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E. Mansat-Krzyzanowska
J. Dantal
M. Hourmant
P. Litoux
J.P. Soulillou
B. Dréno

Frequency of mucosal HPV DNA detection (types 6/11, 16/18, 31/35/51) in skin lesions of renal transplant patients

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E. Mansat-Krzyzanowska
P. Litoux · B. Dréno (✉)
Department of Dermatology,
Hôtel-Dieu, Place A. Ricordeau,
F-44035 Nantes Cedex 01,
France
Fax: + 33 2 40 08 31 17

J. Dantal · M. Hourmant · J. P. Soulillou
Department of Clinical Immunology,
Hôtel-Dieu, Place A. Ricordeau,
F-44035 Nantes Cedex 01, France

Abstract Human papillomaviruses (HPV) probably play a role in the development of skin cancer in renal transplant recipients. Since some mucosal HPV are strongly related to cervical cancer, we compared the frequency of HPV DNA detection (mucosal types 6/11, 16/18, and 31/33/51) in skin cancer of renal transplant recipients (21 lesions) with that in normal subjects without immunodeficiency (21 lesions) and studied the frequency of these same HPV in benign lesions of renal transplant recipients (34 lesions) and normal subjects (30 lesions). An in situ hybridization technique employing cold biotin probes was used. HPV DNA was not significantly ($P = 0.095$) more frequent in malignant skin cancer in renal transplant recipients (42.9 %) than in normal subjects (19.04 %), but was significantly more frequent in benign lesions in renal transplant recipients (32.4 %) than in controls (10 %; $P < 0.05$). These results on a limited number of skin lesions do not allow one to confirm the predominant role of mucosal HPV in the development of skin cancer in renal transplant recipients. HPV interaction with other factors related to the immunosuppressive state may play a role.

Key words Papillomavirus, skin cancer, kidney transplantation · Kidney transplantation, skin cancer, papillomavirus · Skin cancer, papillomavirus, kidney transplantation

Introduction

The frequency of carcinoma in graft recipients is three times that in the general population [13, 19, 22]. The frequency of skin cancer in these patients is 20–30 times as high [10]. In Europe, 40 % of transplant patients develop skin cancer within 20 years after grafting [8, 11] versus 14 years in Australia and New Zealand [20]. Immunosuppression is conducive to various viral infections in these patients [18, 20, 22]. Skin lesions attributable to papillomavirus are found in 87 % of patients 5 years after grafting [24].

This concomitance of cancer and viral skin lesions in transplant patients is indicative of the role of human papillomavirus (HPV) in cutaneous oncogenesis. At the mucosal level, HPV type 16/18 is considered to be involved in the genesis of cervical cancers [33], which are five times as frequent in women with renal transplants [25].

To assess the possible role of mucosal HPV (with normal mucosal tropism; 6/11, 16/18, and 31/35/51) in the development of skin cancer in transplant patients, we used an in situ hybridization technique to study the frequency of HPV DNA in benign and malignant cutaneous lesions in these patients and in a nonimmunosuppressed control population.

Materials and methods

Transplant population

Twenty-one skin cancer biopsies from 13 patients (9 men, 4 women; mean age 53 years, range 35–68 years; mean immunosuppression period 72.3 months, range 11–164 months) were obtained for histopathological study and in situ hybridization. Therapeutic modalities at the time of diagnosis of the skin lesions were as follows: triple therapy [corticosteroids (Cs), azathioprine (AZA), cyclosporin (CyA)] for two patients, Cs + CyA for three patients, AZA + CyA for five patients, Cs + AZA for one patient, and CyA, alone for two patients. Histopathological examination identified 5 in situ, 3 basal cell, and 13 squamous cell carcinomas. Ninety percent of the skin lesions were located in sun-exposed regions of the body (head and neck, back of the hands, forearm).

Thirty-four wart biopsies (26 common, 8 plane) were obtained from 31 patients (19 men, 12 women; mean age 41.2 years, range 11–67 years; mean immunosuppression period 72.2 months, range 3–226 months). The therapeutic modalities at the time of excision were as follows: Cs + AZA + CyA for 11 patients; AZA + CyA for 8 patients; Cs + AZA for 8 patients; Csy + Cs for 2 patients; and CyA alone for 2 patients.

Control population

Twenty-one skin cancers were studied in a control population of 11 men and 10 women (mean age 59.3 years, range 49–70 years), none of whom had received or were receiving immunosuppressant treatment. None had a history of other cancer or evidence of warts or viral skin lesions within the 5 previous years. In 85.7 % of cases, the lesions occurred in sun-exposed regions of the body. Pathological examination identified five in situ, ten basal cell, and six squamous cell carcinomas.

Twenty common and ten plane wart biopsies with typical histology were also obtained from 30 nonimmunosuppressed patients (18 men, 12 women; mean age 38.7 years, range 15–58 years). Ninety percent of these lesions were located in sun-exposed regions of the body.

Technique

An in situ hydridization technique was performed on frozen sections using three cold biotinylated DNA probes [6/11, 16/18, and 31/35/51 (Enzo)] and the following technique.

For the first hybridization step, slides were rehydrated in a 2SSC (sodium chloride-sodium citrate) solution. After heating to 70 °C, the probes were applied (10 μg/ml) to each slide and covered with a silicone coverglass. After denaturation at 95° C and rapid cooling, the slides were incubated overnight in a moisture chamber and then washed in 2SSC solution.

For the second step, a kit (Dako) was used to reveal hybridization. The slides were washed in tris buffered saline (TBS) solution and incubated with streptavidin at room temperature. After another washing in TBS, NBT/BCIP (5-bromo4-chloro-3 indolyl phosphate) was applied in darkness in a moisture chamber. The slides were mounted after a final washing. Interpretation revealed black intracellular granules corresponding to HPV DNA.

Table 1 Different types of carcinomas and warts in transplant and control populations, and the different types of HPV found using an in situ hybridization technique employing cold biotin probes

	Lesions	HPV		
		6/11	16/18	31/35/51
TRANSPLANT PT	21 Skin cancers			
	6 in situ	0	0	3
	3 basal cell	1	1	1
	13 squamous cell	1[a]	0	3
	34 Warts			
	26 common	3	3	3[a]
	8 plane	0	2	1
CONTROLS	21 Skin cancers			
	5 in situ	0	1	0
	10 basal cell	3	0	2[b]
	6 squamous cell	0	0	0
	30 Warts			
	20 common	0	1	1
	10 plane	0	1	0

[a] Associated with another type of HPV in 1 case
[b] Associated with another type of HPV in 2 cases

Statistical analysis

The chi-square test with Yates correction was used for comparative studies.

Results (Table 1)

No age- or sex-related differential criteria were noted for the two patient populations.

Renal transplant patients

Of the 21 skin cancers, 9 (42.9 %) were positive for HPV-DNA: three basal cell (one type 6/11, one type 6/18, and one type 31/35/51), three in situ (all three type 31/35/51), and three squamous cell carcinomas (one type 6/11 and 31/35/51, and two type 31/35/51).

Of the 34 benign lesions, 11 (32.4 %) were positive for HPV-DNA: eight common warts (three type 6/11, two type 16/18, one type 16/18 and 31/35/51, and two type 31/35/51) and three plane warts (two type 16/18 and one type 31/35/51).

Control population

Four out of 21 (19.04 %) skin cancers were positive for HPV DNA: one in situ (type 16/18) and three basal cell

carcinomas (type 6/11, associated with 31/35/51 in two cases).

Three out of 30 (10 %) benign lesions were positive for HPV DNA: two common warts (one type 16/18 and one type 31/35/51) and one plane wart (type 16/18).

The chi-square test with Yates correction showed no difference in the frequency of HPV DNA in carcinomas in the transplant versus the control population($P = 0.095$). However, a significant difference ($P < 0.05$) was found for the frequency of HPV DNA in benign lesions of the transplant versus the control population.

Discussion

Our study demonstrated that oncogenic (types 16/18 and 31/35/51) or nononcogenic (type 6/11) mucosal HPV DNA is identified both in malignant (42.9 %) and benign (32.04 %) lesions in transplant patients. However, a comparison between transplant patients and controls demonstrated an increase in the frequency of mucosal HPV ($P < 0.05$) only in benign lesions of transplant patients. A statistically significant increase in the frequency of HPV DNA in malignant skin lesions of transplant recipients versus the control population was not seen. However, the limited number of lesions in our study does not allow us to draw definitive conclusions.

HPV 16/18 has already been reported in squamous cell carcinoma and Bowen's disease [5, 9, 16, 21, 29] in immunocompetent subjects and transplant patients [22, 27]. It has also been found in warts of transplant patients [5]. Our study confirmed these findings reported in the literature. However, the presence of HPV 16/18 in two common warts in our control population was unexpected.

The presence of HPV 6/11 in benign or malignant skin lesions of transplant patients has been reported only rarely [28]. HPV 6 [3], 11 [7], and both types together [28] have also been noted in malignant skin lesions of nonimmunosuppressed subjects. It is noteworthy that, in our study, HPV 6/11 was not found in any benign lesion in the control population. Although no other study in the literature mentions HPV 31/35/51, its frequency was high in the transplant group (7/9 tumor lesions and 4/11 positive benign lesions) and in the control population (2/4 tumor lesions and 1/3 positive benign lesions). Other HPV, such as HPV 2 [17], 4 [30], 5/8 [1, 14] have been found in skin cancer of transplant patients.

The incidence of squamous cell carcinoma [14] is high in exposed skin of transplant patients; it is 21 times as frequent as in the normal population, and it is associated with a high incidence of HPV infection. This would suggest a relationship between HPV and skin cancer in these patients similar to that between HPV 16/18 and cervical cancer [33]. However, this relationship is not apparent in the results of recent studies. Rudlinger and Grob [23] and Smith et al. [26] did not find HPV DNA respectively in 3 and 28 cutaneous squamous cell carcinomas in transplant patients. Few studies examining skin cancers in the general nonimmunosuppressed population have been carried out, and the results are variable in existing studies. Kawashima et al. [12] used Southern blot to detect HPV in 104 skin cancers, 25 squamous cell carcinomas of the lips, and 23 genital squamous cell carcinomas, reporting percentages of 2 %, 4 %, and 60 %, respectively. Eliezri et al. [6] identified HPV DNA in only 1 of 26 basal cell and 1 of 16 squamous cell carcinomas.

The interpretation of these studies is influenced by the variability in both the methodology (PCR, Southern blot, in situ hybridization) and the choice of probes.

The results of our study confirm the reservations expressed by other authors concerning the exact role of HPV, and more particularly mucosal HPV, in the frequency of skin cancer development in transplant patients. In fact, the absence of a significant difference between carcinoma in our transplant patients and in the control population, as well as the lack of exclusive mucosal HPV infection in skin tumors in immunosuppressed cases, would suggest that "cofactors" associated with HPV are necessary to account for the increased frequency of carcinoma in transplant patients. In renal transplant recipients, most of the carcinomas develop on sun-exposed areas, which suggests that UV light may act as a cocarcinogen.

Later anticancer immunosurveillance in transplant patients could enhance the oncogenic power of HPV, which occurs first at the benign lesion stage. The oncogenic power could also be increased by genetic factors specific to the transplant patient, as recently shown in cases of epidermodysplasia verruciformis [15]. It has been shown that certain HLA types, such as HLA 11, could confer resistance [2] and, on the contrary, that others, such as HLA B27 and HLA DR7 could induce susceptibility to developing skin cancers [4]. Similarly, cutaneous immunodeficiency caused by therapy could induce the development of other viruses acting in synergism with HPV and accounting for the high frequency of mucosal HPV in benign lesions. A decrease in cutaneous immune response has been shown in benign and malignant tumors, together with a reduction in Langerhans cells and keratinocyte expression of ICAM, HLA DR, and b2 microglobulin [31].

Finally, certain therapeutic drugs, such as the azathioprine used in our patients, induce genetic modifications of keratinocytes, thereby favoring the oncogenic activity of papillomaviruses. This therapeutic drugs may play a role by its breakdown into imidazole products that sensitize the skin to sunlight. Cyclosporin may be less oncogenic than azathioprine [32].

Our study does not show a direct role of mucosal HPV in the frequency of skin cancer development in

transplant patients. However, our results may be confirmed in the future by analyzing other cutaneous lesions. In any event, our findings do emphasize the usefulness of investigating the consequences of prolonged immunosuppression with respect to cutaneous immunosurveillance.

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