Oral Oncology 49 (2013) 244–248

Contents lists available at SciVerse ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Molecular etiology of second primary tumors in contralateral tonsils of human papillomavirus-associated index tonsillar carcinomas ☆

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ARTICLE INFO

Article history:
Received 30 May 2012
Received in revised form 10 September 2012
Accepted 10 September 2012
Available online 10 October 2012

Keywords:
Human papillomavirus
Head and neck cancer
Oropharyngeal cancer
Squamous cell carcinoma
Second primary tumor

SUMMARY

Objectives: For patients with tobacco-related head and neck squamous cell carcinoma (HNSCC), the occurrence of a second primary tumor (SPT) is an ominous development that is attributed to a field cancerization effect and portends a poor clinical outcome. The goal of this study was to determine whether patients with human papillomavirus (HPV)-related index tonsillar carcinomas can also develop SPTs in the contralateral tonsil, and to discern the molecular etiology of HPV-related tumor multifocality.
Materials and methods: The surgical pathology archives of The Johns Hopkins Hospital were searched for all patients with primary HPV-related tonsillar squamous cell carcinoma who developed a synchronous or metachronous carcinoma in the contralateral tonsil. The HPV-16 E6 exon was sequenced from each independent cancer site to determine whether the tumor pairs harbored the same or a different HPV-16 variant.
Results: Four patients with bilateral HPV-related tonsillar carcinomas were identified. In every case, the HPV DNA sequences derived from the index tumor and corresponding SPT were 100% concordant, indicating that the index and SPTs were caused by the same HPV-16 variant.
Conclusion: For the small subset of patients with tonsillar carcinomas who develop SPTs in the contralateral tonsil, the index case and the SPT consistently harbored the same HPV variant. This finding suggests that HPV-related tumor multi-focality can be attributed either to independent inoculation events by the same virus, or by migration of HPV-infected cells from a single inoculation site to other regions of Waldeyer's ring.

Introduction

The 20-year cumulative incidence of second primary tumors (SPTs) in head and neck squamous cell carcinoma (HNSCC) is approximately 36%.[1] Risk factors for the development of SPTs are often the same risk factors responsible for the initial primary process, which traditionally have included tobacco and alcohol use. The odds of being diagnosed with a SPT are 2.9 (95% CI 1.8–4.1) times higher in patients who smoke, and 5.2 (95% CI 3.3–7.9) times higher in patients who drink, compared with those who do not use these substances.[2] Human papillomavirus (HPV)-associated HNSCCs (HPV-HNSCC) are less often associated with excessive tobacco and alcohol use and, thus, patients with HPV-HNSCCs are less likely to develop smoking-related SPTs.[3–6] These same patients, however, may be at an elevated risk of developing other HPV-associated carcinomas. Men with HPV-associated oropharyngeal carcinoma are at increased risk for developing HPV-associated anogenital carcinoma, and several recent reports have described patients diagnosed with HPV-HNSCCs who subsequently developed second primary cancers of the head and neck: McGovern et al. described a 46 year-old patient with three synchronous HPV-associated carcinomas of Waldeyer's ring,[7] Roeser et al. described a case of synchronous HPV-associated tonsillar carcinomas,[8] and Singhi et al. observed three patients with HPV-associated oropharyngeal carcinomas in their series of patients with HPV-associated nasopharyngeal carcinomas.[9]

☆ *Grant Support*: This study was supported by the National Institutes of Health/National Institute of Dental and Craniofacial Research (NIH/NIDCR) Head and Neck SPORE Grant P50 DE019032.

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http://dx.doi.org/10.1016/j.oraloncology.2012.09.009

The purpose of this study was to identify patients with human papillomavirus (HPV)-associated index tonsillar carcinomas and a SPT in the contralateral tonsil in order to determine whether these synchronous or metachronous HPV-HNSCC are infected with the same or different variant of HPV. A better understanding of this relationship may provide insight into the mechanisms underlying SPTs in patients with HPV-HNSCC. As the burden of HPV-HNSCC continues to rise, it will become increasingly important to understand the risk of SPT development in this patient population and adopt personalized treatment strategies to manage these virus-related cancers.

Patients and methods

Subjects

This study was approved by the Institutional Review Board of the Johns Hopkins Medical Institutions. Briefly, we searched the Johns Hopkins Hospital pathology database for all patients diagnosed with a primary squamous cell carcinoma of the tonsil between 1999 and 2006. This search yielded 135 patients with a confirmed tissue diagnosis of tonsillar squamous cell carcinoma. We then reviewed all of the pathology reports for these patients to identify those who had synchronous bilateral tonsillar carcinomas or who subsequently developed metachronous tonsillar carcinomas. We were able to identify a total of six cases (or 4% of tonsillar carcinomas). One case was then excluded from the analysis because the tumor blocks were not available for HPV sequencing. Another case was excluded because the tonsillar cancers were not HPV associated as determined by in situ hybridization. The medical records for each of the subjects included in this study were thoroughly reviewed to ensure that each case involved a second primary carcinoma, rather than recurrence. In order to diagnose second primary carcinomas, we utilized a modified version of the criteria that were originally described by Warren and Gates.[10,11] In order to qualify as a SPT, the following criteria were required: (1) each neoplasm must have been anatomically separate; (2) the possibility that a second carcinoma represented a recurrence was excluded (each malignancy must have been separated by time [at least 3 years] and/or anatomical space [at least 2 cm of normal mucosa between each lesion]). To further exclude the possibility of direct tumor extension into adjacent compartments of Waldeyer's ring, we restricted our cases to tonsillar carcinomas separated by a zone (i.e. base of tongue) of clinically uninvolved tissue. Synchronous carcinomas were defined as two geographically separate carcinomas that were diagnosed within a 6-month period. Metachronous carcinomas were defined as geographically separate carcinomas which were diagnosed greater than 6 months after an index carcinoma was diagnosed.

Comparison of HPV-16 sequences and identification of variants

In order to investigate the molecular etiology of SPT development in HPV-HNSCC, we first confirmed the presence of HPV 16 DNA in the index tumor and its paired SPT by a type 16 specific assay which was performed using the in situ hybridization catalyzed signal amplification method for biotinylated probes (DAKO GenPoint, Carpinteria, CA). Subsequently, the viral DNA derived from the index tumor and SPT were sequenced. Paraffin embedded specimens were obtained and processed for genomic DNA extraction. Rather than sequence the entire HPV-16 viral genome in order to identify viral variants, previous research in cervical cancer has demonstrated that specific E6 nucleotide changes are able to correctly identify viral variants with low potential for misclassification.[12,13] Therefore, full-length E6 was amplified using the polymerase chain reaction (PCR), gel purified, and subsequently sequenced. Sequencing data for the primary and secondary tumors were then aligned to compare sequence similarity and to identify HPV variants. Numerous HPV-16 variants have been described in the literature and have been grouped into phylogenic lineages.[14] The HPV-16 variants that were detected in the tumor samples were assigned to a phylogenic class and subclass by detection of characteristic signature gene sequences in the Open Reading Frame (ORF) of E6, as described previously.[12] The prototype sequence of the European lineage was used as the reference for aligning the nucleotide position and comparison of sequences.[15]

Results

Patients

We found that 4% (6 of 135) of patients who were diagnosed with a primary tonsillar carcinoma developed either a synchronous or metachronous SPT in the contralateral tonsil. A total of four subjects met criteria for inclusion and had available tissue for analysis in this study. All patients were male and had a mean age of 59 (range: 44–76 years old). Three of the four identified cases presented with synchronous tonsillar carcinomas, while the remaining case presented with a metachronous tonsillar carcinoma. The first case reported that he had greater than 12 lifetime sexual partners; however, the sexual histories were unavailable for the remaining cases. Three cases presented with a T1 tumor stage for the index carcinoma, while one patient presented with a T3 tumor. The second primary tumors for all cases were staged as early T1 disease or carcinoma in situ. It should be noted that for every case, the SPT occurred at a contralateral site that was distinct from the index tumor; therefore, these carcinomas were classified as a contralateral primary cancer rather than multifocal or multicentric carcinomas. Furthermore, no other secondary HPV-associated cancer in the head and neck or at other sites (anal, penile, etc.) were diagnosed in these patients. All of these head and neck cancer patients are alive and disease free 6–13 years after treatment. Complete demographic and clinicopathological characteristics are shown in Table 1.

HPV-16 DNA sequence analysis

For each case, HPV-16 DNA sequencing from the index and SPTs were found to be 100% concordant in the E6 ORF. For case 1, the isolates obtained from both tumors belonged to the European class of HPV-16. A comparison of the isolates to the reference sequence demonstrated that guanine substitutions occurred at positions 131 and 350, which defines the G131/350G subclass. The prevalence of the G131G variant is approximately 10% in North American cervical specimens.[14] The exact sequence match between the isolates obtained from each independent tumor site suggested that a common viral variant caused the index tumor and the SPT. Similarly, comparison of the HPV-DNA isolates from the other cases again demonstrated consensus genetic sequences between the primary and secondary tumors suggesting infection with the same viral variant. The HPV isolate in case 2 was the European-prototype variant P350G which is prevalent in approximately 30% of North American cervical specimens and the HPV isolates in cases 3 and 4 were Asian-American variants which are prevalent in <1% of North American cervical specimens.[14] Nucleotide sequence variations for each case are depicted in Fig. 1.

Discussion

For head and neck cancer patients, the development of a SPT has considerable implications regarding long-term survival. When