Cutaneous Human Papillomaviruses Persist on Healthy Skin

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Cutaneous human papillomaviruses (HPVs) are frequently found in healthy skin and have also been implicated in non-melanoma skin cancer. For genital HPV types, a persistent infection with one of the high-risk types is a prerequisite for the development of cervical cancer. However, there is only limited data on whether infections with cutaneous HPV types persist over time. Serial forehead swab samples collected from 63 volunteers (42 healthy individuals and 31 renal transplant recipients (RTRs)), sampled 6.3 years (range: 5.0–7.0 years) apart, were analyzed for HPV using general primer PCR, cloning, and sequencing. Among the healthy individuals, the prevalences of HPV were 69% (29/42) at enrolment and 71% (30/42) at follow-up. Among the individuals positive at baseline, 48% (14/29) had a persistent infection. Among the RTRs, 71% (15/21) were positive for HPV at enrolment and 90% (19/21) at follow-up. A persistent infection was detected in 33% (5/15). In total, HPV was detected in 44 of the samples collected at baseline and the same virus was found at follow-up in 43% (19/44). Persistence was not significantly associated with age, sex, immunosuppressive treatment, history of warts, or genus of HPV. We conclude that cutaneous HPV infections commonly persist over several years on healthy skin.

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INTRODUCTION

Over 100 human papillomavirus (HPV) types have been completely sequenced (de Villiers *et al.*, 2004). In addition, sequence information from PCR amplimers (FA amplicons) have identified about 100 additional cutaneous putative HPV types (Forslund *et al.*, 1999, 2004; Antonsson *et al.*, 2000, 2003). Different HPVs infect either mucosal epithelium or the skin. Persistent infection with one of the high-risk genital HPV types, for example, HPV16 or HPV18, is a prerequisite for the development of cervical cancer (Bosch *et al.*, 2002). Cutaneous HPV types have been implicated in non-melanoma skin cancer in patients suffering from the rare inherited genetic disease epidermodysplasia verruciformis (Jablonska and Majewski, 1994). These patients are highly susceptible to infections with a subset of *beta*-papillomaviruses (Kremsdorf *et al.*, 1984). HPV DNA is also detectable in skin carcinomas from both renal transplant recipients (RTRs) (Berkhout *et al.*, 1995; de Jong-Tieben *et al.*, 1995; de Villiers *et al.*, 1997) and immunocompetent individuals (Harwood and Proby, 2002). The role of HPV in these cancers remains to be elucidated, although recent serological studies suggest an association (Karagas *et al.*, 2006).

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Abbreviations: HPV, human papillomavirus; RTR, renal transplant recipient

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Healthy skin harbors a large spectrum of different HPV types (Antonsson *et al.*, 2000, 2003), with more virus being detected in forehead samples than in samples from other body parts such as arms and thighs (Antonsson *et al.*, 2000). However, there is limited data on whether these infections persist in the skin.

In this study, our aims were to explore whether HPV infections of healthy skin are persistently present after a lengthy time span and to explore whether healthy subjects and RTRs have different degrees of viral persistence.

RESULTS

Overall, 71 different HPV types or putative types were detected (36 *gamma*-papillomaviruses, 34 *beta*-papillomaviruses, and one *lambda* papillomavirus) (Table 1). The most commonly detected viruses were HPV20, HPV12, and FA1.1 (detected in 11, eight, and seven samples, respectively) (Table 1). Forty-two viruses were each detected only in a single sample (Table 1).

Among the healthy subjects, the prevalences of HPV were 69% (29/42) in the samples from 1998/1999 and 71% (30/42) in the follow-up samples from 2005. Of the 29 healthy individuals (17 females and 12 males) that were positive for HPV in their first sample, 48% (14/29) were positive for the same HPV type/putative type also in the second sample.

Among the RTRs, 71% (15/21) were positive for HPV in their first sample, and 90% (19/21) were positive at follow-up. Among the 15 RTRs (seven women and eight men) positive for HPV in the first sample, 33% (5/15) had a persistent infection (Table 1).

Overall, 70% (44/63) of the subjects were positive for HPV DNA in their first sample and a persistent HPV infection was

Table 1. HPV types detected on healthy skin among healthy individuals and renal transplant recipients with a persistent HPV infection (a) or with non-persistent infections (b)

(a) Subjects with persistent infection

Age at first sample (years)	Gender	First sample (1998/1999) HPV type/putative type	Genus	Second sample (2005) HPV type/putative type	Genus
36	F	**FA36**	β	**FA36**	β
33	F	**FA23.1**	β	**FA23.1**	β
40	M	**HPV21**	β	**HPV21**	β
33	F	**FA35**	γ	**FA35**	γ
71	F	**FA14**	β	**FA14**	β
47	F	**HPV80**, HPV12	β, β	**HPV80**	β
74	F	**HPV20**, HPV17	β, β	**HPV20**	β
59	F	**HPV20**, FA26.2	β, β	**HPV20**, FA149	β, β
51	F	**HPV12**	β	**HPV12**, FA67, FAIMVS14	β, γ, β
56	F	**HPV20**, FA44	β, γ	**HPV20**, HPV14D, HPV19	β, β, β
49	M	**FA148**, FA51	γ, β	**FA148**	γ
79	F	**HPV14D**, FA26.2	β, β	**HPV14D**	β
38	M	**HPV20**, FA116	β, β	**HPV20**, FA23.1	β, β
47	F	**FA130**, FA31	β, γ	**FA130**, FA20.2	β, γ
61[1]	M	**FA14**	β	**FA14**	β
55[1]	M	**FA122.3**, HPV5b	β, β	**FA122.3**, HPV12, FA158	β, β, β
53[1]	M	**HPV8**, FA68	β, γ	**HPV8**	β
47[1]	F	**FA89**, FA115	γ, γ	**FA89**, FA81, FA7	γ, γ, β
60[1]	F	**FA134**, HPV47	γ, β	**FA134**, HPV80	γ, β

(b) Subjects with non-persistent infection

	HPV type or putative type
First sample (1998/1999)	Genus: *beta*-papillomavirus: HPV8, HPV19, HPV20[2], HPV21[2], HPV24[2], HPV76, FA14, FA25, FA26.2, FA37, FA51.2, FA70, FA119 Genus: *gamma*-papillomavirus: HPV4, HPV50, FA1.1[3], FA13, FA20.3, FA43, FA67, FA79[2], FA145, FA153, FA154, FA155, FA157 Genus: *lambda*-papillomavirus: FA38
Second sample (2005)	Genus: *beta*-papillomavirus: HPV5, HPV8[2], HPV12[4], HPV14D, HPV19[2], HPV20, HPV38b[FA125], HPV47, HPV76, FA18, FA26.2, FA37, FA39, vs92-1 Genus: *gamma*-papillomavirus: HPV50, FA1.1[2], FA1.2, FA6.2[2], FA11, FA30, FA32, FA68, FA81, FA87, FA91, FA106, FA150, FA151, FA152, FA155, FA156, FAIMVS15.1[2]

F, female; HPV, human papillomavirus; M, male.
Persistent HPV types are shown in bold.
[1]Renal transplant recipients.
[2]Detected in two subjects.
[3]Detected in five subjects.
[4]Detected in four subjects.

detected in 43% (19/44) of them (Table 1). Most persistent infections (15/19 (79%)) were *beta*-papillomavirus infections, with HPV20 being the single most common type-specific persistent infection (in 4/19 (21%) of cases) (Table 1). Eighteen subjects were positive for HPV in both samples, but with different types. Seven subjects were HPV positive at enrolment, but negative at follow-up. Twelve subjects were negative for HPV at enrolment but positive at follow-up (Table 1), and seven subjects were negative for HPV at both occasions.

None of the covariates were significantly associated with persistence (Table 2), but HPV isolates within the genus *beta*-papillomavirus tended to persist more than HPV isolates of the genus *gamma*-papillomavirus, 38% (15/39) and 15% (4/26), respectively (Table 1). This tendency is not likely to be attributable to possible different sensitivities for different genera in the PCR detection system, as a large number of *gamma*-papillomavirus isolates were detected (Table 1).

DISCUSSION

We report that cutaneous HPVs commonly persist for many years on healthy skin, with about half (48%) of the healthy individuals being positive for the same HPV type 6 years later. Considering the immunosuppression of the RTRs and the fact that these patients commonly develop skin lesions, for example, squamous-cell carcinoma, with detectable HPV DNA (Berkhout *et al.*, 1995, 2000; de Villiers *et al.*, 1997), it was surprising that persistent HPV infections were not more common in this group than in healthy individuals.

Lack of correlation between presence of warts and HPV persistence may also seem surprising. However, none of the patients had warts on the forehead, where the sample for HPV testing was taken.

The rather high rates of persistence found are still bound to be underestimates. HPV persistence in low copy numbers could have been missed using our general primer PCR system. Also, only three clones per sample were sequenced. This would tend to detect the most abundant infections, again leaving the possibility open that HPV persistence in low copy numbers could have escaped detection.

Previous reports have indicated that the viral DNA contained in virions seems to be more protected against DNA degradation than the human cellular DNA on the skin surface (Antonsson *et al.*, 2003; Hazard *et al.*, 2006), which could have contributed to the high HPV prevalences found also in quite old samples.

Previous studies of cutaneous HPV persistence had a mean follow-up of only 3.2 years and studied only RTRs, not healthy subjects (Berkhout *et al.*, 2000). We report that a high proportion of cutaneous HPV infections are persistently present after even longer time spans and that neither immunosuppression, nor any other investigated covariate, was significantly associated with persistence.

Presence of HPV DNA on the skin could reflect infection, but may also conceivably reflect skin surface contamination of virions shed from productively infected skin at other body sites or even from other subjects. Although repeated contamination with exogenous HPV DNA of the same type cannot be excluded as a reason for persistence, the simplest explanation for the very long-lasting persistence found is that there is an HPV infection at the sampled site and that a noteworthy proportion of cutaneous HPV infections tend to persist for many years.

In conclusion, the knowledge that viral persistence is common in the natural history of cutaneous HPV infections is of interest for understanding the biology of these viruses and may be helpful in the continuing elucidation of their possible role in human skin disorders.

Table 2. Multivariate analysis of parameters affecting a persistent infection of any HPV type

	Subjects with persistence/ total (%)	Crude OR (95% CI)	Adjusted[1] OR (95% CI)
All subjects	19/44 (43)		
Healthy	14/29 (48.3)	1.0 (referent)	1.0 (referent)
RTR	5/15 (33.3)	0.54 (0.12–2.30)	0.84 (0.12–5.74)
Sex			
Female	13/24 (54.2)	1.0 (referent)	1.0 (referent)
Male	6/20 (30.0)	0.37 (0.09–1.48)	0.23 (0.03–1.16)
Age (years)			
<44	5/14 (35.7)	1.0 (referent)	1.0 (referent)
45–54	6/15 (40.0)	1.19 (0.21–7.03)	0.56 (0.04–5.53)
>55	8/15 (53.3)	2.01 (0.37–11.86)	0.94 (0.05–17.9)
Warts at any site			
No	15/33 (45.5)	1.0 (referent)	1.0 (referent)
Yes	4/11 (36.4)	0.69 (0.12–3.37)	0.79 (0.09–6.97)
Previous skin cancer			
No	18/42 (42.9)	1.0 (referent)	1.0 (referent)
Yes	1/2 (50.0)	1.33 (0.02–109)	2.98 (0.009–1,015)
Any previous cancer			
No	17/39 (43.6)	1.0 (referent)	1.0 (referent)
Yes	2/5 (40.0)	0.87 (0.06–8.47)	0.31 (0.004–8.9)
Any allergy			
No	14/30 (46.7)	1.0 (referent)	1.0 (referent)
Yes	5/14 (35.7)	0.64 (0.13–2.77)	0.42 (0.05–2.84)
Eczema			
No	14/35 (40.0)	1.0 (referent)	1.0 (referent)
Yes	5/9 (55.6)	1.85 (0.33–11.1)	2.43 (0.35–20.3)
Genus of HPV			
gamma	2/12 (16.7)	1.0 (referent)	1.0 (referent)
beta	12/20 (60.0)	7.02 (1.08–82.7)	5.94 (0.64–105)
gamma+beta	5/12 (41.7)	3.38 (0.40–45.4)	4.37 (0.33–97.5)

CI, confidence interval; HPV, human papillomavirus; OR, odds ratio; RTR, renal transplant recipient.
Only patients positive for HPV in the first sample were included in the analysis. Questions answered at collection of the first sample.
[1]Adjusted for all parameters in the table.

MATERIALS AND METHODS

Forehead swab samples from RTRs attending the outpatient clinic of nephrology of Malmö University Hospital, Sweden, and sex- and age-matched healthy controls were collected from 1998 to 1999 (Antonsson *et al.*, 2000). In 2005, the subjects who were not

deceased and had not emigrated were invited again. New forehead swab samples could be collected from 21/31 of the invited RTRs (10 females and 11 males, mean age 50 years at first sampling) and from 42/61 of the invited healthy subjects (22 females and 20 males, mean age 47 years). Mean follow-up time was 6.3 years (range: 5.0–7.0 years). Lund University Ethical Committee approved all described studies. The study was conducted according to the Declaration of Helsinki Principles, and all patients gave informed consent.

All subjects answered a questionnaire about presence/absence of warts, eczema, allergy, and history of any cancer at both sampling occasions. To avoid testing samples in any particular order, all samples (collected 1998/1999 and 2005) and 12 internal controls (NaCl) were given new randomized sample numbers and all analyses were performed blinded to any information about the samples.

HPV was detected by general primer PCR using FA primers (Forslund *et al.*, 1999), followed by cloning and sequencing of three clones per sample.

Fifteen new HPV isolates (less than 98% identity to any known HPV sequence) were detected in this study and submitted to GenBank with the following accession numbers: FA145 (DQ418463), FA148 (DQ418464), FA149 (DQ418465), FA150 (DQ418466), FA151 (DQ418467), FA152 (DQ418468), FA153 (DQ418469), FA154 (DQ418470), FA155 (DQ418471), FA156 (DQ418472), FA157 (DQ418473), FA158 (DQ418474), FA159 (DQ418475), FA160 (DQ418476), and FA162 (DQ418477).

Multivariate analysis was performed using LogXact version 6 (Cytel, Cambridge, MA) with RTR/healthy, gender, age, presence/absence of warts, history of cancer/skin cancer, allergy, eczema, and genus of HPV included in the model. Questionnaire data from the baseline visit were used.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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