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Apo-1/Fas (CD95) Gene Polymorphism in Korean Hepatocellular Carcinoma Patients

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It is well known that different expression of Apo-1/Fas (CD95) plays important roles in various tumors and hepatocellular carcinoma (HCC) pathogenesis. Apo-1/Fas mediated apoptosis is one of the important pathways of apoptosis and is known to mediate apoptotic cell death by fas ligand (FasL). To examine the possible relationship between Apo-1/Fas gene polymorphism and HCC susceptibility, *Mva*I restriction fragment length polymorphism (RFLP) of Apo-1/Fas gene was examined in 94 Korean HCC patients and 240 control subjects. No statistically significant difference in the genotypic distribution and allelic frequencies was found between the control and the HCC. It is, therefore, concluded that Apo-1/Fas gene polymorphism is not associated with HCC susceptibility. Further studies are needed in order to clarify the relationships between genotypes of Apo-1/Fas gene and HCC pathogenesis.

Key Words: Hepatocellular carcinoma, Apo-1/Fas (CD95), Polymorphism, PCR-RFLP, Apoptosis

INTRODUCTION

Apoptosis, also known as programmed cell death, is crucial to normal cell function and homeostasis (Schmitz et al, 2000). Apoptotic cell death by cell death receptor of tumor necrosis factor family is an essential and most important process. Apo-1/Fas (CD95) is the best characterized cell death receptor, which is widely expressed in different cells and also is mutated in malignant human diseases (Sharma et al, 2000). In the majority of human tumors, disruption of the balance between cell proliferation and apoptosis has been implicated as a major cause of cancer development (Fan et al, 2001). Changes in apoptosis genes are also known to involve pathogenesis of various diseases. Mutations in various genes are implicated in various human diseases, especially cancers. Among those mutations, several apoptosis genes are associated with cancer developments (Müllauer et al, 2001).

Hepatocellular carcinoma (HCC) is one of the major causes of cancer deaths in Korea (21.3 HCC deaths per one hundred thousands in 2000, Korea National Statistics Office). HCC has been proved to be greatly affected by environmental influences such as virus (Montesano & Hall, 2001) and toxins (Monto & Wright, 2001). In addition, genetic factors are also known as an important contributor to the development of HCC (Rogler & Chisari, 1992).

Involvements of apoptosis in HCC development and progression are well documented (Thorgeirsson et al, 1998), and alterations in Apo-1/Fas and Apo-1/Fas-related molecules have been reported in HCC patients (Lee et al, 2001). Nevertheless, there are no reports about HCC susceptibility and polymorphism of Apo-1/Fas genes. Although Apo-1/Fas is one of the most important molecules mediating apoptosis, there have been few studies about Apo-1/Fas and HCC, including polymorphisms. In this study, we investigated whether the Apo-1/Fas gene polymorphism would affect the HCC susceptibility.

METHODS

Study population

The control group consisted of 240 Koreans without personal or familial history of other disease (mean age of 42.4 ± 0.8, ranging from 21 to 75). The hepatocellular carcinoma patients consisted of 94 unrelated patients previously diagnosed with hepatocellular carcinoma (mean age of 55.3 ± 1.3, ranging from 18 to 80). After explanation of the study, written informed consent was obtained from each subject.

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ABBREVIATIONS: HCC, hepatocellular carcinoma; RFLP, restriction fragment length polymorphism.

Table 1. The *Mva*I polymorphism between Korean control subjects and Korean hepatocellular carcinoma patients

Samples	Genotypes (%)			Allele frequency (%)	
	*1/*1	*1/*2	*2/*2	*1	*2
Control (N=240)	44 (18.3%)	118 (49.2%)	78 (32.5%)	206 (42.9%)	274 (57.1%)
Hepatocellular carcinoma (N=94)	23 (24.5%)	41 (43.6%)	30 (31.9%)	87 (46.3%)	101 (53.7%)
χ^2 value		1.7114		0.6194	
Df		2		1	
p-value		0.425		0.4313	
Odd ratio (95% CI)				1.146 (0.816~1.608)	

Extraction of DNA from blood

Genomic DNA was extracted according to the standard procedure. Blood samples from all subjects were obtained in EDTA tubes. Genomic DNA was extracted using a Whole Blood Genomic DNA Purification kit (CoreBio System Co., Seoul, Korea) and stored at −20°C before use.

Polymerase chain reaction

*Mva*I RFLP was studied according to the method of Huang et al. (1997). The amplified sequence contains promoter region of human Apo-1/Fas gene, and the primers are as follows: 5'-CTACCTAAGAGCTATCTACCGTTC-3' (sense) and 5'-GGCTGTCCATGTTGTGGCTGC-3' (antisense). PCR was carried out using a Perkin Elmer GeneAmp PCR system 9600 (Roche Diagnostics Corporation, Indianapolis, IN, USA). The conditions were as follows; 40 cycles, each consisting of denaturation at 94°C for 30 sec, annealing at 58°C for 1 min, and extension at 72°C for 1 min. The reaction cycles were preceded by a single 5 min denaturation at 94°C and were followed by a 10-min extension at 72°C. PCR products were confirmed by electrophoresis on 1.5% agarose gels.

Restriction fragment length polymorphism (RFLP)

The PCR products of each sample were then digested with the *Mva*I restriction endonuclease (Boehringer Mannheim, Indianapolis, IN, USA), and the resulting products were electrophoresed on 1.5% agarose gels and visualized under UV transilluminator. Two polymorphic alleles, allele *1 (G: fragment of length 166 bp) and allele *2 (A: fragment of length 233 bp) were visualized, depending on the presence of A→G base pair substitution at −670 (Kim et al, 2002) (Fig. 1).

Statistical analysis

For case/control association studies, the significance of observed differences in allelic or genotypic frequencies between HCC patients and control group was determined using χ^2-test. The distribution of the *Mva*I genotypes in HCC was compared with that of the control group using the χ^2test (3×2 contingency table). To compare the allelic frequencies of *1 and *2 of the two groups, a 2×2 contingency (χ^2) test was used. Odds ratios and 95% confidence intervals (CI) were used to quantify any association between the genotype of Fas gene and HCC. The Statistic Analysis System program was used for statistical analysis.

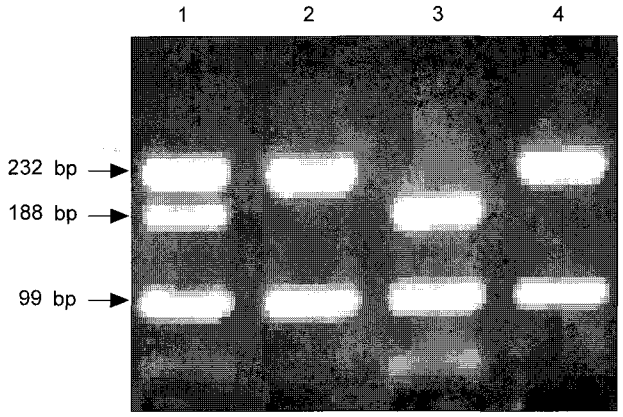

Fig. 1. Genotype analysis of *Mva*I RFLP in Apo-1/Fas (CD95) promoter region. The PCR products were digested with *Mva*I and subjected to electrophoresis in a 1.5% agarose gel, followed by ethidium bromide staining.1. *1/*2 heterozygote; 2 & 4. *2/*2 homozygote; 3. *1/*1 homozygote.

RESULTS

The genotypic distribution and allelic frequencies of *Mva*I polymorphism in the control group and HCC patients group are shown in Table 1. The frequencies of homozygosity for the 1 (*1/*1) and 2 (*2/*2) were 18.3% and 32.5% in the control group, 24.5% and 31.9% in the HCC group, respectively. The frequency of heterozygosity for *1/*2 was 49.2% in the control group, 43.6% in the HCC group, respectively. The allelic frequencies of *1 and *2 were 42.9% and 57.1% in the control group, 46.3% and 53.7% in the HCC group, respectively. Therefore, there was no statistically significant difference in genotypic distribution and allelic frequencies between the control and HCC patients (p=0.415 and 0.4313, respectively).

DISCUSSION

Mutations in apoptosis genes are known to be a pathogenetic factor for human disease (Müllauer et al, 2001). Among the mutations, changes in p53, Bax, bcl-2, and c-IAP2 genes are known to be involved in various

cancers development.

Apo-1/Fas (CD95) is the best-characterized tumor necrosis factor receptor superfamily, which is involved in the regulation of cellular homeostasis during development, differentiation, and cell death. Binding of its natural ligand, Fas ligand (FasL), triggers apoptosis pathway (Peter & Krammer, 1998; Nagata, 1999). Mutations in Apo-1/Fas-mediated pathway can alter the apoptosis and cause malignant transformation of human cells (Maeda & Kamihira, 2001). Studies by Lee et al. (2001) and Park et al. (2001) showed that changes in the expressions of Apo-1/Fas and Apo-1/Fas-related molecules might play important roles in the pathogenesis of HCC and gastric carcinoma

HCC is one of the major cancer types in Korea. Although many factors involved in the pathogenesis of HCC has been suggested, it is not clear which factors involve the development of these cancers. Although both genetic and environmental factors are thought to contribute in carcinogenesis and progression of HCC, HCC has been proved to be more environmentally influenced than other cancers (Rogler & Chisari, 1992; Montesano & Hall, 2001). Because liver detoxifies almost all the exogenous and endogenous compounds, environmental causes of HCC are well documented, and virus-induced carcinogenesis is also extensively studied. Nevertheless, genetic components of HCC carcinogenesis are not well studied. Few genetic polymorphisms are known to be associated with HCC susceptibility. For example, N-acetyltransferase (Yu et al, 2000; Farker et al, 2002), cyclin D1 gene (Zhang et al, 2002), and glutathione-S-transferase μ1 gene (Tiemersma et al, 2001) are known to be associated with HCC susceptibility.

Polymorphic nature of human genes affects the pathophysiology of human disease and determines susceptibility to specific disease (Sud et al, 2001; Alameddine & Zafari, 2002). To date, extensive studies have been focused at this issue to clarify these relations. Polymorphisms of apoptosis genes are the one of main concerns, because of its involvement in the pathogenesis of various diseases (Müllauer et al, 2001). Apo-1/Fas gene polymorphism and its influences on disease have been reported in several studies. Huang et al. (1997) characterized Apo-1/Fas gene and determined distribution of *Mva*I polymorphism in promoter region, and found that GA to GG substitution at −670 nucleotide position in the enhancer region of Apo-1/Fas gene produced *Mva*I RFLP. They also reported that Apo-1/Fas polymorphism was significantly different in SLE patients with photosensitivity (Huang et al, 1999). Other studies focused on immunologic diseases, however, no associations (Lee et al, 2001; Huang et al, 2000).

In summary, although Apo-1/Fas plays an important role in HCC carcinogenesis, there had been no report regarding the relationship between Apo-1/Fas gene polymorphism and HCC. Therefore, we investigated the possible association between Apo-1/Fas gene polymorphism and HCC, however, there was no association. The present result indicates that Apo-1/Fas gene polymorphism is not a determinant of susceptibility to HCC development.

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