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Correspondence and requests for materials should be addressed to J.M.P. (jesusm.paramio@ciemat.es)

* Current Address: Departments of Pediatrics and the Program in Molecular Imaging, Stanford University, Stanford, CA

A Novel Tumor suppressor network in squamous malignancies

Clotilde Costa[1], Mirentxu Santos[1], Carmen Segrelles[1], Marta Dueñas[1], M. Fernanda Lara[1]*, Xabier Agirre[2], Felipe Prosper[2], Ramón García-Escudero[1] & Jesús M. Paramio[1]

[1]Molecular Oncology Unit, Department of Basic Research, CIEMAT (Ed 70A), Ave Complutense 40. 28040 Madrid (Spain), [2]Center for Applied Medical Research, University of Navarra, Ave. Pío XII, 55 E-31008 Pamplona Spain.

The specific ablation of *Rb1* gene in stratified epithelia ($Rb^{F/F}$;K14cre) promotes proliferation and altered differentiation but is insufficient to produce spontaneous tumors. The pRb relative, p107, compensates some of the functions of pRb in these tissues; however, $Rb^{F/F}$;K14cre;$p107^{-/-}$ mice die postnatally. Here we show, using an inducible mouse model ($Rb^{F/F}$;K14creER^{TM}), that p107 exerts specific tumor suppressor functions in the absence of pRb in stratified epithelia. The simultaneous absence of pRb and p107 produces impaired p53 transcriptional functions and reduction of Pten expression, allowing spontaneous squamous carcinoma development. These tumors display significant overlap with human squamous carcinomas, supporting that $Rb^{F/F}$;K14creER^{TM};$p107^{-/-}$ mice might constitute a new model for these malignancies. Remarkably tumor development *in vivo* is partially alleviated by mTOR inhibition. These data demonstrate the existence of a previously unreported functional connection between pRb, Pten and p53 tumor suppressors, through p107, of a particular relevance in squamous tumor development.

The *Rb1* gene product, the pRb protein, exerts essential roles controlling cell cycle progression, differentiation and apoptosis[1]. Accordingly, it plays tumor suppressor functions in multiple tissues, and the disruption of the 'Rb pathway', either by direct *Rb1* gene mutation or, more frequently, via alterations affecting pRb biological functions, is a hallmark of most sporadic human cancers[2]. To analyze *Rb1* roles *in vivo* in adult mice, several tissue specific knock outs have been generated, as mouse models bearing complete *Rb1* gene loss displayed embryonic lethality[3–5]. The constitutive somatic elimination of *Rb1* gene in epidermis ($Rb^{F/F}$;K14cre mice) produced altered proliferation and differentiation, but it was insufficient to promote tumor development[6]. Moreover, upon chemical carcinogenesis protocols, $Rb^{F/F}$;K14cre mice showed reduced tumor incidence and multiplicity as compared to controls. However, the Rb-deficient tumors displayed increased malignancy with high rate of conversion from papillomas to squamous cell carcinomas[7]. This paradoxical observation was explained by an early and acute p53 induction in benign tumor cells, which promoted a selective pressure leading to premature p53 inactivation and increased malignancy[7]. The connection between pRb and p53 in this context was further supported by the findings obtained in mice bearing p53 deletion in stratified epithelia ($p53^{F/F}$;K14cre mice), in which the spontaneous tumor development was accelerated by simultaneous epidermal *Rb1* loss[8]. Remarkably, spontaneous tumors arising in these $pRb^{F/F}$;$p53^{F/F}$;K14cre mice are highly aggressive and display early signs of chromosomal instability[8,9] and high metastatic behavior associated with deregulated miRNA expression[10]. Further, genomic profiling of these spontaneous tumors also revealed a significant overlap with multiple human malignancies distinguished by poor prognosis, altered p53 status and, remarkably, high metastasis incidence[11].

The absence of spontaneous tumors in $Rb^{F/F}$;K14cre mice might suggest that other proteins exert overlapping and/or compensating functions. This seems to be the case of E2F1[12] and p107[13], but not p130[14]. The fact that the $Rb^{F/F}$;K14cre phenotype was aggravated in a $p107^{-/-}$ background, leading to early postnatal death[6], supports the hypothesis that the pRb relative p107 can exert some of the functions of pRb in its absence in epidermis. Importantly, a number of evidences also suggested a possible tumor suppressor role for p107 in absence of pRb[13]. First, double deficient keratinocytes are highly sensitive to Ha-ras-mediated transformation and displayed reduced oncogene-induced premature senescence[13]. Second, transplants of $Rb^{F/F}$;K14cre;$p107^{-/-}$ skin, but not $Rb^{F/F}$;K14cre, invariably developed squamous tumors[13]. And third, the altered behavior of $Rb^{F/F}$;K14cre mice to chemical carcinogenesis is partially alleviated by a reduction of p107 amounts[15]. These findings could also indicate that the absence of p107 affects p53 functions. Indeed, transcriptome analysis of new born epidermis revealed the downregulation of several p53-dependent genes in $Rb^{F/F}$;K14cre;$p107^{-/-}$ mice[13], suggesting the