Simultaneous Inactivation of the p16, p15 and p14 Genes Encoding Cyclin-Dependent Kinase Inhibitors in Canine T-Lymphoid Tumor Cells

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ABSTRACT The p16, p15 and p14 genes are widely known as tumor suppressor genes in human medicine. Although a large number of genetic and epigenetic aberrations in these genes have been reported in human malignancies, canine malignancies have not been well analyzed on the aberrations of these genes. In this study, the full-length complementary DNA (cDNA) of the canine p16 gene was cloned using the 5’ and 3’ rapid amplification of cDNA ends methods. Based on the sequence data, primers specific for p16, p15 and p14 were designed. Using these primers, the expression of p16, p15 and p14 mRNAs could be individually evaluated by reverse transcriptase polymerase chain reaction. Genomic aberrations were also examined using genomic polymerase chain reaction. Two of the 6 canine lymphoid tumor cell lines did not express detectable levels of p16, p15 and p14 mRNAs, and wide-ranging deletions in the p15-p14-p16 genomic locus were suspected. Wide-ranging deletions were also speculated in 2 of 14 dogs with T-cell lymphoid tumors. On the other hand, similar failure of amplification suggesting wide-ranging deletions were not observed in any of the 14 dogs with B-cell lymphoma. Deletion of the p15-p14-p16 genomic locus could be one of the molecular aberrations in canine lymphoid tumor cells.

KEYWORDS canine, lymphoid tumor, p16, tumor suppressor gene.


The p16, p15 and p14 genes are widely known as tumor suppressor genes in human medicine [8, 11]. They are clustered in a region on human chromosome 9 corresponding to mouse chromosome 4, and canine chromosome 11 [9, 40]. The P16 and P15 proteins belong to the inhibitor of cyclin-dependent kinase 4 (INK4) family, and are known as INK4a (cyclin-dependent kinase inhibitor 2A [CDKN2A]) and INK4b (CDKN2b), respectively. By binding directly to CDK4 and CDK6, P16 and P15 block the formation of cyclin D–CDK complexes, resulting in the phosphorylation of retinoblastoma (RB) proteins and G1-phase cell cycle arrest. The p14 gene, which is also known as alternative reading frame (ARF), regulates the cell cycle via an alternative pathway. By binding to the murine double minute 2 (MDM2) protein, P14 inhibits the degradation of P53 by MDM2. Because P53 induces G1-phase cell cycle arrest in conjunction with P21, the outcome of this pathway is similar to that of the P16 and P15 pathways. The human p16 and p14 genes are composed of 3 exons each, and are known to share common exons 2 and 3.

A large number of genetic and epigenetic aberrations of the p16, p15 and p14 genes have been reported in human malignancies. Of these 3 genes, inactivation of p16 has been most frequently found in hematological malignancies, such as lymphoma [2, 3, 5, 6, 10, 16, 17, 20, 24, 36, 44], leukemia [13, 18, 19, 22, 30, 38, 42] and multiple myeloma [25, 32] as well as other non-hematological malignancies of various types [25, 32]. Inactivation of p16 results from 4 types of alterations, namely, homozygous deletion, promoter hypermethylation, loss of heterozygosity and point mutations. Inactivation of p16, occurring mainly due to deletions and methylations [8], was observed in 44% of hematological tumor patients [39]. Furthermore, deletions of the 3 genes occur more frequently in patients with T-cell malignancies than in those with B-cell malignancies [8]. By contrast, patients with B-cell malignancies exhibit a higher incidence of inhibition of expression due to hypermethylation [8].

Partial or whole sequence data of the p16, p15 and p14 open reading frames have been reported. Four reports on the canine p16 gene have been published [7, 9, 21, 27] since the identification of the partial sequence of its exon 2 (GenBank accession no. FJ542309) [21]. In another study [1], the entire coding sequence of the canine p15 gene (GenBank accession no. NM_00114629) was reported. However, the genomic locus harboring p16, p15 and p14 has not been thoroughly examined in dogs. The predicted canine p16, p15 and p14 gene locus, deduced from the corresponding loci in other species, is shown in Fig. 1. Canine p16 and p14 genes are thought to share common exons 2 and 3, with a unique alternatively spliced first exon. Further, the previously reported partial sequence of canine p16 gene exon 2 exhibited high homology to the sequence of p15 gene exon 2. Therefore, the primers designed to amplify exon 2 of the canine p16 gene in previous studies [7, 9, 21, 27] would also amplify the p15 and p14 genes. To examine the expression...
Cell lines were maintained in an atmosphere of 5% CO2. penicillin (100 units/ml) and streptomycin (0.1 mg/ml) were cultivated in RPMI-1640 supplemented with 20% fetal bovine serum (Nichirei biosciences Inc., Tokyo, Japan), 10% fetal bovine serum (Biowest, Nuaillé, France), penicillin (100 units/ml) and streptomycin (0.1 mg/ml). All cell lines were maintained in an atmosphere of 5% CO2.

of p16, p15 and p14 independently, it would be necessary to determine the whole sequence of exon 1 of the canine p16 gene.

Using primers that would amplify not only p16 but also p15 and p14, 2 previous studies reported low or undetectable expression of these 3 genes in canine lymphoma [9, 27]. Partial loss of chromosome 11, on which these genes are located, has also been reported in canine lymphoid malignancies using fluorescent in situ hybridization analysis and comparative genome hybridization [9, 12, 40]. Based on these reports, the p16, p15 and p14 genes are speculated to be partially or completely deleted in canine lymphoid malignancies, similar to human lymphoid tumors.

The first purpose of this study was to determine the complete nucleotide sequence of the canine p16 cDNA, in order to design primers specific to p16, p15 and p14. The second purpose was to examine the aberration of the p16, p15 and p14 genes in the canine lymphoid tumor cell lines and primary tumor cells from dogs with various lymphoid tumors.

MATERIALS AND METHODS

Cells and patient samples: Six lymphoid tumor cell lines derived from dogs with naturally occurring lymphoid malignancies were examined. These included CLBL-1 (multicentric B-cell lymphoma) [34], GL-1 (B-cell acute lymphoblastic leukemia [ALL]) [29], UL-1 (renal T-cell lymphoma) [43], CL-1 (mediastinal T-cell lymphoma) [28], Nody-1 (alimentary lymphoma, and by peripheral blood mononuclear cell (PBMC) separation by gradient centrifugation [43] from the dogs with ALL and CLL. All primary tumor samples were obtained with owners’ informed consent. Full-length cloning of canine p16 complementary DNA: Total RNA was isolated using Illustra RNA Spin Mini (GE Healthcare, Little Chalfont, Buckinghamshire, U.K.), followed by DNase I (Invitrogen) treatment. Complimentary DNA (cDNA) was synthesized using PrimeScript® RT reagent Kit (Takara Bio, Otsu, Japan). All protocols were performed in accordance with the manufacturer’s instructions. To amplify a part of exon 1 of canine p16, we designed degenerative primers, p16 EXON-F94 and p16 EXON-R293 (Table 1), based on the sequence of p16 exon 1 from other species (human, chimpanzee, cattle, mouse and rat). PCR amplification was performed with AmpliTaq gold® 360 (Applied Biosystems, Grand Island, NY, U.S.A.) using normal LN cDNA as template. Cycle conditions included an initial denaturation step at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 3 sec, annealing at 68°C for 30 sec and extension at 72°C for 60 sec, with a final extension step at 72°C for 7 min. The PCR amplification products were electrophoresed on 2% agarose gel (BMBio, Tokyo, Japan), purified using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, U.S.A.) and inserted into the pGEM®–T Easy Vector System (Promega). After transformation, plasmid DNA was extracted using NucleoSpin® Plasmid QuickPure (Macherey-Nagel, Düren, Germany) and subjected to sequence analysis using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and 3130xl Genetic Analyzer (Applied Biosystems).

The 5’ Rapid amplification of cDNA ends (5’ RACE) method was used to determine the full-length sequence of exon 1 of the p16 gene using 5’-full RACE Core Set (TakaraBio). CL-1 cDNA template was generated using the p16 EXON2-R1481 primer phosphorylated at the 5’ end. After circularization using T4 ligase, the cDNA was amplified by nested-PCR using 4 primers: p16 EXON2-F1374 and p16EXON2-F1411, and p16 EXON 1-R169 and p16 EXON

Fig. 1. Predicted gene locus of the p16, p15 and p14 genes on canine chromosome 11 deduced from the gene locus on human chromosome 9. The identified and unidentified exons of p16, p15 and p14 are indicated by white and black squares, respectively. Arrow with asterisk (*) indicates a gap in canine genome database. Locations of the primers in exon 2 of p16 used in previous studies [21] are indicated by daggers (†).
The comparative cycle threshold (Ct) method was used to quantify p16 transcript levels. ΔCt was determined by subtracting the Ct value of RPL13A from the Ct value of the p16 gene. ΔΔCt was calculated by subtracting the ΔCt of the control sample from the ΔCt of the target sample. Normal LN was used as the control sample in this study. The levels of the p16 transcripts relative to that in the normal LN were calculated as $2^{-\Delta\Delta Ct}$. To confirm the amplification efficacy of the primers, a standard curve was generated using serial dilutions of the CL-1 cDNA as template. All samples were evaluated in triplicate.

**Expression analysis of the p15 and p14 genes in canine lymphoid tumor cell lines:** Primers specific for p15, p15 EXON1-F90 and p15 EXON1-R230, and p14, p14 EXON1-F330 and p14 EXON1-R1230, were designed based on the sequences of p15 and p14 registered in GenBank (accession nos. NM_00114629 and FM883643), respectively (Table 1). GAPDH was used as the endogenous control gene (primers GAPDH-F and GAPDH-R; Table 1). Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification was performed for the p15 and p14 genes using AmpliTaq gold® 360. The protocol consisted of an initial denaturation step at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 3 sec, annealing at 60°C for 35 sec and extension at 72°C for 60 sec, with a final extension step at 72°C for 5 min. The PCR product was cloned and sequenced as described above.

**Real-time PCR analysis of p16 expression in canine lymphoid tumor cell lines:** The primers p16 EXON1-F148 and p16 EXON1-R242 were designed to amplify 95 base pairs (bp) of p16 exon 1 based on the p16 sequence determined in this study (Table 1). The mRNA expression in each cell line was measured by real-time PCR using SYBR® Premix Ex Taq (TakaraBio). Ribosomal protein L13A (RPL13A) was used as the endogenous control (RPL13A F and RPL13A R; amplicon length 87 bp, Table 1). The cycle protocol was as follows: denaturation step at 95°C for 10 sec; 40 cycles of denaturation at 95°C for 5 sec and annealing/extension at 60°C for 30 sec; followed by the dissociation step at 95°C for 5 sec, 60°C for 30 sec and 95°C for 15 sec. The PCR amplicons were electrophoresed on 12.5% polyacrylamide gel (ATTO, Tokyo, Japan).

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence (5’–3’)</th>
<th>Genbank no., nucleotide number</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p16 EXON-F94</td>
<td>GGTCGGAGCCCGATTCA</td>
<td>AB675384, nt. 148–169</td>
<td>95</td>
</tr>
<tr>
<td>p16 EXON-R242</td>
<td>ACGGGGTCGGACACAGTT</td>
<td>AB675384, nt. 242–226</td>
<td></td>
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<tr>
<td>RPL13A-F</td>
<td>GCCGGGAAGGTGTAGTCTGT</td>
<td>AJ388525, nt. 87–105</td>
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<tr>
<td>RPL13A-R</td>
<td>GAGAAAGGCGGATGTAATTC</td>
<td>AJ388525, nt. 173–154</td>
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<tr>
<td>p15 EXON1-F90</td>
<td>GCCGGACCTCTCCTGGAAG</td>
<td>NM_00114629, nt. 90–106</td>
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<tr>
<td>p15 EXON1-R230</td>
<td>GCCGGACCTCTCCTGGAAG</td>
<td>NM_00114629, nt. 230–215</td>
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<tr>
<td>p14 EXON1-F330</td>
<td>GCCCTGTTGCTAAGCTGATG</td>
<td>FM883643, nt. 330–349</td>
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<tr>
<td>p14 EXON1-R1230</td>
<td>TGAAGGGTTGCGGACACAGTT</td>
<td>FM883643, nt. 1230–1212</td>
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<tr>
<td>GAPDH-F</td>
<td>TCACCCAGGGCTGTTTTAAAAC</td>
<td>AB038240, nt. 47–67</td>
<td></td>
</tr>
<tr>
<td>GAPDH-R</td>
<td>AGGAAGCATTGCTGCAACTC</td>
<td>AB038240, nt. 448–429</td>
<td></td>
</tr>
</tbody>
</table>

GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; NA, not applicable (primers used for 5’ and 3’ RACE); P, Phosphate group; R represents A or G, S represents C or G.

Table 1. Primer sequences used for full-length complementary DNA cloning and expression analysis of the p16 gene in this study
gold® 360 and the cDNAs of the cell lines as templates in the following protocol: initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 3 sec, annealing at 60°C for 30 sec and extension at 72°C for 60 sec, with a final extension step at 72°C for 7 min. The PCR amplification products were electrophoresed on 12.5% polyacrylamide gel.

Amplification of the p15-p14-p16 gene locus on chromosome 11 by genomic PCR: Genomic DNA was extracted using QIAmp DNA Mini kit (Qiagen, Hilden, Germany). Eight pairs of primers (Table 2) covering a 100-kb region of canine chromosome 11, including the introns and exons of the p16, p15 and p14 genes, were designed (Fig. 2). The primer sequences are listed in Table 2. The GAPDH gene was used as the endogenous control. PCR amplification was performed using AmpliTaq gold® 360 and the DNAs of cell lines and patient samples as templates. The PCR protocol was as follows: initial denaturation at 95°C for 10 min, followed by 35 cycles with denaturation at 95°C for 3 sec, annealing at 60°C for 30 sec and extension at 72°C for 60 sec, with a final extension step at 72°C for 7 min. Electrophoresis was performed on 2% agarose gel. Amplification using distilled water was regarded as negative control.

RESULTS

Full-length cloning of canine p16 cDNA: Using 5’ RACE and 3’ RACE methods, the nucleotide sequence of full-length canine p16 cDNA was determined. A 705-bp entire open reading frame encoding 151 amino acids could be iden-
tified (GenBank accession no. AB675384, nucleotide [nt] 40–492). In the dog genome database, nt no. 166–472 and nt no. 473–705 of canine p16 cDNA were found on canine chromosome 11 separately using BLAST (National Center for Biotechnology Information, Bethesda, MD, U.S.A.) (nt no.166–472, 32195005–32195311; nt no. 473–705, 32192558–32192790). However, no sequence corresponding to the 5' fragment of canine p16 (nt no. 1–165) was found in the dog genome database. Because there is a gap region between exon 1β of the p14 gene and exon 2 of the p16/p14 genes where its exon 1α is expected to be located (Figs. 1 and 2) in the dog genome database, the 5' fragment of canine p16 (nt no. 1–165) was found in this region. Nt no. 1–165 of canine p16 was considered to be its exon 1α, and nt no. 166–472 and nt no. 473–705 were regarded as its exons 2 and 3 from the result of the alignment of the sequence (nt no.166–705) of canine p16 cDNA using BLAST. Therefore, canine p16 gene was assumed to be composed of 3 exons.

The determined full-length cDNA sequence and the deduced amino acid sequence of canine p16 were compared with those of other species. The cDNA sequence of the coding region shared 85, 86, 86, 79 and 76% identities with human, chimpanzee, cattle, mouse and rat sequences, respectively.

Expression analysis of p16, p15 and p14 genes using real-time RT-PCR or RT-PCR: The levels of p16 mRNA were determined using real-time RT-PCR. The levels of p16 mRNA in CLbL-1 and GL-1 cells were lower than those in normal LN cells, and below the limit of detection in UL-1, Nody-1 and Ema cells. The amount of p16 mRNA in CL-1 was approximately 100 fold higher than that in normal LN cells (Fig. 4). Further, the levels of p15 and p14 expression were examined using RT-PCR. The levels of p15 and p14 mRNAs were uniformly high in 4 cell lines (CLbL-1, GL-1, UL-1 and CL-1) compared to those in normal LN cells.
On the other hand, expression of p15 and p14 mRNAs was below the limit of detection in Nody-1 and Ema cells (Fig. 5). Amplification was not observed in these 2 cell lines even when the number of PCR cycles was increased from 35 to 40 (data not shown).

**Amplification of the p15-p14-p16 gene locus by genomic PCR:** To examine deletion in the genomic locus of p16, p15 and p14 genes, we designed 8 primer pairs to amplify each region of the locus (Fig. 2). All the 8 primer pairs designed in the locus amplified the fragments of expected sizes in 4 cell lines (CLbL-1, GL-1, UL-1 and CL-1) and LN cells, whereas no amplification was observed with any of the 8 primer pairs in Nody-1 and Ema cells (Fig. 6).

Primary lymphoid tumor samples were also examined by genomic PCR. Due to limited DNA samples from lymphoid tumor patients, 5 primer pairs covering the exon regions of p16, p15 and p14 were used to examine each gene locus. Faint bands were presumed to arise from non-tumor cells derived from the surrounding normal tissues.

The 14 dogs diagnosed with multicentric high-grade lymphoma and harboring clonal IgH gene rearrangements all exhibited amplifications products of the expected sizes in the regions p15 EXON1, p16 dsEXON2 and p16 usEXON3 (Fig. 7). Amplified bands corresponding to p15 EXON2 and p16 dsEXON1 were faint to absent in 2 (dog nos. 15 and 23), 3 (dog nos. 15, 18, and 23), 5 (dog nos. 15, 18, 19, 20, and 23), 2 (dog nos. 15, 18, and 23) and 2 (dog nos. 15 and 23) dogs, respectively (Fig. 7). In 2 dogs (dog nos. 15 and 23), no or faint bands were detected with any of the 5 primer pairs. The results of genomic PCR in primary lymphoid tumor samples are summarized in Table 3.

**DISCUSSION**

In this study, we identified the full-length nucleotide sequence of the canine p16 gene. As expected, canine p16 was composed of 3 exons, sharing exons 2 and 3 with the p14 gene. The deduced canine p16 amino acid sequence was highly homologous to that of human, chimpanzee and cattle throughout the coding region. On the other hand, the exon 3 sequence exhibited limited identity with that of mouse and rat, although exons 1 and 2 were relatively conserved. Exons
1 and 2 contained 4 ankyrin repeats, which are known to be highly conserved among various species [33]. The ankyrin repeat motif may be important for maintaining the functional conformation of P16.

The cloning of p16 exon 1 enabled us to design primers that distinguished p16 from p15 and p14. Although exon 2 of p15 and p16/p14 was highly homologous, exon 1 of each gene shared only limited identity, and therefore it was possible to design primers specific to each gene. However, the primers specific for p15 and p14 generated several non-specific products, and could not be used for quantification. It was difficult to design another primer for p15, because of the high homology of its exon 2 with that of the p16/p14 genes (98%). Further, polymorphism in exon 1 of the p15 gene was reported in normal dogs [1]. Indeed, the reported polymorphism in p15 exon 1 was also observed in CLBL-1, GL-1, UL-1, CL-1 and LN tissues by sequence analysis (data not shown). Therefore, we did not quantify p15 expression by real-time RT-PCR.

In this study, a part of the p16 nucleotide sequence (GenBank accession no. AB675384, nt 1–91) was obtained from CL-1 cells, but not confirmed in normal cells. Because p16 expression was low in normal cells [38, 45], it was difficult to obtain a clear band from normal tissue by RT-PCR and 5' RACE. Instead, CL-1 cDNA was used as a template for PCR, because this cell line exhibited remarkably high expression of the p16 gene. Further, it was not possible to verify the sequence from the genome database, because p16 exon 1α is located in the gap region. The remaining part of the p16 gene (nt 92–705) was identical between normal LN and CL-1 cells. Although the sequence of 5' end of canine p16 cDNA should be verified in normal LN cells, the data gathered in this study conceivably reflected the sequence of canine normal p16 cDNA.

The amount of p16 mRNA expression was smaller in 5 (B-cell lines; CLBL-1 and GL-1, and 3 T-cell lines; UL-1, Nody-1 and Ema) of the 6 canine lymphoid tumor cell lines, compared to that in normal LN cells. p15 and p14...
mRNAs were also undetectable in 2 lymphoid tumor cell lines (Nody-1 and Ema). On the other hand, p16 mRNA was highly expressed in CL-1 (T-cell line). It has been reported that RB protein becomes inactivated when it is excessively phosphorylated, which elicits high expression of p16 mRNA due to a feedback mechanism [14, 26, 31]. Examination of the phosphorylation status of RB in CL-1 cells will be needed. Furthermore, when some of the cell cycle regulators such as p21, p27, p57 and p53 are inactivated and the progression of cell cycle occurs, over expression of p16 mRNA could be induced as a feedback mechanism. Since mediastinal lymphoma is very rare in dogs, we did not have an opportunity to examine the p16 mRNA expression level in primary tumor samples from dogs with mediastinal lymphoma. In previous studies on the prognosis of canine lymphoma [37], the presence of mediastinal mass was shown as a poor prognostic factor. A further study is needed to examine the relationship between the augmented p16 expression and the disease type or the biologic behavior of lymphoid tumors in dogs.

In Nody-1 and Ema, p16, p15 and p14 mRNAs were not detected. Furthermore, genomic PCR amplification for the p15-p14-p16 locus of the DNA samples with the 8 primer pairs did not generate any product in locus in Nody-1 and Ema. These results suggest the presence of wide-ranging deletions in chromosome 11 in Nody-1 and Ema. To confirm the exact status of the genetic deletions in these 2 cell lines, Southern blot analysis would be necessary. In the cells with such wide-ranging deletion, there should be a simultaneous loss of p16, p15 and p14 resulting in the failure of cell cycle control. Inactivation of these genes, especially in their combinations, was implicated in the tumorigenesis and malignancy from the studies of the in vitro and in vivo mouse model systems with targeted disruption of the corresponding genes [23]. Induction of wild-type p16 into p16-deficient human lymphoma cell lines leads to growth retardation and partial differentiation, indicating that p16 deficiency might contribute to the malignant phenotype [23]. Mice with targeted disruption of both p16/p19 loci developed lymphomas and lymphoid leukemias with a low penetrance, as well as other tumors [35]. From these findings, the simultaneous inactivation of p16, p15 and p14 genes would be also associated with the tumorigenesis and malignancy in canine T-lymphoid tumor cells.

The amounts of p16 mRNA in CLBL-1, GL-1 and UL-1 were shown to be lower than that in normal LN, whereas those of p15 and p14 mRNAs in these cell lines were similar to that in normal LN. If only the amount of p16 mRNA is decreased despite the presence of p15 and p14 mRNAs as shown in normal LN, there is a possibility that deletion around p16 gene exon 1 exists without wide-ranging deletion over p15-p14-p16 locus. However, PCR amplification for the p16 gene exon 1 generated a distinct band in these 3 cell lines using genomic PCR. It is possible that genetic aberrations exist in p16 gene although not detected in this study, or epigenetic mechanism to suppress its expression might be related in these 3 cell lines.

We further examined whether genetic aberrations similar to that in Nody-1 and Ema exist in naturally occurring canine lymphoid tumor cases. Similar failure of amplification of the p15-p14-p16 locus (p15EXON1, p15EXON2, p16dsEXON1, p16dsEXON2 and p16usEXON3) was observed in 2 dogs (dog nos. 15 and 23) with T-cell alimentary lymphoma, although several faint bands conceivably derived from concomitant normal cells were observed in dog no. 23. Such failure of amplification was not observed in any of the 14 multicentric B-cell lymphoma dogs.

Thomas et al. [40] indicated the frequent DNA copy number losses in p15-p14-p16 locus of chromosome 11 in canine T-cell lymphomas but not in B-cell malignancies, using array-based comparative genomic hybridization. Although further studies are needed, deletion of p15-p14-p16 locus would be an important candidate to understand the tumorigenesis in a subtype (possibly high-grade T-cell lymphoma) of canine lymphoid tumors.

In conclusion, the full-length canine p16 cDNA was cloned, and the levels of p16, p15 and p14 expression in canine lymphoid tumor cell lines were determined. Failure of amplification suggesting wide-ranging deletions were observed in 2 canine T-cell lymphoid tumor cell lines lacking p16, p15 and p14 expression. Similar failure of amplification was also found in 2 primary tumor samples from alimentary T-cell lymphoma. Deletion of the p15-p14-p16 genomic locus could be one of the molecular aberrations in canine lymphoid tumor cells.

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