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Strain-Specific Spontaneous and NNK-Mediated Tumorigenesis in *Pten* +/− Mice

Mary Christine Hollander*, Andria R. Balogh*, Jaminelli Liwanag*, Wei Han*, Ritva Ilona Linnoila†, Miriam R. Anver‡ and Phillip A. Dennis*

*Medical Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20889, USA; †Cell and Cancer Biology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20889, USA; ‡Pathology/Histotechnology Laboratory, SAIC-Frederick, National Cancer Institute, Frederick, MD 21702, USA

Abstract

Pten is a negative regulator of the Akt pathway, and its inactivation is believed to be an etiological factor in many tumor types. *Pten* +/− mice are susceptible to a variety of spontaneous tumor types, depending on strain background. *Pten* +/− mice, in lung tumor–sensitive and –resistant background strains, were treated with a tobacco carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), to determine whether allelic Pten deletion can cooperate with NNK in carcinogenesis in lung or other tissues. In lung tumor–resistant C57BL/6 *Pten* +/− or +/+ mice, NNK treatment did not lead to any lung tumors and did not increase the incidence or severity of tumors previously reported for this strain. In contrast, in a lung tumor–susceptible pseudo–A/J strain, there was a dose-dependent increase in lung tumor size in *Pten* +/− compared with +/+ mice, although there was no increase in multiplicity. No other tumor types were observed in pseudo–A/J *Pten* +/− mice regardless of NNK treatment. Lung tumors from these *Pten* +/− mice had *K-ras* mutations, retained Pten expression and had similar Akt pathway activation as lung tumors from +/+ mice. Therefore, deletion of a single copy of Pten does not substantially add to the lung tumor phenotype conferred by mutation of *K-ras* by NNK, and there is likely no selective advantage for loss of the second Pten allele in lung tumor initiation.

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Introduction

Pten is a phosphatase whose inactivation is believed to be an etiological factor in many human tumor types. Although Pten has homology to protein tyrosine phosphatases, its primary substrates are 3′ phosphoinositides. In this respect, Pten acts as an antagonist to the phosphoinositide 3-kinase (PI3K)–Akt pathway, which is frequently activated and associated with poor prognosis in many tumor types [1]. Pten mutation coupled with loss of heterozygosity is found at a high frequency in endometrial, prostate, and high-grade glial tumors [2]. Mutations occur most frequently but not exclusively within the phosphatase domain of the protein, implicating the effects on PI3K–Akt signaling as a primary tumor suppressor function of Pten.

Genetically engineered mice with germline deletion of a single copy of Pten exhibit some of the same tumor types observed in human patients with germline Pten mutation. Loss or reduction of Pten protein is common in non–small cell lung cancer [3], as is Akt activation [4], but it is unclear whether mutant or decreased Pten is a risk factor for tobacco-related neoplasms. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific nitrosamine, which is the most potent carcinogen in tobacco smoke, has been widely used to induce lung tumors in mice [5]. Both NNK treatment and Pten mutation lead to Akt pathway activation *in vitro* and *in vivo* [6,7]. In genetically engineered mouse models, lung-specific deletion of Pten coupled with *K-ras* mutation led to a more malignant lung tumor phenotype, although Akt is most likely a primary target for mutant *K-ras*–mediated lung tumorigenesis [4,8,9].

Abbreviations: PI3K, phosphoinositide 3-kinase; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

Address all correspondence to: Phillip A. Dennis, Medical Oncology Branch, National Cancer Institute, National Institutes of Health, 8901 Wisconsin Ave., Bldg. 8, Rm. 5101, Bethesda, MD 20889. E-mail: dennisp@mail.nih.gov

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Lung carcinogenesis by NNK in mice is highly strain-dependent, and only lung tumors have been described in susceptible strains. However, other cancers have been associated with tobacco use in humans, and there are no mouse models to investigate these. To assess lung carcinogenesis and potentially other tumors types, we treated *Pten+/–* mice in a lung tumor–susceptible (pseudo–A/J) or lung tumor–resistant background (C57BL/6) with NNK. *Pten+/–* pseudo–A/J mice did not show an increase in lung tumor susceptibility, although there was a dose-dependent increase in tumor size. Surprisingly, no other tumors were observed in these mice up to 30 weeks of age. Nearly all lung tumors from NNK-treated *Pten+/–* and *+/+* mice had *K-ras* mutations in exon 1. Similar Akt pathway activation was observed in both groups, although tumors from *Pten+/–* mice showed decreased, but not absent, Pten expression. In a resistant strain background, C57BL/6, *Pten+/–* mice developed adrenal, endometrial, and prostate tumors irrespective of NNK treatment, but no lung tumors. These results indicate that loss of a single copy of Pten does not increase tobacco carcinogen sensitivity, perhaps due to the redundancy of signaling pathways induced by Pten heterozygosity and *K-ras* mutation.

Materials and Methods

Mouse Husbandry and Carcinogen Treatment

Pten+/– mice [10] in a C57BL/6 background (eight backcrosses) were obtained from the Mouse Models of Human Cancer Consortium and had two copies of C57BL/6 *K-ras* allele as determined by polymerase chain reaction (PCR). These mice were backcrossed twice with A/J mice, which are susceptible to lung tumors induced by NNK treatment. Lung tumor–susceptible mice carried two copies of the wild type A/J *K-ras* allele and are referred to as pseudo–A/J. *Pten+/–* pseudo–A/J were crossed with A/J and +/+ and +/– littermates were used for lung tumorigenesis studies. Mice were injected intraperitoneally with NNK (Eagle Pitcher) at 100 mg/kg beginning at 6 weeks of age with either a single dose or three doses at weekly intervals. At 24 weeks after a single dose or 16 weeks after the first of three doses, mice were killed by cervical dislocation, lungs inflated with 10% neutral-buffered formalin, and tissues fixed in the same. The following day, lung lobes were separated and surface lung tumors were counted and measured with a dissecting microscope. Untreated mice were also killed at the same age. *Pten+/–* mice were also crossed with C57BL/6 mice, and the progenies were used for single-dose NNK carcinogenesis but with an end point at 30 weeks after treatment. Data were analyzed using Prism (GraphPad Software, Inc, San Diego, CA) using unpaired *t* tests.

Tissues were processed to paraffin, sectioned, and stained with hematoxylin and eosin. All tissues were evaluated by a board-certified veterinary pathologist. Tissues evaluated included adrenal glands, cervical lymph nodes, spleen, thymus, pancreas, kidneys, lungs, heart, liver, bladder, bone marrow, colon, intestines, brain, and male and female reproductive organs.

Pten genotype was determined by PCR from tail clips [11]. Briefly, 5 mm of the tail tip was lysed overnight in 100 μl of 100 mM Tris, pH 8, 5 mM EDTA, 0.2% SDS, 200 mM NaCl, and 100 μg/ml proteinase K. The following day, 300 μl of water was added, and samples were boiled for 10 minutes before PCR on 1 μl for each sample. *K-ras* allele strain contribution was determined by PCR for Kras_37 [12]. The A/J allele yielded a 91-bp PCR product, whereas the C57BL/6 allele yielded a 128-bp product.

Analysis of K-ras *Mutations*

K-ras mutations were detected in individual lung tumors using the Surveyor Mutation Detection kit (Transgenomic, Inc., Omaha, NE). After paraffin removal with xylene, tumors were excised from unstained 20-μm sections using razor blades and a dissecting microscope. Tumors were identified on adjacent sections stained with hematoxylin and eosin and were clearly visible on unstained sections. Tumor tissue was digested in 50 μl of 100 mM Tris, pH 8.0, 5 mM EDTA, 0.2% SDS, 200 mM NaCl, and 100 μg/ml proteinase K overnight at 55°C. Approximately 50 μl of water was added, samples were boiled, and 5 μl was used for amplification of *K-ras* exons 1 and 2 as described previously [13]. Internal primers were used for a second round of amplification to generate single bands for the mutation assay (exon 1, 5′-gtaaggcctgctgaaaatgact-3′ and 5′-gactgtagagcagcgttacct-3′ generating a product of 146 bp; exon 2, 5′-caagtagtaattgatggagaaacctgt-3′ and 5′-caacttaaacccacctataatggtga-3′ generating a product of 178 bp). Tumor PCR products were mixed with PCR product from A/J mouse tail DNA. Cleavage products of approximately 94 and 52 bp were expected for mutations at codon 12 in exon 1 of *K-ras*, whereas cleavage products of 121 and 67 bp were expected for mutations at codon 61 of exon 2.

Immunohistochemistry for Pten and Akt Pathway Status

Pten, pS473-Akt, and pS235/236 ribosomal S6 expression in lung tumors was determined by immunohistochemistry as described previously [1] with the following exceptions. A Pten rabbit monoclonal antibody (Cell Signaling Technology, Danvers, MA) and an IR800–anti-rabbit secondary (Invitrogen, Carlsbad, CA) was used to quantify Pten. Samples were counterstained with the DNA stain, To-Pro3-iodide (Invitrogen), which emits in the IR700 channel, and were scanned on an Odyssey (Licor Biosciences, Lincoln, NE) infrared scanner at 21-μm resolution. Relative Pten expression was determined as the relative ratio of Pten signal to To-pro3-iodide signal. Tumor locations were verified by comparison to adjacent sections stained with hematoxylin and eosin. Immunohistochemistry for pS473-Akt and pS235/236 ribosomal S6 protein (antibodies 3787 and 4857; Cell Signaling Technology) were performed as described previously. Staining was scored as absent (0), mild (1), moderate (2), or strong (3). Staining index was determined by summing the (fraction of cells staining) × (intensity) as described previously [1].

Results

Development of Lung Tumor–Susceptible and –Resistant Pten+/– *Mice*

Pten+/– mice were treated with a tobacco carcinogen to determine whether allelic loss of Pten might confer increased risk for tumors with a known tobacco-related component. However, strain-specific effects of genetically modified mice are common [14] and *Pten+/–* mice develop a different spectrum of tumors depending on strain background [10,15–17]. In addition, both spontaneous and NNK-induced lung tumors are strain-dependent, with A/J being the most susceptible strain, conferred by the *K-ras* allele [18,19]. To make pure C57BL/6 *Pten+/–* mice susceptible to lung tumorigenesis, they were bred with A/J mice, and *Pten+/–* mice carrying the susceptible A/J *K-ras* allele were selected for this study. These pseudo–A/J and C57BL/6 *Pten+/–* mice were treated with NNK because we expected different tumor spectrums for each background strain.

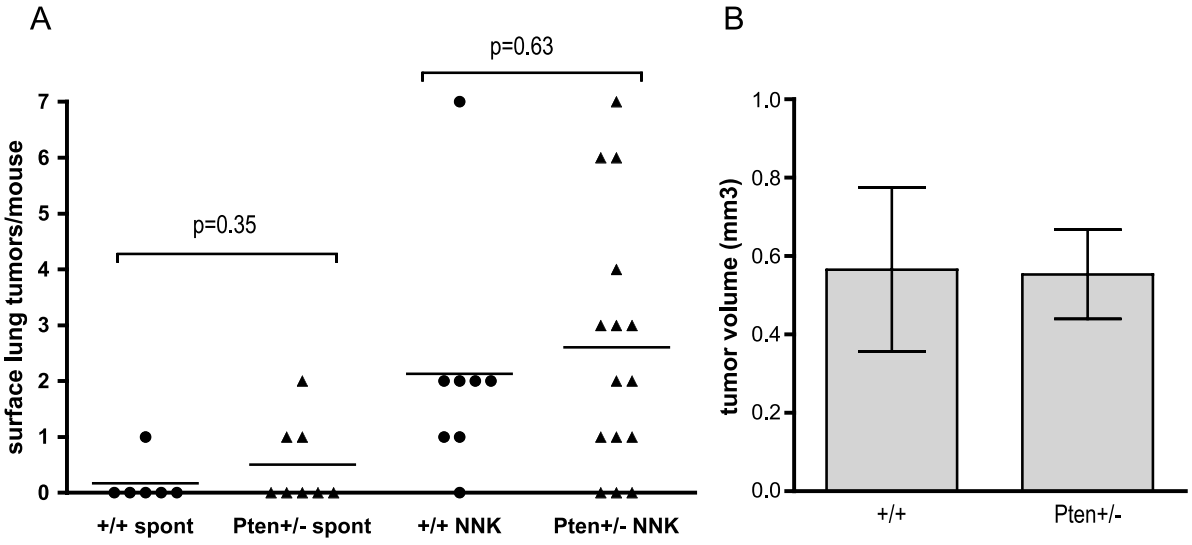

Figure 1. *Pten* +/− mice do not have enhanced susceptibility to either spontaneous or NNK-induced lung tumors. Surface lung tumor multiplicity and tumor size from pseudo–A/J *Pten* +/− and +/+ littermates 24 weeks after treatment with 100 mg/kg NNK at 6 weeks of age or untreated. (A) Each point represents a single mouse. (B) Tumor volume is per individual tumor. The spontaneous cohort consisted of two +/+ females, four +/+ males, two *Pten* +/− females, six *Pten* +/− males. The NNK-treated cohort consisted of four +/+ females, four +/+ males, eight *Pten* +/− females, and seven *Pten* +/− males. All *P* values in this and subsequent figures were obtained using an unpaired *t* test.

Lung Tumorigenesis in Pseudo–A/J Pten+/− *Mice*

Consistent with the inbred A/J strain, pseudo–A/J mice developed both spontaneous and NNK-induced lung tumors, although with somewhat lower multiplicity than observed for inbred A/J mice (Figure 1) [1]. At 24 weeks after a single dose of NNK (at 30 weeks of age), nearly all +/+ and *Pten* +/− mice had developed at least one surface lung tumor, which is in contrast to the spontaneous group, most of which had no tumors. Multiplicity was similar for +/+ and *Pten* +/− mice within both the NNK-treated and untreated groups (Figure 1*A*). In addition, there was no significant difference in tumor size between the *Pten* +/− and +/+ mice (Figure 1*B*). Due to the low lung tumor multiplicity in this study (approximately 2/mouse), a new cohort was treated with three doses of NNK at weekly intervals and killed 16 weeks after the first dose (at 22 weeks of age; Figure 2). More lung tumors were observed in both +/+ and *Pten* +/− groups with three doses of NNK when compared with a single dose (a nine-fold increase for +/+). However, in the three-dose group, *Pten* +/− mice developed significantly fewer lung tumors compared with +/+ littermates (Figure 2*A*). Individual tumor size, however, was increased in the *Pten* +/− mice and averaged 40% larger by

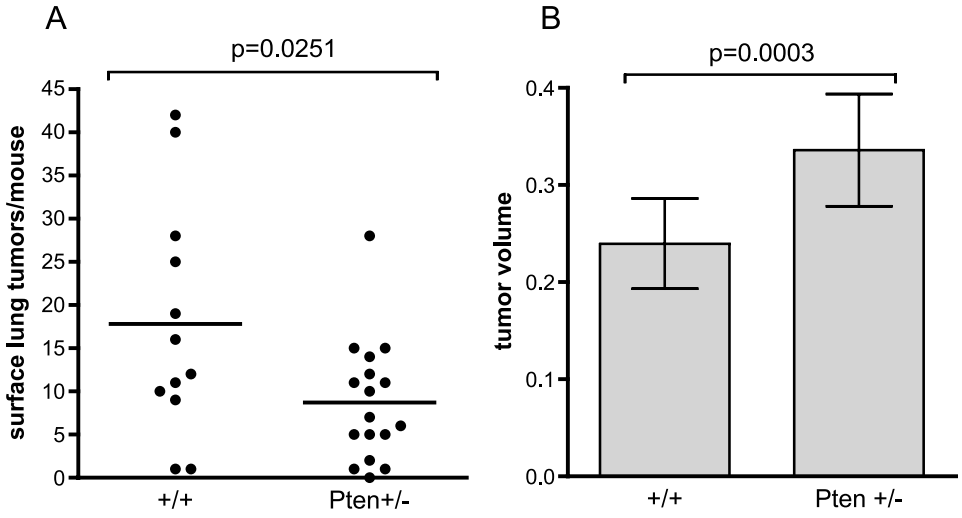

Figure 2. *Pten* +/− mice develop larger but increased numbers of lung tumors with three doses of NNK. Surface lung tumor multiplicity and tumor size from pseudo–A/J *Pten* +/− and +/+ littermates 16 weeks after initial treatment with three doses of 100 mg/kg NNK administered weekly starting at 6 weeks of age. (A) Each point represents a single mouse. (B) Tumor volume is per individual tumor and is presented as mean ± 95% confidence interval for 214 and 148 lung tumors from *Pten* +/+ and +/− mice, respectively. This cohort consisted of 4 +/+ females, 8 +/+ males, 6 *Pten* +/− females, and 11 *Pten* +/− males.

volume (Figure 2*B*). There were no differences between lung tumor incidence, multiplicity, and size between male and female *Pten+/–* mice (data not shown).

Lack of Lung Tumors in Pten+/– *Mice in a Resistant Strain Background*

C57BL/6 is one of the most lung tumor–resistant strains of mice [18]. To determine possible strain-dependent effects of NNK, mice in a C57BL/6 background (more than eight backcrosses with C57BL/6) were treated with a single dose of NNK using an end point at 30 weeks after treatment. No lung tumors were observed in any of these mice, reflecting the relative lung tumor resistance of the background strain (data not shown).

Other Tumor Types in Pten+/– *Mice*

Because loss of Pten expression is associated with other tumor types in addition to lung tumors, a panel of tissues was examined in *Pten+/–* and *+/+* mice in both the pseudo–A/J and C57BL/6 backgrounds. Because *Pten+/–* mice are prone to spontaneous tumors depending on background strain, it was thought that tumors not previously described for *Pten+/–* mice might arise in the pseudo–A/J background. In addition, NNK-induced tumors might arise in tissues other than the lungs for both strains. For these analyses, we included tissues of known tumor development for *Pten+/–* mice as well as tissues where human tumors have a documented tobacco contribution (2004 Surgeon General's Report—The Health Consequences of Smoking). These included adrenal glands, cervical lymph nodes, spleen, thymus, pancreas, kidneys, lungs, heart, liver, bladder, bone marrow, colon, intestines, brain, and male and female reproductive organs. No tumors other than lung tumors were observed for either the *Pten+/–* or *+/+* mice in the pseudo–A/J background with either one or three doses of NNK, at 24 or 16 weeks after NNK injection, respectively. However, 1 of 14 pseudo–A/J *Pten+/–* mice showed a malignant lung adenocarcinoma, whereas no carcinomas were observed in the 11 *+/+* littermate lungs evaluated. This contrasted with the C57BL/6 background in which NNK-treated *Pten+/–* mice did not develop lung tumors.

Fewer *Pten+/–* males than expected were obtained in the C57BL/6 background, which is consistent with previous reports [15]. Consequently, the C57BL/6 NNK-treated *Pten+/–* group consisted of mostly females. All *Pten+/–* mice in this background developed at least one tumor, with or without NNK treatment. Most of these tumors have been described previously in *Pten+/–* mice (Table 1). NNK treatment did not alter tumor/hyperplasia incidence or severity in *Pten+/–* mice, although one of nine NNK-treated mice developed a liver hemangiosarcoma, a malignant tumor type not observed in the spontaneous group or described previously in *Pten+/–* mice. Of 13 C57BL/6 *Pten+/–* mice in the spontaneous and NNK groups, 12 developed adrenal phenochromocytoma by 36 weeks of age. Prostate tumors were observed in all *Pten+/–* males (1/1 with four independent adenomas in the NNK group, 2/2 with adenoma in spontaneous group, one of these with a concurrent carcinoma). Severe lymphoid hyperplasia of the cervical lymph nodes was observed in all *Pten+/–* mice in both NNK and spontaneous groups. Although lymph nodes were generally 1 cm or larger in diameter (data not shown), none were classified as lymphoma on histologic evaluation. In addition, areas of severe uterine hyperplasia were observed in some *Pten+/–* females in the absence of any endometrial tumors, which have been described for *Pten+/–* mice in a CD-1 background [15].

Pten Expression Is Retained in Lung Tumors from Pten+/– *Mice*

Loss of Pten hemizygosity has been reported in several spontaneous tumor types in *Pten+/–* mice [10,15,16,20]. Complete or allelic loss of Pten has been described in human lung tumors [21], although no lung tumors have been reported in *Pten+/–* mice. In NNK-treated *Pten+/–* mice, no increase in lung tumor number was observed, and Pten expression was not lost in 17 lung tumors from eight pseudo–A/J *Pten+/–* mice (Figure 3). Lung tumors (Figure 3*B*) and normal lung tissue (data not shown) from *Pten+/–* mice showed decreased Pten protein expression when compared with *+/+* mice. Although Pten can down-regulate the Akt pathway, decreased Pten levels in *Pten+/–* mice did not seem to affect Akt pathway activation in lungs, because pAkt and pS6 levels were similar in tumors (Figure 3*C*) and normal tissue (data not shown) from *+/+* and *Pten+/–* mice.

Lung Tumors from Pten+/– *Mice Harbor* K-ras *Mutations at the Expected Frequency*

NNK-induced lung tumors in A/J mice have been linked to mutations in *K-ras* [22]. Because Pten allelic loss is associated with lung tumors in humans, it was thought that *Pten+/–* mice might develop NNK-induced lung tumors in the absence of *K-ras* mutations. However, similar frequencies of *K-ras* mutations were found in lung tumors from *Pten+/–* mice compared with tumors from their *+/+* littermates. Of 17 lung tumors from eight *Pten+/–* mice, 15 carried a *K-ras* exon 1 mutation; whereas of 20 lung tumors from eight *+/+* littermates, 15 carried a *K-ras* exon 1 mutation (Figure 3*A*; data not shown). This is similar to published results for exon 1 *K-ras* mutations in lung tumors from NNK-treated A/J mice [23].

Discussion

Although lung cancer is the major tobacco-associated neoplasm, tobacco use contributes to other tumor types in humans. However, in mice, no tobacco carcinogen–associated tumors have been

Table 1. Pathologic Findings from *Pten+/–* and *+/+* Mice.

			NNK	
			Pten+/–	*+/+*
Pseudo–A/J background				
Lung adenoma			11/14*	10/11*
Lung adenocarcinoma			1/14	0/11
	Spontaneous		NNK	
	Pten+/–	*+/+*	*Pten+/–*	*+/+*
C57BL/6 background				
Benign pheochromocytoma	3/4	0/5	8/9	0/5
Malignant pheochromocytoma	1/4	0/5	0/9	0/5
Prostate adenoma	2/2	0/5	1/1	0/5
Prostate carcinoma	1/2	0/5	0/1	0/5
Liver hemangiosarcoma	0/4	0/5	1/9	0/5
Severe lymphoid hyperplasia	4/4	1/5	9/9	0/5
Severe uterine hyperplasia	1/2	0/5	3/7	0/5

In the pseudo–A/J strain background, mice were treated with three doses of NNK and evaluated at 16 weeks after the first injection. In the C57BL/6 strain background, mice were treated with a single dose of NNK and evaluated at 30 weeks after treatment. Values indicate the number of mice affected/total mice evaluated. For prostate and uterine lesions, denominators reflect only males and females, respectively. For prostate, only one of two C57BL/6 *Pten+/–* males treated with NNK had tissue for evaluation.

*From surface lung tumor counts for this group, 16/17 *Pten+/–* and 14/14 *Pten+/+* had visible lung tumors (Figure 2).

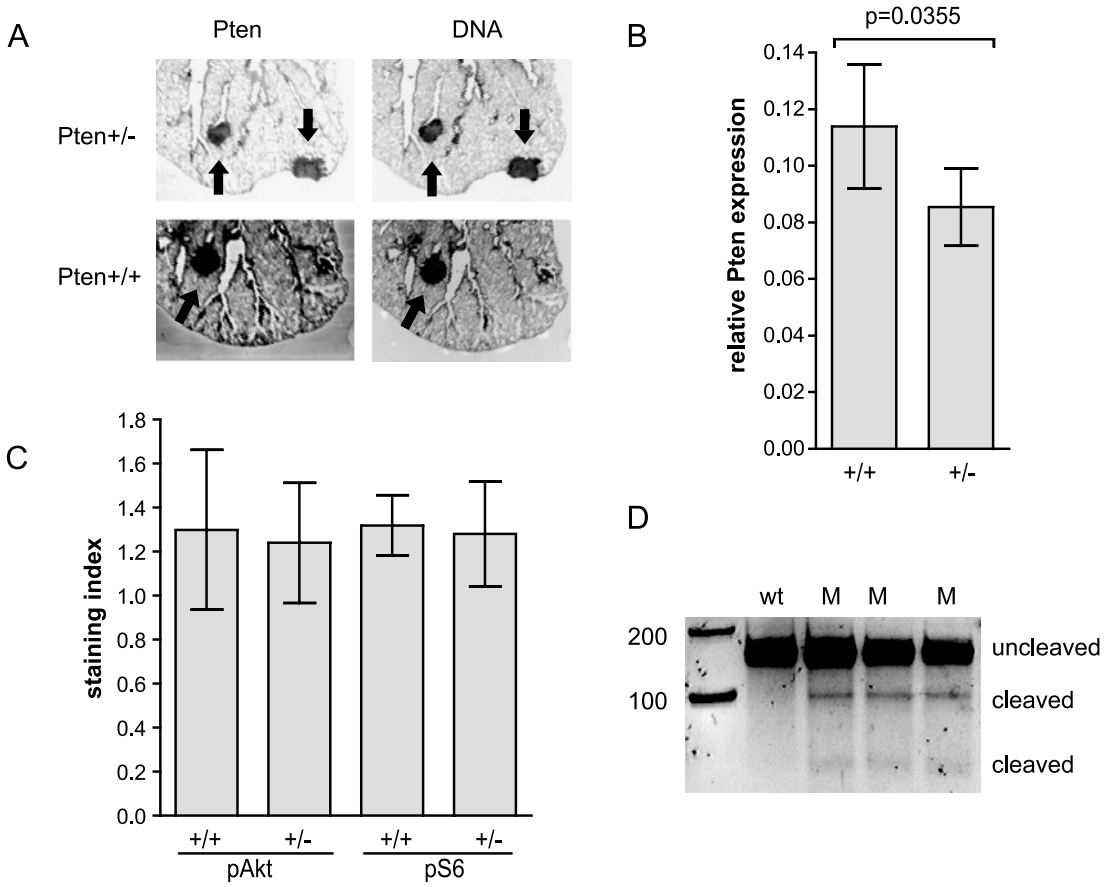

Figure 3. Pten and *K-ras* status of lung tumors from NNK-treated or untreated *Pten +/–* and *+/+* mice. (A) Representative Pten immunohistochemistry staining (visualized using an IR800 secondary antibody) and DNA staining (To-pro3-iodide). Slides were scanned at 1× using a 21-μm resolution on an Odyssey infrared scanner, and portions were enlarged to show detail. Each panel shows approximately one-third of the left lung lobe. Tumors are indicated with arrows. (B) Relative Pten staining from *Pten +/–* and *+/+*, NNK-induced lung tumors. (C) Equivalent Akt pathway activation in lung tumors from *Pten +/–* and *+/+* mice as determined by pS473-Akt and pS235/236 S6 staining. Quantitation methods are described in the Materials and Methods section. (D) *K-ras* mutation was determined by Surveyor Mutation Detection kit. *K-ras* mutation in exon 1 is seen as cleaved DNA in lanes marked "M." Nonmutated *K-ras* does not show any cleaved product in the wild type (wt).

described in tissues other than in the lung. Pten alteration, by loss of heterozygosity, mutation, or methylation, is common in human lung cancer [9,21] and in other tumor types [24]. Humans and mice with germline Pten alteration develop multiple spontaneous tumor types [6,25], but sensitivity to tobacco carcinogens is unknown. For these reasons, *Pten+/–* mice were investigated as a potential model for tobacco carcinogen–induced tumorigenesis in lung and in other tissues. Consistent with previous publications, we found that spontaneous tumor types in *Pten+/–* mice were highly dependent on strain background. The pseudo–A/J strain was resistant to all tumors previously described for *Pten+/–* mice and did not show any spontaneous tumors other than in the lungs. In contrast, no lung tumors were observed in C57BL/6 *Pten+/–* mice, although nearly all developed adrenal tumors, consistent with previous studies for a mixed C57BL/6 background [17]. NNK had no effect on incidence or severity of non–lung lesions in C57BL/6 mice, although a single *Pten+/–* NNK-treated mouse developed a liver hemangiosarcoma, a tumor type not previously described for *Pten+/–* mice. This implies that NNK does not substantially cooperate with Pten allelic loss in tumorigenesis. However, the considerable difference in tumor spectrum between the two strains highlights the effects of other, as yet undetermined genes that can modify tumorigenesis in *Pten+/–* mice. Such phenotypic variation is consistent with the phenotypic spectrum of patients with Cowden disease characterized by germline Pten mutations. In Cowden disease, symptoms and severity vary widely [25], which may reflect the contribution of polymorphisms in other loci that modify the effect of Pten mutation in specific tissues. Overall, strain differences in genetically engineered mice are common and highlight the need to investigate mouse models of human disease in multiple background strains [14].

Decreased or absent Pten protein expression is common in human lung adenocarcinomas and is associated with poor prognosis [3,26]. However, biallelic mutation or loss of Pten have not been associated with *K-ras* mutations in lung tumors. In mice, homozygous deletion of Pten in lung type II cells led to malignant lung tumors starting at 1 year of age and in an increase in urethane-induced lung tumors [8], which have been shown previously to be highly associated with *K-ras* mutation [27]. In another study, homozygous Pten deletion in bronchial epithelium Clara cells did not lead to any spontaneous lung tumors in mice up to 1 year of age but did increase the malignancy of lung tumors in a genetic model of mutant *K-ras* [9]. The two studies described involved *K-ras* mutation coincident with total deletion

of Pten in only specific lung cell types and lung tumors. This is in contrast to the studies described here where one copy of Pten was retained in all tissues and was not lost in any mutant *K-ras*–containing lung tumors. The absence of increased lung tumorigenesis in *Pten+/–* mice supports the hypothesis that decreased Pten expression is involved in the progression of lung tumors rather than in their initiation. This is in contrast to the effects observed in other tissues such as the intestines, breast, and endometrium, where Pten heterozygosity leads to increased tumors [10,15] with frequent loss of the remaining Pten allele [15]. Although normal lung tissues from pseudo–A/J *Pten+/–* mice show decreased expression of Pten protein compared with *+/+* lungs, NNK-induced lung tumors in these mice retain the expression of Pten (Figure 3). Because NNK did not increase lung tumor incidence in *Pten+/–* mice, this suggests either that the loss of the second Pten allele does not occur at a high frequency in lung tissue or that there is no selective advantage for total Pten loss in mouse lung tumors. Because homozygous Pten deletion seems to enhance the malignancy of mutant *K-ras* harboring lung tumors [8,9], it is more likely that loss of the second copy of Pten is an infrequent event in mouse lung tumors. However, the increased size of lung tumors in the three-dose NNK pseudo–A/J group suggests that even deletion of a single copy of Pten may enhance the growth of tumors in the presence of other genetic changes.

The Akt pathway is considered an important target for Pten with loss of Pten phosphatase activity leading to increased PIP3, which activates Akt. Mutant *K-ras* activates PI3K, which also leads to increased PIP3 and Akt activation. Recently, homozygous deletion of PI3K was shown to prevent *K-ras*–induced lung tumors, suggesting that Akt pathway activation is required for *K-ras*–driven lung tumorigenesis [28]. Inhibition of downstream Akt signaling, with the mTOR inhibitor rapamycin, decreased NNK-induced lung tumors by 90% [1]. Here, *Pten+/–* and *+/+* lung tumors showed similar Akt pathway activation (Figure 3) suggesting that *K-ras* mutation alone is enough to activate the pathway to initiate lung tumorigenesis and that deletion of a single copy of Pten does not amplify this signal.

In inbred A/J mice, NNK-induced lung adenocarcinomas were not observed until 25 weeks after treatment [29], and we have found no adenocarcinomas among NNK-induced lung tumors from 15 inbred A/J mice [1]. Although only observed in one mouse, the presence of a malignant lung adenocarcinoma in a *Pten+/–* mouse at only 16 weeks after treatment suggests that loss of even a single allele of Pten may lead to more aggressive tumors or shorter latency to adenocarcinoma after treatment with a tobacco carcinogen. This is also supported by the larger lung tumor size in *Pten+/–* mice treated with three doses of NNK. Studies are ongoing to address whether allelic loss of Pten can lead to increased lung adenocarcinomas at later times and whether this could be accompanied by the total loss of Pten.

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