学 位 論 文 の 要 旨

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学 位 論 文 名　Genetic and Expression Analysis of SNPs in the Human Deoxyribonuclease II: SNPs in the Promoter Region Reduce Its *In Vivo* Activity Through Decreased Promoter Activity

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論 文 内 容 の 要 旨

INTRODUCTION

Deoxyribonuclease II (DNase II; EC 3.1.21.1), one of two distinct types of vertebrate DNases, hydrolyzes double-stranded DNA to 3′-phosphoryl oligonucleotides under acidic conditions in the absence of metal ions. Mice with induced deletion of the DNase II gene (*DNASE2*) could develop a chronic polyarthritis resembling human rheumatoid arthritis (RA), which is characterized by synovitis in diarthrodial joints and erosion of periarticular bone. These facts allow us to assume that single nucleotide polymorphisms (SNPs) in the DNase II gene leading to alterations in *in vivo* DNase activity may be implicated as a genetic factor in the pathophysiology of autoimmune and inflammatory diseases.

In this context, Shin et al. demonstrated that certain SNPs and their haplotypes in the DNase II gene are associated with renal disorders in Korean patients with SLE. Moreover, Rossol et al. reported that homozygous carriers of the *-1951G* allele, the *-1066G* allele, and the *-390A* allele indicate an increased risk of RA in Caucasians. However, there has been no information about any possible association of the levels of *in vivo* DNase II activity, which may be responsible for the pathogenesis of the diseases, with these SNPs. It is indispensable to examine all the related SNPs in the gene that are likely to be responsible for the genetic background of disease susceptibility through reduction of *in vivo* DNase II activity.

In this study, we focused on these 14 SNPs in the gene, reported on the NCBI dbSNP database to be associated to autoimmune diseases; we developed a simple and easy-to-apply technique for

genotyping of these 14 SNPs in *DNASE2* and investigated the distribution of each allele and haplotype in eight Asian, three African, three Mexican, and two Caucasian populations worldwide. Furthermore, the effects of substitution in the protein and mRNA resulting from the SNPs were systematically examined; especially, with regard to five SNPs polymorphic in the Japanese population, we demonstrated the relationship of genotypes/haplotypes with the *in vivo* DNase II and promoter activities.

MATERIALS AND METHODS

Genotyping of the SNPs in the DNase II gene

Genotyping assays for the nine non-synonymous SNPs and five RA-related SNPs, were separately performed using the mismatched PCR-restriction fragment length polymorphism (RFLP) technique. The design of the PCR primers used for the genotyping was based upon the nucleotide sequences of the human *DNASE2*.

Construction and transfection of the expression vectors

A DNA fragment containing the entire coding sequence of human DNase II cDNA was prepared from the total RNA of human spleen by reverse transcriptase-PCR amplification. The amplified fragment was ligated into pcDNA3.1(+) and used as a wild-type construct. Each DNase II cDNA was cloned into a mammalian expression vector and transiently expressed in COS-7 cells.

Reporter assay

Four reporter vectors with haplotypes ACC, GGC, ACA, and GCA of SNP -1951G>A, -1066G>C, and -390A>C were constructed. The human hepatoma, HepG2 was transfected with 1.0 μg of the reporter construct. Both the firefly (*Photinus pyralis*) and sea pansy (*Renilla reniformis*) luciferase activities in the lysates were measured.

Assay for the enzymatic activity

DNase II activity in the cell lysates of the transfected cells and in the serum was assayed by the single radial enzyme diffusion (SRED) method.

The study was approved by the Human Ethical Committee of each institute.

RESULTS AND DISCUSSION

Effect of amino acid-substitution corresponding to the non-synonymous SNPs in *DNASE2* on the DNase II activity

The level of DNase II activity derived from the R39I, H204R, and V206I constructs was similar to that of the wild-type, whereas the A45G, F234S, V300M, and R314L constructs exhibited a significantly decreased level of the DNase II activity, relative to the wild-type. These findings demonstrate that replacement of the amino acid residues at positions 45, 234, 300, and 314 of the

DNase II protein reduced the activity to 20-60% of that of wild type.

Effect of 5 polymorphic SNPs in *DNASE2* on *in vivo* levels of DNase II activity

From the analysis of genotypes and serum DNase II activities in 176 Japanese, we found that the homozygous genotype for the predominant allele at 5 polymorphic SNPs exhibits lower enzyme activities than the homozygous genotype for the counterpart allele. Importantly, -1951G>A, -1066G>C and -390A>C in the promoter region of the gene showed higher effect on *in vivo* levels of DNase II activity than the other 2 SNPs.

Effect of 3 polymorphic SNPs in the upstream region of the DNase II gene on the promoter activity

DNASE2 promoter-luciferase reporter constructs corresponding to four major haplotypes in Japanese populations, GGA, ACC, GGC, and ACA in -1951G>A, -1066G>C, and -390A>C, respectively, were transiently transfected into HepG2 cells, and each luciferase activity was then assayed. The ACC construct showed about 1.7-fold higher activity than the GGA constructs. The replacement of G and G by A and C at positions -1951 and -1066, respectively, might induce an increase of luciferase activity, whereas substitution in -390A>C has a little effect on the activity. Comparing the GCA construct with the ACA, the substitution of *-1951G* from *-1951A* elevated the luciferase activity, supporting such assumption. On the other hand, the promoter activity derived from the GGA was similar to that from the GCA, indicating a slight contribution of -1066G>C on the promoter activity of the DNase II gene. Therefore, it could be clarified that -1951G>A in the promoter region of the gene among RA-related SNPs could primarily alter the promoter activity of *DNASE2*, thereby having a significant effect on the *in vivo* DNase II activity.

CONCLUSION

It seems reasonable to assume that decreased levels of *in vivo* DNase II activity might be implicated in the onset of autoimmune diseases, such as RA. Thus, we could present a reduction of *in vivo* DNase II activity as one of the molecular bases for the etiology of the autoimmune diseases. Since RA is a complex multifactorial and polygenic disorder in which multiple environmental and genetic factors are simultaneously involved, DNase II may be one of the several factors involved in genetic predisposition to the incidence of RA. Especially, a comparison of the promoter activities derived from each haplotype reporter construct allows us to assume that -1951 in the promoter region of the gene among RA-related SNPs could primarily contribute to alteration in the promoter activity of *DNASE2*.