Research Article

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Polymorphisms of DNA repair genes in Korean hepatocellular carcinoma patients with chronic hepatitis B: Possible implications on survival

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Background & Aims: We aimed at determining whether single nucleotide polymorphisms (SNPs) of DNA repair genes influence the development and clinical outcomes of hepatitis B virus (HBV)-associated hepatocellular carcinoma (HCC).

Methods: We evaluated 14 SNPs of eight DNA repair genes in 708 patients with HCC and 388 HBsAg positive controls without HCC. The Kaplan–Meier methods with log-rank test and Cox regression models were used to compare survival of HCC patients according to the genotype.

Results: The SNP of *XRCC4* rs1805377 was significantly associated with decreased risk of HCC development (OR, 0.592; $p = 0.028$) and improved overall survival of patients with HCC (median survival time (MST) of 48, 72, and 89 months for the AA, AG, and GG genotypes, respectively; $p = 0.044$). In addition, SNP of *OGG1* rs1053133 was significantly associated with postoperative recurrence (OR, 0.604; $p = 0.049$), tumor differentiation (OR, 0.571; $p = 0.041$), and improved survival of resected HCC (MST of 55 and 108 months for the GG and GC/CC genotypes, $p = 0.001$). The multivariate analysis showed that *OGG1* rs1052133, *XRCC1* rs25487, *ERCC5* rs2018836, *ERCC5* rs3818356, and *XRCC4* rs1805377 had a significant effect on survival. Moreover, a strong dose-dependent association was observed between the number of putative high-risk genotypes of *OGG1*, *XRCC1*, *ERCC5*, and *XRCC4* with the overall survival. The MST of HCC with $\leqslant 2$ putative high-risk genotypes was significantly prolonged compared to those with $\geqslant 3$ high-risk genotypes (76 *vs.* 46 months, respectively, $p = 0.002$).

Conclusions: Polymorphisms of DNA repair genes play a potential role in the development, progression, and survival of Korean HCC patients with chronic HBV infection.

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Keywords: DNA repair; Polymorphism; HBV; HCC; Survival.

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Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNP, single nucleotide polymorphism; BER, base excision repair; NER, nucleotide excision repair; NHEJ, non-homologous end joining; HBsAg, hepatitis B virus surface antigen; AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; *APEX*, apurinic endonuclease; *OGG*, 8-oxyguanine DNA glycosylase; *XRCC*, X-ray repair complementing defective repair in Chinese hamster cells; *ERCC*, excision repair cross-complementing rodent repair deficiency complementation; *XPA*, xeroderma pigmentosum complementation group A; *XPC*, xeroderma pigmentosum complementation group C.

Introduction

Chronic hepatitis B virus (HBV) infection is one of the established etiologic agents of hepatocelluar carcinoma (HCC) worldwide, which contributes to the development of the majority of HCC cases in Korea [1,2]. However, only a small proportion of chronic HBV carriers develop HCC in their lifetime, suggesting that other risk factors may contribute to the inter-individual variation in susceptibility to hepatocarcinogenesis [3]. In addition, various genetic factors appear to influence the outcome of HBV infections [4–6]. Based on the current knowledge of cancer molecular pathogenesis, polymorphic variations such as single nucleotide polymorphism (SNP) within several gene classes are considered to be important for tumor development.

It is suggested that the majority of HCC cases result from DNA damage caused by hepatitis viruses; this is the major underlying factor that predisposes to the development of HCC [7]. Damage resulting from endogenous or exogenous exposure may be repaired by enzymes encoded by one or more DNA repair pathways. In humans, more than 70 genes are involved in the five main DNA repair pathways: base excision repair (BER), nucleotide excision repair (NER), mismatch repair, homologous recombination repair, and non-homologous end joining (NHEJ). Defective DNA repair can lead to the accumulation of mutations and microsatellite instability in the genome, increasing the chance of malignant transformation [8]. Genetic variation may alter the function of DNA repair proteins, influencing the risk of development and clinical outcomes of HCC.

Although many studies have been published on the associations between SNPs of DNA repair genes and the risk of various cancers [9], few studies have reported on the associations between SNPs of DNA repair genes and HCC. In this study, we investigated the association between selective SNPs of DNA

Cancer

Table 1. SNPs of the DNA repair genes evaluated in this study.

Pathway	Gene	Chromosome	Base change	SNP ID	MAF	HWE
BER	*APEX1*	14q11.2	T to G	rs1130409	0.458	0.467
	OGG1	3p26.2	G to C	rs1052133	0.464	0.242
	XRCC1	19q13.2	G to A	rs25487	0.243	0.574
	XRCC1	19q13.2	G to A	rs25489	0.111	0.643
NER	*ERCC5*	13q32-33.1	G to A	rs2018836	0.287	0.825
	ERCC5	13q33	G to A	rs3818356	0.268	0.643
	XPA	9q22.3	G to A	rs1800975	0.491	0.356
	XPC	3p25	A to C	rs2228001	0.386	0.697
NHEJ	*XRCC4*	5q13-14	G to A	rs1478486	0.149	0.637
	XRCC4	5q13-14	C to G	rs1382376	0.154	0.901
	XRCC4	5q13-14	G to A	rs1478483	0.078	0.674
	XRCC4	5q13-14	C to T	rs963248	0.301	0.285
	XRCC4	5q13-14	A to G	rs1805377	0.266	1
	XRCC5	2q35	G to A	rs3835	0.071	0.822

SNP, single nucleotide polymorphism; BER, base excision repair; NER, nucleotide excision repair; NHEJ, non-homologous end joining; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium.

repair genes and the risk of HCC development and its clinical outcomes.

Patients and methods

Study population

Patients with HCC were recruited consecutively from two hospitals (Asan Medical Center and Ulsan University Hospital) in Korea from 1999 to 2010. A total of 708 Korean patients who were hepatitis B virus surface antigen (HBsAg) carriers with HCC were included. The diagnosis of HCC was made by liver biopsy or the combination of increased alpha-fetoprotein (AFP of $\geqslant$200 ng/ml) and the typical vascular pattern on angiography or dynamic imaging [10]. The control group consisted of 388 inactive HBsAg carriers without HCC who were diagnosed by the absence of hepatitis B virus e antigen (HBeAg), the presence of anti-HBe, <2000 IU/ml of HBV DNA in PCR-based assays, and repeatedly normal alanine aminotransferase levels. Clinical information was collected from the medical records with patients' consent. Tumor progression was defined as postoperative tumor recurrence and distant metastasis. Tumor stage was based on the Barcelona Clinic Liver Cancer (BCLC) staging system [11]. The overall survival time was calculated from the date of tumor resection or the first local treatment to the date of death. The ethical committee of Asan Medical Center and Ulsan University Hospital approved the study and written informed consent was obtained from all the participants.

Selection of candidate genes

SNPs of DNA repair genes were selected that had been previously reported to be associated with cancer risk in the medical literature. Among these SNP sites, 14 SNPs in eight candidate DNA repair genes were selected for testing in this study (Table 1). Four SNPs were selected from BER (*APEX1* rs1130409, *OGG1* rs1052133, *XRCC1* rs25487, and *XRCC1* rs25489), four SNPs from NER (*ERCC5* rs2018836, *ERCC5* rs3818356, *XPA* rs1800975, and *XPC* rs2228001), and six SNPs from NHEJ pathway (rs1478486, rs1382376, rs1478483, rs963248, and rs1805377 of *XRCC4* and *XRCC5* rs3835). All SNPs had minor allele frequencies of >0.05.

Laboratory methods

DNA was extracted from frozen white blood cells using standard methods. Genotype identification was performed with the GenomeLab SNPstream (Ultra-high throughput; UHT), which uses the multiplexed polymerase chain reaction (PCR) in conjunction with tag array single-base extension genotyping (Beckman Coulter, Fullerton, CA, USA) [12]. This system and its accompanying SNPstream software have been described previously by Demomme and Van Oene [13]. In brief, the SNPs of interest were amplified in a 12-pair multiplex PCR under universal conditions (5 mM $MgCl_2$, 75 μM dNTPs, 0.1 unit HotStarTaq DNA Polymerase in a final volume of 5 μl; PCR 94 °C 1 min; 34 cycles 94 °C 30 s, 55 °C 30 s, 72 °C 1 min). Single base extension primers were extended with single TAMRA- or Bodipy-fluorescein-labeled nucleotide terminator reactions (96 °C 3 min, then 45 cycles of 94 °C 20 s, 40 °C 11 s) and then spatially resolved by hybridization with the complementary oligonucleotides arrayed on the 384-well (SNPware Tag array) microplates. The 14 individual SNPs were identified by their position and fluorescent color in each well according to the position of the tagged oligonucleotides. Genotype data were generated on the basis of the relative fluorescent intensities for each SNP. Graphical review and operator adjustment of the genotype clusters were performed to refine fluorescent cut-off values. Genotyping data were obtained from 708 cases and 388 controls; results were reviewed and manually confirmed by experienced researchers.

Statistical analysis

The genotype frequencies of each of the SNPs were tested for Hardy–Weinberg equilibrium in both case and control groups using the Permutation test. The comparison of age between cases and controls was performed using the Student's *t*-test. Categorical variables were compared by the χ^2 test. Unconditional logistic regression, which was used to evaluate a significant association between the distribution of SNPs and clinicopathologic variables, as well as the association with disease (HCC *vs.* controls), was undertaken to estimate the odds ratios (ORs) and their 95% confidence intervals (95% CIs) after adjustment for age and gender. Haplotypes and their population frequencies were estimated by the PHASE software [14,15]. Linkage disequilibrium was determined using the Haploview program [16]. The relation of SNPs to the overall survival was identified using the Kaplan–Meier method with the log-rank test and Cox proportional hazard model. Data analysis was performed using the computer software SAS 8.0 (SAS Inc., Cary, NC, USA). All tests were two tailed and a p <0.05 was considered statistically significant.

Results

Characteristics of the study population

The age and gender distributions of the studied population were similar between HCC cases and controls. The mean age at

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