Molecular cloning of the cDNA of canine homeodomain-interacting protein kinase 2

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The research of p53 is being conducted to find the mechanisms of tumorigenesis and to treat various cancers. Homeodomain-interacting protein kinase2 (HIPK2) is an important factor to regulate p53 and to increase the stability of p53. Activation of HIPK2 leads to the selective phosphorylation of p53, resulting in growth arrest and the enhancement of apoptosis. In this study, the canine HIPK2 cDNA fragments were obtained, and their overlapping regions were aligned to give a total sequence of 3489 bp. The canine HIPK2 cDNA (GenBank accession number; AY800385) shares 93% and 90% sequence identity with those of human and mouse HIPK2, respectively. The canine HIPK2 cDNA contains an open reading frame encoding 1163 amino acid residues and the predicted amino acid sequence has 98% and 96% identity with those of human and mouse, respectively. The deduced amino acid sequence of canine HIPK2 has also all domains' sites compared with human and mouse HIPK2. Therefore, these structural similarities suggested that the canine HIPK2 shares the basic biological functions that HIPK2 exhibit in other species.

Key words: cloning, dog, HIPK2, p53 regulation

Introduction

Homeodomain-interacting protein kinases (HIPKs) constitute a novel family of nuclear protein kinases. Three members of this family, HIPK1, HIPK2 and HIPK3 have been isolated in human and mouse so far but none of those was isolated in dogs. HIPK2 has been described as a homeodomain-interacting protein kinase, which acts as a co-repressor for homeodomain transcription factors [10]. HIPK2 colocalizes with p53 in nuclear bodies and phosphorylates p53.

The tumor suppressor protein p53 is one of the most important regulators of cellular growth functions, such as cell cycle arrest, DNA repair, and apoptosis, and is mutated in about 50% of all human tumors [8]. The p53 is important in the cellular response to cellular stresses, UV, γ-ray, and toxins [2,4,11,16]. Under normal conditions, p53 is a short-lived protein that is highly regulated and maintained at low or undetectable levels [11]. However, after stresses, the activation of p53 coordinates a change in the balance of gene expression leading to growth arrest, DNA repair or apoptosis, and these actions prevent the proliferation of genetically damaged cells. It involves several mechanisms including post-translational modifications such as phosphorylation and acetylation of specific residues in the amino-terminal and carboxy-terminal domains [16,18]. In addition to post-translational modifications, protein-protein interactions and subcellular relocalization also have a role in the activation of p53 [5,17]. The activation of p53 leads to the transcription of several genes whose products trigger different biological outcomes [6].

Activation of HIPK2 leads to the selective phosphorylation of p53 at Ser46, facilitating CBP-mediated acetylation of p53 at Lys382 and promoting p53-dependent gene expression [7]. The HIPK2 enhances the expression of p53 target genes, resulting in growth arrest and the enhancement of apoptosis [3]. Overexpression of HIPK2 leads to an increase of p53 protein expression or stability [19].

The research of p53 is being conducted to find the mechanisms of tumorigenesis and to treat various cancers. Thus, recently the researches are being conducted actively about the structure, function of HIPK2, and the relationship between HIPK2 and p53. In dogs, the gene therapy with p53 in cancer patients is in experimental stage. The study about the nucleotide sequence of canine HIPK2 was performed for the development of cancer therapy because the attack rate of cancer has been increased depending on longevity of pets in veterinary field.

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Fig. 1. Alignment of the nucleotide sequence of canine HIPK2 cDNA with those of human, and mouse counterparts (GenBank accession numbers AF326592 and AF208292). Dots indicate regions of identities in nucleotides. Numbers on left indicate the nucleotide residue position. Gaps were introduced in sequences to maximize alignment (-). This canine HIPK2 cDNA sequence was deposited in the GeneBank nucleotide database under accession number AY800385.
Materials and Methods

Spinal cord preparation

A physically normal, middle-aged, mixed male dog was euthanized with 20 ml of thiopental sodium. Spinal cord was separated and stored until the mRNA extraction was conducted at −70°C freezer.

Total RNA extraction and synthesis of cDNA

The spinal tissue (30 mg) was disrupted in 1.5 ml tube with 350 µl of lysis buffer (Macherey-Nagel, Germany) and was ground with automatic homogenizer. Total RNA was isolated from spinal tissue with RNA extraction kit (Macherey-Nagel, Germany). Full-length first strand cDNA was prepared from total RNA with First Strand cDNA Synthesis Kit (Fermentas, Lithuania). The cDNA was kept in −20°C freezer.

Polymerase chain reaction (PCR) was carried out using the spinal cDNA with primers designed based on conserved region of human and murine nucleotide sequences (GenBank Accession No. AF326592 and AF208292). PCR reaction mixture was consisted with a pair of the primers (1.0 µM each), Taq polymerase (0.75 units; TaKaRa, Japan), 10× PCR buffer (10 µl), dNTP mixture (8 µl), template (1 µg) and deionized water was added to a final volume of 25 µl. Amplification was involved 35 cycles of denaturation (94°C, 1 min.), annealing (45~60°C, 1 min.) and polymerization (72°C, 2 min.) steps.

Cloning and nucleotide sequence analysis

The PCR products were extracted by gel extraction kit-spin (NucleoGen, Korea) and were ligated into pCR2.1-TOPO vector (Invitrogen, USA). The vector was transformed into competent E. coli cells. Plasmid DNAs were isolated with plasmid purification kit (NucleoGen, Korea). The cloned plasmids were committed to TaKaRa-Korea Biomedical, in which ABI PRISM 377 sequencer is used to sequence analysis. The sequences were compared with region of human and murine nucleotide sequences (GenBank Accession No. AF326592 and AF208292).
those of human and murine HIPK2 (GenBank Accession No. AF326592 and AF208292). The amino acid sequence of canine HIPK2 was deduced from nucleotide sequence.

Results

About 30 pairs of primers were designed on the conserved region of human and murine HIPK2 in which 18 pairs of primers were used to find sequence of canine HIPK2. The other primers did not make PCR products or made different sequences products compared with human and murine HIPK2 sequences.

The clones which had overlapping regions were aligned to give a total sequence of 3489 bp as shown in Fig. 1. Canine HIPK2 cDNA sequence elucidated in this study was deposited in the GeneBank nucleotide database under accession number AY800385.

The identity between nucleotide sequence of canine HIPK2 and that of human and murine HIPK2 was 93% and 90%, respectively (Fig. 1). The identity between nucleotide sequence of human and mouse HIPK2 was 90%.

The canine HIPK2 cDNA contained an open reading frame encoding 1163 amino acid residues and the predicted amino acid sequence had 98% and 96% identity with those of human and mouse, respectively (Fig. 2). The nucleotide and amino acid sequences were highly conserved between human, mouse and dog.

Discussion

The nucleotide sequences of canine HIPK2 containing open reading frame region were found. The canine HIPK2 nucleotide sequence was similar to those of human and mouse. The deduced amino acid sequence of canine HIPK2 was also very similar to those of human and murine HIPK2.

The spinal cord was selected because HIPK2 mRNA was detectable in many tissues in human but a relatively high expression was observed in neural tissues, in which there are hippocampus, medulla oblongata, putamen, and so on [20]. Further study is needed to know where canine HIPK2 is expressed highly, using northern blot analysis, dot blot analysis, semi-quantitative RT-PCR [13,20].

HIPK2 contains multiple functional domains: an interaction domain for homeoproteins, a corepressor domain, a PEST sequence, a YH domain in the COOH-terminal and a protein kinase catalytic domain in the N-terminal side [10]. The enhancement of repressor activity of homeoproteins by HIPK2 is conferred by domains within the N-terminal half of the HIPK2. The SRS (nuclear speckle retention signal) that contains PEST sequence and YH domain has a positive and a negative effect on co-repressor activity respectively. It is expected that the functions of canine HIPK2 were similar...
to those of human and murine HIPK2, because the deduced amino acid sequences of canine HIPK2 contained all these domains. For instance, HIPK2 acts as a transcriptional corepressor for homeoproteins and localizes to nuclear speckles. In the N-terminal of the catalytic domain there is a glycine-rich stretch of residues in the vicinity of a lysine residue, which has been shown to be involved in ATP binding. In the central part of the catalytic domain there is a conserved aspartic acid residue which is important for the catalytic activity of the enzyme.

HIPK2 has the function namely activation of transcription mediated by p53 specific promoter elements [19]. Overexpression of HIPK2 leads to an increase of the p53 protein level. The kinase defective mutant of HIPK2 leads to a decrease of p53-induced Mdm2 protein.

In veterinary field, the attack rate of cancer is increasing due to the longevity of pets. So, the researches of cancer and p53 are highlighted and the study of HIPK2 may provide clinical benefits.

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