Unexpected Roles for pRb in Mouse Skin Carcinogenesis

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Abstract

The mouse skin carcinogenesis represents one of the best models for the understanding of malignant transformation, including the multistage nature of tumor development. The retinoblastoma gene product (*pRb*) plays a critical role in cell cycle regulation, differentiation, and inhibition of oncogenic transformation. In epidermis, $Rb^{-/-}$ deletion leads to proliferation and differentiation defects. Numerous evidences showed the involvement of the retinoblastoma pathway in this model. However, the actual role of pRb is still unknown. To study the possible involvement of pRb in keratinocyte malignant transformation, we have carried out two-stage chemical skin carcinogenesis on $Rb^{F19/F19}$ (thereafter $Rb^{+/+}$) and $Rb^{F19/F19}$;*K14Cre* (thereafter $Rb^{-/-}$) animals. Unexpectedly, we found that $Rb^{-/-}$ mice developed fewer and smaller papillomas than the $Rb^{+/+}$ counterparts. Moreover, the small size of the pRb-deficient tumors is associated with an increase in the apoptotic index. Despite this, pRb-deficient tumors display an increased conversion rate to squamous cell carcinomas. Biochemical analyses revealed that these characteristics correlate with the differential expression and activity of different pathways, including E2F/$p19^{arf}$/p53, PTEN/Akt, c-*jun* NH_2-terminal kinase/p38, and nuclear factor-κB. Collectively, our findings show unexpected and hitherto nondescribed roles of pRb during the process of epidermal carcinogenesis. (Cancer Res 2005; 65(21): 9678-86)

Introduction

pRb is a nuclear phosphoprotein that regulates cell cycle progression mainly through the binding to and inhibition of E2F transcription factors (1). Cell cycle progression requires functional inactivation of pRb by phosphorylation. Serial phosphorylation of pRb by cyclin D/cdk4/6 and cyclin E/cdk2 results in the release of pRb-bound E2F, allowing the progression of cells into the S phase (1). Conversely, cell cycle arrest is mediated through the expression of CIP/KIP and INK4 family members of cyclin-dependent kinase (cdk) inhibitors (2). Inactivation of *Rb* has been found in several human tumors including hereditary retinoblastoma, osteosarcoma, small cell lung cancer, bladder, and prostate tumors (3). Recent data also suggest an essential tumor suppressor role for the $pRb^{-/-}$-related proteins p107 and p130 in specific tissues in the absence of pRb (4). Thus far, the vast majority of human tumors display alterations in any of the elements of the so-called *Rb* pathway.

The two-stage mouse skin carcinogenesis is a well-suited model for the understanding of the multistage nature of tumor progression (reviewed in ref. 5). In this system, tumor initiation is accomplished through a single topical application of a carcinogen, typically 7,12-dimethylbenz(a)anthracene (DMBA). This produces an irreversible, genetic inheritable change in the *H-ras* proto-oncogene. Tumor promotion takes place when the initiated cells are expanded due to repeated applications of 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a hyperproliferative stimulus that promotes the generation of papillomas. Finally, although papilloma regression is a common event, in some cases, conversion occurs and papillomas evolve to squamous cell carcinomas (SCC).

Several lines of evidence suggest that the *Rb* pathway is involved in the mouse skin carcinogenesis. First, cyclin D1 expression increases at the very early stages of the process and experiments with *cycD1*-null mice showed its requirement during mouse skin carcinogenesis (6). Second, *cdk4*-deficient mice are resistant to skin tumor development (7), whereas transgenic cdk4 expression in epidermis leads to development of papillomas in DMBA-treated skin without promotion (8). Moreover, mice expressing a mutated form of *cdk4*, which renders this protein insensitive to inhibition by ink4 proteins (R24C mutation), are more susceptible to skin carcinogenesis protocols (9). Third, primary keratinocytes derived from $ink4a^{\Delta 2,3}$;$p21^{waf1}$ doubly deficient mice are more susceptible to $v\text{-}ras^{Ha}$ transformation than their wild-type counterparts (10). Finally, deregulated expression of E2F family members increase the susceptibility to skin tumor development (11–15). Overall, these observations clearly showed that the functional inactivation of pRb is a common event in mouse skin carcinogenesis. Similarly, in human nonmelanoma skin tumors, cyclin D is frequently overexpressed, although the incidence of retinoblastoma gene inactivation is relatively low (16, 17).

Despite these evidences, the actual role of pRb in the mouse skin carcinogenesis has not been established. This is mainly due to the early embryonic lethality displayed by pRb-deficient mice (18–20). To solve this problem, others and we have recently used the Cre/loxP technology to inactivate the *Rb* gene in epidermis (21, 22). pRb-deficient skin is characterized by increased proliferation and altered differentiation leading to epidermal hyperplasia and hyperkeratosis, a phenotype that is further aggravated by loss of *p107* alleles (22) or coexpression of E7 papillomavirus protein (21). These phenotypic alterations are suggestive of a pretumoral state. In this work, we have studied the possible susceptibility of $Rb^{F19/F19}$;*K14Cre* (thereafter $Rb^{-/-}$) mice to the two-stage chemical carcinogenesis. Surprisingly, we found that the absence of pRb leads to reduced tumor development. However, the *Rb*-deficient tumors displayed an increased rate in the malignant

Note: S. Ruiz and M. Santos contributed equally to this work.

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