Differential Expression of p-mTOR in Cutaneous Basal and Squamous Cell Carcinomas Likely Explains their Different Response to mTOR Inhibitors in Organ-transplant Recipients

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Abstract. *Background: Mammalian Target of Rapamycin (mTOR) inhibitors, such as sirolimus and everolimus, have been shown to reduce cutaneous carcinogenesis in organ-transplant recipients requiring for immunosuppressive treatment to prevent from allograft rejection. Clinical observations suggest that cutaneous squamous cell carcinomas (SCC) are more sensitive than basal cell carcinomas (BCC) to the antitumoral effect of these inhibitors. Aim: To investigate if the different response of SCC and BCC to mTOR inhibitors can be explained by differential expression of molecules involved in the mTOR signaling pathway. Materials and Methods: The expression of phospho-mTOR was immunohistocemically studied in specimens of cutaneous SCC and BCC. Results. All 15 SCCs expressed significant cytoplasmic phospho-mTOR immunoreactivity; by contrast, 12/13 BCC were completely negative, only one BCC exhibited weak phospho-mTOR immunoreactivity. Conclusion: The considerably higher expression of phospho-mTOR in SCC compared to BCC is a likely explanation for their higher sensitivity to mTOR inhibitors.*

The mammalian target of rapamycin (mTOR) is a 289-kDa serine/threonine protein kinase belonging to the family of phosphatidylinositol-3 kinase (PI3K)-related kinases. It is phosphorylated at Ser2448 *via* the PI3K/AKT signaling pathway and autophosphorylated at Ser2481. mTOR is the hub of PI3K/AKT/mTOR signaling pathway, acting both as a downstream effector and upstream regulator, and functions in two complexes, mTOR complex 1 (mTORC1) and mTORC2 that play a key role in cell growth and homeostasis (1,2). The regulation of the mTOR pathway is altered in several types of human tumors, so that mTOR has emerged as a target for antitumor therapy (3-5). As a matter of fact, mTOR inhibitors, such as sirolimus (rapamycin) and its analogs (everolimus, temsirolimus, deferolimus, referred to as 'rapalogs'), exert antitumor activity, both directly *in vitro* and *in vivo*, by reduction of cell-cycle progression, leading to cell-cycle arrest at G_1 phase, and by inhibiting the process of tumor (neo)angiogenesis (6-8). Therefore, mTOR inhibitors are already used for the treatment of several types of human cancers, such as advanced renal cancer, breast cancer, subependymal giant-cell tumors, glioblastoma, lymphoma, sarcoma and neuroendocrine tumors, alone or in combination with other chemotherapeutic agents (9, 10). In parallel, sirolimus and everolimus also exert immunosuppressive activities, and by virtue of this are used for preventing allograft rejection in organ transplant recipients (OTR). The combination of both antitumoral and immunosuppressive effects of mTOR inhibitors renders them suitable treatment options for OTR who need immunosuppressive treatments to prevent allograft rejection and who are at risk of developing various malignancies, especially non-melanoma skin cancers (NMSC) (11-13). In fact, along with results from animal studies (14), several clinical studies have shown that converting OTR to mTOR inhibitor-based immunosuppression considerably reduces the risk of development of *de novo* NMSC (15-27). The data currently available suggest that the tumor-preventive effect of mTOR inhibitors is more pronounced for squamous cell carcinoma (SCC) than for basal cell carcinoma (BCC). In the TUMORAPA study, we found that in renal transplant patients with NMSC, the ratio of SCC:BCC decreased from 3.9:1 to 1.4:1 two years after conversion to sirolimus (26). In keeping with this finding, another study found that conversion of renal transplant patients to sirolimus lowered the rate of SCC, but

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not that of BCC (27). We also observed that switching heart-transplant patients to everolimus halved the SCC:BCC ratio (24). It therefore seems that SCC and BCC, despite originating from epidermal keratinocytes, behave differently to mTOR inhibitor-based immunosuppression. We questioned whether this could be due to a different expression of molecules involved in the mTOR signaling pathway in these two types of NMSC. We therefore immunohistochemically investigated the expression of phospho-mTOR, a molecule reflecting the sensitivity of tumors to mTOR inhibitors (28), in a group of cutaneous SCCs and BCCs.

Materials and Methods

Formalin-fixed, paraffin-embedded specimens of SCC (n=15) and BCC (n=13) were retrieved from the archives of the Pathology Department, Ed. Herriot Hospital Group, Lyon. The tumors had been excised from non-immunosuppressed patients and the diagnosis had been made by examination of hematoxylin-eosin-stained sections. A streptavidin-biotin-immunoperoxidase technique was applied on 4-μm thick sections cut from paraffin blocks. The slides were incubated at 60˚C, deparaffinized, dehydrated and rinsed in H_2O_2 solution in 3% methanol. Diaminobenzidine was used as chromogen and Mayer's hematoxylin as counterstain. The antibody used was the rabbit monoclonal IgG clone 49F9 (Cell Signaling Technology, Danvers, MA, USA), produced by immunizing animals with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Ser2448 of human mTOR; it recognizes endogenous mTOR protein when this is phosphorylated at Ser2448.

Results

In non-lesional skin adjacent to tumors, p-mTOR immunoreactivity was observed in the upper viable epidermal cell layers (Figure 1A) and served as an internal positive control. All 15 SCCs expressed diffuse cytoplasmic p-mTOR immunoreactivity in a percentage of cells varying from 30 to 100% (Figure 1B). A total of 12/15 SCCs contained >40% positive cells, and 7/15 of them contained >70% positive cells. The labeling was usually stronger in histologically well-differentiated, keratinizing tumoral areas. Conversely, 12/13 BCC cases exhibited no p-mTOR immunoreactivity (Figure 1C). Only 1/13 BCC exhibited weak reactivity in <5% of cells found around foci of keratinization. The difference between SCC and BCC was statistically highly significant ($p<0.001$ by the χ^2 test).

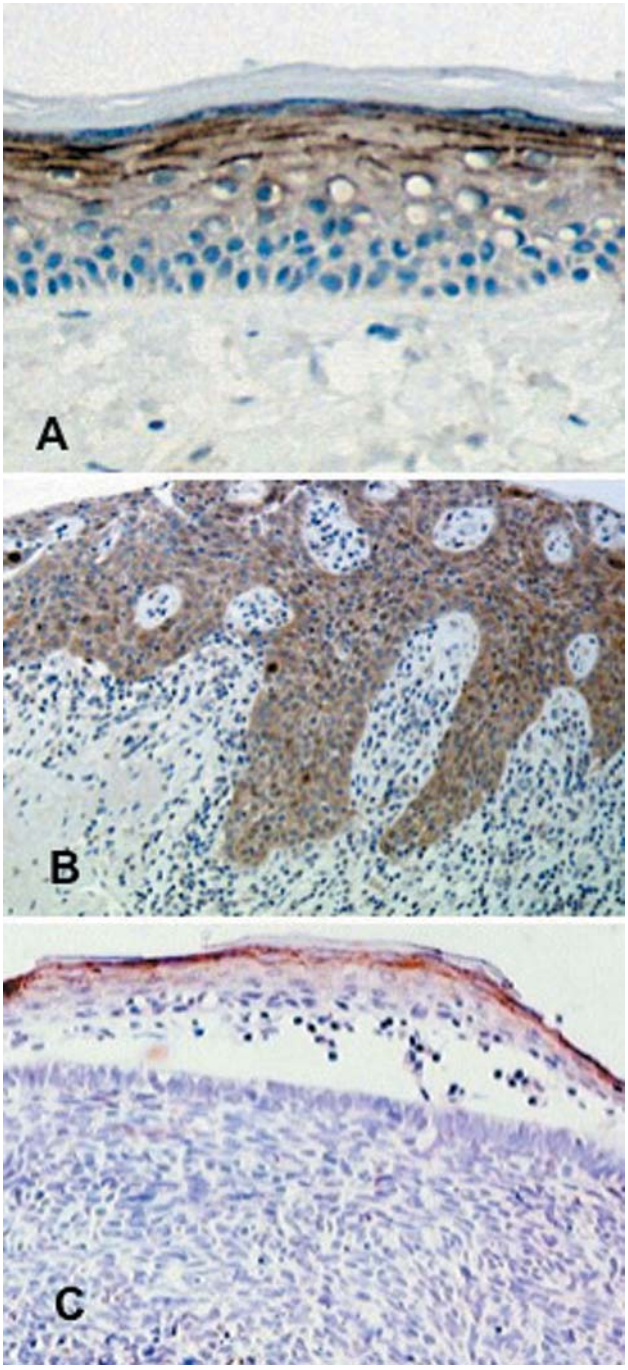

Figure 1. *Phospho-mammalian target of rapamycin is expressed in the cytoplasm of keratinocytes of the upper cell layers in normal epidermis (A) and in the cytoplasm of tumor cells in squamous cell carcinoma (B) but not in basal cell carcinoma (C). Note the reactivity of the upper epidermis serving as an internal positive control in C (original magnification ×250).*

Discussion

Data regarding the expression of p-mTOR in NMSC are to date limited. Two studies found cytoplasmic expression of p-mTOR in SCC (29, 30). In keeping with these studies, we also confirmed p-mTOR expression in all SCC cases we examined. p-mTOR expression in BCC was reported in one study and was found to be much lower compared to SCC (30). Our results, showing that p-mTOR expression is much lower in BCC compared to SCC, are in keeping with the results of these studies. Although the number of specimens

examined in our pilot study is limited, our results show a clear-cut, statistically significant difference between SCC and BCC. As p-mTOR seems to be a biological marker reflecting the sensitivity of tumor cells to mTOR inhibitors (28), the considerably more frequent expression of p-mTOR in SCCs compared to BCCs provides a likely explanation for the fact that the former are more sensitive to the antitumoral effect of mTOR inhibitors than the latter. The predominant effect of mTOR inhibitors on SCC as compared to BCC is clinically interesting since SCCs have a more aggressive course than BCCs (31). We are currently studying a larger number of mediators of the PI3K/AKT pathway in NMSC from OTR developed before and after switching to mTOR inhibitors in order to better-understand the *in vivo* mechanism of the beneficial effect of mTOR inhibitors on cutaneous carcinogenesis.

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