Papillomavirus Research 2 (2016) 52–55

Contents lists available at ScienceDirect

Papillomavirus Research

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

p16(INK4A) expression in invasive laryngeal cancer ☆

Brenda Y. Hernandez [a,*], Mobeen Rahman [b], Charles F. Lynch [c], Wendy Cozen [d], Elizabeth R. Unger [e], Martin Steinau [e], Trevor Thompson [f], Maria Sibug Saber [g], Sean F. Altekruse [h], Marc T. Goodman [i], Amy Powers [j], Christopher Lyu [k], Mona Saraiya [l], The HPV Typing of Cancer Workgroup

[a] University of Hawaii Cancer Center, 701 Ilalo Street #239, Honolulu, HI 96813, USA
[b] University of Hawaii John A. Burns School of Medicine, 651 Ilalo Street #411E, Honolulu, HI 96813, USA
[c] The University of Iowa, College of Public Health, Department of Epidemiology, 145 North Riverside Drive S447 CPHB, Iowa City, IA 52242-2007, USA
[d] University of Southern California, Norris Comprehensive Cancer Center and Keck School of Medicine, Departments of Preventive Medicine and Pathology, 1441 Eastlake Avenue MC 9175, Los Angeles, CA 90089-9175, USA
[e] Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Division of High-Consequence Pathogens and Pathology, Atlanta, GA 30329-4027, USA
[f] Centers for Disease Control and Prevention, 4770 Buford Highway NE, Mailstop F-76, Atlanta, GA 30341-3717, USA
[g] University of Southern California, Norris Comprehensive Cancer Center, 1441 Eastlake Avenue #4420, Los Angeles, CA 90089-9175, USA
[h] National Cancer Institute, Surveillance Research Program, 9609 Medical Center Drive, Room 4E536, Rockville, MD 20850, USA
[i] Cedars-Sinai Medical Center, 8700 Beverly Blvd, Room 1S37, Los Angeles, CA 90048, USA
[j] The Queen's Medical Center, Department of Pathology, 1301 Punchbowl St., Iolani 4, Honolulu, HI 96813, USA
[k] Battelle, 100 Capitola Drive, Suite 200, Durham, NC 27713, USA
[l] Centers for Disease Control and Prevention, 4770 Buford Highway NE, Atlanta, GA 30341-3717, USA

ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form
1 March 2016
Accepted 2 March 2016
Available online 9 March 2016

Keywords:
Larynx
Laryngeal cancer
Human papillomavirus
HPV
P16(INK4A)
P16

ABSTRACT

We examined p16 expression in tumors from a population-based sample of laryngeal cancer cases diagnosed in the U.S. Samples had been previously genotyped for HPV DNA.

Overall, p16 expression was observed in laryngeal tissue from 8 of 101 (7.9%) cases. p16 expression was observed in 2 of 16 (12.5%) cases previously determined to be HPV DNA positive. The two cases dually positive for p16 and HPV DNA were non-keratinizing SCC and papillary SCC tumors that were positive for genotypes 18 and 35/89, respectively. Positivity for p16 and/or HPV DNA was not associated with 5-year survival (log-rank p value = 0.55). Our findings support a limited role of HPV in laryngeal carcinogenesis. p16 is not a reliable surrogate for HPV status in laryngeal cancers and is not a predictor of laryngeal cancer survival.

1. Introduction

Human papillomavirus (HPV) plays an etiologic and prognostic role in oropharyngeal cancer [1–3]. Elevated tumor expression of p16(INK4A) (referred to as p16 hereafter), a cyclin-dependent kinase-4 inhibitor, has been well-characterized in oropharyngeal cancer patients and is strongly correlated with HPV positivity. HPV-positivity combined with expression of p16(INK4A) is strong evidence of biologically relevant infection [4].

Unlike oropharyngeal cancers, an etiologic role of HPV in laryngeal and other malignancies of the head and neck has not been definitively established [1,3]. We recently reported the results of a population-based study to evaluate the genotype-specific prevalence of HPV in invasive laryngeal cancer cases diagnosed in the U.S. [5]. HPV DNA was detected in 31 of 148 (21%) invasive laryngeal cancers; 13 different genotypes were observed. The detection of HPV DNA in tumor tissue, however, is not definitive evidence for causation. The current report examines p16 expression in laryngeal cancer cases in order to further elucidate HPV-related laryngeal cancer development and progression.

☆This project was funded by the Centers for Disease Control and Prevention. The findings and conclusions of this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

* Corresponding author.
E-mail addresses: brenda@cc.hawaii.edu (B.Y. Hernandez), mobeen@hawaii.edu (M. Rahman), charles-lynch@uiowa.edu (C.F. Lynch), wcozen@usc.edu (W. Cozen), eru0@cdc.gov (E.R. Unger), azz9@cdc.gov (M. Steinau), tkt2@cdc.gov (T. Thompson), sibugsab23@gmail.com (M.S. Saber), altekrusesf@mail.nih.gov (S.F. Altekruse), marc.goodman@cshs.org (M.T. Goodman), aapowers@hawaii.edu (A. Powers), Lyuc@Battelle.org (C. Lyu), yzs2@cdc.gov (M. Saraiya).

http://dx.doi.org/10.1016/j.pvr.2016.03.001

2. Methods

This study was approved by the CDC Institutional Review Board (IRB) and the IRBs of the University of Hawaii, University of Iowa, and University of Southern California. All patients were diagnosed in 1993–2004 within the catchment area of three population-based cancer registries [5]. Laryngeal cancer cases were selected from patients with pathologically-confirmed tumors. The majority of cases were squamous cell carcinomas of all subsites including the supraglottis, glottis, and subglottis.

De-identified, clinically annotated formalin-fixed paraffin-embedded (FFPE) tissue specimens were obtained from Residual Tissue Repositories (RTR) affiliated with the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program [6,7]. Through linkage with registry patient and tumor data, tissue specimens were annotated with de-identified demographic, clinical, pathologic, and survival data. Tissue specimens had been previously genotyped for HPV at the CDC laboratories as previously described [5,8] using the Linear Array HPV Genotyping Test for 37 HPV genotypes (LA, Roche Diagnostics, Indianapolis, IN). The INNO-LiPA HPV Genotyping Assay (LiPA, Innogenetics, Gent, Belgium) was also employed for specimens testing negative for HPV and human beta-globin.

2.1. Histologic subtyping by pathologic review

H&E slides of squamous cell carcinoma cases of unspecified subtype, i.e. SCC NOS, were reviewed by a study pathologist (M.R.) for subtype assignment. SCC cases were classified as keratinizing, non-keratinizing, basaloid, verrucous, papillary, and spindle cell.

2.2. p16 immunohistochemistry and pathologic review

p16 expression was evaluated via immunohistochemistry. Sections of tumor tissue were obtained from the same FFPE blocks previously used for HPV genotyping. A p16 mouse monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (dilution 1:400) was used according to the manufacturer's specifications. Slides were read by a study pathologist (M.R.) who was blinded to the HPV status of cases. p16 staining was evaluated based level of staining intensity (mild/weak, moderate, strong), intracellular localization (nuclear, cytoplasmic), staining distribution (patchy, focal, diffuse), and the proportion of tumor cells stained. Specimens exhibiting strong, diffuse nuclear and cytoplasmic staining in $\geq$70% of tumor cells were considered to be definitively positive for p16 based on established criteria [9,10].

2.3. Statistical analyses

Statistical analyses were conducted using SAS version 9.2. Comparison of p16 expression used the Chi-square statistic. Survival was calculated based on the time period from date of diagnosis to date of death or date of last follow-up. Overall five-year survival by p16 and HPV DNA status was evaluated using the Kaplan–Meier method and the log-rank test. All tests were two-sided and a p value <0.05 was considered to be statistically significant.

3. Results

The laryngeal cancer study population has been previously detailed [5]. Tumor tissue specimens from 101 of 148 cases from the prior analysis with sufficient tissue for immunohistochemistry were included in the present study. SCC subtypes included 49 (48.5%) keratinizing, 17 (16.8%) non-keratinizing, 9 (8.9%) papillary, 3 (3%) basaloid, 2 (2.0%) spindle cell, and 1 (1.0%) verrucous. A total of 19 (18.8%) of cases remained classified as unspecified SCC and 1 case was a small cell carcinoma.

Eight of the 101 (7.9%) of laryngeal tumors were considered to be positive for p16 based on the criteria of strong, diffuse p16 staining of the nucleus and cytoplasm in $\geq$70% of tumor cells. Thirty-two cases which exhibited strong, diffuse nuclear and cytoplasmic staining in fewer than 70% of tumor cells and were not considered to be p16 positive. Table 1 compares p16 and HPV DNA status by histologic subtype. Basaloid SCC tumors exhibited the largest proportion of p16 positive tumors (2 of 3). All 3 basaloid tumors were HPV DNA negative. HPV positivity was highest in non-keratinizing (4 of 17) and papillary (2 of 9) SCC tumors. Only 2 of the 26 non-keratinizing and papillary SCC cases were positive for p16 expression.

In total, p16 expression was observed in 2 of 16 (12.5%) HPV DNA positive laryngeal cancer cases (Table 2). One p16-positive case was a non-keratinizing SCC positive for HPV 18 (Fig. 1). The second p16 positive case was a papillary SCC positive for both HPV 35 and 89. Both p16/HPV DNA positive cases were localized tumors of the glottis diagnosed in males under age 50. The 14 HPV positive laryngeal cancer cases without p16 expression included glottal and supraglottal tumors of all stages diagnosed in males and females primarily aged 50 and older. Overall survival was evaluated in the 95 cases with vital status and follow-up information. p16 was not associated with 5-year survival when measured based on p16 expression alone (log-rank p value=0.84) or positivity for either p16 and/or HPV DNA (log-rank p value=0.55) (Fig. 2).

4. Conclusions

Our findings support a limited role of HPV in laryngeal carcinogenesis. Fewer than 10% of all laryngeal tumors expressed p16 and p16 expression did not strongly correlate with HPV DNA status. In total, only a fraction (2%) of laryngeal cancers were positive for both p16 and HPV DNA. We previously observed HPV DNA in over 1 in 5 invasive laryngeal cancers. However, detection of HPV DNA alone is not indicative of a clinically relevant infection. In HPV-induced carcinogenesis, the E7 oncoprotein binds and inactivates the retinoblastoma tumor suppressor gene product, pRb [11]. As pRb is a negative regulator of p16, its inactivation results in overexpression of p16 [11]. Therefore, HPV-positivity combined with p16 expression is strong evidence of biologically relevant infection [4]. Our findings of limited correlation of p16 with HPV DNA status contrasts with the few studies that have examined

Table 1
p16(INK4A) expression and HPV DNA status by histology of invasive laryngeal tumors.

Histology	p16		HPV DNA	
	No. positive	No. negative	No. positive	No. negative
SCC keratinizing (n=49)	3	46	8	41
SCC non-keratinizing (n=17)	1	16	4	13
SCC papillary (n=9)	1	8	2	7
SCC basaloid (n=3)	2	1	0	3
SCC spindle cell (n=2)	0	2	0	2
SCC verrucous (n=1)	0	1	1	0
SCC NOS (n=19)	1	18	1	18
Small cell carcinoma NOS (n=1)	0	1	0	1
Total	8	93	16	85