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ORIGINAL ARTICLE

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Association of human papillomavirus infections with cutaneous tumors in immunosuppressed patients

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Abstract Besides immunosuppression and UV radiation, human papillomavirus (HPV) infection was also suggested to be involved in the development of non-melanoma skin cancer, the most common malignancy after transplantation. In this study we used a comprehensive PCR assay to analyze the prevalence of individual HPV types in different skin lesions from transplant and non-transplant patients. HPV DNA was detected more frequently in squamous cell carcinomas (SCCs) of transplant recipients (75%) than the same lesion was in non-immunosuppressed patients (47%). Similar HPV prevalences were found in cutaneous warts (91% vs 94%), pre-malignant skin tumors (38% vs 36%), and normal skin specimens (17% vs 16%) of both patient populations. Overall, more than 40 different HPV types were identified. HPV types 5 and 8 were found more frequently in SCCs (26%) than in pre-cancerous (5%) or benign lesions (1%). All HPV 5- and HPV 8-positive SCCs were from immunosuppressed patients, indicating that infection with HPV 5 and HPV 8 may present an increased risk of SCC development in these patients.

Keywords Immunosuppression · Skin cancer · Cutaneous warts · Carcinogenesis · Papillomavirus

Introduction

Skin cancer is the most common malignancy diagnosed in organ-transplant recipients, representing 40% of all malignancies after transplantation [24]. Up to 40% of renal-transplant recipients develop non-melanoma skin cancer within 15 years of transplantation [4]. These cancers predominantly represent squamous cell carcinomas (SCCs) and, to a lesser extent, basal cell carcinomas (BCCs).

Viral warts (verrucae vulgares) are even more frequent after transplantation, occurring in over 90% of transplant recipients. Clinical and histological analysis has shown the progression of viral warts to dysplastic lesions and invasive SCCs in immunosuppressed patients [5]. Thus, viral warts that are usually considered as being benign lesions in immunocompetent patients are of different prognostic importance in immunosuppressed patients.

While verruca vulgaris lesions are known to be induced by human papillomavirus (HPV), the role of these viruses for the development of non-melanoma skin cancers is not clear. UV radiation is considered to be the most important pathogenic factor for cutaneous SCCs. In organ-transplant recipients, immunosuppression represents another important risk factor. The possible involvement of HPV in skin carcinogenesis is supported by the detection and persistence of HPV DNA in a large number of skin tumors from transplanted patients [3, 8, 9, 18].

With respect to management of skin care of transplant recipients, the association of HPV infection with

skin cancer development would provide a basis for both preventive (vaccination) and therapeutic (anti-viral/anti-tumor treatment) measures. Especially with respect to vaccination, it is important that we identify the most relevant HPV types associated with skin cancer.

In this study, we analyzed the prevalence of HPV types in different benign, pre-malignant, and malignant skin tumors as well as in normal skin from transplant and non-transplant patients. In order to identify all HPV types present in the lesions, we designed a comprehensive PCR system to detect all characterized genital and cutaneous HPV types.

Patients and methods

Patients

The number of specimens analyzed for each skin lesion is shown in Table 1. Patient data relating to age, gender, and history of immunosuppressive treatment are also given. Skin biopsies of different cutaneous lesions and normal skin were obtained from patients treated at the Department of Dermatology, University of Kiel, between September 1996 and June 2001. In addition, 20 verruca vulgaris specimens were obtained from 19 patients from the Department of Urology, Grosshadern, University of Munich.

Plasmids

To determine the detection limits of different primer pairs for individual HPV types, we used recombinant plasmids, harboring 26 different HPV genomes, as HPV reference sequences. Plasmids containing HPV 6, 11, 16, and 18 were provided by U. Reischl (University of Regensburg, Germany). Plasmids harboring the genomes of the HPV 2, 3, 4, 5, 8, 19, and 20 sequences were a kind gift from H. Pfister and P. Fuchs (University of Cologne, Germany). Plasmids containing HPV 1, 9, 10, 12, 14, 15, 17, 21, 22, 23, 28, 29, 36, 49, and 50 were kindly provided by G. Orth (Institute Pasteur, Paris, France).

DNA isolation from snap-frozen tissue specimens

Punch biopsy specimens 4 mm in diameter were obtained, with sterile equipment being used in each case. We then cut the biopsies with single-use scalpels to prevent carry-over contamination in subsequent DNA isolation and PCR amplification. DNA was isolated from roughly half of the tissue specimens by protein digestion with proteinase K, phenolization, and ethanol precipitation [19]. The nucleic acids were recovered by centrifugation and finally dissolved in 50 µl TE-buffer (10 mmol/l Tris/HCl pH 7.5, 1 mmol/l EDTA). For PCR experiments, 5 µl of this DNA solution containing approximately 1 µg of DNA were used.

HPV DNA detection

In order to include the whole spectrum of HPV types in the amplification procedure, we employed several degenerate primer pairs encompassing different groups of HPV types. Based on the phylogenetic relationship of HPV types, five sets of consensus primers, all derived from the L1 gene, were selected to detect mucosal HPV types (homology group A), epidermodysplasia verruciformis (EV)-associated HPV types (group B1) as well as cutaneous HPV types of groups A2, B2, and E1 [6]. The sequences of primers are shown in Table 2.

Amplification with primers MY9/MY11 and MYN9/MYN10 (nested PCR primer) was shown to detect a broad spectrum of mucosal HPVs [13, 15]. The PCR reactions were carried out as described in these studies.

By amplification of serial dilutions of recombinant plasmids containing HPV 6, 11, 16, and 18, the sensitivity for the MY-nested PCR was 10–100 viral genome copies. Some mucosal HPV types such as HPV 32 or HPV 57 were not amplified by the MYN-primers. These HPV types were detected only by MY-primers in a standard PCR. The sensitivity of these primers to detect mucosal HPV types ranges between 1 fg (HPV 16, 18) and 100 fg (HPV 32, 72), corresponding to 100–10,000 genome copies [10].

We used primers CP65/CP70 and CP66/CP69 in a nested PCR to detect the complete set of EV-associated HPV types, according to the protocol described by Berkhout et al. [2]. Differently from this, we used an Mg^{2+} concentration of 2.5 mmol/l, and the nested PCR consisted of 25 instead of 30 cycles of amplification. Under these conditions, a minimum of 100–500 viral genomes of HPV types 5 and 8 can be detected, which corresponds to the originally described sensitivity range of 1–10 fg [2].

Some of the wart-associated HPV types, such as HPV 1, 2, 7, 10, or 27, can be detected by the MY- or CP-nested PCRs when recombinant HPV plasmids are used. Other wart HPVs were not amplified efficiently by these PCR assays. Therefore, additional consensus primers for HPV 4, 48, 50, 60, and 65 (C4F/C4R), HPV 3, 10, 28, 29, and 77 (CN3F/CN3R), as well as HPV 1, 41, and 63 (CN1F/CN1R) described by Harwood et al. [10], were applied. The amplifications were performed with 45 cycles of the following temperature profile: 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C, with the same reaction mixture as described for the

Table 1 Clinical and anamnestic data of patients with different skin lesions

Skin lesion	Number		Age in years	Gender		Immunosuppression		
	Specimens	Patients	Mean (range)	Male	Female	No	Tx[a]	Other[b]
Verruca vulgaris	81	70	43 (3–72)	58	12	45	19	6
Bowen's disease[c]	36	26	67 (43–85)	18	8	18	7	1
Actinic keratosis	28	20	67 (51–91)	14	6	8	12	0
Cutaneous SCC	42	31	65 (23–91)	27	4	14	11	6
Normal skin[d]	63	63	64 (20–89)	25	38	56	6	1

[a]Immunosuppressive treatment after organ transplantation ($n=53$)
[b]HIVinfection ($n=1$),epidermodysplasia verruciformis ($n=2$), steroid treatment due to immunopathological diseases ($n=3$), psoriasis patients treated with psoralen/UVA ($n=2$), cytotoxic treatment of patients with other cancers ($n=6$)
[c]Only casesof extra-genital localization
[d]Thirteen of 63normal skin specimens were from patients with adjacent non-melanoma skincancers

Table 2 Sequences of oligonucleotides used as probes and primers and length ofspecific PCR products (Y = C/T, M = C/A, W = A/T, K = G/T, R = A/G, S = C/G, V = G/A/C,H = A/T/C)

Primer	Sequence (5-3)	Length (bp)
MY09	CGT CCM ARR GGA WAC TGA TC	
MY11	GCM CAG GGW CAT AAY AAT GG	450
MYN9	GTT ACT GTK GTW GAY ACY AC	
MYN10	TCY TTT ARA TYA ACM TYC CA	370
CP65	CAR GGT CAY AAY AAT GGY AT	
CP70	AAY TTT CGT CCY ARA GRA WAT TGR TC	460
CP66	AAT CAR MTG TTT RTT ACW GT	
CP69	GWT AGA TCW ACA TYC CAR AA	380
CN1F	AAT ARG TTW GAT GAT GCW GAA	
CN1R	AKR TAR TCW GGA TAT TTG CA	320
CN3F	AAC TCT AAY ATW GCA CAT G	
CN3R	CAV GTR CSY TGG CAA ATA TC	270
C4F	GGA GAT ACA GAA AAT CCT	
C4R	SHA TCT CCA TAG ATA TCT TT	330
β-Globin 1	CAA CTT CAT CCA CGT TCA CC	
β-Globin 2	GAA GAG CCA AGG ACA GGT AC	262
Probe		
GOP-Y	GOP-Y1	GAC ACC ACA CGK AGY ACY AAT AT
	GOP-Y2	GAC AAY ACR CGW AAC ACY AAT TT
	GOP-Y3	GAY AAC ACW MGR AAY ACW AAT TT
	GOP-Y4	GAT AAT ACM MGR AAT ACM AAC TT
	GOP-Y5	GAT AAY ACC AGA RRM AYC AGC WT
	GOP-Y6	GAY ACT ACC CKC AGT ACC AAY MT
	GOP-Y7	GAT AAY ACT CRT ART AYR AAT TT
GOP-Z	GOP-Z1	GAY ACT ACM CGY AGT ACY AAC
	GOP-Z2	GAY ACY ACA CGC AGY ACC AAY
	GOP-Z3	GAY ACT ACY CGC AGT ACY AAT
	GOP-Z4	GAY ACM ACT CGT AGT YAC WAT
	GOP-Z5	GAY ACT ACY AGA AGY ACT AAY

MY-PCR [15]. By analyzing serial dilutions of recombinant plasmids containing HPV 1, HPV 3, and HPV 4, we detected viral DNA at a level of 100–500 copies, using primers CN1F/CN1R, CN3F/CN3R, and C4F/C4R, respectively.

HPV typing

Typing of amplified HPV DNA was performed by RFLP analysis and subsequent hybridization with a generic oligonucleotide probe, as has been described previously [14, 15]. The reliability of this HPV typing system has been confirmed by an inter-laboratory comparison with HPV detection and HPV typing [16].

Two mixtures of oligonucleotide probes (GOP-Z and GOP-Y) were used for hybridization of restriction enzyme fragments. GOP-Z consisted of five degenerate oligonucleotides and was designed to hybridize with PCR products of mucosal HPV types (>90% homology to all known mucosal HPVs). GOP-Y represents a mixture of seven degenerate oligonucleotides with at least 90% homology to all known cutaneous HPVs (except HPV type 41). GOP-Y and GOP-Z were derived from the same HPV L1 region located immediately downstream from, and partly overlapping, the inner upstream primers and thus could be used for hybridization analysis of both standard and nested PCR products. The sequences of all probes are given in Table 2.

RFLP was used for HPV typing of PCR products obtained with MY- and CP-primers. In cases of inconclusive patterns that could not be related to any known HPV type, we used direct sequencing of the products to identify the HPV type(s). PCR products obtained with primer pairs CN1F/CN1R, CN3F/CN3R, and C4F/C4R were analyzed by direct sequencing. Prior to sequencing, the PCR products were separated from non-incorporated nucleotides and primers by spin column chromatography. We sequenced the PCR products using fluorescence-labeled di-deoxynucleotides (DNA Sequencing Kit, Perkin-Elmer/Applied Biosystems). Analyses of the sequencing products were performed on an ABI Prism 377 DNA sequencer. The DNA sequences obtained from the PCR products were compared with the available HPV sequences in the EMBL and Genbank databases using the BLAST search program [1].

Control PCRs

To control integrity of DNA isolated from tissue specimens and/or presence of PCR-inhibitory substances in these preparations, we performed β-globin DNA PCR in each specimen. To prevent and monitor contamination of specimens with viral DNA, we strictly followed standard precautions concerning spatial separation of pre- and post-PCR steps, aliquotation of reagents, and single use of scalpels for processing tissue specimens [12]. A set of negative controls (water and porcine liver) was included during all steps of the DNA isolation and amplification procedure.

Results

Comparison of the frequency of HPV in biopsies from different skin lesions

To determine the prevalences of HPV DNA in different skin lesions, we considered only specimens with successful β-globin DNA amplification. HPV DNA was

detected by any one of the PCR assays in 76 of 81 (94%) verruca vulgaris lesions obtained from 66 of 70 (94%) patients. Among pre-malignant lesions HPV was detected in 14 of 36 (39%) extra-genital Bowen's disease lesions from ten of 26 (38%) patients and in 11 of 28 (39%) actinic keratosis specimens from ten of 20 (50%) patients. In cutaneous SCCs HPV DNA was detected in 29 of 42 (69%) specimens obtained from 19 of 31 (61%) patients, while HPV DNA was detected in only 16% of the normal skin tissues.

Within the group of normal skin tissues, 13 specimens were taken from skin areas adjacent to non-melanoma skin cancers also analyzed for HPV DNA. In these biopsy pairs of lesional and non-lesional skin, HPV DNA was also detected more frequently in tumor biopsies: six of 13 tumor samples (46%) in contrast to two of 13 normal samples (15%) were HPV DNA positive. The two HPV-positive normal skin specimens were from patients with HPV-positive skin cancers, containing the same HPV type in the lesional specimen.

Frequency of HPV DNA in skin tumors from immunosuppressed and non-immunosuppressed patients

HPV prevalence was also compared in skin tumors from immunosuppressed transplant recipients and non-immunosuppressed patients. As shown in Fig. 1, HPV DNA was detected more frequently in SCCs from transplant patients (75%) than in SCCs from non-immunosuppressed patients (47%). This difference did not reach statistical significance ($P = 0.20$), maybe due to the small number of cases analyzed. When other patients under immunosuppressive treatment due to immunopathological (systemic lupus erythematosus and psoriasis) or neoplastic diseases (mycosis fungoides and colon cancer) were also considered, the rate of HPV-positive SCC specimens increased to 81%. In pre-malignant lesions (Bowen's disease, actinic keratosis), viral warts (verruca vulgaris), and normal skin, the rate of HPV DNA-positive specimens was similar in transplant recipients and non-immunosuppressed patients (Fig. 1).

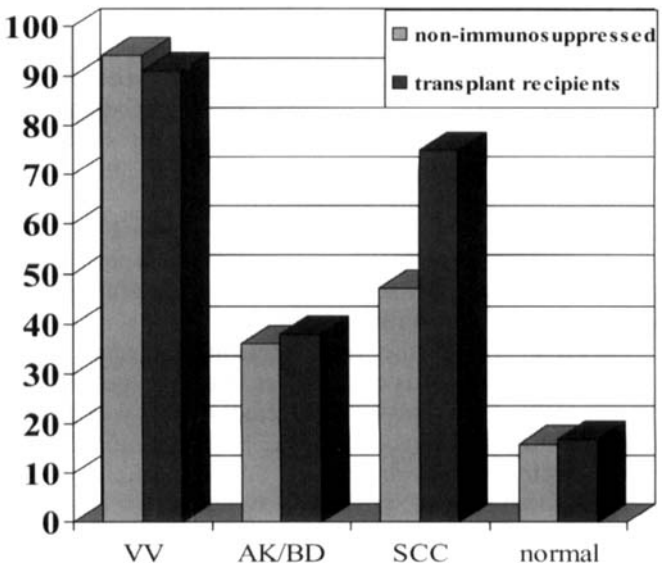

Fig. 1 Frequency of human papillomavirus DNA in skin tumors from non-immunosuppressed patients and immunosuppressed transplant recipients (*VV* verruca vulgaris, *AK* actinic keratosis, *BD* Bowen's disease). Number of specimens analyzed were, VV: non-immunosuppressed $n = 51$, transplant recipients $n = 22$; AK and BD: non-immunosuppressed $n = 36$, transplant recipients $n = 26$; cutaneous SCC: non-immunosuppressed $n = 15$, transplant recipients $n = 16$; normal skin: non-immunosuppressed $n = 56$, transplant recipients $n = 6$

In a number of specimens, more than one HPV type was detected by the PCR assays. These biopsies with multiple HPV types were obtained predominantly from immunosuppressed transplant recipients. As shown in Table 3, 52% of all HPV-positive skin tumor specimens from immunosuppressed patients contained more than one HPV type, in contrast to 19% of the skin tumors from non-immunosuppressed patients.

Spectrum of HPV types in skin tumors from immunosuppressed and non-immunosuppressed patients

Overall, 44 different HPV types, including both cutaneous and genital HPVs, were detected in the skin biopsies analyzed. In Table 4 the frequency of all iden-

Table 3 Proportion of multiple HPV types in HPV-positive skin tumors ofimmunosuppressed and non-immunosuppressed patients (*n* HPV-positive specimens)

Skin lesion	Non-immunosuppressed patients		Immunosuppressed patients			
			Transplant recipients		All	
	n	multiple HPV	*n*	multiple HPV	*n*	multiple HPV
Verruca vulgaris	48	10 (21%)	20	13 (65%)	28	17 (61%)
Pre-malignant lesions	13	1 (8%)	10	5 (50%)	12	5 (42%)
Cutaneous SCC	7	2 (28%)	12	4 (33%)	22	7 (32%)
All	68	13 (19%)	42	22 (52%)	62	29 (47%)

tified HPV types in different skin lesions from immunosuppressed and non-immunosuppressed patients is summarized. Due to the plurality of HPV types, it was difficult to identify any individual HPVs predominating in particular skin tumors of immunosuppressed and non-immunosuppressed patients. However, the distribution of some HPVs is remarkable:

(1) In verruca vulgaris lesions, HPV types 1, 2, 3, 4, 10, 27, and 57 were the dominating HPV types. These viruses were not detected in any of the pre-malignant and malignant skin tumors analyzed.

(2) HPV 57 was the most frequently detected HPV type ($n = 13$). The virus occurred as two subtypes (HPV 57 and HPV 57b) that were detected in viral warts with similar overall prevalences, but with reverse frequencies in lesions from immunosuppressed and non-immunosuppressed patients. While HPV 57 was detected in 17% and 2% of viral warts in patients with and without immunosuppressive treatment, respectively, the opposite prevalences were found for HPV 57b (3% vs 12%) (Table 4).

(3) HPV types 5 and 8 were detected in a total of 15 specimens that, for the most part, represented SCCs (73%). All of these HPV 5- and HPV 8-positive SCC specimens were obtained from immunosuppressed patients. Neither virus was detected in SCCs in patients without immunosuppressive treatment (Table 4).

All other identified HPV types were present only in small numbers of specimens. Thus, no additional individual HPV types could be characterized that may be preferentially associated with any of the skin tumors. Therefore, HPV types were clustered into groups according to their DNA homology. As expected, the classical wart-associated viruses of homology groups A2, A4, B2, and E1 predominated in verruca vulgaris lesions, but were only rarely detected in pre-malignant and malignant skin tumors. The mucocutaneous HPV types 7, 40, and 72 were also detected almost exclusively in viral warts.

The rate of specimens containing EV-HPVs of group B1a was higher in SCCs than in pre-malignant lesions and verrucae vulgares, related to the distribution of HPV 5 and HPV 8 described above. The prevalence of other HPVs of this homology group did not differ significantly among the different skin lesions. Viruses of homology group B1b and genital HPVs were detected with similar frequencies in the skin tumors analyzed.

When skin tumors from immunosuppressed and non-immunosuppressed patients were compared, both genital HPVs and EV-HPVs of group B1a (mainly HPV 5 and HPV 8) were associated more frequently with tumors from immunosuppressed patients (Table 4).

Discussion

A comprehensive PCR assay was used to analyze the prevalence of different HPV types in cutaneous lesions. The suitability of this assay for detection of a broad range of HPV types in cutaneous lesions was supported by the detection of HPV in 94% of viral warts. Similar findings were recently reported by Harwood et al. [10], who were able to detect HPV DNA in all 51 analyzed wart specimens of renal transplant recipients by using an even more extensive nested PCR assay. In previous studies, up to 40% of verruca vulgaris lesions were HPV-DNA-negative [21, 22, 23]. The sensitivity of HPV detection is clearly improved by our PCR system and should be useful to reliably detect HPV in other cutaneous lesions with high sensitivity.

We detected HPV DNA with increasing frequencies in normal skin (16%), pre-malignant lesions (39%), and cutaneous SCCs (69%), indicating an association of HPV infection with SCC development. A rate of HPV DNA detection higher in SCCs than in actinic keratosis was also described by Berkhout et al. [3]. In other studies, however, the prevalence of HPV in SCC biopsies was not higher than in pre-cancerous lesions [7, 11].

Among cutaneous SCCs, HPV DNA was detected more frequently in tumor specimens from immunosuppressed patients. If only transplant recipients are considered, 12 of 16 (75%) SCC specimens obtained from eight of 11 (73%) patients were HPV-positive in contrast to seven of 15 (47%) SCCs from six of 14 (43%) non-immunosuppressed patients. However, the numbers of patients and specimens were too low for a statistically significant difference to be found. The rate of HPV-positive SCCs increased to 81% when other non-transplanted patients receiving immunosuppressive treatment for other reasons (cytostatic treatment due to other cancers or immunosuppressive treatment of psoriasis or autoimmune diseases) were also considered. Although the degree of immunosuppression is different from that in transplant recipients, the immune system of these patients is also impaired.

Our results confirm the higher prevalence of HPV DNA in non-melanoma skin cancers of patients under immunosuppression reported by Harwood et al. [11]. In contrast to that paper we did not find a higher rate of HPV-positive pre-malignant lesions in immunosuppressed compared with non-immunosuppressed patients. The discrepancy may be related to differences in mean age, immunosuppressive treatment, and stages of the disease in both patient populations. It should be considered that older patients not treated with immunosuppressive drugs may not always be truly immunocompetent, due to age-related impairment of immune functions.

The high prevalence of HPV DNA in tumor biopsies as well as the persistence of HPV infections in benign, pre-malignant, and malignant skin lesions of renal transplant recipients [3] suggests an association of HPV with non-melanoma skin cancer, especially in transplant patients. Although the exact role of HPV in the devel-

Table 4 Frequency of individual HPV types in skin lesions of immunosuppressed (*IS*) and non-immunosuppressed (*NIS*) patients (*NS* normalskin, *VV* verruca vulgaris,*AK* actinic keratosis, *BD* extra-genital Bowen's disease, *NID* HPV type not identified)

Homology group	HPV type	Prevalence in skin biopsies									
		NS		VV		AK/BD		SCC		All tumors	
		IS $n=6$	NIS $n=57$	IS $n=30$	NIS $n=51$	IS $n=28$	NIS $n=36$	IS $n=27$	NIS $n=15$	IS $n=85$	NIS $n=102$
E1	1	0	0	3	4	0	0	0	0	3	4
	41	0	0	1	0	0	0	0	0	1	0
	All	0	0	4	4	0	0	0	0	4 (5%)	4 (4%)
B2	4	0	0	4	2	0	0	0	0	4	2
	48	0	0	0	1	0	0	0	0	0	1
	65/vs205–1	0	0	2	0	0	0	2	0	4	0
	All	0	0	6	3	0	0	2	0	8 (9%)	3 (3%)
A2	3	0	0	2	3	0	0	0	0	2	3
	10	0	0	1	4	0	0	0	0	1	4
	28	0	0	2	0	0	0	0	0	2	0
	29	0	0	1	0	0	0	0	0	1	0
	All	0	0	6	7	0	0	0	0	6 (7%)	7 (7%)
A4	2	0	0	1	4	0	0	0	0	1	4
	27	0	0	2	5	0	0	0	0	2	5
	57	0	0	5	1	0	0	0	0	5	1
	57b	0	0	1	6	0	0	0	0	1	6
	All	0	0	9	16	0	0	0	0	9 (11%)	16 (16%)
B1a	5	0	0	1	0	0	1	6	0	7	1
	8	0	0	0	0	1	1	5	0	6	1
	14	0	0	0	1	2	0	1	0	3	1
	20	0	0	2	0	2	0	0	1	4	1
	21	0	0	0	1	0	0	0	0	0	1
	25	0	1	0	1	0	0	0	1	0	2
	36	0	0	0	0	1	1	0	3	1	4
	RTRX5	0	0	1	2	0	0	0	0	1	2
	All	0	1	4	5	6	3	12	5	22 (26%)	13 (13%)
B1b	9	0	0	1	1	0	0	0	0	1	1
	15	0	0	0	0	1	2	3	0	4	2
	17	0	0	0	2	0	1	0	0	0	3
	22	0	0	1	0	1	0	1	0	3	0
	23/23b	0	0	1	0	0	0	0	1	1	1
	37/37v	0	0	0	1	0	0	0	0	0	1
	38	0	0	1	0	0	0	0	1	1	1
	HPVX10b	0	0	0	1	0	0	0	0	0	1
	DL473	0	0	0	0	1	1	0	0	1	1
	HF8	0	1	0	0	0	0	1	0	1	0
	All	0	1	4	5	3	4	5	2	12 (14%)	11 (11%)
NID		0	3	5	6	0	1	0	0	5	10
All EV		0	5	13	16	9	8	17	7	39 (46%)	31 (30%)
Mucocutaneous HBV	7	0	0	0	3	0	0	0	0	0	3
	40	0	0	0	0	1	0	0	0	1	0
	72	0	0	0	1	0	0	0	0	0	1
	All	0	0	0	4	1	0	0	0	1 (1%)	3 (3%)
Genital HPV	6	1	1	1	2	1	1	1	1	3	4
	11	0	0	1	1	0	0	3	0	4	1
	16	3	0	1	1	2	3	3	0	6	4
	31	0	0	0	0	2	0	0	0	2	0
	33	0	0	0	0	0	0	0	1	0	1
	35	0	0	0	0	1	0	0	0	1	0
	58	0	0	0	0	0	0	1	0	1	0
	62	0	0	0	1	0	0	0	0	0	1
	70	0	0	1	0	0	0	1	0	2	0
	73	0	0	1	0	0	0	0	0	1	0
	NID	0	0	1	1	0	0	0	0	1	1
	All	4	1	5	5	6	4	9	2	21 (25%)	12 (12%)

opment of cutaneous malignancies under immunosuppression is at present unclear, viral infection provides an important basis for preventive and therapeutic strategies against skin tumors. The development of virus-like particles (VLPs) as anticancer vaccines has become an interesting approach to the prevention of HPV-induced cancer [20]. To establish a vaccination strategy against skin tumors, it is important that we identify the most common HPV types present in viral warts and those associated with SCC development.

Our preliminary data on the frequency of individual HPV types in different skin lesions indicate that HPV types 5 and 8 may present an increased risk for SCC development in immunosuppressed patients, since these HPV types were found more frequently in SCC lesions than in the pre-cancerous or benign lesions of these patients. HPV 5 and 8 were also shown to predominate in SCCs of patients with EV, a rare inherited disease associated with a deficiency in cellular immunity [17]. Thus, the combination of impaired cellular immunity and infection with HPV types 5 or 8 seems to present an important risk for the development of SCCs in both patients with EV and patients under immunosuppressive treatment. Compared with SCCs of EV patients, associated with HPV 5 or 8 in more than 90%, the rate of HPV 5 and 8 in SCCs from immunosuppressed patients in our study is smaller (26%), indicating other HPV types that may also be involved. Due to the low prevalence, no other particular HPV types preferentially associated with non-melanoma skin cancer were identified in our study. Classical wart-associated HPV types, however, were rarely detected in SCCs and pre-malignant skin tumors, indicating that these viruses were not associated with the development of SCCs.

By comparing the spectrum of HPV types in skin tumors of immunosuppressed and non-immunosuppressed patients, we detected EV-HPV types 5 and 8 of subgroup B1a and genital HPVs more frequently in tumors of immunosuppressed patients. HPV types 5 and 8 were found in 11 of 27 (41%) SCCs in immunosuppressed patients, but in none of 15 SCCs in non-immunosuppressed patients. Among verrucae vulgares, HPV 57 was detected more frequently in lesions in immunosuppressed patients, while HPV 57b and, to a lesser extent, HPV types 2, 10, and 27 were more frequent in verruca of non-immunosuppressed patients. The higher detection rate of HPV types 5, 8, and 57 in skin lesions of patients under immunosuppressive treatment indicates the importance of cellular immune reactions in the control of these HPV types. It may also point towards immunomodulatory functions of these HPVs that, in addition to immunosuppressive drugs, finally result in a net impairment of cellular immune reactions no longer warranting control of local viral infections.

EV-associated HPV types were found with similar prevalences in cutaneous warts of immunosuppressed and non-immunosuppressed patients (43% vs 29%). These frequencies are different from a recent study by Harwood et al. [10], who reported EV-HPVs in 74% of verruca vulgaris lesions in transplant recipients, but only in one of eight cutaneous warts in immunocompetent patients. These authors used four different nested primer pairs for EV-HPV DNA amplification in order to identify mixed infections with EV-HPVs. The different results in these two studies may, therefore, relate to different EV-HPV PCR assays. On the other hand, eight specimens from immunocompetent patients was a rather low number in the study by Harwood et al. [10]. The rate of genital/mucosal HPVs in cutaneous warts of immunosuppressed patients was similar in each study (25% and 26%), but in both studies no particular genital HPV type was found to predominate in warts of these patients. The detection of genital HPVs in about one-fourth of cutaneous warts indicates that the tissue tropism of genital HPVs might not be as stringent as previously assumed.

Until recently, the management of transplant patients with or without HPV infections was not very different. Some centers for dermatological care of transplant recipients have now begun to monitor patients with cutaneous warts (viral warts) at closer intervals (four times a year) [24]. Based on our preliminary data on HPV 5 and HPV 8 prevalence in SCCs, early treatment of benign and pre-malignant skin lesions associated with these viruses should be considered. These two HPV types would also represent primary candidates for the development of VLP-based vaccines against skin tumors.

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