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Gene Profiling Approaches Help to Define the Specific Functions of Retinoblastoma Family in Epidermis

M. Fernanda Lara, Ramón García-Escudero, Sergio Ruiz, Mirentxu Santos, Marta Moral, Ana B. Martínez-Cruz, Carmen Segrelles, Corina Lorz, and Jesús M. Paramio*

Molecular Oncology Unit, Biomedicine Division, CIEMAT, Madrid, Spain

The epidermal-specific ablation of *Rb* gene leads to increased proliferation, aberrant differentiation, and the disengagement of these processes in vivo and in vitro. These differences in phenotype are more severe with the loss of p107, demonstrating the functional compensation between pRb and p107. As p107 and p130 also exert overlapping functions in epidermis, we have generated $Rb^{F19/F19}$K14*cre*;*Rbl2*−/− (pRb−;p130−) mice to analyze possible functional redundancies between pRb and p130. The epidermal phenotype was very similar between pRb− and pRb−;p130− mice, suggesting that pRb and p130 activities are not redundant in epidermis. Importantly, we can correlate the proliferation differences with specific changes in gene expression between pRb−, pRb−;p107− and pRb−;p130− primary keratinocytes using microarray analysis, and explain the phenotypes in the context of altered E2F expression and functionality. Our findings support a model in which the distinct retinoblastoma family members, in conjunction with E2F members, play a central role in regulating epidermal homeostasis through specific or overlapping activities.

Key words: skin; pRb; microarray; transgenic mice; p130

INTRODUCTION

In recent years multiple evidences have demonstrated that the retinoblastoma family of proteins (pRb, p107, and p130) exert essential roles in development through the regulation of several aspects of differentiation programs such as terminal cell cycle exit, maintenance of the postmitotic state and induction of tissue-specific gene expression [1,2]. Most of these data have been inferred from the phenotypic alterations displayed by deficient murine models. Mice lacking either p107 or p130 are viable and normal, whereas mice deficient in both p107 and p130 die at birth with defects in endochondral bone development associated with inappropriate cell cycle exit [3,4]. This finding demonstrates that the possible functions of p107 can be carried out by p130 and vice versa, and these overlapping roles cannot be carried out by pRb, the only remaining member in these animals [3,4]. Mice lacking pRb die in utero and display defects in erythroid, neuronal and lens fiber cell differentiation [5–7], whereas mice doubly mutant for pRb and p107 show an aggravated *Rb*-null phenotype, indicating the existence of shared functions for these two proteins [4]. Similarly, pRb may have overlapping functions with p130 that are not shared with p107 [8]. Interestingly, it has been reported that tumor development by loss of pRb is suppressed by its homologs p107 and p130 in a tissue-specific manner [9]. Consistent with the notion of shared functionality, knockout mouse embryo fibroblasts lacking all three *Rb* family members are immortal and do not respond to senescence inducing signals, although do not behave as tumoral cells [10,11].

The skin, one of the biggest organs of the body, plays essential protective tasks. These functions reside in the epidermis, the external epithelium that covers the mammalian organism, and requires the fine tuning of a characteristic differentiation program and the coupling between proliferation and differentiation processes [12–14]. Proliferative cells are confined to a single basal layer and the non-proliferative differentiating cells are located in the suprabasal layers [14]. Epidermal cells are shed from the skin at their final stage of differentiation; this process requires a robust machinery ensuring complete renovation of the cells in the tissue whilst

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Abbreviations: BrdU, bromodeoxyuridine; Cdk, cyclin dependent kinase; SAM, Significance analysis of microarray.

M. Fernanda Lara, Ramón García-Escudero, and Sergio Ruiz contributed equally to this work.

Sergio Ruiz's present address is Centro de Investigación del Cáncer and Instituto de Biología Molecular y Celular del Cáncer, CSIC-University of Salamanca, E-37007 Salamanca, Spain.

*Correspondence to: Ave Complutense 22, Madrid E-28040, Spain.

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