Skin Carcinoma Arising From Donor Cells in a Kidney Transplant Recipient

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Abstract

The incidence of skin cancer is increased in transplant recipients. UV radiation, papillomaviruses, and immunosuppression participate in the pathogenesis of these tumors. In addition, donor cells may leave the grafted organ, reach peripheral tissues and either induce immune phenomena or possibly take part in tissue remodeling. Herein, we investigated the possible involvement of donor cells in the development of skin tumors in kidney allograft recipients. We analyzed a series of 48 malignant and benign cutaneous tumors developing in 14 females who had been grafted with a male kidney. The number of male cells was measured on microdissected material by quantitative PCR for Y chromosome. In the samples with high levels of male cells, fluorescent in situ hybridization (FISH) with X and Y probes and/or immunofISH with anticytokeratin antibodies were carried out. Male cells were detected in 5/15 squamous cell carcinomas and Bowen disease (range 4-180 copies), 3/5 basal cell carcinomas (91-645), 6/11 actinic keratosis (7-102), 2/4 keratoacanthoma (22-41), and 2/5 benign cutaneous lesions (14-55). In a basal cell carcinoma specimen with a high number of male cells, FISH showed that most cells within the tumoral buds were XY. In this lesion, immunofISH showed the presence of XY cytokeratin-positive cells indicating that the tumor nests contained male keratinocytes. In contrast, in other female transplants, male cells present in the tumors were not epithelial. In conclusion, stem cells originating from a grafted kidney may migrate to the skin, differentiate, or fuse as epithelial. In conclusion, stem cells originating from a grafted kidney may migrate to the skin, differentiate, or fuse as epithelial. In conclusion, stem cells originating from a grafted kidney may migrate to the skin, differentiate, or fuse as epithelial.

Introduction

Solid graft transplantation is associated with an increased risk of neoplasms, essentially cutaneous carcinomas, and to a lower degree, lymphomas (1-3). In kidney graft recipients, the relative risk of development of skin carcinomas is 10 to 250 times higher than in matched controls (4). UV-induced mutations (5, 6), human papillomavirus infections (7, 8), and immunodepression have been implicated in the pathogenicity of these tumors. Occurrence of carcinomas is also correlated with human leukocyte antigen B mismatching between donor and recipient (9) suggesting the involvement of immunologic phenomena. In solid organ transplant recipients, hematopoietic donor cells are frequently found in recipient blood and microchimeric cells are found in different tissues (10, 11). This phenomenon may be involved in low-grade graft versus host disease (12), or in contrast, in specific tolerance towards the donor (13). Besides, plasticity of hematopoietic stem cells has been shown with possible differentiation of CD34-positive transferred cells into epithelial cells such as keratinocytes (14). Similarly, fetal microchimerism may participate in tissue repair or remodeling because a fully differentiated thyroid follicle was composed of male XY cells in a female patient—previously pregnant with a male baby—who had a progressively enlarging goiter (15). Thus, donor cells which circulate in the recipient body may have immunomodulatory activity and/or differentiate into various tissues.

To investigate the possible involvement of these two phenomena in transplant recipient tumor development, we measured the amount of donor-derived cells in benign and malignant skin specimens from kidney transplant recipients. Fluorescent in situ hybridization (FISH) on specimens displaying high amounts of donor cells allowed us to find one basal cell carcinoma (BCC) deriving from the donor.

Materials and Methods

Specimens. A series of 48 routine cutaneous biopsies from 14 female recipients grafted with a male kidney were collected for this study. Paraffin-embedded sections of different thicknesses were used for microdissection or FISH, or FISH together with immunohistochemistry (immuno-FISH). In addition, control specimens corresponding to BC cells from nongrafted females (n = 8), from nevi obtained from prepubertal girls (n = 8), and from male inflammatory skin, nevi, and BCC (n = 5) were also analyzed.

Evaluation of the Density of Inflammatory Infiltrate. Because the peripheral blood of transplant recipients may also contain donor cells, we evaluated the inflammatory infiltrate found in these tumors. Skin cancers arising in grafted recipients are known to disclose less dense inflammatory infiltrates when compared with similar tumors in immunocompetent individuals (16). Thus, finding differences in microchimerism in skin samples could simply reflect differences in inflammation with passive recruitment of donor circulating cells within the skin tumors. Blinded evaluation of the inflammatory infiltrate was done by one of us (J. Kanitakis). The density was graded as discrete (1), median (2), or intense (3).

Microdissection. Microdissection was done on thick paraffin-embedded 8-µm sections. Special precautions were taken to prevent PCR contamination. Sections were carried out by a single female technician, each time using a new slide box and a sterile knife. Sections were deparaffinized in toluene for 10 minutes, rinsed in four successive ethanol solutions (pure, 75%, 50%, and 30%) and finally in water. After drying, the sections were incubated in 2.5% glycerol for 3 minutes. In order to obtain identical volumes of microdissected tissue in all specimens, a 4 mm diameter surface was delineated with a 4 µm punch biopsy. Then, using the tip of a sterile
needle (25-gauge), the 4 mm surface was gently scraped until detachment. The pooled material attached to the needle was transferred into an Eppendorf tube and subjected to the digestion process described below.

**Real-time Quantitative PCR.** Scraped material from the tissue sections was digested with 50 mmol Tris-HCl, 1 mmol EDTA, Tween 20 0.5%, 200 μg/mL proteinase K in a final volume of 50 μL at 37°C overnight followed by denaturation at 95°C for 20 minutes. Ten microliters of the digested material was amplified in a 25 μL volume with the following SRY primers: 245R, 5'-CCC CCA GTC ACA GTC TAT T-3' and 109F, 5'-Tgg Cgt TTA Ggt CAA ATT CGC-3', the probe being 142T-Fam/Tamra: 5'-AgC AgT AgA gCA gTC Agg gAg gCA gA-3.

To enable quantitation, a scale was constructed by digestion of serial dilutions of male cells into 2,000 female cells (the average number of amplifiable genomes recovered from 1/5 of the digested tissue sections). The scales were amplified in the same experiments as the experimental samples to compute absolute values for SRY copies. Real-time quantitative PCR was carried out in an ABI prism 7700 apparatus. The scales were amplified in the same experiments as the experimental samples to compute absolute values for SRY copies. Real-time quantitative PCR was carried out in an ABI prism 7700 apparatus. The scales were amplified in the same experiments as the experimental samples to compute absolute values for SRY copies.

**Fluorescent In situ Hybridization.** Samples harboring more than 20 copies of Y sequence by PCR as well as a few negative controls were analyzed by FISH when material was available. FISH was carried out as described (17). Briefly, 5-μm skin sections were deparaffinized and then rehydrated in ethanol series. DNA was denatured by incubation in 0.2 mol/L HCl for 15 minutes. After incubation with proteinase K, the slides were treated with 4% formaldehyde and dehydrated. The XY cocktail probes (Vysis, Downers Grove, IL) was applied and after closure with rubber cement, the probes and the DNA were denatured and incubated overnight at 42°C. The next day, counterstaining was done with 0.03 μg/mL 4',6-diamidino-2-phenylindole. The percentage of male cells was quantified by counting the intranuclear X,Y spots in several fields using the following formula:

$$\frac{100Y}{X-Y^2}$$

**Results**

**In Allograft Recipients, Microchimerism is Found in All Categories of Skin Specimens.** To detect the presence of male cells in allograft recipient skin tumors, DNA was recovered from the tumor tissue sections and subjected to Y sequence-specific PCR amplification. Amplification curves of the samples were

![Figure 1. SRY real-time PCR analysis of male cells diluted in female cells. The experiment shows that one to two male cells are detected. The curves corresponding to the prepubertal female nevus are not presented because there was no signal.](image-url)
compared with those of serial dilutions of male into female cells enabling absolute quantification of the male copy number. Numerous negative controls [without DNA and prepubertal female skin samples (nevi)] were included in all experiments to show the absence of PCR contamination. The Y detection sensitivity was one to two copies. Because the number of amplifiable genomes was 500 to 3,000 cells as judged by amplifying a one-copy autosomal gene (data not shown), the microchimerism sensitivity detection limit was between 0.2% and 0.03%. In addition, analysis of female peripheral blood lymphocytes as well as of eight prepubertal females skin samples (nevi) and eight BCC from nongrafted females showed the absence of any Y signal, confirming the specificity of quantitative PCR which has been previously validated by others (19).

The complete results are shown in Fig. 2. Amplifiable DNA was present in 40/48 specimens. Male cells were detected in 5/15 squamous cell carcinoma/Bowen specimens, 3/5 BCC, 6/11 actinic keratosis, 2/4 keratoacanthoma, and 2/5 benign lesions. In the positive samples, the ranges of male copy numbers were 4 to 180 in squamous cell carcinomas, 91 to 645 in BCC, 7 to 102 in actinic keratosis, 22 to 41 in keratoacanthoma, and 14 to 55 in benign lesions. In the eight nongrafted females with BCC or nevi, male cells were never detected (Fig. 2), confirming the specificity of the procedure.

There was no statistically significant differences in the number of male cells as well as the number of Y-positive samples between the groups (Kruskal-Wallis test).

The mean inflammatory infiltrate densities were 2.3 in the patients with squamous cell carcinoma/Bowen (range, 1-3), 2.4 in
those with BCC (1.5-3), 2.35 in the actinic keratosis group (1–3),
2.33 in keratoacanthoma (2-2.5), and 1 within benign lesions. As
expected, the degree of inflammation was significantly lower in
the benign lesions (Kruskal-Wallis test; \( P < 0.03 \)) but was not
different between all the other groups of malignant or
premalignant lesions.

A Specimen of Cutaneous Basal Cell Carcinoma Seems to be
Derived From Donor Cells. One specimen (gr9) revealed a very
high level of Y sequences by PCR (645 Y sequence; see Fig. 2),
suggesting that this peculiar dorsal BCC could have been derived
from male keratinocytes. In order to confirm this hypothesis, FISH
for Y and X chromosome was done. In gr9 (Fig. 3A and B), many
XY cells were seen in tumoral buds, the average percentage of male
cells in nine fields (taking into account both the tumor buds and
the overlying epidermis) was 40 ± 10% (mean ± SE). Of note,
normal overlying epidermis only displayed XX cells (Fig. 3A). The
count of tumoral buds alone showed that there was sometimes up
to 105% of cells displaying XY genotype. In control male skin
(Fig. 3C), the percentage of male cells was 103 ± 5%. In two other
allografted female samples in which PCR had not found
Y sequences, FISH was also negative for Y chromosome
(an example is shown in Fig. 3D). To more precisely show that
the tumor epithelial cells were derived from male cells, immuno-
FISH with anticytokeratin antibody was carried out. Several
cytokeratin-positive cells within dermal nests seemed to be XY,
showing that malignant keratinocytes of this BCC gr9 were male
(Fig. 4A and B, to compare with histology, D). In a classic sample
of male BCC, a very similar picture was found (Fig. 4C); not all
the cells displayed Y chromosome. Substitution of anticyto-
keratin antibody by IgG showed the absence of any staining.

Figure 4. Immuno-FISH using X,Y cocktail and
anticytokeratin AE1/AE3 antibody. A and B, gr9 showing cytokeratin-positive buds, several XY
cells being present in these cytokeratin buds (white arrows). Yellow arrow, a nonepithelial,
cytokeratin-negative, male cell. C, result of a male
nongrafted BCC showing results similar to gr9. D, the
H&E-stained section of gr9 with typical dermal
nests of BCC under the epidermis. E, specimen of a
female patient allografted with a male kidney
and with Y sequence positive by PCR. XY cells do not
appear to be surrounded by cytokeratin blue
staining, indicating that these male cells were not
epithelial (yellow arrow). F, actinic keratosis
developing in the same female as gr9 but at another
skin site. Presence of a few XY cells surrounded
by cytokeratin within proliferating epidermis shows
male keratinocytes in this dysplastic lesion
(white arrows).
demonstrating the specificity of the immune reaction (data not shown). Interestingly, some nonepithelial XY cells were seen between buds in gr9, probably corresponding to inflammatory infiltrating cells (Fig 4B, yellow arrow). Of note, the female bearing the gr9 BCC had nine different skin samples analyzed initially by PCR. Besides gr9, two other specimens (gr42 and gr55) displayed male sequences by PCR. One of these, an actinic keratosis (gr55) with 47 sequences of Y DNA, showed the presence of some cytokeratin-positive XY cells (Fig. 4F). In contrast, six other specimens (two actinic keratosis, two squamous cell carcinomas, one keratoacanthoma, and one BCC) from this woman were negative by PCR. Two of these were nevertheless analyzed in situ and FISH was also negative, demonstrating the correspondence between the two techniques. Therefore, the presence of male cells is restricted to some sites in this female recipient.

The analysis by immuno-FISH of six other lesions (gr27, gr28, gr65, gr68, and gr110) obtained from other female patients, which showed the presence of male cells by PCR revealed the presence of noncytokeratin-positive XY cells (an example is shown in Fig. 4E, yellow arrows). Combination of FISH and CD45-common leukocyte antigen-staining was done and also showed the presence of CD45+ microchimeric cells (Fig. 5).

**Discussion**

These results show that in one patient, a skin carcinoma in a kidney recipient derived—at least largely—from the donor. Indeed, dissected material from a BCC section revealed a very high level of male sequences. Using FISH, male cells were found in the tumor nests, but not in the normal epidermis, suggesting clonal expansion of a single donor cell. Finally, immuno-FISH showed in this tumor a picture similar to the control male BCC with nests containing several cytokeratin-positive male cells. All these data show that epithelial tumoral buds were indeed largely composed of male keratinocytes in a female patient. In another specimen obtained from another site of the skin of the same female and featured by an actinic keratosis, a dysplastic neoplastic lesion, male keratinocytes were found within the proliferating epidermis. In contrast, seven other cutaneous specimens issued from the same recipient did not reveal the presence of male epithelial cells, indicating that the presence of male keratinocytes within this female was not a generalized finding. Of note, because the study was done on archival specimens, we could not test the presence of donor cells within the recipient’s peripheral blood lymphocytes. However, such findings are frequently described in kidney transplants, reaching levels up to 70% of cases in the literature (19, 20). In accordance, in some skin specimens, there were some donor cells expressing CD45.

BCC is a primitive carcinoma of the skin derived from keratinocytes. It is a locally invading process and metastases are extraordinarily rare events despite prolonged evolution. Only few cases of metastatic BCC are reported, these being mainly in lymph nodes or in surrounding soft tissues (21). The hypothesis that gr9 could be a metastatic BCC transferred from a donor BCC located within the kidney is unlikely, not only because such metastasis are exceptional, but also because the histopathology of gr9 was typical of a primitive BCC (22). In addition, the gr9 BCC developed 17 years after transplantation, this delay seems too long for a metastatic evolution. Importantly, male keratinocytes were present in an actinic keratosis at another cutaneous site in the same recipient’s skin. Actinic keratosis is a neoplastic dysplastic lesion which may evolve in a BCC but in contrast can never derive from a BCC. Of note, other skin cancers, including BCC, arising at other sites of this female patient were exclusively composed of female cells, demonstrating that this patient was prone to multiple primitive tumors. Thus, gr9 appears as a male BCC that could not have originated from an adoptively transferred tumor.

Several publications recently reported the fate of chimeric cells in recipient’s tissues. When female patients were grafted with male peripheral blood hematopoietic stem cells, male cytokeratin-positive keratinocytes were found within the normal skin (14). In transplanted hearts without any rejection or inflammation, 9% to 10% of myocytes, arterioles, and capillaries were in fact issued from the host (23). In the same way, in grafted kidneys, 23% to 38% of mesenchymal cells originated from the recipient rather than the donor (24). Such seeding does not result only from allogeneic transplantations, but also from pregnancies. Indeed in a female, liver male cells—precisely analyzed by PCR—showed that these originated from a stillbirth which occurred 20 years earlier (25). The presence of fetal mesenchymal stem cells in the marrow of females has recently been shown (26), while evidence for the presence of fetal cells differentiated in several types of peripheral organs in mothers of male babies born several years or decades before were also obtained (27).

The kidney recipient female with the gr9 and gr55 tumors had never been pregnant but was transfused 18 years before. The donor cells which are implicated in carcinogenesis were therefore most probably provided by her male grafted kidney rather than by blood transfusion. In this observation, the donor cells adopted the host tissue phenotype. Whether the donor cells giving rise to keratinocytes are hematopoietic or tissue-specific stem cells (28) is unknown.
Our results show that donor cells, displaying a keratinocyte phenotype, may undergo cancer transformation in the host. Cancer transformation of these cells is possible. Indeed, these cells will behave in the recipient's epidermis as other keratinocytes. Events implicated into cancer transformation in the skin of solid graft recipients are mainly mutations induced by UV radiation. Human papillomavirus infection may augment this process by impairment of the process of DNA repair or by prevention of apoptosis after exposure to UV radiation (5–8). Keratinocytes issued from the transdifferentiation of donor stem cells may therefore undergo transformation under the influence of the same factors. Alternatively, these donor-derived male keratinocytes may be fused cells. Indeed, a recent study have shown that, in vitro, stem cells could fuse with differentiated cells leading to overdiploid cells which adopted the differentiated phenotype (29). Because archival specimens were fixed, karyotypic analysis was not done, we cannot exclude that such fusion may have occurred here. Overdiploid cells would be—because of their genetic abnormalities—prone to undergo cancer transformation.

A recent study interestingly showed that Kaposi's sarcoma cells in renal transplant recipients harbored in five out of eight samples, markers of the donor demonstrating that such neoplastic cells may frequently derive from donors (30). However, Kaposi's sarcoma progenitor cells are possibly hematopoietic cells. Therefore, although the trafficking of donor hematopoietic cells in skin lesions of grafted patients is a frequent phenomenon—as illustrated in the Kaposi's sarcoma study (30) and in this study (in most of our samples, male cells were not epithelial)—the presence of donor-derived epithelial tumoral cells remains a rare event.

In conclusion, after transplantation, there are donor cells that can seed (or fuse with) the skin epithelium where they may rarely undergo neoplastic transformation. Future studies targeting the origin of cancers in other situations with putative microchimerism, such as pregnancy or RBC transfusion, may help to determine if such new possibilities exist.

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References

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