Mutation of Lkb1 and p53 Genes Exert a Cooperative Effect on Tumorigenesis

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Abstract

Peutz-Jeghers syndrome (PJS) is a dominantly inherited disorder characterized by gastrointestinal hamartomatous polyps and mucocutaneous melanin pigmentation. Germ line mutations in Lkb1 cause PJS. We have generated mice carrying an Lkb1 exon 2 to 8 deletion by gene targeting in embryonic stem cells. Heterozygotes develop gastric hamartomas that are histologically similar to those found in humans with PJS. Lkb1 is also reportedly a mediator of p53-dependent apoptosis. To explore the potential combined effects of p53 and Lkb1 alterations on tumorigenesis, we carried out a series of matings with Lkb1+/− and p53 null mice to generate Lkb1+/−/p53−/− and Lkb1−/−/p53−/− mice. Similar to the Lkb1+/− mice, gastrointestinal hamartomas have also been detected in the mice with these two genotypes. The Lkb1+/−/p53−/− mice displayed a dramatically reduced life span and increased tumor incidence compared to the mice with either Lkb1 or p53 single gene knockout. The time to onset of polyposis in Lkb1+/−/p53−/− mice is ~2 months earlier than Lkb1+/−/p53−/− and Lkb1−/−/p53−/− mice, whereas the latter two show a similar time to onset which is ~6 months of age. These results strongly suggested that mutations of p53 and Lkb1 gene cooperate in the acceleration of tumorigenesis. (Cancer Res 2005; 65(24): 11297-303)

Introduction

Peutz-Jeghers syndrome (PJS) is a unique autosomal dominant syndrome characterized by cutaneous hypermelanocytic macules and hamartomatous polyp development in the gastrointestinal tract. Patients with PJS also have a dramatically increased risk for the development of a variety of different neoplasias (1–3). The syndrome is caused by germ line mutations in the LKB1/STK11 gene encoding serine threonine kinase 11 (4–6). Approximately 70% of clinically defined PJS families harbor germ line Lkb1 mutations (7, 8), and the majority of these mutations are inactivating. Also, somatic Lkb1 mutations have been reported in a variety of carcinomas, such as malignant melanoma (9), pancreatic cancer (10), and lung adenocarcinoma (11, 12).

Numerous studies have indicated a tumor-suppressive function for LKB1. This gene has been implicated in the regulation of multiple biological processes and signaling pathways. Restoring LKB1 activity into cancer cell lines defective for its expression results in a G1 cell cycle arrest (13) by inducing the expression of p21/Waf1/Cip1 through a p53-dependent mechanism (14). The involvement of LKB1 in upstream signaling to AKT and the PI3 kinase pathway (15) indicates the importance of this gene as a regulator of cell growth. It has also been reported that LKB1 (XEEK1) regulates Wnt signaling in vertebrate development (16). Shaw et al. (17) showed that LKB1 directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Another study (18) shows that LKB1 regulates 13 kinases of the AMPK subfamily. Taken together, these data suggest that LKB1 plays an important role in tumorigenesis.

The p53 gene is the most intensively studied tumor suppressor gene, which produces a protein product that functions as a transcription factor. Close to half of all human cancers have p53 gene mutations, indicating that loss of its function is a critical event in tumor progression (19). Studies of p53 mouse models have revealed a key function of p53 in inhibition of cell proliferation and induction of cell apoptosis. A p53 null allele has been generated in mice by several groups and the conclusions are similar (20–23). Jacks and colleagues (20) constructed a mouse strain on a C57BL/6 background carrying a p53 mutant allele, which deleted ~40% of the p53 coding region and completely eliminated synthesis of the p53 protein. Mice homozygous for this mutant allele are viable but highly predisposed to malignancy and most of them die by 6 months of age. Mice heterozygous for this mutant allele are also susceptible to tumors, predominantly sarcoma, osteosarcoma, and lymphomas; ~50% of these mice die by 18 months of age. Studies on these mice lacking one or two copies of the p53 gene have provided invaluable insight into the process of tumorigenesis.

There is evidence to suggest that LKB1 plays a key role in p53-mediated apoptosis (24). Loss of heterozygosity (LOH) at 17p as well as overexpression of p53, indicative of p53 gene mutation, was seen in adenocarcinomas associated with PJS (25). In order to study the interaction between LKB1 and p53, we generated heterozygous mice with a targeted inactivating mutation of Lkb1, and then crossed this line with the p53 null mice mentioned above (20) to obtain double knockout mice for Lkb1 and p53 genes. As our data show, gastrointestinal hamartomatous polyps occur with similar frequency and the onset of polyposis occurs at roughly the same age in both Lkb1+/− and Lkb1−/−/p53−/− mice. In contrast, the doubly heterozygous mice have a dramatically increased incidence of tumors characteristic of p53 knockout mice. Furthermore, the time to onset of polyposis in Lkb1+/−/p53−/− is ~2 months earlier than Lkb1+/−/p53−/− and Lkb1−/− mice. All these results suggest that Lkb1 and p53 cooperate synergistically in tumorigenesis. Thus, our mouse models of human PJS should be useful in future studies aimed at revealing the mechanisms of action of LKB1, as well as developing novel preventive and therapeutic strategies.

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Materials and Methods

Mice. Mice were housed at the Department of Veterinary Medicine and Surgery, The University of Texas M.D. Anderson Cancer Center. All experiments described in this article were conducted according to an approved Institutional Animal Care and Use Committee protocol. p53 null mice from a C57BL/6j background (20) were kindly provided by Dr. Guillermirna Lozano (Department of Molecular Genetics, M.D. Anderson Cancer Center, Houston, TX).

Cloning of mouse Lkb1 gene fragments by PCR. A clone (clone 82) was obtained after screening the mouse library λ DASH-2 by hybridization with mouse Lkb1 exon 1 PCR product. Sequencing showed that clone 82 contains exon 1 and part of intron 1 of the mouse Lkb1 gene. Sequencing also allowed us to identify a forward primer (CJ), 5'-TGTTCTCGGTGCGGAG-TGACACA-3', which was used along with a reverse primer (exon 2R, 5'-CTGTTCTCCTCATTGATGAC-3') to amplify the intron 1 fragments. Also, the regions of exons 2 to 8 (2-8F, 5'-GAGGAGGAGGAGGAGGAGATC-3', 3 arm R, 5'-TCCATCCGACCTCCTCTC-3') were amplified separately by using the normal mouse genome as template. A TOPO XL PCR Cloning Kit (Invitrogen, San Diego, CA) was used to obtain a clone (clone B38) for the exon 2 to 8 fragment, and a clone (clone 13) for the intron 8 fragment, respectively. After sequencing, clone 13 was found to be 7894 bp in length (data not shown).

Generation of Lkb1 knockout mice. The 5' homologous arm was cloned into the pBluescript II SK plasmid vector by ligating together the following three fragments one by one: a loxP sequence (5'-ATAAATCTGTA-TAGCATACATTATACGAAGTTAT-3'), the fragment resulting from clone 1 digested with 

Kpn I and 

Apa I (Fig. 1A), the targeting vector was sequenced to verify that no errors had been produced in the process of 

cloning. AB-1 embryonic stem cells were transfected with the linearized 

genomic DNA as template. A TOPO XL PCR Cloning Kit (Invitrogen, San Diego, CA) was used for survival curve analysis and tumor-free survival analysis. Kaplan-Meier survival analysis, STATA software (StataCorp, College Station, TX) was used for survival curve analysis and tumor-free survival analysis. Kaplan-Meier survival analysis, STATA software (StataCorp, College Station, TX) was used for survival curve analysis and tumor-free survival analysis. Statistic significance of genotypes was determined using built-in analysis for survival curves consisting of a log rank test yielding a

P

value.

Loss of heterozygosity analysis. Genomic DNA from paraffin embedded tissue was extracted using QIAamp DNA mini Kit (Qiagen Inc., Valencia, CA). Two sets of primers were used to detect the targeted Lkb1 allele (primer a, 5'-TGTTCTCGGTGCGGAG-TGACACA-3', and primer e, 5'-ACAGGGAGCTACACTAAAG-3') and the wild-type Lkb1 allele (primer 3 arm F, 5'-GAGGAGGAGGAGGAGGAGATC-3', 3 arm R, 5'-TCCATCCGACCTCCTCTC-3', and primer e, respectively). The multiplex PCR was done for 10 minutes at 94°C followed by 30 cycles at 94°C for 30 seconds, 58°C for 45 seconds, and 72°C for 1 minute, and a final extension step at 72°C for 10 minutes.

Results

Generation of Lkb1 conditional null and exon 2 to 8–deleted knockout mice. The Lkb1loxP+ allele with three loxP sites flanking the region of exons 2 to 8 and an adjacent neomycin resistance gene cassette (Fig. 1A), was introduced into mouse embryonic stem cells by homologous recombination. The chimeric mice derived from four recombinant embryonic stem cell clones transmitted the Lkb1loxP allele to their offspring (Fig. 1C). We established two independent mutant mouse lines from embryonic stem cell clones 5B and 5C, both of which were heterozygous for the Lkb1loxP allele
(Fig. 1D). Mice of these two lines were bred and used for further analysis. To obtain Lkb1 exon 2 to 8-deleted (hereafter called knockout) mice, the Lkb1<sup>3loxP/+</sup> mouse was crossed with the C57BL/6J inbred CMV-Cre transgenic mouse. Three different genotypes such as Lkb1<sup>+/−</sup>, Lkb1<sup>flxed neo/+</sup>, and Lkb1<sup>3loxP/+</sup> resulted from the above mating (Fig. 1B). The Lkb1<sup>+/−</sup> genotypes of the offspring were screened by PCR and by Southern blot analysis (Fig. 1E). No homozygous mutant pups were obtained by intercrossing the heterozygote, indicating that Lkb1<sup>−/−</sup> mice were embryonic lethal. Further studies showed that the Lkb1<sup>−/−</sup> embryo was not viable after 9.5 days postcoitum. This is consistent with previous reports on Lkb1 knockout mice by other groups (27–29).

**Survival and polyposis in Lkb1<sup>+/−</sup> mice.** Lkb1<sup>+/−</sup> mice were found to have reduced life spans with ~50% dying by 14 months compared with their wild-type littermates (Fig. 2A). Their abdomens became increasingly distended as they aged. Complete necropsies were done on 46 Lkb1<sup>+/−</sup> mice that became moribund and were sacrificed at ages ranging from 6 to 18 months. At necropsy, gastrointestinal polyps were identified in Lkb1<sup>+/−</sup> mice as early as 6 months of age and in ~75% of mice at age of 6.5 months. Most polyps were located at the junction of the pylorus and duodenum, and a small number of polyps were found in the antrum or fundus of the stomach. No polyps were found in other parts of the gastrointestinal tract including the large intestine (Table 1). In the majority of animals that we examined, polyps originating from the pylorus were large, whereas polyps located in the fundus and antrum were smaller. The large polyps in the pylorus protruded into the duodenum, resulting in gross distension and obstruction of the duodenum (Fig. 3A and B). These obstructions, which mimic the clinically acute obstruction seen in many cases of human PJS, seem to explain the high mortality of the Lkb1<sup>+/−</sup> mice.

Histologic evaluation indicated that all of the polyps were hamartomas (Fig. 3F). The histologic characteristics of these polyps had an extensive arborizing smooth muscle component and normal glandular epithelium. Histologic comparison of polyps derived from Lkb1<sup>+/−</sup> mice (Fig. 3F) with polyps resected from human patients with PJS (Fig. 3E) revealed striking similarities.

Out of 13 Lkb1<sup>+/-</sup> mice with complete histologic assessment of all of the organs, 9 (69%) exhibited organomegaly of spleen, lung, liver, stomach, and intestine. No other neoplasms or tumors were observed.

**Survival and polyposis in Lkb1<sup>+/−</sup>/p53<sup>+/+</sup> and Lkb1<sup>+/−</sup>/p53<sup>−/−</sup> mice.** As mentioned above, current evidence suggests that Lkb1 regulates p53-dependent apoptosis, and loss of p53 is an important factor in tumor development. To determine whether Lkb1 and p53 mutations act synergistically in tumorigenesis, we mated Lkb1 heterozygous knockout mice with p53 homozygous knockout mice (20) to generate a series of mice heterozygous for both mutations (Lkb1<sup>+/−</sup>/p53<sup>+/−</sup>). A population of 129 mice carrying a mutant allele of both Lkb1 and p53 were generated

### Table 1. Effect of the p53 mutation on location of polyps in Lkb1<sup>+/−</sup> mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of mice</th>
<th>Total Polyp location</th>
<th>Stomach and duodenum*</th>
<th>Large intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lkb1&lt;sup&gt;+/−&lt;/sup&gt;</td>
<td>48</td>
<td>46</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>p53&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>38</td>
<td>35</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lkb1&lt;sup&gt;+/−&lt;/sup&gt;/p53&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lkb1&lt;sup&gt;+/−&lt;/sup&gt;/p53&lt;sup&gt;+/−&lt;/sup&gt;</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*Most of the polyps located at the junction of the pylorus and duodenum.

†These mice were sacrificed at 6 to 18 months of age once they became moribund. No polyps were detected in 2 of 48 mice, which are 6.5 and 8 months old, respectively.

‡These mice were sacrificed at 7 to 14 months of age once they became moribund. No polyps were detected in 3 of 38 mice, which are 7, 8, and 8 months old, respectively.

¶These mice were sacrificed at 4 to 6 months of age once they became moribund. No polyps were detected in one of eight mice, which is 6 months old.
and monitored. Lkb1+/−/p53+/− mice were found to have reduced life spans with ∼50% dying by 10 months (Fig. 2B). The median age of survival of Lkb1+/−/p53+/− mice (10 months) was dramatically lower than that of mice heterozygous for the Lkb1 or p53 mutation alone (14 and 18 months, respectively).

Similar to Lkb1+/− mice, Lkb1+/−/p53+/− mice had enlarged abdomens due to hamartomatous polyps in the pylorus and duodenum (Fig. 3C). Mice that became moribund were euthanized and subjected to a complete necropsy. Ninety-two percent (35 out of 38) of the moribund Lkb1+/−/p53+/− mice ranging in age from 7 to 14 months showed polyps in their gastrointestinal tract. The majority of these mice had polyps in the stomach and/or duodenum (Table 1). One mouse also displayed colonic polyps. We compared the time to onset of polyposis observed in Lkb1+/− to that seen in Lkb1+/−/p53+/− mice at different time points. Polyps could not be detected in the mice younger than 5.5 months (four out of four), but were detected in 60% of mice (three out of five) at 6.5 months. This result is similar to the time to onset of polyposis observed in Lkb1+/− mice. In addition, both intensity (data not shown) and the histologic appearance (Fig. 3G) of the polyps in Lkb1+/−/p53+/− mice were very similar to those in Lkb1+/− mice. Thus, it is very possible that loss of one allele of p53 is not enough to accelerate the polyposis. To further evaluate whether p53 plays a role in polyposis, a cohort of 20 mice nullizygous for p53 and containing one mutant Lkb1 allele (Lkb1+/−/ p53−/−) were generated and monitored. The median age of survival of Lkb1+/−/p53−/− mice was ∼4.5 months which is very similar to their littermate p53−/− mice (Fig. 2C). Necropsy studies revealed that gastrointestinal hamartomatous polyps (Fig. 3D) in Lkb1+/−/p53−/− mice were identified as early as 4.3 months. This is ∼2 months earlier than those for Lkb1+/−/p53+/− mice, no time difference was observed between the Lkb1+/− and Lkb1+/−/p53+/− mice. Although the histologic appearance of these polyps from Lkb1+/−/p53+/− mice (Fig. 3H) are very similar to the polyps identified in the Lkb1+/− and Lkb1+/−/p53+/− mice, the difference in the polyps locations were observed as two out of seven mice also had polyps in their large intestines. These findings suggested that the knockout p53 gene in the Lkb1+/− mice increases the time to onset of polyposis and influence the distribution of polyps.

Tumorigenesis in Lkb1+/−/p53+/− and Lkb1+/−/p53−/− mice. In addition to the gastrointestinal polyps, a variety of tumors were also found in the Lkb1+/−/p53+/− and Lkb1+/−/p53−/− mice (Table 2; Fig. 4). Twenty-three (61%) of 38 Lkb1+/−/p53+/− mice developed a total of 30 tumors. Among them, 13 (43%) of these tumors were osteosarcomas, 3 (10%) were sarcomas, and 6 (20%) were lymphomas. These tumor types are the characteristic tumor types detected in the mice heterozygous for p53. In Lkb1+/−/p53−/− mice, osteosarcoma is the predominant one, which is very similar to the dominant tumor type reported previously (20–23) as well as in their p53−/− littermate presented here. Also, we did a tumor-free survival analysis on the Lkb1+/−/p53+/− and p53−/− mice that underwent necropsy. The median age of tumor-free survival for Lkb1+/−/p53+/− mice was 11 months, whereas for p53−/− mice, it was ∼17 months (Fig. 5A), this difference was statistically significant (P < 0.01) suggesting that Lkb1+/−/p53+/− mice had a significant increase in tumor incidence compared with the p53−/− mice.

Most importantly, we observed a pancreatic carcinoma, a lung carcinoma, a breast carcinoma, a small intestinal adenocarcinoma, and two intestinal adenomas in these Lkb1+/−/p53−/− mice (Fig. 4). It was reported that PJS patients are predisposed to develop cancer...
of the pancreas, intestine, breast, and other organs. Our results suggest that heterozygosity for p53 increases tumor incidence in these organs. Thus, Lkb1<sup>+/−</sup>/p53<sup>+/−</sup> mice probably provide a better model for PJS. To test whether there is LOH of Lkb1 involved in these PJS-related tumors in Lkb1<sup>+/−</sup>/p53<sup>+/−</sup> mice, we analyzed the status of the wild-type copy of Lkb1 gene in three carcinomas from pancreas, small intestine, and mammary gland, and one adenoma in the small intestine, as well as an osteosarcoma and a lymphoma by allele-specific PCR analysis. We found that two of the six tumors (Fig. 5b, lanes 2 and 5) displayed a decrease in intensity of staining for the PCR product of the wild-type allele relative to the knockout allele suggesting LOH. Whereas in the rest of the samples, both wild-type and knockout alleles are comparably amplified, suggesting that the wild-type allele was retained.

The Lkb1<sup>+/−</sup>/p53<sup>+/−</sup> mice were highly susceptible to tumors at an early age, particularly malignant lymphoma (Table 2). Carcinoma of the pancreas, mammary gland, and small intestines, observed in Lkb1<sup>+/−</sup>/p53<sup>+/−</sup> mice at >10 months of age, were not observed in the Lkb1<sup>+/−</sup>/p53<sup>−/−</sup> mice, probably due to the severely compromised life span.

Together, these findings showed that the loss of the p53 gene accelerates the time to onset of polyposis in Lkb1<sup>+/−</sup> mice and the added deletion of the Lkb1 gene dramatically increased tumor incidence in p53<sup>−/−</sup> mice and strongly suggests that mutation of both Lkb1 and p53 genes did have a cooperative effect on tumorigenesis.

### Table 2. Tumor spectrum from Lkb1<sup>+/−</sup>/p53<sup>+/−</sup>, Lkb1<sup>+/−</sup>/p53<sup>−/−</sup>, and control mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age (mo)*</th>
<th>Tumor type</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>10.8, 12.0, 16.8, 12.7</td>
<td>osteosarcoma</td>
</tr>
<tr>
<td></td>
<td>18.2</td>
<td>lymphoma (spleen)</td>
</tr>
<tr>
<td>Lkb1&lt;sup&gt;−/−&lt;/sup&gt;/p53&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>9.2, 8.3, 8.6, 9.9, 9.8, 12.0, 7.1, 10.2, 9.9, 11.0, 8.8, 12.6&lt;sup&gt;+&lt;/sup&gt;, 7.6&lt;sup&gt;−&lt;/sup&gt;, 7.6&lt;sup&gt;+&lt;/sup&gt;, 12.4&lt;sup&gt;+&lt;/sup&gt;, 12.6&lt;sup&gt;+&lt;/sup&gt;, 11.2, 7.6, 12.0, 12.6&lt;sup&gt;+&lt;/sup&gt;, 11.0&lt;sup&gt;+&lt;/sup&gt;, 11.0&lt;sup&gt;+&lt;/sup&gt;, 10.9, 12.6&lt;sup&gt;+&lt;/sup&gt;</td>
<td>osteosarcoma, lymphoma (mediastinum), lymphoma (lung), lymphoma (small intestine), sarcoma, adenocarcinoma (small intestine), anaplastic carcinoma (pancreas), anaplastic metastatic lung, adenoma (small intestine), adenocarcinoma mammary gland, lymphoma (mediastinum), osteosarcoma vertebra, osteosarcoma metastatic lung, osteosarcoma metastatic liver, osteosarcoma metastatic spleen, osteosarcoma metastatic kidney, glioblastoma (brain), lymphoma (spleen), lymphoma (lung), lymphoma (liver), lymphoma (heart), lymphoma (kidney), sarcoma (unclassified), fibrosarcoma, anaplastic sarcoma (unclassified)</td>
</tr>
<tr>
<td>p53&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>9.9</td>
<td>osteosarcoma</td>
</tr>
<tr>
<td></td>
<td>8.0&lt;sup&gt;+&lt;/sup&gt;</td>
<td>lymphoma (spleen)</td>
</tr>
<tr>
<td></td>
<td>8.0&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>osteosarcoma metastatic lung</td>
</tr>
<tr>
<td></td>
<td>8.0&lt;sup&gt;+&lt;/sup&gt;</td>
<td>osteosarcoma metastatic liver</td>
</tr>
<tr>
<td>Lkb1&lt;sup&gt;−/−&lt;/sup&gt;/p53&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>6.0, 4.5, 5.7&lt;sup&gt;+&lt;/sup&gt;, 6.0, 4.5, 5.7&lt;sup&gt;+&lt;/sup&gt;, 5.7&lt;sup&gt;+&lt;/sup&gt;, 5.7&lt;sup&gt;+&lt;/sup&gt;, 5.7&lt;sup&gt;+&lt;/sup&gt;, 5.7&lt;sup&gt;+&lt;/sup&gt;, 5.7&lt;sup&gt;+&lt;/sup&gt;, 5.7&lt;sup&gt;+&lt;/sup&gt;, 5.7&lt;sup&gt;+&lt;/sup&gt;, 5.7&lt;sup&gt;+&lt;/sup&gt;, 5.7&lt;sup&gt;+&lt;/sup&gt;</td>
<td>osteosarcoma, lymphoma (spleen), lymphoma (lung), lymphoma (liver), lymphoma (heart), lymphoma (kidney), sarcoma (unclassified), fibrosarcoma, anaplastic sarcoma (unclassified)</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>osteosarcoma</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>lymphoma (spleen)</td>
</tr>
<tr>
<td></td>
<td>5.3</td>
<td>osteosarcoma</td>
</tr>
</tbody>
</table>

NOTE: <sup>+</sup>, <sup>−</sup>, <sup>+</sup>, <sup>−</sup>, <sup>+</sup>, <sup>−</sup>, <sup>+</sup>/<sup>−</sup> symbols represent individual mice where multiple tumors were detected in the same mouse, respectively. Each age corresponds to a different mouse.

### Discussion

In the present study, we developed an Lkb1<sup>+/−</sup> mouse line. It serves as a model for PJS, as hamartomatous polyps were found in the gastrointestinal tract of heterozygous mutant mice, and histologically, the polyps of Lkb1<sup>+/−</sup> mice are strikingly similar to those of polyps found in human patients with PJS. However, the distribution of polyps is different in PJS patients compared with Lkb1<sup>+/−</sup> mice. In human patients with PJS, polyps can arise anywhere in the gastrointestinal tract (30–32). In the present study, the polyps in Lkb1<sup>+/−</sup> mice were located predominantly at the pylorus. Interestingly, mice with either Lkb1<sup>+/−</sup>/p53<sup>+/−</sup> or Lkb1<sup>+/−</sup>/p53<sup>−/−</sup> genotypes showed a shift in the distribution of polyps compared with the Lkb1<sup>+/−</sup> genotype, suggesting that defective p53 can influence the distribution of polyps. Furthermore, it has been shown here that loss of both alleles of p53 accelerate the polyposis by ~2 months, although the time to onset of polyposis (~6 months) in Lkb1<sup>+/−</sup>/p53<sup>−/−</sup> mice was not very different from that in Lkb1<sup>−/−</sup> mice. Taken together, these findings suggest that the interaction between these two tumor suppressor genes in the gastrointestinal tract had a cooperative effect on polyposis.

LKB1 has been reported as a mediator of p53-dependent apoptosis and a player in cell cycle arrest. Therefore, to evaluate the joint effects of the Lkb1 and p53 genes on tumorigenesis, Lkb1<sup>+/−</sup>/p53<sup>−/−</sup> and Lkb1<sup>−/−</sup>/p53<sup>−/−</sup> mice were generated. Hamartomatous polyps in the gastrointestinal tracts as well as...
various other types of tumors have been detected in mice with these two genotypes. Compared with the Lkb1+/−/C0 mice present here as well as the Lkb1+/−/p53+/− mice reported by other groups (28, 29, 33), Lkb1+/−/p53+/− mice showed a dramatically increased incidence of various types of tumors including malignant cancers of the pancreas, mammary gland, and intestinal organs, which are the most common tumor types seen in PJS. Thus, the Lkb1+/−/p53+/− mouse line provides a model for tumor formation for PJS. Although the tumor spectrum in Lkb1+/−/p53+/− mice is not identical to that observed in human patients with PJS, this is the first mouse model of PJS in which a significant incidence of various types of tumors was observed. The Lkb1+/−/p53−/− mice presented here also showed various types of tumors which are characteristic tumor types detected in p53-deficient mice. However, no PJS-predisposed cancer type was observed, which was most likely due to the severely compromised longevity. Meanwhile, the survival curve of the Lkb1+/−/p53−/− and p53−/− are very similar to each other, suggesting that LKB1 and p53 may function in the same apoptotic pathway. Recently, Jones et al. (34) reported that AMPK activation induces phosphorylation of p53 on serine 15 and this phosphorylation is required to initiate AMPK-dependent cell cycle arrest in response to the glucose deprivation. Meanwhile, Shaw et al. (17) showed that LKB1 directly activates AMPK and regulates apoptosis in response to energy stress. These findings suggest that regulation of p53-dependent apoptosis by LKB1 is likely to occur through the activation of AMPK. Further studies need to be done to elucidate the possible linkage between the Lkb1, AMPK, and p53. Several mouse cross-breeding studies have shown cooperation between p53 and other tumor suppressor genes or oncogenes (35, 36). For example, mutation of both Rb and p53 (Rb+/−/p53−/−) produces mice that have shorter life spans and develop a variety of tumors not found in mice with single gene mutations. This also happened in Lkb1+/−/p53−/− mice. Furthermore, it is very possible that LKB1 is in the p53-dependent pathway, loss of one wild-type allele of Lkb1 and p53 both contribute to the tumorigenesis because the p53-dependent pathway is not entirely inactive and the efficiency of the pathway could be decreased through partial loss of LKB1. Together, our data suggests that Lkb1 and p53 cooperate in the acceleration of tumorigenesis.

Figure 4. Representative H&E staining of the tumor detected in the Lkb1+/−/p53−/− mice. A to E, original magnification, ×250. A, adenocarcinoma in small intestine; B, adenocarcinoma in mammary gland; C, anaplastic carcinoma in pancreas; D, lymphoma in spleen; E, osteosarcoma.

Figure 5. Effects of Lkb1 mutation on the tumorigenesis of p53−/− mice. A, tumor-free survival analysis of Lkb1+/−/p53−/− and p53−/− mice (P < 0.01); dashed line, Lkb1+/−/p53−/− mice; dotted line, p53−/− mice. B, Lkb1 allele-specific PCR analysis on DNA from normal mouse tail (N), adenoma in small intestine (1), adenocarcinoma in small intestine (2), adenocarcinoma in mammary gland (3), anaplastic carcinoma in pancreas (4), lymphoma in spleen (5) and osteosarcoma (6). Lanes 2 to 6 correspond to the samples describe in Fig. 4A-E, respectively; WT, wild-type allele; KO, knockout allele.
In conclusion, we have described different Lkb1−/− mouse lines with or without p53 mutation for human PJS and have shown that the Lkb1 and p53 genes cooperate in tumorigenesis. Our findings also suggest that our Lkb1+/− and Lkb1−/−/p53−/− mice will be an important and useful model in which to elucidate the molecular mechanisms underlying PJS polyposis and tumorigenesis. Crossing the Lkb1 knockout mouse with other cancer-prone mouse strains will also help to establish the mechanism of action of Lkb1 by revealing genetic interactions between Lkb1 and other genes important in tumor development.

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References

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