

## Genetic Deletion of $p21^{WAF1}$ Enhances Papilloma Formation but not Malignant Conversion in Experimental Mouse Skin Carcinogenesis

Wendy C. Weinberg,<sup>1</sup> Ester Fernandez-Salas, David L. Morgan, Aryaman Shalizi, Elena Mirosch, Eric Stanulis, Chuxia Deng, Henry Hennings, and Stuart H. Yuspa

Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research [W. C. W., A. S.], Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute [D. L. M., E. F.-S., H. H., S. H. Y.], and Laboratory of Biochemistry and Metabolism, National Institute of Diabetes and Digestive and Kidney Disorders [C. D.], NIH, Bethesda, Maryland 20892, and ROW Sciences, Inc., Rockville, Maryland 20878 [E. S., E. M.]

### Abstract

Tumor suppression by p53 is believed to reside in its ability to regulate gene transcription, including up-regulation of  $p21^{WAF1}$ . In  $p53(-/-)$  mice, chemical- or oncogene-induced skin tumors undergo accelerated malignant conversion. To determine the contribution of the  $p21^{WAF1}$  gene product to epidermal carcinogenesis, animals  $+/+$ ,  $+/-$ , and  $-/-$  for a null mutation in the  $p21^{WAF1}$  gene were treated once with 25 nmol 7,12-dimethylbenz[a]anthracene, followed by 5  $\mu$ g of TPA two times/week for 20 weeks. Papilloma frequency was higher in the  $p21^{WAF1}$ -deficient mice. However, the frequency of malignant conversion was similar among all three genotypes. After TPA treatment, all genotypes developed epidermal hyperplasia, although the labeling index was lower in  $p21^{WAF1} (-/-)$  epidermis compared with  $p21^{WAF1} (+/+)$ . Furthermore, the expression of differentiation markers was the same across genotypes in untreated or TPA-treated epidermis. Similar frequencies of malignant conversion were also observed in an *in vitro* assay. Thus,  $p21^{WAF1}$  suppresses early stages of papilloma formation but not malignant progression in mouse skin carcinogenesis, and decreased levels of  $p21^{WAF1}$  do not account for the enhanced malignant conversion of p53 null epidermal tumors.

### Introduction

The cyclin-dependent kinase inhibitor  $p21^{WAF1}$  (CIP1/CAP20/mda6/sdi1) was one of the first identified targets of p53-mediated gene transcription (1). Up-regulation of  $p21^{WAF1}$  after DNA damage participates in cell cycle arrest (2). Up-regulation has also been documented in cells undergoing differentiation, senescence, and apoptosis, all processes that may negatively influence tumor formation or progression (2–5). Both p53-dependent and -independent regulation of this gene product have been described (2, 4, 6, 7). In keratinocytes it has been also suggested that splice variants of p53 show differential regulation of target gene activation, including  $p21^{WAF1}$  (8). A role for  $p21^{WAF1}$  as a downstream effector of p53-mediated tumor suppression is supported by its ability to block proliferation of p53 deficient tumor cells *in vitro* and *in vivo* (1, 9, 10). Furthermore, both basal and induced levels of  $p21^{WAF1}$  are lower in p53-deficient cells (11, 12). However, the function of  $p21^{WAF1}$  as a tumor suppressor is unclear. Mutations in the gene are rarely observed in human cancers (2), and although a majority of mice homozygous for a null mutation in the  $p53$  gene develop spontaneous tumors by 6 months of age (13, 14),  $p21^{WAF1}$  null mice do not exhibit any predisposition to spontaneous tumor formation (15). In addition, previous studies from our laboratory have demonstrated that p53-deficient keratinocytes, but not  $p21^{WAF1}$ -deficient keratinocytes, display

an increased growth rate *in vivo* after transduction with the  $v-ras^{Ha}$  oncogene and grafting to nude mouse hosts, compared with wild-type keratinocytes (12, 16). Furthermore, in this protocol, keratinocytes derived from all  $p21^{WAF1}$  genotypes formed papillomas with similar malignant conversion frequencies (16), whereas p53-deficient keratinocytes had a markedly increased conversion frequency over control keratinocytes (12). To test directly whether  $p21^{WAF1}$  levels can influence skin tumor formation and progression in an intact animal model, we have examined the incidence and malignant conversion of epidermal papillomas formed in mice expressing a null mutation in the  $p21^{WAF1}$  gene after a two-stage carcinogenesis protocol. We demonstrate that  $p21^{WAF1}$ -deficient mice display an increased predisposition to tumor formation after exposure to chemical carcinogens; however, the benign tumors formed in  $p21^{WAF1}$ -deficient mice do not display an increased malignant potential compared with those arising in  $p21^{WAF1} (+/+)$  mice. These results contrast to earlier studies of chemically induced epidermal carcinogenesis in p53 null mice; thus, the contribution of altered  $p21^{WAF1}$  expression to tumorigenesis is distinct from that of disruption of p53-mediated tumor suppression.

### Materials and Methods

**Animals.** Mice differing in  $p21^{WAF1}$  gene dose were generated by homologous recombination, as described previously (15). The background strain of the mice used in these studies was 129/SvEv. Breeding pairs of mice differing in  $p53$  gene dose were obtained from Robert S. Sikorski (National Cancer Institute, Rockville, MD; Ref. 13). DNA was isolated from tail samples by standard phenol-chloroform extraction, and  $p53$  or  $p21^{WAF1}$  gene status was determined by PCR using primers described previously (Refs. 12 and 16; Recombinant DNA Laboratory, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD). In experiment 1, a total of 34  $p21^{WAF1} (+/+)$ , 71  $p21^{WAF1} (+/-)$ , and 37  $p21^{WAF1} (-/-)$  mice, 6–10 weeks of age, were treated following the two-stage carcinogenesis protocol described below. In experiment 2, 15  $p21^{WAF1} (+/+)$ , 27  $p21^{WAF1} (+/-)$ , and 17  $p21^{WAF1} (-/-)$  mice, 5–7½ months of age, were treated. The first tumors were observed at week 11.

**Animal Treatments/Tumor Induction Protocols.** For tumor induction, mice were initiated with a single dose of 25 nmol DMBA<sup>2</sup> (6.4  $\mu$ g; Acros Organics, Somerville, NJ), applied in 200  $\mu$ l of acetone to the dorsal surface 1 day after shaving. TPA (5  $\mu$ g in 200  $\mu$ l of acetone; Alexis Corp., San Diego, CA) was applied twice weekly for 20 weeks, beginning 1 week after initiation. This dose of TPA was determined to sustain continuous hyperplasia in the 129/SvEv strain at 3 weeks of treatments, and the dosage and schedule of treatments were as described by Reiners and Kameshwar (17). In experiment 2, a small number of control mice of each genotype were treated with DMBA or TPA alone. The occurrence of papillomas and carcinomas was monitored by gross appearance and recorded weekly for each mouse. Tumors were measured between 43 and 46 weeks of the study for experiment 1 and between 34 and 37 weeks for experiment 2, and tumor volumes were calculated as

Received 1/22/99; accepted 3/19/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> To whom requests for reprints should be addressed, at Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, NIH, 30 Convent Drive MSC4340, Building 30, Room 211, Bethesda, MD 20892-4340. Phone: (301) 594-5270; Fax: (301) 402-0823; E-mail: wweinberg@yoda.nidcr.nih.gov.

<sup>2</sup> The abbreviations used are: DMBA, 7,12-dimethylbenz[a]anthracene; BrdUrd; 5-bromo-2'-deoxyuridine; TPA, 12-O-tetradecanoylphorbol-13-acetate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

length  $\times$  width  $\times$  height of each individual tumor, as measured with calipers. At 52 weeks, or if the animal became moribund, mice were euthanized and tumor samples were collected in 70% ethanol or buffered formalin solution. Tumors with a gross carcinoma phenotype and a sampling of papillomas were confirmed by histological analysis of H&E-stained sections. In some experiments mice were treated either once with TPA (5  $\mu$ g) and euthanized 48 h later, or treated three times at 3–4-day intervals and euthanized 3 days after the final treatment. Additional mice were pretreated with 50  $\mu$ g of dexamethasone 5 min before TPA treatment. Control animals were treated in parallel with acetone alone. For determination of *in vivo* labeling index, mice received i.p. injections of 200–250 mg/kg BrdUrd (Sigma Chemical Co., St. Louis, MO) in saline 1 h before being euthanized.

**Immunostaining.** BrdUrd incorporation was detected in ethanol-fixed sections by sequential incubation with mouse antibody to BrdUrd (Becton Dickinson, San Jose, CA) and biotinylated goat antimouse IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Labeled nuclei were visualized with AEC chromogen (BioGenex, San Ramon, CA) and counterstained with Meyer's Hematoxylin (Sigma Chemical Co.). Differentiation-specific proteins keratin 1 and 10, loricrin, and filaggrin were recognized by incubation with monospecific rabbit polyclonal antibodies (BabCO, Richmond, CA) and biotinylated goat antirabbit IgG (Kirkegaard and Perry Laboratories), using AEC (BioGenex) or 3,3'-diaminobenzidine (Sigma Chemical Co.) as chromogen.

**In Vitro Conversion Assay.** Keratinocytes from *p53* null or *p21<sup>WAF1</sup>* null mice and isogenic controls were isolated from epidermis of newborn mice of each genotype as described previously (12, 16), plated on matrix-coated dishes (18), and cultured in medium containing 0.05 mM Ca<sup>2+</sup>, 8% fetal bovine serum (Gemini Bioproducts, Inc., Calabasas, CA), and 1% penicillin-streptomycin (Life Technologies, Inc., Grand Island, NY). A previously described assay for malignant conversion potential was followed (19). Each group consisted of at least 5 and up to 14 dishes, and the experiment was repeated twice with essentially the same results.

## Results

Mice 6–10 weeks of age, differing in *p21<sup>WAF1</sup>* gene dose, were treated once with DMBA as initiating agent and promoted twice weekly with 5  $\mu$ g of TPA. Animals were scored each week for papilloma and carcinoma formation. As shown in Fig. 1, in experiment 1 when young mice were used, the average papilloma yield was ~3-fold higher in mice homozygous for a null mutation in the

*p21<sup>WAF1</sup>* gene compared with wild-type control mice, although heterozygotes demonstrated an intermediate response. The latency period for tumor eruption was the same in all groups. Survival was similar across genotypes up to 40 weeks of treatment (Fig. 1, bottom). The decline in the number of surviving *p21<sup>WAF1</sup>* (+/-) and (-/-) mice past 40 weeks presumably reflects the increased tumor burden on these animals, resulting in increased mortality. Because age can modulate tumor development, the study was also performed in mice 5–7½ months of age, as shown in experiment 2. The increased papilloma formation in *p21<sup>WAF1</sup>* null mice was observed in these mice as well, although the differences were smaller, and the heterozygotes responded more similarly to the wild-type controls. The difference in results between experiments 1 and 2 seems to reflect an increase in tumor formation in older wild-type animals, rather than a decrease in tumor incidence in null or heterozygous mice. No tumors were observed in older mice of any genotype treated with a single dose of DMBA or 20 weeks of TPA promotion alone. There seemed to be a trend for increased mean volume of tumors with decreasing *p21<sup>WAF1</sup>* gene dose (data not shown); however, due to large-size variability among tumors, the median tumor size was not statistically significantly different across genotypes (Kruskal-Wallis test).

To determine whether *p21<sup>WAF1</sup>* deficiency causes an imbalance in cell proliferation *in vivo* that might contribute to the increased papilloma yield, mice received injections of BrdUrd 1 h before being euthanized, and BrdUrd-labeled nuclei were counted. The *in vivo* labeling index of untreated adult skin was similar among the three genotypes (Table 1), with slight variations depending on the hair cycle. Mice of all genotypes developed hyperplasia after a single treatment with the tumor promoter TPA (Fig. 2), and this was sustained after three treatments. The basal cell labeling index was variable within and between animals of each genotype after TPA treatment, presumably reflecting differences in the uniformity of treatment. However, *p21<sup>WAF1</sup>*-deficient epidermis displayed a moderately, but significantly, lower labeling index compared with *p21<sup>WAF1</sup>*(+/+), in contrast to expectations if the absence of *p21<sup>WAF1</sup>* led to increased responsiveness to TPA (Table 1). Thus, an increased

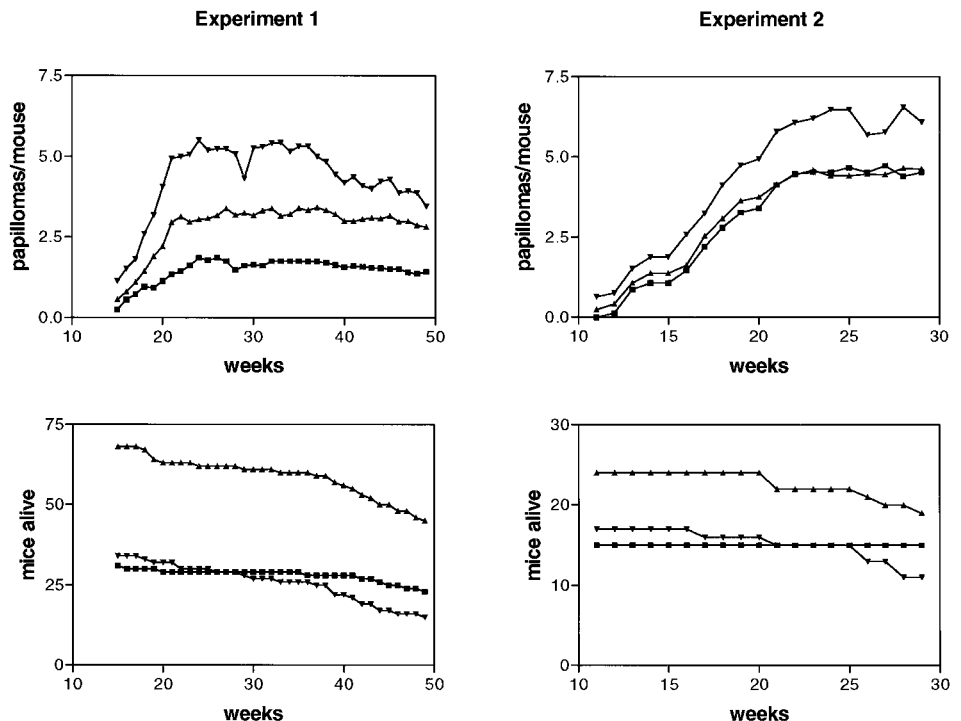


Fig. 1. *p21<sup>WAF1</sup>*-deficient mice display increased papilloma yield after chemical carcinogenesis. Mice 6–10 weeks of age (*Experiment 1*) and 5–7½ months of age (*Experiment 2*) were initiated with DMBA (25 nmol) and promoted twice weekly for 20 weeks with 5  $\mu$ g of TPA, by topical application to dorsal skin. Animals were examined weekly for tumor formation (*top*), and the number of animals alive in each group was documented (*bottom*). ■, *p21<sup>WAF1</sup>*(+/+); ▲, *p21<sup>WAF1</sup>*(+/-); ▼, *p21<sup>WAF1</sup>*(-/-).

Table 1 p21<sup>WAF1</sup> deficiency does not increase labeling index of untreated or TPA-treated adult epidermis

	Labeling index (number of BrdUrd positive cells/ 100 basal cells)		
	p21 <sup>WAF1</sup> genotype		
	+/+	+/-	-/-
Age of mice <sup>a</sup>			
10–12 weeks	3.7 (2) <sup>c</sup>	2.3 (2)	2.7 (3)
19–22 weeks	3.0 (1)	5.3 (1)	4.5 (1)
25–29 weeks	2.9 (2)	2.0 (2)	3.0 (2)
Treatment <sup>b</sup>			
TPA once	22.2 (2)	13.1 <sup>d</sup> (2)	17.7 <sup>d</sup> (2)
TPA 3 times	26.9 (2)	25.7 (3)	18.8 <sup>d</sup> (3)

<sup>a</sup> Control mice were analyzed at each of three ages, and 750–2300 nuclei were counted for each group.

<sup>b</sup> Mice were treated with TPA, as noted, and 750–3400 nuclei were counted for each genotype and treatment.

<sup>c</sup> Number in parentheses indicates number mice/genotype analyzed.

<sup>d</sup> Significantly different from p21<sup>WAF1</sup> (+/+) (two-tailed  $P < 0.0001$  by  $\chi^2$  test).

There was no statistically significant difference in labeling index of untreated skins of p21<sup>WAF1</sup>-deficient mice compared with p21<sup>WAF1</sup> (+/+), summed across all age groups, by Mantel-Haenszel extension of  $\chi^2$  test.

sensitivity to growth-promoting effects of TPA in epidermis of the deficient mice does not explain the higher papilloma incidence of this genotype. Moreover, topical exposure to 100  $\mu\text{g}$  of DMBA did not stimulate or inhibit DNA synthesis in any genotype when BrdUrd incorporation was examined at 8 h after application (data not shown). The expression of differentiation-specific markers in untreated epidermis of all three genotypes was identical, and strong suprabasal expression of keratins 1 and 10, filaggrin, and loricrin was also consistent across genotypes after one or three exposures of TPA (Fig. 2).

Tumor promotion can be blocked by topical treatment with dexamethasone (20), and glucocorticoids have been shown to up-regulate p21<sup>WAF1</sup> (21). To determine whether this sensitivity to steroids could influence promotion in different p21<sup>WAF1</sup> genotypes, 50  $\mu\text{g}$  of dexamethasone was applied to dorsal skin of p21<sup>WAF1</sup> (+/+), (+/-), and (-/-) mice before TPA treatment. All genotypes responded equally to dexamethasone inhibition of TPA-induced hyperplasia (data not shown).

Skin tumor induction in p53 (-/-) mice is characterized by rapid appearance of carcinomas and a high frequency (43%) of malignant conversion (22). To assess if this characteristic is common to p21<sup>WAF1</sup>-deficient mice, tumors arising in each genotype were observed over 52 weeks for conversion to a malignant phenotype. Like p53 (-/-) mice, an increased percentage of p21<sup>WAF1</sup> null mice developed carcinomas compared with wild-type control mice (Table 2). However, because the papilloma yield was substantially increased in the p21<sup>WAF1</sup>-deficient mice, the percentage of papillomas converting to carcinomas was low and not significantly different across genotypes (Table 2). The conversion frequency in these mice was similar to that seen in multiple strains of conventional mice, including 129/SvEv (17). In no instance was a mouse observed to develop more than one carcinoma, despite the increased papilloma burden of p21<sup>WAF1</sup>-deficient mice.

The ability of initiated p21<sup>WAF1</sup>-deficient cells to convert to malignancy was also evaluated in an *in vitro* model in which keratinocytes were transduced with a retrovirus encoding an activated *ras* oncogene and exposed to 3  $\mu\text{g}/\text{ml}$  MNNG for 1 h as a converting agent, followed by selection in medium containing 0.5 mM  $\text{Ca}^{2+}$ . The elevated  $[\text{Ca}^{2+}]$  will inhibit growth of benign but not malignant keratinocytes (19). No colonies formed in keratinocytes from mice differing in p21<sup>WAF1</sup> gene dose in the absence of MNNG. After exposure to MNNG, an average of <1 focus developed/dish [0.8, 0.3, and 0.1 in p21<sup>WAF1</sup> (+/+), (+/-), and (-/-) cultures, respectively].

Thus, consistent with the *in vivo* results, there was no increased tendency toward focus formation in p21<sup>WAF1</sup>-deficient cultures.

## Discussion

Previous studies from our laboratory have demonstrated that p21<sup>WAF1</sup> deficiency does not recapitulate the malignant phenotype associated with p53 loss of function in keratinocytes transduced with the *v-ras*<sup>Ha</sup> oncogene and grafted onto nude mouse hosts (16). In contrast, Missero *et al.* (23) have reported an enhanced growth potential and malignant phenotype of p21<sup>WAF1</sup> null keratinocytes reminiscent of those observed in our previous study of p53 null mice (12). The xenograft approach used in these studies does not allow the discrimination of tumor multiplicity, and the outcome of such experiments could be influenced by a number of technical factors, such as the level of expression of exogenous *ras* oncogene, and stromal influences. Therefore, in the present study, we have used an intact animal model to evaluate the contribution of p21<sup>WAF1</sup> gene dose to both tumor development and progression. The results presented here demonstrate that p21<sup>WAF1</sup> loss enhances the formation of benign epidermal papillomas after exposure to chemical carcinogens, suggesting that p21<sup>WAF1</sup> normally acts to suppress tumor formation. Tumors did not arise in any experimental genotype treated with DMBA or TPA only, suggesting that p21<sup>WAF1</sup> deficiency will not substitute for initiation or promotion. Despite an increase in benign tumor formation, we found no increased frequency of malignant conversion in these tumors, consistent with our earlier grafting studies (16).

The increased incidence of benign tumors after carcinogen exposure observed in this study is similar to that observed in transgenic mice expressing a gain of function p53 mutant targeted to the epidermis, where p21<sup>WAF1</sup> expression was also decreased in the resulting papillomas (24). However, chemical induction of skin tumors on p53 null mice resulted in a low papilloma yield relative to wild-type controls (22). In addition, benign skin tumor formation is inhibited when p53 null mice are bred with mice carrying an activated *v-ras* transgene targeted to the epidermis (25). Together, these results suggest that some p53 function may be required for papilloma formation, whereas other functions, such as p21<sup>WAF1</sup> induction, may inhibit papilloma formation. In contrast, epidermal tumors induced by both chemicals and UVB arising in a p53 null background demonstrate a high malignant potential (22, 26), suggesting that alternate p53-mediated actions regulate initiation, premalignant progression, and malignant conversion.

Although basal p21<sup>WAF1</sup> levels are decreased in p53 null keratinocytes (12), p21<sup>WAF1</sup> levels can be regulated in epidermal keratinocytes independently of p53, after induction of terminal differentiation (4, 6) and during TPA promotion (25). Because cell cycle arrest is linked to the onset of the differentiated phenotype, a loss of this potential growth regulatory control might be expected to affect the commitment of cells to terminal differentiation and, thus, influence the neoplastic phenotype. It has been reported previously that p21<sup>WAF1</sup> null keratinocytes display an increased proliferation potential *in vitro* and that expression of differentiation-specific markers is blocked in the absence of or by overexpression of p21<sup>WAF1</sup> (23, 27). However, other investigators have reported no change in differentiation-specific gene expression in normal human keratinocytes overexpressing p21<sup>WAF1</sup> (28). In this study, we found no evidence *in vivo* of an increase in cell proliferation in p21<sup>WAF1</sup>-deficient epidermis, as determined by BrdUrd incorporation of untreated and TPA-treated skin. Furthermore, untreated and TPA-treated mice of each genotype showed similar expression patterns of differentiation markers *in vivo*.

Because p21<sup>WAF1</sup> deficiency does not seem to increase the *in vivo*



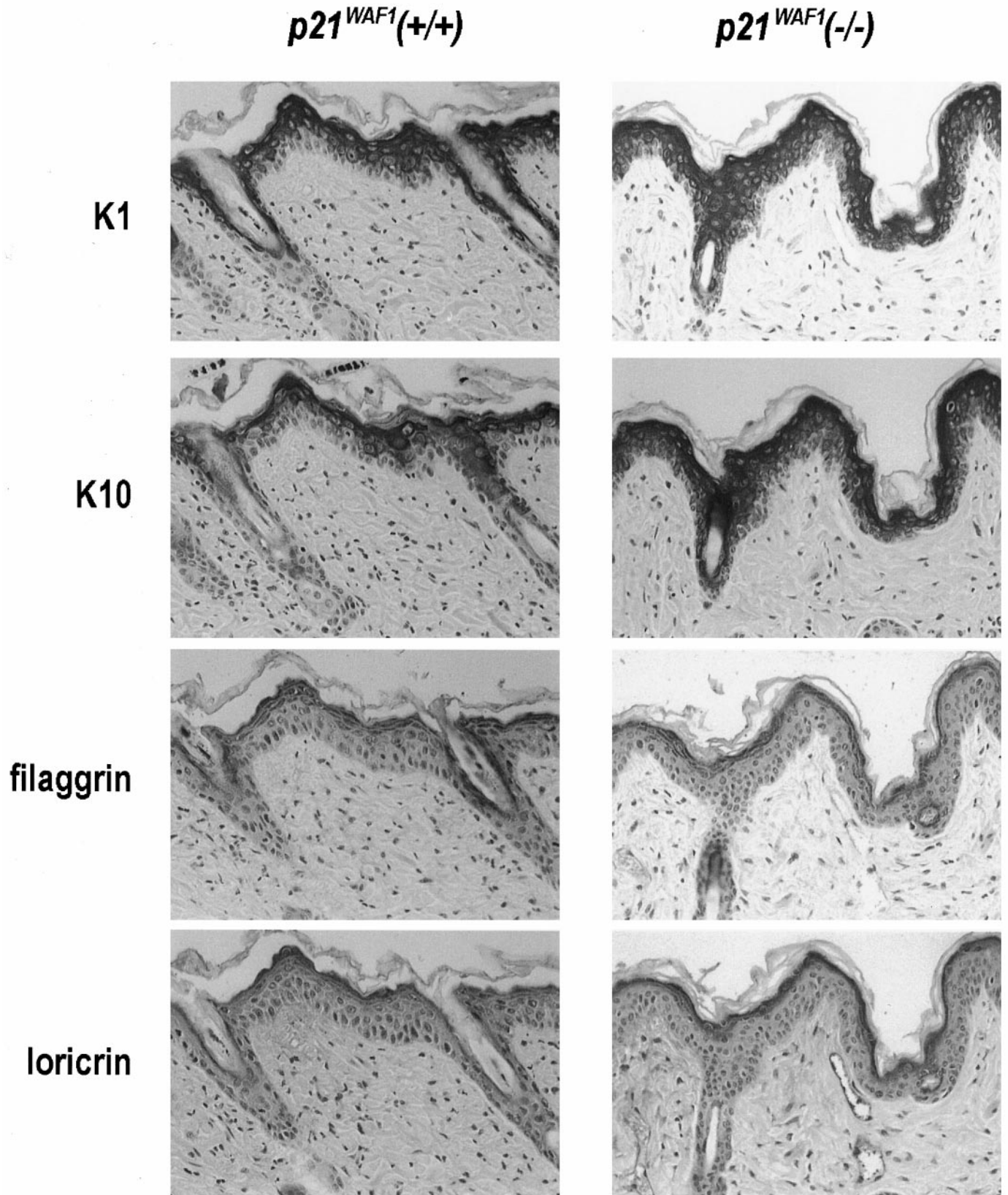


Fig. 2. *In vivo* expression of differentiation markers is independent of p21<sup>WAF1</sup> genotype. Skins from adult mice sacrificed 48 h after treatment with 5  $\mu$ g of TPA were fixed in 70% ethanol and processed for immunohistochemical staining of keratin 1 (K1), keratin 10 (K10), filaggrin, and loricrin ( $\times 200$ ).

Table 2 Papillomas arising in p21<sup>WAF1</sup>-deficient mice do not demonstrate increased frequency of malignant conversion

p21 <sup>WAF1</sup> genotype	% mice with papillomas	Maximum no. of papillomas observed	% mice with carcinomas <sup>a</sup>	Conversion frequency (no. of carcinomas/maximum no. of papillomas × 100)
Experiment 1 <sup>b</sup>				
+/+	56	61	11 (19)	3.3
+/-	76	217	20 (54)	5.1 [p = 0.74] <sup>c</sup>
-/-	81	174	27 (30)	4.6 [p = 1.00]
Experiment 2 <sup>b</sup>				
+/+	100	75	7 (15)	1.3
+/-	82	97	27 (22)	6.2 [p = 0.14]
-/-	100	97	18 (17)	3.1 [p = 0.63]

<sup>a</sup> Number of mice with carcinomas/tumor-bearing mice × 100; number in parentheses indicates maximum number tumor bearing mice in each genotype (mice at risk).

<sup>b</sup> Mice in experiments 1 and 2 were 6–10 weeks and 5–7½ months of age, respectively, at time of initial treatment.

<sup>c</sup> Comparison of conversion frequency in p21<sup>WAF1</sup> (+/+) versus genotype shown (two-tailed *P* from Fisher's exact test).

proliferation rate or alter the expression of differentiation markers in untreated or TPA-treated epidermis, the increased benign tumor incidence observed in p21<sup>WAF1</sup>-deficient mice may reflect a specific effect of p21<sup>WAF1</sup> deficiency on initiated cells. In addition to its participation in cell cycle arrest mediated by inhibition of cyclin-dependent kinase activity, the p21<sup>WAF1</sup> protein interacts with proliferating cell nuclear antigen and, through this interaction, can influence both replicative DNA synthesis and nucleotide excision repair (reviewed in Ref. 2). p21<sup>WAF1</sup>-deficient tumor cell lines have been reported to display DNA repair defects (2). If such a defect is present in normal keratinocytes, the increased incidence of papillomas in p21<sup>WAF1</sup>-deficient mice may reflect decreased repair of carcinogen-induced DNA damage, resulting in a growth advantage to a higher proportion of initiated cells. Additional experiments are required to clarify whether p21<sup>WAF1</sup> influences *in vivo* tumor formation directly via its ability to inhibit cyclin-dependent kinase activity, its interaction with proliferating cell nuclear antigen affecting DNA replication or repair processes, or some other mechanism.

## Acknowledgments

We thank Drs. Nathaniel Rothman and Robert Tarone for assistance with statistical analyses and the Cellular Imaging Core (National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD) for processing fixed samples and H&E staining.

## References

- El-Deiry, W. S., Tokino, W. S., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. WAF1, a potential mediator of p53 tumor suppression. *Cell*, 75: 817–825, 1993.
- El-Deiry, W. S. p21/p53, cellular growth control and genomic integrity. *Curr. Top. Microbiol. Immunol.*, 227: 121–137, 1998.
- Weinberg, W. C., Azzoli, C. G., Chapman, K., Levine, A. J., and Yuspa, S. H. p53-mediated transcriptional activity increases in differentiating epidermal keratinocytes in association with decreased p53 protein. *Oncogene*, 10: 2271–2279, 1995.
- Missero, C., Calautti, E., Eckner, R., Chin, J., Tsai, L. H., Livingston, D. M., and Dotto, G. P. Involvement of the cell-cycle inhibitor *Cip1/WAF1* and the E1A-associated p300 protein in terminal differentiation. *Proc. Natl. Acad. Sci. USA*, 92: 5451–5455, 1995.
- Gervais, J. L., Seth, P., and Zhang, H. Cleavage of CDK inhibitor p21(Cip1/Waf1) by caspases is an early event during DNA damage-induced apoptosis. *J. Biol. Chem.*, 273: 19207–19212, 1998.
- Parker, S. B., Eichele, G., Zhang, P., Rawls, A., Sands, A. T., Bradley, A., Olson, E. N., Harper, J. W., and Elledge, S. J. p53-independent expression of p21<sup>Cip1</sup> in muscle and other terminally differentiating cells. *Science (Washington DC)*, 267: 1024–1027, 1995.
- Liu, M., and Pelling, J. C. UV-B/A irradiation of mouse keratinocytes results in p53-mediated WAF/CIP1 expression. *Oncogene*, 10: 1955–1960, 1996.
- Wu, Y., Huang, H., Miner, Z., and Kulesz-Martin, M. Activities and response to DNA damage of latent and active sequence-specific DNA binding forms of mouse p53. *Proc. Natl. Acad. Sci. USA*, 94: 8982–8987, 1997.
- Eastham, J. A., Hall, S. J., Sehgal, I., Wang, J., Timme, T. L., Yang, G., Connell-Crowley, L., Elledge, S. J., Zhang, W. W., Harper, J. W., and Thompson, T. C. *In vivo* gene therapy with p53 or p21 adenovirus for prostate cancer. *Cancer Res.*, 55: 5151–5155, 1995.
- Chen, J., Willingham, T., Shuford, M., Bruce, D., Rushing, E., Smith, Y., and Nisen, P. D. Effects of ectopic overexpression of p21<sup>WAF1/CIP1</sup> on aneuploidy and the malignant phenotype of human brain tumor cells. *Oncogene*, 13: 1395–1403, 1996.
- El-Deiry, W. S., Harper, J. W., O'Connor, P. M., Velculescu, V. E., Canman, C. E., Jackman, J., Pietenpol, J. A., Burrell, M., Hill, D. E., and Wang, Y. WAF1/CIP1 is induced in p53-mediated G<sub>1</sub> arrest and apoptosis. *Cancer Res.*, 54: 1169–1174, 1994.
- Weinberg, W. C., Azzoli, C. G., Kadiwar, N., and Yuspa, S. H. p53 gene dosage modifies growth and malignant progression of keratinocytes expressing the v-ras<sup>Ha</sup> oncogene. *Cancer Res.*, 54: 5584–5592, 1994.
- Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S., and Bradley, A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumors. *Nature (Lond.)*, 356: 215–221, 1992.
- Jacks, T., Remington, L., Williams, B. O., Schmitt, E. M., Halachmi, S., Bronson, R. T., and Weinberg, R. A. Tumor spectrum analysis in p53-mutant mice. *Curr. Biol.*, 4: 1–7, 1994.
- Deng, C., Zhang, P., Harper, W., Elledge, S. J., and Leder, P. Mice lacking p21<sup>CIP1/WAF1</sup> undergo normal development, but are defective in G<sub>1</sub> checkpoint control. *Cell*, 82: 675–684, 1995.
- Weinberg, W. C., Montano, N. E., and Deng, C. Loss of p21<sup>CIP1/WAF1</sup> does not recapitulate accelerated malignant conversion caused by p53 loss in experimental skin carcinogenesis. *Oncogene*, 15: 685–690, 1997.
- Reiners, J. J., Jr., and Kameshwar, P. S. Susceptibility of 129/SvEv mice in two-stage carcinogenesis protocols to 12–0-tetradecanoylphorbol-13-acetate promotion. *Carcinogenesis (Lond.)*, 18: 593–597, 1997.
- Kawamura, H., Strickland, J. E., and Yuspa, S. H. Association of resistance to terminal differentiation with initiation of carcinogenesis in adult mouse epidermal cells. *Cancer Res.*, 45: 2748–2752, 1985.
- Morgan, D., Welty, D., Glick, A., Greenhalgh, D., Hennings, H., and Yuspa, S. H. Development of an *in vitro* model to study carcinogen-induced neoplastic progression of initiated mouse epidermal cells. *Cancer Res.*, 52: 3145–3156, 1992.
- Strawhecker, J. M., and Pelling, J. C. Inhibition of mouse skin tumorigenesis by dexamethasone occurs through a Ha-ras-independent mechanism. *Carcinogenesis (Lond.)*, 13: 2075–2080, 1992.
- Corroyer, S., Nabeyrat, E., and Clement, A. Involvement of the cell cycle inhibitor CIP1/WAF1 in lung alveolar epithelial cell growth arrest induced by glucocorticoids. *Endocrinology*, 138: 3677–3685, 1997.
- Kemp, C. J., Donehower, L. A., Bradley, A., and Balmain, A. Reduction of p53 gene dosage does not increase initiation or promotion but enhances malignant progression of chemically induced skin tumors. *Cell*, 74: 813–822, 1993.
- Missero, C., Di Cunto, F., Kiyokawa, H., Koff, A., and Dotto, G. P. The absence of p21<sup>CIP1/WAF1</sup> alters keratinocyte growth and differentiation and promotes ras-tumor progression. *Genes Dev.*, 10: 3065–3075, 1996.
- Wang, X. J., Greenhalgh, D. A., Jiang, A., He, D., Zhong, L., Medina, D., Brinkley, B. R., and Roop, D. R. Expression of a p53 mutant in the epidermis of transgenic mice accelerates chemical carcinogenesis. *Oncogene*, 17: 35–45, 1998.
- Greenhalgh, D. A., Wang, X. J., Donehower, L. A., and Roop, D. R. Paradoxical tumor inhibitory effect of p53 loss in transgenic mice expressing epidermal-targeted v-rasHa, v-fos, or human transforming growth factor α. *Cancer Res.*, 56: 4413–4423, 1996.
- Li, G., Tron, V., and Ho, V. Induction of squamous cell carcinoma in p53-deficient mice after ultraviolet irradiation. *J. Invest. Dermatol.*, 110: 72–75, 1998.
- Di Cunto, F., Topley, G., Calautti, E., Hsiao, J., Ong, L., Seth, P. K., and Dotto, G. P. Inhibitory function of p21Cip1/WAF1 in differentiation of primary mouse keratinocytes independent of cell cycle control. *Science (Washington DC)*, 280: 1069–1072, 1998.
- Harvat, B. L., Wang, A., Seth, P., and Jetten, A. M. Up-regulation of p27Kip1, p21WAF1/Cip1 and p16Ink4a is associated with, but not sufficient for, induction of squamous differentiation. *J. Cell Sci.*, 111: 1185–1196, 1998.

# Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

## Genetic Deletion of p21<sup>WAF1</sup> Enhances Papilloma Formation but not Malignant Conversion in Experimental Mouse Skin Carcinogenesis

Wendy C. Weinberg, Ester Fernandez-Salas, David L. Morgan, et al.

*Cancer Res* 1999;59:2050-2054.

**Updated version** Access the most recent version of this article at:  
<http://cancerres.aacrjournals.org/content/59/9/2050>

**Cited articles** This article cites 28 articles, 13 of which you can access for free at:  
<http://cancerres.aacrjournals.org/content/59/9/2050.full#ref-list-1>

**Citing articles** This article has been cited by 21 HighWire-hosted articles. Access the articles at:  
<http://cancerres.aacrjournals.org/content/59/9/2050.full#related-urls>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, use this link  
<http://cancerres.aacrjournals.org/content/59/9/2050>.  
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.