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ORIGIN OF SQUAMOUS AND BASAL CELL CARCINOMAS IN RECIPIENTS OF A KIDNEY ALLOGRAFT

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Abstract

Introduction

Renal allograft recipients have a significantly increased risk of developing a malignancy, of which non-melanoma skin cancers are the most common. After transplantation, donor-derived cells are frequently found in the recipient's circulation or peripheral tissues. It was recently shown in kidney transplant recipients with Kaposi sarcomas that tumor cells were of donor origin. We investigated the presence of donor-derived cells in squamous and basal cell carcinomas of kidney allograft recipients.

Methods

Forty-two tumor specimens from 17 female recipients of a male renal allograft were included. In situ hybridization of the Y chromosome was performed to detect donor-derived male cells. In a number of tumor specimens, real-time quantitative PCR (qPCR) was performed on microdissected tumor tissue for the Y chromosome-specific gene SRY.

Results

No male cells were detected by in situ hybridization of the Y chromosome in any of the skin tumor specimens. Also by qPCR of the SRY gene, no evidence of donor-derived tumor cells was obtained.

Conclusion

The chances of donor-derived progenitor cells developing into a neoplasm may differ according to the progenitor cell phenotype. Whereas it has been shown convincingly that donor-derived cells of hematopoietic origin may develop into Kaposi sarcoma, the present study shows no evidence of donor-derived progenitor cells developing into skin carcinomas.

Introduction

Malignancy occurs fourfold more frequently in renal transplant recipients than in a normal control population.[1,2] The increase in incidence differs between tumors,[2-5] but the risk is dramatically increased for some specific cancers, namely skin cancers, Kaposi sarcoma, and non-Hodgkin lymphoma.[2-4] Non-melanoma skin cancers are the most common de novo malignancies in kidney transplant recipients,[3,6] ultimately affecting 50 percent or more of white transplant recipients.[7,8] Cumulative incidence of developing a malignancy increases with increasing time after transplantation,[2,5] which led to immunosuppressive therapy being considered the main factor responsible for the increased incidence of malignancies after transplantation.[9,10]

With tumors developing in recipients after solid organ transplantation, the question arises whether the tumor cells are of donor- or recipient origin, as hematopoietic donor cells are frequently found in the recipient's circulation and donor-derived cells have been identified in several tissues.[11-13] Reports of malignancies of donor origin developing in allograft recipients are scarce.[14] In Kaposi sarcoma it was determined in a small group of 5 patients that the tumor cells were of donor origin.[15] The aim for the present study was to investigate whether squamous cell carcinomas (SCC) or basal cell carcinomas (BCC) developing after kidney transplantation derive from cells of donor or recipient origin.

Material and methods

Patients and tissue specimens

Malignant skin tumor specimens that developed in female recipients after transplantation of a kidney allograft from a male donor were identified in the Pathology database of the Leiden University Medical Center (LUMC). Renal allograft transplantations had been performed at the LUMC during the period 1971-2002. Both biopsy and excision specimens of SCCs and BCCs were included. A total of 42 tumors, 28 squamous cell carcinomas and 14 basal cell carcinomas, were included into this study. All tumor specimens were re-evaluated and they were diagnosed as infiltrative tumors of either squamous cell or basal cell origin.

In situ hybridization

To detect male cells, in situ hybridization of the Y chromosome was performed as described earlier,[16] with a DIG-labeled Y chromosome-specific DNA probe.[17] To verify the quality of the tumor tissue samples for the detection of sex chromosomes, in situ hybridization of the X chromosome was performed according to the same method, using an X chromosome-specific DNA probe.[18] A skin tissue sample from a male subject that served as a positive control for the in situ hybridization of both the X and Y chromosome showed a positive signal in 661 of 953 (69%) and 544 of 926 nuclei (59%), respectively. All tumor specimens examined showed satisfactory X chromosome staining.

DNA isolation

In 6 tumor specimens from 5 patients DNA analyses were performed. Formalin-fixed and paraffin-embedded tissue sections (4 µm thick) were stained with hematoxylin and eosin (HE) for standard morphologic analysis of tumor areas. The tumor area was then selected on a 10 µm thick tissue section stained with HE and tissue microdissection of this area was performed. DNA was extracted from the microdissected tumor areas using the NucleoSpin Tissue Kit (Machery Nagel, Düren, Germany).

Real time quantitative Polymerase-Chain-Reaction (qPCR) analysis

Real time qPCR analyses were performed of the Y chromosome-specific gene SRY to detect donor-derived male DNA and a non gender-related DNA fragment of 150 bp (ATPase Calcium transporting plasma membrane 4), to confirm the presence and quality of DNA in each sample. Quantitative PCR analyses were performed using an iCycler (Bio-Rad Laboratories, Veenendaal, the Netherlands) with iQ SYBR Green Supermix. The PCR conditions were used as described in iCycler guidelines. The primer sequences used for the SRY gene were 5'-CGC ATT CAT CGT GTG GTC TCG-3' (forward) and 5'-GCC TGT AAT TTC TGT GCC TCC TG-3' (reverse), amplifying a fragment of 159 bp. Dilution series of DNA extracted from male and female renal tissue specimens served in the qPCR of the SRY as positive and negative controls, respectively, and as positive control for the control gene. All PCR analyses contained a blank (water without DNA).

Results

We investigated 42 skin cancer tissue specimens, 28 SSCs and 14 BCCs that developed in 17 female recipients of male allografts (median 2 tumor specimens per woman, range 1-11). Clinical features of all women are summarized in Table 1. The first renal transplantation was performed on average at the age of 37 years, and mean time between first transplantation and time of tissue collection was 18 years (range 4-28 years). Ten women were transplanted once, five women were transplanted twice and two women were transplanted three times. Three re-transplanted women had received one allograft from a female donor; these were the 1st transplantations of patients 7 and 12, and the 2nd transplantation of patient 5. Nine women had more than one tissue specimen investigated. In 5 cases both biopsy and excision specimens from the same tumor were investigated (see Table 1).

Table 1. Clinical features of the renal transplant patients

		Age at transplantation (yrs)			
Patient	Primary disease	1st	2nd	3rd	Skin tumors
1	Reflux nephropathy	48			1 BCC
2	Proliferative glomerulonehritis	45			1 BCC
3	Chronic glomerulonephritis	39	54		1 SCC
4	Chronic interstitial nephritis	60			1 BCC
5	Polycystic kidney disease	39	45#		1 BCC
6	Unknown	38			1 SCC
7	HUS	20#	31	39	1 SCC
8	Chronic glomerulonephritis	39	52		4 BCC
9	Chronic glomerulonephritis	49			2 BCC
10	Polycystic kidney disease	49			2 SCC
11	Chronic interstitial nephritis	34			3 SCC
12	Polycystic kidney disease	60#	61		1 BCC
13	MPGN	30			11 SCC
14	Familial nephropathy	24	27	32	2 BCC
15	HUS	24	42		3 SCC
16	Chronic glomerulonephritis	38			1 BCC, 4 SCC
17	Alport Syndrome	39			2 SCC

HUS, hemolytic uremic syndrome; MPGN, membranoproliferative glomerulonephritis
BCC, basal cell carcinoma; SCC, squamous cell carcinoma. #, transplantation of a female renal allograft

In situ hybridization

After in situ hybridization of the Y chromosome, no male cells were detected in the tissue sections, neither in the tumor areas nor in the normal, adjacent skin (Figure 1). In situ hybridization of the X chromosome showed positive signals in all tissue specimens.

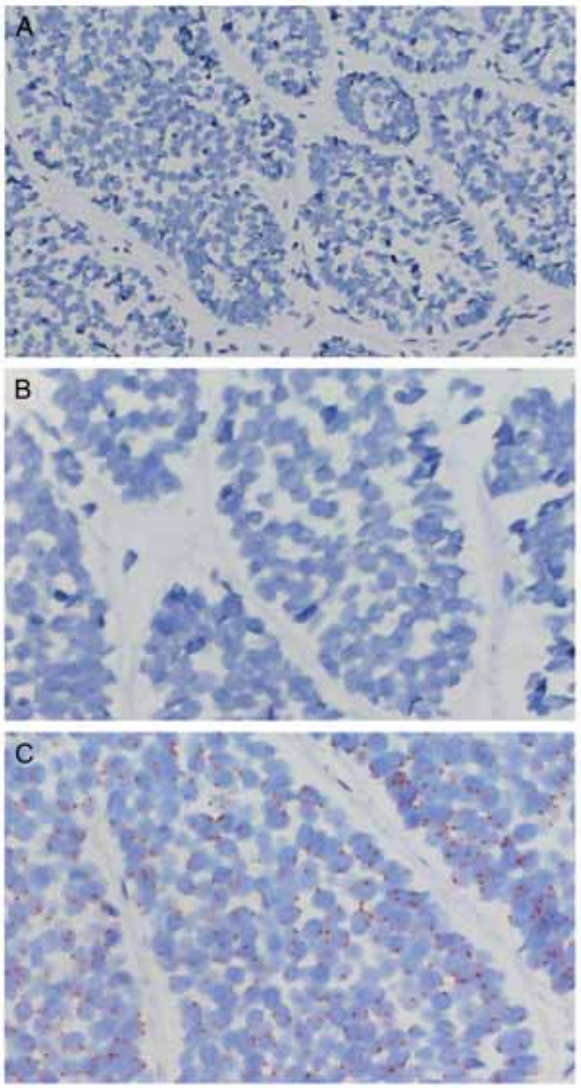

Figure 1. In situ hybridization was performed of the Y and X chromosomes. Basal cell carcinoma of patient 1 after in situ hybridization of the Y chromosome (A), which shows no Y chromosome-positive cells (B), but abundant X chromosome-positive cells are present, as indicated by the red-brown dots (C). Original magnification: 200x (A), 400x (B and C).

Real time qPCR of SRY

Real time quantitative PCR of the Y chromosome-specific gene SRY was performed on 6 different tumor specimens from 5 patients (Table 2). In none of the six tumor sections Y chromosome-sequences were detected. Male and female control DNA samples were consistently positive and negative for Y chromosome-specific sequences, respectively. The sensitivity for the detection of male DNA corresponds to an equivalent of 10 cells per DNA sample. All blank controls were negative. All six DNA samples showed good amplification of the control gene, confirming the presence and quality of DNA in each sample

Table 2. Tumor specimens with results for both ISH Y and qPCR of the SRY gene

Tissue specimen	Patient	ISH Y	qPCR SRY#
Basal cell carcinoma	2	negative	negative
Basal cell carcinoma	4	negative	negative
Squamous cell carcinoma	11	negative	negative
Squamous cell carcinoma	13	negative	negative
Squamous cell carcinoma	13	negative	negative
Basal cell carcinoma	14	negative	negative

ISH Y, in situ hybridization of the Y chromosome. #, performed on microdissected tumor sections

Discussion

The aim of this study was to investigate the origin of the tumor cells in both basal and squamous cell carcinomas in recipients of a kidney allograft. We used two different techniques to determine the origin of SCCs and BCCs in female recipients of a male renal allograft, i.e. in situ hybridization of the Y chromosome and quantitative real-time PCR for the Y chromosome-specific gene SRY. Results from both sets of experiments show that in the investigated samples tumor cells from both SCCs and BCCs are of recipient origin.

Two studies on the origin of skin tumors developing after kidney transplantation were previously published. Barozzi and colleagues demonstrated in 5 of 8 tissue specimens that Kaposi sarcomas developing after kidney transplantation originate from donor origin

using several molecular, cytogenetic, immunohistochemical and immunofluorescence methods.[15] Aractingi et al.[19] investigated non-melanoma skin cancer specimens from female recipients of a male allograft. They identified male cells by real time-quantitative PCR of the Y chromosomal-specific gene SRY and fluorescence in situ hybridization of the Y chromosome, combined with cytokeratin immunostaining. Male DNA sequences were detected in 5 of 15 squamous cell carcinomas/Bowen disease and 3 of 5 basal cell carcinomas. It was not investigated in depth how the results obtained in the PCR analysis related to Y chromosome-positive epithelial cells that were actually part of the tumors. Only one basal cell carcinoma revealed a high number of male cells in the tumor buds, of which some were cytokeratin positive.[19] Moreover, whether male cells were from donor origin was not further investigated. Whereas Aractingi et al. found male DNA in 40% of 20 investigated samples, our results show complete lack of male donor-derived cells in 42 skin tumors.

In line with the previously performed studies, we anticipated that at least in some tumor specimens selected for the present study, male cells would be present. However, none of the investigated samples in the present study contained male cells. Clinically, characteristics of the patients and tumors from our study were consistent with those of previously reported subjects and specimens in allograft recipients. Sixty-seven percent of the tumors were squamous cell carcinomas, and this is consistent with the ratio of squamous cell to basal cell carcinomas in transplant recipients (4:1).[20] Tumor excision occurred several years after transplantation (mean: 18 years), consistent with the widely acknowledged time-related increase in the incidence of post-transplant malignancies.[2,5]

There are two important factors which may have influenced our results. First, the number of SCCs and BCCs that we investigated was larger than that in the study by Aractingi et al, but in our study a relatively large proportion of tumors were derived from a small group of women: nine of 17 women had two or more tumor specimens investigated (up to 11 specimens for one woman). In five cases, biopsy and excision specimens from the same tumor were investigated. It is possible that SCCs or BCCs of donor origin only develop in particular donor-recipient related circumstances. For example, certain HLA combinations between donor and recipient cells predispose for donor-derived tumor

cells escaping the recipient's immuno surveillance: Bouwes Bavinck et al.[21] observed that homozygosity for HLA-DR shows a significant risk of developing a squamous cell carcinoma, although they did not investigate the origin of the skin tumor

Second, detection of the Y chromosome by in situ hybridization may underestimate the number of male cells in tumor specimens, because chromosomal instability is a frequently occurring event in tumors.[22] It is possible that male tumor cells show loss of Y chromosome, as has been described in a minor subset of BCCs and SCCs,[23,24] leading to a false negative result. However, if a significant number of tumor specimens would be of donor origin, it is unlikely that all samples would be affected by loss of the Y chromosome. The sensitivity of the qPCR assay was assessed and calculations based on results of qPCR of the SRY gene compared to a control gene show that male DNA would have been detected if 10% of DNA tested was male (data not shown). As qPCR was performed on microdissected tumor sections, this proportion of male DNA should be present in the DNA samples if the tumors were of donor origin.

Immunosuppressive therapy is considered the main cause for the substantial increase in the risk of developing a post-transplant malignancy,[9,10] because of a diminished capacity of T cells to kill virus-infected and neoplastic cells. Many of the frequently occurring cancers after transplantation are associated with infection by oncogenic viruses, such as human papillomavirus (in the case of skin and cervical cancers),[25,26] Epstein-Barr virus (in the case of lymphoproliferative disorders),[27] and human herpesvirus 8 (in Kaposi sarcoma).[28] For Kaposi sarcoma evidence was found that the donor-derived tumor cells were already infected with human herpesvirus 8 before transplantation.[29]

In the present study, we did not find donor-derived cells in 42 squamous and basal cell carcinomas, by using two different techniques. Involvement of donor-derived cells in tumorigenesis may only occur in certain tumors: it is possible that chances of donor-derived progenitor cells developing into a neoplasm differ according to the progenitor phenotype. Whereas it has been shown convincingly that donor-derived cells of hematopoietic origin may develop into Kaposi sarcoma, the present study shows no evidence of donor-derived progenitor cells developing into skin carcinomas.

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