Multiple Skin Cancers in a Renal Transplant Recipient: A Patient Report with Analyses of Human Papillomavirus and Human Polyomavirus Infection

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ABSTRACT

Skin cancer is an important complication in renal transplant recipients. Associations of transplant-related skin tumor with ultraviolet radiation, age at transplantation, type of immunosuppressant drug administered, and viral infection have been reported; however, the details remain unclear. We report a 61-year-old man who had underwent renal transplantation at 38 years of age and developed multiple skin tumors or squamous cell carcinomas (SCCs). Polymerase chain reaction (PCR) analyses of the patient's 12 tumors for viral DNAs of cutaneous or mucosal human papillomavirus (HPV) and 6 human polyomaviruses (MCPyV, trichodysplasia spinulosa-associated, BK, JC, KI and WU polyomaviruses) only detected cutaneous HPV-DNA in only 5 of the tumors; no other viruses were detected. Real-time PCR showed high loads of cutaneous HPV in 3 SCCs and very low loads of MCPyV in 9. Immunohistochemistry revealed no tumor cell expression for MCPyV-large T-antigen or mucosal HPV. Our report not only reconfirmed the association of cutaneous HPV5 with skin cancer in renal transplant recipients in previous studies but also showed no relevant association of 6 human polyomaviruses and mucosal HPV with skin tumors.

Key words human papillomavirus; human polyomavirus; polymerase chain reaction renal transplantation; immunosuppression; skin cancer

Skin cancer is an important complication in renal transplant recipients. Associations with mainly ultraviolet radiation, age at transplantation, type of immunosuppressant drug administered, and viral infection have been reported,[1, 2] although the details remain unclear. Numerous studies have investigated the relationship between viral infection and skin cancer in renal transplant recipients.[3, 4] however, these studies followed the same patient for several years and only few patients were examined for multiple viral infections.[5, 6] We describe a renal transplant recipient who repeatedly presented with multiple skin cancers, in particular, squamous cell carcinoma (SCC). Twelve tumors were resected from the patient in a span of 8 years (approximately 16–23 years after transplantation) and were examined for viral infection using PCR, real-time PCR and immunohistochemistry analyses. We examined the presence of viruses which have been reported in skin tumors including Merkel cell polyomavirus (MCPyV),[5] cutaneous human papillomavirus (cutaneous HPV),[6] mucosal human papillomavirus (mucosal HPV)[7] and trichodysplasia spinulosa-associated polyomavirus (TSPyV).[8] Furthermore, we also examined the presence of several polyomaviruses including BK polyomavirus (BKPyV),[9] JC polyomavirus (JCPyV),[9] KI polyomavirus (KIPyV)[10, 11] and WU polyomavirus (WUPyV).[12, 13]

This report presents the patient and the results of examination for viruses previously described with a review of literature.

PATIENT REPORT

This report was approved by the Institutional Review Board of Faculty of Medicine, Tottori University, Yonago 683-8503, Japan.

Patient report

A 61-year-old man who had undergone renal transplantation because of chronic renal failure at 38 years of age who has since then under been immunosuppressant therapy with azathioprine and cyclosporine presented a red, dome-shaped, rapidly enlarging tumor on the back of his left hand of 25-mm diameter. The tumor was sur-

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Abbreviations: BCC, basal cell carcinoma; BCPyV, BC polyomavirus; FFPE, formalin fixed, paraffin embedded; HPV, human papillomavirus; JCPyV, JC polyomavirus; KA, keratoacanthoma; KIPyV, KI polyomavirus; LT, large tumor antigen; MCPyV, Merkel cell polyomavirus; PCR, polymerase chain reaction; SCC, squamous cell carcinoma; SPF, short PCR fragment; TSPyV, trichodysplasia spinurosa-associated polyomavirus; VP1, viral protein 1; WUPyV, WU polyomavirus

gically resected, and was histopathologically diagnosed as keratoacanthoma (KA). The patient had no relevant family history (such as epidermodysplasia verruciformis Lewandowsky-Lutz[14]), smoking history or occupational exposure to carcinogens including pitch, tar, and arsenic. The first onset of skin lesions was 7 years after renal transplantation, involving multiple tumor formations on his trunk and limbs. Blood analyses showed no detectable abnormalities except for a slight elevation in lactic acid dehydrogenase levels (218 IU/L; standard value: 106–211 IU/L). Since then, various skin lesions have occurred, concentrating on the sun-exposed areas, including the head and the forearms (Fig. 1). The lesions were treated with liquid nitrogen and lesions not effectively eliminated were surgically excised. Histopathologically, the lesions consisted of benign tumors such as KA, verruca vulgaris, Seborrheic keratosis, and malignant tumors such as SCC in situ, and SCC (Fig. 2). In this case, non-typical tumor formation is one of the characteristics. The findings of tumors are not always exactly like the typically correct findings that are described in text books. Therefore, the pathological findings itself or descriptive term had to be adapted.

Samples and DNA extraction

Twelve skin tumors excised from the patient were obtained from the archive of Yoka Hospital (Yabu, Japan). All cases were reviewed by pathologists, and the diagnoses were confirmed (Table 1). Serial sections of all samples were used for hematoxylin and eosin staining and DNA extraction. DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) samples of skin tumors using QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. The concentrations of the extracted DNA were measured using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

Immunohistochemstry

To detect MVPyV large tumor antigen (LT) and mucosal HPV viral protein 1 (VP1) antigen, immunohistochemistry was performed using mouse monoclonal antibody CM2B4 (diluted at 1:200; Santa Cruz Biotechnology, Santa Cruz, CA) and mouse monoclonal antibody K1H8 (1:100; Dako, Glostrup, Denmark) as primary antibody, respectively. Tissue sections were deparaffinized

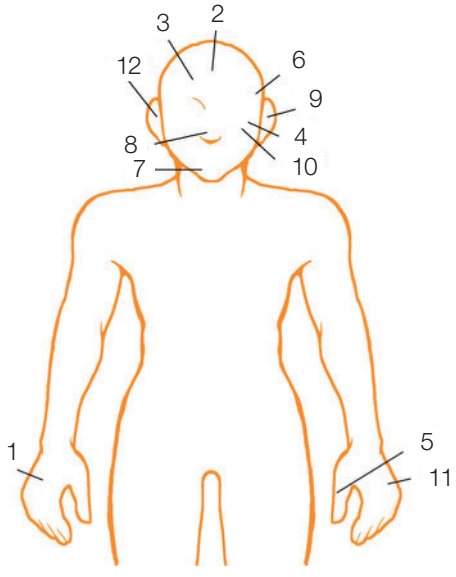

Fig. 1. Locations of each tumor development. Tumors were focused on sun-exposed areas including the head and forearm. Macroscopic and microscopic images of tumors are shown at Fig. 2.

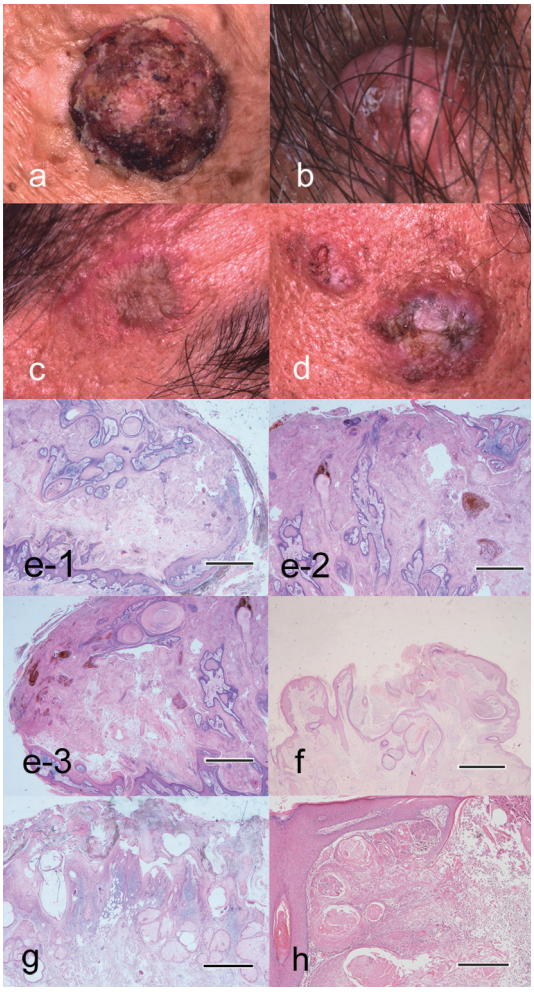

Fig. 2. Macroscopic images (**a**–**d**) and microscopic images (**e**–**h**) are shown, respectively. **a**, **e-1**, **e-2**, **e-3**, tumor of No. 1 (KA); **b**, **f**, tumor of No. 12 (Seborrheic keratosis); **c**, **g**, tumor of No. 3 (SCC in situ); **d**, **h**, tumor of No. 4 (SCC) (haematoxylin and eosin stain). Bar of **e-1**, **e-2**, **e-3**, **f**, 2000 μm; **g**, **h**, 500 μm, respectively.
KA, keratoacanthoma; SCC, squamous cell carcinoma.

Table 1. Tumors from the patient

No.	Years after transplantation	Location	Size (mm)	Diagnosis
1	16	Dorsal surface of right hand	28	KA
2	20	Forehead	10	SCC
3	20	Right forehead	12	SCC in situ
4	20	Left cheek	16	SCC
5	20	Dorsal surface of Left 1st digit	15	Verruca vulgaris
6	21	Left outer canthus	10	SCC
7	21	Right lower jaw	13	SCC
8	21	Right side of nose	5	Verruca vulgaris
9	23	Left ear pinna	12	SCC
10	23	Left cheek	9	SCC in situ
11	23	Dorsal surface of left hand	20	SCC
12	23	Front surface of right ear	9	Seborrheic keratosis

KA, keratoacanthoma; SCC, squamous cell carcinoma.

and rehydrated. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 5 min. Antigen retrieval was performed by incubating the sections in citrate buffer (pH 6.0) for 10 min at 95 °C. After applying the primary antibody, sections were incubated overnight at 4 °C and then thoroughly washed in phosphate-buffered saline. Peroxidase-conjugated goat anti-mouse IgG was applied as the secondary antibody. Sections were incubated for 60 min at room temperature and then washed in phosphate-buffered saline. Diaminobenzidine was used as the chromogen. But no immunoreactivity to MCPyV and mucosal HPV was detected in any of the tumor samples.

PCR

To detect viral DNA in each tumor sample, PCR was carried out using primers described elsewhere.[8–13, 15–18] The PCR products were approximately 100 base pairs in length (Table 2). To detect mucosal HPV, a mixture of short PCR fragment (SPF) 1A, 1B, 1C and 1D was used as forward primer and a mixture of SPF2B and 2D as reverse primer. PCR was performed using 0.25 U of TaKaRa Ex Taq HS (Takara Bio, Ohtsu, Japan), 2.5 pmol of each deoxyribonucleoside triphosphate, 2.0 pmol Mg^{2+}, 5 pmol of each primer pair and 10 ng of genomic DNA, generating a total volume of 10 pL per sample. Amplifications consisted of an initial denaturation for 4 min at 95 °C and 40 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 52 °C (for SPF primers) or at 59 °C (for the other primers), an extension step for 1 min at 72 °C and then, and a final extension for 4 min at 72 °C. PCR products were electrophoresed, stained with ethidium bromide, and visualized under UV light using UVP BioDoc-It System (UVP, Upland, CA).

Sequencing

To determine the sequence of the TaqMan probe used in quantitative PCR, sequencing was performed before quantitative PCR because the primers targeting the cutaneous and mucosal HPV were consensus primers for each type of HPV. The PCR products were purified before sequencing using NucleoSpin Gel and PCR Clean-up (Takara Bio) according to the manufacturer's protocol. Sequencing was performed using BigDye Terminator v3.1 Cycle Sequencing (Thermo Fisher Scientific) on an Applied Biosystems 3130XL Genetic Analyzer (Thermo Fisher Scientific), and the resulting DNA sequences were compared against the reference sequences of GenBank (National Center for Biotechnology Information, National Institute of Health, Bethesda, MD). The PCR results are shown in Table 3. Cutaneous HPV was detected in 5 of the 12 sample tumors. Sequence analysis identified cutaneous HPV as HPV5. MCPyV was detected in 9 of the 12 sample tumors. DNAs of mucosal HPV, BKPyV, JCPyV, TSPyV, KIPyV and WUPyV were not detected in any of the tumor samples.

Real-time PCR

To determine MCPyV and cutaneous HPV copy number in each of the samples examined as positive for these viruses at PCR, real-time PCR was performed using ABI PRISM 7900HT Sequence Detection System (Thermo Fisher Scientific). The PCR mixture to determine viral copy number contained 5.0 pL of THUNDERBIRD Probe qPCR Mix (Toyobo, Osaka, Japan), 3 pmol of the same primer pair as that of PCR, 2 pmol of fluorescein-labeled TaqMan probe, 0.2 pL of 50× ROX reference dye (Toyobo) and 10 ng of genomic DNA, in total volume of 10 pL per sample. The RNAse P gene was used as the internal control. The PCR mixture to determine RNAse P copy number contained 5.0 pL of THUNDERBIRD Probe qPCR Mix (Toyobo), 0.5 pL of 20× TaqMan copy number reference assay (Thermo Fisher Scientific), 0.2 pL of 50 × ROX reference dye (Toyobo) and 10 ng of genomic DNA, in a total volume of 10 pL for each of the samples. Amplifications consisted of an initial denaturation for 1 min at 95 °C and 45 cycles of denaturation for 15 sec at 95 °C, annealing for 45 sec at 60 °C. Standard curves were drawn using positive controls of each of the viruses. The results of quantitative PCR analysis are shown in Table 4. TaqMan probes used in real-time PCR consisted of the universal probe library 22 (for MCPyV) and universal probe library 35 (for HPV 5), respectively.

Table 2. Primers for PCR

Virus	Protein	Product (bp)	Sequence (5'–3')
MCPyV	LT	76	F: AGGTTGACGAGGCCCCTAT
			R: TTCCCGAAGCTGAATCCTC
Mucosal HPV	VP1	66	SPF1A: GCICAGGGICACAATAATGG
			SPF1B: GCICAGGGICATAACAATGG
			SPF1C: GCICAGGGICATAATAATGG
			SPF1D: GCICAAGGICATAATAATGG
			SPF2B: GTIGTATCIACAACAGTAACAAA
			SPF2D: GTIGTATCIACTACAGTAACAAA
Cutaneous HPV	E1	117	F: ACTGACCAAAGCTGGAAATC
			R: TCTTGCAGAGCATTGAAACG
BKPyV	VP1	127	F: GCAGCTCCCAAAAAGCCAAA
			R: CTGGGTTTAGGAAGCATTCTA
JCPyV	VP1	101	F: AGAAAAGGAGAAAGGAAGGACCC
			R: TCTGTAATTGAGTCAACCCCAGTTT
TSPyV	NCCR	103	F: TCATACTGCCACAAACACAGGAAG
			R: AGAACACAGAGCGGGAGGATG
	VP1	143	F: AGTCTAAGGACAACTATGGTTACAG
			R: ATTACAGGTTAGGTCCTCATTCAAC
	LT	121	F: TGTGTTTGGAAACCAGAATCATTTG
			R: TGCTACCTTGCTATTAAATGTGGAG
KIPyV	VP1	100	F: GGAAATACAGCTGCTCAGGAT
			R: CTTTGATACTTGAACCGCTTTCCTT
WUPyV	LT	77	F: TGTTGCATCCATTTGTTACATTCA
			R: GAAAGAACTGTTAGACAAATATATAG

E1, E1 protein; F, forward; HPV, human papillomavirus; JCPyV, JC polyomavirus; KIPyV, KI polyomavirus; LT, large tumor antigen; NCCR, non-coding control region; PCR, polymerase chain reaction; R, reverse; SPF, short PCR fragment; VP1, viral protein 1; WUPyV, WU polyomavirus.

Table 3. The results of PCR for viral DNAs

No.	Diagnosis	BKPyV	JCPyV	TSPyV NCCR	VP1	LT	KIPyV	WUPyV	MCPyV	Mucosal HPV	Cutaneous HPV
1	KA	–	–	–	–	–	–	–	–	–	–
2	SCC	–	–	–	–	–	–	–	–	–	+
3	SCC in situ	–	–	–	–	–	–	–	+	–	+
4	SCC	–	–	–	–	–	–	–	+	–	+
5	Verruca vulgaris	–	–	–	–	–	–	–	+	–	–
6	SCC	–	–	–	–	–	–	–	+	–	–
7	SCC	–	–	–	–	–	–	–	+	–	–
8	Verruca vulgaris	–	–	–	–	–	–	–	+	–	–
9	SCC	–	–	–	–	–	–	–	–	–	–
10	SCC in situ	–	–	–	–	–	–	–	+	–	–
11	SCC	–	–	–	–	–	–	–	+	–	+
12	Seborrheic keratosis	–	–	–	–	–	–	–	+	–	+

BCPyV, BC polyomavirus; HPV, human papillomavirus; JCPyV JC polyomavirus; KA, keratoacanthoma; KIPyV, KI polyomavirus; LT, large tumor antigen; MCPyV, Merkel cell polyomavirus; NCCR, non-coding control region; SCC, squamous cell carcinoma; TSPyV, trichodysplasia spinurosa-associated polyomavirus; VP1, viral protein 1; WUPyV, WU polyomavirus.

Large copy numbers of HPV 5 were observed in 3 (No. 3, 4 and 11) of the 12 tumor samples, and very low copy numbers of MCPyV were detected in 9 of 12 samples.

Table 4. The result of quantitative real-time PCR analysis

No.	Diagnosis	MCPyV (copy/cell)	Cutaneous HPV (copy/cell)
1	KA	–	–
2	SCC	–	ND
3	SCC in situ	0.000482	1.83
4	SCC	0.00340	146.0
5	Verruca vulgaris	0.0000531	–
6	SCC	0.00500	–
7	SCC	0.00103	–
8	Verruca vulgaris	0.000329	–
9	SCC	–	–
10	SCC in situ	0.00306	–
11	SCC	0.000286	1.69
12	Seborrheic keratosis	0.0466	ND

–, not tested because negative at PCR; HPV, human papillomavirus; KA, keratoacanthoma; MCPyV, Merkel cell polyomavirus; ND, not detected; SCC, squamous cell carcinoma.

DISCUSSION

Skin cancer is an important complication in renal transplant recipients. Incidence varies according to geographic latitudes; Naldi et al.[19] reported that the incidence was approximately 5% after 5 years and 10% after 10 years in Italy. Bouwes et al.[20] reported an incidence of 3% after 5 years and 16% after 11 years in Netherlands, and 25% after 5 years and 45% after 11 years in Australia. These results suggest the association of ultraviolet radiation with skin cancer in renal transplant recipients. In the population undergoing transplantation, the standardized morbidity ratio compared with immunocompetent people is 60–250 times for SCC and 10–40 times for basal cell carcinoma (BCC).[21] In addition, the SCC:BCC ratio is reversed compared with the ratio of 1:4 in the immunocompetent people.

In Japan, the incidence of skin cancer is low. Arichi et al.[22] examined 429 renal transplant recipients for 25 years and reported 9 cases (2%) of skin cancers. On the other hand, Imao et al.[23] (2007) reported the overall incidence of malignancy in renal transplant recipients was 6.8% (25/366 patients); however, no skin cancer cases have been documented in this population.

In the present report, we examined 12 tumors from a 61-year-old man who previously underwent renal transplantation and presented with multiple skin cancers. In this case, non-typical tumor formation is one of the characteristic. The findings of tumors are not always correctly typical findings which are described in the text books. Therefore, the pathological findings itself or descriptive term had to be adapted (ex. SCC in situ). Neoplastic lesions developed on sun-exposed area. This suggests that the sun exposure is one of the important factor of tumor formation. In addition, we assumed that viral infection is another important factor coming from immunosuppression. Then we examined for viral effects using PCR assay and immunohistochemical assay.

At first, we tried immunohistochemical assay; however, since significant results were not obtained, we carried out PCR assay. The high viral load of cutaneous HPV (HPV5) was detected in 3 of the 12 tumors. HPV5 has been often detected in skin cancer in renal transplant recipients; Barr et al.[6] detected HPV5/8 in 15 (60%) of 25 SCCs from 202 renal allograft recipients who had undergone kidney transplantations and were monitored over 3 years. In our case, although cutaneous HPV was not necessarily detected in all tumors, all 3 tumors from which cutaneous HPV was detected were SCCs, suggesting that cutaneous HPV affects the development of skin cancer rather than the incidence.

In terms of the association of MCPyV with skin cancer in renal transplant recipients, Mertz et al.[6] examined 17 renal transplant recipients and 3 patients under long-term dialysis with skin tumors and detected MCPyV-DNA by PCR in 2 patients with Bowen's disease. On the other hand as for MCPyV in nontumor tissue, Matsushita et al. reported that MCPyV is prevalent in humans and was detected most frequently in the skin of 41 autopsy cases.[24] We detected MCPyV-DNA in 9 of 12 samples. But MCPyV is considered to have no relation to tumorigenesis in our patient, since their copy numbers were very low.

In terms of the relationship between the type of immunosuppressant and the incidence of skin cancer, previous studies have shown that patients immunosuppressed with azathioprine and cyclosporine showed higher rates of skin cancer than those on tacrolimus,[20, 23] although the finer details are currently under scrutiny.

Our data suggests that there is a relationship between cutaneous HPV in skin cancer and renal transplantation. Cutaneous HPV is influenced by other factors such as ultraviolet radiation, which increases the incidence and the development of skin cancer in renal transplant recipients.

Rogers et al.[25] reported acquired epidermodysplasia verruciformis (epidermodysplasia verruciformis like syndrome). There is a common background such as immunodeficiency. However, in this case, tumors are not always flat warts (verruca plana), and few reports about

a very long follow up of many and multiple tumor formation in the same patient after renal transplantation, as this case, is observed. Therefore, the relation of this case to acquired epidermodysplasia verruciformis is not always clear. Further studies are needed to better understand the etiology of this malignancy.

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The authors declare no conflict of interest.

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