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ORIGINAL ARTICLE

Molecular determinants of Akt-induced keratinocyte transformation

C Segrelles[1,5], M Moral[1,5], M Fernanda Lara[1], S Ruiz[1,3], M Santos[1], H Leis[1,4], R García-Escudero[1], AB Martínez-Cruz[1], J Martínez-Palacio[1], P Hernández[1], C Ballestín[2] and JM Paramio*[,1]

[1]*Department of Cell and Molecular Biology, CIEMAT, Madrid, Spain and* [2]*Department of Pathology, Hospital '12 de Octubre', Madrid, Spain*

The PI3K/PTEN/Akt signaling pathway has emerged in recent years as a main player in human cancers, increasing proliferation and decreasing apoptosis of transformed cells, and thus becoming a potential target for therapeutic intervention. Our previous data have demonstrated that Akt-mediated signaling is of a key relevance in the mouse skin carcinogenesis system, one of the best-known models of experimental carcinogenesis. Here, we investigated the involvement of several pathways as mediators of Akt-induced increased proliferation and tumorigenesis in keratinocytes. Tumors produced by subcutaneous injection of Akt-transformed keratinocytes showed increased Foxo3a phosphorylation, but no major alterations in $p21^{Cip1/WAF1}$, $p27^{Kip1}$ or mdm2 expression and/or localization. In contrast, we found increased expression and nuclear localization of ΔNp63, β-catenin and Lef1. Concomitantly, we also found increased expression of c-myc and CycD1, targets of the β-catenin/Tcf pathway. Such increase is associated with increased phosphorylation and stabilization of c-myc protein as well as increased translation of c-myc and CycD1 due to mTOR activation. Using immunohistochemistry approaches in samples of oral dysplasias and human head and neck squamous cell carcinomas, we confirmed that increased Akt activation significantly correlates with increased ΔNp63 and CycD expression, c-myc phosphorylation and nuclear accumulation of β-catenin. Collectively, these results demonstrate that Akt is able to transform keratinocytes by specific mechanisms involving transcriptional and post-transcriptional processes.
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Introduction

Aberrant activation of the PI3K/PTEN/Akt pathway has been widely implicated in many human cancers. Multiple oncogenic insults can increase Akt activity, affecting a number of putative substrates, which includes a plethora of different signaling intermediates whose activity primarily modulates cell death and proliferation (reviewed in Lawlor and Alessi, 2001; Vivanco and Sawyers, 2002; Luo *et al*., 2003). As a consequence, possible therapies targeting the Akt pathway alone, or in conjunction with other chemotherapeutics, might be suitable cancer treatments (Luo *et al*., 2003). However, this possibility should be preceded by a complete characterization of Akt responses in model systems. Mouse cancer models have been invaluable in understanding the process of tumorigenesis: they allow defined tests of tumor genetics, close analysis of tumor pathology and validation of therapeutic targets. The mouse skin carcinogenesis system is one of the best-characterized models and has provided an important instrumental framework for understanding many of the concepts currently applied to human cancers. This system displays many parallelisms with certain human tumors, such as head and neck squamous cell carcinomas (HNSCC) (reviewed in Slaga *et al*., 1996; Yuspa *et al*., 1996). Others and we have provided evidence on the particular relevance of the PI3K-Akt pathway in mouse skin carcinogenesis (Santos *et al*., 2002; Segrelles *et al*., 2002; Suzuki *et al*., 2003). However, it is important to consider that many genetic events and general changes in the expression profile occurred during the malignant progression of mouse skin tumors, thus making it difficult to ascertain what possible targets are fundamental and what are secondary outcome of the process. As a consequence, possible models that show increased tumorigenic properties upon a limited and controlled number of experimental alterations are also relevant. We have used the PB keratinocyte cell line for these purposes. These cells were obtained from a chemically induced mouse skin papilloma, but they do not show increased EGFR expression nor mutations in *Ha-ras* gene (Yuspa *et al*., 1986; Casanova *et al*., 2002). Moreover, when PB keratinocytes are used in xenograft experiments, they are poorly tumorigenic: few tumors are generated, and those obtained display very long latency, reduced growth rate and highly differentiated

*Correspondence: Dr JM Paramio, Department of Cell and Molecular Biology, CIEMAT (ed. 7), Ave. Complutense 22, Madrid E-28040, Spain.
E-mail: jesusm.paramio@ciemat.es
[5]These two authors contributed equally to this work.
[3]Current address: Cancer Research Center and Institute of Cell and Molecular Biology of Cancer, CSIC-University of Salamanca, P Universidad de Coimbra s/n, Salamanca, Spain.
[4]Current address: Genomics and Pharmacoproteomics Program, FVIB-CIPF, Camino de las Moreras, Valencia, Spain.
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