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Association between the Urokinase Plasminogen Activation System Polymorphisms and NSCLC in Chinese Population

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Abstract
Aim: To evaluate the influence of uPA and uPAR gene polymorphisms in the susceptibility to NSCLC.
Methods: A total of 500 NSCLC patients and 500 healthy controls were recruited and matched according to age and gender. The SNPs distributed in uPA and uPAR gene were selected for genotyping. The association between genotype and NSCLC risk was evaluated by computing the OR and 95% CI with multivariate unconditional logistic regression analyses.
Results: Patients with the uPAR rs344781 T allele had a reduced risk of developing SCC patients (OR = 0.742; 95% CI = 0.579 - 0.950; P = 0.0176), especially a risk of developing male SCC patients (OR = 0.722; 95% CI = 0.546 - 0.954; P = 0.0219). And the uPAR rs344781 C/C allele homozygote was associated with an increased risk of SCC in patients (OR = 1.713; 95% CI = 1.145 - 2.563; P = 0.0083). However, neither allele frequencies nor genotype frequencies in uPA rs4065 were associated with NSCLC (P > 0.05).
Conclusions: Our results revealed that genetic polymorphisms of uPAR rs344781 were associated with the risk of developing SCC (squamous cell carcinoma) in a Chinese population.

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Introduction

Extracellular Matrix (ECM) degradation mediated by the urokinase plasminogen activation (uPA) system is an important mechanism involved in both physiological and pathological tissue remodeling[1,2]. Many experimental and clinical studies have demonstrated the association of uPA system activity with cancer invasion and metastasis, including lung cancer[3,4]. Members of uPA system, including uPA and its receptor (uPAR), play critical roles in proteolysis, migration, invasion, and metastasis[5,6]. The median levels of uPA and uPAR expression are higher in lung tumor tissues than the adjacent lung parenchyma.

Lung cancer is the leading cause of cancer death around the world, due to its high mortality and morbidity[7]. In China alone, the incidence and mortality associated with lung cancer are estimated to be 0.7 and 0.6 million cases, respectively[8]. Pathologically, genetic and environmental interactions play a key role in the development and progression of lung cancer[9]. However, these interactions are dependent on genetic variations or single nucleotide polymorphisms (SNPs). Recently, one study in Taiwan has shown that genetic polymorphisms of the uPA rs4065 C/T and uPAR rs344781 (-516 T/C) were associated with the susceptibility and severity of NSCLC[10]. Although Taiwan and the Chinese mainland are similar in some ways, their living environment and diet were in different ways. In this study, the relationship between SNPs of uPA, uPAR, and NSCLC risk on the Chinese mainland were investigated, and the impact of these SNPs on susceptibility and clinicopathological characteristics of NSCLC were also evaluated.

Materials and Methods

Study population

In this study, we recruited 500 NSCLC patients and 500 unrelated age-matched healthy controls from The Zhejiang Cancer Hospital, Hangzhou, China between March, 2011 and April, 2012. All cases and controls were Chinese Han origin and lived in Zhejiang Province, China. Participants had no history of previous primary cancer other than lung cancer. The controls were independent lung-related diseases to avoid any probable interference from overlapping genes. Current smoker or former smoker or non-smokers were included. All subjects provided their informed consent approved by the Ethic Committee of Zhejiang Cancer Hospital.

SNPs selection and genotyping

uPA rs4065 and uPAR rs344781 were selected according to a previous study[11]. DNA was extracted from whole blood by AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA). The uPA SNP and uPAR SNP of interest were then genotyped using the SEQUENOM Mass ARRAY matrix-assisted laser desorption ionization-time of flight mass spectrometry platform (Sequenom, San Diego, CA). PCR primers and single base extension primers were designed using Assay Designer's software version 3.0 (Sequenom) and synthesized by Sangon Biotech (Shanghai, China). The sequences of forward and reverse primers were as follows: 5´-ACGTTG-GATGAAGAGACTGGGAAGATAGGC-3´ and 5´-ACGTTG-GATGGCCTGAGGGTAAAGCTATTG-3´ for uPA rs4065 (104 bps); 5´-ACGTTGGATGCACATTCCTTTAACATTTACC-3´ and 5´-ACGTTGGATGAACACTTAACCCTTGCTTT-3´ for uPAR rs344781 (115 bps).

Statistical analysis

All statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). Hardy-Weinberg equilibrium (HWE) was carried out for all SNPs, cases and controls were compared using the $\chi 2$ test with a P-value of < 0.001 being considered statistically significant. The $\chi 2$ test was used to assess the frequencies of the selected allele and genotype between the cases and controls. The association between SNPs and NSCLC risk was analyzed by computing the odds ratio (OR) and 95% confidence interval (CI) from multivariate unconditional logistic regression analysis. A two-sided $P < 0.05$ was considered statistically significant.

Results

500 patients (350 males and 150 females) and 500 healthy controls (259 males and 240 females, and gender information for one control subject was missed) were of Chinese Han origin. For NSCLC patients, 331 had adenocarcinoma (ADC), and 169 had squamous-cell carcinomas (SCC); 280 male and 21 female patients were smokers or former smokers, while 189 male and 14 female of the controls were smokers or former smokers. The studied population were within HWE ($P = 0.17109$ for uPA rs4065; $P = 0.14444$ for uPAR rs344781, respectively).

The allele frequency of uPA rs4065 was 12.6% (T) and 87.4% (C) in NSCLC patients, 12.7% (T) and 87.3% (C) in ADC patients, 12.4% (T) and 87.6% (C) in SCC patients, and 12.5% (T) and 87.5% (C) in controls. No statistical differences in allele frequencies of these four SNPs were found between the case and control subjects ($P > 0.05$). Stratification by gender revealed no significant difference in allele frequencies ($P > 0.05$). (Table 1,2,3). Allele frequency of uPAR rs344781 was 51.8% (T) and 48.2% (C) in NSCLC patients, 54.2% (T) and 45.8% (C) in ADC patients, 47.0% (T) and 53.0% (C) in SCC patients, and 54.5% (T) and 45.5% (C) in controls. In patients with SCC, the allele frequencies in uPAR rs344781 were significantly different from the controls (OR = 0.742; 95% CI = 0.579 - 0.950; $P = 0.0176$). We then stratified by analysis of gender, the allele frequencies in uPAR rs344781 were significantly different between male SCC patients and male controls (OR = 0.722; 95% CI = 0.546 - 0.954; $P = 0.0219$). The T allele was found to be associated with a lower risk for SCC development, and it may be a potential lower-risk marker for male SCC patients in China. (Table 1,2,3).

Table 1: Allele frequency of uPA and uPAR SNPs in NSCLC patients and healthy controls.

Gene Allele	N S C L C N=500(%)	Controls N=500(%)	P value	OR(95%CI)	M NSCLC N=350(%)	M Controls N=259(%)	P value	OR(95%CI)	F NSCLC N=150(%)	F Controls N=240(%)	P value	OR(95%CI)
uPA rs4065												
T	126(12.6)	125(12.5)			86(12.3)	63(12.2)			40(13.3)	62(12.9)		
C	874(87.4)	875(87.5)	0.9462	1.009(0.775 -1.315)	614(87.7)	455(87.8)	0.9481	1.012(0.715 -1.431)	260(86.7)	418(87.1)	0.8667	1.037(0.677 -1.589)
uPAR rs344781												
T	518(51.8)	545(54.5)			359(51.3)	285(55.0)			159(53.0)	258(53.7)		
C	482(48.2)	455(45.5)	0.2263	0.897(0.753 -1.070)	341(48.7)	233(45.0)	0.1969	0.861(0.685 -1.081)	141(47.0)	222(46.3)	0.8381	0.97(0.727 -1.296)

NSCLC, Non-small cell lung cancer; M, Male; F, Female.

Genotype frequencies of uPA rs4065 genotype were 76.2% (C/C), 1.4% (T/T) and 22.4% (C/T) in NSCLC patients, 76.1% (C/C), 1.5% (T/T) and 22.4% (C/T) in ADC patients, 76.3% (C/C), 1.2% (T/T) and 22.5% (C/T) in SCC patients, 75.8% (C/C), 0.8% (T/T), and 23.4% (C/T) in the controls. No statistical differences in allele frequencies of these four SNPs were found between the case and control subjects ($P > 0.05$). Stratification by gender revealed no significant difference in genotype frequencies ($P > 0.05$). Genotype frequencies of uPAR rs344781 were 22.8% (C/C), 26.4% (T/T) and 50.8% (C/T) in NSCLC patients, 19.9% (C/C), 28.4% (T/T) and 51.7% (C/T) in ADC patients, 28.4% (C/C), 22.5% (T/T) and 49.1% (C/T) in SCC patients, 18.8% (C/C), 27.8% (T/T), and 53.4% (C/T) in the controls. There were statistically significant differences for the C/C, T/T, and C/T genotypes between

SCC patients and controls (*P* = 0.0263). When analyzing the association between genotypes and the risk of SCC, logistic regression analysis revealed that the uPAR rs344781 C/C allele homozygote was associated with an increased risk of SCC in patients (OR = 1.713; 95% CI = 1.145 - 2.563; *P* = 0.0083). (Table 4,5,6).

Table 2: Allele frequency of uPA and uPAR SNPs in ADC patients and controls.

Gene Allele	ADC N=331(%)	Controls N=500(%)	P value	OR(95%CI)	M ADC N=189(%)	M Controls N=259(%)	P value	OR(95%CI)	F ADC N=142(%)	F Controls N=240(%)	P value	OR(95%CI)
uPA rs4065												
T	84(12.7)	125(12.5)			47(12.4)	63(12.2)			37(13.0)	62(12.9)		
C	578(87.3)	875(87.5)	0.9095	1.017(0.757 -1.367)	331(87.6)	455(87.8)	0.9026	1.026(0.685 -1.535)	247(87.0)	418(87.1)	0.9646	1.01 (0.653 -1.563)
uPAR rs344781												
T	359(54.2)	545(54.5)			208(55.0)	285(55.0)			151(53.2)	258(53.7)		
C	303(45.8)	455(45.5)	0.9137	0.989(0.812 -1.205)	170(45.0)	233(45.0)	0.9983	1 (0.766 -1.306)	133(46.8)	222(46.3)	0.8763	0.977(0.728 -1.311)

ADC, adenocarcinoma; M, Male; F, Female.

Table 3: Allele frequency of uPA and uPAR SNPs in SCC patients and controls.

Gene Allele	SCC N=169(%)	Controls N=500(%)	P value	OR(95%CI)	M SCC N=161(%)	M Controls N=259(%)	P value	OR(95%CI)	F SCC N=8(%)	F Controls N=240(%)	P value	OR(95%CI)
uPA rs4065												
T	42(12.4)	125(12.5)			39(12.1)	63(12.2)			3(18.8)	62(12.9)		
C	296(87.6)	875(87.5)	0.9716	0.993(0.684 -1.443)	283(87.9)	455(87.8)	0.9827	0.995(0.650 -1.524)	13(81.3)	418(87.1)	0.4964	1.556(0.431 -5.615)
uPAR rs344781												
T	159(47.0)	545(54.5)			151(46.9)	285(55.0)			8(50.0)	258(53.7)		
C	179(53.0)	455(45.5)	0.0176	0.742(0.579 -0.950)	171(53.1)	233(45.0)	0.0219	0.722(0.546 -0.954)	8(50.0)	222(46.3)	0.7673	0.86(0.318 -2.330)

SCC, squamous cell carcinoma; M, Male; F, Female.

Table 4: Genotypes of uPA and uPAR SNPs in NSCLC patients and controls.

Gene Allele	NSCLC N=500(%)	Controls N=500(%)	P value	OR(95%CI)	M NSCLC N=350(%)	M Controls N=259(%)	P value	OR(95%CI)	F NSCLC N=150(%)	F Controls N=240(%)	P value	OR(95%CI)
uPA rs4065												
C/C	381(76.2)	379(75.8)			269(76.9)	198(76.4)			112(74.7)	180(75.0)		
T/T	7(1.4)	4(0.8)			5(1.4)	2(0.8)			2(1.3)	2(0.8)		
C/T	112(22.4)	117(23.4)	0.6273		76(21.7)	59(22.8)	0.7271		36(24.0)	58(24.2)	0.8925	
T / T+C/T	119(23.8)	121(24.2)	0.8823	1.022(0.765 -1.366)	81(23.1)	61(23.6)	0.906	1.023(0.700 -1.495)	38(25.3)	60(25.0)	0.9411	0.982(0.614 -1.572)
C / C+C/T	493(98.6)	496(99.2)	0.3631	1.761(0.512 -6.053)	345(98.6)	257(99.2)	0.4525	1.862(0.358 -9.676)	148(98.7)	238(99.2)	0.6335	1.608(0.224 -11.539)
uPAR rs344781												
C/C	114(22.8)	94(18.8)			85(24.3)	53(20.5)			29(19.3)	41(17.1)		
T/T	132(26.4)	139(27.8)			94(26.9)	79(30.5)			38(25.3)	59(24.6)		
C/T	254(50.8)	267(53.4)	0.297		171(48.9)	127(49.0)	0.4368		83(55.3)	140(58.3)	0.8079	
TT+CT	386(77.2)	406(81.2)	0.1192	1.276(0.939 -1.733)	265(75.7)	206(79.5)	0.2653	1.247(0.845 -1.838)	121(80.7)	199(82.9)	0.5732	1.163(0.687 -1.969)
CC+CT	368(73.6)	361(72.2)	0.6185	0.932(0.705 -1.231)	256(73.1)	180(69.5)	0.3241	0.837(0.587 -1.193)	112(74.7)	181(75.4)	0.8676	1.041(0.650 -1.667)

NSCLC, Non-small cell lung cancer; M, Male; F, Female.

Table 5: Genotypes of uPA and uPAR SNPs in ADC patients and controls.

Gene allele	ADC N=331(%)	Controls N=500(%)	P value	OR(95%-CI)	M ADC N=189(%)	M Controls N=259(%)	P value	OR(95%-CI)	F ADC N=142(%)	F Controls N=240(%)	P value	OR(95%CI)
uPA rs4065												
C/C	252(76.1)	379(75.8)			145(76.7)	198(76.4)			107(75.4)	180(75.0)		
T/T	5(1.5)	4(0.8)			3(1.6)	2(0.8)			2(1.4)	2(0.8)		
C/T	74(22.4)	117(23.4)	0.5988		41(21.7)	59(22.8)	0.7014		33(23.2)	58(24.2)	0.8541	
T/T+C/T	79(23.9)	121(24.2)	0.9125	1.018(0.736-1.410)	44(23.3)	61(23.6)	0.9466	1.015(0.652-1.581)	35(24.6)	60(25.0)	0.9387	1.019(0.630-1.648)
C/C+C/T	326(98.5)	496(99.2)	0.3326	1.902(0.507-7.135)	186(98.4)	257(99.2)	0.4173	2.073(0.343-12.528)	140(98.6)	238(99.2)	0.5936	1.7(0.237-12.203)
uPAR rs344781												
C/C	66(19.9)	94(18.8)			39(20.6)	53(20.5)			27(19.0)	41(17.1)		
T/T	94(28.4)	139(27.8)			58(30.7)	79(30.5)			36(25.4)	59(24.6)		
C/T	171(51.7)	267(53.4)	0.872		92(48.7)	127(49.0)	0.9972		79(55.6)	140(58.3)	0.8515	
T/T+C/T	265(80.1)	406(81.2)	0.6834	1.076(0.757-1.528)	150(79.4)	206(79.5)	0.9646	1.011(0.635-1.607)	115(81.0)	199(82.9)	0.6335	1.14(0.666-1.950)
C/C+C/T	237(71.6)	361(72.2)	0.8508	1.03(0.756-1.403)	131(69.3)	180(69.5)	0.9664	1.009(0.672-1.515)	106(74.6)	181(75.4)	0.8666	1.042(0.645-1.682)

ADC, adenocarcinoma; M, Male; F, Female.

Table 6: Genotypes of uPA and uPAR SNPs in SCC patients and controls.

Gene allele	SCC N=169(%)	Controls N=500(%)	P value	OR(95%-CI)	M SCC N=161(%)	M Controls N=259(%)	P value	OR(95%-CI)	F SCC N=8(%)	F Controls N=240(%)	P value	OR(95%CI)
uPA rs4065												
C/C	129(76.3)	379(75.8)			124(77.0)	198(76.4)			5(62.5)	180(75.0)		
T/T	2(1.2)	4(0.8)			2(1.2)	2(0.8)			0(0.0)	2(0.8)		
C/T	38(22.5)	117(23.4)	0.8792		35(21.7)	59(22.8)	0.8682		3(37.5)	58(24.2)	0.6742	
T/T+C/T	40(23.7)	121(24.2)	0.8889	1.03(0.684-1.551)	37(23.0)	61(23.6)	0.893	1.032(0.648-1.645)	3(37.5)	60(25.0)	0.4243	0.556(0.129-2.394)
C/C+C/T	167(98.8)	496(99.2)	0.6476	1.485(0.270-8.182)	159(98.8)	257(99.2)	0.6297	1.616(0.225-11.590)	8(100.0)	238(99.2)	0.7954	
uPAR rs344781												
C/C	48(28.4)	94(18.8)			46(28.6)	53(20.5)			2(25.0)	41(17.1)		
T/T	38(22.5)	139(27.8)			36(22.4)	79(30.5)			2(25.0)	59(24.6)		
C/T	83(49.1)	267(53.4)	0.0263		79(49.1)	127(49.0)	0.0753		4(50.0)	140(58.3)	0.8299	
T/T+C/T	121(71.6)	406(81.2)	0.0083	1.713(1.145-2.563)	115(71.4)	206(79.5)	0.057	1.555(0.985-2.453)	6(75.0)	199(82.9)	0.5607	1.6189(0.315-8.301)
C/C+C/T	131(77.5)	361(72.2)	0.1757	0.753(0.500-1.136)	125(77.6)	180(69.5)	0.0689	0.656(0.416-1.035)	6(75.0)	181(75.4)	0.9785	1.023(0.201-5.204)

SCC, squamous cell carcinoma; M, Male; F, Female.

Discussion

In the current study, we investigated the association between SNPs (uPA rs4065 and uPAR rs344781) and the risk of developing NSCLC. Indeed, we found that patients with the uPAR rs344781 T allele had a reduced risk of developing SCC patients, especially a reduced risk of developing male SCC patients. And the uPAR rs344781 C/C allele homozygote was associated with an increased risk of SCC in patients. However, neither allele frequencies nor genotype frequencies in uPA rs4065 were associated with NSCLC.

Tumor invasion and metastasis require proteolytic enzymes that destroy the extracellular matrix and enhance the formation of novel blood vessels[11]. The uPA system is thought to play a role in several different processes important to tumor progression including tissue remodeling, chemotaxis, tumor invasion, dissemination, proliferation, and angiogenesis[12,13]. The binding of uPA to uPAR increases the efficiency of plasminogen activation and also serves to localize these proteolytic cascades to the migrating or invading edge of cells[5,14,15]. Binding of uPA and vitronectin also promotes cell adhesion and cell migration. In addition, uPAR also interacts with various cell surface receptors such as integrins, growth factor receptors and endocytic receptor 180[16-18]. These interactions activate diverse signaling pathways including FAK, Src, MAPK and PI3K, leading to EMT, cell proliferation, cell migration and the expression of pro-cancer genes[5,19,20]. These signaling pathways play import-

ant roles in NSCLC[21-24]. In accordance with these studies, our study indicated uPAR maybe susceptibility gene involved in NS-CLC development. In this study, the uPAR rs344781 polymorphism was inversely associated with the development of lung SCC in a Chinese population. C/C homozygote individuals had a 1.713-fold increased risk in developing NSCLC compared to T/T homozygote or C/T heterozygote individuals. In addition we found that T allele had a 0.742-fold reduced risk of developing SCC patients, and a 0.722-fold reduced risk of developing male SCC patients.

Lung cancer is a complex disease resulting from environmental factors, genetic factors, and their interactions[25,26]. The possibility that the relationship between the uPA/uPAR genotypes with NSCLC susceptibility in the study is an ethnic-dependent observation cannot be entirely excluded because multiple risk factors and etiology contribute to the pathophysiology of NSCLC development. Our results are not in agreement with those found in a study by Chuen-Ming Shih[10]. Together with earlier studies[27-29], these differences may primarily be attributed to the different living environments.

Conclusion

In conclusion, our results revealed that genetic polymorphisms of uPAR rs344781 were associated with the susceptibility NSCLC. The results of this study uncover the significant relationship between genetic polymorphisms of uPAR with the susceptibility of NSCLC. However, there are three limitaions in our study. First, the small sample size may be a limitation of the present study. Second, we found that uPAR rs344781 polymorphisms were associated with the development of SCC in a Chinese population, would be better illustrated the effect of uPAR rs344781 polymorphisms in SCC cell proliferation if we preformed functional studies of uPAR rs344781 polymorphisms *in vitro*. Third, population stratification may have led to a bias.

Conflict of interest: None declared.

Author's contributions: Yongjun Zhang and Jianzhong Gu designed this study, performed the statistical analysis, and drafted the manuscript. Yingying Shen carried out the collection of samples and helped to draft the manuscript.

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