Highly aggressive human papillomavirus-related oropharyngeal cancer: clinical, radiologic, and pathologic characteristics

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Objective. Although the majority of human papillomavirus (HPV)-positive oropharyngeal squamous cell carcinomas have a favorable prognosis, we search for markers of poor prognosis by carefully examining a subset of highly aggressive cases.

Study design. Seven patients with HPV-positive oropharyngeal cancer who presented with non-pulmonary distant metastasis or developed distant metastasis posttreatment were identified. Eight control cases were chosen which responded well to treatment. Pathologic and radiologic studies were reviewed and compared.

Results. Two cases displayed a small cell carcinoma (SmCC) component upon pathologic review. Biomarker analysis revealed lower expression of NOTCH1 in the aggressive cohort in comparison to controls ($P = .04$). Cases showed a predominance of clustering of lymph nodes, extracapsular spread, and central tumor necrosis.

Conclusion. Although most HPV-related oropharyngeal cancers display a positive prognosis, it is evident that there is a subset, which behaves more aggressively. This early investigation identifies pathologic and radiologic features that may help to predict this behavior. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:327-335)

Head and neck cancer is the sixth most common cancer worldwide, with an annual burden of over 500,000 cases.[1] Recently, molecular and epidemiologic data have established that high-risk human papillomavirus (HPV) is a causative factor for a subset of head and neck squamous cell carcinomas (HNSCCs).[2,3] HPV, particularly type 16, is most closely associated with HNSCC of the oropharynx (oropharyngeal squamous cell carcinoma, OPSCC), where it is found in 40%-60% of these tumors.[3] Of most concern is the fact that incidence of oropharyngeal HPV-related cancers has been significantly increasing over the last 40 years.[3-6] Current evidence points to an increase in sexual promiscuity beginning in the 1960s as the impetus for this trend.[1] Interestingly, these HPV-positive cancers have a distinct clinical and biological signature from their HPV-negative counterparts. In contrast to HPV-negative tumors associated with older age and tobacco or alcohol exposure, HPV-positive tumors are associated with a younger age and increased number of sexual partners.[5-7] Notably, there is an increased survival associated with HPV-positive OPSCC, partly due to increased sensitivity to chemotherapy (CH) and radiation.[3,8] This increased chemoradiosensitivity is likely secondary to production of oncogenes *E6* and *E7* by HPV resulting in preservation of apoptotic pathways.[8,9] Currently, there is no standard treatment for dealing with HPV-positive tumors, although clinical trials are examining the possibility of de-escalation therapy for HPV-positive OPSCC. In these trials, the goal is to maximize treatment response, while minimizing deleterious effects of chemoradiotherapy by using milder treatment agents or doses for the HPV-positive patients.[1]

Although the majority of HPV-positive OPSCC may have a favorable prognosis, it is evident that there is a subset within this group which displays much more aggressive behavior and leads to poor clinical outcome.[10-13] Recently, 2 articles in the pathology

Statement of Clinical Relevance

This article brings awareness to all practitioners involved with human papillomavirus-positive oropharyngeal cancers that there is a subset that behaves very aggressively. It shows the benefit of multimodality research and paves the way for future research to characterize markers of aggression.

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literature have shown that small cell carcinoma (SmCC) of the oropharynx is associated with HPV and that some of these tumors have adjacent squamous cell carcinoma (SCC) components.[11,12] Further, SmCC of the oropharynx is known to widely disseminate and follow a very aggressive clinical course.[12] This could imply that there may be an aggressive subset of HPV-positive OPSCC cases in which an SmCC component exists.

On the basis of the existing literature, we also chose 2 biomarkers for investigation. Expression of p53 has been shown to correlate with disease-specific survival and overall survival (OS) in some studies, but its clinical prognostic significance remains controversial.[10] The NOTCH1 signaling pathway has been studied in HPV-positive cervical cancers and shown to act as a tumor suppressor, which can repress HPV E6/E7 expression and limit carcinogenesis.[14,15] In those with cervical cancer, high NOTCH1 expression has been correlated to less aggressive tumors.[15] More recently, 2 pioneer studies using exome sequencing of tumors from head and neck cancer patients provided strong evidence that NOTCH1 functions as a tumor suppressor in head and neck cancer as well. These studies found that 11%-15% of head and neck cancer patients harbor mutations in NOTCH1 and that NOTCH1 deregulation was a major driver of head and neck cancer carcinogenesis.[16-18] Radiologic studies may also add information as to the aggression of the tumor.[19-24] Currently, there is a lack of literature which has examined such cases in a multidisciplinary manner. Accordingly, the purpose of our study is to carefully examine a known subset of patients with HPV-positive OPSCC that had very aggressive clinical features and attempt to elucidate the pathologic, clinical, and radiologic features that could help explain this unexpected behavior.

PATIENTS AND METHODS

Cases

Approval for this study was obtained from The Ohio State University Office of Responsible Research Practices Cancer Institutional Review Board. Patients who were treated by the Head and Neck cancer comprehensive team at the James Cancer Hospital from 2005 to 2012 known by the authors to have highly aggressive cancers of the oropharynx were included in this study. *Highly aggressive*, in this context, was defined as cancers which had distant metastases in unusual sites (non-pulmonary) at presentation or within 1 year after initial treatment during their clinical course. Cases which did not have biopsy proven results or where metastasis was limited to the lungs were excluded. Our rationale for this selection is that patients with advanced HNSCC who have distant metastasis to non-pulmonary sites or multiple sites have worse OS than those with limited pulmonary metastasis. Specifically, in a recent study with 127 patients, those with lung metastases had a median OS of 26 months, compared to 21 months with liver metastases, 14 months in patients with multiple metastatic locations, and 13 months with metastases to the skeletal system.[25] Because our study was designed as a pilot study, our search for cases was not exhaustive, instead authors were asked to identify any patients they had treated which met the above inclusion criteria. HPV-status was checked from electronic medical records. If HPV-status was unknown, it was assayed by p16 immunohistochemistry (IHC) and HPV high-risk chromogenic in situ hybridization (CISH). Cases were only included if they were deemed HPV-positive, based on a positive result by either of these tests. Representative blocks were chosen for immunohistochemical staining. Medical records were also reviewed to document patient age, sex, tobacco exposure, primary site, clinical course, and clinical outcome.

Controls

For comparison, we identified a set of control patients who had HPV-positive oropharyngeal cancers and favorable outcome. Eight patients were selected who were treated at the James Cancer Hospital from 2005 to 2012. These patients had complete response to primary therapy and had no evidence of distant metastases throughout their course of treatment. These cases were found from a tissue microarray, which had been previously assembled as described before.[26] Please see Table I for features of control cases.

Immunohistochemistry

Immunohistochemical studies were performed on 4-μm sections on positively charged slides cut from formalin-fixed and paraffin embedded tissue. Slides with specimens were then placed in an oven at 60 °C for 1 h, cooled, and deparaffinized and rehydrated through xylenes and graded ethanol solutions to water.

For synaptophysin and chromogranin stains, slides were placed on a Dako Autostainer, immunostaining system (Dako North America Incorporated, Carpinteria, CA, USA). The detection system used was a labeled streptavidin−biotin complex (Dako) applied using standard protocols. The primary antibodies and dilution factors are as follows: synaptophysin (clone 27G12; Leica Microsystems, Bannockburn, IL, USA; 1:100); chromogranin (clone DAK-A3; Dako; 1:200). For p16 staining, slides were placed on a Benchmark XT automatic staining system (Ventana Medical Systems, Tucson, AZ, USA) and viewed with iView DAB detection system (Ventana). The primary antibodies used were p16 (clone INK4a; MTM Laboratories, Heidelberg, Germany; prediluted).