Impacts of *AURKA* genetic polymorphism on urothelial cell carcinoma development

Chia-Hung Huang[1,2,#], Chih-Jung Chen[3,4,#], Pei-Ni Chen[5], Shian-Shiang Wang[4,6], Ying-Erh Chou[1,4,7], Sheng-Chun Hung[1,4,6], Shun-Fa Yang[1,7,*]

[1]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[2]Division of Nephrology, Department of Internal Medicine, Lin Shin Hospital, Taichung, Taiwan
[3]Department of Surgical Pathology, Changhua Christian Hospital, Changhua, Taiwan
[4]School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[5]Institute of Biochemistry, Microbiology and Immunology, Chung Shan Medical University, Taichung, Taiwan
[6]Division of Urology, Department of Surgery, Taichung Veterans General Hospital, Taichung, Taiwan
[7]Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan

[#]These authors contributed equally to the work.

[*]**Address correspondence to:** Shun-Fa Yang, Ph.D. Institute of Medicine, Chung Shan Medical University, Taichung 402, Taiwan; Tel: +886-4-24739595 ext. 34253; Fax: +886-4-24723229; E-mail: ysf@csmu.edu.tw

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Running Title: *AURKA* polymorphism in UCC

Abstract

Urothelial cell carcinoma (UCC) is the most common primary malignancy of the urinary system and the second-most common type of renal cell carcinoma. Aurora kinase A (AURKA), a serine/threonine kinase, has a critical role in centrosome duplication, spindle assembly checkpoint, and cytokinesis. Here, we determined the correlation between UCC susceptibility and *AURKA* polymorphisms. We used real-time polymerase chain reaction to compare the genotype distributions and allelic frequencies of four single-nucleotide polymorphisms (SNPs) of *AURKA*, namely rs1047972, rs2273535, rs2064863, and rs6024836, between 431 UCC cases and 862 healthy controls. Logistic regression models demonstrated that the G allele of rs2064863, a genetic polymorphism of AURKA, exhibited a significant protective effect against UCC among the 862 nonsmokers. Moreover, patients with rs2064863 G allele exhibited a slightly lower risk of lymph node metastasis and those with rs6024836 G allele exhibited a lower risk of distant metastases. Our study suggested that several variants of *AURKA* SNPs rs2064863 and rs6024836 may serve as critical predictors for the clinical status of UCC.

Keywords: Urothelial cell carcinoma; Aurora kinase A; Susceptibility; Single nucleotide polymorphism

Introduction

Urothelial cell carcinoma (UCC), also called transitional cell carcinoma, includes carcinomas affecting the bladder, urethra, ureters, and renal pelvis [1]. UCC is the most common type of bladder cancer, accounting for more than 90% of all bladder cancers, and it poses a challenge to radiologists [2]. Invasive urothelial carcinomas grow from the tissue lining of the bladder into the deeper layers of the bladder wall, and they can extend from the kidney collecting system to the bladder [2]. UCC of the bladder is the fifth-most common cancer in the United States [3]. For patients with metastatic UCC, cisplatin-based chemotherapy, the standard first-line treatment, provides a median overall survival of 13–16 months [4]. This finding emphasizes the need for expanding the armamentarium of reasonable treatment strategies for advanced UCC.

Aurora kinase A (AURKA) is a cell cycle-regulated serine/threonine kinase involved in mitotic spindle formation, chromosome amplification and segregation, aneuploidy, G2/M phase transition during cell cycle, and malignant transformation [5]. AURKA may play a role in cancer development and progression as well as tumorigenesis, and it is frequently overexpressed or mutated in a wide range of human cancers, including lung, liver, prostate, cervix, ovary, and bladder cancers [5-8]. In patients with melanoma, AURKA overexpression is correlated with shortened

survival and poor prognosis and driven by the activation of forkhead box M1 and mitogen activated protein kinase/extracellular-signal-regulated kinase, as noted in BRAF-mutated melanoma cells [9]. AURKA overexpression is also associated with pathological stage and distant metastasis. Inhibition of this overexpression results in the regulation of epithelial–mesenchymal transition in hepatocellular carcinoma, which in turn limits cell invasion [8]. We previously demonstrated that betel nut chewing combined with C-A-T haplotypes of *AURKA* increased the risk of oral squamous cell carcinoma and that the *AURKA* single-nucleotide polymorphism (SNP) rs2064863 increased the risk of advanced-stage tumor development [10]. T/T homozygote carriers of *AURKA* rs1047972 are at a higher risk of hepatocellular carcinoma than C/C homozygote wild-type carriers are [11]. *AURKA* rs2273535 is also associated with a high risk of breast cancer in Asians, whereas in Caucasians, *AURKA* rs1047972 is associated with a low risk of breast cancer risk [12]. Although *AURKA* SNPs increase the risk of cancer, including liver cancer, oral cancer, gastric cancer and breast cancer [10-13], their association with UCC remains unclear. In this case–control study, we assessed this relationship by investigating of four *AURKA* SNPs, namely rs1047972, rs2273535, rs2064863, and rs6024836, in Taiwanese patients with UCC.

Materials and methods

Study participants and ethics statement

We analyzed 431 patients with UCC (272 men and 159 women; mean age=68.6 ± 11.8 years) from the Taichung Veterans General Hospital, Taichung, Taiwan, between 2010 and 2015. We also included healthy controls that had no history of cancer at any site. All research participants were provided with a written description of the study including questions regarding their demographic characteristics. Their personal information was recorded based on their responses. This study was approved by the Institutional Review Board (IRB) of Taichung Veterans General Hospital (IRB no. CF11094), and written informed consent was obtained from all participants before the study was performed. Whole-blood samples collected from the patients and controls were placed in tubes containing ethylenediaminetetraacetic acid (EDTA), immediately centrifuged, and finally, frozen at −80 °C for future DNA extraction.

AURKA SNP selection

For this study, four *AURKA* SNPs, namely rs1047972, rs2273535, rs2064863, and rs6024836, with minor allele frequencies >5% were selected from the International HapMap Project data. The selected SNPs were associated with tumor progression, including that in liver cancer, breast cancer and oral cancers [10, 12, 14].

DNA extraction and genotype determination

Total genomic DNA was extracted from whole blood by using QIAamp DNA blood mini kits based on silica spin column capture (Qiagen, Valencia, CA, USA) for DNA isolation. DNA was eluted from the columns, dissolved in TE buffer, and quantified according to measurements of the optical density at 260 nm. Each final

prepared specimen was stored at −20 °C and used as a template for quantitative polymerase chain reaction (PCR) analysis. Evaluation of *AURKA* polymorphisms were performed using TaqMan SNP genotyping assays with the ABI StepOne Real-time PCR System as previously described [10, 15]. The SNPs were further analyzed using SDS (version 3.0; Applied Biosystems, Foster City, CA, USA).

Statistical analysis

The Hardy–Weinberg equilibrium of the distribution of the genotypes of each SNP was estimated using the chi-square test. The distributions of demographic characteristics and genotype frequencies were compared between healthy controls and UCC cases by using Fisher's exact test and Mann–Whitney *U* test. Odds ratio (ORs) and their 95% confidence intervals (CIs) were estimated using logistic regression models. The relationship between genotype frequencies and UCC risk was assessed according to adjusted ORs (AORs) with 95% CIs derived from multiple logistic regression models. $p < 0.05$ indicated statistically significant differences. Data were analyzed using SAS (version 9.1, 2005; SAS Institute Inc., Cary, NC).

Results

Patient characteristics and distribution of UCC

The demographic characteristics of the participants were statistically analyzed; the results are summarized in Table 1. In total, 431 patients with UCC and 862 healthy controls (mean age ± standard deviation, 68.6 ± 11.8 and 57.2 ± 10.0 years, respectively) were included. The mean age of patients with UCC significantly differed from that of the control groups ($p<0.001$). No significant differences were observed in the distributions of gender and cigarette smoking between patients and controls. Approximately 54.5% of the patients had been diagnosed with nonmuscle invasive tumor (stage pTa–pT1).

Associations between *AURKA* SNPs and UCC

The genotype distributions and allelic frequencies of AURKA polymorphisms of patients with UCC and control participants are denoted in Table 2. In our recruited control group, *AURKA* genotype distribution revealed that the most frequent alleles were homozygous C/C for rs1047972, homozygous T/T for rs2273535 and rs2064863, and heterozygous A/G for rs6024836. No significant differences were observed in the allele and genotype frequencies of rs1047972, rs2273535, rs2064863, or rs6024836 between the patients and controls (Table 2).

Genotype distribution of *AURKA* SNPs among 862 nonsmokers

We investigated genotype distributions of the four AURKA SNPs among 862 nonsmokers (300 patients with UCC and 562 controls; Table 3). After age and gender were controlled, among the 862 nonsmokers, individuals with the G/T genotype of rs2064863 demonstrated a significantly lower risk of UCC than those with the T/T genotype (AOR = 0.669, 95% CI 0.454–0.986, $p< 0.05$; Table 3). Moreover, the individuals with the G/T+G/G genotype of rs2064863 were at a significantly lower risk of UCC compared with those with the T/T genotype (AOR = 0.649, 95% CI 0.447–0.944, $p< 0.05$; Table 3). We did not observe significant differences in the allele and genotype frequencies of rs1047972, rs2273535, or rs6024836 between the patients with UCC and controls.

Associations between rs2064863 and clinical status of UCC

The role of AURKA rs2064863 in the clinicopathological status of patients with UCC was assessed. To this end, we estimated the distribution frequency of clinical statuses, including tumor clinical stage, primary tumor size, lymph node involvement, distant metastases, and histopathological grading, as well as the frequency of *AURKA* genotypes in 431 patients with UCC. Among the 431 patients with UCC, patients with

rs2064863 exhibited a 0.418-fold lower risk of developing a lymph node metastasis status (N1 + N2) of UCC (95% CI 0.191–0.917) than did patients with wild-type *AURKA* (p= 0.030). No significant difference was observed between these patient groups with regard to clinical stage, tumor size, distant metastases, or cell differentiation.

Associations between rs6024836 and clinical status of UCC

We further evaluated the associations of rs6024836 with various clinical characteristics of patients with UCC, including clinical tumor stage, tumor size, lymph node involvement, distant metastases, and histopathologic grading. The results indicated that the patients with rs6024836 exhibited a 0.257-fold lower risk of distant metastases (M1) of UCC (95% CI 0.079–0.832) than did patients with wild-type *AURKA* (p= 0.023). No significant differences between these patient groups, with regard to clinical tumor stage, tumor size, lymph node involvement, or cell differentiation, were noted.

Discussion

Mitosis-regulating AURKA overexpression is strongly associated with cancer progression [16, 17], and genetic susceptibility is critical in various cancer types. Identifying the specific AURKA gene involved in susceptibility to cancer may aid the management of cancer risk. The genotypic polymorphisms of mitosis-regulating *AURKA* have been described. Asian carriers of *AURKA* rs2273535 are at a high risk of breast cancer, but Caucasian carriers are not [18]. *AURKA* rs2273535 may increase susceptibility to UTUC [19]. Moreover, individuals who received alisertib for metastatic urothelial carcinoma, with the AA genotype of rs1047972 have longer progression-free survival compared with individuals with the wild-type TT genotype [20]. *AURKA* rs2273535 (T91A) is significantly associated with high oral cancer risk [21]. Although studies have demonstrated that *AURKA* SNPs are associated with a relatively high risk of tumors, few studies have addressed the relationship between AURKA genetic variants and UCC. In the present study, we did not observe an association between UCC risk and rs1047972, rs2273535, or rs6024836. Our data provided evidence that among nonsmokers, the carriers of at least one G allele (G/T or G/G) in rs2064863 were at a lower risk of UCC than were wild-type T/T carriers (Table 3). These results suggested that genetic variation in *AURKA* may be associated

with UCC susceptibility and may interact with smoking. Furthermore, different *AURKA* SNPs have different roles in cancer susceptibility and progression.

Patients with UCC and increased *AURKA* mRNA expression in tumor tissues not only exhibit high rates of cancer metastasis and high-grade tumors but also have relatively low overall and UCC-specific survival [22]. *AURKA* overexpression also promotes cancer metastasis, increases drug resistance, and is associated with poor prognosis [23-26]. In a study on 786 men with oral cancer, patients with the GG genotype and G allele of *AURKA* rs2064863 were at a 1.365-fold higher risk of stage III or IV oral cancer than were those with the wild-type AA genotype [10]. Other reports on hepatocellular carcinoma have indicated that patients with at least one A allele (A/T or A/A genotype) in *AURKA* rs2273535 were less likely progress to stage III or IV (0.593-fold) and develop large tumors (0.591-fold)) [11]. Our study demonstrated that patients with UCC and with the G/G genotype and G allele carriers of *AURKA* rs2064863 were at a low risk of lymph node metastases; however, this relationship did not extend to the clinical tumor stage, tumor size, metastasis to distant organs, or cell differentiation (Table 4). Similarly, the G/G genotype and G allele carriers of *AURKA* rs6024836 were associated with a low risk of distant metastases of UCC, but this relationship did not extend to the clinical tumor stage, tumor size, lymph node involvement, or cell differentiation (Table 5). These findings suggest that

patients with at least one G allele in *AURKA* rs2064863 or rs6024836 are less likely to experience lymph node metastases or distant metastases, respectively.

In conclusion, several SNP variants of *AURKA* are associated with susceptibility to and clinicopathologic status of UCC. Our data provided evidence that carriers of the G/T and G/G allele of *AURKA* rs2064863 were at a lower risk of UCC than wild-type carriers of the T/T allele. Thus, the AURKA SNPs may be significant predictors of UCC occurrence and reliable biomarkers of disease progression and metastasis in patients with UCC.

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

Variable	Controls (N=862) n (%)	Patients (N=431) n (%)	p value
Age (yrs)			<0.001
≦65	697 (80.9)	166 (38.5)	
>65	165 (19.1)	265 (61.5)	
Mean ± S.D.	57.2 ± 10	68.6 ± 11.8	<0.001
Gender			0.365
Female	296 (34.3)	159 (36.9)	
Male	566 (65.7)	272 (63.1)	
Tobacco consumption			0.113
No	562 (65.2)	300 (69.6)	
Yes	300 (34.8)	131 (30.4)	
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)	

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

Table 2. Genotype Distributions of AURKA Gene Polymorphisms in 862 Controls and 431 Patients with UCC.

Variable	Controls (N=862) n (%)	Patients (N=431) n (%)	OR (95% CI)	AOR (95% CI)
rs1047972				
CC	658 (76.3%)	319 (74%)	1.000 (reference)	1.000 (reference)
TC	194 (22.5%)	106 (24.6%)	1.127 (0.859-1.479)	1.136 (0.831-1.553)
TT	10 (1.2%)	6 (1.4%)	1.238 (0.446-3.435)	1.599 (0.495-5.167)
TC+TT	204 (23.7%)	112 (26%)	1.132 (0.868-1.478)	1.156 (0.851-1.570)
rs2273535				
TT	409 (47.4%)	190 (44.1%)	1.000 (reference)	1.000 (reference)
AT	355 (41.2%)	192 (44.5%)	1.164 (0.910-1.489)	1.055 (0.798-1.396)
AA	98 (11.4%)	49 (11.4%)	1.076 (0.733-1.580)	0.938 (0.599-1.470)
AT+AA	453 (52.6%)	241 (55.9%)	1.145 (0.908-1.445)	1.031 (0.791-1.344)
rs2064863				
TT	591 (68.6%)	306 (71%)	1.000 (reference)	1.000 (reference)
GT	243 (28.2%)	114 (26.5%)	0.906 (0.697-1.177)	0.791 (0.586-1.067)
GG	28 (3.2%)	11 (2.6%)	0.759 (0.373-1.545)	0.505 (0.220-1.160)
GT+GG	271 (31.4%)	125 (29%)	0.891 (0.692-1.147)	0.758 (0.568-1.013)
rs6024836				
AA	363 (42.1%)	173 (40.1%)	1.000 (reference)	1.000 (reference)
AG	376 (43.6%)	201 (46.6%)	1.122 (0.874-1.439)	1.143 (0.860-1.519)
GG	123 (14.3%)	57 (13.2%)	0.972 (0.677-1.397)	1.035 (0.684-1.564)
AG+GG	499 (57.9%)	258 (59.9%)	1.085 (0.857-1.373)	1.117 (0.854-1.460)

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

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. Genotype Distributions of AURKA Gene Polymorphisms among 862 non-smokers.

Variable	Controls (N=562) n (%)	Patients (N=300) n (%)	OR (95% CI)	AOR (95% CI)
rs1047972				
CC	434 (77.2%)	224 (74.7%)	1.000 (reference)	1.000 (reference)
TC	122 (21.7%)	72 (24%)	1.143 (0.820-1.595)	1.167 (0.775-1.759)
TT	6 (1.1%)	4 (1.3%)	1.292 (0.361-4.624)	2.266 (0.493-10.414)
TC+TT	128 (22.8%)	76 (25.3%)	1.150 (0.830-1.594)	1.209 (0.810-1.805)
rs2273535				
TT	263 (46.8%)	135 (45%)	1.000 (reference)	1.000 (reference)
AT	239 (42.5%)	132 (44%)	1.076 (0.799-1.448)	0.913 (0.638-1.306)
AA	60 (10.7%)	33 (11%)	1.071 (0.668-1.719)	0.808 (0.448-1.456)
AT+AA	299 (53.2%)	165 (55%)	1.075 (0.811-1.424)	0.892 (0.635-1.253)
rs2064863				
TT	382 (68%)	215 (71.7%)	1.000 (reference)	1.000 (reference)
GT	162 (28.8%)	76 (25.3%)	0.834 (0.605-1.147)	**0.669 (0.454-0.986)**
GG	18 (3.2%)	9 (3%)	0.888 (0.392-2.012)	0.496 (0.180-1.364)
GT+GG	180 (32%)	85 (28.3%)	0.839 (0.617-1.141)	**0.649 (0.447-0.944)**
rs6024836				
AA	231 (41.1%)	122 (40.7%)	1.000 (reference)	1.000 (reference)
AG	254 (45.2%)	145 (48.3%)	1.081 (0.801-1.458)	1.055 (0.735-1.515)
GG	77 (13.7%)	33 (11%)	0.811 (0.511-1.289)	0.715 (0.407-1.255)
AG+GG	331 (58.9%)	178 (59.3%)	1.018 (0.766-1.354)	0.975 (0.691-1.375)

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

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 and gender.

Table 4. Distribution frequency of the clinical status and AURKA rs2064863 genotype frequencies in 431 UCC patients.

Variable	AURKA (rs2064863)			
	TT (%) (n=306)	GT+GG (%) (n=125)	OR (95% CI)	p value
Stage				
Non muscle invasive tumor (pTa–pT1)	163 (53.3%)	72 (57.6%)	1.000 (reference)	
Muscle invasive tumor (pT2–pT4)	143 (46.7%)	53 (42.4%)	0.839 (0.551-1.277)	0.413
Tumor T status				
Ta	62 (20.3%)	28 (22.4%)	1.000 (reference)	
T1-T4	244 (79.7%)	97 (77.6%)	0.880 (0.532-1.458)	0.620
Lymph node status				
N0	263 (85.9%)	117 (93.6%)	1.000 (reference)	
N1+N2	43 (14.1%)	8 (6.4%)	**0.418 (0.191-0.917)**	**0.030**
Metastasis				
M0	294 (96.1%)	123 (98.4%)	1.000 (reference)	
M1	12 (3.9%)	2 (1.6%)	0.398 (0.088-1.806)	0.233
Histopathologic grading				
Low grade	35 (11.4%)	18 (14.4%)	1.000 (reference)	
High grade	271 (88.6%)	107 (85.6%)	0.768 (0.417-1.414)	0.396

Bold font indicates statistical significance ($p < 0.05$).
The odds ratio (OR) with their 95% confidence intervals were estimated by logistic regression models.

Table 5. Distribution frequency of the clinical status and AURKA rs6024836 genotype frequencies in 431 UCC patients.

Variable	AURKA (rs6024836)			
	AA (%) (n=173)	AG+GG (%) (n=258)	OR (95% CI)	p value
Stage				
Non muscle invasive tumor (pTa–pT1)	91 (52.6%)	144 (55.8%)	1.000 (reference)	
Muscle invasive tumor (pT2–pT4)	82 (47.4%)	114 (44.2%)	0.879 (0.597-1.293)	0.512
Tumor T status				
Ta	37 (21. 4%)	53 (20.5%)	1.000 (reference)	
T1-T4	136 (78.6%)	205 (79.5%)	1.052 (0.656-1.688)	0.833
Lymph node status				
N0	147 (85%)	233 (90.3%)	1.000 (reference)	
N1+N2	26 (15%)	25 (9.7%)	0.607 (0.337-1.091)	0.095
Metastasis				
M0	163 (94.2%)	254 (98.4%)	1.000 (reference)	
M1	10 (5.8%)	4 (1.6%)	**0.257 (0.079-0.832)**	**0.023**
Histopathologic grading				
Low grade	19 (11%)	34 (13.2%)	1.000 (reference)	
High grade	154 (89%)	224 (86.8%)	0.813 (0.447-1.478)	0.497

Bold font indicates statistical significance ($p < 0.05$).
The odds ratio (OR) with their 95% confidence intervals were estimated by logistic regression models.