Aberrations of the p53 tumor suppressor gene in various tumors in dogs

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Objective—To evaluate aberrations of the p53 tumor suppressor gene in naturally developing tumors in dogs.

Sample Population—Tumor specimens from 15 dogs with various tumors, including malignant lymphoma (7 dogs), monocytic leukemia (1), mammary gland adenoma (1), mammary gland benign mixed tumor (1), rhabdomyosarcoma (1), colon cancer (1), and osteosarcoma (3).

Procedure—Aberrations of the p53 gene in these tumor tissues were examined by reverse transcriptase-polymerase chain reaction and single-strand conformation polymorphism analysis, using 3 fragments that covered the entire open reading frame of the canine p53 gene, followed by nucleotide sequencing of the abnormal bands.

Results—Point mutations, deletions, and insertions resulting in a number of amino acid substitutions of wild-type p53 were detected in 7 of the 15 tumor specimens from dogs with malignant lymphoma, monocytic leukemia, rhabdomyosarcoma, colon cancer, and osteosarcoma. Of these 7 dogs, 2 had aberrations of the p53 gene on both alleles, whereas 5 had aberrations of the p53 gene on 1 allele and consequently lacked the wild-type p53 transcript. Many of the aberrations of the p53 gene detected in these tumors were located in the transactivation, DNA binding, and oligomerization domains.

Conclusions and Clinical Relevance—Various naturally developing tumors in dogs often have inactivation of the p53 tumor suppressor gene, which may be 1 of the multiple step-wise genetic changes during tumorigenesis. This study indicates that p53 is a guardian of the cell cycle. Thus, loss of p53 function is considered to be associated with tumorigenesis because of impaired regulation of the cell cycle.

The p53 tumor suppressor gene and its aberrations are considered to be a molecular basis for tumorigenesis. Inactivation of p53 is associated with a broad spectrum of tumors in humans.1 Mutations of the p53 gene are evident in 50 to 55% of all malignancies in humans, including breast cancer, osteosarcoma, colon cancer, and lung cancer.2,3 Furthermore, p53-null mice are susceptible to tumors when they are extremely young.4,5 One function of p53 is to act as an inducible transcriptional factor after DNA damage.6 Another function of p53 is to regulate the cell cycle by inducing P21WAF1, which binds to and inhibits the cyclin-cyclin dependent kinase complex.7 This function is known as p53-mediated G1 arrest. Also, p53 affects induction of apoptosis by inducing bax.8 More recently, it was reported that p53-null cells were not blocked in the G2 phase when they were treated with mitotic spindle inhibitors.9 These observations indicate that p53 is a guardian of the cell cycle. Thus, loss of p53 function is considered to be associated with tumorigenesis because of impaired regulation of the cell cycle.

Neoplastic diseases affecting various tissues, including the mammary gland, skin, and components of the lymphohematopoietic and musculoskeletal systems, are common in dogs and are 1 of the major problems in companion animals.10,11 Although neoplasms in dogs seem to be clinically similar to those in humans, knowledge about the molecular basis for tumorigenesis in dogs is scant. The study reported here was conducted to evaluate aberrations of the p53 tumor suppressor gene in a diverse range of naturally developing tumors in dogs, using a polymerase chain reaction (PCR) technique.

Materials and Methods

Amplification and nucleotide sequencing of wild-type canine p53 cDNA—Total RNA extracted from the spleen of a clinically normal dog was used to generate single-strand cDNA, using a reverse transcriptase-PCR (RT-PCR). Primers were synthesized on the basis of sequences conserved among human,12 cat,13 and mouse14 p53 genes, which would cover the entire coding region for canine p53 (Appendix). Primer pairs 1 and 2, 3 and 4, and 5 and 6 were used for amplifying the 5′, central, and 3′ portions of canine p53 cDNA, respectively. These PCR-amplified fragments were cloned into a plasmid vector and sequenced by use of the dyeoxy chain termination method.15

Tumor specimens—Tumor specimens were obtained from 15 dogs with tumors admitted to the Veterinary Medical Center at the University of Tokyo. Tumor tissues from 10 dogs were obtained during necropsy, and tissues from 5 dogs were obtained during surgical resection of the tumors. Tumors examined in the study included malignant lymphoma (7 dogs), monocytic leukemia (1), mammary gland adenoma (1), mammary gland benign mixed tumor (1),...
rhabdomyosarcoma (1), colon cancer (1), and osteosarcoma (3). Nonaffected control tissues such as brain, skeletal muscle, liver, and myocardium also were obtained from each tumor-bearing dog. All tissues were rapidly frozen in liquid nitrogen and stored at –80 °C for subsequent RNA extraction.

**PCR single-strand conformation polymorphism (SSCP) analysis**—To prepare cDNA samples for PCR-SSCP analysis, total RNA samples were extracted with phenol and chloroform and then reverse-transcribed with avian myeloblastosis virus RT. The entire open reading frame of canine p53 cDNA was divided into 3 parts for PCR amplification. Three pairs of primers were used for amplifying the 5’ (pair 7 and 8; nucleotides –25 to 441), central (pair 9 and 10; nucleotides 735 to 1,178), and 3’ (pair 11 and 12; nucleotides 341 to 778) portions of canine p53 cDNA. The 5’ end of each primer (100 μM) was labeled with γ-32P-dATP (50 pmol, 259 TBq/mmol) and 5 units of polynucleotide kinase in 5 μl of buffer. After denaturation at 94 °C for 3 minutes, 35 cycles of the reaction (60 °C for 2 minutes (annealing and polymerization) and 94 °C for 20 seconds (denaturation)) were performed, followed by a final extension procedure at 72 °C for 10 minutes; all reactions were performed in a thermal cycler. Reaction products were mixed with a solution (100 μl) containing 95% formamide, 20 mM EDTA, and 0 to 5% glycerol. Electrophoresis was performed at 20 W for 2 to 2.5 hours. Temperature was controlled by use of a cooling fan and an aluminum plate. Gels were dried on filter paper and exposed to a radiographic film at –80 °C for 2 to 18 hours.

**Sequencing of abnormal bands**—The DNA extracted from the fragments that had mobility shifts were amplified by PCR, using the same primer pair as for PCR-SSCP. Resulting products were cloned into a plasmid vector. Plasmid DNA was extracted with a commercial plasmid kit and sequenced by use of the dideoxy chain termination method.

**Southern blot analysis**—Tissue samples stored at –80 °C were homogenized in liquid nitrogen and incubated overnight at 37 °C in a lysis buffer containing 20 μg of proteinase K/ml and 10 μg RNase A/ml. These samples were extracted with phenol and chloroform and then precipitated with ethanol. The DNA samples digested with restriction endonucleases were separated by electrophoresis in an 0.8% agarose gel and transferred to a hybridization membrane. Membranes were hybridized overnight at 60 °C with α-32P-labeled canine p53 cDNA probe in a hybridization solution containing 5× SSC (1× SSC contains 0.15M NaCl and 0.015M sodium citrate), 1% sodium dodecyl sulfate, 50 mM tris-HCl (pH 7.6), and 100 μg of salmon testis DNA/ml, and 5× Denhardt solution. After hybridization, filters were washed 3 times with a solution containing 0.3× SSC and 1% sodium dodecyl sulfate (55 C for 30 minutes) and subjected to autoradiography.

**Results**

**Nucleotide sequence of canine p53 cDNA**—The DNA fragments corresponding to the 5’, central, and 3’ portions of canine p53 cDNA were amplified with PCR, separated, extracted from agarose gels, and cloned into plasmid vectors. The nucleotide sequence spanned by these 3 overlapping DNA fragments was 1,247 base pairs (bp) and contained the 5’ noncoding region (46 bp), a long open reading frame (1,146 bp), and the 3’ noncoding region (35 bp). Sequences of the canine p53 cDNA obtained in this study had 79.7, 86.0, 71.8, and 72.7% similarity in amino acid sequence with those of human, cat, mouse, and rat homologues, respectively. Domains I to V that are conserved among many vertebrate species were highly conserved in the sequence of canine p53 cDNA. Domains of transactivation, DNA binding, and oligomerization as well as the nuclear localization signal also were well-conserved in canine p53 cDNA.

**PCR-SSCP analysis of tumor tissues in dogs**—A PCR-SSCP analysis was performed on tissues from 13 dogs with various naturally developing tumors. For these reactions, 3 pairs of primers were used to amplify the 3 overlapping fragments (fragments A, B, and C, comprising nucleotides –25 to 441, 341 to 778, and 735 to 1,178, respectively) that covered the entire open reading frame of canine p53 (Fig 1). Two bands that had a mobility shift were detected in fragment B in a dog with malignant lymphoma (Fig 2). However, 1 shifted band was detected in each lane of fragment A in 2 other dogs with malignant lymphoma. In the dog with monocytic leukemia, band shifts were detected in fragments B and C, whereas in the dog with rhabdomyosarcoma, shifted bands were detected in fragments A and C. In the dog with colon cancer, 4 bands with mobility shift were detected in fragments A and B, and a band shift was detected in fragment C. In 1 of the dogs with osteosarcoma, 4 shifted bands were detected in fragment A, and 1 broad band was shifted in fragment C. In another dog with osteosarcoma, shifted bands were detected in fragments A and B, and in the third dog with osteosarcoma, a broad band was shifted in fragment C. As a result, bands with a mobility shift in PCR-SSCP analysis for

![Diagram](image-url)
p53 mRNA were detected in 9 tumors, but they were not detected in the other 6 tumors.

Sequence determination of mutated p53 in tumor tissues in dogs—Nucleotide sequences were determined for the shifted bands that corresponded to the mutated p53 gene (Table 1, Fig 3). The tumor sample for 1 of the dogs with malignant lymphoma had a missense point mutation resulting in a substitution of Arg237 for Pro; however, samples for 2 other dogs with malignant lymphoma did not have any mutation in sequencing analysis of the shifted bands. In the dog with monocytic leukemia, there were 4 missense point mutations (substitutions of Ala191 for Asp, Ser229 for Phe, Leu296 for Pro, and Gln342 for Arg) and a silent point mutation at nucleotide 941 (from A to G). In the dog with rhabdomyosarcoma, 2 missense point mutations (substitutions of Phe96 for Leu and Ser354 for Pro), 2 silent point mutations at nucleotides 1,059 and 1,138 (from C to T and A to G, respectively), and a deletion of 1 base (G) at nucleotide 383 in codon 128 resulted in substitution of 29 amino acids (Cys128 to Val156) and introduction of a stop codon at codon 156. A tumor sample from the dog with colon cancer had 2 alleles with differing mutations; 1 allele had 4 missense point mutations (substitutions of Asn29 for Asp, Phe155 for Leu, Glu314 for Asp, and Met328 for Arg) and a 3-base insertion of GGT between nucleotides 609 and 610, whereas another allele had 2 deletions of bases (G at nucleotide 736 and GG at nucleotides 748 and 749), which resulted in substitutions of 4 amino acids and a deletion of 1 amino acid (from Glu246-Asp247-Ser248-Ser249-Gly250 to Lys-Thr-Pro-Val). In 1 of the dogs with osteosarcoma, there were 3 missense point mutations (substitutions of Glu28 for Gly, Pro37 for Thr, and Leu50 for Arg) and a deletion of 1 base at nucleotide 110 in codon 37 that resulted in substitutions of 25 amino acids and the introduction of a stop codon in codon 31.
Table 1—Nucleotide sequences of abnormal bands that had a mobility shift, as determined by use of polymerase chain reaction (PCR) and single-strand conformational polymorphism (SSCP) analysis of canine p53 cDNA

<table>
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<tr>
<th>Dog</th>
<th>Type of tumor</th>
<th>Fragments*</th>
<th>Mutations</th>
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<tbody>
<tr>
<td>1</td>
<td>Malignant lymphoma</td>
<td>+ + +</td>
<td>CGG to CCG, Arg&lt;sup&gt;107&lt;/sup&gt; to Pro</td>
</tr>
<tr>
<td>8</td>
<td>Monocytic leukemia</td>
<td>+ + +</td>
<td>GCC to GAC, Ala&lt;sup&gt;107&lt;/sup&gt; to Asp; TCC to TTC, Ser&lt;sup&gt;29&lt;/sup&gt; to Phe; CTG to CCG, Leu&lt;sup&gt;28&lt;/sup&gt; to Pro; GGA to GGG, Gly&lt;sup&gt;55&lt;/sup&gt; to Gly(silent); CAG to GGD, Gln&lt;sup&gt;55&lt;/sup&gt; to Arg</td>
</tr>
<tr>
<td>11</td>
<td>Rhabdomyosarcoma</td>
<td>+ + +</td>
<td>TTC to CTC, Phe&lt;sup&gt;83&lt;/sup&gt; to Leu; G (nt366) deletion, Cys&lt;sup&gt;258&lt;/sup&gt;-Val&lt;sup&gt;258&lt;/sup&gt; to Ser&lt;sup&gt;110&lt;/sup&gt;stop; CAC to CAT, His&lt;sup&gt;239&lt;/sup&gt; to His(silent); GAA to GAG, Gln&lt;sup&gt;285&lt;/sup&gt; to Gln(silent)</td>
</tr>
<tr>
<td>12</td>
<td>Colon cancer</td>
<td>+ + +</td>
<td>AAC to GAC, Asn&lt;sup&gt;288&lt;/sup&gt; to Asp; TCC to TTC, Phe&lt;sup&gt;179&lt;/sup&gt; to Leu; GGT insertion (between nt906-910), Val insertion (between codons 291-295); G (nt738) and GG (nt748, nt749) deletions, Glu&lt;sup&gt;259&lt;/sup&gt;-Asp&lt;sup&gt;259&lt;/sup&gt;-Ser&lt;sup&gt;259&lt;/sup&gt;-Ser&lt;sup&gt;259&lt;/sup&gt;, Gly&lt;sup&gt;383&lt;/sup&gt; to Lys&lt;sup&gt;383&lt;/sup&gt;-His&lt;sup&gt;383&lt;/sup&gt;-Pro&lt;sup&gt;383&lt;/sup&gt;-Glu&lt;sup&gt;383&lt;/sup&gt;-Asp&lt;sup&gt;383&lt;/sup&gt;-Glu&lt;sup&gt;383&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>Osteosarcoma</td>
<td>+ + +</td>
<td>GAG to GGG, Glu&lt;sup&gt;283&lt;/sup&gt; to Gln&lt;sup&gt;283&lt;/sup&gt;-Gly&lt;sup&gt;283&lt;/sup&gt; to Gly; C deletion (nt110), Cys&lt;sup&gt;128&lt;/sup&gt;-Asn&lt;sup&gt;128&lt;/sup&gt; to Cys&lt;sup&gt;128&lt;/sup&gt;-stop&lt;sup&gt;1&lt;/sup&gt;; CCA to ACA, Pro&lt;sup&gt;271&lt;/sup&gt; to Thr; CTC to GCC, Leu&lt;sup&gt;291&lt;/sup&gt; to Arg</td>
</tr>
<tr>
<td>14</td>
<td>Osteosarcoma</td>
<td>+ + +</td>
<td>CCT to CTT, Pro&lt;sup&gt;179&lt;/sup&gt; to Leu; CGT to CAT, Arg&lt;sup&gt;283&lt;/sup&gt; to His; TCC to CCC, Ser&lt;sup&gt;283&lt;/sup&gt; to Pro; C insertion (between nt293-294); Cys&lt;sup&gt;272&lt;/sup&gt;-Lys&lt;sup&gt;272&lt;/sup&gt;stop&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>Osteosarcoma</td>
<td>+ + +</td>
<td>CTC to CCC, Leu&lt;sup&gt;283&lt;/sup&gt; to Pro; GAG to GGG, Glu&lt;sup&gt;55&lt;/sup&gt; to Gly; AAT to GAT, Asp&lt;sup&gt;251&lt;/sup&gt; to Asp; AAG to AGD, Lys&lt;sup&gt;251&lt;/sup&gt; to Arg</td>
</tr>
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*DNA fragments A, B, and C were obtained by PCR-SSCP analysis. + = Abnormal bands detected. – = Only normal bands detected.

In another dog with osteosarcoma, 3 missense point mutations (substitutions of Pro<sup>63</sup> for Leu, Arg<sup>107</sup> for His, and Ser<sup>110</sup> for Pro) and an insertion of 1 base between nucleotides 383 and 384 resulted in substitutions of 24 amino acids and introduction of a stop codon in codon 151 (Cys<sup>128</sup>-Lys<sup>151</sup> for Cys<sup>128</sup>-stop<sup>151</sup>). In the third dog with osteosarcoma, 4 missense point mutations (substitutions of Leu<sup>283</sup> for Pro, Glu<sup>259</sup> for Gly, Asn<sup>285</sup> for Asp, and Lys<sup>285</sup> for Arg) were observed. Unaffected tissues obtained from each tumor-bearing dog were found to contain only the wild-type p53 transcript, as determined by use of PCR-SSCP analysis.

Southern hybridization analysis of the p53 gene in tumor tissues of dogs—To screen for possible structural changes in the p53 gene in naturally developing tumors in dogs, tumor DNA samples were digested with EcoRI or BamHI and subjected to long blotted hybridization analysis, using a 1,158-bp canine p53 probe that had been amplified by use of primers 13 and 14. The p53 gene was detected as a 23-kbp EcoRI DNA fragment or a 6.0-kbp BamHI fragment in lymph node DNA from a clinically normal dog. The EcoRI and BamHI fragments hybridized with the dog p53 probe were detected in all tumor samples examined in this study, indicating that there was not an apparent homozygous deletion of p53 gene in any of the tumor samples.

Discussion
Several studies on cloning of the canine p53 gene have been reported. In 4 of those studies, investigators described partial sequences of canine p53 genomic clones, and investigators in 4 other studies reported sequences of the partial and full-length cDNA clones. In the study reported here, we independently cloned and sequenced a full-length canine p53 cDNA. The nucleotide sequence reported here was completely identical to those of 3 other partial clones of canine p53 reported elsewhere. However, there were a few differences between the canine p53 sequence in our study and those in other reports. Codon 325 of canine p53 in our study was CTC (Leu<sup>259</sup>), which differed from the corresponding codon GCC (Arg<sup>259</sup>) reported in 2 other studies. We sequenced 5 clones of canine p53 in our study and found that codon 325 was CTC (Leu<sup>259</sup>) in 3 clones but GCC (Arg<sup>259</sup>) in 2 clones (data not shown), indicating DNA polymorphism in canine p53 at nucleotide 974. In another study, codon 272 was reported as TCT (Ser<sup>272</sup>) instead of ACT (Thr<sup>272</sup>), which was reported in our study and other studies. Comparison of the nucleotide sequence in our study with that of another full-length canine p53 cDNA revealed many exceptional differences, including 7 nucleotides in the open reading frame and 10 nucleotides in the 3' non-coding region. Because we sequenced 3 clones of canine p53 cDNA derived from splenic samples collected from 5 dogs, and we obtained the same results for all 5, except for the polymorphic sequence at nucleotide 974, the sequence determined in the study reported here seems to be representative of the sequence of canine p53 cDNA.

In our study, we found a total of 30 mutations of p53 cDNA in 7 naturally developing tumors in dogs, including osteosarcoma, lymphoma, monocytic leukemia, rhabdomyosarcoma, and colon cancer. The most notable event was that multiple mutations were detected in most of these tumors, especially in the dog with colon cancer, which had 7 aberrations of the p53 gene, including 4 point mutations, 1 deletion, and 1 insertion. Results obtained in this study differed vastly from those in other reports dealing with the p53 mutation in tumors of dogs, in which 1 missense point mutation had been generally found in each tumor with an aberration of the p53 gene. In the dogs...
with multiple p53 mutations, it can be considered that there may be p53 mutation in the early phase of tumorigenesis, resulting in genetic instability that may result in an accumulation of p53 gene mutations.

Reports on mutational analyses of the p53 gene in tumors of dogs revealed that point mutations, insertions, and deletions were detected in tumors of dogs, similar to results for tumors in humans. Mayr et al. reported a missense mutation (ACT to TCT, Thr→Ser) of the p53 gene was detected in a dog with a papilloma. They also reported that 1 of 10 dogs with mammary gland tumors had 1 missense point mutation at codon 249 (CGG to TGG, Arg→Leu). The second report revealed that 3 of 9 mammary tumor cell lines included missense point mutations (Cys→Phe, Gly→Ala, and Tyr→Asp, respectively). The third report revealed that 4 of 17 osteosarcomas had missense point mutations (Pro→Leu, Cys→Try, and Arg→His) and a 3-base insertion (GTG insertion at codon 218). Chu et al. reported that 6 of 40 mammary gland carcinomas included 6 different missense point mutations, although none of the benign tumors had a p53 mutation. Veldhoen et al. reported that 2 of 8 malignant lymphomas had missense point mutations (Arg→Stop, Val→Met, respectively) and 1 had a 2-base insertion at codon 247, resulting in amino acid substitution and generation of a stop codon.

The accumulated number of p53 mutations in tumors of dogs has reached 49 (19 mutations reported elsewhere and 30 mutations reported here). Of the 49 mutations in tumors of dogs, 37 were found in the central region of the canine p53 gene corresponding to exons 4 to 8 of the human p53 gene. Although the entire coding region of the p53 gene was examined for aberrations in our study, only the central region corresponding to exons 4 to 8 was analyzed previously. The frequency of p53 mutations in tumors of dogs in our study was higher than those in other reports, probably because we examined the entire open reading frame of p53 cDNA or because tumor types with frequent p53 mutations were examined in our study. Among the 49 mutations of the p53 gene found to this point in tumors of dogs, only 1 of them was found at the same site in different tumors. An insertion (Val between codon 205 and codon 206) detected in a dog with colon cancer in our study also was detected in a dog with osteosarcoma. In all previous reports on p53 mutations in tumors of dogs, a single mutation was found in each tumor or cell line. On the contrary, multiple aberrations were detected in 6 tumors in the study reported here. The reason for this difference is unknown, but genetic instability attributable to the functional inactivation of p53 or another mechanism may explain the reason for the multiple aberrations of the p53 gene in tumors of dogs.

Aberrations of the p53 gene have been detected in 50 to 60% of tumors in humans. Most p53 mutations reported in tumors of humans are clustered in 4 of the 5 evolutionary highly conserved domains of the p53 protein localized in exons 4 to 8. Moreover, mutational hot spots have been identified in the human p53 gene (Arg175, Gly245, Arg248, Arg249, Arg273, and Arg282). The nucleotide sequences of these codons often include methylated CGG or CGC (CpG) and the transition substitution is generated by deamination of the methylated cytosine. For these reasons, mutations of the p53 gene in tumors of humans commonly have been reported at CpG sites. Meanwhile, of the 49 p53 mutations that have been identified in tumors of dogs, only 7 were found at CpG sites. To understand the difference in the patterns of p53 gene mutation between humans and dogs, it will be necessary to examine the aberration of the p53 gene in a large number of tumors from dogs. Studies on aberrations of the p53 gene in tumors of humans has revealed mutations characteristic to some peculiar tumors. For example, it was reported in 1 study that many of the p53 mutations in smokers with lung cancer were at Arg157, Arg248, or Arg249. A mutation of the p53 gene at Arg248 was frequently detected in hepatocellular carcinomas of mice induced by administration of aflatoxin B1. Although naturally developing mammary gland tumors, lymphomas, and osteosarcomas in dogs have been examined for aberrations of the p53 gene, its characteristic mutation for each type of tumor in dogs has not been identified.

In humans with colon cancer, 75 to 80% of the tumors examined have lost both wild-type p53 alleles (1 through deletion and the other through point mutation). Loss of a normal allele manifests itself as a loss in heterozygosity of chromosome 17p, which includes the locus of the p53 gene. Tumor samples from a dog with colon cancer and a dog with osteosarcoma in the study reported here had 4 aberrant bands, as determined by use of PCR-SSCP analysis, indicating aberrations in the p53 gene on both alleles. Five other samples with p53 aberrations had 2 abnormal bands and lacked normal bands derived from the wild-type p53 transcript in the SSCP analysis. Analysis of these data indicated that the normal p53 transcript was lost in these tumors. Southern blot analysis did not reveal a homozygous deletion of the p53 gene in these tumors with an aberrant p53 transcript. However, direct evidence does not currently exist for the loss of a normal allele coding the wild-type p53. Additional research, using restriction fragment-length polymorphism or microsatellite markers at the locus of the p53 gene of the canine genome, is needed to clarify the allelic status of the p53 gene in tumors of dogs.

A complex molecular mechanism may contribute to tumorigenesis in dogs, but there have been only a small number of reports on aberrations of oncogenes or tumor suppressor genes other than the p53 gene in tumors of dogs. Investigators have indicated aberrations of ras family genes in tumors of dogs (K-ras mutation in a dog with non–small-cell lung cancer and c-N-ras mutation in a dog with lymphoma). Multiple-step tumorigenesis through mutation of p53 in conjunction with several abnormalities of ras, myc, and other oncogenes or tumor suppressor genes has been documented for several tumors, especially carcinomas in humans with colon
It is necessary to investigate aberrations of other tumor suppressor genes and oncogenes in parallel with the abnormality of the p53 gene in naturally developing tumors of dogs to enable us to understand the molecular mechanism of tumorigenesis.

The p53 gene is necessary for apoptosis initiated by DNA damage and important for control of the cell cycle. Mice lacking a functional p53 tumor suppressor gene, which were generated by gene targeting, and humans with inherited germ-line p53 mutations or LiFraumeni syndrome are at high risk of developing various tumors. A germline mutation of the p53 gene was detected in a dog with lymphoma. Loss of the wild-type p53 may result in accumulation of mutations in several genes that finally cause tumors through a multiple-step tumorigenic process, similar to the situation in humans with carcinomas of the colon. This is supported by frequent aberrations of the p53 gene in a number of cancers in humans and the tumors of dogs observed in our study.

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<th>Primers</th>
<th>Nucleotide positions</th>
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<tr>
<td>1</td>
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<td>5</td>
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Appendix

References


