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Research Paper

Effects of *HMGB1* Polymorphisms on the Susceptibility and Progression of Hepatocellular Carcinoma

Bin Wang[1], Chao-Bin Yeh[2,3], Ming-Yu Lein[4,5], Chen-Ming Su[6], Shun-Fa Yang[7,8], Yu-Fan Liu[8,9,✉], Chih-Hsin Tang[4,10,11,✉]

1. Department of Hepatobiliary Surgery, Affiliated Dongyang Hospital of Wenzhou Medical University, Dongyang, Zhejiang, China
2. Department of Emergency Medicine, School of Medicine, Chung Shan Medical University, Taichung, Taiwan
3. Department of Emergency Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan
4. Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan
5. Division of Hematology and Oncology, Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan
6. Department of Biomedical Sciences Laboratory, Affiliated Dongyang Hospital of Wenzhou Medical University, Dongyang, Zhejiang, China
7. Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
8. Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan
9. Department of Biomedical Sciences, Chung Shan Medical University, Taichung, Taiwan
10. Department of Pharmacology, School of Medicine, China Medical University, Taichung, Taiwan
11. Department of Biotechnology, College of Health Science, Asia University, Taichung, Taiwan

✉ Corresponding authors: Chih-Hsin Tang, PhD. E-mail: chtang@mail.cmu.edu.tw; Graduate Institute of Basic Medical Science, China Medical University. Yu-Fan Liu, PhD. E-mail: yfliu@csmu.edu.tw; Department of Biomedical Sciences, Chung Shan Medical University, Taichung, Taiwan.

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Abstract

Hepatocellular carcinoma (HCC) is a malignancy of liver and a leading cause of cancer mortality worldwide. Its management is compounded by biological and clinical heterogeneity. These interindividual genetic variations can modulate the effects of HCC treatment. High-mobility group box protein 1 (HMGB1) is a well investigated, ubiquitous nuclear protein found in eukaryotic cells that plays a multiple biological roles such as DNA stability, program cell death, immune response, and furthermore in cancer progression. In this report, we examined *HMGB1* single nucleotide polymorphisms (SNPs) with multiple risk factors related to HCC susceptibility and clinicopathological characteristics. Four *HMGB1* SNPs (rs1412125, rs2249825, rs1045411, and rs1360485) were assessed by using a TaqMan SNPs Genotyping in 324 patients with HCC and in 695 cancer-free controls. The results showed that *HMGB1* SNP rs1045411 with CT or at least one T alleles has lower risk of HCC than wild-type (CC) carriers. Moreover, *HMGB1* SNP rs1412125 with TT allele has a higher risk of distant metastasis compared with patients carrying at least one C allele. The present study is the first report to discuss the risk factors associated with *HMGB1* SNPs in HCC progression in Taiwan.

Key words: *HMGB1*; HCC; SNP; Susceptibility

Introduction

Worldwide, hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third leading cause of cancer-related deaths [1]. The global incidence of HCC varies considerably, with particularly high rates in Southeast Asia and sub-Saharan Africa, and lower, but increasing rates, in North America and most of Europe [2]. In Taiwan, HCC is the second leading cause of cancer-related deaths [3, 4]. Enormous studies have indicated that high percentage of HCC progress with chronic liver disease. The progression of HCC is a multiple process which is affected by hepatitis B virus or hepatitis C virus infection, liver fibrosis and cirrhosis, alcohol addiction and hereditary [5, 6].

High-mobility group box protein 1 (*HMGB1*) is a highly conserved, well studied, ubiquitous nuclear protein that is found in mammals [7, 8]. *HMGB1* has DNA binding domains which helps stabilizing nuclear homeostasis and DNA repair [9]. *HMGB1* is also expressed in cytosol and secreted into the

extracellular space. Therefore, *HMGB1* has enormous biological functions and serves as key component in enormous diseases such as inflammatory diseases and tumor [10-14].

Genetic variation plays a crucial role in an individual's susceptibility and progression to cancer. Currently, genotyping single nucleotide polymorphism (SNPs) of a population and comparing the distribution frequency of SNPs among subgroups (e.g., patients and controls) is commonly used to evaluate the risk and prognosis of a cancer [15]. Accumulating evidence suggests an association between SNPs in certain genes and HCC susceptibility. For example, specific SNPs in cathepsin B, the enhancer of zeste 2 (EZH2) gene and plasminogen activator inhibitor contribute to the development of HCC [16-18].

HMGB1 is implicated in HCC development and progression [19]. However, the correlation between *HMGB1* SNPs, HCC risk and prognosis is poorly discussed. Therefore, we investigated a case-control study of four SNPs of *HMGB1* to examine the correlation between these four SNPs and HCC susceptibility and clinicopathologic characteristics.

Materials and Methods

Enrollment of participants and collection of specimens

This study consisted of 324 patients of histologically confirmed HCC from 2007 to 2012 at the Chung Shan Medical University Hospital, Taiwan. All 695 control subjects were recruited at the same hospital without previous cancer history. All the subjects in the study were Han Chinese with the same ethnicity. The patients with HCC were staged according to the Tumor size, Lymph Nodes affected, Metastases (TNM) staging system developed by the American Joint Committee on Cancer (2002) [20]. The questionnaire survey was performed with study subjects to obtain information of sociodemographic characteristics, cigarette smoking and alcohol consumption status. All clinicopathological characteristics were verified by chart review. Diagnosis of liver cirrhosis was assessed by biopsy, MRI, CT or biochemical evidence of liver parenchymal damage with endoscopic esophageal or gastric varices.

The blood samples which obtained from the controls and HCC patients were stored in EDTA tubes, centrifuged immediately and stored at −80°C. The Institutional Review Board of Chung Shan Medical University Hospital and informed written consent of all subjects were obtained before this study.

Genomic DNA extraction

Total genomic DNA from whole blood specimens were isolated by QIAamp DNA blood mini kits (Qiagen, Valencia, CA), following the manufacturer's instructions. DNA was dissolved in TE buffer [10 mM Tris (pH 7.8), 1 mM EDTA] and stored at −20°C until performing Real-time quantitative PCR analysis.

Real-time quantitative PCR

Total four SNPs of *HMGB1* (rs1412125, rs2249825, rs1045411, and rs1360485) were examined by using TaqMan SNPs Genotyping Assays (Applied Biosystems, Warrington, UK), according to the manufacturer's protocols. For the study, genotyping was performed in a blinded fashion without clinical data, and 10% of assays were repeated from different batches for monitoring genotyping quality. Several cases of each genotype were further examined by the DNA sequence analysis to validate results from the PCR analysis [21, 22].

Statistical analysis

Genotype distribution of each SNP was used to assess Hardy–Weinberg equilibrium (HWE) and confirmed by chi-square analysis. The distributions of demographic characteristics between control individuals and patients with HCC were examined by using Mann–Whitney U test and Fisher's exact test. The correlation between genotype frequencies, HCC cancer risk and clinicopathologic characteristics were assessed by adjusted odds ratios (AORs) with 95% confidence intervals (CIs). Multiple logistic regression models were utilized to calculate the estimates after controlling for age, gender, alcohol consumption, and tobacco use. A p value of <0.05 was considered statistically significant. Data were analyzed using SAS statistical software (Version 9.1, 2005; SAS Institute Inc., Cary, NC).

Results

Sociodemographic characteristics and clinical parameters for both the healthy controls and patients with HCC are shown in Table 1. HCC patients were predominantly male with a mean age of approximately 63 years. A significant association was observed between HCC development and alcohol consumption ($p<0.001$), whereas no such significant between-group difference was found in the distribution of tobacco use ($p=0.738$) between healthy controls and patients with HCC. To reduce possible interference of confounding variables, AORs with 95% CIs were estimated by multiple logistic regression models after controlling for age and gender in each comparison.

Genotype distributions and the association between HCC and healthy controls with *HMGB1* polymorphisms are shown in Table 2. All selected gene markers in our control group were statistically analyzed and proved to be in the Hardy-Weinberg equilibrium (p>0.05). The alleles with the highest distribution frequency at *HMGB1* rs1412125, rs2249825, rs1045411, and rs1360485 in both HCC patients and controls were homozygous T/T, homozygous C/C, homozygous C/C, and homozygous T/T. Individuals carrying CT or CT + TT at rs1045411 showed a 0.716-fold (95% CI: 0.533-0.961, p <0.05) and a 0.724-fold (95% CI: 0.546-0.961, p <0.05) lower risk of HCC. Individuals with polymorphisms at rs1412125, rs2249825 and rs1360485 showed no reduction in HCC risk compared with wild-type individuals.

HMGB1 genotypes in HCC patients were evaluated to clarify the role of *HMGB1* polymorphisms in regard to clinical TNM stage, primary tumor size, lymph node metastasis, distant metastasis, vascular invasion, Child–Pugh grade, presence of an HBV or HCV infection, and liver cirrhosis. No significant differences were observed between *HMGB1* rs1412125 genotypes and clinicopathlogic status, except distant metastasis (OR: 0.309; 95% CI: 0.099–0.960; p<0.05), as shown in Table 3.

AFP, AST, and ALT are common clinical pathological markers of HCC [23]. We therefore examined potential associations between the *HMGB1* gene polymorphisms and levels of several serum markers of HCC. No significant associations were found between the levels of these HCC clinical pathologic markers and genotypes of any *HMGB1* SNPs in HCC patients (Table 4).

Table 1. The distributions of demographical characteristics and clinical parameters in 695 controls and 324 patients with HCC.

Variable	Controls (N=695)	Patients (N=324)	p value
Age (yrs)	Mean ± S.D.	Mean ± S.D.	
	52.17 ± 10.11	62.83 ± 11.77	p<0.001*
Gender	n (%)	n (%)	
Male	570 (82.0%)	233 (71.9%)	
Female	125 (18.0%)	91 (28.1%)	p <0.001*
Alcohol consumption			
No	578 (83.2%)	201 (62.0%)	
Yes	117 (16.8%)	123 (38.0%)	p<0.001*
Tobacco consumption			
No	402 (57.8%)	191 (59.0%)	
Yes	293 (42.2%)	133 (41.0%)	p =0.738
Stage			
I+II		214 (66.0%)	
III+IV		110 (34.0%)	
Tumor T status			
≤T2		217 (67.0%)	
>T2		107 (33.0%)	
Lymph node status			
N0		313 (96.6%)	
N1+N2		11 (3.4%)	
Metastasis			
M0		306 (94.4%)	
M1		18 (5.6%)	
vascular invasion			
No		267 (82.4%)	
Yes		57 (17.6%)	

Mann-Whitney U test or Fisher's exact test was used between controls and patients with HCC.

* p value < 0.05 as statistically significant

Table 2. Distribution frequency of *HMGB1* genotypes in 695 controls and 324 patients with HCC.

Variable	Controls (N=695) n (%)	Patients (N=324) n (%)	OR (95% CI)	AOR (95% CI)
rs1412125				
TT	374 (53.8%)	173 (53.4%)	1.00	1.00
TC	275 (39.6%)	130 (40.1%)	1.022 (0.776-1.346)	0.833 (0.583-1.190)
CC	46 (6.6%)	21 (6.5%)	0.987 (0.571-1.705)	0.889 (0.456-1.732)
TC+CC	321 (46.2%)	151 (46.6%)	1.017 (0.781-1.325)	0.841 (0.600-1.181)
rs2249825				
CC	521 (75.0%)	235 (72.5%)	1.00	1.00
CG	163 (23.5%)	83 (25.6%)	1.129 (0.831-1.533)	1.380 (0.940-2.024)
GG	11 (1.5%)	6 (1.9%)	1.209 (0.442-3.309)	1.262 (0.362-4.399)
CG+GG	174 (25.0%)	89 (27.5%)	1.134 (0.842-1.528)	1.371 (0.994-1.993)
rs1045411				
CC	425 (61.2%)	223 (68.8%)	1.00	1.00
CT	239 (34.4%)	89 (27.5%)	0.710 (0.530-0.951)* p=0.022	0.716 (0.533-0.961)* p=0.026
TT	31 (4.4%)	12 (3.7%)	0.738 (0.372-1.465)	0.794 (0.398-1.584)
CT+TT	270 (38.8%)	101 (31.2%)	0.713 (0.539-0.944)* p=0.018	0.724 (0.546-0.961)* p=0.025
rs1360485				
TT	399 (57.4%)	192 (59.3%)	1.00	1.00
TC	257 (37.0%)	188 (36.4%)	0.954 (0.723-1.260)	1.086 (0.764-1.543)
CC	39 (5.6%)	14 (4.3%)	0.746 (0.396-1.407)	0.935 (0.438-1.998)
TC+CC	296 (42.6%)	132 (40.7%)	0.927 (0.709-1.211)	1.065 (0.760-1.493)

The odds ratios (ORs) and with their 95% confidence intervals (CIs) were estimated by logistic regression models. The adjusted odds ratios (AORs) with their 95% confidence intervals (CIs) were estimated by multiple logistic regression models after controlling for age, gender, alcohol and tobacco consumption. * p value < 0.05 as statistically significant.

Table 3. Odds ratio (OR) and 95% confidence interval (CI) of clinical status and *HMGB1* rs1412125 genotypic frequencies in 324 HCC patients.

Variable	Genotypic frequencies			
	TT (N=173)	TC+CC (N=151)	OR (95% CI)	p value
Clinical Stage				
Stage I/II	115 (66.5%)	99 (65.6%)	1.00	p=0.862
Stage III/IV	58 (33.5%)	52 (34.4%)	1.041 (0.657-1.651)	
Tumor size				
≦ T2	116 (67.1%)	101 (66.9%)	1.00	p=0.975
> T2	57 (32.9%)	50 (33.1%)	1.007 (0.633-1.602)	
Lymph node metastasis				
No	167 (96.5%)	146 (96.7%)	1.00	p=0.938
Yes	6 (3.5%)	5 (3.3%)	0.953 (0.285-3.188)	
Distant metastasis				
No	159 (92.3%)	147 (97.4%)	1.00	p=0.033*
Yes	14 (8.1%)	4 (2.6%)	0.309 (0.099-0.960)	
Vascular invasion				
No	139 (80.3%)	128 (84.8%)	1.00	p=0.297
Yes	34 (19.7%)	23 (15.2%)	0.735 (0.411-1.313)	
Child-Pugh grade				
A	130 (75.1%)	116 (76.8%)	1.00	p=0.725
B or C	43 (24.9%)	35 (23.2%)	0.912 (0.547-1.522)	
HBsAg				
Negative	99 (57.2%)	88 (58.3%)	1.00	p=0.848
Positive	74 (42.8%)	63 (41.7%)	0.958 (0.616-1.490)	
Anti-HCV				
Negative	93 (53.8%)	78 (51.7%)	1.00	p=0.705
Positive	80 (46.2%)	73 (48.3%)	1.088 (0.703-1.685)	
Liver cirrhosis				
Negative	31 (17.9%)	34 (22.5%)	1.00	p=0.303
Positive	142 (82.1%)	117 (77.5%)	0.751 (0.436-1.295)	

The ORs with analyzed by their 95% CIs were estimated by logistic regression models.

> T2: multiple tumor more than 5 cm or tumor involving a major branch of the portal or hepatic vein(s)

* p value < 0.05 as statistically significant.

Table 4. Association of *HMGB1* genotypic frequencies with HCC laboratory status.

Characteristic	α-Fetoprotein [a] (ng/mL)	AST [a] (IU/L)	ALT [a] (IU/L)	AST/ALT ratio [a]
rs1412125				
TT	4083.0 ± 1321.3	128.7 ± 15.9	122.6 ± 17.8	1.50 ± 0.13
TC+CC	2310.6 ± 1012.3	148.9 ± 30.0	109.3 ± 18.4	1.50 ± 0.10
p value	0.298	0.540	0.607	0.987
rs2249825				
CC	2412.9 ± 751.6	149.5 ± 21.9	125.6 ± 16.9	1.46 ± 0.10
CG+GG	5485.8 ± 2363.3	107.9 ± 13.7	92.1 ± 13.1	1.60 ± 0.14
p value	0.106	0.257	0.243	0.456
rs1045411				
CC	2953.9 ± 900.8	147.6 ± 22.9	121.1 ± 17.4	1.50 ± 0.11
CT+TT	3916.5 ± 1857.3	117.4 ± 14.6	106.0 ± 14.8	1.50 ± 0.13
p value	0.599	0.390	0.584	0.973
rs1360485				
TT	2804.9 ± 916.4	146.7 ± 23.0	129.3 ± 19.9	1.49 ± 0.12
TC+CC	3914.6 ± 1604.6	125.6 ± 22.1	97.6 ± 12.0	1.51 ± 0.11
p value	0.521	0.526	0.224	0.870

Mann-Whitney U test was used between two groups.

[a] Mean ± S.E.

Discussion

HCC is one of the most common and lethal malignancies in the world, so preventing its occurrence and reducing its mortality rate are amongst the most important challenges faced by public healthcare. Major etiologies for HCC in Taiwan include infection with HBV or HCV, alcohol consumption, a history of liver cirrhosis, and family history of HCC [24, 25]. The data in Table 1 indicate that the ratios of tobacco smokers/nonsmokers in

control subjects (42.2: 57.8) and HCC patients (41:59) were almost equal. However, the number of subjects who had consumed alcohol among patients with HCC was higher (38%) than that among controls (16.8%). Increasingly, reports are providing evidence showing that alcohol consumption is a risk factor for HCC [26, 27]. Our results imply that the risk of developing HCC is higher with alcohol than it is with smoking.

Accumulating evidence has shown that progressive genomic changes can potentially alter cell phenotypes and assist preneoplastic lesions to develop into HCC. Genetic polymorphisms and somatic mutations are associated with the risk of HCC [28], while multiple gene alterations (e.g., allelic deletions, insertions, polymorphisms, mutations and methylation changes) cause genetic and molecular aberrations, which are also able to lead to the formation of HCC [29]. The genetic component is therefore widely acknowledged to be a pivotal factor for HCC risk. Thus, extensive genetic information and statistical comparisons of genetic variations between patients and healthy subjects has become an accepted and practical means of searching for genetic markers that predict the risk and pathological development of HCC.

HMGB1 plays multiple roles with inside or outside of cell, such as chromatin stabilization, DNA repair, gene transcription, program cell death regulation, and immune response. In recent studies, *HMGB1* has been implicated in tumor progression such as colon, breast, oral, and lung cancer [30-32]. Previous review has indicated the role of *HMGB1* in HCC progression [19]. Wang et al., also has been summarized the HMGB1 signaling pathway in HCC development [33]. In addition, Bi et al., found that HMGB1 upregulated with HCC progression [34]. However, the correlation between *HMGB1* polymorphisms and HCC progression is never discussed.

In present work, four *HMGB1* SNPs were assessed by SNP genotyping in 324 patients with HCC and in 695 healthy controls. The result indicated that CT or CT + TT at rs1045411 polymorphism is significantly correlated with reduced HCC risk. In contrast, polymorphisms at rs1412125, rs2249825 and rs1360485 showed no reduction in HCC risk compared with healthy controls. We further examined the correlation between rs1045411 polymorphism and clinicopathlogic status but showed no significant differences (Supplementary Data Table S1). It is well demonstrated that polymorphisms in the 3′-flanking region of a gene could regulate gene expression [35]. The rs1045411 polymorphism locates in the 3′-flanking region and maybe affect *HMGB1* gene expression. Our data indicated that individuals with HCC carrying at least one C allele at rs1412125 showed a lower risk of distant metastasis. This SNP is indicated to regulate binding of transcription factors for example GATA-1, GATA-2, GATA-3, and Lmo2 because its location in CCAAT box of DNA binding motif [36]. The detail functional of rs1412125 has not been discussed in present study. More experiments should been perform to elucidated the role of *HMGB1* polymorphism in HCC progression.

The present study provides novel insight about the SNPs in *HMGB1* on HCC susceptibility and clinicopathology. However, the limitation of this work is lacked patient's survival data. Therefore, whether *HMGB1* polymorphisms link survival of HCC patients are needs further examination. In addition, larger study which contains more individuals is needed to examine the functions of *HMGB1* polymorphisms in HCC progression. In conclusion, this report first provides a correlation between *HMGB1* polymorphisms and HCC risk. Our investigation of *HMGB1* polymorphisms may provide novel insight to develop it as helpful prognosis marker for HCC treatment.

Supplementary Material

Table S1. http://www.medsci.org/v13p0304s1.pdf

Acknowledgments

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Competing Interests

All of the authors confirm that they have no financial or personal relationships with other people or organizations that could inappropriately influence this work.

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