Research Paper

Effect of *HMGB1* Polymorphisms on Urothelial Cell Carcinoma Susceptibility and Clinicopathological Characteristics

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Abstract

The high mobility group box 1 gene (*HMGB1*) plays a prominent role in cancer progression, angiogenesis, invasion, and metastasis. This study explored the effect of *HMGB1* polymorphisms on clinicopathological characteristics of urothelial cell carcinoma (UCC). In total, 1293 participants (431 patients with UCC and 862 healthy controls) were recruited. Four single-nucleotide polymorphisms (SNPs) of *HMGB1* (rs1412125, rs1360485, rs1045411, and rs2249825) were assessed using TaqMan real-time polymerase chain reaction assay. The results indicated that individuals carrying at least one T allele at rs1045411 had a lower risk of UCC than those with the wild-type allele [adjusted odds ratio = 0.722, 95% confidence interval (CI) = 0.565–0.924]. Furthermore, female patients with UCC carrying at least one T allele at rs1045411 were at a lower invasive tumor stage than those with the wild-type allele [odds ratio (OR) = 0.396, 95% CI = 0.169–0.929], similar to nonsmoking patients (OR = 0.607, 95% CI = 0.374–0.985). In conclusion, this is the first report on correlation between *HMGB1* polymorphisms and UCC risk. Individuals carrying at least one T allele at rs1045411 are associated with a lower risk of UCC and a less invasive disease in women and nonsmokers.

Key words: high mobility group box 1, polymorphism, urothelial cell carcinoma

Introduction

Bladder cancer (BC) is the seventh most common cancer in men and the 11th most common cancer in both sexes worldwide, with an incidence of 9.0 and 2.2 per 100,000 person-years in men and women, respectively [1]. More than 95% BCs are urothelial cell carcinomas (UCCs) and constitute the most frequent malignant tumors of the urinary tract [2]. Overall, 50%–70 % of patients with UCC experience a recurrence within 5 years; of them, 10% progress into invasive disease, a highly aggressive malignancy that causes mortality [3].

In Taiwan, BC is the ninth and 16th most common cancer in men and women, respectively, with a male-to-female predominance ratio of 2.6:1 [4]. The geographic characteristic of BC is particularly noted in an arseniasis-endemic area in Taiwan, which is associated with black foot disease and has contributed to a higher incidence of UCC, skin cancer, and lung cancer [5].

Environmental factors, such as tobacco use and exposure to aromatic amines, lead to carcinogenesis of BC in well-established data [6-8]. Both genetic factors

and single-nucleotide polymorphisms (SNPs) are pivotal in BC tumorigenesis and progression, such as an oncogene or a tumor suppressor gene [9-12]. Our previous studies have revealed an association between *CA9*, *ICAM1*, and *EZH2* polymorphisms and UCC susceptibility and clinicopathological characteristics, which may be a potential marker that indicates tumor treatment and progression [13-15].

The high mobility group box 1 protein (HMGB1), belonging to the high mobility group protein family, demonstrates rapid mobility in sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels; it was first purified from calf thymus nuclei in the 1970s. It contains two 80-amino acid DNA-binding domains and a negatively charged C-terminus [16, 17]. HMGB1 functions as a chromatin structural protein in the nucleus and as a proinflammatory cytokine extracellularly [18]. In the nucleus, it acts as a DNA chaperone or a nonhistone DNA-binding protein that can bend DNA and promote the formation of complexes comprising several transcription factors [19]. Extracellular HMGB1 occurs if HMGB1 is passively leaks from cells when cell membrane integrity is lost during necrosis; thus, it is a reliable indicator for necrosis. Extracellular HMGB1 binds with high affinity to the receptor for advanced glycation end products; it is a potent mediator of inflammation [20]. Furthermore, HMGB1 overexpression is an indicator for malignant tumorigenesis, proliferation, invasion, and migration [21-24].

HMGB1 overexpression in UCC is associated with tumor progression and angiogenesis [25, 26], whereas HMGB1 knockdown is associated with suppressed cell growth, migration, and induced cell apoptosis, and it is sensitive to radiotherapy and cisplatin-based chemotherapy [27, 28]. Although HMGB1 is a poor prognosis factor, it is a powerful cytokine released by UCC cells, which directs host immune responses and potentiates the cytotoxicity effects of bacillus Calmette–Guérin vaccine, which is the standard treatment for nonmuscle-invasive BCs [29]. Therefore, HMGB1 acts as a potential molecule marker for predicting prognosis and treatment response.

Single-nucleotide polymorphisms (SNPs) are the most common type of DNA sequence variation influencing the progression of various diseases [30]. The effect of *HMGB1* polymorphisms on clinicopathological features of cancers, Including lung cancer, gastric cancer, hepatocellular carcinoma, uterine cancer, and oral squamous cell carcinoma, has been reported [31-35]. Nevertheless, few studies have investigated the association between *HMGB1* variants and UCC risk and prognosis. The present study investigated the relationship of four *HMGB1* SNPs, namely rs1412125, rs2249825, rs1045411, and rs1360485, with UCC susceptibility and clinicopathological characteristics.

Materials and Methods

Subjects and Specimen Collection

The study recruited 431 patients (272 men and 159 women, with a mean age of 68.60 years) in 2011-2016 at Taichung Veterans General Hospital in Taichung, Taiwan. All patients have pathology proved urothelial cell carcinoma of urinary bladder. For the control group, during the same study period, 862 ethnic individuals were enrolled and entered the physical examination. Approval was obtained from the Institutional Review Board (IRB) of Taichung Veterans General Hospital (IRB No. CF11094). We used a questionnaire to obtain information on patient exposure to tobacco consumption for both cases and controls.

HMGB1 SNP selection

We included four *HMGB1* SNPs (rs1412125, rs2249825, rs1045411, and rs1360485) in the HapMap Chinese Han Beijing population. Moreover, these four *HMGB1* genetic polymorphisms were selected based on their potential involvement in the several cancer types [31, 33, 36]. The specific heterozygosity frequencies using the East Asian population of HMGB1 rs1412125, rs2249825, rs1045411, and rs1360485 were 44.4 %, 28.0 %, 33.5 % and 36.3 %, respectively.

Genomic DNA extraction

Total genomic DNA from whole blood specimens were isolated by QIAamp DNA blood mini kits (Qiagen, Valencia, CA) as previously described [37]. DNA was dissolved in TE buffer and stored at −20°C until performing Real-time quantitative PCR analysis.

Real-time quantitative PCR

Total four SNPs of HMGB1 were examined by using TaqMan SNPs Genotyping Assays (Applied Biosystems, Warrington, UK), according to the manufacturer's protocols as previously described [31, 33].

Statistical analysis

The Mann-Whitney U-test was used to compare differences in distributions of patient demographic characteristics between the control and UCC groups. Differences between the two groups were considered significant if p values < 0.05. The adjusted odds ratios (AORs) and 95% confidence intervals (CIs) of the association between clinicopathological characteristics and genotype frequencies were assessed using multiple logistic regression models, after controlling for other covariates. Data were analyzed with SAS

statistical software (vers. 9.1, 2005; SAS Institute, Cary, NC).

Results

The patient characteristics and clinical parameters are listed in Table 1. The study population was Taiwanese, with a predominance of men with UCC (n = 272, 63.1%). At diagnosis, 235 patients had nonmuscle-invasive BC (54.5%) and 196 had muscle-invasive BC (45.5%). Furthermore, 378 and 53 patients had high- and low-grade tumors, respectively. Lymph node status (n = 51, 11.8%) and metastasis (n = 14, 3.2%) were evaluated through contrast-enhanced computed tomography. To diminish possible interference, we estimated the adjusted odds ratios (AORs) with their 95% confidence intervals (CIs) by using multiple logistic regression models, with adjustments for age, sex, and tobacco consumption.

Table 2 lists the distribution frequency of *HMGB1* genotypes in the 862 healthy controls and 431 patients with UCC. In both groups, the highest distribution frequencies was demonstrated by homozygous TT at rs1412125 and rs1360485 and by homozygous CC at rs1045411 and rs2249825. The patients carrying at least one T allele at rs1045411 (CT or TT) presented a lower UCC risk than did a those carrying the wild type (CC; AOR = 0.722, 95% CI = 0.565–0.924, *p* = 0.010). Furthermore, the CC genotype at rs1412125 also showed a decreased UCC risk compared with the wild type after adjustment (AOR = 0.555, 95% CI = 0.341–0.902, *p* = 0.018).

Regarding the tumor stage, female patients with UCC carrying rs1045411 CT+TT demonstrated a lower risk of stage T1–T4 UCC than did those carrying the wild type [odds ratio (OR) = 0.396, 95% CI = 0.169-0.929, *p* = 0.030; Table 3]. Furthermore, rs1045411 CT+TT appeared to be a protective factor in nonsmoking patients: it significantly reduced the muscle-invasive UCC risk (OR = 0.607, 95% CI = 0.374–0.985, *p* = 0.043). There was no significant difference in lymph node status, metastasis, or histopathologic grading between the two groups in a subgroup analysis.

Discussion

We examined the association between *HMGB1* SNPs and susceptibility to and clinicopathological features of UCC. The results revealed that patient carrying at least one T allele at rs1045411 had a significantly lower risk of UCC than did those carrying a wild type (AOR = 0.722). Furthermore, this SNP demonstrated a less invasive cancer stage in women and nonsmokers in subgroup analysis, which appeared to have a protective factor. The CC mutation

at rs1412125 also led to a lower UCC risk (AOR = 0.555).

Table 1. The distributions of demographical characteristics in 862 controls and 431 patients with UCC.

Variable	Controls (N=862)	Patients (N=431)	p value
Age (yrs)	Mean ± S.D.	Mean ± S.D.	
	57.18 ± 9.99	68.60 ± 11.81	p<0.001
Gender			
Male	566 (65.7%)	272 (63.1%)	
Female	296 (34.3%)	159 (36.9%)	p =0.365
Tobacco consumption			
No	562 (65.2%)	300 (69.6%)	
Yes	300 (34.8%)	131 (30.4%)	p =0.113
Stage			
Non muscle invasive tumor (pTa–pT1)		235 (54.5%)	
Muscle invasive tumor (pT2–pT4)		196 (45.5%)	
Tumor T status			
Ta		90 (20.9%)	
T1-T4		341 (79.1%)	
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%)	

Mann-Whitney U test was used between controls and patients with UCC.

Accumulating evidence has indicated that genetic susceptibility and familial factors, along with environmental factors, may affect BC risk and incidence. A study reported that family history of cancer among first degree relatives, particularly among relatively young patients, was associated with shared environmental exposure, a potential confounding factor [38]. Genome-wide association studies have also identified several possible susceptibility genetic loci associated with BC risk [39-41]. Furthermore, smoking is the most well-known risk factor for BC, causing BC in 50%–65% and 20%–30% of male and female patients, respectively—regardless of being current and former smokers [42, 43]. The difference in the prevalence among both sexes has also been established in a population-based study, which demonstrated a higher prevalence in men, possibly because of a higher frequency of tobacco use and increased exposure to chemicals [44].

HMGB1 mRNA and protein overexpression is associated with poorer prognosis with various cancer types [24]. Higher *HMGB1* expression is also found in several malignancies, including lung cancer, hepatocellular carcinoma, gastric cancer, uterine cancer, and oral squamous cell carcinoma [31-35]; thus, HMGB1 is an oncoprotein. However, because its

paradoxical roles as a tumor suppressor and an oncogenic factor have been reported, the role of HMGB1 in cancer progression remains unclear [45]. Intracellularly, HMGB1 is a highly conserved chromosomal protein acting as a DNA chaperone, whereas extracellularly, it acting with cytokines, chemokines, and growth factors [45].

HMGB1 overexpression has been associated with poor prognosis, progression, and angiogenesis in BC in both clinical and animal studies [25, 26]. In the present study, four *HMGB1* SNPs were assessed in patients with UCC and healthy controls, with the results indicating that CT or CT+TT at rs1045411 is significantly associated with lower UCC risk. This result is consistent with our previous study on hepatocellular carcinoma and oral squamous cell carcinoma, in which we observed that CT and CT+TT at rs1045411 was associated with lower risk of malignancy than was the wild type [31, 33]. The 3'-untranslated region of *HMGB1* covers 2 kb, where rs1045411 is located, which might be the region most sensitive to microRNA epigenetic regulation. Furthermore, compared with the T allele, the C allele creates a slight kink in the *HMGB1* mRNA structure, resulting in a less negative free-energy state and less stable hybridization and thus altering the *HMGB1* mRNA stability and increasing susceptibility to malignant progression [35, 46]. This finding also suggests the synergistic protective effect of at least one T allele at rs1045411 in women and nonsmokers—established by the lower UCC risk noted in comparison with that in men and smoker, respectively; thus, compared with the wild type, the presence of at least one T allele at rs1045411 is potentially associated with a less aggressive UCC. In addition, individuals with CC at rs1412125 were associated with less UCC risk after adjustment. This echoes our previous finding that one C allele at rs1412125 is associated with less distal metastasis risk in hepatocellular carcinoma [31]. This SNP, situated at a transcription factor binding site, may be one of the most common elements in eukaryotic promoters [47, 48]. Although the actual mechanism of action of rs1412125 is unclear, the C allele potentially also reduces lung cancer risk [34].

To our knowledge, no study has examined *HMGB1* SNPs with regard to UCC susceptibility and clinicopathology. However, this study has several limitations. First, treatment and survival data were lacking; these data may aid in enhanced interpretation of *HMGB1* SNP mechanism in a larger cohort study. Second, a study using larger sample size is required to confirm the actual function and statistical significance of our results. A large-scale study verifying the association of *HMGB1* SNP with UCC risk is warranted.

Table 2. Distribution frequency of *HMGB1* genotypes in 862 controls and 431 UCC patients.

Variable	Controls (N= 862) n(%)	Patients(N= 431) n (%)	OR (95% CI)	AOR (95% CI)
rs1412125				
TT	448 (52.0%)	231 (53.6%)	1.00	1.00
TC	336 (39.0%)	175 (40.6%)	1.010 (0.793-1.287)	1.015 (0.792-1.301)
CC	78 (9.0%)	25 (5.8%)	0.622 (0.386-1.002)	**0.555 (0.341-0.902)***
TC+CC	414 (48.0%)	200 (46.4%)	0.937 (0.743-1.181)	0.922 (0.727-1.169)
rs1360485				
TT	474 (55.0%)	255 (59.2%)	1.00	1.00
TC	327 (37.9%)	154 (35.7%)	0.875 (0.685-1.118)	0.874 (0.680-1.123)
CC	61 (7.1%)	22 (5.1%)	0.670 (0.402-1.117)	0.646 (0.384-1.088)
TT+CC	388 (45.0%)	176 (40.8%)	0.843 (0.667-1.066)	0.837 (0.659-1.064)
rs1045411				
CC	503 (58.4%)	283 (65.7%)	1.00	1.00
CT	304 (35.3%)	127 (29.5%)	**0.743 (0.576-0.956)***	**0.732 (0.565-0.949)***
TT	55 (6.4%)	21 (4.8%)	0.679 (0.402-1.145)	0.668 (0.392-1.139)
CT+TT	359 (41.6%)	148 (34.3%)	**0.733 (0.576-0.932)***	**0.722 (0.565-0.924)***
rs2249825				
CC	606 (70.3%)	299 (69.4%)	1.00	1.00
CG	233 (27.0%)	121 (28.1%)	1.053 (0.812-1.365)	1.018 (0.780-1.327)
GG	23 (2.7%)	11 (2.5%)	0.969 (0.466-2.015)	0.871 (0.414-1.834)
CG+GG	256 (29.7%)	132 (30.6%)	1.045 (0.813-1.344)	1.004 (0.776-1.299)

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. Note: * and Bold text indicated a significant association with p value <0.05.

Table 3. Distribution frequency of the clinical status and *HMGB1* rs1045411 genotype frequencies in 159 female patients with UCC.

Variable	HMGB1 (rs1045411)		OR (95% CI)	p value
	CC (%) (n=103)	CT+TT (%) (n=56)		
Stage				
Non muscle invasive tumor (pTa–pT1)	50 (48.5%)	34 (60.7%)	1.00	
Muscle invasive tumor (pT2–pT4)	53 (51.5%)	22 (39.3%)	0.610 (0.315-1.182)	p=0.142
Tumor T status				
Ta	12 (11.7%)	14 (25.0%)	1.00	
T1-T4	91 (88.3%)	42 (75.0%)	0.396 (0.169-0.929)	**p=0.030** *
Lymph node status				
N0	92 (89.3%)	49 (87.5%)	1.00	
N1+N2	11 (10.7%)	7 (12.5%)	1.195 (0.436-3.277)	p=0.729
Metastasis				
M0	102 (99.0%)	54 (96.4%)	1.00	
M1	1 (1.0%)	2 (3.6%)	3.778 (0.335-42.613)	p=0.250
Histopathologic grading				
Low grade	9 (8.7%)	5 (8.9%)	1.00	
High grade	94 (91.3%)	51 (91.1%)	0.977 (0.311-3.069)	p=0.968

Note: * and Bold text indicated a significant association with p value <0.05.

Table 4. Distribution frequency of the clinical status and *HMGB1* rs1045411 genotype frequencies in 300 UCC patients with non-smoker.

Variable	HMGB1 (rs1045411)			
	CC (%) (n=194)	CT+TT (%) (n=106)	OR (95% CI)	p value
Stage				
Non muscle invasive tumor (pTa–pT1)	99 (51.0%)	67 (63.2%)	1.00	
Muscle invasive tumor (pT2–pT4)	95 (49.0%)	39 (36.8%)	0.607 (0.374-0.985)	**p=0.043***
Tumor T status				
Ta	34 (17.5%)	27 (25.5%)	1.00	
T1-T4	160 (82.5%)	79 (74.5%)	0.622 (0.351-1.102)	p=0.102
Lymph node status				
N0	174 (89.7%)	94 (88.7%)	1.00	
N1+N2	20 (10.3%)	12 (11.3%)	1.111 (0.520-2.371)	p=0.786
Metastasis				
M0	191 (98.5%)	104 (98.1%)	1.00	
M1	3 (1.5%)	2 (1.9%)	1.224 (0.201-7.445)	p=0.826
Histopathologic grading				
Low grade	23 (11.9%)	13 (12.3%)	1.00	
High grade	171 (88.1%)	93 (87.7%)	0.962 (0.466-1.988)	p=0.917

Note: * and Bold text indicated a significant association with p value <0.05.

In conclusion, this is the first report to correlate *HMGB1* polymorphisms with UCC risk. Individuals carry at least one T allele at rs1045411 had a lower UCC risk and less invasive disease; most of this population was female and nonsmoking.

Acknowledgments

Competing Interests

The authors have declared that no competing interest exists.

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