicated by dark blue dotlike staining within the cytoplasm of tumor cells. Copy number can be estimated by comparison to the controls, which are xenograft controls that have known copy numbers. Internal negative controls were examined for each tissue section.

RESULTS
There were a total of 22 patients in this series. There were 14 men and 8 women. The average age of the patients was 64 years. In no case did the multiple samples have a different HPV status. The most common anatomic sites included tongue (8 cases), tonsil (7 cases), larynx (6 cases), and hypopharynx (1 case). Subsites of the larynx included 3 supraglottic cancers and 3 glottic cancers. All tongue cases involved the mobile tongue. The tumor (T) stagings of these 25 samples were: T1 = 5, T2 = 8, T3 = 4, and T4 = 5. The neck (N) stagings were: N0 = 17, N1 = 2, N2b = 5, and N2c = 1. A total of 24 samples were stage M0, whereas 1 sample was stage M1. A total of 2 of 22 patients (aged 52 and 58 years) had positive detection for high-risk HPV probes (10%). Both of these samples were tonsillar tumors, and both were nonkeratinizing squamous carcinomas (29% of tonsil carcinomas were high-risk HPV positive). The tumors were diffusely positive for HPV, with the staining being positive in the majority of the tumor (Figures 1 and 2). No tumors were positive for low-risk HPV. The HPV-negative tumors (Figures 3 and 4) included 2 additional nonkeratinizing squamous carcinomas, 2 basaloid squamous cell carcinomas, and 18 conventional keratinizing squamous cell carcinomas. Of the conventional squamous carcinomas, 2 were well differentiated, 7 were moderately differentiated, and 9 were poorly differentiated. The Table summarizes the demographic data.

There are clear associations, however, between tonsillar tumors and HPV, and particularly with basaloid squamous cell carcinoma.25 Our results confirm this association, as 2 of 7 tonsillar carcinomas were positive for high-risk HPV. The overall rate of HPV in our series of nonsmokers and nondrinkers was 10%, which was lower than the reported 50% in a similar series of nonsmokers,2 although these assays were performed using a PCR-based assay. One explanation may be that the average age of our patients was 64 years, thus being less likely to exhibit HPV in squamous cell carcinoma than younger patients. Interestingly, we did not observe any HPV positivity in cases of squamous cell carcinoma outside of the tonsils.

The literature does suggest a strong relationship between basaloid squamous cell carcinomas and HPV positivity. In our results, the tonsillar carcinomas that were positive for HPV showed nonkeratinizing squamous morphology. The tumors that were negative for HPV included a subset that were of a nonkeratinizing and basaloid morphology, but the majority were conventional keratinizing squamous cell carcinoma.

The method of detection of HPV may be partially responsible for the wide variation in reported incidence of HPV in squamous carcinomas. Detection of virus can be performed using immunohistochemistry, ISH, or PCR-based assays. In situ hybridization is thought to reflect one of the most accurate methods, since the technique allows for visualization of HPV within the cells, and it may also be used to identify integrated HPV. Because PCR is an amplification-based procedure, it is considered by some investigators to be an overly sensitive technique for detection of HPV. There is a recognized risk of contamination inherent in PCR-based assays, and this makes these techniques particularly prone to false-positive results. Sources of false-positive results include HPV from adjacent squamous intraepithelial lesions, latent infections, or laboratory contamination.21 The direct visualization of HPV within cancer cells in ISH methods, along with the ability of the assay to be used on any type of sample (fresh, paraffin-
One concern that does arise in interpreting HPV ISH is how much of a tumor needs to be positive to propose an HPV-related etiology? In our experience, low-risk HPV-related lesions in the head and neck, such as respiratory papillomatosis, are not uniformly positive for HPV and, in fact, show very patchy staining. In the high-risk HPV-positive tumors in this series and in our other experiences using this assay diagnostically, diffuse positivity for HPV is usually seen. Therefore, these high-risk HPV-associated lesions may be more uniformly positive than the low-risk associated benign counterpart lesions.

CONCLUSIONS

This study showed a low rate of HPV in carcinomas from nonsmokers/nondrinkers in our patient population. The only cases of HPV were detected in tonsillar tumors. The study also demonstrates the utility of new, commercially available, well-validated probe sets for low- and high-risk HPV determination that can be used in clinical paraffin-embedded tissue samples. There are significant advantages of the ISH technique in terms of visualization and localization of HPV and a high sensitivity that is coupled with a high specificity.

References

5. Weinberger PM, Yu Z, Halfyry BC, et al. Molecular classification identifies a...


