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Short Report

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Detection of circulating tumor human papillomavirus DNA before diagnosis of HPV-positive head and neck cancer

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Abstract

Human papillomavirus (HPV), most commonly HPV16, causes a growing subset of head and neck squamous cell carcinomas (HNSCCs), including the overwhelming majority of oropharynx squamous cell carcinomas in many developed countries. Circulating biomarkers for HPV-positive HNSCC may allow for earlier diagnosis, with potential to decrease morbidity and mortality. This case-control study evaluated whether circulating tumor HPV DNA (ctHPVDNA) is detectable in prediagnostic plasma from individuals later diagnosed with HPV-positive HNSCC. Cases were participants in a hospital-based research biobank with archived plasma collected ≥6 months before HNSCC diagnosis, and available archival tumor tissue for HPV testing. Controls were biobank participants without cancer or HPV-related diagnoses, matched 10:1 to cases by sex, race, age and year of plasma collection. HPV DNA was detected in plasma and tumor tissue using a previously validated digital droplet PCR-based assay that quantifies tumor-tissue-modified viral (TTMV) HPV DNA. Twelve HNSCC patients with median age of 68.5 years (range, 51-87 years) were included. Ten (83.3%) had HPV16 DNA-positive tumors. ctHPV16DNA was detected in prediagnostic plasma from 3 of 10 (30%) patients with HPV16-positive tumors, including 3 of 7 (43%) patients with HPV16-positive oropharynx tumors. The timing of the plasma collection was 19, 34 and 43 months before cancer diagnosis. None of the 100 matched controls had detectable ctHPV16DNA. This is the first report that ctHPV16 DNA is detectable at least several years before diagnosis of HPV16-positive HNSCC for a subset of patients. Further investigation of ctHPV16DNA as a biomarker for early diagnosis of HPV16-positive HNSCC is warranted.

KEYWORDS
circulating tumor DNA, head and neck cancer, human papillomavirus, oropharynx cancer, tumor-tissue-modified viral (TTMV) DNA

Abbreviations: ctHPVDNA, circulating tumor HPV DNA; ddPCR, digital droplet PCR; frag, fragments of DNA; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; OPSCC, oropharynx squamous cell carcinoma; TTMV DNA, tumor tissue-modified viral DNA.

What's new?

Circulating tumor human papillomavirus (HPV) DNA (ctHPVDNA) is associated with tumor burden and response to treatment in HPV-positive head and neck squamous cell carcinoma (HNSCC). In this case-control study, ctHPVDNA was detected in plasma greater than 3 years prior to diagnosis of HPV-positive HNSCC. This is the first study to demonstrate that ctHPVDNA is detectable prior to clinical development of an HPV-positive cancer and thus, may allow for earlier diagnosis of HPV-positive HNSCC.

1 | INTRODUCTION

Human papillomavirus (HPV) now causes the overwhelming majority of oropharyngeal squamous cell carcinomas (OPSCCs) in the United States and other developed countries, and is also detected in a minority of head and neck squamous cell carcinomas (HNSCCs) from other anatomical sites such as the oral cavity and larynx.[1,2] The incidence of OPSCC in the United States has been rising since the early 2000s, and is projected to continue rising for the next several decades.[3,4] Indeed, OPSCC has surpassed cervical cancer as the most common HPV-related cancer in the United States.[5] The vast majority of HPV-positive HNSCCs are caused by HPV type 16.[6]

Earlier detection of HPV-positive HNSCC may potentially decrease treatment-related morbidity and mortality for a rapidly growing patient population. Circulating tumor HPV DNA (ctHPVDNA) is a dynamic biomarker that has been detected using ultrasensitive PCR methods in plasma from approximately 90% of p16-positive OPSCC patients (where p16-positive is a surrogate for HPV-positive), correlates strongly with response to treatment, and has excellent predictive value for disease recurrence after treatment.[7-10] Whether, and for how long, ctHPVDNA is detectable before HPV-positive HNSCC is unknown. Herein, we report that genotype-specific tumor-tissue-modified virus (TTMV) ctHPVDNA is detectable in plasma several years prior to the diagnosis of HPV-positive HNSCC.

2 | MATERIALS AND METHODS

2.1 | Patients and eligibility

This was a retrospective matched case-control study of participants in the Mass General Brigham (MGB) Biobank who later were diagnosed with HNSCC (cases), compared to participants without HNSCC (controls). Eligible cases had banked plasma samples collected at least 6 months prior to HNSCC diagnosis, and available archival tumor material to ascertain/confirm HPV tumor status. Ten controls per case were randomly selected, matched on age and calendar year at time of plasma collection, race and sex. Controls with any known cancer or HPV-associated diagnoses (eg, cervical HPV infection or precancer) were excluded. The MGB Biobank is a hospital-based cohort research study ongoing since 2010 at MGB hospital sites, described by Karlson et al and Weiss et al.[11,12] Plasma from MGB Biobank participants is isolated from whole blood collected in EDTA using a centrifuge at 2465*g*. Plasma aliquots of 0.5 mL are stored at −80°C. None of the plasma aliquots used in our study were previously thawed. Plasma sample volumes in our study were approximately 1 mL (median 1.1 mL, range, 0.9-2 mL).

2.2 | Tumor tissue and plasma testing

Digital droplet PCR (ddPCR) analysis of TTMV-HPV DNA from deidentified formalin-fixed, paraffin-embedded tumor samples and deidentified archival plasma samples was performed using the NavDx assay by Naveris Inc. (Natick, Massachusetts), a CLIA-certified, CAP-accredited independent laboratory, according to methods adapted from Chera et al.[7,8] NavDx uses ddPCR to detect and quantify DNA produced during the fragmentation of integrated and/or episomal HPV DNA from malignant epithelial cells undergoing cell death. Algorithmic analysis of ddPCR data yields the TTMV score. The TTMV score is designed to distinguish tumor-derived from infectious virus-derived DNA by leveraging tumor-specific fragmentation patterns, which are known to be specific to tissue of origin,[13] and has high analytic sensitivity and specificity for the detection of DNA from HPV-driven cancers.[7]

In brief, DNA was extracted as previously described.[7,8,14] Sample quality was confirmed using ddPCR for a region of the *ESR1* gene. TTMV-HPV DNA from one of five high-risk HPV subtypes (16, 18, 31, 33 and 35) was quantified by ddPCR using a set of primers and probes in each reaction that span multiple amplicons for individual HPV strains. Analysis of droplets was performed using the K-Nearest Neighbors algorithm to identify clusters associated with specific amplicons and DNA fragment sizes. The counts for each of the clusters were summed in a weighted linear combination to create the normalized TTMV score. Circulating TTMV-HPV DNA in plasma is reported herein as ctHPVDNA with units of fragments (frag)/mL, while TTMV-HPV DNA in tumor samples is reported as such with units of frag/ng of tumor tissue DNA. Plasma and tumor samples were first tested for TTMV-HPV16 DNA. If TTMV-HPV16 DNA was not detected or was <10 frag/mL or frag/ng, ddPCR testing for four additional HPV types (18, 31, 33 and 35) was performed. All samples were tested on plates with positive and negative control DNA. Multiple control plasma samples for one case (5 of 10 controls for Case 1) were unexpectedly positive for ctHPV33DNA on initial testing. Given the

rarity of HPV33,[6,15] additional aliquots were obtained of the same archival plasma samples. Re-analysis showed that all were negative for ctHPV33DNA. Therefore, this was considered a spurious result.

2.3 | Data analysis

Demographic and cancer-related data were extracted from medical records. Descriptive statistics were reported as number, percentage (%) or median, range. The 95% confidence intervals (CI) for proportions were determined using binomial exact calculations. Proportions and medians were compared using Fisher's exact and Wilcoxon Rank-sum tests, respectively, with two-sided *P*-value <.05 considered statistically significant.

3 | RESULTS

3.1 | Cases and tumor HPV status

Among 154 total HNSCC patients with archived plasma in the MGB Biobank, 15 had plasma collected at least 6 months prior to HNSCC diagnosis. Three of the fifteen did not have available archived tumor tissue. Thus, 12 individuals diagnosed with HNSCC between May 2015 and January 2020 were eligible for study inclusion. Case characteristics are displayed in Table 1. Ten cases were white men, and two were white women. Median age was 68.5 years (range, 51-87 years). Oropharynx was the most common primary tumor site (N = 7, 58%). Archival tumor tissue testing revealed TTMV-HPV16 DNA positivity in 10 of the 12 tumors (83%), including all seven OPSCCs. The remaining two tumors were TTMV-HPV DNA-negative.

3.2 | Plasma ctHPVDNA

Plasma samples among cases were collected a median of 30.5 months prior to HNSCC diagnosis (range, 17-76 months). ctHPV16DNA was detected in plasma samples from 3 of the 10 patients with HPV16-positive HNSCCs (30%, 95% CI = 7%-65%, Figure 1), including three of the seven patients with HPV16-positive OPSCC (43%, 95% CI = 10%-82%), but was not detected for the two patients with HPV-negative HNSCCs. Plasma samples from the three patients with detectable prediagnostic ctHPV16DNA were collected 19, 34 and 43 months before HPV16-positive OPSCC diagnosis (Figure 1). Other types of ctHPVDNA (18, 31, 33 and 35) were not detected among patients who were ctHPV16DNA-negative.

All three patients with detectable ctHPV16DNA had nodal metastases, compared to four of seven patients with no detectable ctHPV16DNA despite HPV16-positive tumors (Table 1). There was not a statistically significant association of plasma ctHPVDNA positivity with either the presence of nodal metastases ($P = .48$) or median tumor TTMV-HPV16 DNA frag/ng ($P = .91$) among patients with HPV16-positive tumors.

None of the 100 matched controls had detectable plasma ctHPV16DNA. Controls were also negative for ctHPVDNA from other assayed HPV types (see Section 2.2).

4 | DISCUSSION

This is the first report, to the authors' knowledge, that ctHPVDNA can be detected prior to clinical diagnosis of HPV-positive HNSCC. ctHPV16DNA was detected using the NavDx TTMV assay in prediagnostic plasma from three of seven (43%) HPV16-positive OPSCC patients, with a lead time up to 43 months, compared to 0 of 100 controls. Despite a small sample size, these findings augment our understanding of the natural history of HPV-positive OPSCC and the dynamics of ctHPVDNA, and contribute to a rationale for future investigations of biomarker-based early detection of HPV-positive OPSCC.

ctHPVDNA was detected in prediagnostic plasma from some, but not all, patients with HPV-positive HNSCCs in our study. This may be due in part to the technical limitations of working with archival samples, which were not processed specifically for ctHPVDNA detection[16]; however, it likely also reflects the natural history of the included cases. HPV-positive OPSCCs have a favorable prognosis, and at least a subset demonstrate an indolent growth pattern.[17] Thus, the three cases with detectable ctHPVDNA, all of whom had oropharyngeal tumors with nodal metastases, may have had slowly growing disease with intravascular shedding of ctHPVDNA for years prior to clinical detection. Of note, more advanced nodal disease at the time of OPSCC diagnosis has been previously associated with higher levels of ctHPVDNA.[7] In contrast, cases without detectable ctHPVDNA may simply have had more rapidly growing tumors, or may fall among the subset of HPV-positive tumors without detectable ctHPVDNA at the time of diagnosis.[7,8]

Seropositivity to the HPV16 E6 antigen is another biomarker that has been observed in plasma collected before diagnosis of HPV16-positive OPSCC.[18] HPV16 E6 antibody titers are stable after seroconversion, which occurs up to several decades before OPSCC diagnosis, and change little after treatment.[18,19] It is conceivable that HPV16 E6 seropositivity indicates a history of persistent HPV16 mucosal infection at high risk for malignant transformation, while detectable ctHPV16DNA reflects the development of invasive disease and shedding of tumor DNA into the circulation. The relationship between these biomarkers in the prediagnostic setting is unknown, but remains an area of active investigation.

Screening for HPV-positive OPSCC is not yet feasible for several reasons,[4] most prominently its relatively low overall incidence resulting in a low positive predictive value for even an ideal biomarker.[4] However, even among high-risk populations for whom rising incidence may justify screening, another important barrier to screening is the lack of targetable premalignant lesions analogous to cervical precancers.[4] Our data indicate that ctHPVDNA positivity may potentially serve as a surrogate "precancer," for example, a dynamic target known to vary with tumor burden,[7-9] allowing for the study of early interventions to prevent progression to clinical disease.

TABLE 1 Characteristics of head and neck cancer cases[a]

ID	Sex	Age (years)	Smoking status	Tumor site	Tumor stage[b]	Year of Cancer Dx	Clinical HPV tumor testing[c]	Months from Plasma to Cancer Dx	Tumor tissue HPV16 DNA[c,d] (frag/ng)	Plasma ctHPV16 DNA[d] (frag/mL)
1	M	65	Former	BOT	T3N0	2016	P16+	76	590	0
2	M	74	Never	BOT	T4aN2c	2017	P16+	43	262	11
3	M	69	Never	BOT	T2N1	2019	P16+, HPV16/18+	44	3656	0
4	M	70	Never	BOT	T2N1	2019	P16+	34	10 517	24
5	M	67	Never	Tonsil	T1N2c	2018	HPV16+	19	2105	23
6	M	87	Never	Tonsil	T2N1	2019	P16+	27	82	0
7	M	76	Current	BOT	T2N2b	2019	HPV16+	23	31	0
8	M	58	Never	Lateral tongue	T2N0	2019	Not tested	74	447	0
9	F	51	Never	Lateral tongue	T2N0	2015	Not tested	23	15 417	0
10	F	59	Current	Supraglottis	T3N2c	2018	Not tested	18	41 503	0
11	M	68	Former	Epiglottis	T1N0	2019	Not tested	46	0	0
12	M	82	Former	Lateral tongue	T2N0	2018	Not tested	17	0	0

Abbreviations: BOT, base of tongue; Dx, diagnosis; frag, fragment (of DNA); HPV, human papillomavirus.
[a]All participants were white.
[b]AJCC 7th edition tumor stage. All patients were M0.
[c]Clinical testing refers to any testing that was performed for HPV tumor status during the course of initial clinical diagnosis and treatment, as abstracted from medical records.
[d]Tumor tissue-modified viral HPV DNA.

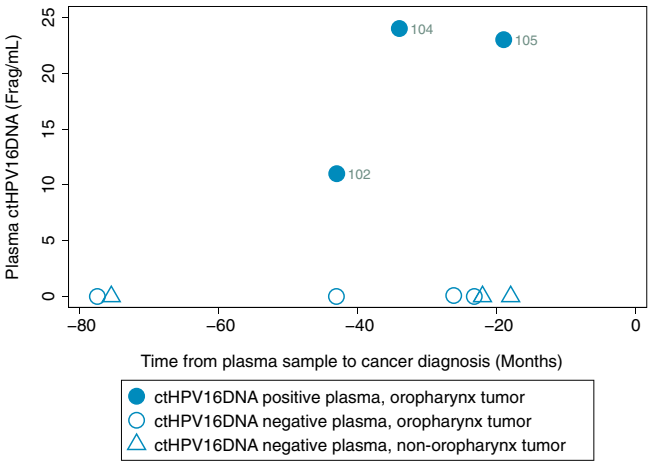

FIGURE 1 Plasma circulating tumor HPV16 DNA in pre-diagnostic specimens from patients later diagnosed with TTMV-HPV16-positive head and neck squamous cell carcinoma. Zero months represents time of diagnosis. Solid shapes represent plasma samples positive for ctHPV16DNA, which are labeled with ID numbers corresponding to Table 1, while empty shapes represent plasma sample negative for ctHPV16DNA. Circles represent oropharynx tumors and triangles represent non-oropharynx tumors. ctHPV16DNA refers to TTMV-DNA fragments/mL plasma. ctHPV16DNA, circulating tumor HPV16 DNA; frag/mL, fragments per mL; TTMV, tumor tissue-modified viral DNA [Color figure can be viewed at wileyonlinelibrary.com]

The generalizability of our study is limited by a small sample size of just 10 HPV16-positive HNSCC patients. In addition, other ctHPVDNA assays may have yielded different results than the TTMV assay used herein.

In summary, this is the first report that ctHPV16DNA is detectable in plasma up to several years prior to the diagnosis of HPV16-positive OPSCC for a subset of patients. We provide proof-of-concept for future larger studies evaluating biomarker-based early diagnosis of HPV-positive HNSCC.

CONFLICT OF INTEREST

Daniel Faden holds equity in Illumina, receives consulting fees from Merck, Noetic and Focus on Boston and receives research funding from Bristol Myers Squibb and Foundation Medicine. Ravindra Uppaluri serves on a Merck head and neck cancer advisory board. Glenn J Hanna receives research funding from Bristol-Myers Squibb, Elevar, Exicure, GlaxoSmithKline, Kartos Therapeutics, Kite Pharma, NatKwest/Altor Bioscience, Regeneron, Sanofi Genzyme and V Foundation; and serves as a consultant or on advisory boards for Bicara, Bio-Rad, Bristol-Myers Squibb, Exicure, General Catalyst, Kura, Maverick Therapeutics, Merck, Naveris, Prelude, Regeneron, Remix, Sanofi Genzyme and Boxer Capital. Phil Stephens, Charlotte Kuperwasser and Sunil Kumar are employees of and stockholders in Naveris. Eleni Rettig, Shaiba Sandhu, Kristine Wong, William Faquin, Chloe Warinner, Jeremy Richmon, Mark Varvares, Rosh Sethi and Herve Sroussi have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Eleni M. Rettig: Conceptualization, Investigation, Supervision, Writing—original draft. Daniel L. Faden: Conceptualization, Investigation, Resources, Writing—review & editing. Shaiba Sandhu: Investigation. Kristine Wong: Resources, Writing—review & editing. William C. Faquin: Resources, Writing—review & editing. Chloe Warinner:

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ETHICS STATEMENT

Our study was approved by the Mass General Brigham Institutional Review Board. All participants provided informed consent prior to enrollment in the Mass General Brigham Biobank.

DATA AVAILABILITY STATEMENT

The data that support the findings of our study are available from the corresponding author upon reasonable request.

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