HPV Positive Squamous Cell Carcinoma of the Oropharynx. Are we Observing an Unusual Pattern of Metastases?

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Abstract To describe the clinical, histopathologic, immunohistochemical and molecular features of human papilloma virus (HPV)+ squamous cell carcinomas of the oropharynx that had an atypical clinical course. **Methods and Results:** Four patients with HPV+ oropharyngeal squamous cell carcinoma (OPSCC) were identified retrospectively based on unanticipated clinical behavior. The histopathology, immunohistochemistry and molecular studies of both the primary tumor and the metastases were analyzed to look for any predictors to explain the clinical course. The four patients were all male (average age 55) who presented initially with a neck mass and had stage IVA disease. Three of the primary tumors were nonkeratinizing squamous cell carcinoma and one case was a hybrid tumor of both high-grade squamous cell carcinoma and nonkeratinizing type,

and all were p16 and HPV 16/18 positive. All patients received concurrent chemoradiation as primary therapy and had a complete response. Disease-free survival ranged from 7 to 15 months and metastases in 3 patients occurred only in bone, including the sternum, humerus, clavicle, and vertebrae. In one patient, distant metastases were identified in the pancreas, liver, lung and skull base. All metastatic lesions were nonkeratinizing morphology and were p16 and/or HPV positive. Two patients died of their disease, one patient is alive with disease and one patient is disease-free. Although infrequent, unanticipated clinical outcomes can occur in HPV-related OPSCC including distant metastases and bone-only metastases. The tumor morphology of the metastases was comparable to the primary and retained p16 and HPV expression.

Keywords HPV · Oropharynx · Metastases

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Introduction

Over the last few decades, clear evidence has emerged indicating that human papilloma virus (HPV)-associated tumors of the oropharynx (OP) are responsible for the marked increase in the incidence of squamous cell carcinoma in this region [1]. More than 50 % of all oropharyngeal squamous cell carcinomas (OPSCC) are associated with high-risk HPV (mostly HPV 16) and are positive for p16 by immunohistochemistry (IHC) [2, 3]. This disease appears to have a distinct molecular, epidemiological, and clinical behavior. Multiple retrospective series have shown that patients with p16-related OPSCC have a better prognosis compared to patients with HPV/p16-negative tumors [2, 4, 5].

Patients with OPSCC are generally younger, and are usually not tobacco or heavy alcohol users. Despite

improved overall survival among patients with HPV/p16+ tumors compared to HPV/p16–OPSCC [6–9], the rate of distant metastasis remains equivalent among the two groups. It has become apparent to us, and recently reported by others [10], that we may be observing a peculiar pattern of distant metastases sometimes involving bone-only sites in HPV/p16+ OPSCC.

We add an additional 4 cases of atypical metastases of HPV/p16+ OPSCC. Herein, we describe the histopathology and immunohistochemistry of the primary tumor, as well as the metastases, to determine if there are any unique pathologic findings that correspond to the unusual clinical behavior.

Methods

Patients with OPSCC with atypical clinical behavior were identified by treating oncologists at Winship Cancer Institute, Emory University, Atlanta, GA between 2009–2011. Of eight cases identified, only four cases where formalin-fixed, paraffin-embedded (FFPE) pathology material was available from both the primary tumor and metastases were included in this study. The medical records of the patients were reviewed with regard to: the patient's history, age, gender and race; radiographic findings; and treatment and outcome. The histologic specimens were analyzed for morphology (keratinizing, nonkeratinizing with maturation, and non-keratinizing) [11], cytologic features including pleomorphism and mitotic figures, and perineural and lymphovascular invasion. The primary tumors were evaluated for p16 by IHC and high-risk HPV by in situ hybridization (ISH). The metastatic tumors were also evaluated for p16 and/or HPV positivity. For HPV testing, hybridization was demonstrated using a biotinylated HPV 16/18 DNA probe (DAKO, Carpinteria, CA, USA) and GenPoint™ catalyzed signal amplification system

(DAKO) according to the supplier's instructions using 3, 3'-diaminobenzidine (DAB). Positive HPV staining was identified as punctate staining in the nuclei (distinct dot-like intranuclear signal) and both strong, diffuse positivity and extremely focal staining were considered positive [4]. The positive control was a known HPV+ OPSCC, and both a positive DNA and a negative control were run for each specimen.

For p16 evaluation, FFPE tissue blocks or cell blocks were obtained and five micrometer sections were cut and deparaffinized. Antigen retrieval was performed and tissue sections were incubated with a mouse monoclonal antibody against p16 (CINtec Histology Kit, MTM Laboratories, Heidelberg, Germany) according to the manufacturer's instructions. A known p16 positive OPSCC was used as a positive control. The p16 IHC was considered positive if more than 75 % of the tumor cells were strongly positive in the nucleus and/or cytoplasm as reported by others [4].

Institutional review board approval was obtained for this study.

Results

Demographic Data

All patients were white males aged from 46 to 66 (mean, 55 years) with an initial presentation of a neck mass (Table 1). The base of tongue was the primary site in 3 cases and the tonsil in 1 patient. All patients received concurrent chemoradiation as the initial treatment.

The pathologic specimens were analyzed histologically for cytologic features, p16 IHC and/or HPV ISH findings (Table 2). The metastatic sites that were biopsied were also reviewed and evaluated for p16 and/or HPV.

The individual details of the four patients are described below.

Table 1 Clinical characteristics

	Age (y)	Gender	Race	Initial presentation	1° site	T	N	Stage	ECS ^a	Treatment/response	Disease-free survival (months)	Overall survival (months)
Case 1	65	Male	W	2–3 month history bilateral neck nodes	BOT	4	2c	IVA	No	Concurrent CRT: CR	10	NED, 22
Case 2	66	Male	W	2 week history of left neck mass	BOT	1	2c	IVA	Yes	Concurrent CRT: CR	9	DOD, 28
Case 3	47	Male	W	2 month history of left sided neck mass	Tonsil	1	2b	IVA	Yes	Concurrent CRT: CR	15	AWD, 18
Case 4	46	Male	W	1 month history of left sided neck mass	BOT	1	2c	IVA	No	Concurrent CRT: CR	7	DOD, 30

y years, W white, BOT base of tongue, CRT chemoradiation, CR complete response, NED no evidence of disease, AWD alive with disease, DOD dead of disease

^a ECS: extracapsular spread of cervical lymph nodes based on radiographic imaging

Table 2 Pathologic findings and pattern of distant metastases in HPV+ OPSCC

	Tumor morphology	Lymphovascular invasion	Perineural invasion	Mitoses per 10HPF	Synchronous 2nd primary	Site of distant mets
Case 1	NKSCC	Yes	No	30	Supraglottis	Sternum
Case 2	Hybrid: SCC & NKSCC	No	No	47	No	Spine: T8, T9, L1, L2, with progressive vertebrae disease
Case 3	NKSCC	No	No	37	No	Clavicle, humerus, ribs, sacrum, spine: thoracic and lumbar
Case 4	NKSCC	Yes	No	52	No	Lung, pancreas, liver, skull base, diaphragmatic soft tissue, paraesophageal

OPSCC oropharyngeal squamous cell carcinoma, HPF high-power field, NKSCC nonkeratinizing squamous cell carcinoma

Case 1

A 65-year-old white male with a 20 pack-year history of tobacco use, but who discontinued 25 years ago and used alcohol socially, presented with a 2- to 3-month history of bilateral lymphadenopathy. A CT scan demonstrated numerous bilateral necrotic lymph nodes, the largest being 4.5 cm on the right side and 3.5 cm on the left side, although radiographically not suspicious for extracapsular spread (ECS). A 4.4 × 3.0 cm area mass involving both right and left tongue base and extending caudally to the epiglottis and involving the extrinsic tongue muscles was identified. During direct laryngoscopy, a second primary tumor <1.5 cm in the right posterior aryepiglottic fold was identified. This was noncontiguous with the tongue base mass. A biopsy from the tongue base revealed nonkeratinizing squamous cell carcinoma with extensive lymphovascular invasion (Fig. 1a, b). The tumor cells were positive for HPV 16/18 by ISH and diffusely positive for p16 by IHC and the patient was clinically staged as T4, N2c (Stage IVA) (Fig. 1c, d). The tumor from the aryepiglottic fold showed similar histology. He received combined modality therapy with external beam radiation and cisplatin at 100 mg/m². The patient noted tinnitus following cycle 2 of cisplatin and therefore treatment was changed to weekly carboplatin and paclitaxel. A restaging CT scan obtained 2 months after completion of therapy revealed no evidence of residual disease. However, a surveillance PET done 4 months after completion of therapy showed FDG-uptake within the right base of tongue in addition to a single metastatic-appearing lesion in the left sternum (Fig. 1d). The patient underwent a biopsy of his tongue base, which revealed scarring and chronic inflammation. The patient declined receiving systemic therapy and had a partial sternotomy confirming metastatic squamous cell carcinoma. The tumor demonstrated a cystic architecture composed of nonkeratinizing sheets and nests of basaloid tumor cells with sharply defined borders similar to cystic metastasis to cervical lymph nodes often

described in HPV +OPSCC (Fig. 1e, f). The metastatic tumor was strongly p16+ by IHC as well as HPV16+ by ISH confirming the diagnosis. He received external beam radiation to the same area and remains disease-free 13 months after metastectomy, 20 months after completion of chemoradiation therapy, and 22 months after initial diagnosis.

Case 2

A 66-year-old white male noted a left neck mass that had been present for 2 weeks. The patient denied tobacco use and used alcohol socially. A fine needle aspiration (FNA) biopsy was positive for metastatic squamous cells, which were diffusely positive for p16 by IHC and HPV16/18 by ISH. A CT examination showed a 1.7 cm left base of tongue lesion with a 2.7 cm left neck mass and 2.0 cm right neck mass consistent with a conglomeration of lymph nodes with necrotic centers radiographically suspicious for ECS. A biopsy of the tongue base showed a markedly anaplastic carcinoma adjacent to areas of more typical nonkeratinizing squamous cell carcinoma (Fig. 2a). Repeat staining of the primary tumor was also diffusely positive for p16 by IHC as well as HPV16+ by ISH including the anaplastic areas (Fig. 2b). He was clinically staged T1N2c (Stage IVA) SCC and received concurrent radiation therapy sensitized with cisplatin and cetuximab on a clinical protocol. The patient developed an anaphylactic reaction to the cetuximab during his first infusion and cetuximab was subsequently discontinued but completed two cycles of cisplatin in conjunction with radiation. A restaging PET/CT 2 months after completion of therapy was negative for disease. Nine months after completion of therapy, a new site of metastasis was identified involving the T9 vertebral body. A biopsy confirmed metastatic nonkeratinizing squamous cell carcinoma which was p16+. He received stereotactic radiosurgery to T9, and subsequently underwent systemic therapy with 4 cycles of TIC (paclitaxel, ifosfamide and carboplatin) chemotherapy. A lesion at L2

◀ **Fig. 1** High-power photomicrograph of HPV+ nonkeratinizing squamous cell carcinoma from the tongue base (a). Prominent lymphovascular invasion was identified in the biopsy specimen (arrows) (b) and showed strong immunoreactivity for p16 (c) and positive dot-like nuclear signal for HPV 16/18 by in situ hybridization (arrows) (d). PET scan revealed a single metastatic bone lesion in the sternum (e). The bone metastasis demonstrated a cystic architecture similar to cystic metastasis to cervical lymph nodes often described in HPV-associated oropharyngeal squamous cell carcinomas (f)

was noted 6 months later and was confirmed to be of metastatic nature via a CT-guided biopsy. Additional biopsy-proven metastatic sites involved L1 and T8, all showing a nonkeratinizing pattern with p16+ (Fig. 2c, f, d). He underwent stereotactic radiation to these sites. The

patient continued to have slowly progressive disease to the vertebrae and died of disease 28 months after his initial diagnosis and 19 months after his metastatic diagnosis.

Case 3

A 47-year-old otherwise healthy white male presented with a 2-month history of a 3 cm left neck mass. He denied tobacco use and used alcohol socially. FNA biopsy of the neck mass was positive for metastatic cells that were diffusely positive for p16 by IHC and positive for HPV by ISH. Radiographic imaging did not reveal a primary site but was suspicious for ECS of the cervical lymph node.

Fig. 2 Biopsy from the base of tongue shows a hybrid tumor with areas of typical nonkeratinizing squamous cell carcinoma adjacent to areas with marked pleomorphism and keratin production (a). Both morphologic components were strongly immunoreactive for p16 (b).

A core biopsy from a thoracic vertebrae demonstrates a nonkeratinizing squamous cell carcinoma with no squamous differentiation (c) which shows strong immunoreactivity for p16 (d)

Examination of the tonsillectomy specimen showed a 0.3 cm nonkeratinizing squamous cell carcinoma which also was diffusely positive for p16 (Fig. 3a, b). The patient was clinically staged as T1N2b (Stage IVA). He received concurrent external beam radiation and three cycles of cisplatin dosed at 100 mg/m². A post-treatment CT scan was negative for persistent or new disease. However, 15 months after initial presentation, the patient experienced back pain. A PET-CT of the body demonstrated numerous lytic, hypermetabolic lesions of the bones including the right clavicle, left humerus, right 4th and 5th ribs, sacrum and the thoracic and lumbar spine (Fig. 3c, d). The primary tumor site was negative for disease by imaging as were the cervical lymph nodes. The patient received palliative

chemotherapy consisting of 4 rounds of carboplatin, taxotere and cetuximab, however, has had continued progression of disease as evidenced by PET-CT imaging 18 months after initial presentation.

Case 4

A 46-year-old otherwise healthy white male presented with a 1-month history of a right-sided neck mass. The patient smoked 4–6 cigars a year and drank 2 glasses of wine daily. Radiographic imaging was positive for a 2 × 1.8 cm area mass of the right base of tongue, as well as right and left cervical lymph nodes measuring 2.2 and 1.4 cm in greatest dimension, respectively, without imaging findings of ECS.

Fig. 3 A 0.3 cm nonkeratinizing squamous cell carcinoma from the tonsil (**a**) which showed strong immunoreactivity for p16 (**b**) and was positive for HPV 16/18 (not shown). PET imaging revealed

numerous bony metastases, including the humerus (*arrow*) (**c**) and vertebrae (*arrow*) (**d**) 15 months after completion of concurrent chemoradiation

A biopsy of the tongue base was positive for nonkeratinizing squamous cell carcinoma which was diffusely positive for p16 by IHC and HPV16 by ISH (Fig. 4a, b). Focally, lymphovascular invasion was identified. The patient was clinically staged as T1N2c (Stage IVA) and treated with concurrent external beam radiation and two cycles of cisplatin (100 mg/m²). A restaging CT 2 months after treatment was negative for persistent or new disease. However, a surveillance PET-CT, 7 months after initial presentation, showed multiple lung nodules and a biopsy was positive for nonkeratinizing squamous cell carcinoma with prominent lymphovascular invasion which was diffusely positive for p16 and HPV, consistent with metastatic disease (Fig. 4c, d). The patient received 3 cycles of TIC chemotherapy and remained disease-free until 25 months after his initial diagnosis when he experienced pancreatitis. Radiographic imaging revealed a mass at the head of the pancreas, as well as paraesophageal and diaphragmatic soft tissue masses. FNA biopsies of the liver and pancreas were positive for nonkeratinizing cancer and was strongly p16+ (Fig. 4e). Two months later, a 2.5 cm nasopharyngeal mass extending to the clivus was noted on imaging, but this was not biopsied. The patient died 3 months later, 30 months after his initial presentation.

Pathologic Features

Three of the four cases of OPSCC had a nonkeratinizing morphology with <10 % squamous maturation. Case 2 had hybrid morphology with areas of obvious squamous differentiation alongside more typical nonkeratinizing morphology. The areas of squamous differentiation had markedly pleomorphic appearance with numerous atypical mitoses. Mitotic activity of the primary tumor ranged from 30 to 52 per 10 high-power fields. Prominent lymphovascular invasion was noted in the primary tumors from Cases 1 and 4 and lymphovascular invasion was seen in the mediastinal biopsies from Case 4. All metastases had nonkeratinizing morphology and were positive for p16 and/or HPV. Only one patient had a synchronous tumor of the same histologic type (Case 1) and no patients developed a second primary.

Discussion

Many retrospective and prospective studies have demonstrated that HPV related OPSCC have improved clinical outcome despite the advance stage at presentation [1, 5], particularly in comparison to HPV-OPSCC. Huang et al [10] recently described, in a retrospective cohort study, unusual clinical behavior in p16+ OPSCC treated with radiotherapy [10]. This included distant metastases (DM)

to multiple organs and unusual sites. The tumors were not tested for HPV as p16 was used as the surrogate marker for HPV. In addition the primary tumor morphology and the pathology of the DM were not described.

To our knowledge, this is the only other report describing atypical behavior in HPV+ OPSCC. In this study, we identified four patients who all presented with the typical clinical presentation of HPV+ OPSCC and yet had unusual outcomes. Three patient's tumors were of nonkeratinizing morphology while one patient had a mixture of nonkeratinizing and squamous differentiation (hybrid). Lymphovascular invasion was readily identified in Cases 1 and 4 but perineural invasion was not seen. All tumors were mitotically active ranging from 30 to 52 mitoses/10 high-power field. Case 1 had a synchronous lesion in the supraglottis showing similar tumor morphology. All tumors strongly expressed p16 by IHC. Primary tumors were positive for HPV16/18 with ISH. On review of the metastatic lesions, the tumor morphology in all cases was nonkeratinizing squamous cell carcinoma. This was evident even in Case 2 where the primary tumor had hybrid morphology with markedly anaplastic areas. The metastatic deposits retained strong p16 expression and when performed, were positive for HPV by ISH. Interestingly, the bone metastasis to the sternum in Case 1 demonstrated a cystic pattern similar in nature to the cystic metastatic pattern to cervical lymph nodes often described in HPV-associated OPSCC. None of the clinical or pathologic parameters evaluated were predictive of an atypical clinical course. No patient had a second primary, and no patient had local recurrence.

Huang et al [10] described in their OPSCC cohort that patients with HPV+ tumors had a lower incidence of other malignancies compared to HPV-OPSCC, and attributed these findings to lower exposure to tobacco [10]. In addition both synchronous and metachronous second primaries were significantly greater in HPV-OPSCC compared to HPV+ OPSCC. The 3 synchronous tumors in the HPV+ cohort all occurred in the head and neck mucosa (supraglottic, post-cricoid, and nasopharynx) as did our synchronous tumor in the supraglottis from Case 1 [10]. Some of the unique findings in our 4 cases are the rapidity and location of DM. The interval from completion of therapy to onset of DM ranged from 4–11 months (7 months, mean). Distant metastases occurred in 10 % of HPV+ OPSCC cohort in Huang et al [10] and the median onset of DM was 18 months (2–52 months) and 63 % of DM occurred in the lungs [10]. However, in this cohort 5 % of HPV+ OPSCC had multiple organ dissemination. In the current study, bone-only metastases occurred in 3 cases and in 2 of these patients, multiple skeletal sites were involved. In case 4 no bone metastases were observed, however, widespread metastases to numerous organs including lung, liver, and pancreas were noted.

◀ **Fig. 4** Low-power photomicrograph showing nonkeratinizing squamous cell carcinoma of the base of tongue (**a**) which was strongly immunoreactive for p16 (**b**). A biopsy of a lung nodule showed morphology similar to the primary tumor (**c**) and p16 immunostain highlights the prominent lymphovascular invasion (**d**). A fine needle aspiration of a FDG-avid lesion on PET at the head of the pancreas shows a neoplasm with similar morphology to the primary tumor along with ductal epithelial cells (*arrows*) (**e**). The tumor cells were positive for HPV 16/18 by in situ hybridization (*arrows*) (**f**)

Albeit an unusual occurrence, awareness of atypical clinical behavior of some HPV+ OPSCC is important on several levels. Consideration should be given to the choices and frequency of radiologic exams for post-therapy surveillance for patients treated for OPSCC, as well as the choices of radiologic exams for initial staging of these patients. Pathologists need to be aware of these unusual presentations and confirm the pathology with p16 and/or HPV to rule out a second primary tumor. Before instituting de-escalation of chemotherapy and/or radiation therapy, improved understanding of the biology of HPV+ disease and the reasons underlying these observed patterns of distant and unusual metastases is prudent.

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