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

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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 multifunctional receptor 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 (1704Q > T), rs1800624 (−374T > A), rs1800625 (−429T > C), and rs2070600 (Gly82Ser), as well as one 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, 4.409 and adjusted odds ratio [AOR], 2.568; 95% CI, 1.418–4.653). 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.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies in the world and the third most common cause of cancer-related death. The incidence of HCC is constant worldwide with the highest rates in Southeast Asia and Sub-Saharan Africa. Although it is estimated that 70%–90% of HCC develops within an established background of chronic liver disease, 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. 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. 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. 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. 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 pathophysiologic processes, including inflammatory disorders, renal disease, Alzheimer disease, diabetic arteriosclerosis, tumorgenesis, and metastasis.

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. 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.

Abbreviations: HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, RAGE = receptor for advanced glycosylation endproducts, SNP = single-nucleotide polymorphism.
and the resultant induction of multiple inflammatory mediators under hypoxic conditions. 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. 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. 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. The 5 polymorphisms include rs1800625, rs1800624, and the 63 bp deletion (−407 to −345) in the 5’ flanking region, rs2070600 in exon 3, and rs1800623 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 rs1800623 (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) allele polymorphisms were assessed with PCR as described previously. 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-CCTGGGTTTAGTTGAGAATT-3; Reverse: 5-ATTCAATGCCCTTTGGGACAAGA-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 (ORs) 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. 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 and 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.

| Variable                        | Controls (N = 300) | Patients (N = 265) | P Value |
|--------------------------------|-------------------|-------------------|---------|
| Age, y                         | Mean ± SD         | Mean ± SD         | P = 0.793 |
| Male                           | 62.75 ± 10.33     | 62.99 ± 11.97     |         |
| Female                         | 62.43 ± 10.32     | 62.99 ± 11.97     |         |
| Alcohol consumption            |                   |                   |         |
| No                             | 207 (69.0%)       | 188 (70.9%)       |         |
| Yes                            | 93 (31.0%)        | 77 (29.1%)        |         |
| Tobacco consumption            |                   |                   |         |
| No                             | 174 (58.0%)       | 170 (64.2%)       |         |
| Yes                            | 126 (42.0%)       | 95 (35.8%)        |         |

TABLE 1. Distributions of Demographical Characteristics in 300 Controls and 265 Patients With HCC
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, yet a correlation of RAGE gene polymorphisms with hepatocarcinogenesis remains undefined. Four SNPs, rs184003 (1704G > A), 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 (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 = 0.100). 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

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**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         |
|         |                          | 44 (16.6%)              | 2.565 (1.492–4.409) | 2.568 (1.418–4.653) |
|         |                          | 5 (1.9%)                | 6.412 (0.744–55.289) | 8.074 (0.850–76.729) |
|         |                          | 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         |
|         |                          | 49 (18.5%)              | 0.713 (0.474–1.074) | 0.828 (0.526–1.304) |
|         |                          | 6 (2.3%)                | 0.786 (0.268–2.303) | 0.825 (0.238–2.862) |
|         |                          | 55 (20.8%)              | 0.720 (0.487–1.066) | 0.824 (0.534–1.283) |
| rs2070600 |                          |                          |             |              |
| GG       | 179 (59.7%)              | 160 (60.4%)              | 1.00        | 1.00         |
|         |                          | 89 (33.2%)              | 0.879 (0.619–1.249) | 0.824 (0.551–1.231) |
|         |                          | 17 (6.4%)               | 2.113 (0.916–4.874) | 1.908 (0.737–4.938) |
| GA       | 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         |
|         |                          | 91 (34.3%)              | 1.434 (0.997–2.061) | 1.608 (0.997–2.562) |
|         |                          | 11 (4.2%)               | 1.276 (0.540–3.017) | 1.139 (0.425–3.049) |
| GA       | 121 (40.3%)              | 105 (39.6%)              | 0.971 (0.693–1.360) | 0.903 (0.614–1.329) |
| 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/Ins + 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.
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-429T, 374G82SerG1704) being chosen as the reference. We found that all these common RAGE haplotypes exhibit significant associations with increased susceptibility to HCC (T-429T, 374G82SerG1704): OR, 1.569; 95%CI, 1.187–2.073; T-429T, 374G82SerT1704: OR, 1.721; 95%CI, 1.290–2.295; C-429T, 374G82SerG1704: OR, 3.109; 95% CI, 2.042–4.732 with the exception of T-429A, 374G82SerG1704. As the risk for HCC 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. 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, 345) of hepatitis B virus surface antigen, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, OR = odds ratio.
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. 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. Alterations in the proportion of a particular isoform to the full-length RAGE have been proposed to act as a prognostic marker for 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 Polymorphisms in the Risk of HCC

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

| Characteristic | Variable | Controls (N = 600) n, % | Patients (N = 530) n, % | OR (95% CI) | P Value |
|---------------|----------|------------------------|------------------------|-------------|---------|
| rs1800625 TT  | G3/G3    | 285 (47.5%)            | 181 (34.2%)            | Reference   |         |
| rs1800625 TC  | G3/G2    | 110 (18.3%)            | 121 (22.8%)            | 1.732 (1.260–2.381) | 0.001   |
| rs1800625 CC  | G2/G2    | 94 (15.7%)             | 113 (21.3%)            | 1.893 (1.359–2.636) | <0.001  |
| rs1800624 TT  | A3/A3    | 67 (11.1%)             | 60 (11.3%)             | 1.410 (0.950–2.093) | 0.087   |
| rs1800624 TC  | A3/A2    | 19 (3.2%)              | 54 (10.2%)             | 4.475 (2.569–7.795) | <0.001  |
| rs1800624 CC  | A2/A2    | 25 (4.2%)              | 25 (4.2%)              | —           | —       |

CI = confidence interval, HCC = hepatocellular carcinoma, OR = odds ratio, RAGE = receptor for advanced glycosylation endproducts.

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

| Variable | Controls (N = 600) n, % | Patients (N = 530) n, % | OR (95% CI) | P Value |
|----------|------------------------|------------------------|-------------|---------|
| r2070600 TT  | G3/G3                 | 285 (47.5%)            | 181 (34.2%)            | Reference   |         |
| r2070600 TC  | G3/G2                 | 110 (18.3%)            | 121 (22.8%)            | 1.732 (1.260–2.381) | 0.001   |
| r2070600 CC  | G2/G2                 | 94 (15.7%)             | 113 (21.3%)            | 1.893 (1.359–2.636) | <0.001  |
| rs2070600 TT  | A3/A3                 | 67 (11.1%)             | 60 (11.3%)             | 1.410 (0.950–2.093) | 0.087   |
| rs2070600 TC  | A3/A2                 | 19 (3.2%)              | 54 (10.2%)             | 4.475 (2.569–7.795) | <0.001  |
| rs2070600 CC  | A2/A2                 | 25 (4.2%)              | 25 (4.2%)              | —           | —       |

CI = confidence interval, HCC = hepatocellular carcinoma, OR = odds ratio, RAGE = receptor for advanced glycosylation endproducts.

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nonsynonymous SNP that contributes to increased affinity for ligand binding,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 (\textasciitilde 429T \textgreater 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.\textsuperscript{49} In normal circumstances, the basal level of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of RAGE expression is low in all tissues, except the lung.\textsuperscript{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.\textsuperscript{50–52} These suggest that increased RAGE expression by either genetic or other factors may confer the transformation of
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