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Technical report

Characterization of canine BCL6 cDNA and detection systems for its protein expression

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ABSTRACT

BCL6 is known to be a key molecule in germinal center (GC) formation of lymph nodes, and its expression profiles have been implicated in the prognosis of diffuse large B-cell lymphoma in humans. The present study was carried out to characterize canine BCL6 cDNA and to indicate the technical methods for detection of the BCL6 protein in dog tissues. The deduced amino acid sequence of canine BCL6 showed close homology to that of human BCL6 (96.3%), especially in the zinc-finger motifs and POZ (poxvirus and zinc finger) domain with complete identity. Immunoblot analysis of a canine lymph node with an anti-human BCL6 monoclonal antibody revealed a band of 80 kDa. Immunohistochemical staining using the same antibody produced positive reactions in the cells exclusively localized in the GC of a canine lymph node. This study will be useful for the molecular classification of canine B-cell lymphomas with different prognoses.

The human BCL6 gene encodes a 95-kD nuclear sequence-specific transcriptional repressor (Deweindt et al., 1995; Chang et al., 1996; Seyfert et al., 1996). It is mainly expressed in the nucleus of B-cells in germinal centers (GCs) (Cattoretti et al., 1995). BCL6 has received much attention with regard to the classification and prognosis of human non-Hodgkin's lymphoma (NHL). The most common type of human NHL are diffuse large B-cell lymphomas (DLBCL), accounting for 30–40% of adult NHL (Jaffe, 1998). A comprehensive gene expression profiling study identified 2 molecularly distinct forms of DLBCL: GC-like DLBCL characterized by the expression of genes normally expressed in GC B-cells and activated B-cell (ABC)-like DLBCL characterized by the expression of genes normally induced during in vitro activation of B-cells. The overall survival time in patients with GC-like DLBCL was reported to be significantly longer than that in patients with ABC-like DLBCL (Alizadeh et al., 2000). The BCL6 gene is considered to be one of the hallmarks of GC-like DLBCL, and its expression is known to be strongly indicative of the survival in patients with DLBCL (Lossos et al., 2001).

Lymphoma is one of the most common malignant tumors in dogs, accounting for 83% of all hematopoietic tumors and 7–24% of all neoplasms (Jacobs et al., 2002; Kaiser, 1981). Since the representative histologic type of canine lymphoma is DLBCL (Vezzali et al., 2010), it has been utilized as an attractive model for human DLBCL (Jamadar-Shroff et al., 2009). Although human and canine DLBCL share many similarities, molecular approaches to predict the prognosis of canine lymphoma are still lacking. It can be speculated that measurement of BCL6 gene expression would be useful for predicting the prognosis of canine patients with DLBCL as indicated in humans.

To our knowledge, cloning of canine BCL6 cDNA and its protein detection system have not been reported. As the first step to examine BCL6 gene expression in dogs with B-cell lymphoma, we molecularly cloned and characterized

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canine BCL6 cDNA and assessed the technical methods to detect the BCL6 protein in dog tissues.

A popliteal lymph node was surgically resected from a healthy adult beagle dog. This procedure was conducted in accordance with the guidelines of the Animal Care Committee of the Graduate School of Agricultural and Life Sciences, the University of Tokyo.

Total RNA was extracted from the normal lymph node with a commercial available kit (RNAqueous, Ambion, TX, USA) and treated with a DNA-free kit (DNase I, Invitrogen, CA, USA) to prevent contamination by genomic DNA. Reverse transcription of RNA was performed with a cDNA synthesis kit (PrimeScript RT-PCR Kit, Takara, Shiga, Japan). Template cDNA was amplified by PCR with DNA polymerase (Phusion High-Fidelity DNA Polymerase, Finnzymes, Espoo, Finland) using the following primer pairs: 5′-TGA ATC AAG GCA TTG GGT GA-3′ (forward) and 5′-TTT GGG TAG ATT CTG AGA AGG GA-3′ (reverse). These primers were designed on the basis of the predicted sequence of canine BCL6 (GenBank accession number; XM_545248). The PCR product was analyzed by 1% agarose gel electrophoresis and purified from the agarose gel with a commercially available kit (Wizard SV Gel and PCR Clean-Up System, Promega Corporation, WI, USA). Following A-tailing, the purified PCR product was cloned into a T/A cloning vector (pGEM-T Easy) (Promega Corporation). Plasmid DNA was extracted with a plasmid purification kit (NucleoSpin Plasmid QuickPure, MACHEREY-NAGEL, Duren, Germany) and sequenced by use of the dideoxy chain termination method with 4 primers; the 5′- and 3′-end were identified with 2 primers complimentary to T7 and SP6 promoter sites of the vector and the inner site was identified with 2 primers complimentary to the predicted sequences of canine BCL6 (nucleotides 500-519: 5′-CAA GAC ATC ATG GCC TAT CG-3′, nucleotides 1026-1044: 5′-GTC TGG TTA GTC CAC AGA G-3′, GenBank accession number; XM_545248), which were designed to overlap the each fragment. Eight independent clones of the PCR product were sequenced to avoid errors in sequence analysis.

A protein sample was extracted from the normal canine lymph node using lysis buffer (50 mM Tris pH 7.5, 100 mM NaCl, 1.0% NP-40, 10% Glycerol, 1 mM EDTA), analyzed by SDS-PAGE using 12.5% gels, and blotted onto a PVDF membrane (Amersham Hybond-P PVDF membrane, GE Healthcare, NJ, USA). The membrane was blocked with a commercially available blocking buffer (ECL Advance blocking agent, GE Healthcare). Immunoblotting was carried out with a rabbit monoclonal anti-human BCL6 antibody (clone: EP529Y; Abcam, MA, USA) diluted at 1:3000 in Tris-HCL buffer saline with 0.1% Tween 20 (TBS-T) by incubation for 1 h at room temperature. After washing in TBS-T, the membrane was incubated with horseradish peroxidase-labeled goat anti-rabbit IgG (Abcam) diluted at 1:12000 in TBS-T for 1 h at room temperature. Immunodetection was performed by chemiluminescence (ECL Advance, GE Healthcare) according to the manufacturer's protocol.

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Canine BCL6 MASPADSCIQ FTRHASDVLL NLNRLRSRDI LTDVVIVVSR EQFRAHKTVL 50
Human BCL6  .......... .......... .......... .......... .......... 50
Canine BCL6 MACSGLFYSI FTDQLKCNLS VINLDPEINP EGFCILLDFM YTSRLNLREG 100
Human BCL6  .......... .......... .......... .......... .......... 100
Canine BCL6 NIMAVMATAM YLQMEHVVDT CRKFIKASEA EMVPAIKPPR EEFLNSRMLM 150
Human BCL6  .......... .......... .......... ...S...... .......... 150
Canine BCL6 PQDIMAYRGR EVVENNLPLR NAPGCESRAF APSLYSGLST PPASYPVYGH 200
Human BCL6  .......... .......... S......... .......... .....SM.S. 200
Canine BCL6 LPVSSFLFSD EELRDARMPV ANPFPKERAL PCDSARPIPG DYSRPAMEIS 250
Human BCL6  .....L.... ..F..V.... .......... .......V.. E....TL.V. 250
Canine BCL6 PNVCHSSIYS PKEAAPEETH SDMHYGVAEG PKPAVPSARN APYFPCDKAS 300
Human BCL6  ......N... ...TI...AR .....S.... L...A..... .......... 300
Canine BCL6 KEEERPSSED EIALHFEPPS APLNRKGLVS PQSPQKSDCQ PNSPTESCSS 350
Human BCL6  .......... .........N .......... .......... .......... 350
Canine BCL6 KNACILQTSG SPPAKSPTDP KACNWKKYKF IVLNSLNQNA KPEGPEQAEL 400
Human BCL6  .......A.. .......... .......... .......... .......... 400
Canine BCL6 GRLSPRAYTA PPACQPPMEP ESLDLQSPTK LSASGEDSTI PQASRLNNIV 450
Human BCL6  .......... .......... .N........ .......... .......... 450
Canine BCL6 NRSLTGSPRS SSESHSPLYL HPPKCTSCGS QSPQHAEMCL HTAGPTFPEE 500
Human BCL6  ...M...... .........M .......... .......... .......... 500
Canine BCL6 MGETQSEYSD SSCENGAFFC NECDCRFSEE ASLKRHTLQT HSDKPYKCDR 550
Human BCL6  .......... .......... .......... .......... .......... 550
Canine BCL6 CQASFRYKGN LASHKTVHTG EKPYRCNICG AQFNRPANLK THTRIHSGEK 600
Human BCL6  .......... .......... .......... .......... .......... 600
Canine BCL6 PYKCETCGAR FVQVAHLRAH VLIHTGEKPY PCEICGTRFR HLQTLKSHLR 650
Human BCL6  .......... .......... .......... .......... .......... 650
Canine BCL6 IHTGEKPYHC EKCNLHFRHK SQLRLHLRQK HGAITNTKVQ YRVSATDLPP 700
Human BCL6  .......... .......... .......... .......... .......... 700
Canine BCL6 ELPKAC 706
Human BCL6  ...... 706
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Fig. 1. Comparison of the deduced amino acid sequence of canine BCL6 cDNA (GenBank accession number; HM008708) with that of its human counterpart (GenBank accession number; NP_001124317.1). The amino-terminal POZ domain is boxed with a solid line. Six zinc-finger motifs are boxed with dashed lines. Dots indicate identical residues.