Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

Research Article

Cancer Epidemiology, Biomarkers & Prevention

Case–Control Study of Cutaneous Human Papillomaviruses in Squamous Cell Carcinoma of the Skin

Michelle R. Iannacone[1], Tarik Gheit[6], Tim Waterboer[7], Anna R. Giuliano[1], Jane L. Messina[2,3,4], Neil A. Fenske[3,4], Basil S. Cherpelis[4], Vernon K. Sondak[2], Richard G. Roetzheim[5], Kristina M. Michael[7], Massimo Tommasino[6], Michael Pawlita[7], and Dana E. Rollison[1]

Abstract

Background: Cutaneous human papillomavirus (HPV) infection may be a risk factor for squamous cell carcinoma (SCC) of the skin.

Methods: To investigate the association between cutaneous HPV and SCC, a case–control study was conducted, including 173 SCC cases from a university dermatology clinic and 300 controls that screened negative for skin cancer. Serum antibodies against cutaneous HPV types in genera alpha, beta, gamma, mu, and nu were measured. Tumor tissue from 159 SCC cases was tested for the presence of DNA for genus-beta HPV types. Using logistic regression ORs and 95% confidence intervals (CI) were estimated for the associations between SCC and cutaneous HPV infection, adjusting for age and sex. The Bonferroni method was used to account for multiple comparisons.

Results: SCC was positively associated with seropositivity to any genus-beta HPV type (OR, 1.93; 95% CI, 1.23–3.02), particularly with types in species-1 (OR, 1.86; 95% CI, 1.22–2.85). Type-specific associations with SCC were observed for HPV 8 (OR, 1.80; 95% CI, 1.14–2.84), 17 (OR, 1.59; 95% CI, 1.02–2.49) and HPV 10 from genus-alpha (OR, 2.24; 95% CI, 1.04–4.85). None of the type-specific associations remained statistically significant after correction for multiple comparisons. When DNA-positive SCC cases were compared with controls, strong serologic associations were observed for HPVs 5 (OR, 3.48; 95% CI, 1.27–9.59), 17 (OR, 3.36; 95% CI, 1.29–8.72), and 24 (OR, 3.79; 95% CI, 1.24–11.5).

Conclusion: Genus-beta HPV infections were associated with SCC in our study population.

Impact: Identifying the role of cutaneous HPV infection in SCC may lead to improved characterization of high-risk individuals and the development of novel prevention strategies. *Cancer Epidemiol Biomarkers Prev; 1–11. ©2012 AACR.*

Introduction

Squamous cell carcinoma (SCC) of the skin is the second most frequently occurring cancer among Caucasians in the United States, and the incidence continues to increase each year (1). Established risk factors for SCC include UV radiation exposure, older age, light skin, and immunosuppression (2). Emerging evidence suggests that cutaneous human papillomavirus (HPV) infection may also be a risk factor for SCC (3–8). HPV types that infect cutaneous epithelia have been identified from genera alpha, beta, gamma, mu, and nu (9). Presence of antibodies against one or more of the genus-beta HPV types as a group has been associated with SCC in several case–control studies (3–8) and type-specific associations with SCC have also been observed with HPV 8 (5, 8, 10), 15 (11), 17 (11), and 38 (5, 11). Estimates of HPV DNA prevalence in SCC tissues from immunocompetent individuals range from 20% to 48% (10, 12–16). To our knowledge, only one study has reported findings on the relationship between HPV seroreactivity and SCC in conjunction with detection of HPV DNA in the skin cancer tumor itself, and in that study, DNA-positive SCC cases were more likely to be seropositive for any HPV type than DNA-negative SCC cases (3).

This is the first case–control study in a U.S. population to investigate the association between SCC and seroreactivity to cutaneous HPV types belonging to 5 different genera. In addition, serologic associations between beta HPV types and SCC were further stratified by the presence of DNA of these HPV types in the tumor tissues.

Authors' Affiliations: [1]Department of Cancer Epidemiology, [2]Cutaneous Oncology Program, Moffitt Cancer Center; Departments of [3]Pathology and Cell Biology, [4]Dermatology & Cutaneous Surgery, and [5]Family Medicine, University of South Florida College of Medicine, Tampa, Florida; [6]International Agency for Research on Cancer, Lyon, France; and [7]Infection and Cancer Program, German Cancer Research Center Deutsches Krebsforschungszentrum, DKFZ, Heidelberg, Germany

Corresponding Author: Dana E. Rollison, Department of Cancer Epidemiology, Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612. Phone: 813-745-6530; Fax: 813-745-6525; E-mail: dana.rollison@moffitt.org

doi: 10.1158/1055-9965.EPI-12-0032

Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

Materials and Methods

Study design and population

A clinic-based case–control study was conducted in Tampa, FL to investigate the association between cutaneous HPV infection and SCC of the skin. The study design and population have been previously described in detail (17). Briefly, histologically confirmed SCC cases were recruited from the University of South Florida (USF; Tampa, FL) Dermatology clinic ($n = 191$). Control subjects were recruited from the USF Family Medicine and Moffitt's Lifetime Cancer Screening and Prevention (LCS) clinics. All control participants included in the current study underwent a full-body skin cancer screening exam, were negative for current signs of skin cancer, and had no history of any type of cancer ($n = 281$). If a patient had a suspicious lesion detected during the skin screening exam that was later determined to be benign based on pathology review, the patient was also included as a control ($n = 77$). If a patient's screen-detected lesion was histologically confirmed to be an SCC then that patient was included as a case ($n = 6$). Study participants were ages 18 to 80 and were eligible to participate regardless of immune status. Nine SCC cases (4.7%) reported a history of organ transplantation.

Participants completed a self-administered questionnaire on skin cancer risk factors, and blood samples were obtained from 174 (90.6%) cases and 340 (95.0%) controls. With the exception of 2 non-White controls, the current analysis was restricted to White participants. The final sample size for the analysis of cutaneous HPV seroreactivity was 173 SCC cases and 300 controls. From patients with SCC undergoing surgical excision, a 3-mm punch of the residual SCC tumor was obtained and flash frozen in liquid nitrogen. Analyses were restricted to tumor specimens that tested positive for β-globin, corresponding to 180 SCC tumors from 159 individual patients, including 19 patients who contributed tissues from distinct, concurrent tumors. The final sample size for analyses including cutaneous HPV seroreactivity and DNA status consisted of 146 SCC cases and 300 controls. Written informed consent was provided by all study participants and all study procedures were approved by the Institutional Review Board at the USF.

HPV antibody measurement

Sera were tested for antibodies to the major capsid protein L1 of cutaneous HPV type(s) within genera alpha (2, 3, 7, 10, 27, 57, and 77); beta (5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, 96, and 107); gamma (4, 48, 50, 65, 88, 95, 101, and 103); mu (1); and nu (41). Sera were also tested for antibodies to the VP1 capsid protein of 2 human polyomaviruses, JC virus (JCV) and KI virus (KIV), to test the specificity of associations observed between cutaneous HPV and SCC.

The antibody detection method used is based on glutathione *S*-transferase (GST) capture ELISA (18, 19) in combination with fluorescent bead technology (Luminex; refs. 20, 21), as previously described. Individual cutoff values to define HPV type–specific seropositivity were applied as described previously to allow for the direct comparison of cutaneous HPV seroprevalence across studies that used the same assay (4, 22).

HPV DNA detection

DNA extraction from frozen SCC tumor tissues was carried out using the Qiagen BioRobot EZ1 with the EZ1 DNA tissue kit according to the manufacturer's instructions (Qiagen). Briefly, frozen tissues were incubated in proteinase K and a buffer G2 (Qiagen) at 56°C until the tissue was completely lysed. To monitor the possible occurrence of cross-contamination between the different specimens during DNA extraction, tubes containing buffer only were also included.

HPV DNA was measured in all samples using an assay that detects 25 genus-beta HPV types (5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93, and 96), based on the same techniques used to measure mucosal HPV types (23, 24). The assay combines 2 different techniques: multiplex PCR using HPV type–specific primer for amplification of viral DNA and array primer extension (APEX) for typing with 2 type-specific probes each.

Statistical analysis

Skin cancer risk factors were compared between SCC cases and controls using the χ^2 test, and independent associations between these factors and case–control status were estimated by calculating ORs and 95% confidence intervals (CI) using conditional logistic regression with adjustment for all other factors. The same approach was used to compare factors between controls who were seropositive for at least one cutaneous HPV type and controls who were seronegative for all HPV types. HPV type–specific seroprevalence was calculated as the proportion of patients who tested positive for antibodies to a given HPV type. Genus-specific HPV seroprevalence was calculated as the proportion of patients who tested seropositive for at least one HPV type in a given genus. Cutaneous HPV seropositivity to any type was defined as testing seropositive to at least one of the 33 cutaneous HPV types tested across the 5 genera. Logistic regression was used to calculate ORs and 95% CIs to estimate the associations between SCC and (i) type-specific cutaneous HPV seropositivity, (ii) genus-specific seropositivity, and (iii) the number of types in a given genus for which an individual tested seropositive. Tests for trend in risk for SCC associated with seropositivity for increasing number of cutaneous HPV types within a given genus were conducted by assigning ordinal values to each category and including the ordinal variable in the logistic regression model. The Bonferroni method was used to account for multiple comparisons, reducing the statistical significance level for SCC associated with genus-specific HPV seropositivity to $P < 0.01$ and type-specific HPV seropositivity to $P < 0.002$.

Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

Tumor tissues obtained from SCC cases were classified as positive or negative for the presence of HPV DNA for types in genus-beta. The 19 cases who contributed more than one SCC tumor tissue were considered HPV DNA–positive if at least one of the tumor tissues provided tested positive for genus-beta HPV DNA. Logistic regression was then used to calculate the OR and 95% CI for the association between seropositivity to any genus-beta HPV type and SCC, stratified by the presence or absence of DNA to any genus-beta HPV type in the tumor tissue (i.e., genus-beta HPV–positive vs. genus-beta HPV–negative). This analysis was restricted to the genus-beta HPV types included in both the antibody and PCR assays (5, 8, 20, 24, 36, 9, 15, 17, 23, 38, 75, 76, 92, and 96). Type-specific concordance was calculated among the SCC cases as the proportion that tested seropositive for a given HPV type and had DNA in their tumor tissue corresponding to the same HPV type. Using logistic regression, additional stratified analyses were then conducted to compare genus-beta type–specific HPV seropositivity between controls and SCC cases that had DNA in their tumors corresponding to the same HPV type for which antibodies were detected.

All factors listed in Table 1 were considered as potential confounders. With the exception of age and sex, none of the factors listed in Table 1 altered the ORs and corresponding 95% CIs for the associations between SCC and cutaneous HPV infection (measured by serology or DNA) by more than 10%. Therefore, all associations were adjusted for age (as a continuous variable) and sex only. To rule out the possibility of residual confounding by age, an age-restricted analysis was conducted by restricting both cases and controls to those aged 40 to 69. In addition, sex-stratified ORs and 95% CIs were estimated for the associations between genus-specific HPV seropositivity and SCC, also restricted to cases and controls aged 40 to 49. Analyses were conducted with and without the 9 SCC cases who reported a history of organ transplantation with similar results, thus, all cases were included. Analyses were conducted using the SAS statistical software package (version 9.2; SAS Institute).

Results

The distribution of demographic, lifestyle, and skin cancer risk factors among SCC cases and controls is presented in Table 1. Compared with controls, SCC cases were significantly more likely to be older, male, and less educated, and to have light eye and hair color, past occupational sunlight exposure, history of blistering sunburn, cutaneous sensitivity to sunlight exposure, poor tanning ability, and a history of alcohol consumption and smoking (Table 1). Demographic, lifestyle, and skin cancer risk factors were not associated with cutaneous HPV serostatus for any HPV type among the controls (Table 2).

Associations between genus-specific HPV seroreactivity and SCC are presented in Table 3. SCC was significantly associated with seropositivity to any genus-beta HPV type (OR, 1.93; 95% CI, 1.23–3.02), with increasing risk observed with an increase in the number of genus-beta HPV types for which an individual tested seropositive up to 8 types; seropositivity for >8 types showed no additional risk for SCC. Similar associations with SCC were not observed for seropositivity to HPV type(s) in genus alpha, gamma, mu, or nu or the polyomaviruses JCV and KIV (Table 3).

Analyses restricted to cases and controls ages 40 to 69 years yielded associations between cutaneous HPV seroreactivity and SCC of similar magnitude as those observed for the total study population. Specifically, SCC was significantly associated with seropositivity for at least one HPV type in genus-beta (OR, 2.05; 95% CI, 1.21–3.46) but not with genus-alpha (OR, 1.15; 95% CI, 0.69–1.89), gamma (OR, 1.25; 95% CI, 0.77–2.02), mu (OR, 1.20; 95% CI, 0.74–1.97), or nu (OR, 1.06; 95% CI, 0.49–2.27). In addition, age-restricted, sex-stratified analyses showed similar associations between genus-specific cutaneous HPV seropositivity and SCC among males (genus-alpha: OR, 1.04; 95% CI, 0.54–2.01; beta: OR, 2.15; 95% CI, 1.07–4.29; gamma: OR, 1.02; 95% CI, 0.54–1.91; mu: OR, 1.05; 95% CI, 0.56–1.97; nu: OR, 1.10; 95% CI, 0.43–2.77) and females (genus-alpha: OR, 1.35; 95% CI, 0.62–2.94; beta: OR, 2.17; 95% CI, 0.93–5.07; gamma: OR, 1.69; 95% CI, 0.80–3.60; mu: OR, 1.59; 95% CI, 0.72–3.51; nu: OR, 1.03; 95% CI, 0.27–3.98).

Seroprevalence was greatest for HPV type 4 in genus-gamma for both SCC cases (43.4%) and controls (34.3%); however, seropositivity to HPV 4 was not significantly associated with SCC (Table 4). HPV 10 was the single type from genus-alpha significantly associated with SCC (OR, 2.24; 95% CI, 1.04–4.85; Table 4). Within genus-beta, seroprevalence was greatest for HPV types 49, 8, and 17 among cases and types 49, 17, 15, and 9 among controls with significant associations observed with SCC for types 8 (OR, 1.80; 95% CI, 1.14–2.84) and 17 (OR, 1.59; 95% CI, 1.02–2.49; Table 4). None of the type-specific associations corresponded to a P value of less than 0.002, the significance level adjusted for multiple comparisons using the Bonferroni method. At the species level, SCC was significantly positively associated with seropositivity for HPV types in genus-beta species 1 (OR, 1.86; 95% CI, 1.22–2.85; $P = 0.004$). No significant associations were observed for genus-beta species 2 to 5 (Table 4) and no significant associations with SCC were observed for individual HPV types in genera gamma, mu, or nu (Table 4).

Ninety-six SCC cases (66%) were DNA positive for at least one genus-beta HPV type tested. Seropositivity to at least one of the genus-beta HPV types tested was associated with overall DNA positivity of genus-beta HPV types in SCC (OR, 2.81; 95% CI, 1.53–5.16; data not shown). Seropositivity to any genus-beta HPV type was not associated with SCC among those cases with tumors negative for HPV DNA (OR, 0.98; 95% CI, 0.53–1.84; data not shown). On the basis of these observations, a decision was made to investigate genus-beta type–specific correlations between seroantibodies and DNA presence in the

Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

Table 1. Association between demographic, lifestyle, and skin cancer risk factors and SCC cases and controls

	Controls ($N = 300$)	SCC ($N = 173$)		
Variable	***n* (%)**	***n* (%)**	**OR (95% CI)[a]**	***P*b**
Age [mean (SD)], y	55.4 (11.7)	64.4 (9.9)	1.09 (1.06–1.11)	<0.0001[c]
Age, y				
18–39	28 (9.3)	4 (2.3)	1.00 (reference)	
40–49	54 (18.0)	10 (5.8)	2.10 (0.48–9.32)	
50–59	104 (34.7)	35 (20.2)	2.62 (0.66–10.37)	
60–69	85 (28.3)	67 (38.7)	6.13 (1.59–23.58)	
70–80	29 (9.7)	57 (33.0)	17.13 (4.19–70.07)	<0.0001
Sex				
Female	186 (62.0)	59 (34.1)	1.00 (reference)	
Male	114 (38.0)	114 (65.9)	3.07 (1.70–5.52)	<0.0001
Education, y				
>12	267 (90.2)	121 (78.6)	1.00 (reference)	
≤12	29 (9.8)	33 (21.4)	3.40 (1.64–7.06)	0.001
Eye color				
Dark brown	81 (27.3)	20 (13.1)	1.00 (reference)	
Blue	85 (28.6)	62 (40.5)	1.66 (0.78–3.51)	
Green	48 (16.2)	24 (15.7)	2.44 (1.05–5.65)	
Hazel	48 (16.2)	30 (19.6)	1.60 (0.70–3.67)	
Light brown	35 (11.8)	17 (11.1)	0.92 (0.35–2.46)	0.01
Hair color				
Black/brown	234 (78.3)	102 (66.2)	1.00 (reference)	
Blonde/red	65 (21.7)	52 (33.8)	1.50 (0.82–2.75)	0.01
Job in sun for ≥3 mo				
No	216 (72.7)	78 (50.7)	1.00 (reference)	
Yes	81 (27.3)	76 (49.4)	1.64 (0.93–2.88)	<0.0001
History of blistering sunburn				
No	95 (32.0)	35 (22.9)	1.00 (reference)	
Yes	202 (68.0)	118 (77.1)	1.19 (0.66–2.12)	0.04
Skin reaction to season's first sun exposure				
Tan or no change in skin color	44 (14.8)	20 (13.1)	1.00 (reference)	
Mild sunburn turns to a tan	136 (45.6)	45 (29.4)	1.06 (0.49–2.29)	
Sunburn with or without blisters	118 (39.6)	88 (57.5)	1.48 (0.67–3.29)	0.001
Skin reaction to repeated sun exposure				
It tans easily	174 (59.0)	56 (36.6)	1.00 (reference)	
It can tan if you work at it	99 (33.6)	72 (47.1)	3.01 (1.62–5.59)	
It is unable to tan	22 (7.5)	25 (16.3)	2.47 (0.96–6.38)	<0.0001
Alcohol consumption				
No drinks in past year	38 (12.8)	31 (20.3)	1.00 (reference)	
≥1 drink in the past year	260 (87.3)	122 (79.7)	0.93 (0.47–1.82)	0.04
Ever smoked 100 cigarettes				
No	150 (50.2)	47 (30.7)	1.00 (reference)	
Yes	149 (49.8)	106 (69.3)	1.82 (1.07–3.08)	<0.0001

NOTE: The distribution of demographic, lifestyle, and skin cancer risk factors among SCC cases and controls and reports the χ^2 *P* value for case–control differences in these factors. In addition, the ORs and 95% CIs are provided as estimates of the associations between demographic, lifestyle, and skin cancer risk factors and SCC with adjustment for all other factors in the table.

[a]Adjusted for all factors included in the table.

[b]*P* value for χ^2 test.

[c]*P* value for Wilcoxon rank-sum test.

Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

Table 2. Distribution of demographic, lifestyle, and skin cancer risk factors by cutaneous HPV serostatus among controls ($N = 300$)

	HPV serostatus			
	Negative ($N = 44$)	Positive ($N = 256$)		
Variable	***n* (%)**	***n* (%)**	**OR[a] (95% CI)[a]**	***P*[b]**
Age [mean (SD)], y	56.2 (11.6)	55.2 (12.5)	0.98 (0.95–1.02)	0.45[c]
Age, y				
18–39	6 (13.6)	22 (8.6)	1.00 (reference)	
40–49	6 (13.6)	48 (18.8)	3.69 (0.89–15.23)	
50–59	12 (27.3)	92 (35.9)	2.95 (0.83–10.42)	
60–69	15 (34.1)	70 (27.3)	1.15 (0.32–4.11)	
70–80	5 (11.4)	24 (9.4)	1.07 (0.22–5.25)	0.53
Sex				
Female	28 (63.6)	158 (61.7)	1.00 (reference)	
Male	16 (36.4)	98 (38.3)	1.12 (0.47–2.69)	0.81
Education, y				
>12	40 (90.9)	227 (90.1)	1.00 (reference)	
≤12	4 (9.1)	25 (9.9)	1.16 (0.34–3.99)	0.86
Eye color				
Dark brown	14 (33.3)	71 (27.8)	1.00 (reference)	
Blue	7 (16.7)	41 (16.1)	0.50 (0.19–1.33)	
Green	4 (9.5)	44 (17.3)	0.61 (0.20–1.85)	
Hazel	5 (11.9)	30 (11.8)	1.70 (0.48–6.09)	
Light brown	12 (28.6)	69 (27.1)	1.25 (0.34–4.57)	0.78
Hair color				
Black/brown	39 (88.6)	195 (76.5)	1.00 (reference)	
Blonde/red	5 (11.4)	60 (23.5)	2.98 (0.99–8.98)	0.08
Job in sun for ≥3 mo				
No	33 (78.6)	183 (71.8)	1.00 (reference)	
Yes	9 (21.4)	72 (28.2)	1.16 (0.46–2.96)	0.36
History of blistering sunburn				
No	18 (41.9)	77 (30.3)	1.00 (reference)	
Yes	25 (58.1)	177 (69.7)	1.90 (0.89–4.06)	0.13
Skin reaction to season's first sun exposure				
Tan or no change in skin color	5 (11.6)	39 (15.3)	1.00 (reference)	
Mild sunburn turns to a tan	26 (60.5)	110 (43.1)	1.22 (0.36–4.12)	
Sunburn with or without blisters	12 (60.5)	106 (41.6)	0.56 (0.19–1.70)	0.11
Skin reaction to repeated sun exposure				
It tans easily	27 (64.3)	147 (58.1)	1.00 (reference)	
It can tan if you work at it/unable to tan	15 (35.7)	106 (41.9)	0.66 (0.28–1.55)	0.45
Alcohol consumption				
No drinks in past year	7 (15.9)	31 (12.2)	1.00 (reference)	
≥1 drink in the past year	37 (84.1)	223 (87.8)	0.82 (0.24–2.67)	0.5
Ever smoked 100 cigarettes				
No	25 (56.8)	125 (49.0)	1.00 (reference)	
Yes	19 (43.2)	130 (51.0)	1.83 (0.84–3.99)	0.34

NOTE: The distribution of demographic, lifestyle, and skin cancer risk factors by cutaneous HPV serostatus in the controls and reports the χ^2 *P* value for differences in the these factors between controls who were cutaneous HPV seropositive for any type versus seronegative for all types. In addition, the ORs and 95% CIs are provided as estimates of the associations between demographic, lifestyle, and skin cancer risk factors and cutaneous HPV serostatus with adjustment for all other factors in the table.

[a]Adjusted for all factors listed in table.

[b]*P* value for χ^2 test.

[c]*P* value for Wilcoxon rank-sum test.

Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

Table 3. Associations between genus-specific HPV seroreactivity and SCC cases and controls

	Controls ($N = 300$)	SCC ($N = 173$)		
HPV genus	***n* (%)**	***n* (%)**	**OR (95% CI)[a]**	***P*[b]**
Alpha				
Seronegative	194 (64.7)	108 (62.4)	1.00 (reference)	ref.
Seropositive	106 (35.3)	65 (37.6)	1.11 (0.72–1.71)	0.64
1 HPV type	59 (19.7)	30 (17.3)	0.91 (0.52–1.59)	0.75
≥2 HPV types	47 (15.7)	35 (20.2)	1.35 (0.77–2.34)	0.29
			$P_{trend} = 0.39$	
Beta				
Seronegative	119 (39.7)	46 (26.6)	1.00 (reference)	ref.
Seropositive	181 (60.3)	127 (73.4)	1.93 (1.23–3.02)	0.004
1 HPV type	47 (15.7)	32 (18.5)	1.88 (1.01–3.49)	0.05
2–3 HPV types	51 (17.0)	32 (18.5)	1.93 (1.04–3.58)	0.04
4–8 HPV types	36 (12.0)	29 (16.8)	2.50 (1.30–4.83)	0.006
>8 HPV types	47 (15.7)	34 (19.7)	1.60 (0.86–3.00)	0.14
			$P_{trend} = 0.04$	
Gamma				
Seronegative	145 (48.3)	66 (38.2)	1.00 (reference)	ref.
Seropositive	155 (51.7)	107 (61.9)	1.34 (0.88–2.04)	0.17
1 HPV type	63 (21.0)	43 (23.7)	1.17 (0.68–2.00)	0.58
≥2 HPV types	92 (30.7)	66 (38.2)	1.47 (0.92–2.36)	0.11
			$P_{trend} = 0.11$	
Mu				
Seronegative	204 (68.0)	107 (61.9)	1.00 (reference)	ref.
Seropositive	96 (32.0)	66 (38.2)	1.41 (0.91–2.18)	0.13
Nu				
Seronegative	266 (88.7)	152 (87.9)	1.00 (reference)	ref.
Seropositive	34 (11.3)	21 (12.1)	0.91 (0.48–1.74)	0.78
Control antigens				
JCV				
Seronegative	108 (36.0)	45 (66.0)	1.00 (reference)	ref.
Seropositive	192 (64.0)	128 (74.0)	1.40 (0.89–2.20)	0.15
KIV				
Seronegative	24 (8.0)	12 (6.9)	1.00 (reference)	ref.
Seropositive	276 (92.0)	161 (93.1)	0.78 (0.34–1.75)	0.54

NOTE: The ORs and 95% CIs for the associations between genus-specific HPV seroreactivity and SCC, along with the *P* value for trend for SCC risk associated with seropositivity to an increasing number of HPV types within a given genus. In addition, for comparison purposes, the ORs and 95% CIs for the associations between 2 human polyomaviruses (JCV and KIV) and SCC are also presented.

[a]OR and 95% CI adjusted for age and sex.

[b]*P* value for β-coefficient corresponding to HPV term in logistic regression model.

tumor for the same HPV types. Among the 96 SCC cases with HPV DNA–positive tumors, 41.7% tested seropositive for antibodies to the same HPV type detected in the tumor tissue (data not shown). Concordance between the specific HPV type for which DNA was observed in the tumor and the HPV type for which circulating antibodies were observed ranged from 12.5% for type 9 to 54.5% for type 17 (Table 5). Compared with controls, SCC cases who were DNA positive for the following HPV types had significantly greater seroprevalence for the same HPV types: HPV 5 (OR, 3.48; 95% CI, 1.27–9.59), 24 (OR, 3.79; 95% CI, 1.24–11.5), and 17 (OR, 3.36; 95% CI, 1.29–8.72; Table 5). SCC cases with tumors that tested DNA negative for all genus-beta HPV types were significantly less likely to be seropositive for HPV types 20, 36, 9, 15, 38, and 75 than controls (Table 5).

Discussion

Observations from the current case–control study support the association between cutaneous HPV seropositivity and SCC. Seropositivity to HPV 10 was the single type from genus-alpha associated with SCC. Seropositivity for

Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

Table 4. Association between type-specific HPV seropositivity and SCC

	Controls ($N = 300$)	SCC ($N = 173$)		
HPV genus/type	***n* (%)**	***n* (%)**	**OR (95% CI)[a]**	***P*[b]**
Any HPV type	256 (85.3)	161 (93.1)	2.50 (1.21–5.15)	0.01
Alpha				
2	29 (9.7)	15 (8.7)	0.77 (0.37–1.61)	0.49
3	36 (12.0)	24 (13.9)	1.21 (0.65–2.24)	0.55
7	22 (7.3)	15 (8.7)	1.09 (0.50–2.35)	0.83
10	17 (5.7)	19 (11.0)	2.24 (1.04–4.85)	0.04
27	33 (11.0)	29 (16.8)	1.78 (0.97–3.26)	0.06
57	22 (7.3)	16 (9.2)	1.42 (0.66–3.07)	0.37
77	47 (15.7)	33 (19.1)	1.36 (0.79–2.35)	0.27
Beta (β)				
Any β_1	100 (33.3)	85 (49.1)	1.86 (1.22–2.85)	0.004
5	38 (12.7)	35 (20.2)	1.68 (0.95–2.96)	0.07
8	69 (23.0)	63 (36.4)	1.80 (1.14–2.84)	0.01
20	63 (21.0)	51 (29.5)	1.41 (0.88–2.27)	0.16
24	37 (12.3)	36 (20.8)	1.63 (0.93–2.86)	0.09
36	52 (17.3)	40 (23.1)	1.13 (0.68–1.90)	0.64
Any β_2	143 (47.7)	91 (52.6)	1.30 (0.86–1.97)	0.21
9	75 (25.0)	51 (29.5)	1.17 (0.74–1.85)	0.51
15	76 (25.3)	50 (28.9)	0.93 (0.58–1.49)	0.77
17	77 (25.7)	61 (35.3)	1.59 (1.02–2.49)	0.04
23	61 (20.3)	42 (24.3)	1.23 (0.75–2.02)	0.42
38	74 (24.7)	42 (24.3)	0.94 (0.58–1.52)	0.79
107	48 (16.0)	31 (17.9)	0.91 (0.52–1.58)	0.73
Any β_3	101 (33.7)	73 (42.2)	1.38 (0.90–2.11)	0.14
49	89 (29.7)	65 (37.6)	1.36 (0.88–2.11)	0.16
75	46 (15.3)	31 (17.9)	1.04 (0.60–1.81)	0.89
76	54 (18.0)	41 (23.7)	1.24 (0.74–2.05)	0.41
β_4 (92)	50 (16.7)	37 (21.4)	1.29 (0.76–2.18)	0.35
β_5 (96)	45 (15.0)	44 (25.4)	1.64 (0.97–2.76)	0.06
Gamma				
4	103 (34.3)	75 (43.4)	1.49 (0.97–2.28)	0.07
48	64 (21.3)	48 (27.7)	1.56 (0.96–2.53)	0.07
50	47 (15.7)	27 (15.6)	0.90 (0.51–1.59)	0.72
65	78 (26.0)	55 (31.8)	1.20 (0.76–1.90)	0.42
88	20 (6.7)	14 (8.1)	1.23 (0.55–2.73)	0.61
95	67 (22.3)	40 (23.1)	0.97 (0.60–1.59)	0.92
101	33 (11.0)	24 (13.9)	1.20 (0.64–2.26)	0.57
103	13 (4.3)	9 (5.2)	0.92 (0.35–2.41)	0.86
Mu (1)	96 (32.0)	66 (38.2)	1.41 (0.91–2.18)	0.13
Nu (41)	34 (11.3)	21 (12.1)	0.91 (0.48–1.74)	0.78

NOTE: The ORs and 95% CIs for the associations between type-specific HPV seropositivity and SCC.

[a]OR and 95% CI adjusted for age and sex.

[b]*P* value for β-coefficient corresponding to HPV term in logistic regression model.

cutaneous HPV types in genus-beta overall, and for types 8 and 17 specifically, was significantly associated with SCC in this clinic-based case–control study. In addition, correlations between seropositivity and DNA positivity for the same genus-beta HPV type were observed for HPV types 5, 24, and 17. SCC cases with tumors negative for any genus-beta HPV type were less likely to be seroprevalent for HPV types 20, 36, 9, 15, 38, and 75 than controls.

The positive association observed between SCC and seropositivity to HPV types in genus-beta is consistent with 2 (7, 8) of 5 case–control studies (3, 5, 7, 8, 16) including one study from the United States (7). Type-

Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

Table 5. Association between genus-β type–specific HPV seropositivity and SCC, stratified by HPV DNA status of the same HPV type in the tumor

		SCC HPV type-specific DNA status			
		Positive ($N = 96$)		Negative ($N = 50$)	
Beta (β) Species/type[a]	Controls (N = 300) *n* (%)	*n* (%)	OR (95% CI)[b]	*n* (%)	OR[b] (95% CI)[b]
β_1					
HPV 5					
Seronegative	262 (87.3)	13 (61.9)	1.00 (reference)	44 (88.0)	1.00 (reference)
Seropositive	38 (12.7)	8 (38.1)	3.48 (1.27–9.59)	6 (12.0)	0.87 (0.34–2.25)
HPV 8					
Seronegative	231 (77.0)	10 (58.8)	1.00 (reference)	36 (72.0)	1.00 (reference)
Seropositive	69 (23.0)	7 (41.2)	2.04 (0.69–6.01)	14 (28.0)	1.26 (0.63–2.53)
HPV 20					
Seronegative	237 (79.0)	6 (54.5)	1.00 (reference)	46 (92.0)	1.00 (reference)
Seropositive	63 (21.0)	5 (45.5)	2.74 (0.77–9.74)	4 (8.0)	0.31 (0.11–0.91)
HPV 24					
Seronegative	263 (87.7)	11 (61.1)	1.00 (reference)	48 (96.0)	1.00 (reference)
Seropositive	37 (12.3)	7 (38.9)	3.79 (1.24–11.5)	2 (4.0)	0.24 (0.05–1.08)
HPV 36					
Seronegative	248 (82.7)	10 (55.6)	1.00 (reference)	46 (92.0)	1.00 (reference)
Seropositive	52 (17.3)	8 (44.4)	2.63 (0.92–7.50)	4 (8.0)	0.32 (0.11–0.96)
β_2					
HPV 9					
Seronegative	225 (75.0)	7 (87.5)	1.00 (reference)	46 (92.0)	1.00 (reference)
Seropositive	75 (25.0)	1 (12.5)	0.31 (0.04–2.72)	4 (8.0)	0.24 (0.08–0.70)
HPV 15					
Seronegative	224 (74.7)	7 (50.0)	1.00 (reference)	46 (92.0)	1.00 (reference)
Seropositive	76 (25.3)	7 (50.0)	2.55 (0.81–8.01)	4 (8.0)	0.23 (0.08–0.66)
HPV 17					
Seronegative	223 (74.3)	10 (45.5)	1.00 (reference)	41 (82.0)	1.00 (reference)
Seropositive	77 (25.7)	12 (54.5)	3.36 (1.29–8.72)	9 (18.0)	0.68 (0.31–1.49)
HPV 23					
Seronegative	239 (79.7)	30 (76.9)	1.00 (reference)	46 (92.0)	1.00 (reference)
Seropositive	61 (20.3)	9 (23.1)	1.26 (0.54–2.94)	4 (8.0)	0.36 (0.12–1.07)
HPV 38					
Seronegative	226 (75.3)	17 (65.4)	1.00 (reference)	47 (94.0)	1.00 (reference)
Seropositive	74 (24.7)	9 (34.6)	1.36 (0.55–3.38)	3 (6.0)	0.20 (0.06–0.67)
β_3					
HPV 75					
Seronegative	254 (84.7)	4 (80.0)	1.00 (reference)	49 (98.0)	1.00 (reference)
Seropositive	46 (15.3)	1 (20.0)	1.27 (0.14–12.0)	1 (2.0)	0.11 (0.01–0.80)
β_4					
HPV 92					
Seronegative	250 (83.3)	7 (77.8)	1.00 (reference)	45 (90.0)	1.00 (reference)
Seropositive	50 (16.7)	2 (22.2)	1.27 (0.24–6.65)	5 (10.0)	0.56 (0.21–1.51)
β_5					
HPV 96					
Seronegative	255 (85.0)	4 (57.1)	1.00 (reference)	46 (92.0)	1.00 (reference)
Seropositive	45 (15.0)	3 (42.9)	3.47 (0.72–16.6)	4 (8.0)	0.42 (0.14–1.28)

NOTE: The ORs and 95% CIs for the associations between genus-β type–specific HPV seropositivity and SCC, stratified by the presence of HPV DNA to the same genus-beta type in the SCC tumor tissue itself.

[a]No tumor specimens were DNA positive for HPV 76. Therefore, concordance analyses were not conducted for HPV 76.

[b]OR and 95% CI adjusted for age and sex.

Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

specific associations observed with SCC for HPV types 8 and 17 in genus-beta agree with findings from case–control studies in the United States (7), the Netherlands (5), and Australia (8) for HPV type 8 and with findings from case–control studies in the United States (7) and Italy (25) for HPV type 17. In contrast to previous case–control studies, SCC was not associated with seropositivity to HPV types 5 (6), 15 (7, 11), and 38 (5, 11), as well as with types 20, 24, 9, 49, 75, 76, 92, and 96 (7). Neither of 2 published cohort studies (4, 26) observed statistically significant increased risks of SCC associated with HPV seropositivity for types in genera alpha (4), beta (4, 26), gamma (4), mu (4), or nu (4).

A positive dose–response between seropositivity to increasing numbers of HPV types in genus-beta and SCC was observed in the current study population, consistent with 3 (7, 8, 27) of 4 (7, 8, 25, 27) previous case–control studies. However, in a recent prospective cohort study (26), an increased risk of SCC was not observed among individuals who were seropositive to at least one genus-beta HPV type or multiple types. Furthermore, neither of the 2 prospective studies reporting increased risks of SCC associated with baseline cutaneous HPV seropositivity observed statistically significant type-specific associations with any individual type from genus-beta (4, 26).

The single type-specific association with SCC observed in this study for a cutaneous HPV type in a genus other than beta was with HPV type 10 in genus-alpha, consistent with the 2 studies in the literature previously reporting on HPV type–specific associations outside of genus-beta (4, 11). HPV 10 has been associated with benign skin lesions (28) but displays weak transforming activities by *in vitro* experimental models (29), thus, its direct involvement in skin carcinogenesis remains unclear.

Among SCC cases from Sweden and Austria (3), 58% tested DNA positive for at least 1 HPV type in genus-beta compared with 66% in SCC cases from the current study. In addition, a low overall concordance between HPV DNA and seropositivity for the same HPV type (19%) was observed (3) compared with the current study with an overall type-specific concordance of 41.7%. In addition, among participants in the current study, individual type-specific concordance was greater for genus-beta HPV types 5, 8, and 15 but lower for types 9 and 24 as compared with participants from Sweden/Austria (3). The observed difference in overall concordance between HPV types identified from the tumor and circulating antibodies may be explained by a difference in the number of genus-beta HPV types examined, (8 types in the Swedish/Austrian population compared with 13 types in the current study population). Individual type-specific variations in concordance may be explained by differences in the laboratory techniques used to test for the presence of HPV DNA in the tissue specimens. In addition, Andersson and colleagues obtained biopsies from the malignant lesion and adjacent healthy skin and subsequently classified SCC cases as DNA positive if either of the biopsies tested positive for beta HPV DNA (3) in contrast to the current study that defined DNA positivity based on detection in the SCC tumor only.

It was unexpected to observe statistically significant inverse associations between seropositivity and SCC among HPV DNA–negative cases. One explanation may simply be that SCC cases with tumors negative for HPV DNA and subsequently low antibody seroprevalence compared with controls may have a different risk profile for disease. In terms of previous findings, these data may explain the low-risk estimates observed in previous studies for the associations between cutaneous HPV seropositivity and SCC when HPV DNA status of the tumor has not been accounted for. In addition, the observations from the current study may underlie the discrepant findings presented across case–control studies. Further research is needed to delineate the role of cutaneous HPV antibodies as either a marker of prevalent and cleared infections or a marker of current active infections and their subsequent association with SCC risk.

The current study provides evidence for the association between genus-beta HPV and SCC, however, the exact mechanism by which cutaneous HPV is associated with SCC remains unclear. It has been hypothesized that the effects of genus-beta HPV on UV-induced DNA damage and apoptosis could lead to the accumulation of mutations which predispose to SCC formation (30). Evidence from *in vitro* and *in vivo* studies have shown that *E6* and *E7* proteins from certain cutaneous HPV types are capable of inhibiting UV-induced cell-cycle check points, apoptosis, and DNA repair machinery (31–36). Observations from epidemiologic studies have shown statistically significant interactions between sun-related factors and genus-beta HPV seropositivity in relation to SCC (27, 37, 38). In addition, HPV 38 *E6* and *E7* proteins have displayed transforming properties in primary human keratinocyte cells sufficient to deregulate cell-cycle control and growth arrest (39). Taken together, these findings support a role for cutaneous HPV as a cofactor in SCC carcinogenesis, in contrast to the direct role of mucosal HPV infections in the carcinogenesis of cervical cancer.

Clinic based case–control study populations are often not representative of the general population. However, cases and controls were recruited from clinics that serve the same underlying populations, thus preserving internal validity. Because a considerable proportion of the skin cancer screening patients were confirmed to be free of skin cancer only after completion of the follow-up visit with a dermatologist, there was at time lag from study consent to verification of case–control status. As such, matching controls to cases was logistically challenging and significant case–control differences in age and sex resulted. However, all analyses were adjusted for age and sex. Furthermore, the analysis restricted to those aged 40 to 69 yielded similar results as those obtained from the entire study population. In addition, similar associations between genus-specific HPV seropositivity and SCC were observed between males and females in the restricted age range. Therefore, the observed associations between

Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

cutaneous HPV seropositivity and SCC are not likely due to residual confounding by age and/or sex. Adjustment for multiple comparisons reduced the statistical significance of most of the observed associations, thus, chance cannot be ruled out as an explanation for these findings. However, multiple comparisons is a methodologic challenge faced by all epidemiologic studies of cutaneous HPV infections, given the sheer number of types potentially implicated in SCC. Benign lesions were not obtained for control subjects that completed dermatologic follow-up, as such, for comparison purposes it was not possible to test the benign lesions for the presence of HPV DNA. If in fact cutaneous HPV is associated with benign skin lesions then the observed results would be attenuated given that a subset of the controls were diagnosed with such lesions.

Several study strengths should also be noted. The current study is the first to present cutaneous HPV genus and type-specific associations outside of genus-alpha and -beta and to incorporate comparisons with genus-beta HPV DNA reactivity in SCC tumor tissues in a U.S. population. Only one study in the published literature has presented similar results among residents of Sweden and Austria (3). This is a major strength of the current study compared with previously published case–control studies of cutaneous HPV serology in SCC, allowing the unique opportunity to investigate specificity of serologic response and the presence of DNA for the same HPV type. In addition, the laboratory used to test for cutaneous HPV seropositivity has been used in most of the seroepidemiologic studies of cutaneous HPV published to date (4, 6, 7, 11, 22, 25, 40, 41), allowing for direct comparison across studies. Finally, the full-body skin examination conducted on all control subjects is another study strength, as it eliminated misclassification of SCC case–control status that can result by inclusion of apparently healthy individuals who in fact have a prevalent, undiagnosed SCC. Of note, 6 of the SCC cases included in the current study were screen-detected.

The current study confirms previous findings supporting the association between genus-beta seropositivity and SCC. In addition, genus-beta type–specific associations with SCC are supported by the observed correlation between HPV seropositivity and tumor DNA positivity for specific types in genus-beta. However, additional longitudinal studies need to be conducted to better understand the direction of the association between cutaneous HPV infection and SCC to rule out the possibility of reverse causality.

Disclosure of Potential Conflicts of Interest

T. Waterboer is an employee for Boehringer Ingelheim. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A.R. Giuliano, M. Pawlita, D.E. Rollison
Development of methodology: T. Waterboer, J.L. Messina, K.M. Michael, M. Pawlita, D.E. Rollison
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.R. Iannacone, T. Waterboer, J.L. Messina, N.A. Fenske, B.S. Cherpelis, V.K. Sondak, R.G. Roetzheim, K.M. Michael, M. Pawlita, D.E. Rollison
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.R. Iannacone, A.R. Giuliano, V.K. Sondak, M. Tommasino, M. Pawlita, D.E. Rollison
Writing, review, and/or revision of the manuscript: M.R. Iannacone, T. Waterboer, A.R. Giuliano, J.L. Messina, N.A. Fenske, B.S. Cherpelis, V.K. Sondak, R.G. Roetzheim, K.M. Michael, M. Pawlita, D.E. Rollison
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.R. Iannacone, D.E. Rollison
Study supervision: V.K. Sondak, D.E. Rollison
Development of methods for the detection of papillomaviruses (ICB, IARC): T. Gheit

Acknowledgments

The authors thank the supporting staff at USF and LCS for their assistance with patient recruitment, especially Kristen Jonathan, Jill Weber, and Carolyn Gerow.

Grant Support

This work was supported by a James and Esther King New Investigator grant awarded to DER (06NIR-08) by the Florida Department of Health (FL DOH). This research was also supported in part by the Miles for Moffitt Foundation Funds.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 12, 2012; revised May 25, 2012; accepted June 11, 2012; published OnlineFirst June 15, 2012.

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277–300.
2. Saladi RN, Persaud AN. The causes of skin cancer: a comprehensive review. Drugs Today (Barc) 2005;41:37–53.
3. Andersson K, Waterboer T, Kirnbauer R, Slupetzky K, Iftner T, de Villiers EM, et al. Seroreactivity to cutaneous human papillomaviruses among patients with nonmelanoma skin cancer or benign skin lesions. Cancer Epidemiol Biomarkers Prev 2008;17:189–95.
4. Casabonne D, Michael KM, Waterboer T, Pawlita M, Forslund O, Burk RD, et al. A prospective pilot study of antibodies against human papillomaviruses and cutaneous squamous cell carcinoma nested in the Oxford component of the European Prospective Investigation into Cancer and Nutrition. Int J Cancer 2007;121:1862–8.
5. Feltkamp MC, Broer R, di Summa FM, Struijk L, van der Meijden E, Verlaan BP, et al. Seroreactivity to epidermodysplasia verruciformis-related human papillomavirus types is associated with nonmelanoma skin cancer. Cancer Res 2003;63:2695–700.
6. Karagas MR, Nelson HH, Sehr P, Waterboer T, Stukel TA, Andrew A, et al. Human papillomavirus infection and incidence of squamous cell and basal cell carcinomas of the skin. J Natl Cancer Inst 2006;98:389–95.
7. Karagas MR, Waterboer T, Li Z, Nelson HH, Michael KM, Bavinck JN, et al. Genus beta human papillomaviruses and incidence of basal cell and squamous cell carcinomas of skin: population based case-control study. BMJ 2010;341:c2986.
8. Struijk L, Hall L, van der Meijden E, Wanningen P, Bavinck JN, Neale R, et al. Markers of cutaneous human papillomavirus infection in individuals with tumor-free skin, actinic keratoses, and squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 2006;15:529–35.

Published OnlineFirst June 15, 2012; DOI: 10.1158/1055-9965.EPI-12-0032

9. Bernard HU, Burk RD, Chen Z, van Doorslaer K, Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 2010;401:70–9.
10. Forslund O, Ly H, Reid C, Higgins G. A broad spectrum of human papillomavirus types is present in the skin of Australian patients with non-melanoma skin cancers and solar keratosis. Br J Dermatol 2003;149:64–73.
11. Waterboer T, Abeni D, Sampogna F, Rother A, Masini C, Sehr P, et al. Serological association of beta and gamma human papillomaviruses with squamous cell carcinoma of the skin. Br J Dermatol 2008;159: 457–9.
12. Forslund O, Iftner T, Andersson K, Lindelof B, Hradil E, Nordin P, et al. Cutaneous human papillomaviruses found in sun-exposed skin: Beta-papillomavirus species 2 predominates in squamous cell carcinoma. J Infect Dis 2007;196:876–83.
13. Harwood CA, Surentheran T, McGregor JM, Spink PJ, Leigh IM, Breuer J, et al. Human papillomavirus infection and non-melanoma skin cancer in immunosuppressed and immunocompetent individuals. J Med Virol 2000;61:289–97.
14. Pfister H, Fuchs PG, Majewski S, Jablonska S, Pniewska I, Malejczyk M. High prevalence of epidermodysplasia verruciformis-associated human papillomavirus DNA in actinic keratoses of the immunocompetent population. Arch Dermatol Res 2003;295:273–9.
15. Stockfleth E, Nindl I, Sterry W, Ulrich C, Schmook T, Meyer T. Human papillomaviruses in transplant-associated skin cancers. Dermatol Surg 2004;30:604–9.
16. Termorshuizen F, Feltkamp MC, Struijk L, de Gruijl FR, Bavinck JN, van Loveren H. Sunlight exposure and (sero)prevalence of epidermodysplasia verruciformis-associated human papillomavirus. J Invest Dermatol 2004;122:1456–62.
17. Rollison DE, Guiliano A, Messina JL, Fenske NA, Cherpelis BS, Sondak VK, et al. Case-control study of Merkel cell polyomavirus infection and cutaneous squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 2012;21:74–81.
18. Sehr P, Muller M, Hopfl R, Widschwendter A, Pawlita M. HPV antibody detection by ELISA with capsid protein L1 fused to glutathione S-transferase. J Virol Methods 2002;106:61–70.
19. Sehr P, Zumbach K, Pawlita M. A generic capture ELISA for recombinant proteins fused to glutathione S-transferase: validation for HPV serology. J Immunol Methods 2001;253:153–62.
20. Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO, et al. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. Clin Chem 2005;51: 1845–53.
21. Waterboer T, Sehr P, Pawlita M. Suppression of non-specific binding in serological Luminex assays. J Immunol Methods 2006;309:200–4.
22. Michael KM, Waterboer T, Sehr P, Rother A, Reidel U, Boeing H, et al. Seroprevalence of 34 human papillomavirus types in the German general population. PLoS Pathog 2008;4:e1000091.
23. Gheit T, Billoud G, de Koning MN, Gemignani F, Forslund O, Sylla BS, et al. Development of a sensitive and specific multiplex PCR method combined with DNA microarray primer extension to detect Betapapillomavirus types. J Clin Microbiol 2007;45:2537–44.
24. Gheit T, Landi S, Gemignani F, Snijders PJ, Vaccarella S, Franceschi S, et al. Development of a sensitive and specific assay combining multiplex PCR and DNA microarray primer extension to detect high-risk mucosal human papillomavirus types. J Clin Microbiol 2006;44:2025–31.
25. Waterboer T, Neale R, Michael KM, Sehr P, de Koning MN, Weissenborn SJ, et al. Antibody responses to 26 skin human papillomavirus types in the Netherlands, Italy and Australia. J Gen Virol 2009;90: 1986–98.
26. Plasmeijer EI, Pandeya N, O'Rourke P, Pawlita M, Waterboer T, Feltkamp MC, et al. The Association between Cutaneous Squamous Cell Carcinoma and Betapapillomavirus Seropositivity: a Cohort Study. Cancer Epidemiol Biomarkers Prev 2011;20:1171–7.
27. Bouwes Bavinck JN, Neale RE, Abeni D, Euvrard S, Green AC, Harwood CA, et al. Multicenter study of the association between beta-papillomavirus infection and cutaneous squamous cell carcinoma. Cancer Res 2010;70:9777–86.
28. Kremsdorf D, Jablonska S, Favre M, Orth G. Human papillomaviruses associated with epidermodysplasia verruciformis. II. Molecular cloning and biochemical characterization of human papillomavirus 3a, 8, 10, and 12 genomes. J Virol 1983;48:340–51.
29. Boxman IL, Mulder LH, Noya F, de Waard V, Gibbs S, Broker TR, et al. Transduction of the E6 and E7 genes of epidermodysplasia-verruciformis-associated human papillomaviruses alters human keratinocyte growth and differentiation in organotypic cultures. J Invest Dermatol 2001;117:1397–404.
30. Tomlins C, Storey A. Cutaneous HPV5 E6 causes increased expression of Osteoprotegerin and Interleukin 6 which contribute to evasion of UV-induced apoptosis. Carcinogenesis 2010;31: 2155–64.
31. Dong W, Arpin C, Accardi R, Gissmann L, Sylla BS, Marvel J, et al. Loss of p53 or p73 in human papillomavirus type 38 E6 and E7 transgenic mice partially restores the UV-activated cell cycle checkpoints. Oncogene 2008;27:2923–8.
32. Dong W, Kloz U, Accardi R, Caldeira S, Tong WM, Wang ZQ, et al. Skin hyperproliferation and susceptibility to chemical carcinogenesis in transgenic mice expressing E6 and E7 of human papillomavirus type 38. J Virol 2005;79:14899–908.
33. Jackson S, Harwood C, Thomas M, Banks L, Storey A. Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. Genes Dev 2000;14:3065–73.
34. Jackson S, Storey A. E6 proteins from diverse cutaneous HPV types inhibit apoptosis in response to UV damage. Oncogene 2000;19: 592–8.
35. Simmonds M, Storey A. Identification of the regions of the HPV 5 E6 protein involved in Bak degradation and inhibition of apoptosis. Int J Cancer 2008;123:2260–6.
36. Viarisio D, Mueller-Decker K, Kloz U, Aengeneyndt B, Kopp-Schneider A, Grone HJ, et al. E6 and E7 from beta HPV38 cooperate with ultraviolet light in the development of actinic keratosis-like lesions and squamous cell carcinoma in mice. PLoS Pathog 2011;7: e1002125.
37. Hall L, Struijk L, Neale RE, Feltkamp MC. Re: Human papillomavirus infection and incidence of squamous cell and basal cell carcinomas of the skin. J Natl Cancer Inst 2006;98:1425–6.
38. Iannacone MR, Wang W, Stockwell HG, O'Rourke K, Giuliano AR, Sondak VK, et al. Sunlight exposure and cutaneous human papillomavirus seroreactivity in basal cell and squamous cell carcinomas of the skin. J Infect Dis. 2012 Jun 1. [Epub ahead of print].
39. Caldeira S, Zehbe I, Accardi R, Malanchi I, Dong W, Giarre M, et al. The E6 and E7 proteins of the cutaneous human papillomavirus type 38 display transforming properties. J Virol 2003;77:2195–206.
40. Iannacone MR, Michael KM, Giuliano AR, Waterboer T, Pawlita M, Rollison DE. Risk factors for cutaneous human papillomavirus seroreactivity among patients undergoing skin cancer screening in Florida. J Infect Dis 201:760–9.
41. Rollison DE, Pawlita M, Giuliano AR, Iannacone MR, Sondak VK, Messina JL, et al. Measures of cutaneous human papillomavirus infection in normal tissues as biomarkers of HPV in corresponding nonmelanoma skin cancers. Int J Cancer 2008;123:2337–42.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

Case–Control Study of Cutaneous Human Papillomaviruses in Squamous Cell Carcinoma of the Skin

Michelle R. Iannacone, Tarik Gheit, Tim Waterboer, et al.

Cancer Epidemiol Biomarkers Prev Published OnlineFirst June 15, 2012.