Human Papillomavirus-16 Associated Adenocarcinoma NOS of Base of Tongue

Bayardo Perez-Ordoñez · Jonathan C. Irish ·
Eugene S. Yu · Maura L. Gillison

Received: 24 July 2012 / Accepted: 1 October 2012 / Published online: 10 October 2012
© Springer Science+Business Media New York 2012

Abstract The causative role of human papillomavirus (HPV) in a subset of squamous cell carcinomas of the oropharynx (OSCC) is now well established with HPV-16 being detected in approximately 92 % of HPV-positive OSCC in North America. Recently HPV has also been found in some non-squamous oropharyngeal carcinomas including adenocarcinomas, neuroendocrine carcinomas, and undifferentiated “lymphoepithelioma-like” carcinomas. Herein, we report a HPV-16 positive adenocarcinoma NOS arising in the base of the tongue of a non-smoker, 64 year-old male. To our knowledge, this is the first time this association has been clearly documented. The adenocarcinoma

was diffusely positive for p16 and HPV-16 was demonstrated by in situ hybridization and linear array analysis. Q-PCR demonstrated a viral load of 4 copies per cancer cell, whereas qRT-PCR for E6/E7 transcript levels were 30 copies per 1,000 human ribosomal protein large P0 (RPLP0) reference gene. The presence of HPV in non-squamous oropharyngeal carcinomas raises the possibility that HPV is also involved in the carcinogenesis of some uncommon carcinomas of the region.

Keywords Human papillomavirus (HPV) · HPV-16 · Adenocarcinoma · Oropharynx · Base of tongue

B. Perez-Ordoñez (✉)
Department of Pathology, Toronto General Hospital,
University Health Network, 200 Elizabeth Street,
Toronto, ON M5G 2C4, Canada
e-mail: Bayardo.Perez-Ordóñez@uhn.ca

B. Perez-Ordoñez
Department of Laboratory Medicine and Pathobiology,
University of Toronto, Toronto, ON, Canada

J. C. Irish
Department of Otolaryngology-Head and Neck Surgery/Surgical
Oncology, Wharton Head and Neck Program, Princess Margaret
Hospital, University Health Network, Toronto, ON, Canada

E. S. Yu
Joint Department of Medical Imaging of Mount Sinai Hospital,
University Health Network, Toronto, ON, Canada

E. S. Yu
Department of Medical Imaging, University of Toronto,
Toronto, ON, Canada

M. L. Gillison
Department of Viral Oncology, The Ohio State University
Comprehensive Cancer Center, Columbus, OH, USA

Introduction

The causative role of human papillomavirus (HPV) in a subset of squamous cell carcinomas of the oropharynx (OSCC) is now well established [1]. HPV is detected in approximately 60–70 % of OSCC in North America [1–3] with HPV-16 found in 92 % of HPV-positive OSCC [4]. HPV is a small double-stranded DNA virus which contains its coding information in three major regions: the early region (E1–8), responsible for transcription, plasmid replication, and transformation; the late region coding the major (L1) and minor (L2) capsid proteins; and the long-control region (LCR) which regulates viral transcription and replication [5]. Two viral oncogenes, E6 and E7, are found in the early region. E6 binds and targets p53 for degradation, while E7 inactivates the retinoblastoma (RB) family of proteins. The inactivation of p53 and RB results in aberrant cell proliferation, inhibition of apoptosis, cellular immortalization and genomic instability [6, 7].

HPV-positive OSCC appears to be a distinctive disease compared to HPV-negative OSCC. Patients with HPV-positive

OSCC are on average 5 years younger than HPV-negative patients, have higher lifetime number of vaginal-sex and oral-sex partners, higher prevalence of oral HPV infection, and lower incidence of alcohol and tobacco use [8]. HPV status in OSCC is also a powerful prognostic indicator with HPV-positive OSCC patients having a 58 % reduction in the risk of death when compared to patients with HPV-negative OSCC [2, 9]. Pathologically, most HPV-positive OSCCs exhibit non-keratinizing or basaloid morphology [10–13]. Recently, HPV has also been found in adenosquamous carcinomas [14], neuroendocrine carcinomas [15, 16] and undifferentiated “lymphoepithelioma-like” carcinomas of the oropharynx [17, 18]. Although the number of cases described is still small, HPV-associated neuroendocrine carcinomas of the oropharynx are aggressive malignancies [15, 16].

Herein, we describe the presence of HPV-16 in a high-grade, non-salivary gland-type adenocarcinoma of the base of the tongue. To our knowledge, this is the first time this association has been clearly documented. The identification of transcriptionally active HPV in several types of non-squamous carcinomas of the oropharynx suggests a role for the virus in the etiology of at least a portion of non-squamous carcinomas of this region.

Materials and Methods

The initial diagnostic biopsy and subsequent surgical specimen were routinely fixed in neutral buffered formalin. Representative sections were processed as per standard protocols, embedded in paraffin, sectioned at 5 μ and stained with hematoxylin and eosin. Selected sections of tumor were stained with PAS, PAS-D, mucicarmine and antibodies listed in Table 1.

In situ hybridization (ISH) was performed in a representative section of tumor using the GenPoint ISH amplification system (Dako, Carpinteria, CA) which can detect a single integrated copy of HPV-16 DNA [19]. The presence of HPV DNA was also investigated by Linear Array HPV Genotyping Test (Hoffmann-La Roche, Basel, Switzerland) [20]. HPV-16 viral load and E6/E7 RNA transcripts levels were measured using quantitative real time PCR and (qPCR) reverse transcriptase PCR (qRT-PCR) using human ribosomal protein large P0 (RPLP0) as reference gene [21].

Results

Clinical Findings

The patient was a previously healthy, 64 year-old male, with an 8-month history of slowly progressive dysphagia,

Table 1 Antibodies and concentrations used

Antibodies	Concentration	Source
Keratin 7 (CK7)	1/200	Dako
Keratin 20 (CK20)	1/100	Dako
High-molecular weight keratin (HMWK)	Prediluted	Novocastra
Keratin 19 (CK19)	1/50	Novocastra
Epithelial membrane antigen (EMA)	Prediluted	Ventana
Monoclonal carcinoembryonic antigen (mCEA)	1/5,000	Biomed
p16	1/50	BD Pharmigen
p53	Prediluted	Ventana
p63	1/50	Vector
Chromogranin	Prediluted	Ventana
Synaptophysin	Prediluted	Ventana
CDX-2	1/30	Biocare Medical
MUC-2	1/50	Novocastra
MUC-5AC	1/50	Novocastra
MUC-6	1/50	Novocastra
Thyroid transcription factor-1 (TTF-1)	1/20	Novocastra
Thyroglobulin	Prediluted	Cell Marque
S100	Prediluted	Ventana

odynophagia, and hemoptysis. The patient was a lifetime non-smoker who only used alcohol occasionally. Physical examination revealed an exophytic and friable mass involving the left tongue base, left glossotonsillar sulcus, left vallecula, and left lateral pharyngeal wall (Fig. 1). After a biopsy rendered a diagnosis of high-grade adenocarcinoma, a transhyoid supraglottic laryngectomy with resection of the left tongue base and a left neck dissection was performed.

The surgical specimen demonstrated a high-grade adenocarcinoma, not otherwise specified (NOS) involving the left base of tongue and left tonsil. The tumor measured 4.9 $\times$ 4.2 $\times$ 0.8 cm. Four of fifty-one lymph nodes in the left neck dissection were positive for adenocarcinoma (4/51). Final pathologic staging was pT3 N2b M0. The patient received adjuvant radiotherapy and is alive with no disease 6 months after diagnosis.

Light Microscopy

The tumor predominantly involved the submucosa and skeletal muscle (Fig. 2a); however, there was also a mucosal component interspersed with non-neoplastic squamous epithelium (Fig. 2b). The squamous epithelium immediately adjacent to the adenocarcinoma, showed slight perinuclear halos suggestive of HPV-related changes; however no

Fig. 1 Axial T2 weighted images with fat saturation demonstrate a mildly hyperintense mass involving the left tonsil with contiguous extension along the left tongue base (*arrows*). Sagittal T2 weighted

image shows the component of tumor along the tongue base (*long arrow*) and the continuous disease extending onto the vallecula (*short arrow*)

definitive dysplasia, or carcinoma in situ was present (Fig. 2b). The tumor was composed of closely packed large, medium, and small glands with prominent cribriform or “back-to-back” architecture (Fig. 2c). The individual tumor cells were columnar and exhibited amphophilic to pale eosinophilic cytoplasm and high nucleo-cytoplasmic ratio. The nuclei were pseudostratified and contained vesicular chromatin with occasional small to medium nucleoli. The mitotic rate was high with more than 40 mitotic figures (MF)/10 HPFs. Numerous apoptotic cells, tumor necrosis and perineural invasion were present. No squamous differentiation or mucous cells were noted. PAS and mucicarmine stains demonstrated luminal mucin only.

Immunohistochemistry

The tumor cells showed diffuse staining for keratin 7 (CK7) (Fig. 2d), p16 (Fig. 2e), monoclonal carcinoembryonic antigen (CEA), and epithelial membrane antigen (EMA). Staining for CK7 and p16 clearly separated the adenocarcinoma from the adjacent non-neoplastic squamous epithelium (Fig. 2d, e). There was patchy and variable expression of high-molecular weight keratin (34BE12). Less than 1 % of tumor cells were positive for MUC-5AC, chromogranin and synaptophysin. CDX-2, keratin 19 (CK19), keratin 20 (CK20), S100, MUC-2, MUC-6, p53, p63, thyroid transcription factor-1 (TTF-1), and thyroglobulin were negative.

The areas of non-neoplastic squamous epithelium showing “koilocytic” changes were negative for p16.

ISH and Molecular Findings

ISH demonstrated diffuse, multiple punctuate nuclear dots confirming the presence of integrated HPV-16 in the adenocarcinoma (Fig. 2f). Similarly to CK7 and p16, ISH clearly separated the tumor cells from the non-neoplastic squamous epithelium which was negative. Linear array analysis was positive for HPV-16. Quantitative-PCR demonstrated a viral load of 4 copies per cancer cell, whereas qRT-PCR for E6/E7 transcript levels were 30 copies per 1,000 RPLPO control.

Discussion

Herein we describe an HPV-positive, high-grade, non-salivary gland type adenocarcinoma of the base of the tongue. The tumor was diffusely positive for p16 and the presence of transcriptionally active HPV-16 was confirmed by Linear Array, in situ hybridization, q-PCR, and E6/E7 qRT-PCR. To our knowledge, this is the first reported and well documented example of an HPV-positive adenocarcinoma NOS arising in the oropharynx. Salivary gland-type carcinomas and other types of adenocarcinomas of the oropharynx are unusual, the most common histologic types

Fig. 2 Adenocarcinoma involving the surface of the base of the tongue. Note the remaining normal crypts and lymphoid component ($\times 16$) (a). Surface involvement demonstrating the transition from adenocarcinoma to non-neoplastic squamous epithelium. The squamous epithelium shows perinuclear halos reminiscent of koilocytes ($\times 200$) (b). The adenocarcinoma was composed of back-to-back

glands with scanty intervening stroma. The glands were lined by columnar cells with pseudostratified nuclei ($\times 100$) (c). The tumor was diffusely positive for CK7 (d). Tumor showing diffuse nuclear and cytoplasmic staining for p16 ($\times 100$) (e). In situ hybridization for HPV16 showing coarse nuclear signal indicative of high-copy number ($\times 200$) (f)

being mucoepidermoid carcinoma, adenoid cystic carcinoma, and adenocarcinoma NOS [22–25]. Other rare variants include colonic and signet-ring cell types [26, 27]. The real prevalence of HPV in oropharyngeal adenocarcinoma has not been investigated and is therefore, unknown.

Most studies and reports of oropharyngeal adenocarcinomas have been surgical or pathologic series published before the causative role of HPV in the carcinogenesis of OSCC had been elucidated. In a recent study, Boland et al. [28] investigated the expression of p16 expression and the

presence of HPV in adenoid cystic carcinoma of different head and neck sites. Using ISH for high-risk HPV, the authors found that only 2 of 25 (8 %) cases were positive for HPV. The HPV-positive cases were high-grade adenoid cystic carcinomas of the nasal cavity. The two oropharyngeal tumors included in the study; one in the base of tongue and the second, soft palate; were negative for HPV. In a study of 28 high-grade sinonasal adenocarcinomas, Stelow et al. [29] described an HPV-associated high-grade sinonasal adenocarcinoma with 4 additional tumors tested for p16 being negative. The authors stated in the Discussion section of their report, that they had seen oropharyngeal carcinomas associated with HPV showing some glandular differentiation; however, this finding was not clearly documented in any of the cited reports [13, 18].

The precise origin of this HPV-associated adenocarcinoma is unclear. The tumor demonstrated a pure “ductal” phenotype and lacked light microscopic and immunophenotypic features of a specific “salivary gland-type” carcinoma. The presence of a surface component is suggestive of surface origin; however, the bulk of the tumor was submucosal which argues for an origin from the ductal portion of normal seromucous glands.

The recent reports describing the presence of HPV in oropharyngeal neuroendocrine carcinomas [15, 16], adenosquamous carcinomas [14], and undifferentiated carcinomas [17, 18], and now adenocarcinoma—raise the possibility that HPV is involved in the carcinogenesis of, not only squamous cell carcinoma, but other uncommon carcinomas of the region. This situation resembles the role played by HPV in the carcinogenesis of uterine cervix where HPV is found not only in squamous cell carcinoma [30], but also in adenocarcinomas [31], and neuroendocrine carcinomas [32, 33].

In summary, we describe a rare case of HPV-positive adenocarcinoma NOS of the oropharynx in a non-smoker male. Additional studies are required to determine the true prevalence and clinical significance, including prognosis, of HPV in adenocarcinomas of the oropharynx.

References

- Vidal L, Gillison ML. Human papillomavirus in HNSCC: recognition of a distinct disease type. *Hematol Oncol Clin N Am*. 2008;22:1125–42.
- Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 2010;363:24–35.
- Kreimer AR, Clifford GM, Boyle P, et al. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev*. 2005;14:467–75.
- Jordan RC, Lingen MW, Perez-Ordóñez B, et al. Validation of methods for oropharyngeal cancer HPV status determination in US cooperative group trials. *Am J Surg Pathol*. 2012;36:945–54.
- IARC. Monographs on the Evaluation of Carcinogenic Risk to Humans, Vol 90. *Human papillomaviruses*. Lyon: World Health Organization; 2007.
- Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. *Nat Rev Cancer*. 2010;10:550–60.
- Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. *Nat Rev Cancer*. 2011;11:9–22.
- D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med*. 2007;356:1944–56.
- Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst*. 2008;100:261–9.
- Chernock RD, El-Mofty SK, Thorstad WL, et al. HPV-related nonkeratinizing squamous cell carcinoma of the oropharynx: utility of microscopic features in predicting patient outcome. *Head Neck Pathol*. 2009;3:186–94.
- Chernock RD, Lewis JS Jr, Zhang Q, et al. Human papillomavirus-positive basaloid squamous cell carcinomas of the upper aerodigestive tract: a distinct clinicopathologic and molecular subtype of basaloid squamous cell carcinoma. *Hum Pathol*. 2010;41:1016–23.
- Begum S, Westra WH. Basaloid squamous cell carcinoma of the head and neck is a mixed variant that can be further resolved by HPV status. *Am J Surg Pathol*. 2008;32:1044–50.
- Stelow EB, Jo VY, Stoler MH, et al. Human papillomavirus-associated squamous cell carcinoma of the upper aerodigestive tract. *Am J Surg Pathol*. 2010;34:e15–24.
- Masand RP, El-Mofty SK, Ma XJ, et al. Adenosquamous carcinoma of the head and neck: relationship to human papillomavirus and review of the literature. *Head Neck Pathol*. 2011;5:108–16.
- Bishop JA, Westra WH. Human papillomavirus-related small cell carcinoma of the oropharynx. *Am J Surg Pathol*. 2011;35:1679–84.
- Kraft S, Faquin WC, Krane JF. HPV-associated neuroendocrine carcinoma of the oropharynx: a rare new entity with potentially aggressive clinical behavior. *Am J Surg Pathol*. 2012;36:321–30.
- Carpenter DH, El-Mofty SK, Lewis JS Jr. Undifferentiated carcinoma of the oropharynx: a human papillomavirus-associated tumor with a favorable prognosis. *Mod Pathol*. 2011;24:1306–12.
- Singhi AD, Stelow EB, Mills SE, et al. Lymphoepithelial-like carcinoma of the oropharynx: a morphologic variant of HPV-related head and neck carcinoma. *Am J Surg Pathol*. 2010;34:800–5.
- Huang CC, Qiu JT, Kashima ML, et al. Generation of type-specific probes for the detection of single-copy human papillomavirus by a novel in situ hybridization method. *Mod Pathol*. 1998;11:971–7.
- Sandri MT, Riggio D, Salvatici M, et al. Typing of human papillomavirus in women with cervical lesions: prevalence and distribution of different genotypes. *J Med Virol*. 2009;81:271–7.
- Gillison ML, D'Souza G, Westra W, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst*. 2008;100:407–20.
- Iyer NG, Kim L, Nixon IJ, et al. Factors predicting outcome in malignant minor salivary gland tumors of the oropharynx. *Arch Otolaryngol Head Neck Surg*. 2010;136:1240–7.
- Eveson JW, Cawson RA. Tumours of the minor (oropharyngeal) salivary glands: a demographic study of 336 cases. *J Oral Pathol*. 1985;14:500–9.
- de Vries EJ, Johnson JT, Myers EN, et al. Base of tongue salivary gland tumors. *Head Neck Surg*. 1987;9:329–31.
- Chuiwa H, Sakamoto K, Umeno H, et al. Minor salivary gland carcinomas of oral cavity and oropharynx. *J Laryngol Otol Suppl*. 2009;31:52–7.

26. Bell D, Kupferman ME, Williams MD, et al. Primary colonic-type adenocarcinoma of the base of the tongue: a previously unreported phenotype. *Hum Pathol*. 2009;40:1798–802.
27. Veit JA, Reichelt U, Tesche S. Signet ring cell adenocarcinoma of the oropharynx: presentation of a rare case and review of the literature. *Auris Nasus Larynx*. 2009;36:717–20.
28. Boland JM, McPhail ED, Garcia JJ, et al. Detection of human papilloma virus and p16 expression in high-grade adenoid cystic carcinoma of the head and neck. *Mod Pathol*. 2012;25:529–36.
29. Stelow EB, Jo VY, Mills SE, et al. A histologic and immunohistochemical study describing the diversity of tumors classified as sinonasal high-grade nonintestinal adenocarcinomas. *Am J Surg Pathol*. 2011;35:971–80.
30. Snijders PJ, Steenbergen RD, Heideman DA, et al. HPV-mediated cervical carcinogenesis: concepts and clinical implications. *J Pathol*. 2006;208:152–64.
31. Pirog EC, Kleter B, Olgac S, et al. Prevalence of human papillomavirus DNA in different histological subtypes of cervical adenocarcinoma. *Am J Pathol*. 2000;157:1055–62.
32. Abeler VM, Holm R, Nesland JM, et al. Small cell carcinoma of the cervix. A clinicopathologic study of 26 patients. *Cancer*. 1994;73:672–7.
33. Grayson W, Rhemtula HA, Taylor LF, et al. Detection of human papillomavirus in large cell neuroendocrine carcinoma of the uterine cervix: a study of 12 cases. *J Clin Pathol*. 2002;55:108–14.