MOLECULAR CARCINOGENESIS 47:105–113 (2008)

p107 Acts as a Tumor Suppressor in pRb-Deficient Epidermis

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The specific deletion of *Rb* gene in epidermis leads to altered proliferation and differentiation, but not to the development of spontaneous tumors. Our previous data have demonstrated the existence of a functional compensation of *Rb* loss by *Rbl1* (p107) in as the phenotypic differences with respect to controls are intensified. However, the possible evolution of this aggravated phenotype, in particular in relationship with tumorigenesis, has not been evaluated due to the premature death of the double deficient mice. We have now investigated whether p107 can also act as a tumor suppressor in pRb-deficient epidermis using different experimental approaches. We found spontaneous tumor development in doubly-deficient skin grafts. Moreover, *Rb*-deficient keratinocytes are susceptible to Ha-ras-induced transformation, and this susceptibility is enhanced by p107 loss. Further functional analyses, including microarray gene expression profiling, indicated that the loss of p107, in the absence of pRb, produces the reduction of p53-dependent pro-apoptotic signals. Overall, our data demonstrate that p107 behaves as a tumor suppressor in epidermis in the absence of pRb and suggest novel tumor-suppressive roles for p107 in the context of functional p53 and activated Ras.

Key words: epidermis; tumorigenesis; pRb; p107; Ras; p53; apoptosis; microarray

INTRODUCTION

The retinoblastoma family of proteins (pRb, p107, and p130) plays a crucial role in cell cycle regulation through transcriptional inhibition of E2F-responsive promoters [1,2]. Somatic mutations of the *Rb* gene are associated with specific subsets of human cancers, whereas most human cancers display inactivation of the pRb-dependent regulatory pathway [3] (for a careful discussion see, Ref. [4]). This may suggest that the *Rb* functions are carried out, in its absence, by other proteins. This suggests that the Rb functions are carried out, in its absence, by other proteins, which should also be controlled by the same upstream regulators. This implies that the mechanisms that inactivate pRb also may inactivate these hypothetical proteins, thus leading to complete inactivation of the pathway. The other *Rb* family members, p107 and p130, are plausible candidates. In support of this, the absence of p107 or p130 increments the number and type of tumors displayed by *Rb*+/− mice [5].

Tissue-specific recombination technologies have been used to avoid the embryonic lethality of germ line inactivation of *Rb*. We and others have recently reported that *Rb* gene ablation in epidermis ($Rb^{F19/F19}$; *K14*Cre, thereafter *Rb*−) leads to altered proliferation and differentiation, demonstrating an essential role for pRb in maintaining post mitotic state of terminally differentiated keratinocytes [6,7]. However, mice lacking *Rb* in epidermis do not develop spontaneous tumors [7] and, upon chemical skin carcinogenesis, *Rb*-mice developed fewer and smaller papillomas than control littermates due to increased apoptosis. Nonetheless, the *Rb*-deficient tumors display a more malignant phenotype, with increased conversion to squamous cell carcinomas (SCC), probably due to premature *Trp53* loss [8] (for a careful discussion see Ref. [9]).

The limited tumor spectrum in *Rb*-deficient mice might be attributed to the tumor suppressor activity of the other pRb family members in specific tissues. Indeed, *Rb*+/−;*Rbl1*−/− chimeric mice developed tumors, most frequently in the pituitary gland, coecum, bone, and lymphoid tissue [5]. Similarly, the absence of functional *Rb* in epidermis is partially compensated by the *Rbl1* gene product, p107, as the phenotypic abnormalities in vivo and in vitro become enhanced with the consecutive loss of *Rbl1* alleles [7]. Mice bearing the specific epidermal deletion of *Rb* gene in an *Rbl1*-null background die

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Received 15 March 2007; Revised 22 May 2007; Accepted 22 May 2007

DOI 10.1002/mc.20367