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Original Article

Clinicopathologic analysis of malignant or premalignant cutaneous neoplasms in Japanese kidney transplant recipients

Natsuki Taniguchi[1], Taishi Takahara[1], Takanori Ito[1], Yuki Yamamoto[1], Akira Satou[1], Akiko Ohashi[1], Emiko Takahashi[1], Nagako Maeda[2], Toyonori Tsuzuki[1]

[1]Department of Surgical Pathology, Aichi Medical University Hospital, Nagakute, Japan; [2]Department of Surgical Pathology, Japanese Red Cross Aichi Medical Center Nagoya Daini Hospital, Japan

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Abstract: It is well known that recipients of kidney transplants are at an increased risk of developing malignant or premalignant cutaneous neoplasms (MPCNs) after transplantation. However, the pathogenesis of MPCNs after kidney transplant has not been well-studied in Asian populations. This study aimed to describe the clinicopathologic-characteristics of MPCNs in an Asian population. We retrospectively reviewed the medical records of 1956 patients who received kidney transplants at two hospitals in Japan, between 2003 and 2019. Among these patients, 24 developed 50 MPCN lesions, including 14 squamous cell carcinoma (SCC, 28%), 23 Bowen's disease (BD, 46%), 11 actinic keratosis (AK, 22%), and two basal cell carcinoma (BCC, 4%). No patient had malignant melanoma. The duration from transplantation to the diagnosis was significantly longer for SCC than for BD or AK (P=0.021, 0.036, respectively). Seven patients had multiple MPCNs in sun-exposed areas of skin. Among the 50 MPCNs, 40 (80%) were located in sun-exposed areas, and 10 (20%) were located in sun-protected areas. MPCNs in sun-exposed skin were frequently accompanied by dermal solar elastosis (90%, 36/40). We found high-risk human papillomavirus (HR-HPV) infections in two anogenital lesions (100%, 2/2). In contrast, HR-HPV infections were not detected in any extragenital lesions (0%, 0/30). Our results suggested that, among Japanese recipients of kidney transplant, MPCNs in sun-exposed skin areas may be associated with immunosuppression and ultraviolet exposure.

Keywords: Skin cancer, kidney transplant, UV exposure, human papilloma virus, RNA in situ hybridization

Introduction

Recent advances in the management of kidney transplantations have significantly improved early graft survival rates. However, long-term survival rates have remained unchanged, largely due to long-term complications [1]. One of the most critical long-term complications is the development of malignancies, and skin is one of the sites most frequently involved [2].

Squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and cutaneous malignant melanoma are the most common malignancies in recipients of kidney transplantation. Compared to western populations, the incidences of those malignancies in Asian populations are relatively low [3-5]. Similar to immunocompetent populations, among individuals with kidney transplants, the incidence of malignant cutaneous neoplasms is much higher in western populations than in Asian populations. For example, the cumulative incidences of malignant cutaneous neoplasms in recipients of kidney transplants in the USA and Australia are estimated to be up to 40% and 80%, respectively [6]. In contrast, in a Japanese cohort study, only 0.4% of recipients of kidney transplants developed malignant cutaneous neoplasms, during a median-follow up of 37.5 months [7]. However, the characteristics of malignant cutaneous neoplasms in Asian recipients of kidney transplants are uncertain.

Several risk factors are known to be involved in the development of malignant cutaneous neoplasms after kidney transplantation in western populations. For example, ultraviolet (UV) light

exposure induces gene mutations and promotes skin carcinogenesis [8]. Accordingly, the post-transplant incidence is comparatively higher in countries with high sun exposure, like Australia [8]. Immunosuppression is also known to promote skin carcinogenesis [8]. Human papillomavirus (HPV) is also a risk factor for developing malignant cutaneous neoplasms [8]. High-risk HPV (HR-HPV) and low-risk HPV (LR-HPV) infections are defined according to whether the HPV infection is associated with polymorphisms in *E7*, a viral oncogenic gene associated with retinoblastoma protein (pRb) [9]. As with other malignancies, such as post-transplant lymphoproliferative disorders, immunosuppressive therapies can lead to impaired T-cell-mediated immune responses, which may promote the proliferation of virus-infected neoplastic cells in recipients of kidney transplants [10, 11]. However, the importance of these risk factors may be affected by the patient's ethnicity. For example, it is known that high levels of eumelanin in skin provides protection from UV-induced DNA damage [12]. Moreover, the prevalence of HPV infections in cancer varies across racial groups [13]. These findings suggest that the pathogenesis of malignant cutaneous neoplasms in Asian recipients of kidney transplants may be different from that of western recipients. Thus, we aimed to investigate the pathogenesis of skin cancer in Japanese recipients of kidney transplants.

Herein, we analyzed the clinicopathologic characteristics of malignant or premalignant cutaneous neoplasms (MPCNs) found in 1956 recipients of kidney transplants. We investigated the HR-HPV prevalence to gain a better understanding of the clinical behavior and biologic properties of MPCNs. We analyzed the premalignant lesion, actinic keratosis (AK), because it is known to have some genetic alterations found in invasive carcinomas, and because AK often progresses to invasive carcinoma [14].

Patients and methods

Study subjects

This research was approved by the Ethics of Aichi Medical University Hospital (No. 2020-188) and Japanese Red Cross Aichi Medical Center Nagoya Daini Hospital (No. 1573). We retrospectively reviewed the medical records of 1956 patients that received kidney transplantations in Aichi Medical University Hospital or Japanese Red Cross Aichi Medical Center Nagoya Daini Hospital, between 2003 and 2019. We collected data on patient sex, age, age at transplantation, the duration of dialysis prior to transplantation, cancer history, cause of death, total follow up time, the history of infection, immunosuppression (regimen, use of induction therapy, and duration), incidence of MPCNs (including SCC, Bowen's disease [BD]/SCC in situ, AK, BCC, and melanoma) during the follow-up period, and their clinicopathologic features (location, diagnosis, treatment, and prognosis). The locations of skin lesions were classified as sun-exposed areas and sun-protected areas, as described previously [15]. The histologic diagnoses of MPCNs were based on biopsy or surgical specimens, collected in each hospital. The degree of SCC differentiation was graded as: "well", "moderate" and "poor" [16]. All MPCN specimens were retrieved and reviewed by two pathologists (N.T. and T.T.). The *P*-value was calculated by Kruskal-Wallis test, and $P<0.05$ was considered significant.

Immunohistochemical analysis

After reviewing hematoxylin and eosin (H&E)-stained tissue sections from each patient sample, we selected one representative specimen for the immunohistochemical analysis. Immunohistochemical procedures were performed with the p16 antibody (CINtec® p16 Histology, Ventana, Tucson, AZ) and the Ventana Benchmark Ultra automated stainer, according to the manufacture's protocol. Specimens were regarded as positive for p16, when we detected diffuse nuclear and cytoplasmic staining in more than 80% of tumor cells [17].

RNA in situ hybridization

To detect the HR-HPV, we performed RNA in situ hybridization (ISH) with the HPV HR18 probe (Advanced Cell Diagnostics, Newark, CA), which targeted 18 HR-HPV genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82). To detect the LR-HPV, we performed in situ hybridization with the HPV LR6 probe (Advanced Cell Diagnostics), which targeted 6 LR-HPV genotypes (6, 11, 40, 42, 43, and 44). We performed all RNA ISH with

Table 1. Clinical characteristics of patients who underwent kidney transplantation

Characteristic	Total (n=24)
Male	19 (79.1%)
Dialysis before transplantation	21 (87.5%)
Cancer prior to transplantation	3 (12.5%)
Total follow up time (years)	13 (7-44)
Age at first transplantation (years)	51.5 (14-75)
Age at MPCN diagnosis (years)	68 (50-83)
Time from kidney transplantation to diagnosis (years)	11 (7-38)
Duration of dialysis (years)	3 (0-25)
Total duration of immunosuppression (years)	20.5 (7-44)
Immunosuppression	
Cyclosporine/Tacrolimus	21 (87.5%)
Azathioprine/MMF	21 (87.5%)
Steroids	24 (100%)
Other	5 (20.8%)
Received more than one transplantation	5 (20.8%)
Cancer other than skin cancer after transplantation	4 (16.7%)
Death	4 (16.7%)

Values are the median (%) or median (range), as indicated. MPCN: Malignant or premalignant cutaneous neoplasm, MMF: Mycophenolate Mofetil.

the RNAscope® 2.5 HD reagent kit BROWN (Advanced Cell Diagnostics), according to the manufacturer's protocol. Briefly, tissue sections were baked for 1 h at 60°C and dried. RNAscope® hydrogen peroxide was applied for 10 min at room temperature, then slides were boiled in Target Retrieval solution for 25 min. Next, RNAscope® hydrogen protease was applied for 30 min at 40°C. We added probes specific for *Homo sapiens* peptidylprolyl cis-trans isomerase B and bacterial 4-hydroxyterahydrodipicolinate reductase as positive and negative controls, respectively. The slides were incubated for 2 h at 40°C with the four probes, then the signals were visualized with the RNAscope® 2.5 HD reagent kit BROWN. Finally, all slides were counterstained and evaluated. Positive staining was defined as the appearance of dark-brown, dot-like spots in the cytoplasm and nuclei [18].

Results

Patient characteristics

Among 1956 patients enrolled in this study, 24 developed MPCNs during a median follow-up period of 13 years (range 7-44). The clinical characteristics of patients with MPCNs are summarized in **Table 1**. The male: female ratio was 3.8, and the median age at diagnosis was 68 years (range 50-83). The median time from transplantation to the diagnosis of MPCNs was 11 years (range 7-38). Twenty-one patients (87.5%) received hemodialysis before transplantation. The median duration of dialysis was 3 years (range 0-25). Among the 5 patients that underwent more than one transplantation, 4 (16.7%) received a second kidney transplantation, and one (4.1%) received a liver transplantation after the kidney transplantation. All patients received immunosuppressants, both as induction therapy and as a concomitant therapy. Among these, 18 patients (75%) received a three-drug combination therapy that comprised a calcineurin inhibitor, an anti-metabolite, and steroids. Among the 3 patients (12.5%) with a history of cancer before transplantation, one had stomach cancer, one had kidney cancer, and one had skin cancer (BCC). Among the 4 deaths, one patient died of chronic heart failure and one patient committed suicide during follow-up. No patient died of MPCNs. Among the 4 patients that developed cancers other than skin cancer, two developed pancreatic cancers; one developed dermatofibrosarcoma; and the other developed gastric cancer. Of these, one patient with pancreatic cancer and another patient with dermatofibrosarcoma died of their cancers.

Characteristics of malignant and premalignant cutaneous neoplasms

Among the 24 patients with MPCNs, 9 (37.5%) had multiple MPCNs, and 7 had multiple lesions located in sun-exposed areas. Fifty lesions were identified histologically. The histologic diagnoses were: SCC (n=14, 28%), BD/SCC in situ (n=23, 46%), AK (n=11, 22%), BCC (n=2, 4%), and melanoma (n=0, 0%). As shown in **Table 2**, most of these lesions were in sun-exposed areas (n=40, 80%) in the head and neck (n=31, 62%) and upper limbs (n=9, 18%). Of these, 90% (36/40) were accompanied by dermal solar elastosis. Two patients had skin

Table 2. Locations of malignant and premalignant cutaneous neoplasms

Location	Histology			
	SCC, n=14	BD, n=23	AK, n=11	BCC, n=2
Sun-exposed areas				
Head and Neck	8 (57.1%)	14 (60.8%)	7 (63.6%)	2 (100%)
Nose	0	1 (4.3%)	0	1 (50.0%)
Forehead	1 (7.1%)	0	0	1 (50.0%)
Cheek	6 (42.9%)	5 (21.7%)	2 (18.1%)	0
Ear	1 (7.1%)	6 (26.0%)	3 (27.2%)	0
Temple	0	1 (4.3%)	1 (9.0%)	0
Neck	0	1 (4.3%)	1 (9.0%)	0
Upper limb	3 (21.4%)	2 (8.7%)	4 (36.4%)	0
Sun-protected areas				
Lower limb	1 (7.1%)	3 (13.0%)	0	0
Trunk	0	3 (13.0%)	0	0
Genitalia	2 (14.3%)	1 (4.3%)	0	0

SCC: Squamous cell carcinoma, BD: Bowen's disease, AK: Actinic keratosis, BCC: Basal cell carcinoma.

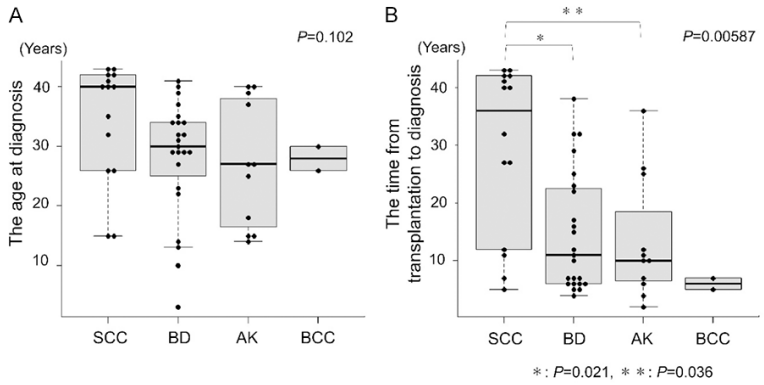

Figure 1. Relationships between histologic diagnosis and patient age or the time elapsed after kidney transplantation. A. Patient age distribution, according to histologic subtypes. No significant difference was observed between histologic subtypes. The *P*-value was calculated by Kruskal-Wallis test (*P*=0.102). B. Time elapsed after kidney transplantation, according to histologic subtype. The *P*-value was calculated by Kruskal-Wallis test (*P*=0.00587). Significant differences were observed in each pair (**P*=0.021, ***P*=0.036). SCC: Squamous cell carcinoma, BD: Bowen's disease, AK: Actinic keratosis, BCC: Basal cell carcinoma.

lesions in the genital area. One had SCCs in the scrotum and anus, which were diagnosed simultaneously, and the other had BD in the vagina. These three lesions were clinically diagnosed as Bowenoid papulosis (BP). Two patients had a solitary BCC lesion on the face, and no recurrence was observed. There was no apparent association between the age at diagnosis and the histologic diagnosis (**Figure 1A**, *P*=0.102). However, the time from transplantation to diagnosis was significantly longer for SCC than for BD or AK (**Figure 1B**, *P*=0.021 and 0.036, respectively).

Characteristics of the skin lesions in the six patients with SCC are summarized in **Table 3**. All six patients were male with multiple MPCNs. All 14 SCC lesions showed well- or moderately differentiated keratinization. Patient A had widespread seborrheic keratosis on the face and upper limbs, and multiple BDs and SCCs had arisen within seborrheic keratosis areas (**Figure 2A**). Intriguingly, another patient had numerous seborrheic keratosis over the whole body, predominantly in sun-exposed areas, and he subsequently developed BD in the right knee. Five patients (all except patient B) had multiple MPCNs in sun-exposed areas. Solar elastosis in the dermis (**Figure 2B**) was observed in 23 (92%) of the 25 lesions in sun-exposed areas. Patient B was clinically diagnosed with BP and had two lesions on the genitalia, which were histologically diagnosed as SCC (**Figure 2C**). Patient C had SCC on the fifth toe of the left foot and left inguinal lymph node metastasis. He had undergone surgery and radiochemotherapy. He subsequently developed a metastatic lesion in the left thigh, three years after the surgery.

We next evaluated the prevalence of HPV infections among patients with MPCNs (**Table 4**). An HPV infection is a well-known risk factor for skin neoplasms in recipients of kidney transplants [8]. Among the 50 MPCN lesions included in this study, unstained slides were available for 33 lesions from 23 patients. We excluded one slide of a BD lesion, because we could not detect an RNA-ISH signal for the positive control probe. Thus, we evaluated 32 lesions for the presence of HPV mRNA transcripts.

Table 3. Clinical and histologic features of patients with squamous cell carcinoma

Patient number	Histologic diagnosis	Lesion number	Age	Sex	Primary disease	Past history	Site of tumor	Tumor size (mm)	Tumor thickness (mm)	Differentiation	Solar elastosis
Patient A	SCC	1	72	M	CKD	None	Right cheek	19×18	2	well	(+)
		2	80				Forehead	30×20	10	moderate	(+)
		3	80				Right arm	20×18	7	well	(+)
		4	81				Left shoulder	40×30	11	well	(+)
		5	82				Right cheek	10×10	1.2	well	(+)
		6	82				Left cheek	40×28	9	well	(+)
		7	83				Left wrist	20×20	8.2	well	(-)
		8	83				Left ear	26×23	7.2	moderate	(+)
	BD	1	62				N.A.	N.A.			(+)
		2	69				Left thigh	13			(-)
		3	72				Right cheek	10×10			(+)
		4	72				Left arm	20×14			(-)
	AK	1	65				Right hand	5			(+)
Patient B	SCC	1	55	M	N.A.	None	Scrotum	40×30	6	well	(-)
		2	55				Anus	40×50	16	well	(-)
Patient C	SCC	1	66	M	CGN	Cerebral aneurysm	Left foot	20×10	N.A.	well to moderate	N.A.*
	AK	1	67				Left preauricular	8×5			(+)
		2	67				Left cheek	5×2			(+)
Patient D	SCC	1	75	M	PKD	None	Left cheek	8×6	2	moderate	(+)
	BD	1	74				Left preauricular	25×8			(+)
		2	4				Right preauricular	17×11			(+)
		3	74				Left preauricular	95×55			(+)
Patient E	SCC	1	66	M	N.A.	None	Right cheek	4	2	well	(+)
	BD	1	70				Forehead	12×8			(+)
Patient F	SCC	1	75	M	N.A.	None	Left preauricular	15×10	7	well	(+)
	BD	1	75				Left cheek	5×5			(+)
		2	80				Left preauricular	12×8			(+)
	AK	1	80				Right preauricular	4			(+)
		2	80				Right neck	12×8			(+)

SCC: Squamous cell carcinoma, BD: Bowen's disease, AK: Actinic keratosis, BCC: Basal cell carcinoma, M: Male, N.A.: Not available, CKD: chronic kidney disease, CGN: Chronic glomerulonephritis, PKD: Polycystic kidney disease, *: Dermal component was not included in the specimen.

HR-HPV was detected in one SCC lesion located at the anus and one BD lesion located in the vagina (2/32, 6.3%). Both of these lesions showed positive p16 immunostaining (**Figure 3A**, **3B**). HR-HPV was not detected in any of the lesions located in non-anogenital areas, (0/30, 0%), but p16 positive staining was frequently observed in HPV HR18-negative MPCNs (14/30, 46.7%; **Figure 3C**). LR-HPV was not detected in this study, regardless of whether the lesion was located in the anogenital area (0/32, 0%).

We also evaluated several histologic features known to be associated with HPV infections. Coarse keratohyalin granules, papillomatosis, and a perinuclear halo have been considered typical histologic features of HPV infection [19] (**Figure 3E**, **3F**). These features were found in both HPV-positive lesions located in the anogenital area. However, these findings were also observed in HPV-negative lesions at the following frequencies: coarse keratohyalin granules (16/30, 53.3%), papillomatosis (10/30, 33.3%), and perinuclear halos (11/30, 36.7%). Melanin deposition, a rare finding in BD, was reported to be associated with HPV-positive cases. Melanin was detected in approximately half of the patients with BD (n=8, 53%; **Figure 3G**).

Discussion

The importance of managing long-term complications in recipients of kidney transplants has grown, due to improvements in early graft survival rates [1]. Previous studies that described the risk of developing malignancies in recipients of kidney transplants showed that skin

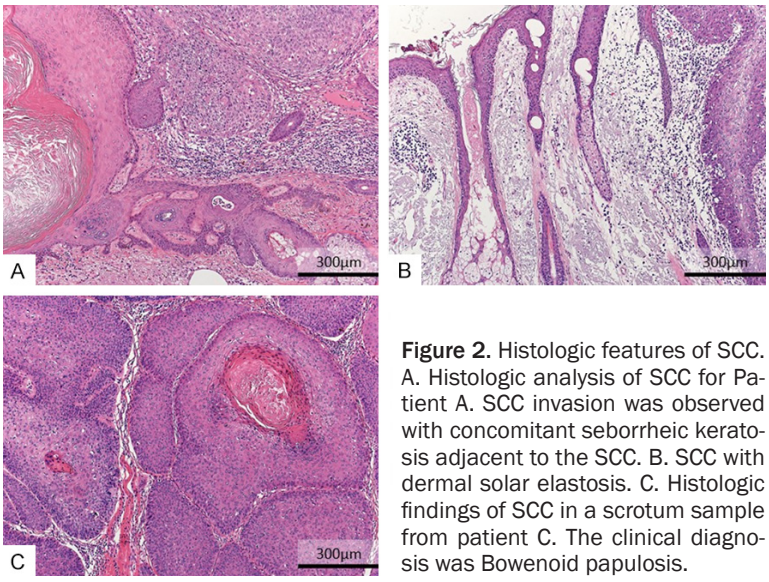

Figure 2. Histologic features of SCC. A. Histologic analysis of SCC for Patient A. SCC invasion was observed with concomitant seborrheic keratosis adjacent to the SCC. B. SCC with dermal solar elastosis. C. Histologic findings of SCC in a scrotum sample from patient C. The clinical diagnosis was Bowenoid papulosis.

Table 4. Histologic features, p16 immunostaining, and prevalence of high-risk human papilloma virus infections in malignant or premalignant cutaneous neoplasms

Feature	HPV HR18 positive (n=2)	HPV HR18 negative (n=30)
Diagnosis		
SCC	1 (50%)	5 (16.7%)
BD	1 (50%)	15 (50%)
AK	0	9 (30%)
BCC	0	1 (3.3%)
Histology		
Keratohyalin granules	2 (100%)	16 (53.3%)
Papillomatosis	2 (100%)	10 (33.3%)
Perinuclear halo	2 (100%)	11 (36.7%)
Pigmentation	0	14 (46.7%)
p16 IHC		
Positive	2 (100%)	14 (46.7%)
Negative	0	16 (53.3%)

SCC: Squamous cell carcinoma, BD: Bowen's disease, AK: Actinic keratosis, BCC: Basal cell carcinoma, IHC: Immunohistochemistry.

was the most common site of involvement [2]. The incidence of MPCNs in recipients of kidney transplants was suggested to be associated with either ethnicity or environmental factors. For example, the cumulative incidence of malignant cutaneous neoplasms in recipients of kidney transplants reached 40 to 60% at 20 years after transplantation in the US and Europe, and it reached 80% in Australia [6]. However, the pathogenesis of malignant cutaneous neoplasms in recipients of kidney transplants has not been well-studied in Asian populations. Our study was the first to describe the clinicopathologic features of MPCNs in recipients of kidney transplants in Japan. Indeed, the incidence of malignant skin neoplasms in recipients of kidney transplants in Asian countries was reported to be much lower than the incidences observed in western countries. Miyazaki et al. reported that, in Japan, only 19 of 4600 (0.4%) recipients of kidney transplants developed malignant skin neoplasms [7]. In our study, 24 of 1956 (1.2%) recipients of kidney transplants developed MPCNs, comparable to the frequency reported by Miyazaki et al. This difference in the incidence of malignant skin neoplasms between estern and Asian populations suggests that the pathogenesis may depend on ethnicity. Interestingly, no patient in our study had malignant melanoma, which is one of the most common malignant skin neoplasms in western countries, in both recipients and non-recipients of kidney transplants. Although the incidence of cutaneous malignancies is markedly increased in recipients of kidney transplants, compared to non-transplant recipients, the degree of increase varies by histologic type. In western countries, the incidence of SCC, BCC, and melanoma in recipients of kidney transplants were reported to be increased by 65-fold, 10-fold, and 3-fold, respectively, compared to their incidences in the general population [6]. Although based on a limited number of cases, our results suggested that, in our Asian population, kidney transplantation increased the susceptibilities to AK, BD, and SCC, but not to BCC or malignant melanoma.

Previous studies have revealed several risk factors for the development of SCC, including con-

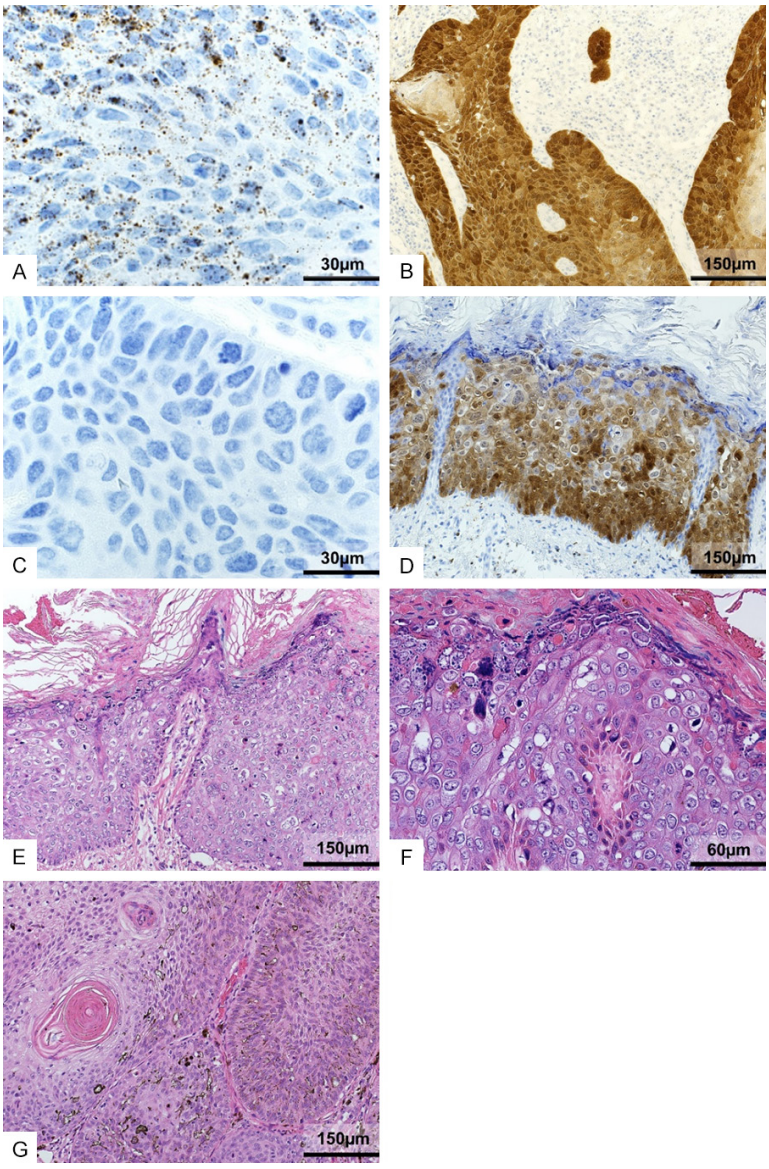

Figure 3. Histology of specimens with or without high-risk human papillomavirus (HR-HPV). (A) A representative case of positive HR-HPV staining. (B) Note the diffuse, strong nuclear and cytoplasmic p16 staining. (C) Representative case of negative HR-HPV staining. (D) This case also showed positive p16 staining. (E-G) HR-HPV negative case with well-known HPV-related histology, including (E) papillomatosis and parakeratosis, (F) perinuclear halos and keratohyalin granules, and (G) pigmentation.

tinued exposure to immunosuppression, exposure to UV light, and HPV infections [3, 8]. Immunosuppressive therapies reduce T-cell numbers and functions, which results in diminished anti-tumor immunity, and thereby, promotes skin carcinogenesis [5]. It is assumed that SCC develops from normal skin, AK, or BD, through the accumulation of genetic alterations induced by UV exposure or aging [20, 21]. Notably, in the present study, the time elapsed after transplantation was correlated with the histologic progression from AK or BD to SCC, whereas patient age was not. Given these findings, we hypothesized that immunosuppressive therapy in the transplant recipients played a more important role in skin carcinogenesis than the accumulation of genetic alterations or age-related immunosenescence. This hypothesis is supported by previous studies, which showed that susceptibility to malignant cutaneous neoplasms was increased by a specific combination of immunosuppressive agents [22, 23].

This study highlighted the contribution of UV exposure to the development of skin carcinogenesis in recipients of kidney transplants. We found that 80% (40/50) of MPCNs arose in sun-exposed areas, and of these, 90% (36/40) were accompanied by dermal solar elastosis. The occurrence of multiple MPCNs in sun-exposed areas of the patients in our study also indicates that UV exposure is involved in skin carcinogenesis. It is well known that UV radiation, particularly UV-B, can induce unique signatures of somatic mutations and promote skin carcinogenesis in immunocompetent patients [21]. Notably, a UV-associated mutational signature is associated with an increase in the neoantigen load, and this increase results in promoting T cell-mediated immune responses, which contribute to tumor cell removal by the host immune system [24]. However, in the microenvironment of SCC, in patients with organ transplants, the immune response is reduced, compared to that observed in immunocompetent patients [25]. Taken

together, these observations suggest that immunosuppressive therapy and UV exposure may promote skin carcinogenesis in a cooperative manner.

The pathogenesis of malignant cutaneous neoplasms in sun-exposed areas seems to be different from the pathogenesis in anogenital areas. Instead of UV exposure, HR-HPV infections played a major role in carcinogenesis in the anogenital area [26]. HPV-encoded proteins, E6 and E7, functionally inactivate tumor-suppressor genes, such as *TP53* and *Rb*, which leads to malignant transformation [9]. HR-HPV was exclusively found in anogenital sites in our study, consistent with a previous study [3]. These trends were also observed in immunocompetent patients, where only a small proportion of extragenital malignant cutaneous neoplasms harbored HR-HPV [26].

Intriguingly, in our study, although MPCNs in sun-exposed areas did not harbor HR-HPVs or LR-HPVs, they exhibited several histologic and immunohistochemical features typical of HPV-related neoplasms. We found that 81.2% (26/32) of the lesions exhibited coarse keratohyalin granules, papillomatosis, skin pigmentation, and/or perinuclear halos, which are all well-known histologic features of HPV-related neoplasms [19]. Moreover, 50% (16/32) of the lesions showed diffuse cytoplasmic or nuclear p16 staining, which is widely performed clinically to detect HPV infections [17, 27]. These findings imply that, in our study, the skin neoplasms in sun-exposed areas might have been infected with HPV subtypes that were not included in our analysis. This hypothesis is supported by the epidermodysplasia verruciformis (EV)-like features uniquely observed in two of our patients, which displayed multiple seborrheic keratoses, accompanied by SCC (**Figure 2A**). In EV, skin neoplasms are induced by HPV-5 or HPV-8 infections [28]; thus, these pathogens might have been involved in skin carcinogenesis in our two patients. However, those pathogens are also detected in normal skin in healthy individuals; consequently, they are not considered necessary for cancer cell maintenance [29]. Instead, when present, these pathogens might cooperate with other oncogenic factors, such as immunosuppressive therapies or UV exposure, to promote skin carcinogenesis.

Our findings have clinical implications for the management of MPCNs in recipients of kidney transplants. BP and BD in sun-exposed areas are generally histologically indistinguishable [30]. In our study, MPCNs in sun-exposed areas showed several characteristics in common with BP, including multiple lesions, HPV-related histology, and strong p16 expression. BP typically has an indolent clinical course, and sometimes, it spontaneously regresses and restores immune suppression [31], as observed in other immunodeficiency-associated neoplasms [10, 11]. In contrast, in kidney transplant recipients, SCC was reported to be more aggressive than in immunocompetent patients [8]. Similarly, in this study, patient C had SCC in sun-exposed areas, which displayed an aggressive clinical course. Furthermore, the MPCN specimens from sun-exposed areas did not have HR-HPV infections, which suggested that they were associated with UV-exposure. Although the number of lesions included in this study was too small to draw definitive conclusions, our results suggest that MPCNs in sun-exposed areas should be treated separately from BPs that arise in anogenital areas. Furthermore, our findings suggest that the location of the lesion is the most critical factor in the differential diagnosis of BD and BP.

In conclusion, we demonstrated that MPCNs occur in sun-exposed areas in Japanese patients, consistent with findings in western patients. Uniquely, we demonstrated that the incidence of MPCNs was lower in our Asian patients than those reported for western populations. Our findings suggest that surveillance for cutaneous neoplasms in recipients of kidney transplants should be tailored according to the patient's race.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Toyonori Tsuzuki, Department of Surgical Pathology, Aichi Medical University Hospital, 1-1, Yazakokarimata, Nagakute, Japan. Tel: +81-561-62-3311; Fax: +81-561-61-3811; E-mail: tsuzuki@aichi-med-u.ac.jp

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