The Bcl-2 family proteins have been identified as a key regulator of apoptosis in many cellular systems. This family is commonly divided into anti-apoptotic and pro-apoptotic members, which share close structural homologies [1, 9].

Bcl-xL, a member of Bcl-2-related anti-apoptosis protein family, acts to regulate mitochondrial membrane potential and blocks the release of cytochrome-C and apoptosis inducing-factor into the cytoplasm [5, 17, 26]. Under various circumstances, the activity of Bcl-xL protein may be regulated through caspase cleavage [2], phosphorylation [4], and intracellular translocation [10]. A recent gene-targeting study has shown that the Bcl-xL gene is essential to the survival of hematopoietic cells and postmitotic neurons in developing mouse embryos [20].

Chemotherapy and radiotherapy induce cancer cell death, in part, by activating apoptotic pathways [11, 12, 27, 28]. The resistance of cancer cells to chemotherapy and radiotherapy might be resulted from alterations in apoptosis promoting mechanisms [24]. In human malignancies, recent observations suggested that Bcl-2 family members, especially Bcl-xL, are involved in the mechanism of resistance to chemotherapeutic agents and radiation [18, 25]. Increased Bcl-xL expression has been observed in some human malignancies, including gastric carcinoma [13], colorectal carcinoma [14], ovarian carcinoma [15], prostate cancer [16], pancreatic cancer [7, 8] and others [3, 6]. As with human malignancies, Bcl-xL would be related to the increase of resistance to the treatments in canine malignancies.

In the present study, to investigate the role of Bcl-xL in canine malignancies, we cloned and sequenced the canine Bcl-xL cDNA and examined the expression of the corresponding mRNA in canine tumor cell lines by reverse transcription-polymerase chain reaction (RT-PCR).

Venous blood was collected from a healthy beagle dog and overlaid on Ficoll-Hypaque (Lymphoprep; specific gravity 1.077, NYCOMED PHARMA AS, Oslo, Norway). These were centrifuged at room temperature (350 × g) for 30 min, and the peripheral blood mononuclear cell (PBMC)-fraction were collected separately.

Total RNAs was extracted from the mononuclear cell-fraction using RNasy total RNA kit (QIAGEN, CA). Reverse transcription of the poly(A)+ RNA was performed with a Omniscript™ Reverse Transcriptase kit (QIAGEN). Oligonucleotide primers to amplify a central region of canine Bcl-xL cDNA were designed based on the human, mouse and rat Bcl-xL sequences (GenBank/EMBL/DDBJ accession Nos.: human Bcl-xL, Z23115; mouse Bcl-xL, U51278; rat Bcl-xL, U72350): forward primer, 5'-AATT-GTCCTCAGACCAACCGGG-3' (nucleotide (nt) 134–153 in human, 102–121 in mouse and 71–90 in rat Bcl-xL cDNA) and reverse primer, 5'-GCTTAGGTGTCAGATCAG-3' (nt 672–653 in human and 609–590 in rat Bcl-xL cDNA). Using these primer pairs, canine Bcl-xL cDNA was amplified from the cDNA of mononuclear cells. The PCR amplifications consisted of pre-denaturation (5 min, 95°C) and 35 cycles of denaturation (1 min, at 94°C), primer annealing (2 min, at 55°C) and polymerization (2 min, at 72°C).

The PCR amplified a single DNA fragment of about 600 bp, and the product was cloned into the pCRII vector (Invitrogen, CA). The plasmid DNAs from each sample were extracted with the Quantum prep kit (BIO RAD, CA) and sequenced by mentioned above.

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**Canine Bcl-xL Gene and Its Expression in Tumor Cell Lines**

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**ABSTRACT.** The canine Bcl-xL gene was cloned and sequenced. Canine Bcl-xL cDNA clone was 1252 bp in length, and encoded 233 deduced amino acids. The predicted canine Bcl-xL amino acid sequence shared 99.6%, 97.0%, 97.9%, 98.7% and 98.3% homology with that of human, mouse, rat, sheep and pig Bcl-xL, respectively. RT-PCR analysis revealed that canine Bcl-xL mRNA was constitutively expressed in CL-1 (canine lymphoma) and GL-1 (canine B cell leukemia) cell lines.

**KEY WORDS:** Bcl-xL, canine, cloning, RT-PCR.
The deduced amino acid sequences encoded by the canine Bcl-xL clone were aligned with amino acid sequences deduced from the human, mouse, rat, sheep (GenBank/EMBL/DDBJ accession no.- AF164517) and pig Bcl-xL (accession no.- AF216205), and found to share 99.6%, 97.0%, 97.9%, 98.7% and 98.3% homology, respectively with these sequences. This result indicated that Bcl-xL genes would be well conserved among mammalian species. Sequence alignment analysis indicated that the domain organization was identical to those of known Bcl-xL proteins, with four BH domains and a putative transmembrane region (Fig. 1) [1, 22, 23].

To investigate Bcl-xL mRNA expression in canine tumor cell line, we performed semi-quantitative RT-PCR. As an internal control, glycerardehyde-3-phosphate dehydrogenase (GAPDH) mRNA was amplified in each sample. We used the following primers: Bcl-xL sense, 5'-GGCCTTTTTCTCTTCTGGTGT-3' (nt. 828-847), and antisence: 5'-CTCTGGGTTGCTGCACTGGTTT-3' (nt. 994-1013); and GAPDH sense, 5'-GGAGAAAGCTGCCAAATGACA-3' and antisense, 5'-ACAAAGGAATGAGCTGACA-3'.

Total RNA was extracted from CL-1 (canine lymphoma) [19], GL-1 (canine B cell leukemia) [21] cell lines and PBMCs of three healthy dogs. For the RT reaction, total RNA (2 µg) was primed with oligo (dT) and reverse-transcribed into cDNA using Omniscript™ Reverse Transcriptase kit (QIAGEN). The conditions for PCR amplification of cDNA were as follows: one cycle at 95°C for 5 min as an initial denaturation step; then, denaturation at 94°C for 1 min, annealing step at 64°C for 1 min, and extension at 72°C for 2 min. The number of cycles was chosen in the middle of the exponential phase of the reaction for each cell line. In some cases, we performed PCR reactions using a higher number of cycles in order to assess the expression of a specific gene as indicated in the respective figure legend. As shown in Fig. 2, Bcl-xL mRNA was constitutively expressed in CL-1 and GL-1 cells. The level of Bcl-xL mRNA was expressed high in CL-1 and GL-1 cells and it was increased compared with non-stimulated PBMCs. In conclusion, the cDNA encoding the full-length canine Bcl-xL was cloned and sequenced. Bcl-xL mRNA was constitutively expressed in canine lymphoma and leukemia cell.
lines, which might be related to the survival and/or the resistance to apoptosis.

Since Bcl-xL antisense oligonucleotides is now used in therapy of human cancer, clinical studies using antisense Bcl-xL alone or in combination with chemotherapy in canine cancer should be performed in the immediate future.

REFERENCES