NOTE *Internal Medicine*

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

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(Received 16 April 2002/Accepted 9 October 2002)

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 acides. 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.

J. Vet. Med. Sci. 65(1): 149–151, 2003

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 ($350 \times g$) for 30 min, and the peripheral blood mononuclear cell (PBMC)-fraction were collected separately.

Total RNAs was extracted from the mononuclear cell-fraction using RNeasy total RNA kit (QIAGEN, CA). Reverse transcription of the poly(A)$^+$ RNA was performed with a Omniscript^{TM} 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'-AATGTCTCAGAGCAACCGGG-3' (nucleotide (nt) 134–153 in human, 102–121 in mouse and 71–90 in rat *Bcl-xL* cDNA) and reverse primer, 5'-GCTCTAGGTGGTCATTCAGG-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-denature (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 dideoxy chain termination method using an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer, CA). A series of 5'-and 3'-RACE-PCR experiments was carried out to determine the full length of cDNA sequences for the canine *Bcl-xL* by using 5'RACE System for Rapid Amplification of cDNA Ends (GIBCOBRL, MD) and 3'RACE system for Rapid Amplification of cDNA Ends user manual (GIBCOBRL, MD). The sequences of gene-specific primers were designed from the sequences of progressively amplified products beginning with the sequence of the canine *Bcl-xL* fragment. The PCR products of 5' and 3' sides of *Bcl-xL* were sequenced by mentioned above.

The full length cDNA clone of *Bcl-xL* (1252 bp) contained a single open reading frame of 699 bp coding a protein of 233 amino acids (GenBank/EMBL/DDBJ accession no,- AB073983). A typical polyadenylation signal (AATAAA) was found in the 3' untranslated region.

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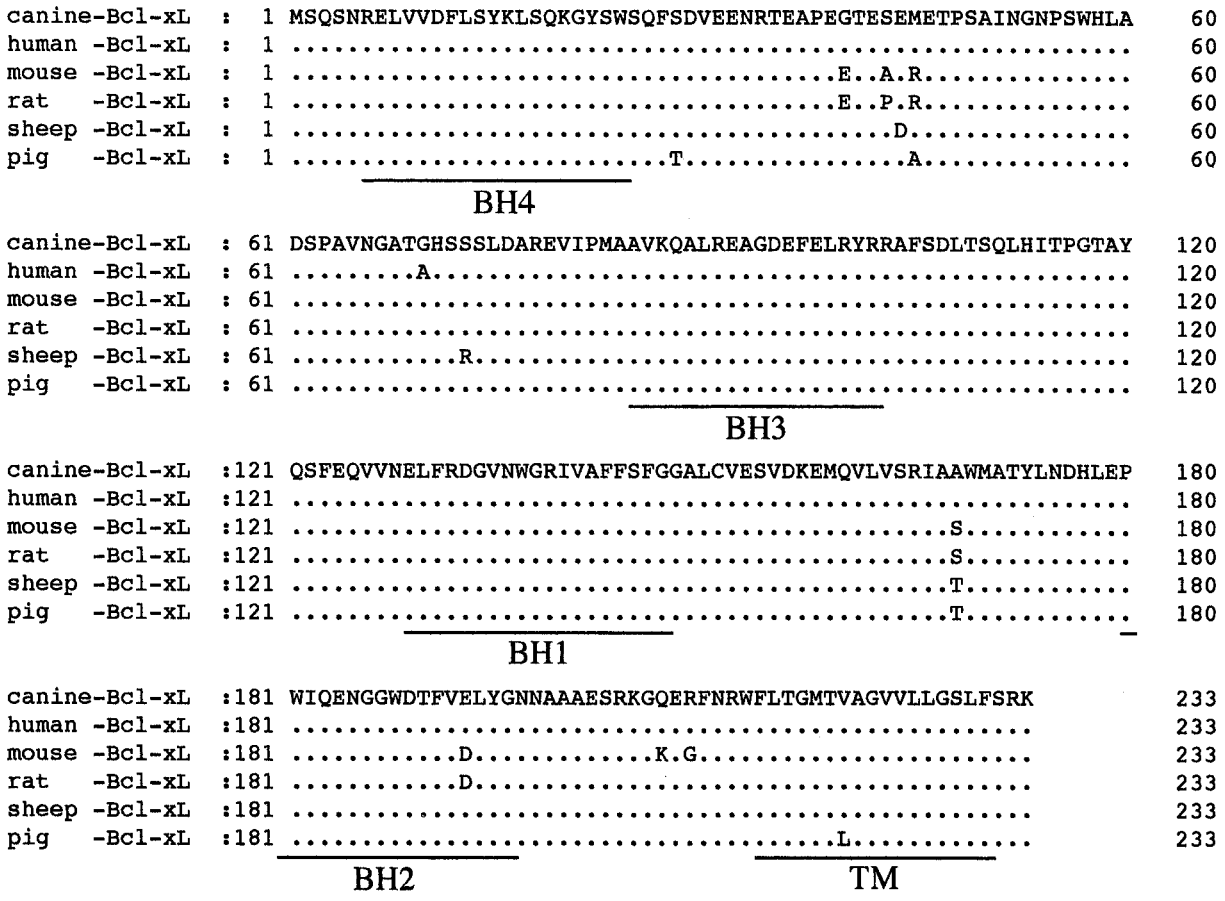

Fig. 1. Alignment of the amino acid sequences of the Bcl-xL proteins. The Bcl-2 homology domain (BH) and transmembrane domain (TM) are underlined and indicated below in bold letters. Areas of amino acid identity between the dog, human, mouse, rat, sheep and pig Bcl-xL are indicated by stippling.

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'-GGCCTTTTTCTCCTTCGGTG-3' (nt. 828-847), and antisence: 5'-CTCTCGGCTGCTGCATTGTT-3' (nt. 994-1013); and *GAPDH* sense, 5'-GGAGAAAGCTGCCAAATATG-3' and antisense, 5'-ACAAGGAAATGAGCTTGACA-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^{TM} 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 is 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

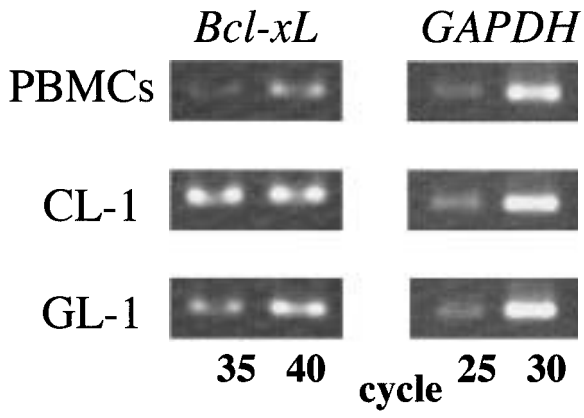

Fig. 2. Expression of *Bcl-xL* gene in PBMCs, CL-1 and GL-1 cells. PBMCs, CL-1 and GL-1 cells were assessed by RT-PCR analysis. PCR amplification of glyceraldehyde -3- phosphate dehydrogenase (GAPDH) was used as an internal control.

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.

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