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Human Papillomavirus-Related Small Cell Carcinoma of the Oropharynx

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Abstract

Human papillomavirus (HPV)-related oropharyngeal squamous cell carcinoma (OPSqCC) represents an important subgroup of head and neck cancer that is characterized by a distinct risk factor profile, a relatively consistent microscopic appearance, and a favorable prognosis. A growing experience with HPV testing of OPSqCCs has uncovered variants that deviate from prototypic HPV-related cancer with respect to morphology but not clinical behavior. In effect, HPV positivity confers a favorable prognosis independent of morphologic subtype. We report 5 cases of HPV-related oropharyngeal carcinomas with well developed features of small cell carcinoma (SCC) to define the prognostic impact of HPV positivity in a tumor type universally regarded as highly aggressive. Four of the SCCs arose in association with a conventional HPV-related OPSqCC. All 5 SCCs were HPV positive by in-situ hybridization. By immunohistochemistry, all 5 cases were p16 positive, synaptophysin positive, and CK5/6 negative. Four of the patients were males. The mean age was 61 years (range 49–67). The SCCs were associated with metastatic spread to distant sites (60%) and poor survival outcomes: 3 patients (60%) died as a result of their disease (mean survival time, 10 months; range, 6–15 months). HPV testing has disclosed a previously unrecognized variant of HPV-related oropharyngeal carcinoma that is microscopically characterized by the small cell phenotype. Recognition of this component, even in association with conventional HPV-related OPSqCC, is important as it may indicate an aggressive phenotype that supersedes HPV positivity as a prognostic indicator.

Keywords

Small cell carcinoma; high grade neuroendocrine carcinoma; human papillomavirus; HPV; HPV-related squamous cell carcinoma; p16; p63; in situ hybridization

Introduction

Small cell carcinoma (SCC) is a high grade neuroendocrine carcinoma. Most occur in the lung, but SCC can arise in extrapulmonary sites including the head and neck.(16,21) In the head and neck, SCC is most commonly encountered in the larynx, but it has also been reported in the sinonasal tract, salivary glands, trachea, oral cavity and oropharynx.

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(11,22,28) Those carcinomas arising in the oropharynx are usually widely disseminated at the time of diagnosis, and tend to follow an aggressive clinical course.(4)

High risk human papillomavirus (HPV), particularly the 16 type, has been established as a causative agent for a significant proportion of oropharyngeal squamous cell carcinomas (OPSqCCs),(10) and the incidence of these HPV-related carcinomas is on the rise.(13,17,20) Importantly, HPV-related carcinoma represents a distinct biological and clinical subtype of OPSqCC that is associated with improved clinical outcomes. HPV-positivity correlates with a lower risk of tumor progression and death, reflecting in part an enhanced sensitivity to ionizing radiation with or without chemotherapy.(3,7,9) Accordingly, HPV positivity is being used in ongoing clinical trials to identify and select “low risk” patients for less intensive multimodality therapy (i.e. de-escalation therapy).

The favorable prognosis conferred by HPV positivity consistently trumps traditional prognostic features predicated on morphology such as tumor grade and histologic subtype. As one example, the detection of HPV in a basaloid squamous cell carcinoma identifies a tumor that departs from the highly aggressive behavior usually associated with the basaloid variant.(5) HPV positivity in SCCs of the oropharynx has not been previously described. The purpose of this study was to determine the HPV status of SCCs arising in this site, and to discern whether HPV-positivity influences clinical behavior.

Materials and Methods

Cases

Study approval was obtained from The Johns Hopkins Medical Institutions Internal Review Board. Cases of SCC arising from the oropharynx were identified from a computerized search of the surgical pathology files of The Johns Hopkins Hospital between 1985 and 2010. For each case, all surgical pathology slides were reviewed to confirm the diagnosis of SCC as defined the 2004 World Health Organization Classification of head and neck tumors.(29) A representative block was chosen for in situ hybridization and immunohistochemical studies. Medical records were reviewed to document patient age, sex, tobacco and alcohol use, primary site of tumor origin, treatment, and patient outcome.

Immunohistochemistry

Immunohistochemical studies were performed on five-micron sections prepared from formalin-fixed and paraffin embedded tissue using standard autostaining protocols on a Ventana Benchmark XT autostainer (Ventana Medical Systems, Inc. Tucson, AZ). Deparaffinization and antigen retrieval (i-view detection system; Ventana) were carried out as an automated program of the above-mentioned stainer. Appropriate positive and negative controls were performed on each run, with appropriate results. The primary antibodies and final dilutions were: thyroid transcription factor-1 (TTF-1) (clone 8G7G3/1; Cell Marque, Rocklin, CA; dilution 1:500); p63 (clone 4A4; Cell Marque; prediluted by manufacturer); synaptophysin (clone 27G12; Leica Microsystems, Bannockburn, IL; prediluted by manufacturer); chromogranin (clone LK2H10; Ventana; prediluted by manufacturer); CK5/6 (clone D5/16 B4; Cell Marque; prediluted by manufacturer); p16 (clone INK4a; MTM Laboratories, Heidelberg, Germany; prediluted by manufacturer); and CAM5.2 (clone B22.1+B23; Cell Marque; prediluted by manufacturer).

For P16, expression was regarded as positive only if strong and diffuse nuclear and cytoplasmic staining was present in ≥70% of the tumor. TTF-1 and p63 demonstrated nuclear positivity while synaptophysin, chromogranin, CAM5.2, and CK5/6 showed cytoplasmic staining. For CAM5.2, the pattern of staining (i.e., diffuse or dot-like) was also recorded.

In situ hybridization

Five-micron sections from formalin-fixed paraffin embedded tumor blocks were evaluated for the presence of HPV DNA by two different methods of in situ hybridization. Each case underwent testing by both an automated protocol utilizing the Ventana HR HPV III probe set (Ventana Medical Systems, Tucson, AZ) and the type 16-specific ISH catalyzed signal amplification method for biotinylated probes (DAKO GenPoint, Carpinteria, CA). For each method, a positive result was defined as the detection of punctate signals localized to tumor nuclei. HPV-16-positive controls included HPV-16 positive OPSqCC cell lines (SiHa and CaSki), while an HPV-16 negative HNSCC served as a negative control.

Results

Nine oropharyngeal SCCs were identified. The clinical features of these SCCs are summarized in Table 1. Five (56%) were found to be HPV-positive. All five HPV-positive cases were from SCCs diagnosed between 2001 and 2010 (2001, 2006, 2009, 2009, 2010), while the four HPV-negative cases were from 1988 to 2009 (1988, 1993, 2007, 2009). Patients ranged in age from 49 to 83. Patients with HPV-related SCCs tended to be younger than those with HPV-unrelated SCCs ((mean 61 years vs. 71 years). Eight (89%) patients were men. Seven (78%) patients were either former (n=4) or current (n=3) smokers. The two patients who never smoked had HPV-related SCCs. For the 5 patients where a detailed history of smoking activity was available, all had 20 or more pack years of smoking (mean, 45 pack years; median, 40 pack years). Four patients presented with neck masses, 2 patients presented with odynophagia, 1 patient presented with a large oropharyngeal mass, 1 patient with an oropharyngeal tumor extending into the nasopharynx presented with nasal obstruction and epistaxis, and 1 patient was incidentally found to have a lung metastasis during hospitalization for a cerebrovascular accident. Five patients were treated with some combination of surgery, radiation therapy, and chemotherapy; 1 was treated with chemotherapy and radiation; 2 received chemotherapy alone, and 1 had surgical removal with no additional therapy. Seven patients developed distant metastases and died as a result of their disease 2–15 months (mean, 9 months) after diagnosis. Two patients with HPV-related SCCs were alive with no evidence of disease at 5 and 20 months of follow-up.

The pathologic and immunohistochemical features of the oropharyngeal SCCs are summarized in Table 2. Five (56%) were HPV positive. Four of the HPV-related SCCs were type 16 positive. In one type-16 negative case, HPV was detected using a broader probe set that includes 11 non-16 high risk types. In 4 cases, the SCC arose in association with a squamous cell carcinoma exhibiting a more conventional HPV morphology (i.e. non-keratinizing and basaloid with tumor infiltrating lymphocytes) (Figure 1). In 3 of these cases, the small cell and non-small cell components were synchronous, and in one case the SCC locally recurred at the site where an HPV-related SqCC had been resected 18 months earlier. Seven tumors were p16 positive by immunohistochemistry including all 5 of the HPV-related SCCs (Figure 2) and 2 HPV-unrelated SCCs. All cases were positive for CAM. 2, usually in a perinuclear dot-like pattern. All cases were positive for synaptophysin, and 7 of 9 were also positive for chromogranin. Two cases were TTF-1 positive. P63 staining was present in 4 of the cases, but staining ranged from focal (less than 5% of cells) (n=3) to diffuse (n=1). CK5/6 was negative in all SCCs, only highlighting areas of squamous differentiation.

Discussion

HPV-related OPSqCC has a characteristic microscopic appearance. These tumors typically take origin from the tonsillar crypts and infiltrate the lymphoid stroma as lobules of immature basaloid cells with minimal cytoplasmic keratinization.(30) Routine HPV testing

of OPSqCCs has expanded the morphologic spectrum of HPV-related cases including the recognition of certain phenotypic variants that deviate from the histologic prototype in predictable ways. These include papillary,(12) lymphoepithelial-like,(25) basaloid squamous,(5) and adenosquamous(18) variants of HPV-related OPSqCC. Although these phenotypic variations may be diagnostically relevant for the way they cause confusion with other cancer types, they do not appear to impact prognosis. The presence of HPV consistently imparts a favorable prognosis, even when detected in more aggressive phenotypes such as the basaloid squamous cell carcinoma.(5)

We report yet another variant of HPV-related oropharyngeal carcinoma that is microscopically indistinguishable from small cell carcinoma of the lung. This variant shares features with small cell carcinoma of the uterine cervix including an association with high risk HPV, expression of neuroendocrine markers by immunohistochemistry, and its frequent co-existence with a non-small cell carcinoma including squamous cell carcinoma.(6,26) In our small series, 80% of the cases arose in association with a synchronous or metachronous HPV-associated squamous cell carcinoma. Most importantly, HPV-related SCC of the oropharynx may share the same aggressive clinical features of its counterpart in the uterine cervix where the small cell phenotype has been associated with early distant spread and poor overall survival.(2,27) In our 5 cases, 3 patients died within 15 months of diagnosis (mean, 10 months) with widely disseminated disease. This aggressive behavior is in sharp contrast to non-small cell carcinomas of the oropharynx where the presence of HPV is associated with 3 year survival rates exceeding 80%.(3) At this point, experience with HPV-related SCCs of the oropharynx is too small to fully appreciate the prognostic implications of the small cell phenotype and to assign a clear treatment algorithm such as therapeutic protocols used to treat SCCs arising in other anatomic sites. While the small cell phenotype does appear to point to a more aggressive behavior, the HPV-related tumors were not uniformly fatal in contrast to the HPV-negative SCCs. At the very least, the presence of a small cell component should disqualify any patient with an HPV-related OPSqCC from being considered for less intensive multimodality therapy (i.e. de-escalation therapy) solely on the grounds of HPV positivity.

For patients with HPV-related cancer of the oropharynx, recognition of the small cell variant and its distinction from HPV-related squamous cell carcinoma is important but not necessarily straightforward. Both tumor types share certain morphologic features including small hyperchromatic cells with scant cytoplasm, and comedonecrosis.(24) Immunohistochemistry may play a role in confirming the presence of a small cell component. Although absence of p63 is used to differentiate small cell carcinoma of the lung from squamous cell carcinoma, (14,31,33) we found that p63 was positive in 4 of the 8 tested SCCs. Cytokeratin 5/6 was a more reliable distinguishing marker. In contrast to OPSqCCs that are consistently cytokeratin 5/6 positive, all of the SCCs were cytokeratin 5/6 negative. These findings support the conclusion of Serrano et al (24) that small cell carcinomas of the head and neck sometimes express p63, and that loss of CK5/6 expression is more specific than loss of p63 expression in distinguishing the small cell phenotype. Only 2 of the 9 SCCs were TTF-1, but TTF1 positivity is very specific for SCC in this setting.(29) All 9 SCCs demonstrated immunohistochemical evidence of neuroendocrine differentiation: All were synaptophysin positive, and 7 were chromogranin positive. For SCCs of the oropharynx, p16 positivity may not be a reliable surrogate marker for HPV infection. We found that p16 was positive in 2 of the 4 SCCs that were HPV negative using an in-situ hybridization approach. This is not altogether unexpected as most small cell carcinomas arising in other sites (e.g. lungs) are often p16 positive due to mechanisms unrelated to HPV infection.(32)

Admittedly, SCC of the oropharynx is a very rare form of head and neck cancer. Most SCCs of the head and neck occur in the larynx, and its description in the oropharynx is limited to a case reports and small case series.(1,4,11,15,19,23,28) The rising overall incidence of HPV-related OPSqCCs suggests the possibility of a parallel upsurge in the appearance of its various subtypes including this small cell variant. In our experience, HPV was only detected in those SCCs diagnosed in the last decade. Conversely, HPV-related SCC was not detected prior to 2001. This time trend mirrors the rising overall incidence of HPV-related OPSqCC and the reversal in the ratio of HPV-positive to HPV-negative cases noted over the past decade.(8)

In summary, we report a subset of HPV-related oropharyngeal carcinoma that demonstrates a small cell morphology. Despite the presence of HPV, this small cell phenotype may indicate a greater propensity for aggressive clinical behavior.

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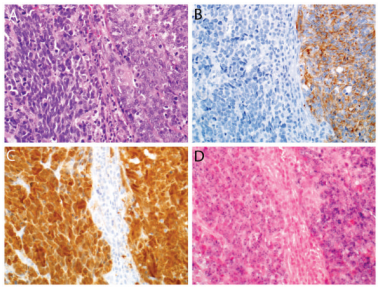

Figure 1.
HPV-related carcinoma with mixed small cell (left) and squamous cell (right) components. In this area of transition, cords of tumor cells with ample cytoplasm, vesicular nuclei and conspicuous nucleoli merge with small cells with scant cytoplasm, hyperchromatic nuclei and absent nucleoli.

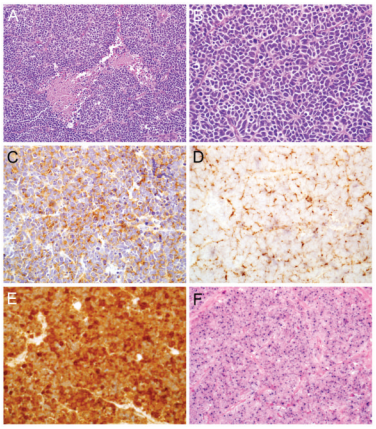

Figure 2.
HPV-related small cell carcinoma of the oropharynx. The tumor consists of sheets of small hyperchromatic cells with zones of necrosis (A). The cells exhibit a high nuclear:cytoplasmic ratio, nuclear molding, and rosette formations (B). The cells are positive for synaptophysin (C), CAM5.2 in a dot-like pattern (D), and p16 (E). HPV in situ hybridization shows hybridization signals localized to the tumor nuclei (F).

Table 1

Clinical characteristics of patients with HPV-related small cell carcinoma of the oropharynx

Case #	HPV status	Year	Site	Age	Sex	Pack years	Presentation	Treatment	Clinical course	Outcome	Time to death/last follow-up (months)
1	+	2001	tonsil	65	F	40	lung metastasis	CT	distant metastases	DWD	6
2	+	2006	tonsil	67	M	80	neck metastasis	Surgery/RT/CT	distant metastases	DWD	15
3	+	2009	soft palate	67	M	20	epistaxis	Surgery/RT/CT	complete response	NED	20
4	+	2009	tonsil	55	M	0	neck metastasis	CT/RT	distant metastases	DWD	9
5	+	2010	tonsil	49	M	0	neck metastasis	CT/RT	complete response	NED	5
6	−	1988	base of tongue	72	M	35	odynophagia	surgery	distant metastases	DWD	12
7	−	1993	base of tongue	66	M	50	neck metastasis	surgery/RT/CT	distant metastases	DWD	12
8	−	2007	tonsil	63	M	Unknown	odynophagia	Surgery/RT/CT	distant metastases	DWD	9
9	−	2009	tonsil	83	M	unknown	oropharyngeal mass	CT	distant metastases	DWD	2

CT, chemotherapy; RT, radiation therapy; DWD, dead with disease; NED, no evidence of disease

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Table 2

In situ hybridization and immunohistochemical findings in HPV-related small cell carcinomas of the oropharynx.

Case	HPV in-situ hybridization		Immunohistochemistry						
	high risk*	type 16	P16	synaptophysin	chromogranin	TTF-1	CAM5.2	p63	CK5/6
1	−	+	+	+	+ (focal)	−	+ (dot-like)	+ (focal)	−
2	+	+	+	+	+ (focal)	+	+ (dot-like)	+ (focal)	−
3	+	−	+	+	−	−	+ (diffuse)	−	−
4	−	+	+	+	+	+	+ (dot-like)	+	−
5	+	+	+	+	−	−	+ (dot-like)	−	−
6	−	−	+	+	+	−	+ (dot-like)	−	−
7	−	−	−	+	+	−	+ (dot-like)	−	−
8	−	−	+	+	+	−	+	ND	ND
9	−	−	−	+ (focal)	+	−	+ (dot-like)	+ (focal)	−

*high risk DNA probe cocktail with affinity to HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66; ND, not done (due to insufficient material).

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