Null Results in Brief

Cancer Epidemiology, Biomarkers & Prevention

Human Papillomavirus DNA Is Rarely Detected in Colorectal Carcinomas and Not Associated with Microsatellite Instability: The Seattle Colon Cancer Family Registry

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Abstract

Background: Persistent infection with oncogenic human papillomavirus (HPV) types-16 and -18 is an established cause of cervical and other cancers. Some studies report detection of oncogenic HPV DNA in colorectal carcinomas, with prevalence estimates as high as 84%. However, other studies report detecting no HPV DNA in colorectal tumors.

Methods: To evaluate the prevalence of HPV in colorectal cancer subsets, we conducted a case–case comparison study. This study included 555 cases of incident colorectal cancer from the Seattle Colon Cancer Family Registry (CCFR), ages 20 to 74 years and diagnosed between 1998 and 2002. Standardized interviews were used to elicit demographics and risk factor data. Tumor DNA was assayed for HPV-16 and -18 DNA using real-time PCR. Microsatellite instability (MSI) status was assessed using a standard 10-marker panel and confirmed with immunohistochemical staining. Prevalence estimates were calculated for the overall sample, and stratified by patient and tumor characteristics. Fisher exact test was used to compare prevalence between strata.

Results: HPV-16 DNA was detected in 2% of colorectal tumors, but no HPV-18 DNA was detected. HPV-16 prevalence did not vary between cases according to sex, age, race, smoking-status, or MSI-status ($P > 0.05$). HPV-16 prevalence in rectal carcinomas was 5% compared with 1% in colon carcinomas ($P = 0.03$).

Conclusions: Among a large sample of colorectal carcinomas, prevalence of HPV-16 and -18 was very low. Prior studies detecting high HPV prevalence in colorectal carcinomas are likely the result of contamination from the anal canal or clinical processing.

Impact: HPV is unlikely to play a large role in colorectal carcinogenesis. *Cancer Epidemiol Biomarkers Prev; 22(2); 317–9. ©2012 AACR.*

Introduction

DNA from oncogenic human papillomaviruses (HPV) types-16 and -18, which cause cervical cancer, anal cancer, and other epithelial cancers (1), has been identified in colorectal cancer tissue samples in 9 studies, with prevalence estimates up to 84% (2). However, several other studies report no HPV DNA in colorectal carcinomas, or adenomatous polyps (2). Therefore, the potential role of HPV in colorectal carcinogenesis is unclear.

HPV DNA is detected in virtually 100% of cervical carcinomas, but other cancers with established etiologic links to HPV have varying prevalence of HPV DNA (1). At sites where less than 100% of tumors are positive for HPV, there is generally morphologic and molecular heterogeneity in tumors that correlate with HPV DNA detection (1). For example, HPV-positive tumors tend to lack mutations in *TP53*, an important oncogene that is often mutated in other carcinomas (3). Also, HPV-related cancers tend to have an early age of onset compared with other cancers, and cofactors, such as cigarette smoking, are important in HPV-associated carcinogenesis (4). Thus, if colorectal cancer has a causal association with HPV, then HPV-positive colorectal tumors would likely correlate with certain epidemiologic and molecular tumor characteristics, such as microsatellite instability (MSI). This subset accounts for approximately 15% to 17% of colorectal cancers, has a low prevalence of *TP53* mutation (5), and is associated with cigarette smoking (6). To evaluate the nature of any association between HPV and colorectal cancer, we conducted a large case–case comparison study of HPV and colorectal cancer subsets.

Materials and Methods

Study population

Incident colorectal cancer cases, ages 20 to 74 years, occurring January 1998 to June 2002, and residing in Washington's King, Pierce, or Snohomish counties, were recruited into the Seattle Colon Cancer Family Registery

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(CCFR; ref. 7). Of the 2,573 eligible cases, 71% agreed to participate and completed the informed consent process. Participation in these analyses was restricted to the 555 cases with local stage disease at diagnosis and tumor DNA available. Demographics and smoking status were collected in a standardized telephone interview.

Microsatellite instability analysis

As previously described, investigators determined MSI-status of tumors using 10 genomic markers (7). Fluorescent dye–tagged PCR fragments were analyzed on an ABI3100 genetic analyzer to classify tumors as MSI-high (≥30% of unstable loci), MSI-stable/low (0% to <30% of unstable loci). MSI results were confirmed with immunohistochemical staining to test for protein expression of MLH1, MSH2, MSH6, and PMS2 ($K = 0.95$; ref. 7).

Real-time PCR for HPV-16 and -18 DNA

We conducted real-time PCR assays to detect HPV-16 and -18 DNA in colorectal carcinoma tissue DNA extracted from formalin-fixed paraffin-embedded blocks using the ABI Prism Sequence Detection System (Applied Biosystems). Specific primers and probes for this assay were previously reported (8). The *β-globin* gene was amplified in the same reaction as HPV-16 and -18 *E7* genes to control for DNA quality.

Statistical analyses

Prevalence of HPV-16 and -18 was calculated for overall, and for subsets of colorectal cancer cases, stratified by age, sex, race, smoking status, anatomic site, and MSI-status. Fisher exact test was used to compare the prevalence of HPV between strata.

Power calculation

In studies detecting HPV DNA in colorectal cancers, HPV prevalence estimates ranged from 31% to 84% (2). Given this range, the power to detect a 50% difference in prevalence between subsets of colorectal cancer by MSI-status ranged from 74% to 99%, using a 2-side test with $\alpha = 0.05$ (Power and Sample Size Program, version 3.0.43, 2009, Vanderbilt University, Nashville, TN).

Results

No HPV-18 DNA was detected in any of the 555 colorectal carcinomas tested. HPV-16 DNA was detected in 13 colorectal carcinoma samples (2% prevalence). HPV-16 prevalence did not vary between cases according to sex, age, race, smoking status, or MSI-status ($P > 0.05$). Prevalence of HPV-16 was higher in rectal compared with colon carcinomas ($P = 0.03$; Table 1).

Table 1. HPV prevalence in colorectal carcinoma tissue, by patient and tumor characteristics: The CCFR, 1998 to 2002

	HPV-16–negative (*N* = 542)	HPV-16–positive (*N* = 13)	Fisher exact *P* value
	N (%)	*N* (%)	
Sex			
Male	303 (97)	10 (3)	
Female	239 (99)	3 (1)	0.16
Age, y			
<55	129 (98)	3 (2)	
≥55	413 (98)	10 (2)	0.63
Race			
Caucasian	503 (97)	13 (3)	
Asian American	18 (100)	0 (0)	
African American	7 (100)	0 (0)	
Other	14 (100)	0 (0)	0.99
Smoking status			
Never	187 (99)	2 (1)	
Former	295 (97)	10 (3)	
Current	60 (98)	1 (2)	0.24
Anatomic location			
Colon	381 (99)	5 (1)	
Rectum	154 (95)	8 (5)	0.03
MSI-status			
MSI-low/stable	456 (97)	12 (3)	
MSI-high	86 (99)	1 (1)	0.70

Discussion

This is the largest study by more than 2-fold to evaluate colorectal carcinomas for the presence of HPV DNA and the first to analyze the correlation between HPV and MSI-status in colorectal cancer. Our results suggest that HPV is unlikely to have an etiologic role in colorectal carcinogenesis. Not only did we observe a low prevalence of HPV in colorectal carcinomas, but there was no association between HPV positive tumors and epidemiologic factors associated with HPV-related cancers, such as early age at diagnosis and cigarette smoking. Also, HPV was not associated with MSI-status, an important molecular marker in colorectal cancer that, similar to cancers associated with HPV, has a low prevalence of *TP53* mutation.

HPV prevalence was associated with rectal tumor location. In the absences of other factors correlating with the epidemiology and biology of HPV, this association is likely a consequence of HPV contamination from the anal canal, a site known to be susceptible to HPV infection and which is situated adjacent to the rectum. In studies that use highly sensitive methods, such as PCR, DNA contamination that can occur during the clinical processing of diagnostic specimens, and from other sites of the body, needs to be considered in the interpretation of results.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A.N. Burnett-Hartman, P.A. Newcomb
Development of methodology: A.N. Burnett-Hartman, P.A. Newcomb
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.N. Burnett-Hartman, Q. Feng, V. Popov, P.A. Newcomb
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.N. Burnett-Hartman
Writing, review, and/or revision of the manuscript: A.N. Burnett-Hartman, Q. Feng, V. Popov, A. Kalidindi, P.A. Newcomb
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Kalidindi
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