Human Pathology (2014) **45**, 310–319

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Human PATHOLOGY

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Original contribution

African Americans with oropharyngeal carcinoma have significantly poorer outcomes despite similar rates of human papillomavirus–mediated carcinogenesis☆,☆☆,★,★★

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Received 21 June 2013; revised 10 September 2013; accepted 13 September 2013

Keywords:
African Americans;
Disparity;
Oropharyngeal;
HPV;
Outcome;
p16;
Oropharynx

Summary We examined racial disparities among 102 oropharyngeal carcinoma (OPC) patients (30 African Americans and 72 whites) comparing rates of transcriptionally active human papillomavirus (HPV)16/18 and $p16^{INK4a}$ overexpression, with times to disease progression and disease-specific survival (DSS). Expression of HPV16/18 transcripts was assessed by reverse transcription and polymerase chain reaction using type-specific *E6/E7* primers; $p16^{INK4a}$ was evaluated by immunohistochemistry. African Americans were significantly more likely to present with high T stage disease and receive nonsurgical treatment. HPV16/18 was present in 63% of patients; no racial differences were observed. Silenced $p16^{INK4a}$ in OPC was significantly more common in African Americans (15/24) than in whites (20/69) (P = .004) and in HPV16+ African Americans (6/24) than in HPV+ whites (2/42) (P = .023). Kaplan-Meier analysis for DSS revealed a protective effect for $p16^{INK4a}$ overexpression (P = .0028; hazard ratio [HR], 0.23), HPV16+ (P = .036; HR, 0.38), and whites (P = .0039; HR, 0.27). Shorter DSS was associated with primary definitive chemoradiation (P = .019; HR, 3.49) and T3/T4 disease (P = .0001; HR, 7.75). A protective effect with respect to disease progression was observed for HPV16+ (P = .007; HR, 0.27), whites (P = .0006; HR, 0.197), and $p16^{INK4a}$ overexpression (P = .0001; HR, 0.116). African Americans with OPC experience poorer outcomes likely due to $p16^{INK4a}$ silencing, higher T stage, and nonsurgical treatment but not lower rates of transcriptionally active HPV16/18.

☆ Drs Isayeva and Xu are cofirst authors.
☆☆ The authors have no financial conflict of interest regarding this publication.
★ This project was funded by the Department of Pathology, University of Alabama at Birmingham.
★★ These data were presented in part at the 101st Annual Meeting, United States and Canadian Academy of Pathology, Vancouver, BC, Canada, March 2012.
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http://dx.doi.org/10.1016/j.humpath.2013.09.006

1. Introduction

Oropharyngeal carcinoma (OPC) represents approximately 20% of all cancers arising within the combined oral cavity and pharynx [1]. Population-based incidence data over the last 6 decades confirm a significantly increased incidence of OPC, especially among women and individuals younger than 60 years [2,3]. Human papillomavirus (HPV) analysis performed on OPC specimens from Surveillance, Epidemiology, and End Results biorepositories demonstrates substantially increased association with HPV over time, from 16.3% to 71.7% between 1984 and 2004 [4]. Currently, approximately 70% to 80% of OPC are associated with high-risk HPV-mediated carcinogenesis; most of these are caused by HPV16 [5–8]. Trends regarding OPC and race are also changing; the incidence of OPC between 1973 and 1995 was significantly higher in African Americans than in whites [2]. This is now reversed due to the decreasing incidence among African Americans and increasing incidence among whites. In 2013, the age-adjusted OPC incidences per 100 000 persons for whites (men, 8.8; women, 1.9) are now higher than for African Americans (men, 7.9; women, 1.6) [9].

It is now well accepted that HPV-mediated OPC is associated with significantly improved outcome as compared with HPV-negative counterparts. A recent meta-analysis pooling 823 OPC patients from 11 studies assessing HPV by polymerase chain reaction (PCR) demonstrated a significant protective effect for overall survival (OS) (relative risk, 0.43); the protective effect is was similar for OPC patients assessed by in situ hybridization (ISH) [10]. Similarly, another recent meta-analysis demonstrated a protective effect for HPV-mediated OPC with respect to disease-specific survival (DSS) (pooled hazard ratio [HR], 0.28) and progression-free survival (pooled HR, 0.40) [11].

In 2009, Settle et al [12] raised the provocative question as to whether lower prevalence of HPV-mediated carcinogenesis was responsible for the poorer OS observed among a subset of African Americans with OPC. These patients were part of a phase III prospective trial for stage III/IV patients treated with primary chemoradiotherapy (TAX 324, n = 539, all head and neck sites) [12]. A major limitation was that HPV status was assessed in only 28 African American study patients with tumors from all anatomical sites. The significance of demonstrating HPV16 in only 1 of 28 specimens from African American patients is uncertain, especially given the lack of information regarding anatomical subsite. In the same year, Sedaghat et al [13] demonstrated no differences between whites and African Americans with OPC with respect to HPV16 or outcome, albeit in a much smaller cohort (n = 49). Similarly, no outcome disparities were found for African Americans with OPC in a matched cohort from Pittsburgh (n = 348) [14]. On the other hand, other groups have published findings similar to the Settle study, suggesting that whites with OPC have better outcomes due to higher rates of HPV-mediated carcinogenesis [15–17]. However, these studies were limited in that (1) the tumor anatomic subsites were not detailed with respect to HPV and race, (2) HPV was not directly assessed, or (3) no distinction was made between driver and passenger HPV infection. "HPV driver infection" refers to transcriptionally active virus, which is associated with $p16^{INK4a}$ overexpression. $p16^{INK4a}$ is a cyclin-dependent kinase (CDK) inhibitor of Rb deactivation through CDK4/CDK6 binding. High-risk HPV E7 can directly bind and inactivate Rb, which de-represses the CDK complex and releases bound $p16^{INK4a}$. Thus, $p16^{INK4a}$ has become a validated surrogate biomarker for transcriptionally active HPV driver infection [18]. By contrast, "HPV passenger infection" is genome positive but transcript negative and does not definitely equate with viral-mediated cancer.

Chernock et al [19] recently published data on a large cohort of OPC patients (n = 174) demonstrating significantly lower rates of HPV DNA, less $p16^{INK4a}$ overexpression, and shorter disease-free survival among African Americans. Their strategy was to combine HPV DNA ISH and $p16^{INK4a}$ immunohistochemistry (IHC) as a surrogate measure for transcriptional activity. One shortcoming of this approach is that ISH is not as sensitive as PCR assays. As geographic and population differences can certainly impact rates of HPV-mediated carcinogenesis, we undertook the first analysis of HPV-mediated OPC in the Deep South. We tested the following 2 hypotheses: (1) African Americans with OPC were significantly less likely to have HPV-mediated disease. (2) Lower rates of HPV-mediated OPC are responsible for poorer disease-free and disease-specific outcomes in African Americans. Our strategy was to determine transcriptional activity by direct assessment of HPV RNA transcripts, rather than using combined DNA/$p16^{INK4a}$ IHC assays. We chose to include HPV18 in our analyses, as it represents one of the more commonly detected genotypes after HPV16 [6,20,21].

2. Materials and methods

This study was approved by the Institutional Review Boards of the University of Alabama at Birmingham (UAB). Consecutive patients diagnosed with primary OPC from 2005 to 2012 were identified from the UAB pathology files. Pathologic diagnoses were confirmed; electronic medical record data regarding patient age, sex, tumor site, self-reported race, TNM stage, and treatment (primary surgery, ± adjuvant treatment, primary chemotherapy/radiation) were abstracted. All available data on smoking and alcohol intake were collected (ever smoked yes/no, pack-years, prior smoking, no. of years quit smoking, any alcohol intake, ounces per week). Unfortunately, the smoking and alcohol data often lacked quantitative detail; therefore, they were analyzed only as categorical variables (yes/no). To identify additional patients of interest (African American patients with oropharyngeal carcinoma), a UAB radiation oncology database commencing in 2004 was also searched.