

## Amplification of a *c-yes-1*-Related Oncogene in Canine Lymphoid Leukemia

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**ABSTRACT.** Genomic DNAs from 4 canine spontaneous lymphoid neoplasms were examined by Southern blot hybridization using 7 kinds of oncogene probes. Analysis using a human *c-yes-1* cDNA probe revealed amplification of the proto-oncogene in a DNA sample from a dog with lymphoid leukemia. Its degree was about 4- to 8- fold in comparison with a control DNA sample from a healthy dog. The genomic abnormality of canine *c-yes-1*-related oncogene may have a role in the tumorigenesis of this neoplasm, although the significance of the structural change in this oncogene remains unclear.—**KEY WORDS:** *c-yes-1*, canine, oncogene.

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In order to act in neoplasia, oncogenes must be activated by gene amplification, overexpression, point mutation, rearrangement, deletion, promoter insertion, or translocation [1, 15]. In humans, amplification of various oncogenes has been reported in several tumors or cell lines derived from neoplasms [13]. Although these oncogenes are known to be well conserved in mammals or even in yeast cells [7, 9], there are few reports of oncogene abnormalities in tumors of domestic animals [9]. The present paper describes amplification of oncogene related to human *c-yes-1* in canine lymphoid leukemia.

Tissue samples from 4 cases of canine lymphoid neoplasms were examined. One was a lymphoid leukemia (CL1) and the other three were lymphosarcomas (CL2-4). The lymph nodes from the affected dogs were collected at biopsy or autopsy and stored at  $-70^{\circ}\text{C}$  until use. As controls, the testis from a clinically normal mongrel dog (control) and human placenta from a Japanese woman (human) were employed.

High-molecular-weight genomic DNA was isolated from 1 to 1.5 g of each specimen as described previously [7, 8]. Cellular DNA samples of 10  $\mu\text{g}$  were digested with restriction endonucleases (*Bam*HI, *Pst*I, *Hind*III, *Xba*I or *Hinc*II; Takara Shuzo Co., Ltd., Kyoto, Japan). The DNA digests were then subjected to electro-phoresis and transferred to a nylon membrane filter (NITRAN-N, Schleicher & Schuell, Dassel, German) as described previously [2, 16]. The filters were hybridized at low stringency, using 7 kinds of human DNA fragments; human *c-yes-1* cDNA [19], human genomic *c-myc* [4], human genomic *c-ros-1* [6], human genomic *c-erbB-2* [11], *v-myc* [20], *v-Ki-ras* [4], and *v-Ha-ras* [3]. These human DNA fragments were labeled with [ $\alpha$ -<sup>32</sup>P]-deoxycytidine-5'-triphosphate (approximately 111 TBq/mmol, ICN Biomedicals Inc., California, U.S.A.) by the primer extension method [5].

The results of autoradiography after Southern blot hybridization using the human *c-yes-1* cDNA probe and *Bam*HI digested genomic DNA are shown in Fig. 1. In the genomic DNAs from the 5 dogs, two bands showing

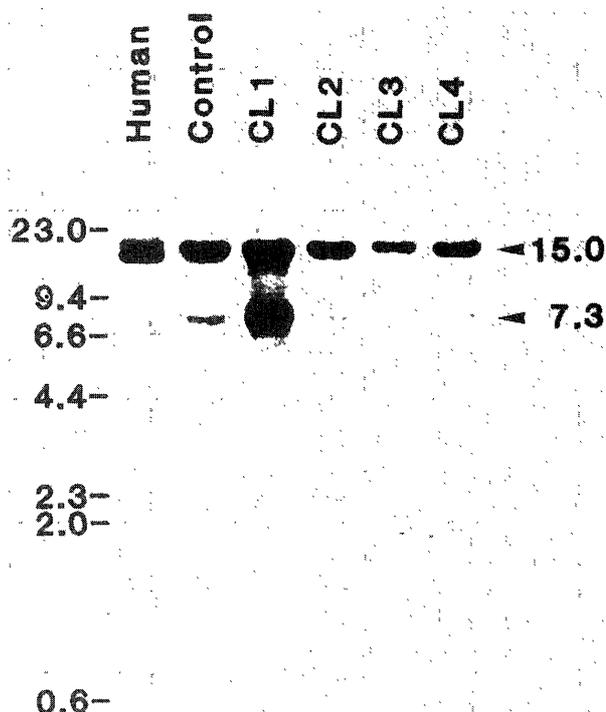


Fig. 1. Southern blot analysis of the genomic DNAs obtained from canine lymphoid neoplasms and normal controls and digested with *Bam*HI, using the human *c-yes-1* cDNA oncogene probe. Numbers on the right are the sizes of the hybridized bands, and numbers on the left are the sizes of  $\lambda$ -DNA fragments digested with *Hind*III for use as size markers; units are kilobase pair.

hybridization with human *c-yes-1* cDNA were found at positions corresponding to approximately 15.0 and 7.3 kilobase pairs (kb). Among these, a markedly amplified band at the 7.3 kb was detected in the genomic DNA from one dog (CL1). When serially diluted amounts of the genomic DNA from CL1 were hybridized under the same conditions as in Fig. 1 with human *c-yes-1* cDNA, the amplified band at 7.4 kb was detectable even in samples diluted 4- or 8-fold (Fig. 2). In the genomic DNA from CL1, amplified bands related to *c-yes-1* were also seen in *Pst*I, *Hind*III, *Xba*I, *Hinc*II, and *Bam*HI-*Hind*III digests, respectively (data not shown). On the other hand, no abnormal structure was found on the same filters using the

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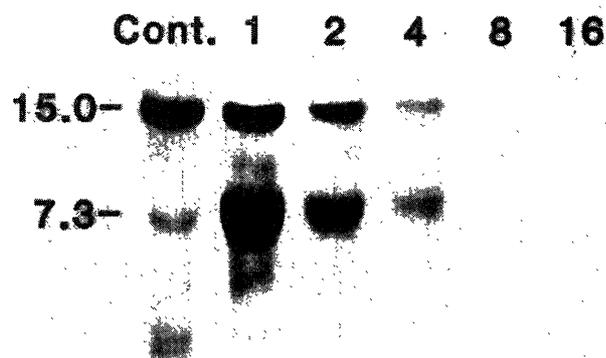


Fig. 2. Southern blot analysis of the serially diluted DNA from the dog CL1. The genomic DNA (10  $\mu$ g) from the CL1 was serially diluted with a 1 mM EDTA disodium and 10 mM Tris-HCl solution (pH 7.5), and the intensity of the *Bam*HI-digested, 7.3 kb *c-yes-1*-related bands in these samples was compared with that in 10  $\mu$ g of the DNA from the normal control dog specimen (Cont.). The reciprocal of the dilution factor is shown above the lane.

other 6 probes (data not shown).

The human *c-yes-1* oncogene encodes a 62 kilodalton protein, p62<sup>*c-yes*</sup>, which is myristylated, associated with membranes, and belongs to the non-receptor-type tyrosine kinase family or *src* oncogene family [11, 12, 17, 18]. Seki *et al.* [10] reported about 4- to 5-fold amplification of the human *c-yes-1* oncogene in a human primary gastric cancer. In addition, more than 2-fold amplification of the human epidermal growth factor gene, is said to be a significant event in human squamous cell carcinoma cell lines [21]. These previous findings suggest that the 4- to 8-fold amplification of *c-yes-1*-related oncogene cannot be ruled out as a possible event related to tumorigenesis of the present canine lymphoid leukemia. However, since the present study is only a preliminary survey with a small number of samples, the significance of the structural change in this oncogene remains unclear.

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