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Switching of Melanocyte Pigmentation Associated with Pituitary Pars Intermedia Tumors in $Rb^{+/-}$ and $p27^{-/-}$ Female Mice with Yellow Pelage

Adrian P. L. Smith, PhD,[1] Martha Henze,[1] Kent G. Osborn, DVM, PhD,[1] John A. Lee, PhD,[2] Donna M. Bortner, PhD,[3] Michael P. Rosenberg, PhD,[4] and Steven I. Reed, PhD[1,*]

As an incidental finding in a study of mammary tumorigenesis, two lines of genetically engineered mice were observed to develop pigmentation changes of the fur. Mice with targeted mutations of the *Rb1* (Rb) and *Cdkn1b* ($p27^{kip1}$) genes were crossed from C57BL/6 (black coat color; eumelanin) and 129Sv (wild-type agouti coat color) backgrounds, respectively, to one with a dominant yellow coat color (phaeomelanin) carrying a transgene for *Agouti* under a keratinocyte specific promoter. Both $Rb^{+/-}$ and $p27^{-/-}$ mice developed pituitary tumors of the pars intermedia that were associated with a switch to black (eumelanic) fur but were not observed in sibling $Rb^{+/+}$ and $p27^{+/+}$ mice. This phenomenon was observed first in the vibrissae and, subsequently one to two weeks later, as periorbital and dorsal patches, and was associated with pituitary lesions larger than four millimeters in the longest dimension. In $Rb^{+/-}$ mice, pigmentation change preceded a moribund state attributable to the tumors by two to four weeks, whereas in $p27^{-/-}$ mice, the pigmentation alteration was earlier, more gradual, and prolonged. The switch from phaeomelanin to eumelanin in the fur is most likely due to out-competition of the agouti gene product by α-melanocyte-stimulating hormone from the pituitary tumors, an effect masked in black or agouti mice.

As an incidental finding during the course of a mammary tumorigenesis study, we noticed dark pigmentation changes in the adults of two lines of genetically engineered mice with yellow fur coloration. The rationale for the study was to examine the oncogenic effects of human cyclin E under a mammary-specific promoter (1) through a mechanism inducing genomic instability (2) yet to be elucidated. Mice with targeted mutations of *Rb1* (Rb) or *Cdkn1b* (p27), the proteins products of which negatively regulate cell cycle progression and are established tumor suppressors, were crossed to a line of mice transgenic for human cyclin E. For each doubly engineered line, we expected loss of heterozygosity for the tumor suppressor in the mammary gland and, hence, increased tumorigenesis. In addition, p27 is an inhibitor of cyclin E-dependent kinase activity; therefore, we anticipated that a deficiency of p27 would increase the oncogenic properties of the cyclin E transgene.

Targeted mutation of *Rb1* in mice does not lead to the phenotype of retinoblastoma susceptibility expected from human studies. Rather, $Rb^{-/-}$ mice die in utero, whereas $Rb^{+/-}$ mice develop pituitary pars intermedia tumors with almost complete penetrance and high incidence of medullary thyroid carcinoma (3-5). Targeted mutation of the p27 gene (*Cdkn1b*) leads to a phenotype of female infertility and multi-organ hyperplasia, including the pituitary pars intermedia (6-8). That $p27^{-/-}$ and $Rb^{+/-}$ mice develop pituitary pars intermedia tumors suggests they are components of overlapping pathways suppressing pars intermedia tumorigenesis, which has been confirmed in mice mutant at both loci (9).

Pars intermedia adenomas in $Rb^{+/-}$ mice are associated with loss of the remaining Rb wild-type allele in cells at an early age (10), and these lesions produce α-melanocyte-stimulating hormone (αMSH) subsequent to a long and variable latency (4). Similarly, pars intermedia hyperplasia appears early in $p27^{-/-}$ mice, usually detectable histologically, at less than 12 weeks of age (8). However, most genetically engineered mice are produced and maintained in genetic strains with dark fur; either black (C57BL/6) or wild-type agouti (129SvJ etc), and the effects of possible αMSH overproduction in the skin are masked or too subtle to detect visually. Indeed, in $Rb^{+/-}$ mice, circulating concentration of αMSH is correlated with tumor progression, up to 50-fold greater than that in control mice (4). Despite this detailed hormone analysis, pigment changes were not observed, presumably due to dark fur coloration.

The wild-type agouti coat pattern in mice is generated by the temporal switching between black (eumelanin) and yellow (phaeomelanin) pigmentation during the growth of each hair. Typically this produces black apical and basal bands, with an intervening subapical yellow band (11). Normal regulation of fur pigmentation is controlled by local expression of the product of the agouti gene (*A*). The wild-type allele is dominant; *A/A* and *A/a* mice are agouti, whereas *a/a* mice are black. Phaeomelanin production is induced by binding of the agouti gene product to the melanocortin 1 receptor (MC1R). The molecular basis for the switching is likely due to temporal competition for the receptor between the agouti gene product and pituitary-derived αMSH (12).

Introduction of the agouti cDNA as a transgene under the K14 keratinocyte-specific promoter induces yellow coat colora-

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[1]The Scripps Research Institute, Department of Molecular Biology, MB-7, 10550 North Torrey Pines Road, La Jolla, California 92037, [2]Department of Histopathology, Rotherham General Hospital, Rotherham, S60 2UD, United Kingdom, [3]GlaxoSmithKline, Research Triangle Park, North Carolina 27709, and [4]Piedmont Research Center, 3300 Gateway Centre, Morrisville, North Carolina 27560.
[*]Corresponding author.

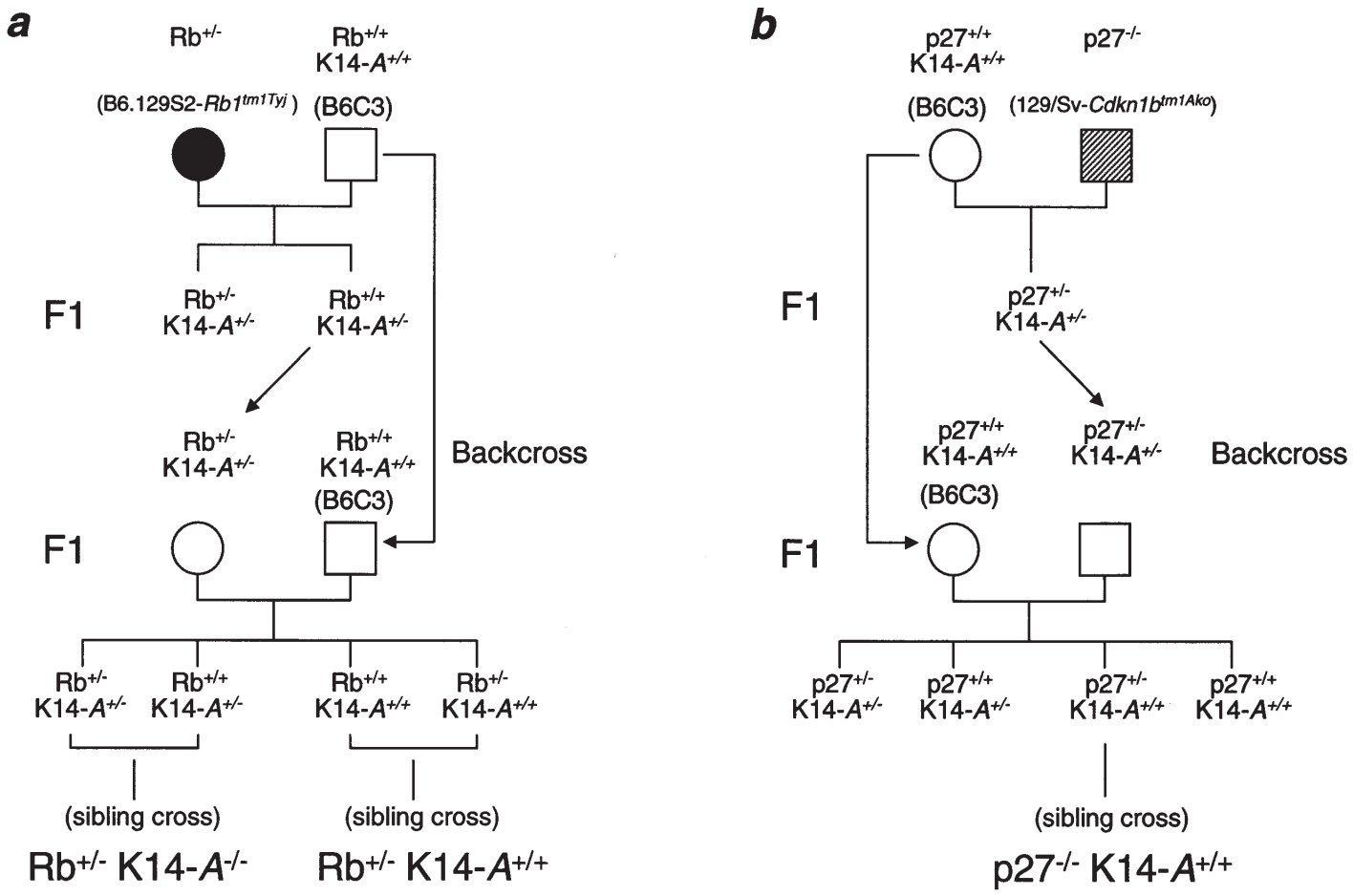

Figure 1. Generation of doubly engineered mice. Mice carrying targeted mutations of the *Rb1* (Rb) and *Cdkn1b* (p27) genes were crossed to mice with the K14-*Aguoti* and BLG-T380A transgenes (denoted K14-*A*), which co-segregate at a single locus of insertion (the location of which is unknown). Mice without the transgene are designated K14-$A^{-/-}$, whereas mice homozygous for the locus of insertion are designated K14-$A^{+/+}$. The locus of transgene insertion is inherited independently of *Rb1* and *Cdkn1b* loci located on chromosomes 14 and 6, respectively. (a) The $Rb^{+/-}$K14-$A^{+/+}$ mice were maintained by sibling crosses of $Rb^{+/-}$K14-$A^{+/+}$ x $Rb^{+/+}$K14-$A^{+/+}$. (b) The $p27^{-/-}$K14-$A^{+/+}$ mice were generated from sibling crosses of $p27^{+/-}$K14-$A^{+/+}$ x $p27^{+/-}$K14-$A^{+/+}$ used to establish a colony as $p27^{-/-}$ mice are infertile. The solid circle denotes black fur coloration, and the hatched square represents agouti. All other mice are yellow.

tion and can be used to track the inheritance of co-integrated transgenes (13). Yellow coat coloration is associated with a number of the many alleles of *Agouti* (14), but confining expression to the skin avoids the 'yellow obese mouse syndrome' (11) of the A^y mutation, a consequence of ectopic expression of *Agouti* (15) interfering with pro-opiomelanocortin (POMC) signaling in the brain (16).

Since agouti coat coloration in mice results from temporal competition for MC1R between the *Agouti* gene product and circulating αMSH, hormonally active melanotroph neoplasms that increase αMSH expression might alter coat coloration. Spontaneous pituitary tumors in mice are common, but usually present in the anterior pituitary gland (pars distalis rather than the pars intermedia, which contains the melanotrophs) and often present with hormonally distinct systemic phenotypes, such as galactorrhea (inappropriate lactation in the absence of pregnancy) associated with prolactinomas. In contrast, proliferative lesions of the pars intermedia are a rare occurrence in wild-type mice, but occur with almost 100% penetrance in $Rb^{+/-}$ (3-5) and $p27^{-/-}$ mice (6-8).

We present evidence that adenomas of the pars intermedia in $Rb^{+/-}$ and $p27^{-/-}$ mice are associated with a switch from phaeomelanin to eumelanin in the fur of yellow mice carrying the K14-*Agouti* transgene.

Case Report

The B6.129S2-*Rb1tm1Tyj* ($Rb^{+/-}$) mice (5) and 129/Sv-*Cdkn1btm1Ako* ($p27^{-/-}$) mice (7) (black and agouti fur coloration, respectively), were crossed (Fig. 1) to a transgenic line in a C57BL/6 × C3H background with dominant yellow hair coloration. The latter were created by co-integration of a transgene for the *Agouti* gene product under the K14 promoter as described (13) (hereafter referred to as K14-*A*) and for a human cyclin E transgene under the mammary-specific β-lactoglobulin promoter (BLG-T380A). In these mice, the expression of the cyclin E transgene, a hyperstable form of cyclin E (17), is confined to the lactiferous mammary epithelium during the final third of pregnancy and lactation and does not influence the development of pituitary neoplasia (data not shown). The locus of insertion for the BLG-T380A/K14-*A* transgene is inherited as a single locus independently of *Rb1* (chromosome 14), *Cdkn1b* (chromosome 6), *Trp53* (chromosome 11), and X or Y (data not shown) and, as such, is designated as K14-$A^{-/-}$, K14-$A^{+/-}$, and

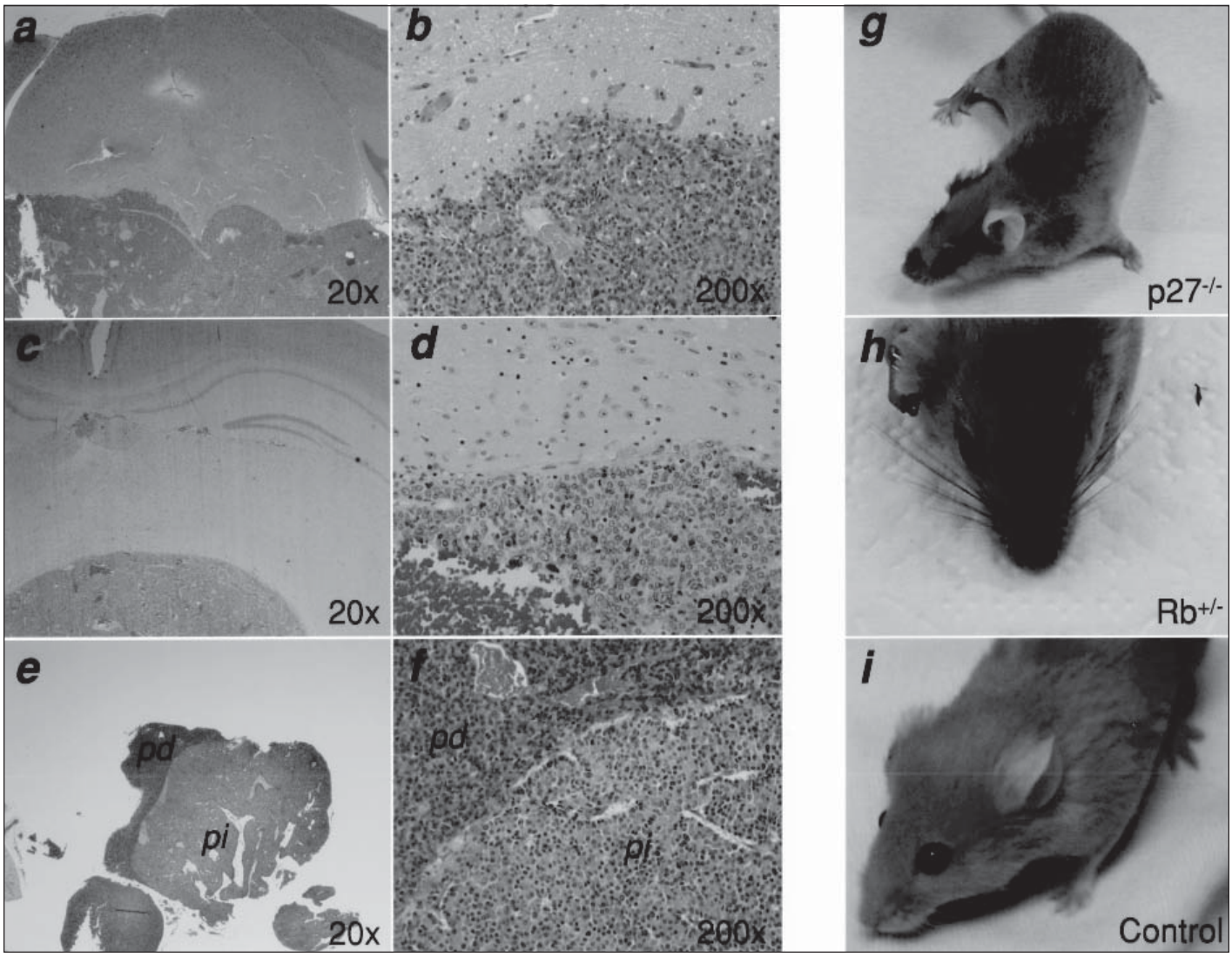

Figure 2. Photomicrographs of pituitary tumor histologic features in $Rb^{+/-}$ K14-$A^{+/+}$ mice at 20× and 200× the original magnification (a-f) and pigmentation changes in $Rb^{+/-}$ K14-$A^{+/+}$ and $p27^{-/-}$ K14-$A^{+/+}$ mice (G-I). (a) and (b)—carcinoma exhibiting invasion of the overlying brain, nuclear atypia, areas of necrosis, and high mitotic rate; (c) and (d)—aggressive adenoma with no invasion of the brain, but with moderate to high mitotic rate, nuclear atypia, and areas of necrosis; (e) and (f)—benign adenoma, with little atypia or necrosis and fewer mitotic figures (pi = pars intermedia, pd = pars distalis); (g)—nine-month-old $p27^{-/-}$ K14-$A^{+/+}$ mouse with extensive periorbital and dorsal eumelanic pigmentation; (h)—necropsy photograph of an $Rb^{+/-}$ K14-$A^{+/+}$ mouse showing typical dark vibrissae with dark supraorbital patches; and (i)—control K14-$A^{+/+}$ mouse showing normal coloration. H&E stain.

K14-$A^{+/+}$ to demonstrate the zygosity of the transgene (although copy number at the locus remains unknown). The coloration of K14-$A^{+/+}$ mice (13) is solid and uniform yellow truncal pelage, with only slightly lighter coloration ventrally, and the vibrissae also are yellow (Fig. 2). This coloration is dominant over the endogenous agouti alleles. The eyes, the melanocytes of which are from a separate embryonic lineage, are black.

As the study was principally of mammary tumorigenesis, only female mice were used. After two pregnancies (to activate the BLG-T380A transgene in the mammary epithelium), each $Rb^{+/-}$ K14-$A^{+/+}$ mouse was aged to one year. Although $p27^{-/-}$ mice are not able to become pregnant (6-8), each $p27^{-/-}$ K14-$A^{+/+}$ mouse was maintained with a male in a breeder cage for two months to induce the BLG-T380A transgene via post-coital prolactin release, then was aged to 18 months. All mice were inspected twice weekly for signs of mammary tumorigenesis or any other notable clinical signs of disease.

At one year, or sooner if the mice became moribund, they were euthanized. Each mouse underwent a full necropsy which included, but was not limited to routine histologic examination of the mammary glands and pituitary gland. Tissues were fixed in neutral-buffered 10% formalin for 24 h, embedded in paraffin, sectioned at 8-µm thickness, and stained with hematoxylin and eosin (H&E) by use of standard methods. Because of the obvious hormonal link between the pituitary pars intermedia and adrenal cortex, the left adrenal gland in each mouse was dissected free from the kidney and perirenal adipose after fixation and was weighed.

A total of 52 $Rb^{+/-}$ K14-$A^{+/+}$ and four $p27^{-/-}$ K14-$A^{+/+}$ mice that also carried the BLG-T380A transgene were studied. Fifty control mice carrying the K14-*A* and BLG-T380A transgenes but also were $Rb^{+/+}$ and $p27^{+/+}$, 50 $Rb^{+/-}$ mice without either transgene

Table 1. Pars intermedia (p.i.) lesion and pigmentation incidence in the mice of the report

Genotype	$Rb^{+/-}$ K14-$A^{+/+}$ (yel)	$Rb^{+/+}$ K14-$A^{+/+}$ (yel)	$p27^{-/-}$ K14-$A^{+/+}$ (yel)	$p27^{+/-}$ K14-$A^{+/+}$ (yel)	$Rb^{+/-}$ K14-$A^{-/-}$ (black)
p.i. lesion	46	0	4	3	46
Pigmentation	14	0	4	0	ND
Pigmentation with p.i. lesion ≥ 4 mm	14	0	4	0	ND
Pigmentation with p.i. lesion < 4 mm	0	0	0	0	ND
p.i. lesion ≥ 4 mm without pigmentation	1	0	0	0	ND
p.i. lesion < 4 mm without pigmentation	31	0	0	3	ND
N	52	50	4	5	50

(hence, had black fur), and five $p27^{+/-}$ K14-$A^{+/+}$ mice (siblings of the $p27^{-/-}$ K14-$A^{+/+}$ mice) also were studied.

All animals of this report were maintained at a density of one to two animals per cage under a 12:12-h light:dark cycle, with ad libitum access to food and water in compliance with, and approval of The Scripps Research Institute (TSRI) Animal Research Committee. Sentinel mice were negative for pinworms, fur mites, and a panel of murine viruses and bacteria that included the following: mouse hepatitis virus, mouse minute virus, mouse parvovirus, Sendai virus, *Mycoplasma pulmonis*, Theiler's murine encephalomyelitis virus, epizootic diarrhea of infant mice (Rotavirus), pneumonia virus of mice, reovirus 3, lymphocytic choriomeningitis virus, ectromelia virus, mouse adenovirus 1, mouse adenovirus 2, polyoma virus, *Encephalitozoon cuniculi*, cilia associated respiratory bacillus, *Clostridium piliforme*, and murine cytomegalovirus. The SRI has maintained AAALAC approval since February, 1986.

Results

At one year of age, 46 of 52 (88%) $Rb^{+/-}$ K14-$A^{+/+}$ mice had developed pituitary pars intermedia lesions, as determined by histologic examination of H&E-stained sections (Table 1). Two tumors were malignant, with unequivocal invasion of the brain; 34 were benign, and were classified as adenomas; and 10 had aggressive cytologic features, such as high mitotic index, nuclear atypia, and necrosis, though invasion of the overlying brain was not evident (Fig. 2A-F). Thirty percent of these mice (n = 14) had patches of dark fur coloration (Fig. 2G-I). Another 7% (n = 3) had galactorrhea and diffuse mammary alveolar hyperplasia without eumelanic pigmentation, although the pituitary tumors morphologically resembled adenomas consistent with pars intermedia origin. In addition, 4% (n = 2) had small tumors and were euthanized because of a moribund state associated with hydrocephalus (no eumelanic patches). Physical measurements of the tumors (longest dimension) were greater in mice with eumelanic patches: mean ± SD, 5.3 ± 1.9 mm (n = 14) versus 2.4 ± 1.3 mm (n = 32) ($P < 0.001$). Pigmentation changes were always associated with pituitary pars intermedia lesions ($\chi^2 = 13.4$, $P < 0.0001$), which always were > 4 mm in the longest dimension, with the exception of one that was largely hemorrhagic. Eumelanic patches were also associated with higher grade lesions: two of two malignant tumors and seven of 10 of the "aggressive" adenomas. Size was also correlated with grade. Pituitary lesions and pigmentation changes were absent in 50 control mice that were K14-$A^{+/+}$ transgenic but wild-type at the Rb locus.

Left adrenal gland mass in $Rb^{+/-}$ K14-$A^{+/+}$ mice did not correlate with size of the pituitary lesion ($r = 0.09$; n = 47) or differ between animals with (3.75 ± 0.69 mg) or without (3.90 ± 0.71 mg) pigmentation changes ($P > 0.5$; n = 13 and 33, respectively). However, there appeared to be mild hypertrophic change in the zona fasciculata of each mouse with pigmentation changes and the cytoplasm appeared more solidly eosinophilic than that in mice without eumelanic patches. Mice with eumelanic patches always contained a vast majority of these cells lacking the finely vacuolated cytoplasm of the normal adrenal cortex zona fasciculata.

The onset of pigment changes in $Rb^{+/-}$ K14-$A^{+/+}$ mice was usually first observed as darkening of the vibrissae, followed two to 14 days later with periorbital and/or dorsolateral patches with few ventral patches. Rarely did the mice progress beyond developing the initial changes before becoming moribund, often within four weeks of the first instance of eumelanic pigmentation. The earliest age at which $Rb^{+/-}$ K14-$A^{+/+}$ mice had pigmentation changes was at nine months.

Only four $p27^{-/-}$ K14-$A^{+/+}$ mice were available for study, but all four had pigmentation changes associated with large pituitary lesions (> 4 mm in the longest dimension; see Table 1). Pigmentation changes were not seen in any of the five $p27^{+/-}$ K14-$A^{+/+}$ sibling mice, three of which had pars intermedia lesions that were small in size (< 4 mm). In contrast to the $Rb^{+/-}$ K14-$A^{+/+}$ mice, eumelanic changes appeared first at approximately six months, and continued with aging. In those mice, almost all of the dorsal pelage usually changed color before the mice became moribund.

Histologic examination in these mice revealed hyperplastic/benign pars intermedia lesions with a generally lower mitotic index than that of the tumors in the $Rb^{+/-}$ K14-$A^{+/+}$ mice and fewer cytologic changes, indicating that these lesions were less aggressive. The adrenal glands of $p27^{-/-}$ K14-$A^{+/+}$ mice were approximately two- to threefold more massive than the $Rb^{+/-}$ K14-$A^{+/+}$ adrenal glands (9.95 ± 0.71 mg) due to medullary hyperplasia, which is part of the multi-organ hyperplasia associated with this genotype and is indicative of it not being due to strain differences (6-8). However, the zona fasciculata of $p27^{-/-}$ K14-$A^{+/+}$ mice with eumelanic pigmentation changes appeared to be composed of a mixture of cells with a solidly eosinophilic cytoplasm, those with the normal finely vacuolated cytoplasm and, in some instances, cells with a cytoplasm containing areas of both kinds.

At 18 months of age, none of the 50 control $Rb^{+/+}$ or five $p27^{+/-}$ mice carrying the K14-$A^{+/+}$ transgene had developed pituitary lesions or pigmentation changes. At one year of age, 46 of 50 (92%) control $Rb^{+/-}$ K14-$A^{-/-}$ mice (without the transgene) had developed pituitary lesions, although pigmentation changes were not evident in these mice with black fur coloration.

Discussion

We report a phenotype of fur pigmentation change associated with melanotroph tumors in $Rb^{+/-}$ and $p27^{-/-}$ mice with yellow pelage. Pituitary lesions or pigmentation changes were not observed in control mice transgenic for K14-*A* and BLG-T380A, but wild type for p27 and Rb or in $p27^{+/-}$ controls. Comparable tumor spectrum, growth kinetics, grade, and cytologic findings were seen in 50 $Rb^{+/-}$ mice without the K14-*A* transgene, in an identical background and in other studies of different genetic backgrounds (3-9). We have not had the opportunity to examine

changes in agouti p27$^{-/-}$ K14-*A*$^{-/-}$ mice that are likely to be subtle (and not reported by others). Therefore, the tumor susceptibility phenotypes of Rb$^{+/-}$ and p27$^{-/-}$ mice appear to be independent of genetic background, and the pigmentation changes that are associated with the pituitary lesions appear to be masked in black (C57BL/6 and C57BL/6xC3H) and agouti (129SvJ) mice. Furthermore, pigmentation changes were associated with pars intermedia lesions > 4 mm in the longest dimension, indicating that the lesion must attain this size before circulating MSH concentration is sufficient to out-compete the effects of K14-*A*$^{+/+}$.

The differences in the pigmentation latency and severity between Rb$^{+/-}$ K14-*A*$^{+/+}$ and p27$^{-/-}$ K14-*A*$^{+/+}$ mice probably reflect the differences in the biological nature of the pituitary lesions. All p27$^{-/-}$ mice develop pituitary hyperplasia at an early age; hence, the earlier onset in pigmentation. It has been reported that 50% of these mice develop overt pars intermedia neoplasia (8), but this was not evident in any of our mice. Indeed the cytologic features suggest a benign biology for these lesions, which is supported by the slower but more profound degree of eumelanic pigmentation and the long latency before the animals become moribund. Conversely, the frequency of adenoma formation in Rb$^{+/-}$ mice is almost 100%, with lifespan restricted largely by the phenotypic neoplastic changes in the pituitary and thyroid parafollicular cells. The pituitary lesions of Rb$^{+/-}$ K14-*A*$^{+/+}$ mice are generally benign, but may have cytologic markers indicative of aggressive biology. Indeed, the switching of pigmentation was associated with larger lesions that tended to be of a more aggressive nature. Furthermore, within a month of development of the initial signs of eumelanic pigmentation, the Rb$^{+/-}$ K14-*A*$^{+/+}$ mice became moribund, with evidence of the more aggressive biology of these lesions, providing little time for further pigmentation to develop.

The observation that only 30% of the Rb$^{+/-}$ K14-*A*$^{+/+}$ mice developed eumelanic patches likely reflects the stochastic nature of adenomatous transformation, conferring apoptotic resistance when refractory to D2 neuron signaling (9). In Rb$^{+/-}$ mice, the single wild-type Rb allele is lost early (< 90 days after birth), but subsequent genetic lesions are a spontaneous process and the latency is long and variable (9 to 12 months). Indeed, some of our Rb$^{+/-}$ K14-*A*$^{+/+}$ mice were euthanized at < 10 months of age due to complications associated with medullary thyroid carcinomas without overt pituitary lesions or pigmentation changes. Furthermore, in three Rb$^{+/-}$ K14-*A*$^{+/+}$ mice, large pituitary adenomas were associated with galactorrhea without pigmentation changes despite histologic morphology ('foamy' lightly eosinophilic cytoplasm) consistent with a pars intermedia origin. In the absence of ancillary diagnostics, we interpret these cases as hormonally inactive pars intermedia adenomas with physical destruction of the bridge with the hypothalamus known to secrete prolactin inhibitory factor. In each of these three cases, histologic examination revealed the presence of some normal pars distalis, in which the mammotrophs are located, that was usually absent in the larger lesions associated with pigmentation changes.

Switching from phaeomelanin to eumelanin in these mice probably occurs as a result of an increase in the ratio of circulating αMSH to local expression of the agouti gene product competing for the MC1R expressed in the melanocytes. The functional tumors of the pars intermedia increase the melanotroph population and increase circulating αMSH concentration (4). Therefore, it appears likely that increased circulating αMSH concentration can effectively out-compete the agouti gene product in the eumelanic patches.

The rostral-to-caudal pattern of pigment changes reflects the molting cycle in mice, which extends craniocaudad and ventrodorsad in waves, as opposed to the mosaic pattern seen in humans and other domestic species (18). This process allows melanocytes to deposit eumelanin in growing hair. This also may explain the more severe eumelanic pigmentation in the p27$^{-/-}$ K14-*A*$^{+/+}$ mice that permits eumelanin deposition over several successive waves, whereas onset of eumelanic deposition in Rb$^{+/-}$ K14-*A*$^{+/+}$ mice is probably only a fraction of one molting. That only certain areas are affected may also be influenced by a number of the many genes' other loci affecting coat patterning (18). Shaving of the coat of K14-*A*$^{+/+}$ mice does not result in eumelanin pigmentation, whereas plucking (which induces new follicle hair growth) does. In a few instances where our Rb$^{+/-}$ K14-*A*$^{+/+}$ and p27$^{-/-}$ K14-*A*$^{+/+}$ mice have developed dorsocervical dermatitis, the areas surrounding the lesions became eumelanic. Clearly, pigmentation can be influenced by local and circulating factors.

Although we have not directly compared melanin deposition in the mice of this report with that in wild-type black mice, we have detected some similarities with hyperpigmentation in humans. In people, hyperpigmentation results from primary adrenal insufficiency (Addison's disease) or is secondary to adrenalectomy (Nelson's syndrome). In both instances, suppression of pituitary activity by cortisol is removed. The adrenal histologic findings, at least in the Rb$^{+/-}$ K14-*A*$^{+/+}$ mice, are consistent with adrenal dysfunction secondary to the formation of pituitary lesions. Whether the normal endocrine feedback loop is broken because the αMSH/adrenocorticotropic hormone (ACTH) overproduction outstrips the adrenocortical cortisol production or the adrenal cortex fails is unclear.

Both αMSH and ACTH are melanocortins derived from the precursor POMC, the effects of which are mediated through the melanocortin receptor family. Differential affinities for each of the five melanocortin receptors and tissue-specific expression of the receptors themselves provide discrete functions for each melanocortin. α-Melanocyte-stimulating hormone has the highest affinity for MC1R and MC4R expressed in the melanocyte and hypothalamus, respectively, whereas the receptor for ACTH is MC2R, which is expressed almost exclusively in the adrenal cortex. It is likely that competition for the MC4R in the hypothalamus by ectopically expressed *Agouti* gene product is responsible for the hyperphagia and obesity associated with the *A*^{y} 'yellow obese mouse syndrome,' and for MC1R in the keratinocytes that results in the coloration phenotype. In the mice of this study, the expression of the agouti gene product was largely restricted to the keratinocytes in the skin (13); therefore, these mice did not show a phenotype of type-II diabetes mellitus.

We conclude that pars intermedia tumors of melanotroph origin in Rb$^{+/-}$ and p27$^{-/-}$ mice increase circulating αMSH concentration, the effects of which are masked in mice with dark coloration but are revealed in yellow K14-*A*$^{+/+}$ mice. Monitoring the degree of pigmentation in these mice provides a means of visually assessing pituitary lesion formation in live mice that is not possible in albino mice or in mice with black or agouti pelage.

Acknowledgments

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