Medicine®

OBSERVATIONAL STUDY

OPEN

Effects of *RAGE* Gene Polymorphisms on the Risk and Progression of Hepatocellular Carcinoma

Shih-Chi Su, PhD, Ming-Ju Hsieh, PhD, Ying-Erh Chou, PhD, Wen-Lang Fan, PhD, Chao-Bin Yeh, MD, PhD, and Shun-Fa Yang, PhD

Abstract: Hepatocellular carcinoma (HCC) is a common malignancy of the liver, whose heterogeneous incidence reflects genetic variations among individuals in the main risk factors. The receptor for advanced glycosylation endproducts (RAGE) is a multiligand receptor and known to be implicated in various pathogenic conditions, such as diabetes, inflammatory disorder, Alzheimer disease, and cancer. In this study, the impact of *RAGE* gene polymorphisms on the susceptibility to hepatocarcinogenesis was explored. Four single-nucleotide polymorphisms (SNPs), rs184003 (1704G > T), rs1800624 (−374T > A), rs1800625 (−429T > C), and rs2070600 (Gly82Ser), as well as 1 gene polymorphism of *RAGE* gene, a 63 bp deletion allele (−407 to −345) were analyzed between 300 cancer-free subjects and 265 HCC cases. We detected a significant association of rs1800625 with the increased risk of HCC (odds ratio [OR], 2.565; 95% confidence interval [CI], 1.492–4.409 and adjusted odds ratio [AOR], 2.568; 95% CI, 1.418–4.653). However, patients who possess at least 1 polymorphic allele of rs1800625 are less prone to develop late-stage (stage III/IV, OR, 0.502; 95% CI, 0.243–1.037; $P = 0.059$ and AOR, 0.461; 95% CI, 0.219–0.970; $P = 0.041$) and large-size tumors (OR, 0.398; 95% CI, 0.183–0.864; $P = 0.017$ and AOR, 0.351; 95% CI, 0.157–0.781; $P = 0.010$). Furthermore, individuals bearing specific haplotypes of 4 RAGE SNPs tested are more inclined to have HCC. In conclusion, our data suggest a correlation of *RAGE* gene polymorphism rs1800625 with the early stage of liver tumorigenesis and implicate its protective role in the progression of HCC.

(*Medicine* 94(34):e1396)

Abbreviations: HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, RAGE = receptor for advanced glycosylation endproducts, SNP = single-nucleotide polymorphism.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies in the world and the third most common cause of cancer-related death.[1] The incidence of HCC is inconstant worldwide with the highest rates in Southeast Asia and Sub-Saharan Africa.[2] Although it is estimated that 70%–90% of HCC develops within an established background of chronic liver disease,[3] the carcinogenesis of HCC is a complex process that is associated with miscellaneous risk factors, including but not limited to exposure of aflatoxin B, chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), excessive consumption of alcohol and tobacco, iron overload, and diabetes.[4] Recently, numerous lines of evidence have revealed that single-nucleotide polymorphisms (SNPs) may be associated with hepatocarcinogenesis independently or in combination with established major risks in defined populations.[5–7] These studies highlight variations within individual genomes that modulate oxidative stress, deoxyribonucleic acid (DNA) repair, iron metabolism, cell signaling, inflammatory, and immune responses as genetic predispositions toward liver tumorigenesis and partly explain the observed differences in the risk of HCC occurrence.

The receptor for advanced glycosylation endproducts (RAGE) is a member of the immunoglobulin superfamily of cell surface molecules whose broad repertoire of ligands potentiates the orchestration of numerous cell signaling pathways to regulate a number of cellular responses.[8] Other than the full-length protein with a wide spectrum of ligand specificities, a great variety of RAGE transcript variants with distinct functions is generated owing to alternative splicing, producing N- and C-terminally truncated isoforms that act as endogenous modulators of the RAGE receptor through competitive ligand binding or by displacing the intact protein in the plasma membrane.[9] The biology of RAGE is largely dictated by the juxtaposition of different forms of RAGE and its ligands at pathologic sites, leading to sustained dysregulation of RAGE-dependent cell responses. Cumulative evidence has indicated that RAGE be implicated in various pathophysiological processes, including inflammatory disorders, renal disease, Alzheimer disease, diabetic arteriosclerosis, tumorigenesis, and metastasis.[10–13]

Since the liver is central to the clearance and catabolism of circulating advanced glycosylation endproducts (AGEs), a cognate ligand of RAGE, the AGE-RAGE axis has been shown to be functionally involved in the development of various liver diseases and liver carcinogenesis.[14,15] Moreover, it has been demonstrated that high-mobility group box 1 (HMGB1), another RAGE ligand, promote the invasion and metastasis of HCC through the activation of RAGE and TLR4 signaling

Editor: Giovanni Tarantino.
Received: February 24, 2015; revised: July 25, 2015; accepted: July 27, 2015.
From the Whole-Genome Research Core Laboratory of Human Diseases, Chang Gung Memorial Hospital, Keelung (S-CS, W-LF); Department of Dermatology, Drug Hypersensitivity Clinical and Research Center, Chang Gung Memorial Hospital, Linkou (S-CS); Cancer Research Center, Changhua Christian Hospital, Changhua (M-JH); Institute of Medicine (M-JH, S-FY); Department of Forensic Medicine, Chung Shan Medical University (Y-EC); Department of Medical Research (Y-EC, S-FY); Department of Emergency Medicine, Chung Shan Medical University Hospital (C-BY); and Department of Emergency Medicine, School of Medicine, Chung Shan Medical University, Taichung, Taiwan (C-BY).
Correspondence: Shun-Fa Yang, Chao-Bin Yeh, Institute of Medicine, Chung Shan Medical University, 110 Chien-Kuo N. Road, Section 1, Taichung 402, Taiwan (e-mail: ysf@csmu.edu.tw, sky5ff@gmail.com).
The authors have no funding and conflicts of interest to disclose.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000001396

and the resultant induction of multiple inflammatory mediators under hypoxic conditions.[16] A function of RAGE in association with hepatocarcinogenesis is further supported by the finding that serum level of soluble RAGE (sRAGE), known as a decoy to avoid interaction of RAGE with its proinflammatory ligands, is inversely correlated with the risk of HCC.[17] However, little is known regarding the *RAGE* gene polymorphisms on the susceptibility to HCC. Here, we performed a hospital-based study to evaluate the impact of gene variations of RAGE on the development of HCC and observed an association of a RAGE SNP (rs1800625) with the risk and progression of HCC.

MATERIALS AND METHODS

Subjects

This study was approved by the institutional review board of Chung Shan Medical University Hospital in Taichung, Taiwan. Subjects, including 265 patients with HCC and 300 cancer-free controls, were recruited in this investigation from 2006 to 2013, and all participants provided informed written consent at enrollment. The diagnoses of HCC were confirmed histologically in all cases. During the same study period, ethnically matched individuals who have neither diagnosed with HCC nor self-reported history of cancer of any sites were enrolled as the controls. The TNM classification of the American Joint Committee on Cancer (AJCC) was used for staging of HCC.[18] Diagnosis of liver cirrhosis was carried out by liver biopsy, abdominal sonography, or biochemical data of hepatic parenchymal damage with endoscopic management of esophageal or gastric varices. Other clinical and pathological parameters, including clinical staging, tumor volume, lymph-node metastasis, distant metastasis, presence of HBV surface antigen (HBsAg), reactivity with antibodies against HCV (anti-HCV), liver cirrhosis, the levels of α-fetoprotein, aspartate aminotransferase, and alanine aminotransferase, were obtained from the chart reviews.

Demographic Information

Data on age, gender, alcohol consumption, and tobacco use were recorded from each participant. Alcohol consumption is defined as having up to an average of more than 2 drinks per day. Tobacco use is defined as current smoking of at least 1 cigarette per day during the latest 3 months.

Polymorphism Selection

In this study, the selection of 5 well-characterized common polymorphisms from *RAGE* gene is based on their wide associations with the development of many types of malignancies.[19–24] The 5 polymorphisms include rs1800625, rs1800624, and the 63 bp deletion (−407 to −345) in the 5′ flanking region, rs2070600 in exon 3, and rs184003 in intron 7.

RAGE Genotyping

Genomic DNA was isolated from 3 mL of blood using QIAamp DNA blood mini kits (Qiagen, Valencia, CA). Assessment of allelic discrimination for the *RAGE* rs1800625 (assay IDs: C_8848033_1), rs1800624 (assay IDs: C_3293837_1), rs2070600 (assay IDs: C_15867521_20), and rs184003 (assay IDs: C_2412456_10) SNP was performed by using the TaqMan assay with an Applied Biosystems StepOne Real-Time polymerase chain reaction (PCR) System (Applied Biosystems, Foster City, CA), and further evaluated with Sequence Detection System version 3.0 software (Applied Biosystems). The total volume of TaqMan assays was 10 μL, containing 5 μL of Master Mix, 0.25 μL of probes, and 10 ng of genomic DNA. The real-time PCR reaction included an initial denaturation step at 95 °C for 10 minutes, followed by 40 amplification cycles, each consisting of 95 °C for 15 seconds and 60 °C for 1 minutes. In addition, the 63 bp deletion (−407 to −345) allelic polymorphisms were assessed with PCR as described previously.[25] In brief, the reaction was initiated at 94 °C for 1.5 minutes, followed by 35 amplification cycles consisting of 15 seconds of denaturation at 95 °C, 30 seconds of annealing at 60 °C, and 30 seconds of elongation at 72 °C, and a final extension at 72 °C for 10 minutes (Forward: 5-CCTGGGTTTAGTTGAGAATTTTTT-3; Reverse: 5-ATTCATGCCTTTGGGACAAGAG-3). The products were separated on a 3% agarose gel and then stained with ethidium bromide. The product sizes are expected to be 327 and 390 bp for the deleted and major allele, respectively.

Statistical Analysis

A goodness-of-fit v2 test was used to evaluate Hardy–Weinberg equilibrium for biallelic markers. The differences in demographic parameters between HCC patients and cancer-free controls were estimated by using Fisher exact test or Mann–Whitney *U* test. The adjusted odds ratios (AORs) with their 95% confidence intervals (CIs) obtained by multiple logistic regression models after controlling for other covariates were used to assess the correlation of genotype frequencies with the risk of liver cancer plus clinical characteristics. The haplotype-based analysis was conducted using the Phase program.[26] A P value < 0.05 was considered significant. The data were processed by using SAS statistical software (Version 9.1, 2005; SAS Institute Inc., Cary, NC).

RESULTS

Characteristics of Study Participants

The statistical analysis of demographic characteristics between 2 study groups (300 normal controls and 265 patients with HCC) is shown in Table 1. Since age and gender were identified as risk factors for the occurrence of HCC,[27,28] age-compatible ($P = 0.793$) and gender-compatible ($P = 0.615$) controls were enrolled in this study to compare with HCC patients. The average age of patients at onset of HCC in this study is 62.99 ± 11.97. Males are more likely to develop HCC

TABLE 1. Distributions of Demographical Characteristics in 300 Controls and 265 Patients With HCC

Variable	Controls (N = 300)	Patients (N = 265)	*P* Value
Age, y	Mean ± SD	Mean ± SD	
	62.75 ± 10.33	62.99 ± 11.97	$P = 0.793$
Gender	n (%)	n (%)	
Male	207 (69.0%)	188 (70.9%)	
Female	93 (31.0%)	77 (29.1%)	$P = 0.615$
Alcohol consumption			
No	174 (58.0%)	170 (64.2%)	
Yes	126 (42.0%)	95 (35.8%)	$P = 0.135$
Tobacco consumption			
No	187 (62.3%)	160 (60.4%)	
Yes	113 (37.7%)	105 (39.6%)	$P = 0.613$

than females. However, neither alcohol consumption nor tobacco use was found to elevate the risk of developing HCC.

Association of *RAGE* Gene Polymorphisms With HCC

A functional role of RAGE expression in the development of HCC has been previously demonstrated,[15] yet a correlation of *RAGE* gene polymorphisms with hepatocarcinogenesis remains undefined. Four SNPs, rs184003 (1704G > T), rs1800624 (−374T > A), rs1800625 (−429T > C), and rs2070600 (Gly82-Ser), as well as 1 gene polymorphism of *RAGE* gene, a 63 bp deletion allele (−407 to −345) that are shown to be widely associated with many types of malignancies were evaluated in this investigation. Genotype frequencies of these 5 RAGE polymorphisms and their correlation with the predisposition to liver cancer are summarized in Table 2. No deviation ($P > 0.05$) from Hardy–Weinberg equilibrium in both case and control groups was achieved for all 5 polymorphisms. To attenuate the possible interference of other confounders, we performed comparisons using OR (with 95% CI) together with AOR (with 95% CI) which was estimated after adjustment for age, gender, alcohol consumption, and tobacco use by multiple logistic regression models. Among *RAGE* gene polymorphisms examined, individuals who carry 1 minor allele of rs1800625 (TC) are more inclined to develop HCC with the OR being 2.565 (95% CI, 1.492–4.409) and AOR being 2.568 (95% CI, 1.418–4.653). As joined with the homozygous genotype (CC) for rs1800625, its correlation with the susceptibility to HCC is fortified (OR, 2.732; 95% CI, 1.614–4.625 and AOR, 2.808; 95% CI, 1.581–4.985). Nevertheless, we failed to detect any significant association of the other 4 RAGE variants individually with the incidence of liver cancer between the 2 groups.

Correlation Between Polymorphic Genotypes of *RAGE* and Clinical Status of HCC

Due to a correlation of rs1800625 with the risk of HCC detected in this investigation, the associations between the *RAGE* gene polymorphisms and clinicopathologic characteristics of HCC patients were further explored (Table 3). We found that patients who carry at least 1 polymorphic allele of rs1800625 (heterozygote or homozygote for the minor allele) are less prone to develop late-stage (stage III/IV, OR, 0.502; 95% CI, 0.243–1.037; $P = 0.059$ and AOR, 0.461; 95% CI, 0.219–0.970; $P = 0.041$) and large-size tumors (OR, 0.398; 95% CI, 0.183–0.864; $P = 0.017$ and AOR, 0.351; 95% CI, 0.157–0.781; $P = 0.010$). However, no significant association of rs1800625 polymorphism with lymph node metastasis, distant metastasis, Child–Pugh classification, prevalence of HBV and HCV infections, and cirrhosis was observed, indicating that rs1800625 variants may negatively regulate the tumor cell proliferation but not invasion and differentiation.

In addition, we also explored the potential association between the *RAGE* gene polymorphisms and the levels of

TABLE 2. Genotype Distributions of *RAGE* Gene Polymorphisms in 300 Controls and 265 Patients With HCC

Variable	Controls (N = 300) n (%)	Patients (N = 265) n (%)	OR (95% CI)	AOR (95% CI)
rs1800625				
TT	277 (92.3%)	216 (81.5%)	1.00	1.00
TC	22 (7.4%)	44 (16.6%)	2.565 (1.492–4.409)*	2.568 (1.418–4.653)*
CC	1 (0.3%)	5 (1.9%)	6.412 (0.744–55.289)	8.074 (0.850–76.729)
TC + CC	23 (7.7%)	49 (18.5%)	2.732 (1.614–4.625)*	2.808 (1.581–4.985)*
rs1800624				
TT	220 (73.3%)	210 (79.2%)	1.00	1.00
TA	72 (24.0%)	49 (18.5%)	0.713 (0.474–1.074)	0.828 (0.526–1.304)
AA	8 (2.7%)	6 (2.3%)	0.786 (0.268–2.303)	0.825 (0.238–2.862)
TA + AA	80 (26.7%)	55 (20.8%)	0.720 (0.487–1.066)	0.828 (0.534–1.283)
rs2070600				
GG	179 (59.7%)	160 (60.4%)	1.00	1.00
GA	112 (37.3%)	88 (33.2%)	0.879 (0.619–1.249)	0.824 (0.551–1.231)
AA	9 (3.0%)	17 (6.4%)	2.113 (0.916–4.874)	1.908 (0.737–4.938)
GA + AA	121 (40.3%)	105 (39.6%)	0.971 (0.693–1.360)	0.903 (0.614–1.329)
rs184003				
GG	208 (69.3%)	163 (61.5%)	1.00	1.00
GT	81 (27.0%)	91 (34.3%)	1.434 (0.997–2.061)	1.608 (0.997–2.562)
TT	11 (3.7%)	11 (4.2%)	1.276 (0.540–3.017)	1.139 (0.425–3.049)
GT + TT	92 (30.7%)	102 (38.5%)	1.415 (0.998–2.005)	1.489 (0.989–2.306)
63 bp deletion				
INS/INS	280 (93.3%)	242 (91.3%)	1.00	1.00
INS/Del	20 (6.7%)	23 (8.7%)	1.331 (0.713–2.482)	1.283 (0.644–2.555)
Del/Del	0 (0%)	0 (0%)	–	–
INS/Del + Del/Del	20 (6.7%)	23 (8.7%)	1.331 (0.713–2.482)	1.283 (0.644–2.555)

The ORs and with their 95% CIs were estimated by logistic regression models. The AORs with their 95% confidence intervals (CIs) were estimated by multiple logistic regression models after controlling for age, gender, tobacco, and alcohol consumption. *P value < 0.05 as statistically significant. AOR = adjusted odds ratio, CI = confidence interval, HCC = hepatocellular carcinoma, RAGE = receptor for advanced glycosylation endproducts, OR = odds ratio.

TABLE 3. Associations Between Polymorphic Genotypes of rs1800625 and Clinicopathologic Characteristics of HCC

Variable	Genotypic Frequencies			
	TT (N = 216) n, %	TC + CC (N = 49) n, %	OR (95% CI) *P* Value	AOR (95% CI) *P* Value
Clinical stage				
Stage I/II	137 (63.4%)	38 (77.6%)	1.00	1.00
Stage III/IV	79 (36.6%)	11 (22.4%)	0.502 (0.243–1.037) $P = 0.059$	0.461 (0.219–0.970) $P = 0.041*$
Tumor size				
≦T2	138 (63.9%)	40 (81.6%)	1.00	1.00
>T2	78 (36.1%)	9 (18.4%)	0.398 (0.183–0.864) $P = 0.017^*$	0.351 (0.157–0.781) $P = 0.010*$
Lymph node metastasis				
No	208 (96.3%)	48 (98.0%)	1.00	1.00
Yes	8 (3.7%)	1 (2.0%)	0.542 (0.066–4.434) $P = 0.562$	0.562 (0.068–4.676) $P = 0.594$
Distant metastasis				
No	207 (95.8%)	46 (93.9%)	1.00	1.00
Yes	9 (4.2%)	3 (6.1%)	1.500 (0.391–5.758) $P = 0.552$	1.681 (0.428–6.601) $P = 0.457$
Child–Pugh grade				
A	167 (77.3%)	36 (73.5%)	1.00	1.00
B or C	49 (22.7%)	13 (26.5%)	1.231 (0.605–2.502) $P = 0.566$	1.210 (0.592–2.474) $P = 0.601$
HBsAg				
Negative	124 (57.4%)	33 (67.3%)	1.00	1.00
Positive	92 (42.6%)	16 (32.7%)	0.653 (0.339–1.258) $P = 0.201$	0.666 (0.341–1.301) $P = 0.234$
Anti-HCV				
Negative	114 (52.8%)	22 (44.9%)	1.00	1.00
Positive	102 (47.2%)	27 (55.1%)	1.372 (0.736–2.558) $P = 0.319$	1.327 (0.703–2.504) $P = 0.383$
Liver cirrhosis				
Negative	53 (24.5%)	9 (18.4%)	1.00	1.00
Positive	163 (75.5%)	40 (81.6%)	1.445 (0.658–3.174) $P = 0.357$	1.521 (0.686–3.371) $P = 0.302$

The ORs with analyzed by their 95% CIs were estimated by logistic regression models. The AORs with their 95% CI were estimated by multiple logistic regression models, after controlling for age, gender, tobacco, and alcohol consumption. >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. AOR = adjusted odds ratio, CI = confidence interval, HBsAg = hepatitis B virus surface antigen, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, OR = odds ratio.

several serum markers of HCC, including α-fetoprotein, alanine transaminase, and aspartate transaminase. As a consequence, no significant difference in the serum levels of these markers was detected between patients who possess at least 1 polymorphic allele and those who do not for any of the RAGE SNPs examined (Table 4).

Association of RAGE Haplotypes With HCC

The relationship of RAGE haplotypes with the risk of developing HCC was also evaluated. The frequency distributions of 5 common RAGE rs1800624, rs1800625, rs184003, and rs2070600 haplotypes are shown in Table 5, with the most frequent haplotype in the controls ($T_{-429}T_{-374}G_{82Gly}G_{1704}$) being chosen as the reference. We found that all these common RAGE haplotypes exhibit significant associations with increased susceptibility to HCC ($T_{-429}T_{-374}A_{82Ser}G_{1704}$: OR, 1.569; 95%CI, 1.187–2.073; $T_{-429}T_{-374}G_{82Ser}T_{1704}$: OR, 1.721; 95%CI, 1.290–2.295; $C_{-429}T_{-374}G_{82Ser}G_{1704}$: OR, 3.109; 95% CI, 2.042–4.732) with the exception of $T_{-429}A_{-374}G_{82Ser}G_{1704}$. As the risk for HCC was associated with these 4 haplotypes, we further determined whether the diplotypes comprising these haplotypes influence the risk of HCC more remarkably than each individual haplotype. As shown in Table 6, a 2.509-fold increase in the risk (95% CI = 1.590–3.959) for HCC was detected in non-TTGG carriers, suggesting a correlation of *RAGE* gene polymorphisms with the susceptibility to liver cancer.

DISCUSSION

A convergence of numerous lines of evidence has indicated that development of HCC is a complex process affected by both inherited and acquired factors. In the present study, we for the first time revealed that the *RAGE* gene polymorphism rs1800625 raises the susceptibility to HCC and serves as a protective factor from developing the late-stage and large-size tumors in the Taiwanese population.

Recently, rs1800625 from the *RAGE* gene is shown to be widely associated with the development or aggressiveness of many types of malignancies, including lung, kidney, and oral cancer.[20,23,24] Among these cancer types, in addition to rs1800625, many *RAGE* gene polymorphisms also play a prominent role in the cancer progression. Here, we observed a significant correlation of rs1800625 with the occurrence of HCC but no difference in genotype frequencies for rs1800624, rs184003, rs2070600, and a 63 bp deletion allele (−407 to −345) of *RAGE* gene between the cases and controls. In contrast, 2 different groups working on the impact of the *RAGE* gene polymorphisms on predisposition to breast cancer failed to

TABLE 4. Association of *RAGE* Genotypic Frequencies With HCC Laboratory Status

Characteristic	AFP*, ng/mL	AST*, IU/L	ALT*, IU/L	AST/ALT Ratio*
rs1800625				
TT	3056.0 ± 900.9	167.3 ± 23.7	140.6 ± 18.8	1.56 ± 0.11
TC/CC	4574.7 ± 2924.7	123.5 ± 26.7	98.0 ± 17.1	1.41 ± 0.19
P value	0.518	0.395	0.290	0.568
rs1800624				
TT	3530.9 ± 1076.6	160.9 ± 23.2	124.8 ± 16.0	1.61 ± 0.12
TA/AA	2595.8 ± 1534.9	152.7 ± 38.3	163.0 ± 44.3	1.25 ± 0.10
p value	0.678	0.867	0.323	0.138
rs2070600				
GG	2504.5 ± 1047.3	153.1 ± 25.5	134.1 ± 22.3	1.44 ± 0.14
GA/AA	4605.1 ± 1649.7	168.6 ± 32.3	130.6 ± 20.3	1.67 ± 0.14
P value	0.260	0.704	0.913	0.263
rs184003				
GG	4364.9 ± 1313.5	156.1 ± 27.7	124.8 ± 19.1	1.56 ± 0.11
GT/TT	1693.8 ± 1074.6	164.1 ± 27.3	145.3 ± 26.9	1.50 ± 0.19
P value	0.153	0.846	0.524	0.782
63 bp deletion				
INS/INS	3581.2 ± 993.3	158.6 ± 20.8	137.2 ± 16.9	1.53 ± 0.10
INS/Del + Del/Del	764.8 ± 611.9	165.8 ± 73.3	85.2 ± 28.9	1.56 ± 0.37
P value	0.384	0.919	0.350	0.940

Mann–Whitney *U* test was used to compare 2 groups. AFP = α-fetoprotein, ALT = alanine transaminase, AST = aspartate transaminase, HCC = hepatocellular carcinoma, RAGE = receptor for advanced glycosylation endproducts.
* Mean ± SE.

demonstrate any evident association between rs1800625 and the risk of breast cancer.[19,29] Our data, together with findings from others, suggest that there is a genetic heterogeneity across various forms of tumors, and these polymorphisms of small effect can cumulatively contribute to tumorigenesis, although different cancer types may share the same risk alleles.

RAGE is a membrane protein that comprises an ectodomain, which is responsible for ligand engagement, a single transmembrane helix, and a cytosolic unstructured tail, which mediates intracellular signaling.[30] Numerous RAGE isoforms that may influence the RAGE signaling are favorably generated by alternative processing of mRNA or proteolytic breakdown of full-length RAGE under certain conditions.[8,31] Alterations in the proportion of a particular isoform to the full-length RAGE have been proposed to act as a prognostic marker for various human diseases.[8] In addition to the presence of multiple variant forms, the actions of RAGE can be mostly explained by its affinity for a broad spectrum of ligands. RAGE ligands consist of several structurally distinct families, including the prototype of High Mobility Group family proteins, high-mobility group protein B1 (HMGB1)/amphoterin,[32] members of the S100/calgranulin protein family,[33,34] and extracellular matrix proteins such as collagen type I and IV,[35] β-amyloid,[36] phosphatidylserine,[37] complement C3a,[38] and some AGEs.[39] Recent observations have suggested that HMGB1, a type of damage-associated molecular pattern molecules (DAMPs), modulates the development and metastasis of HCC through activating RAGE signaling.[16,40–42] Another cognate ligand of RAGE, the AGEs, has also been shown to interact with the RAGE receptor to promote the development of liver carcinogenesis.[15,43] Notably, we observed that RAGE haplotype with the polymorphic allele of Gly82Ser (rs2070600), a

TABLE 5. The Estimated Haplotype Frequencies of 4 Examined Polymorphisms in *RAGE* Gene and the Corresponding Risk for HCC

Variable							
rs1800625 T/C	rs1800624 T/A	rs2070600 G/A	rs184003 G/T	Controls (N = 600) n, %	Patients (N = 530) n, %	OR (95% CI)	*P* Value
T_{-429}	T_{-374}	G_{82Gly}	G_{1704}	285 (47.5%)	181 (34.2%)	Reference	
T_{-429}	T_{-374}	A_{82Ser}	G_{1704}	110 (18.3 %)	121 (22.8%)	1.732 (1.260–2.381)	0.001
T_{-429}	T_{-374}	G_{82Gly}	T_{1704}	94 (15.7%)	113 (21.3%)	1.893 (1.359–2.636)	<0.001
T_{-429}	A_{-374}	G_{82Gly}	G_{1704}	67 (11.1%)	60 (11.3%)	1.410 (0.950–2.093)	0.087
C_{-429}	T_{-374}	G_{82Gly}	G_{1704}	19 (3.2%)	54 (10.2%)	4.475 (2.569–7.795)	<0.001
Others*				25 (4.2%)	1 (0.2%)	—	—

CI = confidence interval, HCC = hepatocellular carcinoma, OR = odds ratio, RAGE = receptor for advanced glycosylation endproducts.
* Others: $T_{-429}T_{-374}A_{82Ser}T_{1704}$ (14; control: 14; patient: 0), $C_{-429}A_{-374}G_{82Gly}G_{1704}$ (8; control: 8; patient: 0), $T_{-429}A_{-374}A_{82Ser}G_{1704}$ (4; control: 3; patient: 1).

TABLE 6. Frequencies of RAGE Haplotype Pairs in Control Subjects and Patients With HCC

Variable	Controls (N = 300) n (%)	Patients (N = 265) n (%)	OR (95% CI)	*P*
TTGG/TTGG	79 (26.3%)	30 (11.3%)	Reference	
TTGG/non-TTGG*	127 (42.3 %)	121 (45.7%)	2.509 (1.539–4.089)	<0.001
non-TTGG/non-TTGG	94 (31.4%)	114 (43.0%)	3.194 (1.935–5.272)	<0.001
TTGG/non-TTGG and non-TTGG/non-TTGG	221 (73.7%)	235 (88.7%)	2.800 (1.770–4.430)	<0.001

CI = confidence interval, HCC = hepatocellular carcinoma, OR = odds ratio, RAGE = receptor for advanced glycosylation endproducts.
* Non-TTGG represents any haplotype other than $T_{-429}T_{-374}G_{82Gly}G_{1704}$ and comprises $T_{-429}T_{-374}A_{82Ser}G_{1704}$, $T_{-429}T_{-374}G_{82Gly}T_{1704}$, $T_{-429}A_{-374}G_{82Gly}G_{1704}$, $C_{-429}T_{-374}G_{82Gly}G_{1704}$, $T_{-429}T_{-374}A_{82Ser}T_{1704}$, $C_{-429}A_{-374}G_{82Gly}G_{1704}$, and $T_{-429}A_{-374}A_{82Ser}G_{1704}$.

nonsynonymous SNP that contributes to increased affinity for ligand binding,[44,45] is significantly correlated with increased risk of HCC (Table 5). Moreover, numerous studies have suggested that rs2070600 is pertinent to many types of malignancies, such as breast, gastric, and colon cancer.[21,46,47] These data, together with our result, indicate a role of the Ser82 allele in hepatocarcinogenesis due to leveraging the affinity between RAGE and its ligands.

Ligand binding not just triggers various cascades of RAGE intracellular signal pathways, but also enhances its expression.[48] Other than changing the ligand affinity or repertoire, alterations in the level of RAGE expression seem to be implicated in enhanced susceptibility to HCC indicated by our result on detecting the association of rs1800625 (−429T > C), a RAGE polymorphism situated in the 5′-flanking region, with the occurrence of HCC (Table 2). This observation is further bolstered by our haplotype analysis (Table 5). A functional study has shown that the polymorphic allele (C) of rs1800625 gives rise to heightened expression of RAGE.[49] In normal circumstances, the basal level of RAGE expression is low in all tissues, except the lung.[30] Nonetheless, under certain pathological conditions, such as diabetes, atherosclerosis, chronic inflammation, or neurodegenerative diseases, RAGE can be upregulated in a cell type- or tissue-restricted manner.[50–52] These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of normal hepatocytes into a malignant state, resulting in a higher risk of HCC. Intriguingly, we found that patients who bear the polymorphic allele of rs1800625 (heterozygote or homozygote for the minor allele) are less inclined to develop late-stage and large-size tumors. This is somewhat in concordance with the finding that hepatic RAGE expression may be relevant to the early tumorigenesis of HCC. Once cancer is established, HCC develops and dedifferentiates step-by-step while the expression of RAGE declines as the tumors grow into a more advanced HCC.[15] Overall, our results presented here demonstrate a functional impact of rs1800625 on alternations in RAGE expression, positively contributing to the early hepatocarcinogenesis but negatively regulating the development and progression of HCC.

Our data indicate an impact of gene variations of RAGE on the development of HCC; however, there are several limitations present in the study. One is that the effects of environmental risks on the susceptibility of liver cancer are limited and may be underestimated because of a potential exclusion of subjects who may have heavy tobacco use previously but are not current smokers or a lack of population stratification based on the amount or duration of alcohol use. Another weakness is that the potential heterogeneity, in term of the severity and subtype of liver cancer or the variety of HCC-related clinical manifestations, such as diabetes, non-alcoholic fatty liver disease, HBV, and HCV infection, within the HCC patients may validly lead to different conclusions about the effects of *RAGE* gene polymorphisms on the risk and progression of HCC. In addition, the results presented in this study may not be able to be extended to other populations unless replication studies are carried out.

Taken together, our results show that SNP rs1800625 of *RAGE* gene causally contributes to an increased risk of HCC. In addition, an inverse association of rs1800625 was detected with the progression of HCC. These findings indicate a novel genetic predisposition to liver tumorigenesis.

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin.* 2012;62:10–29.
2. Blechacz B, Mishra L. Hepatocellular carcinoma biology. *Recent Results Cancer Res.* 2013;190:1–20.
3. Sherman M. Hepatocellular carcinoma: epidemiology, surveillance, and diagnosis. *Semin Liver Dis.* 2010;30:3–16.
4. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet.* 2012;379:1245–1255.
5. Miki D, Ochi H, Hayes CN, et al. Hepatocellular carcinoma: towards personalized medicine. *Cancer Sci.* 2012;103:846–850.
6. Nahon P, Zucman-Rossi J. Single nucleotide polymorphisms and risk of hepatocellular carcinoma in cirrhosis. *J Hepatol.* 2012;57:663–674.
7. Jin F, Xiong WJ, Jing JC, et al. Evaluation of the association studies of single nucleotide polymorphisms and hepatocellular carcinoma: a systematic review. *J Cancer Res Clin Oncol.* 2011;137:1095–1104.
8. Xie J, Mendez JD, Mendez-Valenzuela V, et al. Cellular signalling of the receptor for advanced glycation end products (RAGE). *Cell Signal.* 2013;25:2185–2197.
9. Sterenczak KA, Nolte I, Murua Escobar H. RAGE splicing variants in mammals. *Methods Mol Biol.* 2013;963:265–276.
10. Lee EJ, Park JH. Receptor for advanced glycation endproducts (rage), its ligands, and soluble RAGE: potential biomarkers for diagnosis and therapeutic targets for human renal diseases. *Genom Inform.* 2013;11:224–229.
11. Park S, Yoon SJ, Tae HJ, et al. RAGE and cardiovascular disease. *Front Biosci.* 2011;16:486–497.
12. Sims GP, Rowe DC, Rietdijk ST, et al. HMGB1 and RAGE in inflammation and cancer. *Ann Rev Immunol.* 2010;28:367–388.
13. Ramasamy R, Yan SF, Schmidt AM. RAGE: therapeutic target and biomarker of the inflammatory response – the evidence mounts. *J Leukoc Biol.* 2009;86:505–512.
14. Hyogo H, Yamagishi S. Advanced glycation end products (AGEs) and their involvement in liver disease. *Curr Pharm Des.* 2008;14:969–972.

15. Hiwatashi K, Ueno S, Abeyama K, et al. A novel function of the receptor for advanced glycation end-products (RAGE) in association with tumorigenesis and tumor differentiation of HCC. *Ann Surg Oncol.* 2008;15:923–933.
16. Yan W, Chang Y, Liang X, et al. High-mobility group box 1 activates caspase-1 and promotes hepatocellular carcinoma invasiveness and metastases. *Hepatology.* 2012;55:1863–1875.
17. Moy KA, Jiao L, Freedman ND, et al. Soluble receptor for advanced glycation end products and risk of liver cancer. *Hepatology.* 2013;57:2338–2345.
18. Vauthey JN, Lauwers GY, Esnaola NF, et al. Simplified staging for hepatocellular carcinoma. *J Clin Oncol.* 2002;20:1527–1536.
19. Pan H, He L, Wang B, et al. The relationship between RAGE gene four common polymorphisms and breast cancer risk in northeastern Han Chinese. *Sci Rep.* 2014;4:4355.
20. Pan H, Niu W, He L, et al. Contributory role of five common polymorphisms of RAGE and APE1 genes in lung cancer among Han Chinese. *PloS One.* 2013;8:e69018.
21. Gu H, Yang L, Sun Q, et al. Gly82Ser polymorphism of the receptor for advanced glycation end products is associated with an increased risk of gastric cancer in a Chinese population. *Clin Cancer Res.* 2008;14:3627–3632.
22. Zhang S, Hou X, Zi S, et al. Polymorphisms of receptor for advanced glycation end products and risk of epithelial ovarian cancer in Chinese patients. *Cell Physiol Biochem.* 2013;31:525–531.
23. Su S, Chien M, Lin C, et al. RAGE gene polymorphism and environmental factor in the risk of oral cancer. *J Dent Res.* 2015;94:403–411.
24. Chocholaty M, Jachymova M, Schmidt M, et al. Polymorphisms of the receptor for advanced glycation end-products and glyoxalase I in patients with renal cancer. *Tumour Biol.* 2015;36:2121–2126.
25. Schenk S, Schraml P, Bendik I, et al. A novel polymorphism in the promoter of the RAGE gene is associated with non-small cell lung cancer. *Lung Cancer.* 2001;32:7–12.
26. Stephens M, Scheet P. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. *Am J Hum Genet.* 2005;76:449–462.
27. Venook AP, Papandreou C, Furuse J, et al. The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. *Oncologist.* 2010;15(Suppl 4):5–13.
28. Bruno S, Savojardo D, Almasio PL, et al. Critical reappraisal of risk factors for occurrence of hepatocellular carcinoma in patients with hepatitis C virus. *Hepat Med.* 2011;3:21–28.
29. Hashemi M, Moazeni-Roodi A, Arbabi F, et al. Genotyping of -374A/T, -429A/G, and 63 bp Ins/del polymorphisms of RAGE by rapid one-step hexaprimer amplification refractory mutation system polymerase chain reaction in breast cancer patients. *Nucleosides Nucleotides Nucleic acids.* 2012;31:401–410.
30. Neeper M, Schmidt AM, Brett J, et al. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem.* 1992;267:14998–15004.
31. Kierdorf K, Fritz G. RAGE regulation and signaling in inflammation and beyond. *J Leukoc Biol.* 2013;94:55–68.
32. Hori O, Brett J, Slattery T, et al. The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. Mediation of neurite outgrowth and co-expression of rage and amphoterin in the developing nervous system. *J Biol Chem.* 1995;270:25752–25761.
33. Hofmann MA, Drury S, Fu C, et al. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell.* 1999;97:889–901.
34. Leclerc E, Fritz G, Vetter SW, et al. Binding of S100 proteins to RAGE: an update. *Biochim Biophys Acta.* 2009;1793:993–1007.
35. Demling N, Ehrhardt C, Kasper M, et al. Promotion of cell adherence and spreading: a novel function of RAGE, the highly selective differentiation marker of human alveolar epithelial type I cells. *Cell Tissue Res.* 2006;323:475–488.
36. Yan SD, Chen X, Fu J, et al. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature.* 1996;382:685–691.
37. He M, Kubo H, Morimoto K, et al. Receptor for advanced glycation end products binds to phosphatidylserine and assists in the clearance of apoptotic cells. *EMBO Rep.* 2011;12:358–364.
38. Ruan BH, Li X, Winkler AR, et al. Complement C3a, CpG oligos, and DNA/C3a complex stimulate IFN-alpha production in a receptor for advanced glycation end product-dependent manner. *J Immunol.* 2010;185:4213–4222.
39. Sparvero LJ, Asafu-Adjei D, Kang R, et al. RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation. *J Translat Med.* 2009;7:17.
40. Chen RC, Yi PP, Zhou RR, et al. The role of HMGB1-RAGE axis in migration and invasion of hepatocellular carcinoma cell lines. *Mol Cell Biochem.* 2014;390:271–280.
41. Cheng P, Dai W, Wang F, et al. Ethyl pyruvate inhibits proliferation and induces apoptosis of hepatocellular carcinoma via regulation of the HMGB1-RAGE and AKT pathways. *Biochem Biophys Res Commun.* 2014;443:1162–1168.
42. Pusterla T, Nemeth J, Stein I, et al. Receptor for advanced glycation endproducts (RAGE) is a key regulator of oval cell activation and inflammation-associated liver carcinogenesis in mice. *Hepatology.* 2013;58:363–373.
43. Takino J, Yamagishi S, Takeuchi M. Glycer-AGEs-RAGE signaling enhances the angiogenic potential of hepatocellular carcinoma by upregulating VEGF expression. *World J Gastroenterol.* 2012;18:1781–1788.
44. Hudson BI, Stickland MH, Grant PJ. Identification of polymorphisms in the receptor for advanced glycation end products (RAGE) gene: prevalence in type 2 diabetes and ethnic groups. *Diabetes.* 1998;47:1155–1157.
45. Hofmann MA, Drury S, Hudson BI, et al. RAGE and arthritis: the G82S polymorphism amplifies the inflammatory response. *Genes Immunity.* 2002;3:123–135.
46. Qian F, Sun BL, Zhang WY, et al. Gly82Ser polymorphism of the receptor for advanced glycation end-product (RAGE) potential high risk in patients with colorectal cancer. *Tumour Biol.* 2014;35:3171–3175.
47. Tesarova P, Kalousova M, Jachymova M, et al. Receptor for advanced glycation end products (RAGE) – soluble form (sRAGE) and gene polymorphisms in patients with breast cancer. *Cancer Invest.* 2007;25:720–725.
48. Clynes R, Moser B, Yan SF, et al. Receptor for AGE (RAGE): weaving tangled webs within the inflammatory response. *Curr Mol Med.* 2007;7:743–751.
49. Hudson BI, Stickland MH, Futers TS, et al. Effects of novel polymorphisms in the RAGE gene on transcriptional regulation and their association with diabetic retinopathy. *Diabetes.* 2001;50:1505–1511.
50. Basta G. Receptor for advanced glycation endproducts and atherosclerosis: From basic mechanisms to clinical implications. *Atherosclerosis.* 2008;196:9–21.
51. Yan SF, Ramasamy R, Schmidt AM. Receptor for AGE (RAGE) and its ligands-cast into leading roles in diabetes and the inflammatory response. *J Mol Med.* 2009;87:235–247.
52. Yan SF, Yan SD, Ramasamy R, et al. Tempering the wrath of RAGE: an emerging therapeutic strategy against diabetic complications, neurodegeneration, and inflammation. *Ann Med.* 2009;41:408–422.