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Impact of *RAGE* polymorphisms on urothelial cell carcinoma clinicopathologic characteristics and long-term survival

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Abstract

Objectives: Urothelial cell carcinoma (UCC) is a common malignancy of the urinary tract that is associated with environmental factors and genetic effects. Receptor for advanced glycosylation endproducts (RAGE) plays a vital role in chronic inflammation and tumorigenesis. This study explored the impact of RAGE gene polymorphisms on the clinicopathological status and prognosis of patients with UCC.

Materials and methods: A total of 1,023 participants, including 431 patients with UCC and 592 healthy controls, were recruited for this study. Four polymorphisms of the RAGE gene, including -429T>C (rs1800625), -374T>A (rs1800624), Gly82Ser (rs2070600), and 1704G>T (rs184003), were examined using a real-time polymerase chain reaction with the TaqMan assay.

Results: We found that individuals carrying at least 1 C allele at *RAGE* rs1800625 had a higher risk of developing UCC than those carrying a wild-type allele. Subgroup analysis revealed that among UCC patients aged younger than 65 years, those with a C allele at rs1800625 had a higher incidence of muscle invasive tumor and lymph node invasion. With a median follow-up of 39 months, TT polymorphisms of rs184003 were associated with worse disease-specific survival (HR = 7.58, 95% CI = 2.52−22.77, $P < 0.001$) and all-cause survival (HR = 6.45, 95% CI = 2.19−19.034, $P < 0.001$) in UCC patients aged younger than 65 years.

Conclusion: This study is the first to report a correlation between *RAGE* polymorphisms and UCC risk. Individuals that carry at least 1 C allele at rs1800625 of *RAGE* are associated with a higher risk of UCC development, particularly if they belong to the categories previously identified. TT polymorphism at rs184003 is associated with worse disease-specific survival and overall survival in people aged ≤65 years.

Keywords: Receptor for advanced glycosylation endproducts; Polymorphism; Urothelial cell carcinoma

1. Introduction

Urothelial cell carcinoma (UCC) is the most common malignancy of the urinary tract with 150,350 new diagnoses reported in 2018 and 33,170 deaths in the United States [1]. Among bladder cancers (BCs) developed from urothelium, 95% are derived from UCC, and the global incidence is 9.0 and 2.2 per 100,000 person/years for men and women, respectively, with a mortality rate of 3.2% for men and 0.9% for women [2]. Nonmuscular-invasive disease constitutes 75% of BC diagnoses, and the standard treatment is transurethral resection of the bladder tumor followed by bacillus Calmette-Guerin or intravesical chemotherapy,

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with a 5-year survival rate of approximately 81% [3]. For the other 25% of patients diagnosed with muscular-invasive disease, radical cystectomy or transurethral resection of the bladder tumor followed by combined chemotherapy and radiatiotherapy is the preferred treatment, despite the likelihood of subsequent recurrence or distal metastasis. For this approach, the 5-year survival rate is only 47% [4].

Genetic factors have emerged as potential bio-markers and are associated with the staging and prognosis of BC [5]. For example, TP-53, which is a known tumor suppression gene and activator of DNA repair, is associated with advanced disease, poor response to treatment, and unfavorable outcomes [6]. Recently, several studies have revealed the association between single nucleotide polymorphism (SNP) and the tumorigenesis of BC, which represents a major risk to defined populations [7−9]. Gene variations within individual genomes that regulate telomere maintenance, apoptosis, and inflammation may act as predispositional factors toward the risk of BC tumorigenesis and prognosis [10].

The receptor for advanced glycosylation endproducts (RAGE) is a member of the immunoglobulin superfamily. It acts as a receptor with broad spectrum ligand specificities that regulate the innate immunity [11]. RAGE senses a variety of signaling molecules, including high-mobility group box 1 (HMGB1), advanced glycation end products, S100/calgranulins, phosphatidylserine, C3a, β-amyloid, and advanced oxidation protein products. It triggers the intracellular signaling pathways to control the cellular processes, such as cell proliferation, inflammation, apoptosis, and autophagy, and leads to tumorigenesis [12,13]. Emerging evidence has indicated that RAGE acts as response to cellular damage associated with various pathophysiological processes including inflammatory disorders, Alzheimer's disease, diabetes, renal disease, and malignant disorders such as tumor progression and metastasis [14−16].

The gene of RAGE is located on chromosome 6p21.3 and is composed of 11 exons, 10 introns, and a 3′UTR. It is the multifunctional receptor that mediates responses to cell stress and cell damage [17]. The majority of genetic variants in RAGE are SNPs, and these types of variations can potentially alter the function and expression of RAGE, thus bridging inflammation and carcinogenesis [18,19]. Recent studies have revealed the link between RAGE genetic polymorphisms and various types of cancer development and progression such as lung, breast, ovarian, colorectal, and oral cancers as well as hepatocellular carcinoma [20−25]. However, it has been shown in animal models that blocking the RAGE pathway decreases tumor metastases, tumor growth, and subsequently inhibits tumor proliferation and invasion [26].

One previous study has revealed that mRNA expression of RAGE in UCC cancer tissue is a possible useful biomarker [27]. Nevertheless, the association between RAGE variants and UCC risk and prognosis is limited. The present study investigated the relationships between 4 SNPs (rs1800625, rs1800624, rs2070600, and rs184003) in the RAGE gene and UCC susceptibility and clinicopathologic characteristics.

2. Materials and methods

2.1. Subjects and specimen collection

This study recruited 431 patients at Taichung Veterans General Hospital in Taichung, Taiwan in 2011 to 2015. During the same period, 592 ethnic individuals without malignant history before were enrolled as cancer-free control group. For the UCC group, all patients have pathologic proved UCC of urinary bladder and staging by pathologist according to TNM staging system of the American Joint Committee on Cancer Staging Manual (7th ed.). Histopathologic grading was defined as high grade and low grade papillary tumor according to 2004 WHO grading system. Metastasis status was evaluated base on regular compute tomography during follow-up. In this study, approval (No. CF11094) was obtained from the Institutional Review Board of Taichung Veterans General Hospital.

2.2. Polymorphism selection

Four well-investigated common polymorphisms from RAGE gene were selected for examination based on their association with the development of malignancies and the 4 polymorphisms include rs1800625 (-429T/C), rs1800624 (-374T/A) in promoter region, rs2070600 (Gly82Ser) in exon 3, and rs184003 (1704G>T) in intron 7 [22−25,28,29].

2.3. Genomic DNA extraction

Genomic DNA was isolated from whole blood specimens using QIAamp DNA blood mini kits (Qiagen, Valencia, CA). DNA was dissolved in TE buffer and stored at −20°C until acting Real-time quantitative polymerase chain reaction analysis. Four SNPs of RAGE (rs1800625, rs1800624, rs2070600, and rs184003) were examined using TaqMan SNPs Genotyping Assays (Applied Biosystems, Warrington, UK) with an Applied Biosystems StepOne Real-Time polymerase chain reaction System (Applied Biosystems, Foster City, CA) (Supplementary Figure S1).

2.4. Statistical analysis

Mann-Whitney *U* test and Fisher exact test were used to compare the differences in demographic characteristics between controls and UCC group. The adjusted odds ratios (AORs) with 95% confidence intervals (CIs) for the association between genotype frequencies and clinicopathologic characteristics were estimated by multiple logistic regression models, after controlling for other covariates. End point evaluation for disease specific mortality and all cause

of death were examined using Kaplan Meier survival curve and multiple Cox proportional hazards model after controlling for age, gender and tobacco consumption. A P value <0.05 was considered significant. All data were analyzed with Statistical Analytic System (SAS Institute, Cary, NC) software (vers. 9.1, 2005) for Windows.

3. Results

3.1. Characteristics of study participants

The demographic characteristics of the two participant groups in our study (592 controls and 431 patients with UCC) were analyzed and are shown in Table 1. The mean age of patients with UCC was 68.6 ± 11.8 years, with males being the predominant subgroup ($n = 272$, 63.1%). At diagnosis, 235 and 196 patients had non-muscular-invasive (54.5%) and muscular-invasive (45.5%) tumors, respectively, and the histopathologic report confirmed that 378 (87.7%) and 53 patients (12.3%) had high-grade and low-grade tumors, respectively. In addition, 51 patients (11.8%) had lymph-node-related cancers and 14 patients (3.2%) had confirmed metastasis lesions, as determined by a contrast-enhanced computed tomography survey. To diminish possible interferences, we estimated the AORs with a 95% CI by adjusting for age, sex, and tobacco consumption.

3.2. Association of RAGE gene polymorphisms with UCC

The distribution frequency of *RAGE* genotypes with 4 SNPs in 592 health controls and 431 patients with UCC are shown in Table 2. Genotypic distributions of RAGE rs1800625, rs2070600, and rs184003 conformed to Hardy-Weinberg equilibrium ($P = 0.280$, χ^2 value: 1.167; $P = 0.218$, χ^2 value: 1.514; and $P = 0.965$, χ^2 value: 0.002, respectively). For both groups, the highest distribution frequency was demonstrated by homozygous TT at rs1800625 and rs1800624 and by homozygous GG at rs2070600 and rs184003. Those patients carrying at least 1 C allele at rs1800625 (TC or CC) had a higher UCC risk than those carrying the wild type (AOR = 1.90, 95% CI = 1.21–2.98, $P = 0.005$). In addition, patients with the AA genotype at rs2070600 appeared to have a higher risk of disease after adjustment (AOR = 2.26, 95% CI = 1.06–4.81, $P = 0.035$).

Subgroup analysis further demonstrated the risk of UCC in individuals with different genotype polymorphisms at rs1800625, as shown in Table 3. Individuals carrying at least one C allele at rs1800625 (TC + CC) appeared to have a high risk of disease if they belonged to the following categories: age ≤65 years (AOR = 1.76, 95% CI = 1.04–2.98, $P = 0.035$), female (AOR = 3.96, 95% CI = 1.47–10.65, $P = 0.006$), and no tobacco consumption (AOR = 3.17, 95% CI = 1.77–5.68, $P < 0.001$).

3.3. Correlation between RAGE SNPs and clinical status of UCC

Because the genotype polymorphism of the rs1800625 appeared to be correlated with a risk of UCC in this study, we further examined the association of clinicopathologic characteristics in patients aged younger than 65 years with UCC, as shown in Table 4. In

Table 1
The distributions of demographical characteristics in 592 controls and 431 patients with UCC

Variable	Controls ($N = 592$) n (%)	Patients ($N = 431$) n (%)	P value
Age (yrs)			
≦65	473 (79.9%)	166 (38.5%)	<0.001
>65	119 (20.1%)	265 (61.5%)	
Mean ± S.D.	51.0 ± 15.0	68.6 ± 11.8	<0.001
Gender			
Female	107 (18.1%)	159 (36.9%)	<0.001
Male	485 (81.9%)	272 (63.1%)	
Tobacco consumption			
No	368 (62.2%)	300 (69.6%)	0.014
Yes	224 (37.8%)	131 (30.4%)	
Stage			
Nonmuscle invasive tumor (pTa–pT1)		235 (54.5%)	
Muscle invasive tumor (pT2–pT4)		196 (45.5%)	
Lymph node status			
N0		380 (88.2%)	
N1+N2		51 (11.8%)	
Metastasis			
M0		417 (96.8%)	
M1		14 (3.2%)	
Histopathologic grading			
Low grade		53 (12.3%)	
High grade		378 (87.7%)	

Student's t test or chi-squared test was used between controls and patients with UCC.

Table 2
Genotype distributions of RAGE gene polymorphisms in 592 controls and 431 patients with UCC

Variable	Controls ($N = 592$) *n* (%)	Patients ($N = 431$) *n* (%)	OR (95% CI)	*P* value	AOR (95% CI)	*P* value
rs1800625						
TT	532 (89.9%)	359 (83.3%)	Reference		Reference	
TC	57 (9.6%)	64 (14.8%)	1.66 (1.14−2.44)	$P = 0.009$	1.69 (1.06−2.71)	$P = 0.028$
CC	3 (0.5%)	8 (1.9%)	3.95 (1.04−15.00)	$P = 0.043$	6.54 (1.40−30.52)	$P = 0.017$
TC+CC	60 (10.1%)	72 (16.7%)	1.78 (1.23−2.57)	$P = 0.002$	1.90 (1.21−2.98)	$P = 0.005$
rs1800624						
TT	435 (73.5%)	336 (78.0%)	Reference		Reference	
TA	136 (23.0%)	86 (20.0%)	0.82 (0.60−1.11)	$P = 0.199$	0.83 (0.57−1.20)	$P = 0.317$
AA	21 (3.5%)	9 (2.1%)	0.56 (0.25−1.23)	$P = 0.146$	0.86 (0.32−2.27)	$P = 0.756$
TT + AA	157 (26.5%)	95 (22.0%)	0.78 (0.59−1.05)	$P = 0.101$	0.83 (0.59−1.18)	$P = 0.305$
rs2070600						
GG	361 (61%)	270 (62.6%)	Reference		Reference	
GA	209 (35.3%)	140 (32.5%)	0.90 (0.69−1.17)	$P = 0.416$	0.86 (0.62−1.18)	$P = 0.351$
AA	22 (3.7%)	21 (4.9%)	1.28 (0.69−2.37)	$P = 0.439$	2.26 (1.06−4.81)	$P = 0.035$
GA + AA	231 (39.0%)	161 (37.4%)	0.93 (0.72−1.20)	$P = 0.589$	0.95 (0.70−1.30)	$P = 0.762$
rs184003						
GG	398 (67.2%)	300 (69.6%)	Reference		Reference	
GT	175 (29.6%)	118 (27.4%)	0.90 (0.68−1.18)	$P = 0.431$	1.01 (0.72−1.41)	$P = 0.968$
TT	19 (3.2%)	13 (3.0%)	0.91 (0.44−1.87)	$P = 0.792$	0.99 (0.43−2.27)	$P = 0.986$
GT + TT	194 (32.8%)	131 (30.4%)	0.90 (0.69−1.17)	$P = 0.420$	1.01 (0.73−1.39)	$P = 0.974$

The odds ratio (OR) with their 95% confidence intervals were estimated by logistic regression models.

The adjusted odds ratio (AOR) with their 95% confidence intervals were estimated by multiple logistic regression models after controlling for age, gender and tobacco consumption.

Table 3
Subgroup analysis of polymorphic genotypes of RAGE rs1800625 in 592 controls and 431 patients with UCC

	Controls *n* (%)	Patients *n* (%)	OR (95% CI)	*P* value	AOR (95% CI)	*P* value
Age ≦65[a]						
TT	425 (89.9%)	139 (83.7%)	Reference	$P = 0.037$	Reference	$P = 0.035$
TC + CC	48 (10.1%)	27 (16.3%)	1.72 (1.03−2.86)		1.76 (1.04−2.98)	
Age > 65[a]						
TT	107 (89.9%)	220 (83.0%)	Reference	$P = 0.082$	Reference	$P = 0.078$
TC + CC	12 (10.1%)	45 (17.0%)	1.82 (0.93−3.59)		1.84 (0.93−3.64)	
Female[b]						
TT	101 (94.4%)	132 (83.0%)	Reference	$P = 0.009$	Reference	$P = 0.006$
TC + CC	6 (5.6%)	27 (17.0%)	3.44 (1.37−8.66)		3.96 (1.47−10.65)	
Male[b]						
TT	431 (88.9%)	227 (83.5%)	Reference	$P = 0.035$	Reference	$P = 0.215$
TC + CC	54 (11.1%)	45 (16.5%)	1.58 (1.03−2.43)		1.41 (0.82−2.42)	
Without tobacco consumption[c]						
TT	337 (91.6%)	246 (82.0%)	Reference	$P < 0.001$	Reference	$P < 0.001$
TC + CC	31 (8.4%)	54 (18.0%)	2.39 (1.49−3.82)		3.17 (1.77−5.68)	
With tobacco consumption[c]						
TT	195 (87.1%)	113 (86.3%)	Reference	$P = 0.831$	Reference	$P = 0.365$
TC + CC	29 (12.9%)	18 (13.7%)	1.07 (0.57−2.02)		0.69 (0.31−1.53)	

The odds ratio (OR) with their 95% confidence intervals were estimated by logistic regression models.

The adjusted odds ratio (AOR) with their 95% confidence intervals was estimated by multiple logistic regression models.

[a] Adjusted for gender and tobacco consumption.

[b] Adjusted for age and tobacco consumption.

[c] Adjusted for age and gender.

this subgroup, patients with at least 1 C allele (TC + CC) had a higher incidence of muscular-invasive tumor (OR = 2.63, 95% CI = 1.11−6.27, $P = 0.029$), and lymph-node-related factors (OR = 3.36, 95% CI = 1.31−8.61, $P = 0.012$).

Regarding the risk of disease-specific mortality in 166 patients aged younger than 65 years (Table 5), patients with a TC alleles at rs1800625 had a higher risk of disease-specific mortality (AHR = 2.88, 95% CI = 1.25−6.60). This was also observed in patients with at least one C allele at

Table 4
Distribution frequency of the clinical status and RAGE rs1800625 genotype frequencies in 166 UCC patients aged younger than 65 years

Variable	RAGE (rs1800625)			
	TT (%) ($n = 139$)	TC + CC (%) ($n = 27$)	OR (95% CI)	P value
Stage				
Nonmuscle invasive tumor (pTa–pT1)	79 (56.8%)	9 (33.3%)	Reference	
Muscle invasive tumor (pT2–pT4)	60 (43.2%)	18 (66.7%)	2.63 (1.11–6.27)	0.029
Lymph node status				
N0	121 (87.1%)	18 (66.7%)	Reference	
N1+N2	18 (12.9%)	9 (33.3%)	3.36 (1.31–8.61)	0.012
Metastasis				
M0	135 (97.1%)	24 (88.9%)	Reference	
M1	4 (2.9%)	3 (11.1%)	4.21 (0.89–20.05)	0.070
Histopathologic grading				
Low grade	21 (15.1%)	2 (7.4%)	Reference	
High grade	118 (84.9%)	25 (92.6%)	2.23 (0.49–10.10)	0.300

The odds ratio (OR) with their 95% confidence intervals were estimated by logistic regression models.

Table 5
Risk of disease-specific mortality on Genotype Distributions of RAGE Gene Polymorphisms among 166 UCC patients aged younger than 65 years

Variable	N	Number of disease-specific mortality	HR (95% CI)	AHR (95% CI)
rs1800625				
TT	139	20	Reference	Reference
TC	22	8	**2.86 (1.26–6.50)**	**2.88 (1.25–6.60)**
CC	5	1	1.24 (0.17–9.27)	1.34 (0.17–10.36)
TC + CC	27	9	**2.50 (1.14–5.49)**	**2.55 (1.16–5.62)**
rs1800624				
TT	130	24	Reference	Reference
TA	31	5	0.78 (0.30–2.04)	0.78 (0.296–2.053)
AA	5	0	NA	NA
TT+AA	36	5	0.66 (0.25–1.72)	0.649 (0.246–1.708)
rs2070600				
GG	102	17	Reference	Reference
GA	53	10	1.14 (0.52–2.48)	1.14 (0.52–2.53)
AA	11	2	0.98 (0.23–4.23)	0.97 (0.22–4.25)
GA+AA	64	12	1.11 (0.53–2.32)	1.11 (0.53–2.35)
rs184003				
GG	115	18	Reference	Reference
GT	46	7	1.06 (0.44–2.54)	1.06 (0.44–2.54)
TT	5	4	**7.58 (2.52–22.77)**	**8.36 (2.64–26.53)**
GT+TT	51	11	1.54 (0.73–3.26)	1.54 (0.73–3.27)

Bold font indicates statistical significance ($P < 0.05$).
The hazrds ratio (HR) with their 95% confidence intervals were estimated by Cox proportional hazards model.
The adjusted hard ratio (AHR) with their 95% confidence intervals were estimated by multiple Cox proportional hazards model after controlling for age, gender, and tobacco consumption.
Abbreviation: NA = not available.

rs1800625 (TC + CC) (AHR = 2.55, 95% CI = 1.16–5.62). Furthermore, patients aged younger than 65 years with a TT allele at rs184003 appeared to have a higher risk of disease-specific mortality as compared with the wild type (AHR = 8.36, 95% CI = 2.64–26.53). Furthermore, as shown in Fig. 1A, patients with the TT genotype at rs184003 appeared to have both a poor prognosis and lower disease-specific survival as compared with the wild type (log rank test, $P < 0.001$). Table 6 shows the polymorphisms and risk of all-cause mortality in patients aged younger than 65 years. Patients with a TT allele at 184003 had a higher risk of death (AHR = 6.39, 95% CI = 2.10–19.45), a finding further verified by the Kaplan-Meier survival curve shown in Fig. 1B, indicating that TT polymorphisms at rs184003 were associated with reduced all-cause survival (log rank test, $P < 0.001$). Moreover, the RAGE mRNA levels, pathological stage, tumor size, lymph node status, and overall survival of bladder urothelial carcinoma from The Cancer Genome Atlas data set were assessed. Among patients with bladder urothelial carcinoma, the relative levels of RAGE mRNA were significantly lower in Stage III + IV patients than in Stage I + II patients

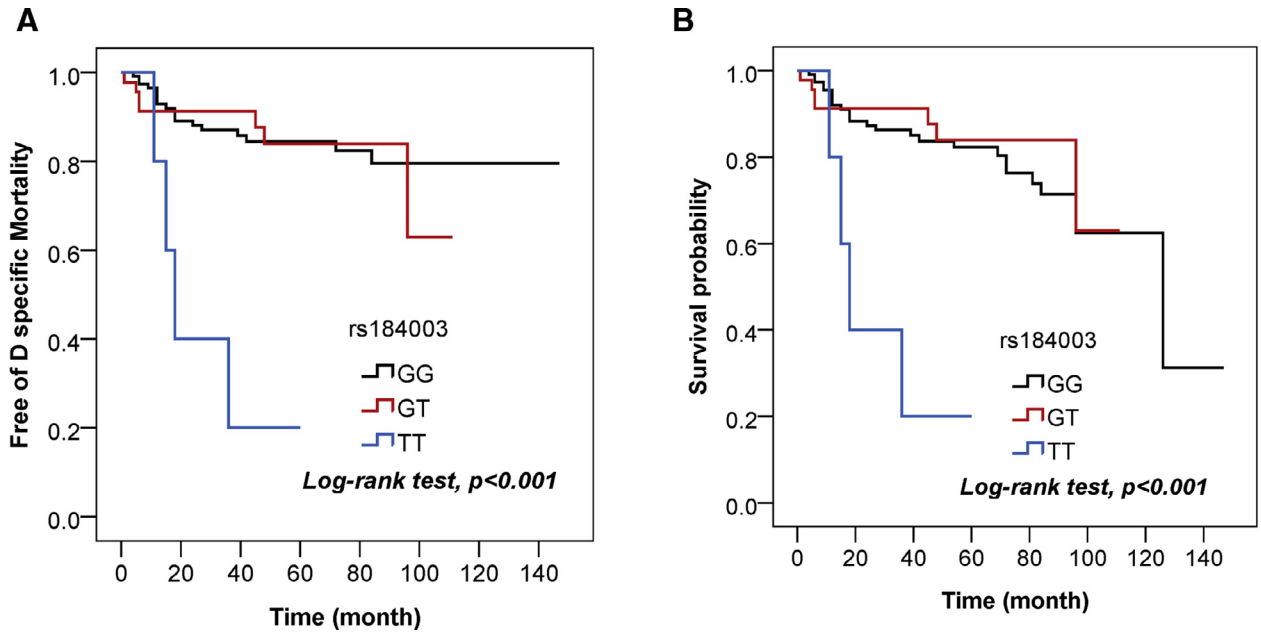

Fig. 1. **Analysis of RAGE polymorphism and survival in urothelial cell carcinoma patients aged younger than 65 years.** (A). Disease specific mortality among the 3 phenotype of polymorphisms at RAGE rs184003 in patients aged younger than 65 years. (B). All-cause mortality among the 3 phenotype of polymorphisms at RAGE rs184003 in patients aged younger than 65 years.

Table 6
Risk of death on Genotype Distributions of RAGE Gene Polymorphisms among 166 UCC patients aged younger than 65 years

Variable	*N*	Number of death	HR (95% CI)	AHR (95% CI)
rs1800625				
TT	139	28	Reference	Reference
TC	22	8	2.13 (0.97–4.70)	2.08 (0.94–4.61)
CC	5	1	0.89 (0.12–6.61)	0.95 (0.13–7.15)
TC + CC	27	9	1.85 (0.87–3.93)	1.84 (0.86–3.92)
rs1800624				
TT	130	30	Reference	Reference
TA	31	6	0.73 (0.30–1.77)	0.74 (0.31–1.78)
AA	5	1	0.65 (0.09–4.77)	0.64 (0.09–4.76)
TT + AA	36	7	0.72 (0.31–1.64)	0.72 (0.32–1.65)
rs2070600				
GG	102	21	Reference	Reference
GA	53	13	1.08 (0.53–2.19)	1.05 (0.51–2.15)
AA	11	3	1.14 (0.34–3.85)	1.20 (0.35–4.08)
GA + AA	64	16	1.09 (0.56–2.12)	1.08 (0.55–2.10)
rs184003				
GG	115	26	Reference	Reference
GT	46	7	0.78 (0.34–1.80)	0.79 (0.34–1.83)
TT	5	4	**6.45 (2.19–19.04)**	**6.39 (2.10–19.45)**
GT + TT	51	11	1.14 (0.56–2.31)	1.15 (0.57–2.35)

Bold font indicates statistical significance ($P < 0.05$).

The hazrds ratio (HR) with their 95% confidence intervals were estimated by Cox proportional hazards model.

The adjusted hard ratio (AHR) with their 95% confidence intervals were estimated by multiple Cox proportional hazards model after controlling for age, gender, and tobacco consumption.

($P = 0.001$; Fig. 2A). The relative levels of RAGE mRNA were also significantly lower in larger tumor size (T3 + T4) and lymph node metastasis, respectively ($P = 0.0037$ and $P = 0.0105$; Fig. 2B and C). In addition, as shown in Fig. 2D, bladder urothelial carcinoma revealed that a low RAGE expression was associated with a poor overall survival ($P < 0.001$). These data suggest that RAGE expression may be involved in UCC progression.

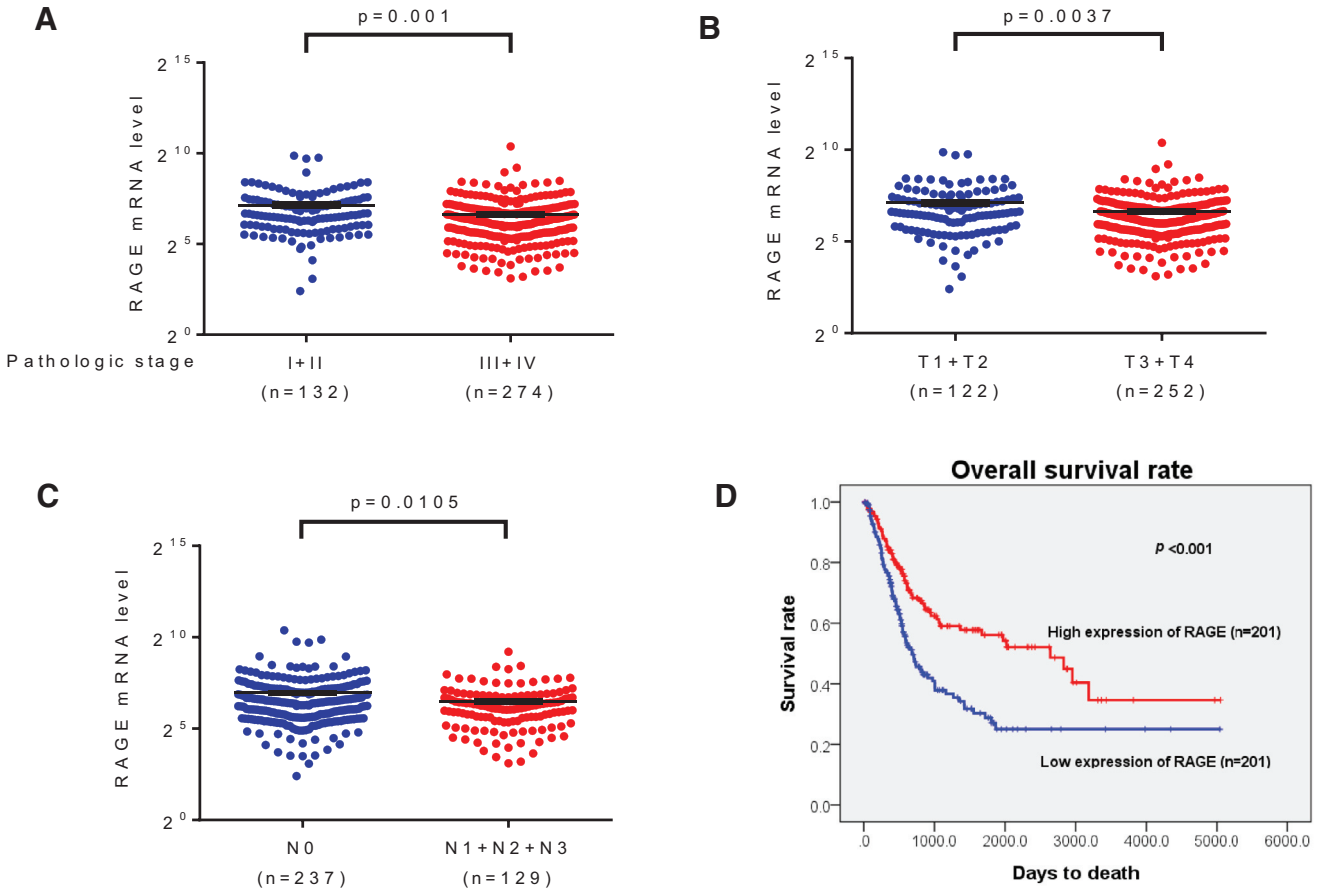

Fig. 2. **RAGE mRNA level of bladder urothelial carcinoma patients from TCGA database.** (A) RAGE levels were compared according to stage. (B) RAGE levels were compared according to tumor size status. (C) RAGE levels were compared according to lymph node status. (D). Effect of RAGE mRNA expression on the overall survival of patients with bladder urothelial carcinoma evaluated using the Kaplan-Meier method. An overall survival curve was produced for overall patients ($n = 402$), with high (red lines) and low (blue lines) RAGE mRNA expression levels. The P values were determined using a log-rank test. (Color version of figure is available online.)

4. Discussion

The development of UCC is a complex process that is affected by both inherited and acquired factors. This study is the first to examine the association between *RAGE* SNPs and the clinicopathologic features of UCC. The results suggest that an individual with at least 1 C allele at rs1800625 is associated with a higher incidence of UCC, and the risk tends to be higher for those aged ≤65 years, females, and those without tobacco use. Moreover, this polymorphism tended to associate with more advanced stages of diseases in patients aged younger than 65 years. In addition, patients aged ≤65 years with a TT polymorphism at rs184003 were associated with a higher risk of disease-specific mortality and all-cause mortality.

RAGE is a transmembrane receptor of the immunoglobulin superfamily, which functions for cell signaling and may be triggered by factors such as HMGB1, advanced glycation end products, S100, phosphatidylserine, β-amyloid, and C3a. RAGE also plays a pivotal role in innate immune response and tumorigenesis [12]. In general, RAGE is highly expressed only in the lungs at readily measurable levels, but it increases quickly during reaction to chronic stress at sites of epithelial cells [30]. Other than the association between RAGE expression and inflammatory conditions such as cardiovascular disease, diabetes, and Alzheimer's disease [31-33], RAGE may be bound by stress-associated ligands and serve as a counter receptor for leukocyte integrins. It also plays a major role in cell adhesion, clustering, and the recruitment of inflammatory cells [34]. Furthermore, it may attach to proteoglycans at the surface of cancer cells and subsequently induce malignant transformation of malignancies and metastasis [35]. Under pathological conditions, this pathway may induce diminished apoptosis, but it also enhances cell autophagy, thus contributing to tumor progression, and cell metastasis [36,37]. Previous studies have demonstrated the expression of RAGE in lung, colorectal, hepatocellular, pancreatic, kidney, and prostate cancers [38–42]. In addition, the expression and up-regulation of RAGE in BC tissue cells is a potential diagnosis or treatment marker [27]. Together with the expression of RAGE, one of the most frequent

RAGE ligands, HMGB1, has been observed to be associated with the clinopathological features, progression, and a prognostic role in chemotherapy and radiotherapy for UCC.

Ligand binding may not be the only trigger of the cascades of RAGE signal pathways. In addition, changing the ligand affinity or repertoire may also alter the RAGE level and subsequently enhance the susceptibility of cancer [25]. Functional studies have revealed that the C allele of rs1800625 may induce the expression of RAGE and be associated with chronic inflammatory conditions [43]. Furthermore, our data suggest that the C allele of rs1800625 tend to associate more with UCC, particularly in individuals without risk factors (i.e., those who are young, female, and do not engage in tobacco consumption). This result is also consistent with our previous study that found that the alteration at rs1800625 (-429T>C) was associated with the development and progression of oral squamous cell carcinoma and hepatocellular carcinoma [24,25]. It also echoed the finding that RAGE expression is a risk factor and potential marker for the development of BC [27]. Nevertheless, in our study, we failed to demonstrate the association between SNPs of rs1800625 and prognosis with respect to disease-specific and all-cause mortality.

Interestingly, we found that young patients with TT polymorphisms at rs184003 were associated with poor prognoses for disease-specific and all-cause survival. Regarding the seventh intron in the RAGE gene, Li et al. showed that TT polymorphisms at rs184003 were associated with higher expressions of RAGE levels in gastric cancer as compared with the wild type [44]. Pan et al. also demonstrated that rs184003 may play a role in the breast cancer progression in northeastern Han Chinese [29]. Although rs184003 is an intronic locus, it might act as a surrogate for other related functional loci and subsequently regulate the expression of RAGE, thereby contributing to tumorigenesis and progression.

AA polymorphisms at rs2070600 also demonstrated a higher risk of UCC as compared with the wild type after adjusting for risk factors, even though it was not correlated with the clinicopathologic stage. Located at exon 3 of *RAGE*, it was thought to have an effect on the functions and promote a change from glycine to serine within the assumed ligand-binding domain of the protein. This change would enhance ligand affinity and subsequently lead to the expression of RAGE [45]. Yang et al. demonstrated SNPs at rs2070600 associated with the risk of development of gastric cancer in the Chinese population, especially for young individuals without risk factors [28]. Su et al. further demonstrated that polymorphic alleles of rs2070600 at RAGE may contribute to increased ligand binding affinity and that they correlated with increased risk of hepatocellular cell carcinoma [25]. Thus, the functional role of RAGE (rs2070600) polymorphisms in the exon 3 region is worth for further investigation, which will be included in our future work.

Our data demonstrate the effect of RAGE gene variations on the development and clinicopathologic features of UCC. However, this study has several limitations. First, the retrospective design, relatively small sample size, and short follow-up period may limit the findings and restrict their application. Although tobacco smoking is a major environmental risk factor for the development of UCC, it has a cumulative effect and we lack the data to differentiate former and current smokers. A large-scale study verifying the association of RAGE SNP with UCC risk is warranted.

This is the first study to report the correlations between *RAGE* polymorphisms and UCC risk. Individuals carrying at least 1 C allele at rs1800625 of *RAGE* are associated with a higher risk of UCC development, particularly those in the following categories: age ≤65 years, female, and no tobacco consumption. TT polymorphisms at rs184003 are associated with reduced disease-specific survival and overall survival in people aged ≤65 years.

Conflict of interest

The authors declared no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2019.02.012.

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