Components of the Rb pathway are critical targets of UV mutagenesis in a murine melanoma model

Karuppiah Kannan*†, Norman E. Sharpless*†‡, Jin Xu*, Ronan C. O'Hagan*, Marcus Bosenberg*§¶, and Lynda Chin*‖**

*Department of Medical Oncology, Dana–Farber Cancer Institute, and §Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115; and ‖Department of Dermatology, Harvard Medical School, Boston, MA 02114

Edited by Philip C. Hanawalt, Stanford University, Stanford, CA, and approved December 4, 2002 (received for review October 21, 2002)

Epidemiological studies support a link between melanoma risk and UV exposure early in life, yet the molecular targets of UV's mutagenic actions are not known. By using well characterized murine models of melanoma, we provide genetic and molecular evidence that identifies components of the Rb pathway as the principal targets of UV mutagenesis in murine melanoma development. In a melanoma model driven by H-RAS activation and loss of $p19^{ARF}$ function, UV exposure resulted in a marked acceleration in melanoma genesis, with nearly half of these tumors harboring amplification of *cyclin-dependent kinase* (*cdk*) *6*, whereas none of the melanomas arising in the absence of UV treatment possessed *cdk6* amplification. Moreover, UV-induced melanomas showed a strict reciprocal relationship between *cdk6* amplification and $p16^{INK4a}$ loss, which is consistent with the actions of UV along the Rb pathway. Most significantly, UV exposure had no impact on the kinetics of melanoma driven by H-RAS activation and $p16^{INK4a}$ deficiency. Together, these molecular and genetic data identify components of the Rb pathway as critical biological targets of UV-induced mutagenesis in the development of murine melanoma *in vivo*.

$p16^{INK4a}$ | $p19^{ARF}$ | UVB | cdk6

Melanoma, the most lethal human skin cancer, shows an alarming rate of increase worldwide and causes >7,000 deaths annually in the United States alone (1–3). Epidemiological evidence has established that a history of sunburn and intermittent exposure to UV light, particularly early in life, promotes melanoma development (4, 5). This epidemiological association is strong and is causally linked by investigations using human skin grafts (6, 7). However, the specific molecular targets, if any, of this environmental carcinogen are not known. Although the finding of C>T point mutations ("UV-signature") of $p16^{INK4a}$ in human melanoma suggested its targeting by this carcinogen in humans (8–10), the observation of a similar C>T mutation bias in glioma (reviewed in ref. 11), a non-UV induced tumor, has called this conclusion into question. Alternatively, the relationship between $p16^{INK4a}$ and melanoma has been explained by the observation that UV light can induce $p16^{INK4a}$ expression in human melanocytes (12, 13), thereby implying a role for $p16^{INK4a}$ in the repair of UV-induced lesions. Finally, several groups have suggested that UV functions in a noncell autonomous manner to facilitate melanoma either by inducing immune suppression (14) or by eliciting the elaboration of tumor-promoting paracrine factors (15). Thus, a definitive UV-$p16^{INK4a}$ link, and the nature of this interaction, has yet to be clarified on either the molecular, physiological, or genetic levels.

In view of the presence of activating B-RAF mutation, a direct signaling surrogate of RAS activation, in ≈70% of human melanomas (16), and RAS mutation in an additional 20% (17), RAS-pathway activation appears to represent a rite of passage for human melanoma. We have previously generated transgenic mice expressing an activated form of human H-Ras (*Tyr-RAS*) in melanocytes that are highly melanoma-prone when introduced onto *Ink4a/Arf*$^{-/-}$ or *p53*$^{-/-}$ backgrounds (18, 19). Importantly, we have shown that RAS-induced melanomas from these models harbor secondary genomic changes detectable by conventional and array-based comparative genomic hybridization (CGH) that are syntenic to known hotspots of human melanomas (ref. 19; L.C. and B. Bastian, unpublished observations), further validating the use of this model in the study of the human disease.

Loss of the *INK4a/ARF* (*CDKN2A*) locus is encountered in ≈50% of human melanomas (20, 21) and, along with activating mutation of B-RAF (16), are the most common genetic lesions of this cancer type. The *INK4a/ARF* locus encodes two distinct proteins, $p16^{INK4a}$ and $p14^{ARF}$ ($p19^{ARF}$ in the mouse), both of which demonstrate tumor suppressor activity in genetically distinct anti-cancer pathways: the "Rb pathway" for $p16^{INK4a}$ and the "p53 pathway" for $p14^{ARF}$ (see below; reviewed in refs. 11 and 22). ARF and $p16^{INK4a}$ have different first exons (1β and 1α respectively) and 5′ regulatory units, but are spliced into a common second exon in alternate reading frames. The cyclin-dependent kinase inhibitor (CKI) $p16^{INK4a}$ is known to inhibit CDK4/CDK6-directed phosphorylation of RB, and loss of $p16^{INK4a}$ permits RB hyperphosphorylation and subsequent de-repression of RB-regulated genes. Also, a regulator of the cell cycle, $p14^{ARF}$ has been shown to be a principal regulator of MDM2, an E3 ubiquitin ligase important in p53 degradation. Consequently, loss of $p14^{ARF}$ (or $p19^{ARF}$) can directly impair the stabilization of p53 in response to certain oncogenic stresses. Several lines of evidence support the view that both $p16^{INK4a}$ and $p14^{ARF}$ play roles in melanoma suppression *in vivo*. First, germline mutations of either exon 1α (affecting $p16^{INK4a}$ only) or exon 1β (targeting $p14^{ARF}$ only) have both been identified in melanoma-prone kindreds (11, 23–25). Also, animals specifically deficient for $p16^{INK4a}$ have a low frequency of carcinogen-induced melanoma that is augmented in the setting of $p19^{ARF}$ haploinsufficiency (26–28). Finally, similar to mice on *Ink4a/Arf*$^{-/-}$ (doubly null for both $p16^{INK4a}$ and $p19^{ARF}$) background (18), *Tyr-RAS* mice deficient for either $p16^{INK4a}$ or $p19^{ARF}$ are susceptible to RAS-induced melanomas (unpublished observations).

Here, these genetically defined mouse models of melanoma were used to explore the role of neonatal UV exposure in promoting melanoma development, and particular emphasis was placed on whether UV-induced melanoma incidence is modulated by the status of the Rb vs. p53 pathways. We found that neonatal UV treatment accelerates melanoma formation in *Tyr-RAS* $p19^{ARF-/-}$ animals. This increase in tumorigenesis is accompanied by *cdk6* amplification, which is mutually exclusive with $p16^{INK4a}$ loss in UV-treated animals and is not seen in tumors from non-UV-treated mice. Most significantly, UV light

This paper was submitted directly (Track II) to the PNAS office.

Abbreviation: CGH, comparative genomic hybridization.

†K.K. and N.E.S. contributed equally to this work.

‡Present address: The Lineberger Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7295.

¶Present address: Department of Pathology, University of Vermont, Burlington, VT 05405.

**To whom correspondence should be addressed at: Dana–Farber Cancer Institute, Harvard Medical School, 44 Binney Street (M413), Boston, MA 02115. E-mail: Lynda_Chin@dfci.harvard.edu.

did not accelerate melanoma formation in *Tyr-RAS* mice lacking $p16^{INK4a}$. These results suggest that components of the Rb pathway are the principal and rate-limiting target(s) of UV's actions in melanoma formation.

Materials and Methods

Mouse Tumor Cohorts. Mice specifically lacking $p19^{ARF}$ were generated by standard techniques with Cre-mediated excision of the neomycin marker embedded in exon 1β (N.E.S. and R. DePinho, unpublished work). The phenotype of this knockout strain is similar to that of a previously published $p19^{ARF}$ KO (29), with the development of spontaneous tumors with a median latency of ≈60 weeks. Experimental cohorts were generated by initially crossing $p19^{ARF-/-}$ (or $p16^{INK4a-/-}$; ref. 26) mice (both strains on FVB N2 background) onto tyrosinase enhancer-promoter-driven $H\text{-}RAS^{V12G}$ transgenic mice (*Tyr-RAS;* FVB N6; ref. 18) followed by heterozygous intercrosses between *Tyr-RAS* $p19^{ARF\pm}$ (or $p16^{INK4a\pm}$) animals. Cohorts were observed for melanoma development daily and moribund animals were killed for necropsy. Tumor tissues were fixed and paraffin-embedded for histopathological and also flash-frozen for subsequent analyses. Genotypes were determined by gene-specific PCR for both $p16^{INK4a}$ (26) and $p19^{ARF}$ (primers and conditions available upon request). The *Tyr-RAS* allele is transmitted on the Y chromosome. To minimize strain variability between these cohorts, FVB males were selected by using a marker-assisted genotyping protocol (30) to generate N3 backcrossed cohorts. In brief, "best" male founders were identified by analyzing 44 loci polymorphic between SvEv and FVB, with >14 males screened per generation. Loci were allelotyped on SYBR-stained 3% Nusieve gels by using PCR primers (The Jackson Laboratory; http://www.informatics.jax.org/, allele and primer lists available on request). Therefore, all animals analyzed in this study were >87.5% for FVB (N3). In Kaplan–Meier analyses, *Tyr-RAS* $p16^{INK4a+/+}$ or *Tyr-RAS* $p19^{ARF+/+}$ littermate controls (i.e., from heterozygous intercrosses) were compared with *Tyr-RAS* $p16^{INK4a-/-}$ or *Tyr-RAS* $p19^{ARF-/-}$ animals, respectively.

Tumor Analysis. Methylation-specific PCR and LOH analysis in exon 1α of $p16^{INK4a}$ were performed as described (19, 26). RNA was isolated from tumor specimens immediately after surgical removal by using Trizol reagents (GIBCO/BRL). For the detection of point mutations, 2 μg of DNase-treated RNA was used for cDNA synthesis (Superscript, Invitrogen). To determine the sequence of *cdk4* and $p16^{INK4a}$, primers spanning the entire ORFs were used to amplify tumor cDNA. All mutations were confirmed by sequencing at least twice. For sequence-tagged site (STS)-PCR and quantitative RT-PCR, primers from the 3′UTR of *cdk6* and *gapdh* were used; STS marker D6mit104 was used as a normal copy number control. PCR was performed by using 100 ng of genomic DNA or 1 μl of RT reaction mix with marker or gene-specific primers for 17 cycles of 95°C for 30 s, 56°C for 1 min, and 72°C for 45 s. PCR products were run on a 1% agarose TBE gel (100 mM Tris/100 mM boric acid/2.0 mM EDTA, pH 8.3) and transferred to Hybond N+ (Amersham Pharmacia). Probes were generated by PCR of pooled normal genomic DNA from the mice of origin for each tumor by using the same primers as for the quantitative PCR analysis. Randomly primed P^{32}-labeled probes were hybridized for 2 h at 65°C in RapidHyb (Amersham Pharmacia). Quantification was performed by using a PhosphorImager. *Rb* and *ink4a* Southern blots were performed as described (refs. 19 and 31; *Rb* probe courtesy of T. Jacks, Boston). *Cdk6* Southern analysis was performed by using an exonic probe generated by PCR (primers available upon request) and was normalized by using a nonamplified genomic DNA probe (32). Total protein lysates were prepared by briefly sonicating the tumor tissues in the presence of RIPA buffer with protease and phosphatase inhibitors and analyzed for $p16^{INK4a}$ as described (26). For cdk4 Western blots and immunoprecipitation, antibody C22 (Santa Cruz Biotechnology) was used. For immunoprecipitation analyses, 1 mg of protein extract was precleared by incubation with protein A Sepharose (Sigma) and preimmune serum and incubated for 2 h with anti-cdk4 antibody. Extracts were incubated for 1 h after the addition of protein A Sepharose. Immunoprecipitated complexes were fractionated by using SDS/PAGE and transferred to poly(vinylidene difluoride) membrane, and probed with anti-$p16^{INK4a}$ (M156, Santa Cruz Biotechnology) or anti-cdk4 antibody.

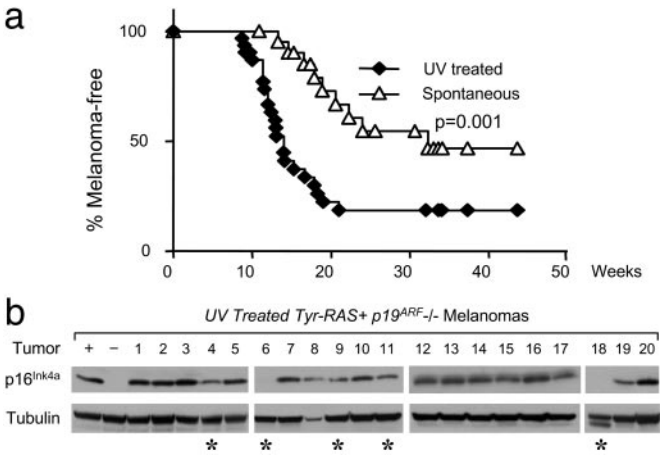

Fig. 1. Loss of $p19^{ARF}$ can cooperate with UV exposure to facilitate melanoma formation. FVB (N3) neonatal mice (1- to 3-day-old pups) were treated with a single dose of total body UV irradiation (9 kJ/M^2) by using an FS20T12 UV lamp (peak emittance in the UVB range, 310 nm). (*a*) *Tyr-RAS* $p19^{ARF-/-}$ mice with ($n = 31$) or without ($n = 22$) neonatal UV exposure were observed for tumor formation. No UV-treated Tyr-RAS $p19^{ARF+/+}$ mice developed melanoma ($n = 18$). Melanoma-free survival is shown, and nonmelanoma tumors (e.g., lymphoma) were censored in this analysis. The survival curves were compared with the log-rank test. (*b*) Western blot analysis of $p16^{INK4a}$ expression in UV-treated *Tyr-RAS* $p19^{ARF-/-}$ melanomas.

Results and Discussion

We examined the impact of $p19^{ARF}$ status in UV-mediated melanoma genesis by subjecting littermate *Tyr-RAS* $p19^{ARF-/-}$ and *Tyr-RAS* $p19^{ARF+/+}$ animals to a single neonatal erythrogenic dose of UVB irradiation as described (33) and followed them for melanoma development. For controls, alternating litters from the same colony were withheld from UVB treatment and observed for spontaneous melanoma development. Although none of the *Tyr-RAS* $p19^{ARF+/+}$ mice, with or without UVB exposure, developed melanoma during a 50-week period of observation, UV-treated *Tyr-RAS* $p19^{ARF-/-}$ animals demonstrated numerous melanomas arising with significantly shorter latency (Fig. 1*a*) relative to untreated *Tyr-RAS* $p19^{ARF-/-}$ controls. Furthermore, in the UV-treated cohort, the multiplicity of tumors was markedly increased from an average of 1.1 tumors per animal in the untreated group to 3.0 melanomas per animal in the UV-treated group ($P < 0.001$). These data demonstrate potent cooperation between UVB and the genetic alterations of activated H-RAS and $p19^{ARF}$ deficiency in melanoma formation.

Although the histopathology of UV-induced and spontaneous tumors were indistinguishable (data not shown), molecular characterization of the $p16^{INK4a}$-pRb axis by candidate gene survey in UV-induced melanomas revealed a shift in the mutational profile from that of spontaneous tumors. As was the case for spontaneous melanomas, $p16^{INK4a}$ methylation, Rb loss, *cdk4* overexpression or point mutation, and *c-myc* overexpression were not detected in melanomas from UV-treated *Tyr-RAS* $p19^{ARF-/-}$ mice (data not shown). In contrast, molecular analysis

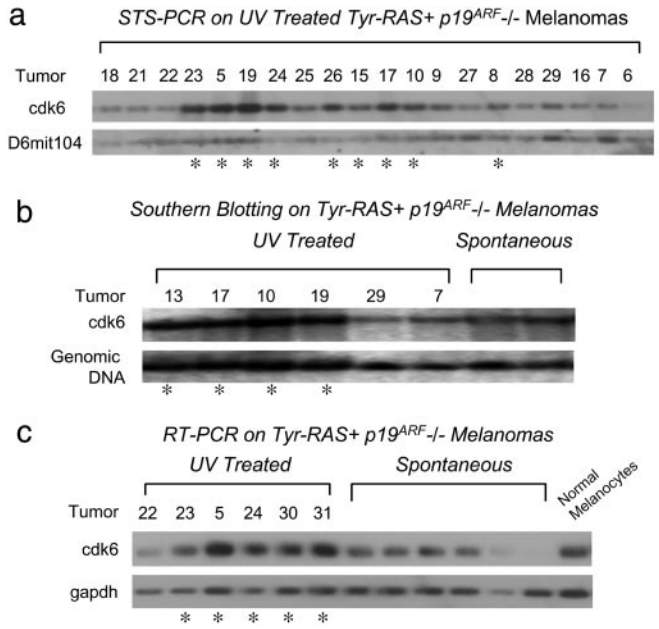

Fig. 2. *Cdk6* is amplified and overexpressed in a subset of UV-treated, but not spontaneous, *Tyr-RAS p19$^{ARF-/-}$* melanomas. Melanomas that harbor *cdk6* amplification do not demonstrate p16^{INK4a} loss. (*a*) STS-PCR analysis of *cdk6* copy number in UV-treated *Tyr-RAS p19$^{ARF-/-}$* melanomas. Signal was normalized to a nonamplified normal copy number control marker (D6mit104). *, Greater than twofold amplification by densitometry. (*b*) Southern blot analysis of *Tyr-RAS p19$^{ARF-/-}$* melanomas by using a *cdk6* probe, normalized to a chromosome 19 genomic DNA probe (32) that is not amplified. *, Greater than twofold amplification by densitometry. (*c*) Quantitative RT-PCR analysis of *cdk6* expression in *Tyr-RAS p19$^{ARF-/-}$* melanomas. *cdk6* signal was normalized to gapdh expression. Nontransformed primary cultured melanocytes isolated from *Ink4a/Arf$^{-/-}$* animals were used as control. *, Greater than twofold overexpression by densitometry.

of the spontaneous tumors showed that Rb pathway inactivation does occur via p16^{INK4a} loss or point mutation in ≈50% of cases (unpublished observations). In tumors from UV-treated *Tyr-RAS p19$^{ARF-/-}$* mice, this frequency of p16^{INK4a} functional loss (as measured by direct sequencing, Western and IP-Western with cdk4) was decreased (8/36 tumors = 22%; Fig. 1*b* and data not shown). Instead, *cdk6* amplification and overexpression (Fig. 2*a–c*) emerged as the principal Rb pathway lesion in UV-induced melanomas. A twofold or greater increase in *cdk6* gene copy number was detected in 16 of 35 (46%) melanomas from UV-treated *Tyr-RAS p19$^{ARF-/-}$* mice, compared with 0 of 22 melanomas from untreated *Tyr-RAS p19$^{ARF-/-}$* mice (Table 1; $P < 0.0001$). A commensurate gene dosage increase of proximal chromosome 5 was documented by an independent method, i.e., array-based CGH profiling of the *Tyr-RAS p19$^{ARF-/-}$* melanomas from UV-treated mice. The minimal region of this amplification was mapped to ≈1 MB, within which resides three annotated genes, one of which encodes *cdk6* (R.C.O.-H., C. Brennan, and L.C., unpublished work). In accord with the

Table 1. Rb pathway status in *Tyr-RAS p19$^{ARF-/-}$* melanomas

Rb pathway status	Spontaneous	UV-treated
p16^{INK4a} Loss	7 of 18	8 of 36*
cdk6 Amplification	0 of 20†	16 of 35*†
Both	0 of 15	0 of 35*

*$P < 0.008$ for lack of association between p16^{INK4a} loss and *cdk6* amplification.
$^{\dagger}P < 0.0001$ for frequency of *cdk6* amplification in spontaneous vs. UV-treated tumors.

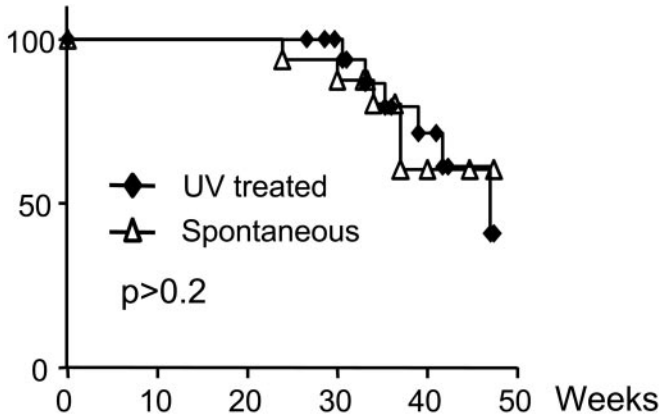

Fig. 3. UV treatment does not accelerate melanoma formation in *Tyr-RAS p16$^{INK4a-/-}$* mice. *Tyr-RAS p16$^{INK4a-/-}$* were randomized to UV exposure as in Fig. 1*a* and observed for tumor formation ($n = 16$ for non-UV-treated, $n = 23$ for UV-treated, $P > 0.2$).

result of the candidate gene survey, proximal chromosome 5 amplification was not detected in spontaneous melanomas from this cohort. Consistent with their known functional overlap in Rb pathway regulation, *p16^{INK4a}* loss and *cdk6* amplification were mutually exclusive (Table 1, $P < 0.008$) among the UV-treated *Tyr-RAS p19$^{ARF-/-}$* melanomas. This strict reciprocal relationship, coupled with the emergence of *cdk6* amplification in the UV-treated cohort, suggests that UVB's melanoma-promoting activities in this model are functionally linked to inactivation of the Rb pathway and are achieved most often by p16^{INK4a} loss or *cdk6* amplification.

To validate this UV-pRB pathway hypothesis by genetic means, we repeated the above UV study by employing *Tyr-RAS p16$^{INK4a-/-}$* animals to reveal whether Rb pathway inactivation, via p16^{INK4a} loss, is functionally equivalent to UVB exposure (Fig. 3). In sharp contrast to *Tyr-RAS p19$^{ARF-/-}$* mice, no cooperation was seen between UVB exposure and p16^{INK4a} loss in this model. Consistent with this lack of cooperation, the mutational profile of the p19^{ARF}-p53 axis in UV-induced melanomas from *Tyr-RAS p16$^{INK4a-/-}$* mice was similar to that of spontaneous melanomas from the same mice (not shown). Therefore, germ-line inactivation of *p16^{INK4a}* eliminated the melanoma-promoting effect of UVB exposure.

The finding that UV exposure cooperates with p19^{ARF} deficiency, but not p16^{INK4a} deficiency, in melanoma formation was unanticipated, given the presumed broad mutagenic action of UV, and suggests several possible explanations. First, this result might reflect a role of p19^{ARF} in the repair of UV-induced damage. Although, when compared with *p53$^{-/-}$* cells, *p19$^{ARF-/-}$* cells are considerably less resistant to agents that induce double-strand breaks (34, 35); *p19$^{ARF-/-}$* mouse embryo fibroblasts do demonstrate a modest resistance to G_1 arrest after IR exposure and increased polyploidy after nocodazole treatment (36). If an impairment of DNA damage checkpoints were to underlie the increase in tumorigenesis of *Tyr-RAS p19$^{ARF-/-}$* mice upon UVB exposure, we would expect to see increased cytogenetic complexity in UV-induced tumors. However, an analysis of these tumors by array-based CGH reveals exactly the opposite: UV-treated tumors are far less complex cytogenetically than spontaneously emerged melanomas (R.C.O.-H., C. Brennan, and L.C., unpublished work). Therefore, UV-light seems to be targeting a specific oncogenic pathway rather than engendering broad, genome-wide DNA damage in UV-treated *Tyr-RAS p19$^{ARF-/-}$* mice. A second interpretation would be that this dose of UVB does not accelerate tumorigenesis by inducing DNA damage, but rather serves as a mitogenic stimulus for melanocytes, as suggested (3), and that p19^{ARF} perhaps plays a

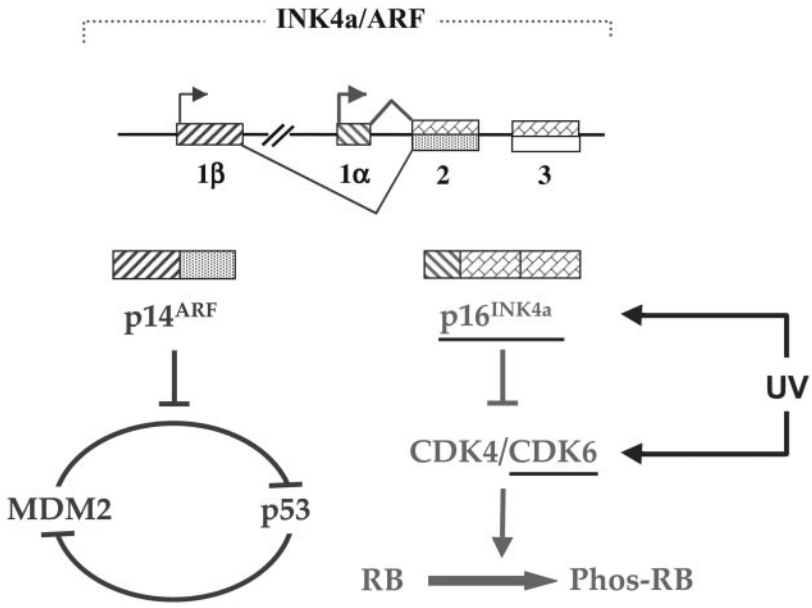

Fig. 4. The Rb pathway is targeted in melanoma by UV light. The *INK4a/ARF* locus encodes two potent regulators of the cell cycle that function in genetically distinct pathways ($p16^{INK4a}$–RB and $p14^{ARF}$–p53). Although inactivation of both pathways seems crucial in melanoma formation, the data in this paper and the human genetics of melanoma have, to date, only identified targeting of components (underlined) of the Rb pathway by UV radiation. These results suggest that RB pathway inactivation by UV light is a major and rate-limiting step in melanoma formation.

role in limiting this mitogenic response. When measured at an age near the time of melanoma emergence, however, melanocyte numbers are not altered in the skin of UV-treated *Tyr-RAS* $p19^{ARF-/-}$mice relative to untreated littermate controls, arguing against such an effect (data not shown). Moreover, neither of these explanations would explain the lack of cooperation between UV exposure and $p16^{INK4a}$ loss, nor the mutually exclusive loss of $p16^{INK4a}$ or amplification of *cdk6* seen in UV-treated *Tyr-RAS* $p19^{ARF-/-}$ tumors. Similarly, the inability of UV to accelerate melanoma development in $p16^{INK4a-/-}$ animals would argue against a general immune suppressive role of UV as an explanation for this observation. For these reasons, we favor an alternative possibility: that UV exposure enhances melanoma risk by inactivating the Rb pathway, primarily via $p16^{INK4a}$ loss or *cdk6* amplification in our model system. However, it is important to note that, because Rb loss was not seen in any tumor, our data do not rigorously exclude an Rb-independent role for $p16^{INK4a}$ and CDK6 in melanoma formation as has been suggested (37).

The most studied types of UV-induced DNA damage are C→T base substitutions at dipyrimidine sites leading to formation of pyrimidine dimers. The findings of such C→T mutation in human melanomas (8–10) has implicated $p16^{INK4a}$ as a target of UV-induced mutagenesis in human. However, UV radiation is known to induce a wide range of other DNA damages, including protein–DNA crosslinks, oxidative base damage, single-strand breaks (38), as well as chromosomal aberrations classically associated with double-strand breaks such as deletion (39). Although point mutations of $p16^{INK4a}$ were not detected in our model system, the emergence of *cdk6* amplification in UV-treated murine melanomas, in light of the biochemical relationship between these two proteins (Fig. 4), is consistent with the observation of UV-signature mutation in human melanoma and provides further molecular support for the $p16^{INK4a}$-pRB pathway as a target of UV's mutagenic action. In addition, CDK6 overexpression has been described in ≈40% of primary human tumors and cell lines (40), and large amplifications of 7q (including *Cdk6* at 7q21–22) have been described in ≈50% of primary tumors (41). Importantly, this cross-species concordance of CDK6 involvement lends support to the view that data derived from genetically defined mouse models of human melanoma could guide the analysis of UV exposure history and CDK6 interactions in human populations.

In summary, this genetic model of melanoma points to Rb pathway inactivation as a major rate-limiting step in UV's melanoma-promoting actions and provides impetus for its comprehensive molecular examination in human cancer. Clearly, our data suggest that such an analysis should consider the possibility of a variety of Rb pathway lesions, including point mutations, genomic amplifications and deletions, and aberrations of expression. Likewise, several known components of the RB pathway (e.g., $p16^{INK4a}$, CDK4, and CDK6, RB) warrant analysis in a well annotated collection of clinical samples with detailed UV exposure history and outcome. As UV exposure likely occurs decades before tumor development, our data would predict that the molecular targeting of RB pathway components might be detectable in dysplatic nevi years before their conversion to frank melanoma. Thus, the observation revealed in this work suggests a rational method of risk stratification in the large and clinically heterogeneous cohort of individuals with significant prior history of sunburn that is at increased risk of melanoma.

We thank G. Merlino and F. Noonan for advice on UV-induced tumorigenesis and N. Bardeesy, G. Merlino, and R. DePinho for critical reading of the manuscript. N.E.S. and M.B. were supported by grants from the National Institutes of Health and the Howard Hughes Medical Institute. This work was supported in part by grants from the National Institutes of Health, the Claudia Adams Barr Program in Cancer Research, and the Rockefeller Brothers Fund (to L.C.). L.C. is a Charles E. Culpeper Medical Scholar.

1. SEER (1998) *SEER Cancer Statistics Review, 1973–1995* (National Cancer Institute, Bethesda).
2. Chin, L., Merlino, G. & DePinho, R. A. (1998) *Genes Dev.* **12,** 3467–3481.
3. Gilchrest, B. A., Eller, M. S., Geller, A. C. & Yaar, M. (1999) *N. Engl. J. Med.* **340,** 1341–1348.
4. Harrison, S. L., MacLennan, R., Speare, R. & Wronski, I. (1994) *Lancet* **344,** 1529–1532.

5. Zanetti, R., Franceschi, S., Rosso, S., Colonna, S. & Bidoli, E. (1992) *Eur. J. Cancer* **28A,** 1172–1176.
6. Atillasoy, E. S., Seykora, J. T., Soballe, P. W., Elenitsas, R., Nesbit, M., Elder, D. E., Montone, K. T., Sauter, E. & Herlyn, M. (1998) *Am. J. Pathol.* **152,** 1179–1186.
7. Berking, C., Takemoto, R., Binder, R. L., Hartman, S. M., Ruiter, D. J., Gallagher, P. M., Lessin, S. R. & Herlyn, M. (2002) *Carcinogenesis* **23,** 181–187.
8. Pollock, P. M., Pearson, J. V. & Hayward, N. K. (1996) *Genes Chromosomes Cancer* **15,** 77–88.
9. Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S. V., Stockert, E., Day, R. S., III, Johnson, B. E. & Skolnick, M. H. (1994) *Science* **264,** 436–440.
10. Peris, K., Chimenti, S., Fargnoli, M. C., Valeri, P., Kerl, H. & Wolf, P. (1999) *J. Invest. Dermatol.* **112,** 825–826.
11. Ruas, M. & Peters, G. (1998) *Biochim. Biophys. Acta* **1378,** F115–F177.
12. Pavey, S., Conroy, S., Russell, T. & Gabrielli, B. (1999) *Cancer Res.* **59,** 4185–4189.
13. Piepkorn, M. (2000) *J. Am. Acad. Dermatol.* **42,** 741–745.
14. Donawho, C. K. & Kripke, M. L. (1991) *Cancer Res.* **51,** 4176–4181.
15. Jamal, S. & Schneider, R. J. (2002) *J. Clin. Invest.* **110,** 443–452.
16. Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M. J., Bottomley, W., *et al.* (2002) *Nature* **417,** 949–954.
17. Herlyn, M. & Satyamoorthy, K. (1996) *Am. J. Pathol.* **149,** 739–744.
18. Chin, L., Pomerantz, J., Polsky, D., Jacobson, M., Cohen, C., Cordon-Cardo, C., Horner, J. W., II, & DePinho, R. A. (1997) *Genes Dev.* **11,** 2822–2834.
19. Bardeesy, N., Bastian, B. C., Hezel, A., Pinkel, D., DePinho, R. A. & Chin, L. (2001) *Mol. Cell. Biol.* **21,** 2144–2153.
20. Flores, J. F., Walker, G. J., Glendening, J. M., Haluska, F. G., Castresana, J. S., Rubio, M. P., Pastorfide, G. C., Boyer, L. A., Kao, W. H., Bulyk, M. L., *et al.* (1996) *Cancer Res.* **56,** 5023–5032.
21. Walker, G. J., Flores, J. F., Glendening, J. M., Lin, A. H., Markl, I. D. & Fountain, J. W. (1998) *Genes Chromosomes Cancer* **22,** 157–163.
22. Sharpless, N. E. & DePinho, R. A. (1999) *Curr. Opin. Genet. Dev.* **9,** 22–30.
23. FitzGerald, M. G., Harkin, D. P., Silva-Arrieta, S., MacDonald, D. J., Lucchina, L. C., Unsal, H., O'Neill, E., Koh, J., Finkelstein, D. M., Isselbacher, K. J., *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93,** 8541–8545.
24. Randerson-Moor, J. A., Harland, M., Williams, S., Cuthbert-Heavens, D., Sheridan, E., Aveyard, J., Sibley, K., Whitaker, L., Knowles, M., Newton Bishop, J. & Bishop, D. T. (2001) *Hum. Mol. Genet.* **10,** 55–62.
25. Rizos, H., Puig, S., Badenas, C., Malvehy, J., Darmanian, A. P., Jimenez, L., Mila, M. & Kefford, R. F. (2001) *Oncogene* **20,** 5543–5547.
26. Sharpless, N. E., Bardeesy, N., Lee, K. H., Carrasco, D., Castrillon, D. H., Aguirre, A. J., Wu, E. A., Horner, J. W. & DePinho, R. A. (2001) *Nature* **413,** 86–91.
27. Sharpless, N. E., Alson, S., Chan, S., Silver, D. P., Castrillon, D. H. & DePinho, R. A. (2002) *Cancer Res.* **62,** 2761–2765.
28. Krimpenfort, P., Quon, K. C., Mooi, W. J., Loonstra, A. & Berns, A. (2001) *Nature* **413,** 83–86.
29. Kamijo, T., Bodner, S., van de Kamp, E., Randle, D. H. & Sherr, C. J. (1999) *Cancer Res.* **59,** 2217–2222.
30. Markel, P., Shu, P., Ebeling, C., Carlson, G. A., Nagle, D. L., Smutko, J. S. & Moore, K. J. (1997) *Nat. Genet.* **17,** 280–284.
31. Williams, B. O., Remington, L., Albert, D. M., Mukai, S., Bronson, R. T. & Jacks, T. (1994) *Nat. Genet.* **7,** 480–484.
32. Sharpless, N. E., Ferguson, D. O., O'Hagan, R. C., Castrillon, D. H., Lee, C., Farazi, P. A., Alson, S., Fleming, J., Morton, C. C., Frank, K., *et al.* (2001) *Mol. Cell* **8,** 1187–1196.
33. Noonan, F. P., Recio, J. A., Takayama, H., Duray, P., Anver, M. R., Rush, W. L., De Fabo, E. C. & Merlino, G. (2001) *Nature* **413,** 271–272.
34. Kamijo, T., Zindy, F., Roussel, M. F., Quelle, D. E., Downing, J. R., Ashmun, R. A., Grosveld, G. & Sherr, C. J. (1997) *Cell* **91,** 649–659.
35. Stott, F. J., Bates, S., James, M. C., McConnell, B. B., Starborg, M., Brookes, S., Palmero, I., Ryan, K., Hara, E., Vousden, K. H. & Peters, G. (1998) *EMBO J.* **17,** 5001–5014.
36. Khan, S. H., Moritsugu, J. & Wahl, G. M. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 3266–3271.
37. Fahraeus, R. & Lane, D. P. (1999) *EMBO J.* **18,** 2106–2118.
38. de Gruijl, F. R., van Kranen, H. J. & Mullenders, L. H. (2001) *J. Photochem. Photobiol. B* **63,** 19–27.
39. Horiguchi, M., Masumura, K. I., Ikehata, H., Ono, T., Kanke, Y. & Nohmi, T. (2001) *Cancer Res.* **61,** 3913–3918.
40. Tang, L., Li, G., Tron, V. A., Trotter, M. J. & Ho, V. C. (1999) *Melanoma Res.* **9,** 148–154.
41. Bastian, B. C., LeBoit, P. E., Hamm, H., Brocker, E. B. & Pinkel, D. (1998) *Cancer Res.* **58,** 2170–2175.