ORIGINAL ARTICLE

Role of HHV-8 and mTOR pathway in post-transplant Kaposi sarcoma staging

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SUMMARY

Kaposi's sarcoma (KS) is one of the most frequent transplant related tumors. Several pathways are involved; however, the impact of the molecular phenotype associated to the tumor stage and the behavior-depending resultant therapy is still unknown. The aim of our study was to analyze the role of HHV-8 and mTOR pathway in tumor stages of skin KS after renal transplantation. Twelve renal transplant recipients with cutaneous KS from five transplant centers (1980–2007) under reduction of immunosuppression or conversion to mTOR inhibitor were included. The expression of HHV-8, PTEN, TGFβ, VEGF, phospho-mTOR, and phospho-P70S6K in tumoral tissue was analyzed. KS lesions were classified as patch, plaque, and nodule state. HHV-8 infection was found in all tissue samples. KS lesions showed high activation of VEGF, p-mTOR and p-P70S6K, low PTEN, and null TGFβ expression. The only pathway activated in a staging-dependent manner was mTOR with higher p-mTOR and p-P70S6K expression in nodule versus patch stage. KS lesions disappeared after 5.24 months in all converted patients without any recurrence in 14.05 years of mean follow-up. The activation of mTOR pathway according to KS stages supports the rational of the mTOR inhibitor in post-transplant Kaposi.

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Introduction

Kaposi's sarcoma (KS) incidence in transplant recipients is dramatically increased 500-fold compared to age-matched controls [1]. KS is a multifocal vascular neoplasm strongly linked to infection by human herpes virus 8 (HHV-8) [2], and seropositive recipients have 50-fold higher risk of KS than uninfected recipients [3–5]. Additionally, other factors have been related with KS; age higher than 60 years old, male sex, end-stage renal disease, and the inherent risk of transmission related with chronic renal disease on dialysis who receive HHV-8 blood transfusions [6,7]. HLA-B mismatching has been shown as a risk factor in the USA, highlighting the role of immunosuppression and immune stimulation in KS pathogenesis [8]. In this context, the high prevalence of KS post-transplant suggests an important role of interactions between viral infection, the immune system, and host factors.

The alteration of the immune surveillance system during the immunosuppressive therapy as well as the pro-angiogenic mechanisms independent of the host immunity favored by calcineurin inhibitors, through vascular endothelial growth factor (VEGF) and transforming growth factor β (TGFβ), has been proposed as oncogenic mechanisms for KS in transplant recipients [9–11]. The traditional approach to managing transplant-associated Kaposi's sarcoma was to reduce or even discontinue immunosuppressive therapy; skin lesions usually regressed; however, it carried a risk of graft rejection. Kaposi's sarcoma generally recurred when immunosuppressive therapy was reintroduced or after a second transplantation [12].

On the other side, Montaner *et al.* [13] proposed another mechanism of carcinogenesis for the development of KS, through a single lytic gene of the HHV-8, viral receptor coupled to the protein G (vGPCR), which is able to produce vascular tumors in mouse models. This receptor activates AKT, which has been recognized as an essential kinase to link PI3K and mammalian target of rapamycin (mTOR) pathways [14].

The mTOR pathway is a critical intracellular route in the regulation of several cellular processes including cell proliferation and survival, cell size and response to nutrient availability, intermediary metabolism, angiogenesis, and tissue invasion [15]. The mTOR acts by directly activating P70S6 kinase (P70S6K), a primary downstream effector of mTOR that regulates protein synthesis. In KS mouse models and AIDS, KS has shown elevated levels of P70S6K, and its level of expression has been used as a predictor of early tumor response to the mTOR inhibition sarcomas in nontransplant patients [16,17].

There are clinical evidences about mTOR inhibition and skin KS regression being safe for the transplant patient without loss of the renal graft [18,19]. Furthermore, several markers have been proposed as markers of the mTOR pathway activity and its activity as a marker or mTOR inhibition responses, including the P70S6K, the phosphatase and tensin homolog deleted on chromosome ten (PTEN), a tumor suppressor protein, and TGFβ.

The aim of our study was to analyze the role of HHV8 and mTOR pathway in tumor staging of KS as a predictor of long-term graft and patient survival and the sensitivity to mTOR inhibitor therapy.

Materials and methods

Study population

HIV-negative renal transplant patients who received a renal graft in five transplant centers in Spain between 1980 and December of 2007 and developed cutaneous KS at least 1 month after transplantation were analyzed. Patients who developed visceral KS and those with cutaneous KS and without enough sample for the complete immunohistochemical analysis were excluded. The immunosuppressive management after KS depended on era and transplanting center: reduce or eliminate calcineurin inhibitor (immunosuppression reduction group) versus conversion to mTOR inhibitor (conversion group). In the reduction group, the calcineurin inhibitor was reduced or eliminated. Conversion strategy was planned within 2 weeks after KS diagnosis. The decision of use a rapamycin loading dose was based on clinical practice of each center to achieve rapamycin blood levels between 4 ng/ml and 8 ng/ml. The patient follow-up was conducted according to each center clinical practice until KS diagnose. Then, a dermatologist performed KS follow-up every 3 months. Transplant physician intensified the follow-up to the immunosuppressive management and achieve immunosuppressive target levels. The follow-up of the study was conducted until September of 2015. The study was approved by the local ethics committees in Spain and was performed in accordance with the declarations of Helsinki and Istanbul.

Histology

The primary tumor specimens were obtained from the tumor bank of each center. Tissue sections (3-μm-thick)

were mounted on HistoBond adhesion microslides, dewaxed, and rehydrated. Hematoxylin/eosin (H/E) was performed in one from every five slices to check the adequate orientation of the preparation. H/E was performed to localize the most important types of cells, vessels, and muscle fibers as well as to differentiate muscle fibers, cells nuclei, fibrin, and collagen (data not shown).

The KS was diagnosed in all cases by histopathological evaluation after H/E staining. KS lesions were classified as patch, plaque, and nodule state.

Immunohistochemistry

Sections (3-μm-thick) mounted on xylene glass slides (Dako, Carpinteria, CA, USA) were used for immunohistochemistry. All procedures were performed as previously published by our group [20,21]. After antigen retrieval carried out by 5 min pressure-cooking in 10 mM sodium citrate buffer (pH 6.0), endogenous peroxidase blocking for 10 min in 3% hydrogen peroxide (Merck, Darmstadt, Germany) was performed before primary antibody incubation.

The primary antibodies were anti-HHV-8 (Adv. Biotechnologies Inc., Columbia, MD, USA), anti-PTEN, anti-phospho-mTOR (Ser 2448) (p-mTOR), and anti-phospho-P70S6K (Thr389) (p-P70S6K) from Cell Signaling (Beverly, MA, USA); anti-TGFβ(Clone TGFB21) and anti-VEGF (Chemicon, Millipore corp., Billerica, MA, USA). The distinct primary antibodies used in this study were incubated overnight at 4 °C except for anti-HHV-8 that was incubated for 60 min at room temperature. Envision system-specific anti-rabbit secondary antibodies, labeled with horseradish peroxidase polymer (Dako, Glostrup, Denmark), were applied for 30 min. All sections were counterstained with Mayer's hematoxylin for 1 min. Immunohistochemical procedures for each antibody were performed at the same time to avoid possible day-to-day variations in staining performance.

Controls for specificity of immunohistochemistry were performed by omitting any essential step of the immunoreaction or by substituting the primary antibody with an equivalent concentration of nonimmune immunoglobulin. The analysis of the samples was performed independently by two different experienced observers blinded to the tumor pattern and to the patient outcome. A semiquantitative method was performed to evaluate extension of positivity of these proteins in the cytoplasm and nucleus of tumoral cells and epidermal cells adjacent to the tumor based on the following categories: (0) absence, (1) low (<25%), and (2) high expression (>25%).

Statistical analysis

Statistical analysis was performed using Graph Pad Prism version 5 (GraphPad Software Inc., San Diego, CA, USA) or the IBM SPSS 19.0 statistics package (IBM, Armonk, NY, USA). Data are expressed as the mean ± standard deviation and were analyzed using nonparametric *t*-test (Mann–Whitney *U*-test). Univariate analysis using the log-rank test was conducted to visualize (Kaplan–Meier curves) and assess graft and patient survival (time from kidney transplantation to graft lost, and to death or last follow-up, respectively) in the longitudinal cohort of patients with KS. All presented *P* values are two-sided, and $P < 0.05$ was considered to be statistically significant.

Results

Immunosuppression and clinical outcome

The mean incidence of cutaneous KS in the five centers analyzed was 0.25% in the period of study. Twelve renal transplant patients with KS lesions were included (Table 1). Eleven patients received calcineurin inhibitor (tacrolimus–cyclosporine: 3–8 patients) treatment in the first month of transplant, and only one patient received an anticalcineurinic-free treatment with antilymphocytic antibodies. Seven patients were on mycophenolic acid and 5 on azathioprine, and all of them were on prednisone as coadjuvant therapy. The median time of KS onset after renal transplantation was 8.5 months (min–max: 5–282 months). The mean age at the time of KS diagnosis was 65 years (range: 49–76 years), and 75% of the patients were male.

The patients developing KS before the year 2000 ($n = 6$) were treated with a reduction of immunosuppression, which caused in all cases loss of the graft (min–max: 1–36 months). In reduction group, calcineurin inhibitors and azathioprine, four and one patients respectively, were discontinued by a short withdrawal interval of 2 weeks, and mycophenolate reduced. Two patients died with functioning graft 2.5 months due to cardiovascular and terminal liver disease. The remaining patients return to dialysis died related to cardiovascular reasons (3/4) or sepsis (1/4). None patient died due to KS. The patients presenting with KS after the year 2000 ($n = 6$) were switched to rapamycin as a strategy for skin KS treatment. In four out six patients the calcineurin inhibitors elimination and mTOR inhibitor introduction were performed within 2 weeks. In two converted patients, rapamycin was introduced with calcineurin inhibitors withdrawn in a longer time. Fifty percentage of patients received rapamycin loading dose, and all patients

Table 1. Patient demographics and baseline characteristics.

	Conversion group (n = 6)	Reduction group (n = 6)	P-value
Mean age at KS ± SD (years)*	67.83 ± 3.37	62.33 ± 8.87	
Male gender, *n* (%)†	4 (66.7)	5 (83.3)	0.963
Smoking habit, *n* (%)†	2 (33.3)	4 (66.7)	0.963
Hypertension, *n* (%)†	6 (100)	5 (83.8)	0.992
Diabetes, *n* (%)†	1 (16.7)	0 (0)	0.992
Dyslipidemia, *n* (%)†	5 (83.8)	6 (100)	0.963
Cardiovascular disease, *n* (%)†	1 (16.7)	2 (33.3)	0.776
Etiology of CKD, *n* (%)‡			
Poliquistosis	3 (50)	0 (0)	0.396
Glomerulopathy	0 (0)	2 (33.3)	
Interstitial nephropathy	2 (33.3)	1 (16.7)	
Unknown	1 (16.7)	3 (50)	
Time on dialysis (months)*	37.89 ± 45.36	27.86 ± 29.62	0.660
Previous transplants, *n* (%)†			
0	5 (83.3)	6 (100)	0.341
1	1 (16.7)	0 (0)	
Immunosuppression at KS†			
CNI, *n* (%)	6 (100)	5 (83.3)	0.341
AZA/MMF, *n* (%)	6 (100)	6 (100)	1.000

KS, Kaposi sarcoma.

**t*-student; Levene = variances.

†Fisher Chi-Square.

‡Pearson Chi-Square; NS, non-statistical significance; CKD, chronic kidney disease; CNI, calcineurin inhibitors; AZA, azathioprine; MMF, mycophenolate.

achieved to the target rapamycin blood levels. Two converted patients lost the graft: one returned to dialysis and the other one died due to mitral insufficiency 8 and 12 years after conversion, respectively. Conversion to mTOR inhibitor significantly increased the time of graft survival compared to patients who were treated with a reduction of immunosuppression (169.4 ± 60.9 months vs. 12.7 ± 17.0 months; $P = 0.0022$) (Fig. 1a). Similar results were found in death-censored graft survival analysis ($P = 0.0029$) (Fig. 1b). Conversion strategy prolonged significantly patient survival compared to reduction strategy (169.4 ± 60.9 months vs. 40.4 ± 54.6 months; $P = 0.0089$) (Fig. 1c).

Human herpes virus-8 expression in Kaposi's sarcoma skin lesions

HHV-8 was detected in all cases with a nuclear expression (Fig. 2a).

Activation of TGFβ, VEGF, and mTOR pathways in Kaposi's sarcoma skin lesions

Transforming growth factor β was not expressed in tumor; however, TGFβ endoluminal staining was found in 11 cases (Figs 2b and d). In contrast, VEGF was highly expressed by KS cells and epidermal cells adjacent to the tumor in all cases (Figs 2c and d).

Considering mTOR pathway, PTEN was expressed in 75% of KS samples. However, this staining was deemed as low in 89% of the cases (Figs 2e and h). The expression of p-mTOR was medium or high in all cases (Figs 2f and h). Positive nuclear staining for p-P70S6K1 was observed in all cases and some of them presented also cytoplasmic staining (Figs 2g and h).

Kaposi's sarcoma stages; comparative analysis of the immunohistochemistry

Patch state of KS lesion was diagnosed in four patients, five cases were lesion in plaque, and three patients showed lesion in nodule state (Fig. 3a). VEGF was highly expressed in all stages of KS lesions. A clinical correlation between the status of the KS and the expression of several mTOR pathway molecules was found. In addition, the differences between low and high stages of KS in mTOR expression were higher in downstream molecules (p-mTOR and p-P70S6K) than PTEN (Fig. 3b).

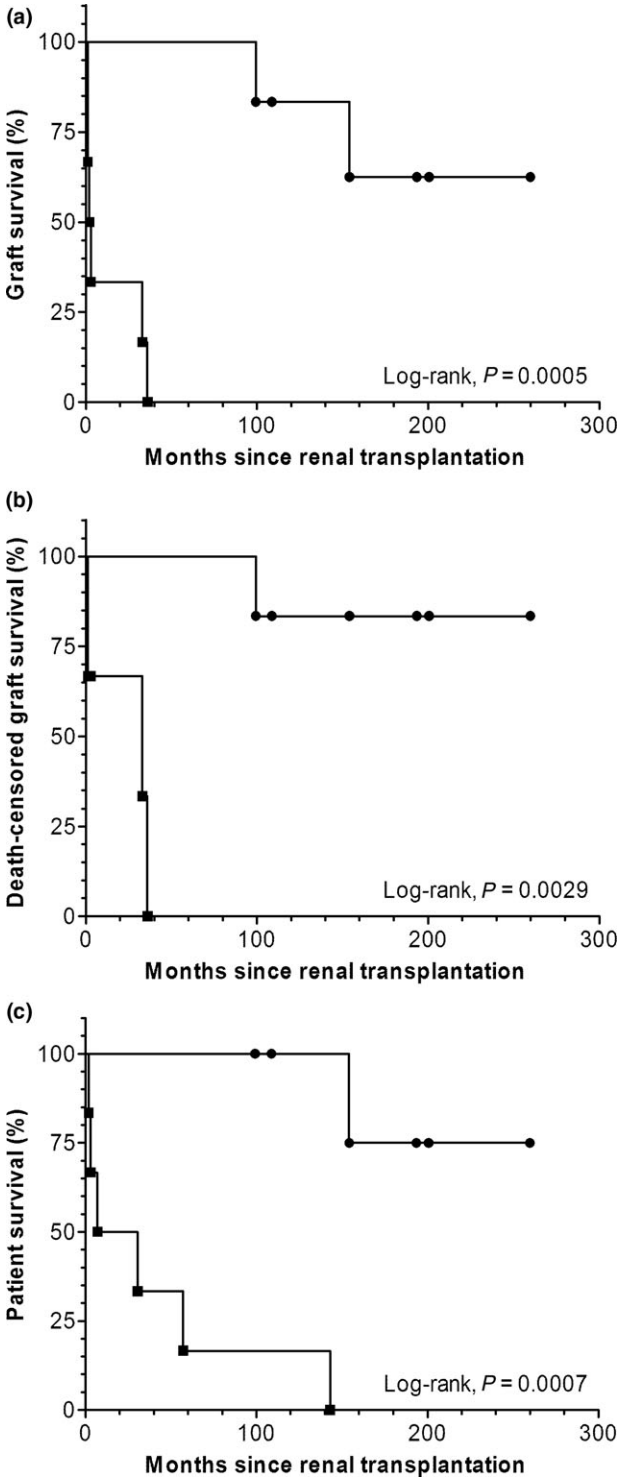

Figure 1 (a) Kaplan–Meier plots of graft survival after renal transplantation. (b) Kaplan–Meier plots of death-censored graft survival after renal transplantation. (c) Kaplan–Meier plots of patient survival after renal transplantation. Reduction group (circle) includes all patients that after KS diagnosis, the calcineurin inhibitor was reduced or even eliminated. Conversion group (square) includes all patients that after KS diagnosis were converted from calcineurin inhibitor to mTOR inhibitor treatment.

Effect of mTOR inhibition on Kaposi's sarcoma skin lesions and long-term follow-up

The cutaneous lesions disappeared macroscopically after 5.24 ± 6.42 months (min–max: 0.9–18.0 months) in all patients converted to mTOR inhibitors. Clinical remission was confirmed in four cases through the histological analysis of another skin specimen from the site of a previous Kaposi's sarcoma lesion. All rebiopsies were negative for Kaposi's sarcoma.

None of patients under mTOR inhibitor treatment presented recurrence of KS lesions, only one patient developed a *de novo* malignancy after 6 years of conversion: an esophageal adenocarcinoma unrelated with KS extensively described by Canha *et al.* [22].

Discussion

Renal transplant patients with Kaposi's sarcoma skin lesions in different transplant centers were analyzed. HHV-8 infection was found in all KS samples, and mTOR pathway was activated in different KS stages.

Previous reports have been shown that tapering immunotherapy in post-transplant KS had benefit in cutaneous lesions; however, the majority of patients lost the graft [23–25]. In contrast, mTOR inhibitor conversion offers complete remission without any detrimental effect on graft function [18,19]. In our reduction group, all grafts were lost within 3 years after transplantation, whereas all patients converted to mTOR inhibitors preserved renal graft function at least 8 years after transplantation (6.5 years after mTOR inhibitor conversion). Cumulative graft survival was 62.5% at 14.08 years of mean follow-up (10.1 years since mTOR inhibitor conversion), rising to 83.3% in death-censored graft survival analysis. In addition, one patient was followed during 260 months (21.7 years) after transplantation, who remained 147 months (14 years) under mTOR inhibitor therapy.

Cancer development reduces patient survival in transplant patients [26,27]. Despite a lack of information about KS-related mortality in transplant patients, a trend to higher mortality in KS renal transplant patients has been reported [28]. In our cohort, 83.3% of patients from the reduction group died before 5 years after transplantation, whereas all patients converted to an mTOR inhibitor at least survived 9 years. Cumulative patient survival showed that 75% patient stayed alive at least 12.8 years, and one patient was followed during 21.7 years after transplantation.

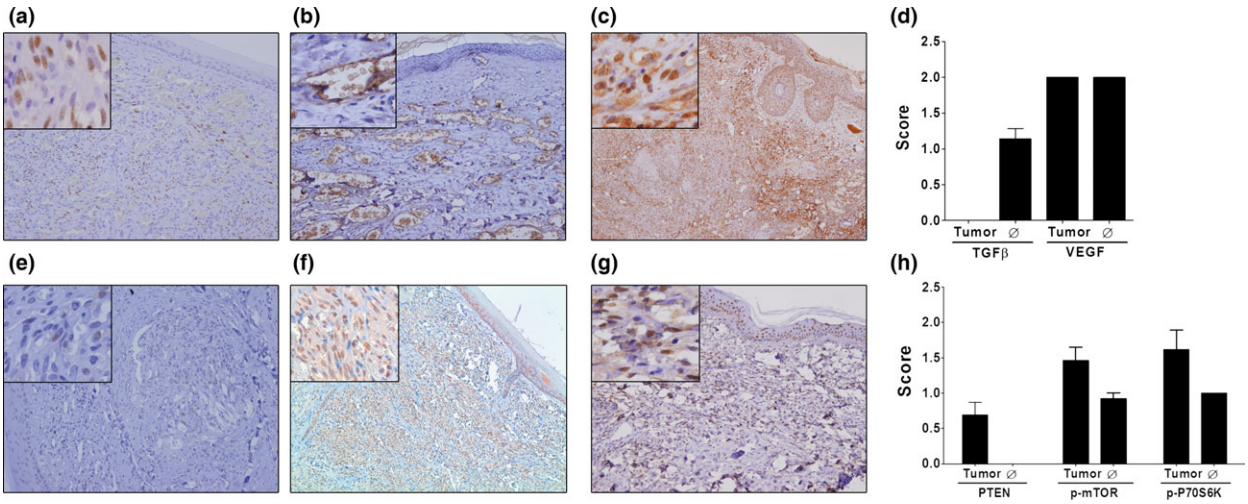

Figure 2 HHV-8 infection and the activation of TGFβ, VEGF, and mTOR pathways in Kaposi's sarcoma skin lesions. (a) Positive immunostaining for HHV-8. (b) Positive immunostaining for TGFβ. (c) Positive immunostaining for VEGF. (d) Quantification of TGFβ and VEGF staining into tumor cells and epidermal cells adjacent to the tumor (Ø). (e) Positive immunostaining for PTEN. (f) Positive immunostaining for p-mTOR. (g) Positive immunostaining for p-P70S6K. (h) Quantification of cytoplasm and nuclear PTEN, p-mTOR, and p-P70S6K staining in tumor and tumor surrounding epidermis (Ø). All images are shown at ×100 magnification. Insets images are higher magnification of staining at ×400.

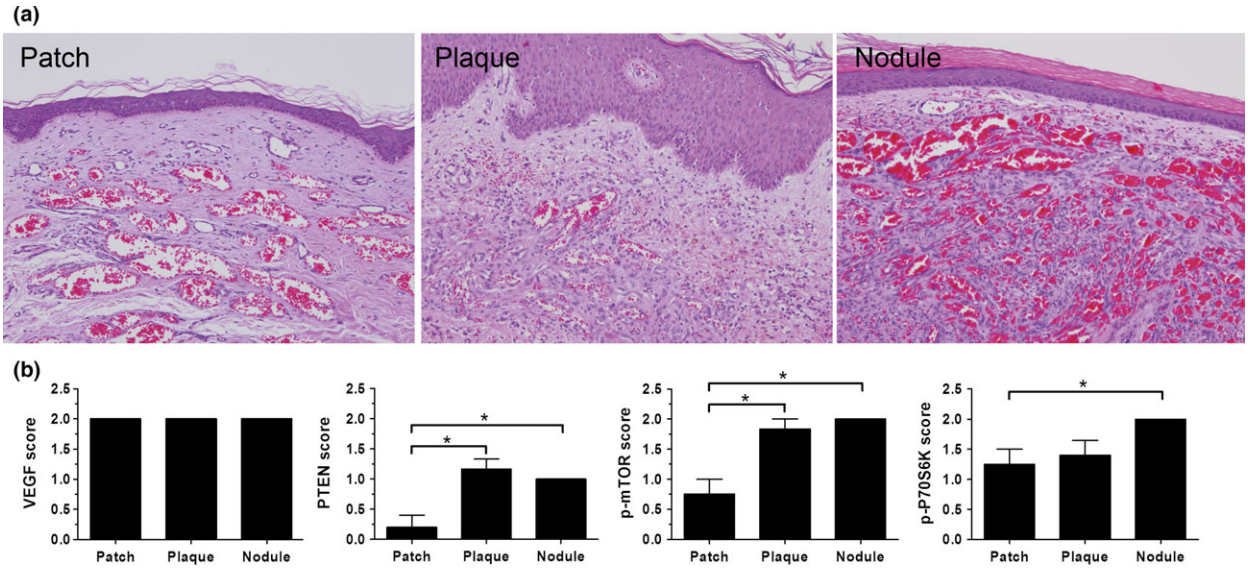

Figure 3 Kaposi's sarcoma skin lesions. (a) Different patterns of Kaposi's sarcoma lesions. A representative image for each stage is shown: patch, plaque, and nodule. All images are shown at ×100 magnification. (b) Immunohistochemical analysis of each stage; VEGF, PTEN, p-mTOR, and p-P70S6K staining. *$P < 0.05$, significant difference compared with patch stage.

However, no KS-related graft loss and death were found. Most patients in the reduction lost the graft or died in a short-term after the diagnosis of KS due to other ongoing complications different to KS. Unfortunately, the study is retrospective, and potential confounding factors were not controlled. In addition, the number of patients did not allow for a multivariate Cox's regression analysis. The prompt death of patients in the reduction group does not allow us to evaluate the total regression of cutaneous KS lesions, although partially regression was observed at that point. However, in conversion group, we observed the complete regression in a short period of time (5.24 months).

On KS development, HHV-8 has been found as a one of the main oncogenic factors. HHV-8 seroprevalence in end-stage renal disease (ESRD) patients is higher than in age and sex-matched healthy controls [29]. The risk of post-transplantation Kaposi's sarcoma

is around 25% among seropositive patients compared with 0.7% among seronegative patients [30]. In renal transplant patients with KS, only 66.6% were HHV8 seropositive in an observational prospective follow-up study [31]. In addition, lack of HHV-8 DNA load correlation between advanced KS lesions and sera in individual patients was reported [32]. In our study, all patients analyzed showed HHV-8 expression in tissue from KS specimens.

Viral receptor coupled to the protein G upregulates VEGF secretion by acting on hypoxia inducible factor (HIF) [33,34]. Inflammation and neovascularization are associated with cytokine dysregulation and mTOR/HIF/VEGF activation in KS development [16,34]. Our results confirm the high expression of VEGF in all stages of KS after renal transplantation. The elevated expression of VEGF may be the result of the viral activation of the mTOR pathway as a response to growth factors and inflammatory mediators [13]. In addition, tumor surrounding epidermis shows high VEGF expression. It was reported that the modification of the microenvironment could lead to different production of VEGF by epithelial cells [35]. The tumor hypoxic microenvironment related with KS can explain the high expression of VEGF in peritumoral epidermis, in contrast to the low VEGF expression observed in nonpathological skin as previously reports [36].

The PTEN tumor suppressor is a central negative regulator of the PI3K/mTOR/HIF/VEGF signaling pathway. Loss of PTEN function plays essential roles in the development and progression of human cancers [37]. KS lesions showed low PTEN expression.

The involvement of mTOR pathway in KS is confirmed by the activation of mTOR and the downstream effector molecule (P70S6K) in their phosphorylated forms [34]. Medium to high expression of p-mTOR and p-P70S6K was found in all KS lesions. Furthermore, for the first time, the expression of the mTOR pathway has been correlated with increasing tumor stages.

PTEN-negative tumors and p-AKT or p-P70S6K1 expressing tumors have demonstrated *in vitro* to be most sensitive to rapamycin treatment, suggesting a determinant role of these molecular markers for selecting appropriate patients for rapamycin therapy [38,39]. A positive p-S6K1 tumoral expression has been associated with recurrence of disease and shorter survival in patients with breast cancer [40]. Iwenofu *et al.* [17] showed a positive correlation between high expression of P70S6k and positive response to mTOR inhibitors treatment and low expression with progressive disease in spite of mTOR inhibitors treatment in sarcomas. Our results are concordant with these investigations.

While cyclosporine treatment in solid organ transplantation can promote cancer progression by a direct cellular effect through TGFβ pathway [10], there are many tumors with genetic defects in TGFβ signaling, especially in colon and pancreatic cancers [41]. Our group reported low expression of TGFβ in colon cancer in renal transplant patients [21]. Regarding Kaposi's sarcoma, we did not detect TGFβ expression in KS skin lesions, which may be linked with the results presented by Di Bartolo *et al.*, where HHV-8 could silence TGFβ pathway through several mechanisms, including downregulation of the TGFβ type II receptor [42]. In addition, those tumors with low expression of TGFβ have been associated with good response to rapamycin treatment [43].

The resistance to mTOR inhibitor treatment on KS lesions has been described, although in our cohort of patients was not observed. Kaposi's sarcoma relapses have seen even with trough levels between 5 and 12 ng/ml[25]. Resistance could be due to mutations of mTOR pathway, which prevent the binding of mTOR inhibitor or provoke the bypass of mTOR regulation through a persistent activation of downstream molecules [44]. The implication of other pathways like cyclooxygenase-2-prostaglandin E2-eicosanoid receptor inflammatory axis or NFκB pathway could be another explanation for mTOR inhibition resistance [45,46].

In conclusion, HHV-8 infection in all tissue samples and the activation of mTOR pathway according to KS stages could explain the benefit of the mTOR inhibitor switch in renal transplant patients who develop KS. The reduced sample size, the lack of randomization, and absence of multivariate analysis to control for possible confounding factors are limitations in the analysis of graft and patient survival. Further prospective and enlarge studies are needed to address the impact of conversion to mTOR inhibitors in graft and patient survival.

Authorship

AH-S and JR: participated in the design and performance of the research, data analysis, and writing of the article. AP and DM-R: participated in the performance of the research and data analysis. AM, AIS-F, PE, MAI, JMC, and AV: participated in the performance of the research and writing of the article. FO, JMC, and IR: participated in the design of the research and writing of the article.

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Conflicts of interest

The authors have declared no conflicts of interest.

REFERENCES

1. Penn I. Cancers in renal transplant recipients. *Adv Ren Replace Ther* 2000; **7**: 147.
2. Chang Y, Cesarman E, Pessin MS, *et al.* Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 1994; **266**: 1865.
3. Parravicini C, Olsen SJ, Capra M, *et al.* Risk of Kaposi's sarcoma-associated herpes virus transmission from donor allografts among Italian posttransplant Kaposi's sarcoma patients. *Blood* 1997; **90**: 2826.
4. Andreoni M, Goletti D, Pezzotti P, *et al.* Prevalence, incidence and correlates of HHV-8/KSHV infection and Kaposi's sarcoma in renal and liver transplant recipients. *J Infect* 2001; **43**: 195.
5. Razonable RR, Zerr DM. HHV-6, HHV-7 and HHV-8 in solid organ transplant recipients. *Am J Transplant* 2009; **9**(Suppl 4): S100.
6. Almuneef M, Nimjee S, Khoshnood K, Miller G, Rigsby MO. Prevalence of antibodies to human herpesvirus 8 (HHV-8) in Saudi Arabian patients with and without renal failure. *Transplantation* 2001; **71**: 1120.
7. Hladik W, Dollard SC, Mermin J, *et al.* Transmission of human herpesvirus 8 by blood transfusion. *N Engl J Med* 2006; **355**: 1331.
8. Mbulaiteye SM, Engels EA. Kaposi's sarcoma risk among transplant recipients in the United States (1993-2003). *Int J Cancer* 2006; **119**: 2685.
9. Shihab FS, Andoh TF, Tanner AM, *et al.* Role of transforming growth factor-beta 1 in experimental chronic cyclosporine nephropathy. *Kidney Int* 1996; **49**: 1141.
10. Hojo M, Morimoto T, Maluccio M, *et al.* Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature* 1999; **397**: 530.
11. Guba M, Graeb C, Jauch KW, Geissler EK. Pro- and anti-cancer effects of immunosuppressive agents used in organ transplantation. *Transplantation* 2004; **77**: 1777.
12. Doutrelepont JM, De Pauw L, Gruber SA, *et al.* Renal transplantation exposes patients with previous Kaposi's sarcoma to a high risk of recurrence. *Transplantation* 1996; **62**: 463.
13. Montaner S, Sodhi A, Molinolo A, *et al.* Endothelial infection with KSHV genes *in vivo* reveals that vGPCR initiates Kaposi's sarcomagenesis and can promote the tumorigenic potential of viral latent genes. *Cancer Cell* 2003; **3**: 23.
14. Sodhi A, Montaner S, Patel V, *et al.* Akt plays a central role in sarcomagenesis induced by Kaposi's sarcoma herpesvirus-encoded G protein-coupled receptor. *Proc Natl Acad Sci USA* 2004; **101**: 4821.
15. Luo J, Manning BD, Cantley LC. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. *Cancer Cell* 2003; **4**: 257.
16. Sodhi A, Chaisuparat R, Hu J, *et al.* The TSC2/mTOR pathway drives endothelial cell transformation induced by the Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor. *Cancer Cell* 2006; **10**: 133.
17. Iwenofu OH, Lackman RD, Staddon AP, Goodwin DG, Haupt HM, Brooks JS. Phospho-S6 ribosomal protein: a potential new predictive sarcoma marker for targeted mTOR therapy. *Mod Pathol* 2008; **21**: 231.
18. Campistol JM, Gutierrez-Dalmau A, Torregrosa JV. Conversion to sirolimus: a successful treatment for posttransplantation Kaposi's sarcoma. *Transplantation* 2004; **77**: 760.
19. Stallone G, Schena A, Infante B, *et al.* Sirolimus for Kaposi's sarcoma in renal-transplant recipients. *N Engl J Med* 2005; **352**: 1317.
20. Gutierrez-Dalmau A, Revuelta I, Ferrer B, *et al.* Distinct immunohistochemical phenotype of nonmelanoma skin cancers between renal transplant and immunocompetent populations. *Transplantation* 2010; **90**: 986.
21. Revuelta I, Moya-Rull D, Garcia-Herrera A, *et al.* Role of oncogenic pathways and KRAS/BRAF mutations in the behavior of colon adenocarcinoma in renal transplant patients. *Transplantation* 2012; **93**: 509.
22. Canha C, Ferreira R, Rovira J, *et al.* A case of esophageal adenocarcinoma on long-term rapamycin monotherapy. *Transpl Int* 2015; **28**: 1240.
23. Veroux M, Puliatti C, Fiamingo P, *et al.* Early *de novo* malignancies after kidney transplantation. *Transplant Proc* 2004; **36**: 718.
24. Boeckle E, Boesmueller C, Wiesmayr S, *et al.* Kaposi sarcoma in solid organ transplant recipients: a single center report. *Transplant Proc* 2005; **37**: 1905.
25. Lebbe C, Euvrard S, Barrou B, *et al.* Sirolimus conversion for patients with posttransplant Kaposi's sarcoma. *Am J Transplant* 2006; **6**: 2164.
26. Farrugia D, Mahboob S, Cheshire J, *et al.* Malignancy-related mortality following kidney transplantation is common. *Kidney Int* 2014; **85**: 1395.
27. Na R, Grulich AE, Meagher NS, McCaughan GW, Keogh AM, Vajdic CM. *De novo* cancer-related death in Australian liver and cardiothoracic transplant recipients. *Am J Transplant* 2013; **13**: 1296.
28. Einollahi B, Lessan-Pezeshki M, Nourbala MH, *et al.* Kaposi's sarcoma following living donor kidney transplantation: review of 7,939 recipients. *Int Urol Nephrol* 2009; **41**: 679.
29. Su CC, Tsai JP, Lin MN, Hsieh TC, Tseng RC, Chu TY. High seroprevalence of human herpesvirus type 8 in patients with end-stage renal disease in Taiwan. *J Clin Virol* 2013; **58**: 89.
30. Ahmadpoor P, Ilkhanizadeh B, Sharifzadeh P, *et al.* Seroprevalence of human herpes virus-8 in renal transplant recipients: a single center study from Iran. *Transplant Proc* 2007; **39**: 1000.
31. Raeisi D, Payandeh M, Madani SH, Zare ME, Kansestani AN, Hashemian AH. Kaposi's Sarcoma after Kidney Transplantation: a 21-Years Experience. *Int J Hematol Oncol Stem Cell Res* 2013; **7**: 29.

32. Pak F, Mwakigonja AR, Kokhaei P, *et al.* Kaposi's sarcoma herpesvirus load in biopsies of cutaneous and oral Kaposi's sarcoma lesions. *Eur J Cancer* 2007; **43**: 1877.
33. Sodhi A, Montaner S, Patel V, *et al.* The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1alpha. *Cancer Res* 2000; **60**: 4873.
34. Jham BC, Ma T, Hu J, *et al.* Amplification of the angiogenic signal through the activation of the TSC/mTOR/HIF axis by the KSHV vGPCR in Kaposi's sarcoma. *PLoS One* 2011; **6**: e19103.
35. Viac J, Palacio S, Schmitt D, Claudy A. Expression of vascular endothelial growth factor in normal epidermis, epithelial tumors and cultured keratinocytes. *Arch Dermatol Res* 1997; **289**: 158.
36. Weninger W, Uthman A, Pammer J, *et al.* Vascular endothelial growth factor production in normal epidermis and in benign and malignant epithelial skin tumors. *Lab Invest* 1996; **75**: 647.
37. Xu D, Yao Y, Jiang X, Lu L, Dai W. Regulation of PTEN stability and activity by Plk3. *J Biol Chem* 2010; **285**: 39935.
38. Noh WC, Mondesire WH, Peng J, *et al.* Determinants of rapamycin sensitivity in breast cancer cells. *Clin Cancer Res* 2004; **10**: 1013.
39. Fang J, Ding M, Yang L, Liu LZ, Jiang BH. PI3K/PTEN/AKT signaling regulates prostate tumor angiogenesis. *Cell Signal* 2007; **19**: 2487.
40. Noh WC, Kim YH, Kim MS, *et al.* Activation of the mTOR signaling pathway in breast cancer and its correlation with the clinicopathologic variables. *Breast Cancer Res Treat* 2008; **110**: 477.
41. Miyaki M, Kuroki T. Role of Smad4 (DPC4) inactivation in human cancer. *Biochem Biophys Res Commun* 2003; **306**: 799.
42. Di Bartolo DL, Cannon M, Liu YF, *et al.* KSHV LANA inhibits TGF-beta signaling through epigenetic silencing of the TGF-beta type II receptor. *Blood* 2008; **111**: 4731.
43. Gadir N, Jackson DN, Lee E, Foster DA. Defective TGF-beta signaling sensitizes human cancer cells to rapamycin. *Oncogene* 2008; **27**: 1055.
44. Huang S, Houghton PJ. Mechanisms of resistance to rapamycins. *Drug Resist Updat* 2001; **4**: 378.
45. Paul AG, Chandran B, Sharma-Walia N. Cyclooxygenase-2-prostaglandin E2-eicosanoid receptor inflammatory axis: a key player in Kaposi's sarcoma-associated herpes virus associated malignancies. *Transl Res* 2013; **162**: 77.
46. Gonzalez-Pardo V, D'Elia N, Verstuyf A, Boland R, Russo de Boland A. NFkappaB pathway is down-regulated by 1alpha,25(OH)(2)-vitamin D(3) in endothelial cells transformed by Kaposi sarcoma-associated herpes virus G protein coupled receptor. *Steroids* 2012; **77**: 1025.