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Mutation of Canine Tumor Suppressor Gene p53 in a Mammary Gland Adenocarcinoma and a Malignant Mast Cell Tumor

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Abstract : To identify mutations in exons 5 to 8 of the p53 tumor suppressor gene, we have analysed in 12 spontaneous canine tumors. In a malignant mast cell tumor, a 1 base pair alteration AGT → AGC (silent point mutation, serine) in codon 249 in exon 8 was detected. And the mammary gland adenocarcinoma was found to have a mis-sense point mutation (CCT → TCT) in codon 285 in exon 8.

Key words **:** dog, mutation, p53, tumor

Introduction

Numerous studies have been focused on the investigation of the significant role of the p53 tumor suppressor gene in the tumorigenesis of human and canine cancers[1,3,4,11]. Its product, wild-type p53 protein acts as an inducible transcriptional factor after DNA damage, a negative regulator of cellular proliferation, the induction of apoptosis in stressed cells, the direction of DNA repair, and the regulation of other transcription factors[4,13,14]. It is believed that the mutation of p53 gene associated with tumorigenesis because of uncontrolled the cell cycle. Abnormalities of this gene have been documented in spontaneous canine tumors studied, including thyroid carcinoma, oral papilloma, mammary tumors, osteosarcoma, circumanal gland adenoma, lymphoma[2,3,8,9,15]. But, the data related to p53 mutations in canine neoplasms is limited.

To identify mutations in the highly conserved regions of canine p53 tumor suppressor gene, we have studied in twelve cases of spontaneous canine tumors.

Materials and Methods

Twelve biopsy samples and peripheral blood were obtained from dogs referred to the Veterinary Medical Teaching Hospital at the Seoul National University, for diagnosis and treatment. Tissue blocks of each tumor were frozen in liquid nitrogen immediately after surgical removal and stored at -70°C for DNA extraction. Adjacent sections were fixed in 10% formalin and routine histopathologic examination was performed. The examined tumors were benign mammary tumors (6), a mammary gland adenocarcinoma (1), a sertoli cell tumor (1), a malignant mast cell tumor (1), a plasmacytoma (1), a liposarcoma (1), and a lymphosarcoma (1) (Table 1). Genomic DNA was extracted from tumor specimens and blood using standard techniques[10].

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PCR oligonucleotides (primers) for amplification of the p53 fragments and PCR condition were designed on the basis of previously published sequencing data[3,4] and used to generate exon 5, exon 6, exon 7 and exon 8 fragments, the highly conserved regions of canine p53 gene (Table 2). These primers (20 pmol each) were mixed with 50 ng of canine genomic DNA, 1.5 mM MgCl2, 200 μM dNTP's, 1 unit Taq-polymerase (Core Taq), and 10× of the company's PCR buffer, in a final volume of 20 μl. PCR was carried out for 35 cycles of denaturation (94°C, 30 sec), annealing (53°C, 30 sec), and polymerization (72°C, 30 sec) steps, followed by a final incubation for 5 min at 72°C. The obtained PCR product was run on a 1.5% agarose gel electrophoresis to check the specificity of the reaction.

The PCR product was gel-purified using Jetsorb gel extraction kit (Genomed, Wielandstr, Germany) on a 1.5% agarose gel. Double stranded DNA was sequenced according to the dideoxy chain termination method using an Auto Read Sequencing Kit (ALFwin Sequence Analyser 2.00, Amersham Pharmacia Biotech, USA). Sequence analysis was performed at least twice, using independently amplified and subcloned PCR products to exclude PCR artifacts.

Results

The mutations of canine p53 gene were detected in two samples among investigated 12 tumor samples (Table 3).

In the sample of malignant mast cell tumor (Fig 1) localized on the left cheek of a 10 year-old female Chihuahua (VMTH005), a 1 base pair alteration AGT → AGC (silent point mutation, serine) in codon 249 of exon 7-8 junction was detected. But, the mutation was not present in the peripheral blood cells.

The mammary gland adenocarcinoma (Fig 2) of a 8 year-old female Maltese (VMTH010) was found to have a mis-sense point mutation (CCT → TCT) in codon 285 of exon 8. This changes gives rise to the amino acid change proline → serine. It was not detected in the peripheral blood cells.

Table 1. Characteristics of various canine tumors

ID	Breed	Histological tumor type	Age	Sex
VMTH001	Mixed	Benign mammary tumor	10 y	F
VMTH002	Pekinese	Sertoli cell tumor	4 y	M
VMTH003	Poodle	Benign mammary tumor	12 y	F
VMTH004	Papillon	Benign mammary tumor	10 y	F
VMTH005	Mixed	Malignant Mast cell tumor*	10 y	F
VMTH006	Jindo	Benign mammary tumor	7 y	M
VMTH007	Maltese	Lymphosarcoma	10 y	F
VMTH008	Yorkshire terrier	Benign mammary tumor	7 y	F
VMTH009	Yorkshire terrier	Liposarcoma	5 y	F
VMTH010	Maltese	Mammary gland adenocarcinoma*	8 y	M
VMTH011	Maltese	Benign mammary tumor	11 y	F
VMTH012	Maltese	Benign mammary tumor	9 y	F

*Tumor with mutation of p53 gene

Table 2. Primers used to amplify and sequence the canine p53 gene

Product location	Product Size (bp)	Primer sequences (5' to 3')	T (a) (°C)
Exon 5	249	GAGGAATTCCTGTCCATCTGTCCT (S) AGACTCGAGGCCTTGTCCCATCTG (AS)	50
Exon 6	204	GAGGAATTCTCCCCGATGGCTCTT (S) AGACTCGAGTCAACTCCCGCCTCA (AS)	52
Exon 7	235	GAGGAATTCCTGGGCCTACCTTCT (S) AGACTCGAGCAGAGGAGATTCCAC (AS)	50
Exon 8	193	GAGGAATTCCTCTCTTCTCACCTG (S) AGACTCGAGCCTTCACCTCCTCTT (AS)	53

Table 3. Identified mutations and normal sequence of the canine p53 gene

ID	Codon	Nucleotide	Amino acid
VMTH005	249	AG<u>T</u> → AG<u>C</u>	Ser → Ser
VMTH010	285	<u>C</u>CT → <u>T</u>CT	Pro → Ser

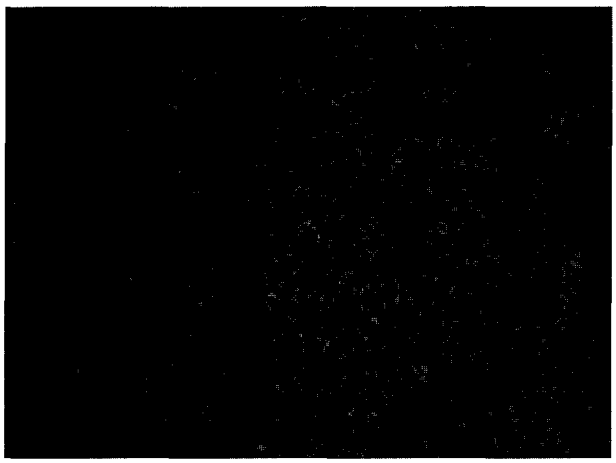

Fig 1. Histopathological sections from VMTH005 diagnosed as a malignant mast cell tumor. H&E, ×200.

Fig 2. VMTH010 was diagnosed as a mammary gland adenocarcinoma by histopathological examination. H&E, ×200.

According to human codon numbering[1], these equivalent to human codon numbers 261 and 297. Repeated sequence analysis on each of these p53-mutated tumors was performed twice in order to look for PCR errors.

Discussion

The p53 tumor suppressor protein plays a central role in

the regulation of cell proliferation, genomic stability, and programmed cell death[13,14]. But the p53 gene mutation lead to an amino acid substitution in the protein and may contribute to deregulated cell growth and tumor resistance to chemotherapy[14]. P53 mutations are the most common genetic alterations found in human tumors, including cancers of the breast, lung, colon, osteosarcoma and others[7,11]. And the investigations on the role of p53 mutation in the carcinogenesis of spontaneous canine tumors have been performed[2,3,5,8-9]. This suggests that the role of wild type p53 protein in preventing tumor formation and progression may be similar in both humans and dogs. In the present study, p53 mutation was demonstrated in two cases out of twelve patients. Patient VMTH 010 with the mammary gland adenocarcinoma displayed mis-sense point mutation in codon 285 (human codon positions 297) of exon 8. This point mutation (CCT → TCT) results in a substitution from proline to serine. The great majority of the mutations in 53 gene are mis-sense like this[2]. Recently, similar p53 gene mutations in canine mammary tumors have been identified. These p53 mutations were located at human codon numbers 21, 22, 24, 82, 102, 116, 138, 175, 176, 236, 245, 249 within exon two, four, five, and seven[5,6,13,16]. And also nonsense, splicing, and frameshift mutations in exon 4, 5, 6, and 7 of the p53 gene have been detected in canine mammary tumors[1]. Although not directly investigated, the mutation of p53 indicated that allelic loss of the p53 gene may have also occurred during tumorigenesis.

And the case of malignant mast cell tumor was found to have a point mutation in codon 249 (human codon numbers 261) of exon 7-8 junction. However, this mutation is silent and it does not appear to have played a role in the development of the tumor.

All of two cases was transition point mutation and was detected in exon 8. G:C → A:T transitions are the major point mutations (47%) of all p53 gene identified in human cancers[12]. But the transversions in which a purine is replaced by a pyrimidine or vice versa are rare. In a variety of human tumors, greater than 90% of mis-sense mutations in p53 span highly conserved regions II-V, DNA binding domain[1,11]. In canine tumors, the majority of alterations clustered too in four of five conserved domains localized between exons 5-8, and this part is well known for harboring "hot spots" in dog and human tumors[1].

Our results demonstrate that mutation of the p53 gene occurs in canine mammary gland adenocarcinoma and malignant mast cell tumor. The detection of mutation in canine p53 gene could be used for database for diagnostic markers and therapy of neoplastic disease in dogs. But more extensive knowledge of the p53 gene associated cancers in dogs is urgently needed. In addition further sequencing data are now required on p53 tumour suppressor gene modifications associated with tumourigenesis in different disease stages.

Conclusion

To identify mutations in the highly conserved regions of canine p53 tumor suppressor gene, we have studied in twelve cases of spontaneous canine tumors. In a malignant mast cell tumor, a 1 base pair alteration AGT → AGC (silent point mutation, serine) in codon 249 in exon 8 was detected. And the mammary gland adenocarcinoma was found to have a mis-sense point mutation (CCT → TCT) in codon 285 in exon 8. This changes gives rise to the amino acid change proline → serine. The detection of mutation in canine p53 gene could be used for database for diagnostic markers and therapy of neoplastic disease in dogs. But more extensive knowledge of the p53 gene associated cancers in dogs is urgently needed.

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개의 유선암종과 악성 비만세포 종양에서 발생한 종양억제 유전자 p53의 변이

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초 록 : 개에서 자연적으로 발생한 12예의 종양에 대해, 종양 억제 유전자 p53의 변이와의 관계를 확인해 보았다. 종양조직에서 일반적인 방법으로 DNA를 추출하여, PCR 기법으로 p53을 증폭하여 염기서열을 확인한 결과, 개의 유선암종 예에서 exon 8의 codon 285에서 CCT → TCT (proline → serine)로 점변이 된 것이 확인되었다. 또한 악성 비만세포 종양 예에서도 exon 8의 codon 249에서 AGT → AGC로 점변이 된 것이 확인되었으나 silent point mutation (serine)으로 판명되었다. 이상의 결과를 토대로 개의 유선암종과 악성 비만세포 종양에서 종양억제 유전자 p53의 변이가 확인되었으며, 이는 종양의 형성과 관련된 p53의 역할이나 종양의 치료 및 예후 판정에 p53을 활용하는 연구의 초석이 되리라 사료되며, 차후 이 유전자에 대한 광범위한 연구가 지속되어야 하리라 생각된다.