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Research Paper

Impact of the Receptor for Advanced Glycation End Products Genetic Polymorphisms on the Progression in Uterine Cervical Cancer

Chung-Yuan Lee[1,2,3,#], Soo-Cheen Ng[4,5,#], Yi-Hsuan Hsiao[4,6], Shun-Fa Yang[1,7], Chun-Fang Hsu[1], Po-Hui Wang[1,4,5,7,✉]

1. Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
2. Department of Obstetrics and Gynecology, Chiayi Chang Gung Memorial Hospital Chiayi, Taiwan
3. Department of Nursing, Chang Gung University of Science and Technology, Chiayi Campus, Chiayi, Taiwan
4. School of Medicine, Chung Shan Medical University, Taichung, Taiwan
5. Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taichung, Taiwan
6. Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan
7. Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan

[#]These authors contributed equally to this work.

✉ Corresponding author: Po-Hui Wang, MD, PhD. Institute of Medicine, Chung Shan Medical University, 110, Section 1, Chien-Kuo North Road, Taichung, 40201, Taiwan. Tel.: 886-4-24739595 ext. 21721; Fax: 884-4-24738493; E-mail: ginhow84921344@yahoo.com.tw

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Abstract

To date, few studies have explored the effects of single nucleotide polymorphisms (SNPs) of the receptor for advanced glycation end products (RAGE) in uterine cervical cancer. Therefore, we conducted this study to investigate the involvement of RAGE SNPs in cervical cancer. In total, 117 patients with cervical invasive cancer, 84 with precancerous lesions, and 320 normal women were recruited consecutively. Real-time polymerase chain reaction was used to examine the genotypic frequencies of RAGE SNPs. The results indicated that among the four RAGE SNPs, only the GT/TT genotype of rs184003 was distributed differently between patients with cervical neoplasias and the normal controls, with GG as a reference. Moreover, cervical cancer patients with genotypes TA/AA in rs1800624 exhibited a lower risk of parametrium invasion, moderate-to-poor cell differentiation, and pelvic lymph node metastasis. In conclusion, RAGE SNPs rs1800624 was associated with some clinicopathological variables in cervical cancer.

Key words: RAGE, single nucleotide polymorphism, uterine cervical cancer, recurrence-free survival, overall survival

Introduction

In Taiwan, uterine cervical invasive cancer was the second most common type of gynecological cancer according to a 2013 annual cancer registry report. It was also reported to be the third most prevalent cancer among Taiwanese women. Cervical carcinogenesis is usually considered a continuum of neoplastic transition from low-grade cervical intraepithelial neoplasia (CIN 1; histologically, mild dysplasia) to high-grade CINs [including CIN 2 (histologically, moderate dysplasia) and CIN 3 (including severe dysplasia and carcinoma *in situ*)] to invasive cancer [1, 2]. If mitoses and immature cells occupy the lower third of the cervical epithelium, the lesion is categorized as CIN 1. In CIN 2 and CIN 3, mitoses and immature cells occupy the middle and upper thirds of the epithelium, respectively. Moreover, the Bethesda system designates low- and high-grade squamous intraepithelial lesions as the cytologic counterparts of these lesions [3, 4]. Approximately 10% of LSIL and 20%–30% of HSIL may progress to invasive cancer of uterine cervix [1, 2].

The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface molecules, the corresponding RAGE gene of which is located in chromosome 6p21.3 [5, 6]. It is expressed in many tissues, including the liver, heart, and kidney [7]. Thus, RAGE is involved in various pathophysiological processes, such as inflammation, and in cardiovascular disease, Alzheimer disease, carcinogenesis, and metastasis [8-11]. RAGE may bind with advanced glycation end products (AGEs), high-mobility group box 1 protein (HMGB1) [11], and certain S100/calgranulin family members [12, 13]. Another crucial ligand of RAGE may be glycosaminoglycan, which often attaches to proteoglycans on the surface of tumor cells and is critical in malignant transformation and cancer metastasis [14].

If a different single nucleotide develops in the shared sequence of a gene in more than 5% of the population for a species, a single nucleotide polymorphism (SNP) is identified [15]. Genetic polymorphisms are probably related to the development and occurrence of certain diseases, such as cancer. Genetic variants may affect the promoter activity and gene expression [16-19]. SNPs have a modifying function on genetic expression and are related to an increased risk of ovarian and breast tumorigenesis [20]. Cumulative evidence has demonstrated that more than 50 types of genetic polymorphisms are present in the region of the RAGE gene, the majority of which are SNPs [21, 22]. To our knowledge, few studies have associated the genetic distribution of RAGE polymorphisms with the progression of cervical cancer and patient prognosis. Therefore, we investigated the relationships between RAGE genetic variants, cervical tumorigenesis, clinicopathological characteristics, and the recurrence-free and overall survival of patients with cancer in Taiwan.

Materials and Methods

Participants

In total, 521 women (117 invasive cancer, 84 precancerous lesions of the uterine cervix, and 320 normal controls) were recruited. The cancer stages of the 117 women with cervical invasive cancer were categorized according to the 2009 International Federation of Gynecology and Obstetrics Classification. The patients with cervical cancer were treated through standard protocols at the Department of Obstetrics and Gynecology in Chung Shan Medical University Hospital, Taiwan from August 1993 to August 2014. Cervical punch biopsy under colposcopy, large loop excision of the transformation zone, and total abdominal or vaginal hysterectomy were performed on the 84 patients with precancerous lesions. The diagnoses of lesions were confirmed on the basis of the pathological reports before the treatment commenced. All individuals were Taiwanese women living in Central Taiwan. The Institutional Review Board of Chung Shan Medical University approved this study (CSMUH IRB: CS14014). All participants provided informed consent.

Blood sample collection and genomic DNA extraction

Blood samples were collected from all participants and placed into Vacutainer tubes containing ethylenediaminetetraacetic acid. QIAamp DNA blood mini kits (Qiagen, Valencia, CA, USA) were used extract DNA from white blood cells as previously described [23]. The products were then used as polymerase chain reaction templates.

Single nucleotide polymorphisms (SNPs) by real time-PCR and genotyping

Four RAGE genetic variants were evaluated based on the data of International HapMap Project and based on their wide associations with the development of various types of cancer [24-27]. Genotypes of RAGE SNPs 1704G>T (rs184003) (C_2412456_10), −374T>A (rs1800624) (C_3293837_1), −429T>C (rs1800625) (C_8848033_1), and Gly82Ser (rs2070600) (C_15867521_20) were determined by ABI StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), and analyzed with SDS vers. 3.0 software, as described previously [24, 28].

Statistical analysis

Analysis of variance was used to examine age difference among patients with cervical invasive cancer, those with precancerous lesions, and the controls for multiple comparisons. Then, a post hoc analysis was performed for detecting significant differences using the Scheffe test. The relationships between the distribution of RAGE SNPs and incidence of cervical neoplasias (including invasive cancer and precancerous lesions) were assessed through chi-squared or Fisher's exact tests. Logistic regression modeling was performed to analyze the genotypic distributions of the RAGE SNPs before and after controlling for age between the patients with cervical neoplasias and controls. However, a multinomial logistic regression model was used for comparing patients with invasive cancer, those with precancerous lesions, and controls. Odds ratios (ORs) and ORs adjusted for age (AORs) and their 95% confidence intervals (CIs) were calculated.

The chi-squared or Fisher's exact tests were

applied to relate the various clinicopathological factors to RAGE genetic polymorphisms. The patients were followed to calculate the recurrence-free and overall survival between primary surgery and recurrence, death, or end of the study (December 4, 2017) by using the Kaplan–Meier curve model in a univariate analysis for RAGE SNPs and clinico-pathological variables. In the multivariate analysis of survival time, a Cox proportional hazard model with a forward stepwise approach was performed to examine the effects of RAGE SNPs and various clinicopathological variables on recurrence-free and overall survival. Hazard ratios (HRs) and their 95% CIs were determined thereafter. A p value of <0.05 indicated statistical significance. SPSS (version 22.0) and WinPepi (version 10.0) were employed for the statistical analyses.

Results

Significant differences were observed for the age distribution between patients with cervical neoplasias and controls (48.8 ± 13.5 vs. 44.0 ± 10.2 years, p < 0.001). The age difference between patients with cervical invasive cancer and those with precancerous lesions (54.1 ± 12.3 vs. 41.7 ± 11.6 years) and between those with cervical invasive cancer and controls (54.1 ± 12.3 vs. 44.0 ± 10.2 years) were significant (both p <0.001). However, no significant differences in age distribution were observed between patients with precancerous lesions and controls (41.7 ± 11.6 vs. 44.0 ± 10.2 years, p = 0.208).

Association of RAGE genetic variant frequencies with uterine cervical neoplasias

The genotypic distributions of RAGE SNPs in women with cervical neoplasias and controls are summarized in Table 1. The genotypic distribution of RAGE SNP rs184003 satisfied the Hardy-Weinberg equilibrium in the normal controls [p>0.05, χ^2 value: 3.82 < 5.99, degree of freedom=2]. Distributions of other RAGE SNPs rs1800624, rs1800625 and rs2070600 all conformed to this equilibrium (p>0.05, χ^2 value: 0.06; p>0.05, χ^2 value: 0.01 and p>0.05, χ^2 value: 3.43, respectively). The GT/TT genotypes of RAGE SNP rs184003 tended to be distributed differently between patients with cervical neoplasias and controls when GG was used as a reference (OR = 0.69, 95% CI = 0.47–1.00, p = 0.051; Table 1). After controlling for age, women carrying GT/TT has a lower risk of cervical neoplasias (AOR = 0.65, 95% CI = 0.44–0.96, p = 0.031; Table 1). However, no significantly differences was noted in the frequencies for other RAGE SNPs, namely rs1800624, rs1800625, and rs2070600, between patients with cervical neoplasias and controls or between patients with cervical neoplasias and controls, even after controlling for age.

Relationships of RAGE genetic variant frequencies with uterine cervical carcinogenesis

When the cervical neoplasias group were reclassified into invasive cancer and precancerous lesions subgroups, differences in the GT/TT genotypes of rs184003 were not observed among patients with cervical invasive cancer and precancerous lesions as well as controls, with GG as a reference (p = 0.135; Table 2). After controlling for age, women with GT/TT did not have a lower risk of precancerous lesions and invasive cancer of the uterine cervix (AOR = 0.65, 95% CI = 0.38–1.10, p = 0.110 and AOR = 0.67, 95% CI = 0.41–1.08, p = 0.101, respectively; Table 2). The distributions of other RAGE SNPs rs1800624, rs1800625 and rs2070600 did not significantly differ between patients with cervical invasive cancer, those with precancerous lesions, and controls.

Table 1. Genetic variant distributions of the receptor for advanced glycation end products gene in Taiwanese women with neoplasias of the uterine cervix and normal controls.

Variables	Normal controls (n =320)	Cervical neoplasias[a] (n=201)	ORs (95% CIs)[a]	p values	AORs (95% CIs)[b]	Adjusted p values
rs184003						
GG[c]	196	140	1.00	0.156	1.00	
GT	116	57	0.69 (0.47-1.01)		0.64 (0.43-0.96)	0.029
TT	8	4	0.70 (0.21-2.37)		0.80 (0.23-2.76)	0.722
GG[c]	196	140	1.00	0.051	1.00	
GT/TT	124	61	0.69 (0.47-1.00)		0.65 (0.44-0.96)	0.031
GG/GT[c]	312	197	1.00	0.774	1.00	
TT	8	4	0.79 (0.24-2.67)		0.92 (0.27-3.16)	0.895
rs1800624						
TT[c]	242	155	1.00	0.393	1.00	
TA	72	39	0.85 (0.55-1.31)		0.88 (0.56-1.37)	0.571
AA	6	7	1.82 (1.60-5.52)		1.67 (0.54-5.15)	0.372
TT[c]	242	155	1.00	0.698	1.00	
TA/AA	78	46	0.92 (0.61-1.40)		0.95 (0.62-1.44)	0.79

Variables	Normal controls (n =320)	Cervical neoplasias[a] (n=201)	ORs (95% CIs)[a]	*p* values	AORs (95% CIs)[b]	Adjusted *p* values
TT/TA[c]	314	194	1.00	0.252	1.00	
AA	6	7	1.89 (0.63-5.70)		1.72 (0.56-5.27)	0.345
rs1800625						
TT[c]	270	181	1.00	0.153	1.00	
TC	48	19	0.59 (0.34-1.04)		0.61 (0.34-1.09)	0.094
CC	2	1	0.75 (0.07-8.29)		0.88 (0.08-10.12)	0.917
TT[c]	270	181	1.00	0.064	1.00	
TC/CC	50	20	0.60 (0.34-1.04)		0.62 (0.35-1.09)	0.098
TT/TC[c]	318	200	1.00	1.000	1.00	
CC	2	1	0.80 (0.07-8.83)		0.94 (0.08-10.79)	0.957
rs2070600						
GG[c]	189	111	1.00	0.462	1.00	
GA	121	80	1.13 (0.78-1.63)		1.17 (0.80-1.70)	0.427
AA	10	10	1.70 (0.69-4.22)		1.60 (0.62-4.09)	0.331
GG[c]	189	111	1.00	0.388	1.00	
GA/AA	131	90	1.17 (0.82-1.67)		1.20 (0.83-1.73)	0.331
GG/GA[c]	310	191	1.00	0.285	1.00	
AA	10	10	1.62 (0.66-3.97)		1.50 (0.59-3.80)	0.392

Statistical analysis: logistic regression model or chi-square or Fisher's exact tests.

[a]Cervical neoplasias included precancerous lesions and invasive cancer of the uterine cervix.

[b]The adjusted *p* values as well as adjusted odds ratios and their 95% confident intervals were calculated by logistic regression model after controlling age.

[c]Used as a reference for comparison to calculate the odds ratios of other genotypes. 95% CIs, 95% confidence intervals.

Table 2. Genetic variant distributions of the receptor for advanced glycation end products gene in Taiwanese women with uterine cervical invasive cancer or precancerous lesions and normal controls.

Variables	Normal controls (n =320)	Pre-cancerous lesions (n = 84)	Invasive cancer (n = 117)	*p* values	AORs (95% CIs)[a]	Adjusted *p* values	AORs (95% CIs)[b]	Adjusted *p* values
rs184003								
GG[c]	196	60	80	0.379	1.00		1.00	
GT	116	22	35		0.64 (0.37-1.10)	0.109	0.66 (0.40-1.08)	0.098
TT	8	2	2		0.77 (0.16-3.77)	0.752	0.81 (0.16-4.23)	0.806
GG[c]	196	60	80	0.135	1.00		1.00	
GT/TT	124	24	37		0.65 (0.38-1.10)	0.110	0.67 (0.41-1.08)	0.101
GG/GT[c]	312	82	115	1.000	1.00		1.00	
TT	8	2	2		0.89 (0.18-4.31)	0.884	0.94 (0.18-5.82)	0.937
rs1800624								
TT[c]	242	61	94	0.389	1.00		1.00	
TA	72	19	20		1.06 (0.59-1.89)	0.847	0.77 (0.43-1.38)	0.387
AA	6	4	3		2.97 (0.80-10.96)	0.103	1.04 (0.24-4.62)	0.954
TT[c]	242	61	94	0.415	1.00		1.00	
TA/AA	78	23	23		1.20 (0.69-2.06)	0.524	0.80 (0.46-1.38)	0.416
TT/TA[c]	314	80	114	0.287	1.00		1.00	
AA	6	4	3		2.93 (0.80-10.73)	0.105	1.10 (0.25-4.85)	0.899
rs1800625								
TT[c]	270	76	105	0.365	1.00		1.00	
TC	48	8	11		0.49 (0.21-1.14)	0.098	0.75 (0.36-1.56)	0.441
CC	2	0	1		u.a	u.a	2.12 (0.16-27.74)	0.568
TT[c]	270	76	105	0.179	1.00		1.00	
TC/CC	50	8	12		0.47 (0.21-1.09)	0.078	0.79 (0.39-1.62)	0.526
TT/TC[c]	318	84	116	1.000	1.00		1.00	
CC	2	0	1		u.a	u.a	2.20 (0.17-28.86)	0.548
rs2070600								
GG[c]	189	49	62	0.607	1.00		1.00	
GA	121	32	48		1.05 (0.64-1.74)	0.842	1.26 (0.79-2.03)	0.333
AA	10	3	7		1.15 (0.30-4.38)	0.833	1.84 (0.57-5.88)	0.306
GG[c]	189	49	62	0.518	1.00		1.00	
GA/AA	131	35	55		1.06 (0.65-1.73)	0.814	1.31 (0.83-2.07)	0.254
GG/GA[c]	310	81	110	0.334	1.00		1.00	
AA	10	3	7		1.13 (0.30-4.22)	0.855	1.67 (0.53-5.25)	0.381

Statistical analysis: multinomial logistic regression or chi-square or Fisher's exact tests.

[a]Adjusted *p* values and adjusted odds ratios with their 95% CIs were calculated using multinomial logistic regression models after controlling age between patients with cervical precancerous lesions and control women.

[b]Adjusted *p* values and adjusted odds ratios with their 95% CIs were estimated using multinomial logistic regression models after controlling age between patients with cervical invasive cancer and control women.

[c]Used as a reference for comparison to estimate the odds ratios of other genotypes.

AORs, adjusted odds ratios; 95% CIs, 95% confidence intervals; u.a., unavailable.

Table 3. Relationships of genotypic distribution of receptor for advanced glycation end products genetic varivants rs184003 and rs1800624 with clinicopathological parameters of the patients with invasive cancer of uterine cervix.

	rs184003				rs1800624			
Variable	**GG[b]**	**GT/ TT**	***p* value**	**ORs (95% CIs)**	**TT[b]**	**TA/ AA**	***p* value**	**ORs (95% CIs)**
Clinical stage			0.129				0.103	
stage I[b]	49	17		1.00	50	16		1.00
≥ stage II	26	17		1.88 (0.76-4.65)	38	5		0.41 (0.11-1.32)
Pathologic type			0.968				0.759	
squamous cell carcinoma[b]	62	28		1.00	73	17		1.00
adenocarcinoma	13	6		1.02 (0.29-3.26)	15	4		1.15 (0.25-4.24)
Cell grading			0.337				0.034[c]	
well (grade 1)[b]	17	5		1.00	14	8		1.00
moderate & poor (grades 2/3)	58	29		1.70 (0.53-6.46)	74	13		0.31 (0.10-1.03)
Stromal invasion depth			0.596				0.175	
≤10 mm[b]	45	18		1.00	48	15		1.00
> 10 mm	30	15		1.25 (0.50-3.09)	39	6		0.49 (0.14-1.51)
Tumor diameter[b]			0.312				0.092	
≤ 4cm	48	18		1.00	50	16		1.00
> 4cm	28	16		1.52 (0.62-3.73)	39	5		0.40 (0.11-1.28)
Parametrium			0.418				0.023[c]	
no invasion[b]	55	22		1.00	58	19		1.00
invasion	21	12		1.43 (0.54-3.66)	31	2		0.20 (0.02-0.92)
Vagina			0.279				0.153	
no invasion[b]	60	24		1.00	66	18		1.00
invasion	15	10		1.67 (0.58-4.61)	23	2		0.32 (0.03-1.52)
Pelvic lymph node			0.848				0.035[c]	
no metastasis[b]	55	24		1.00	60	19		1.00
metastasis	21	10		1.09 (0.40-2.88)	29	2		0.22 (0.02-1.01)

Statistical analyses: chi-square or Fisher's exact tests, [c]$p<0.05$

[a]Some clinicopathological data could not be obtained from the patients with cervical invasive cancer due to incomplete medical charts or records.

[b]As a reference. ORs, odds ratios; 95% CIs, 95% confidence intervals.

Association of RAGE genetic polymorphisms with clinicopathological variables in patients with cervical cancer

Because the women with GT/TT genotype of rs184003 demonstrated lower risk of cervical neoplasias (with GG as a reference), we investigated the association of this RAGE SNP with clinicopathological variables in the patients with cervical cancer. However, no association between rs184003 and clinicopathological characteristics was noted (Table 3). Regarding the various clinicopathological factors of RAGE genetic variants, cervical cancer patients with TA/AA genotypes in rs1800624 exhibited lower risk of parametrium invasion (OR = 0.20, 95% CI = 0.02–0.92, $p = 0.023$) and tended to have moderate-to-poor cell differentiation (OR = 0.31, 95% CI = 0.10–1.03, $p = 0.034$) and pelvic lymph node metastasis (OR = 0.22, 95% CI = 0.02–1.01, $p = 0.035$) compared with those with genotype TT (Table 3). No significant associations were noted between other RAGE SNPs and clinicopathological variants (data not shown).

Univariate analysis and Kaplan–Meier curve models for recurrence-free and overall survival of patients with cervical cancer

We next evaluated the effects of rs1800624 and various clinicopathological parameters on the recurrence-free and overall survival of patients with cervical cancer. In the univariate analysis, we found no association of rs1800624 with patient survival ($p = 0.156$ and 0.204 for recurrence-free and overall survival, respectively; Table 4). However, the following were significantly associated with recurrence-free survival: cancer stage ($p = 0.002$), stromal invasion depth ($p = 0.002$), tumor diameter ($p = 0.013$), parametrium invasion ($p = 0.016$), vagina invasion ($p = 0.022$) and pelvic lymph node metastasis ($p = 0.002$; Table 4). Furthermore, the following were significantly associated with overall survival: cancer stage ($p = 0.002$), cell grading ($p = 0.046$), stromal invasion depth ($p = 0.003$), tumor diameter ($p = 0.007$), parametrium invasion ($p = 0.001$) and lymph node metastasis ($p = 0.001$; Table 4).

Multivariate analysis and Cox proportional hazard models for recurrence-free and overall survival of patients with cervical cancer

In a multivariate analysis, we observed no association between rs1800624 and patient survival ($p = 0.409$ and 0.330 for recurrence-free and overall survival, respectively; Table 5). A more advanced cancer stage was the only independent predictor of a less favorable recurrence-free survival for cervical cancer patients (HR = 3.86, 95% CI = 1.56–9.55, $p = 0.004$; Table 5). Only pelvic lymph node metastasis

could independently predict poorer overall survival (HR = 3.30, 95% CI = 1.35–8.08, p = 0.009; Table 5). Other RAGE SNPs exhibited no influence on the recurrence-free and overall survival of patients with cervical cancer in univariate and multivariate analyses (data not shown).

Discussion

RAGE is activated by binding with AGEs, HMGB1, and S100 proteins in cancer cells [12, 13]. Ligand formation not only initiates intracellular signal transduction but also upregulates RAGE expression [29]. The interactions of RAGE with various ligands have crucial roles in cancer pathogenesis and progression [30], through cell proliferation and invasion occur [31]. The binding of RAGE with S100A9 is essential in cervical cancer development [32]. Tian et al. also reported that S100A7 bound to RAGE to promote the migration, invasion and metastasis of human cervical cancer cells [33]. In addition, SNPs may exert a modifying function on gene expression. We inferred that RAGE may be involved in cervical carcinogenesis. The GT/TT genotype of rs184003 appeared to prevent these patients from susceptibility to cervical neoplasias. Yue et al. demonstrated a significant association between RAGE SNP rs1800624 and the risk of breast cancer in a Han Chinese population [34]. However, this protective effect was found to disappear after cervical neoplasias were subdivided into invasive and precancerous lesions subgroups, even after controlling for age. Moreover, no other RAGE SNPs were involved in cervical carcinogenesis.

Su et al. revealed that RAGE SNP rs1800625 is involved in the formation of oral squamous cell carcinoma [24]. It is also associated with early-stage liver carcinogenesis, and its protective role has been implicated in hepatocellular carcinoma progression [28]. In contrast to our findings, Xu et al. suggested that RAGE Gly82Ser polymorphism (rs2070600), interacting with human papillomavirus (HPV) infection, was associated with cervical cancer development in a Chinese population [35]. The authors revealed a different distribution of rs2070600 between patients with cancer and controls by using a stratification analysis for HPV infection. However, this difference was not observed between the smaller non–HPV-infected patients with cancer and non–HPV-infected controls. Furthermore, they also could not demonstrate an association of 1704G>T (rs184003), −374T>A (rs1800624), and −429T>C (rs1800625) with susceptibility to cervical cancer. Although HPV infection is a prominent cause of cervical cancer, many infected women do not have invasive cancer. Therefore, HPV infection itself is an inadequate indicator for cervical cancer. Other cofactors are required to interact with HPV infection for cervical invasive cancer formation.

This study has two limitations, which may explain the differences among results. First, data on HPV infection were limited. This may be partially attributed to the conservative attitude of Taiwanese women. Although HPV infection rates in HSIL and invasive cancer of uterine cervix were reported to be 84.3%–100% according to a study by the Taiwan Cooperative Oncologic Group [36], normal controls in Taiwan did not undergo HPV tests if their Pap smear reports were normal because HPV tests are not covered under the National Health Insurance program. Second, having multiple partners is a variable that probably had a substantial influence on the results in this study; however, this information frequently withheld by our participants, possibly because of the conservative attitude.

Our results revealed that among four RAGE SNPs, rs1800624 was the only genetic variant associated with clinicopathological characteristics of cervical cancer. The patients with cervical cancer patients with the TA/AA genotypes of rs1800624 had a lower risk of parametrium invasion, moderate-to-poor cell differentiation, and pelvic lymph node metastasis. To our knowledge, no study has reported the association of RAGE SNPs with clinicopathological variables of cervical cancer. By contrast, in Taiwanese patients with oral cancer, the RAGE SNP rs1800625 indicated increased cancer risk; it was also associated with late-stage and large-size tumors in a Taiwanese population [24].

Table 4. Univariate analysis for the effects of receptor for advanced glycation end products (RAGE) genetic polymorphism and various clinicopatholgical parameters on the recurrence-free survival and overall survival of the patients with uterine cervical cancer

	Recurrence-free survival		Overall survival	
Variable	***p* value**	**HR & 95% CI[b]**	***p* value**	**HR & 95% CI[b]**
RAGE genetic polymorphism				
rs1800624 TA/AA vs TT[a]	0.156	0.35 (0.08-1.49)	0.204	0.39 (0.09-1.67)
Clinicopathological characteristics				
Stage				
≥ stage II vs stage I[a]	0.002	4.05 (1.67-9.83)	0.002	4.31 (1.70-10.91)
Cell grading				
moderate & poor (grades 2/3) vs well (grade 1)[a]	0.084	3.61 (0.84-15.48)	0.046	7.74 (1.04-57.78)

	Recurrence-free survival		Overall survival	
Variable	***p* value**	**HR & 95% CI[b]**	***p* value**	**HR & 95% CI[b]**
Stromal invasion depth				
> 10 mm vs ≤10 mm[a]	0.002	4.01 (1.65-9.70)	0.003	4.15 (1.62-10.61)
Tumor diameter				
> 4 cm vs ≤ 4cm[a]	0.013	2.87 (1.25-6.58)	0.007	3.26 (1.39-7.62)
Parametrium				
invasion vs no invasion[a]	0.016	2.71 (1.21-6.07)	0.001	3.76 (1.66-8.49)
Vagina				
invasion vs no invasion[a]	0.022	2.59 (1.15-5.85)	0.394	1.47 (0.61-3.59)
Pelvic lymph node				
metastasis vs no metastasis[a]	0.002	3.67 (1.64-8.22)	0.001	4.06 (1.80-9.16)

Statistical analyses: Kaplan-Meier curve model

[a]As a comparison reference

[b]HR, hazard ratio and 95% CI, 95% confidence interval for RAGE genetic variant and clinicopathological variables, compared to their respective controls.

Table 5. Multivariate analysis for the effects of receptor for advanced glycation end products (RAGE) genetic polymorphism and various clinicopatholgical parameters on the recurrence-free survival and overall survival of the patients with uterine cervical cancer

	Recurrence-free survival		Overall survival	
Variable	*p* value	HR & 95% CI[b]	*p* value	HR & 95% CI[b]
RAGE genetic polymorphism				
rs1800624 TA/AA vs TT[a]	0.409	0.54 (0.12-2.36)	0.330	0.36 (0.04-2.85)
Clinicopathological characteristics				
Stage				
≥ stage II vs stage I[a]	0.004	3.86 (1.56-9.55)	>0.05	u.a.
Pelvic lymph node				
metastasis vs no metastasis[a]	>0.05	u.a.	0.009	3.30 (1.35-8.08)

[a]As a comparison reference

[b]HR, hazard ratio and 95% CI, 95% confidence interval for RAGE genetic polymorphism and clinicopathological variables, compared to their respective controls.

u.a.: unavailable

Because rs1800624 was associated with some clinicopathological variables, we analyzed its impact on patient survival. Our univariate and multivariate analysis results indicated no association of rs1800624 with patient survival. Few studies have reported the effect of rs1800624 on cervical cancer prognosis. Yamaguchi et al. revealed that rs1800624 and rs1800625 were not associated with 5-year survival rates in patients with metastatic lung adeno-carcinoma; however, the authors demonstrated that RAGE SNP rs2070600 was independently related to systemic inflammation and predicted 5-year mortality in these patients by using a multivariate Cox proportional hazard model [27]. Our relatively small sample size and long inclusion time interval of some patients may have limited the applicability of our results. By using univariate analysis, more advanced stage, deep stromal invasion, large tumor size, parametrium and vagina invasion, as well as pelvic lymph node metastasis were found to be crucial variables in determining the recurrence-free survival of patients with cervical cancer. In addition, more advanced stage, cell grading, deep stromal invasion, large tumor size, parametrium invasion, as well as pelvic lymph node metastasis were predictive of overall survival in our study. By using a multivariate Cox proportional hazard analysis after including rs1800624, we revealed that only a more advanced cancer stage was an independent predictor of recurrence and only pelvic lymph node metastasis a critical predictor of overall survival in patients with cervical cancer. This corroborates the finding of other studies that lymph node metastasis is the most crucial prognostic variable for death in patients with cervical cancer [37, 38]. Kamura et al. demonstrated that the 5-year survival rate significantly decreased from 85%–90% to 30%–50% in cervical cancer patients with positive pelvic lymph nodes [39].

In conclusion, RAGE SNPs are not associated with a susceptibility to precancerous lesions and invasive cancer of uterine cervix in Taiwanese women. However, cervical cancer patients with the TA/AA genotypes of rs1800624 exhibit a lower risk of parametrium invasion, moderate-to-poor cell differentiation, and pelvic lymph node metastasis. However, in this study, no RAGE SNP is associated with patient survival; only pelvic lymph node metastasis could independently predict poor overall survival in our patients.

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Competing Interests

The authors have declared that no competing interest exists.

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