Human c-yes-1-Related Proto-Oncogene in Clinically Normal Dogs

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ABSTRACT. Human c-yes-1-related canine proto-oncogene in genomic DNAs from 21 clinically normal dogs was analyzed by Southern blot hybridization. The present study indicated that this proto-oncogene was well conserved in clinically normal dogs, however, there were structural changes in some dogs. These changes were different in each individuals and detected in low frequency.—**KEY WORDS:** canine, c-yes-1, oncogene.

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A human cellular *yes* oncogene, c-yes-1, has been identified as a homologue of the v-yes oncogene of avian sarcoma virus [12], and is expressed in many tissues [4, 5, 11], especially in the brain [7-9]. As well as in human beings, cloning and sequencing of c-yes proto-oncogenes from chickens and amphibians have been performed [6, 10, 13]. We have observed human c-yes-1-related proto-oncogenes in clinically normal dogs [2] and structural changes in a canine mammary gland tumor [3] by Southern blot hybridization. The purpose of the present study is to investigate precise restriction patterns of human c-yes-1-related canine proto-oncogenes using a great number of normal dog specimens, and to clarify whether the structural changes observed in our previous report [2] occur frequently or rarely in normal dogs.

Testes of 21 adult clinically normal dogs were collected at Miyazaki Prefectural Animal Health Center, Japan. The samples were stored at -70°C. Human placenta was used as a control.

High-molecular-weight genomic DNAs were isolated from 1-1.5 g of each testis specimen as described [2]. The samples of 10 µg genomic DNA were digested with one or two restriction endonucleases; *EcoRI*, *HindIII*, *BamHI* or *PstI* (Takara Shuzo Co., Ltd., Kyoto) and subjected to electrophoresis on 0.8% agarose gels, then transferred to nylon membrane filters (Nytran-N, Schleicher & Schuell, Dassel, Germany) as described [2]. The molecular-weight-marker DNA fragments were obtained by digestion of λ-phage DNA (Takara Shuzo Co., Ltd., Kyoto) with *HindIII*. The high-molecular-weight DNAs on the filters were hybridized with a probe of human c-yes-1 cDNA *HindIII-EcoRI* 0.55 kilobase pairs (kb) [10] (Supplied by Dr. M. Shibuya, the Department of Genetics, Institute of Medical Science, The University of Tokyo) as described [2].

In *EcoRI* digests, two major bands were detected at 7.0 and 2.9 kb from all of the 21 specimens, and an extra band was observed at approximately 9.5 kb from dog No. 13 (Fig. 1A). In *HindIII* digests, three bands were detected at approximately 5.4, 3.7 and 2.6 kb from 20 specimens. In dog No. 17, the 5.4 kb-band was replaced by a 5.1 kb-band, and two other bands were the same as those of the other specimens (Fig. 1B). In *BamHI* digests, approximately 14.0 kb-band was encountered from all of the specimens. There was also a band at approximately 6.4

kb, although this was not clearly demarcated. In dogs No. 17 and 21, an extra band was found at approximately 2.4 kb (Fig. 1C). In *PstI* digests, two major bands were detected at approximately 4.3 and 2.1 kb from all of the specimens.

In order to obtain further details about the unique bands, genomic DNAs from dogs No. 13 and 17, and also from dog No.16 as a control specimen, digested with *HindIII-EcoRI* or *HindIII-BamHI*, was hybridized with the c-yes-1 probe. In the *HindIII-EcoRI* digests, 4.0, 2.1, 0.8 kb-bands from No. 16, and 3.4, 2.1, 0.8 kb-bands from No. 13 and No. 17 samples were detected. In *HindIII-BamHI* digests, the same bands of 2.8, 2.1 and 1.7 kb were found in all of the three specimens.

The human c-yes-1-related canine proto-oncogene was detected in genomic DNAs from all of the clinically normal dogs used in this study, and unique bands were detected in 3 dogs. These unique bands were different in each individuals and detected in low frequency. To our knowledge, genomic DNA polymorphism of a human epidermal growth factor receptor gene can be detected as normal phenotypes in human beings, and both or either of the two bands are detected at the same rate in *HindIII* digests of all human specimens [1].

In our study, one of the unique bands closely resembled the abnormal structural band of a canine mammary tumor specimen as described by Miyoshi *et al.* [3], although the molecular weight was slightly different due to variation in the migration of the DNA fragments used as molecular weight markers during electrophoresis. Therefore, it also seems to be possible that these structural changes may have tumorigenic potential. Although the dogs showing these changes were clinically normal. The changes might act as initiator or promoter, if they survive.

It is unclear whether the changes observed in the present study represent DNA polymorphisms as normal phenotypes or were related to tumorigenesis. In order to determine whether these structural changes are located in functional regions (i.e. exon or regulatory region for transcription) or non-functional regions (i.e. intron), molecular cloning of the canine c-yes oncogene are now in progress. The molecular cloning and sequencing of this oncogene will help to resolve the relationship between the observed structural changes and tumorigenesis in the dogs.

Fig. 1. Hybridization of genomic DNAs from clinically normal dogs using the human *c-yes-1* probe. The DNA samples were digested with *EcoRI* (1A), *HindIII* (1B) and *BamHI* (1C). Molecular weight markers, obtained by digestion of λ -phage DNA with *HindIII*, are shown on the left. C = human placental DNA specimen.

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