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Association between polymorphisms in the *XRCC1* gene and the risk of non-small cell lung cancer

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ABSTRACT. Here, we have reported a case-control study investigating the association between *XRCC1* codons Arg194Trp, Arg280His, and Arg399Gln and the development of NSCLC. NSCLC patients (N = 245) and healthy controls (N = 257) were randomly selected from the Huaihe Hospital between March 2012 and August 2014. DNA extracted from the patient and control blood samples were subjected to polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to assess the genotyping of *XRCC1* Arg194Trp, Arg280His, and Arg399Gln. Multivariate logistic regression analyses revealed an association between the expression of the AA genotype and A allele genotypes and a significantly increased risk of NSCLC, compared to the GG genotype [95% confidence interval (CI); Odd's ratio (OR) = 2.82 (1.141-5.86) and 1.67 (1.17-2.37), respectively]. The potential association between the A allele of *XRCC1* Arg399Gln and the risk of NSCLC was more evident in smokers (95%CI; OR = 1.70; 1.11-2.63). In conclusion, the *XRCC1* Arg399Gln polymorphism was found to be associated with increased risk of NSCLC, especially in tobacco smokers.

Key words: XRCC1; Polymorphism; Non-small cell lung cancer; Multivariate logistic regression analysis

INTRODUCTION

Lung cancer is a main cause of cancer related mortality worldwide (Siegel et al., 2012). NSCLC is known to be caused by a complex, multistep, and multifactorial process, and NSCLC is strongly related to environmental factors such as chemical carcinogens results from tobacco use and occupational exposure (Rodríguez et al., 2000; Tardon et al., 2005; Siegel et al., 2012). However, only a number of individuals who are exposed to the risk factors of NSCLC develop NSCLC, which suggested that hereditary factors may be involved in the development of NSCLC.

The chemical carcinogens may cause indirectly to DNA damage by inactivation of enzymes that are involved in DNA repair, or directly, by generating DNA strand breaks and base damage that can result in severe mutations leading to cancer (Hoeijmakers, 2001). The base excision repair (BER) is one of the most important DNA repair process involved in maintaining genome integrity (Wood et al., 2001). The DNA repair enzyme XRCC1 is an important protein of the BER pathway, and it serves as a molecular scaffold for most other members of the BER short-patch pathway (Tudek et al., 2007; Maynard et al., 2009). The *XRCC1* gene undergoes three important polymorphisms, and they are at codons Arg194Trp, Arg280His, and Arg399Gln.

Previous studies have investigated the role of *XRCC1* codons Arg194Trp, Arg280His, and Arg399Gln in the risk of NSCLC; however, the results remain inconsistent (Zienolddiny et al., 2006; Jiang et al., 2010; Kim et al., 2010; Qian et al., 2011; Vaezi et al., 2011; Nakhaei et al., 2013; Sun et al., 2013; Du et al., 2014; Kang et al., 2015). In our study, we have conducted a hospital-based case-control study, investigating the association between *XRCC1* codons Arg194Trp, Arg280His, and Arg399Gln and the development of NSCLC.

MATERIAL AND METHODS

Subjects

This study included 209 consecutive primary NSCLC patients. NSCLC was confirmed in all patients by computed tomography (CT) or pathological examination via bronchoscopy, and they were selected from the Huaihe Hospital of Henan University between March 2012 and August 2014. The inclusion criteria of included patients with NSCLC were those who were untreated with preoperative chemotherapy or radiotherapy, and did not exhibited secondary or recurrent tumors. Finally, 245 patients with NSCLC agreed to participate into our study, and the participation rate was 91.08%.

A total of 295 control samples were randomly selected from individuals who came to undergo routine health examinations at the health examination clinic of the Huaihe Hospital of Henan University between March 2012 and August 2014. All controls were lack of cancers. Finally, 257 controls agreed to participate in this study, and the pariticipation rate was 87.12%.

Demographic characteristics on the NSCLC patients and controls were collected from a standardized questionnaire using face-to-face interviews. The demographic characteristics included age, gender, smoking status, drinking status and family history of cancer. The histology of NSCLC was obtained from pathology reports. The will of the NSCLC patients and controls to participate in our study was determined based on the answers provided in a predesigned questionnaire. This protocol was approved by the Clinical Research Ethics Committee of the Huaihe Hospital of Henan University.

DNA extraction and genotype analysis

Blood samples (5 mL) were obtained from all NSCLC patients and controls; the samples were stored at -20°C until further use. Genomic DNA was extracted from peripheral blood using the TIANamp Blood DNA Kit (Tiangen, Beijing, China), according to the manufacturer protocols. Genotyping of *XRCC1* Arg194Trp, Arg280His, and Arg399Gln were performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) process. The primers and probes specific for XRCC1 Arg194 Trp, Arg280His, and Arg399Gln were designed using the Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA). For *XRCC1* Arg194 Trp, the forwards and reverse primers were 5'-GCCAGGGCCCCTCCTTCAA-3' and 5'-TACCCTCAGACCCACGAGT-3', respectively. For *XRCC1* Arg280His, the forwards and reverse primers were 5'-CAGTGGTGCTAACCTAATC-3' and 5'-AGTAGTCTGCTGGCTCTGG-3', respectively. For *XRCC1* Arg399Gln, the forwards and reverse primers were 5'-CAGTGGTGCTAACCTAATC-3' and 5'-AGTAGTCTGCTGGCTCTGGG-3', respectively. The PCR reaction was conducted at t 95°C for 1 min for the initial denaturation, 40 cycles at 95°C for 20 s, 60°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 5 min.

Statistical analysis

All statistical tests were conducted using the SPSS software for Windows version 16.0 (IBM, Armonk, NY, USA). The statistical differences in demographic and clinical characteristics between NSCLC patients and controls were assessed by a χ^2 test and a Student's *t*-test. Departures from Hardy-Weinberg equilibrium (HWE) for *XRCC1* Arg194Trp, Arg280His, and Arg399Gln were tested using Fisher's exact test in the controls. The association between *XRCC1* Arg194Trp, Arg280His, and Arg399Gln polymorphisms and risk of NSCLC patients were calculated using logistic regression models. The results were expressed using odd's ratio (OR) and 95 % confidence interval (CI). P values less than 0.05 were considered to denote significant association.

RESULTS

The demographic and clinical characteristics of the NSCLC patients and controls are summarized in Table 1. Of the 245 confirmed patients with NSCLC, 173 were males and 72 were female patients. The 257 control subjects comprised of 166 males and 91 female subjects. As expected, no significant differences were observed between patients with NSCLC and controls in terms of the sex, age, and drinking status. NSCLC patients were more likely to be smokers, compared to the control subjects (χ^2 = 39.09, P value < 0.001). Of 245 patients with NSCLC, 214 patients (87.35%) were in the III-IV tumor stage, 101 (41.22%) were squamous cell carcinoma, and 144 (58.78%) were adenocarcinoma.

The genotype frequencies of *XRCC1* Arg194 Trp, Arg280His and Arg399Gln are summarized in Table 2. By χ^2-test, we found that the observed genotype frequencies of *XRCC1* Arg194Trp, Arg280His and Arg399Gln in the control samples agreed with the Hardy-Weinberg equilibrium, and the P values were 0.20, 0.69 and 0.82, respectively. By χ^2-test, there was significant difference between the genotype frequencies of *XRCC1* Arg399Gln between the NSCLC patients and controls (χ^2 = 10.46, P = 0.005). The results of the multivariate logistic regression analysis revealed the association of individuals expressing the AA genotype and A allele of *XRCC1* Arg399Gln with a significantly increased risk of NSCLC, compared to the GG genotype; the OR (95%CI) for these individuals was determined to be 2.82 (1.141-5.86) and 1.67 (1.17-2.37), respectively.

The association between the *XRCC1* Arg399Gln polymorphism and risk of NSCLC was stratified based on variables such as age, sex, and tabacco smoking (Table 3). Multivariate logistic regression analysis revealed that the potential association between the A allele of *XRCC1* Arg399Gln and the risk of NSCLC was more evident in ever smokers (95%CI; OR = 1.70; 1.11-2.63).

Table 1. Demographic and clinical characteristics of NSCLC patients and control subjects.

Variables	Patients N = 245	%	Controls N = 257	%	χ^2 or t-test	P value
Age, years						
≤60	102	41.63	102	39.69		
≥60	143	58.37	155	60.31	0.2	0.66
Gender						
Female	72	29.39	91	35.41		
Male	173	70.61	166	64.59	1.32	0.15
Tobacco smoking						
Never	87	35.51	163	63.42		
Ever	158	64.49	94	36.58	39.09	<0.001
Alcohol drinking						
Never	138	56.33	157	61.09		
Ever	107	43.67	100	38.91	1.17	0.28
Stage						
I-II	31	12.65				
III-IV	214	87.35				
Histology						
Squamous cell carcinoma	101	41.22				
Adenocarcinoma	144	58.78				

Table 2. Logistic regression analysis of the association between polymorphisms in the *XRCC1* gene and increased risk of NSCLC.

XRCC1	Patients	%	Co[illegible]	%	[illegible]s Ratio (95% Confidence Interval)[1]	P value
Arg194 Trp						
Arg/Arg	99	47.[illegible]	1[illegible]	[illegible]78	1.0 (Ref.)	-
Arg/Trp	90	2.[illegible]	[illegible]	41.43	1.35 (0.89-2.06)	0.14
Trp/Trp	[illegible]	10.0[illegible]	[illegible]	8.10	3.15 (1.32-8.09)	0.004
Arg/Trp + Trp/Trp	111	52.86	10[illegible]	49.52	1.52 (1.02-2.28)	0.03
Arg280His						
Arg/Arg	100	[illegible].62	109	51.90	1.0 (Ref.)	-
Arg/His	[illegible]	41.43	82	39.05	1.16 (0.76-1.77)	0.48
His/His	[illegible]3	[illegible].[illegible]	19	9.05	1.32 (0.64-2.73)	0.41
Arg/His+ His/H[illegible]	[illegible]	[illegible]2.38	101	48.10	1.19 (0.79-1.77)	0.38
Arg399Gln						
Arg/Arg	[illegible]	74.29	164	78.10	1.0 (Ref.)	-
Arg/Gln	34	16.19	30	14.29	1.19 (0.67-2.12)	0.52
Gln/Gln	20	9.52	16	7.62	1.31 (0.62-2.82)	0.44
Arg/Gln + Gln/Gln	54	25.71	46	21.90	1.23 (0.77-1.99)	0.36

[1]Adjusted for sex, age, and tobacco smoking.

Table 3. Interaction between the *XRCC1* Arg194Trp polymorphism and demographic characteristics in the risk of NSCLC.

Characteristics	Arg194 Trp				OR (95%CI)	P value
	Arg/Arg		Arg/Trp + Trp/Trp			
	Patients	Controls	Patients	Controls		
Age, years						
≤55	45	50	51	49	1.16 (0.63-2.11)	0.52
≥55	54	56	60	55	1.13 (0.65-1.97)	0.44
Sex						
Female	26	30	32	34	1.09 (0.50-2.36)	0.82
Male	73	76	79	70	1.17 (0.73-1.90)	0.49
Cigarette smoking						
Never	42	63	26	68	0.57 (0.30-1.09)	0.07
Current or former	57	43	85	36	1.78 (1.01-3.24)	<0.05

DISCUSSION

NSCLC associated with several environmental factors such as tobacco carcinogens (Tardon et al., 2005; Siegel et al., 2012). Carcinogenic compounds exert their effect causing direct or indirect DNA alteration. Repair of DNA damage capacity which is under genetic control may be an important endogen factor influencing NSCLC susceptibility. In our study, we investigated the role of XRCC1 Arg194Trp, Arg280His, and Arg399Gln in increasing the risk of NSCLC in a Chinese population. In this study, we discovered an interaction between the *XRCC1* Arg399Gln polymorphism and increased risk of NSCLC, as well as an interaction between tobacco smoking and risk of NSCLC.

Mutations in *XRCC1* gene may lead to decrease or loss of its DNA repair capacity and confer the variation in susceptibility to diverse malignant tumors among individuals. *XRCC1* Arg194 Trp, Arg280His and Arg399Gln polymorphisms are the most extensively studies SNPs. Numerous epidemiological studies have indicated that polymorphisms in the *XRCC1* gene may modify the risk of several types of cancers, including endometrial cancer, breast cancer, gastric cancer, glioma, and colorectal cancer (Ramadan et al., 2014; Forat-Yazdi et al., 2015; Mutlu et al., 2015; Wang et al., 2015a; Wang et al., 2015b; Zhu et al., 2015). However, previously performed meta-analyses have reported no association between polymorphism in the *XRCC1* gene and the risk of thyroid carcinoma, head and neck cancer, or colorectal cancer (Li et al., 2014; Wu et al., 2014; Qin et al., 2015).

In our study, we found that a significant association between polymorphism in the *XRCC1* Arg399Gln and risk of NSCLC (Butkiewicz et al., 2001; Gao et al., 2003; Popanda et al., 2004; Zienolddiny et al., 2006; Kim et al., 2010; Natukula et al., 2013; Du et al., 2014; Kang et al., 2015). Natukula et al. (2013) reported that Gln/Gln and Arg/Gln of *XRCC1* Arg399Gln may influence cancer susceptibility in NSCLC patients, especially in males and smokers. Du et al. (2014) investigated the correlation between *XRCC1* genes and the risk of NSCLC in a case-control study, they discovered that the Arg194Trp and Arg399Gln genetic variations in *XRCC1* were associated with the risk of NSCLC. Zienolddiny et al. (2006) discovered a correlation between *XRCC1* Arg194Trp, Arg280His and Arg399Gln gene polymorphisms and increased risk of NSCLC. However, some studies reported in consistent results. Butkiewicz et al. (2001) did not discover any significant associations between *XRCC1* Arg399Gln polymorphism and risk of NSCLC. Gao et al. (2003) also did not revealed any significant correlations between *XRCC1* Arg399Gln polymorphism and risk of NSCLC. Kang et al. (2015) discovered that the *XRCC1* Arg399Gln polymorphism was not associated with the risk of NSCLC. A recent meta-analysis conducted by Qian et al. (2011) revealed that genetic variations in *XRCC1* Arg399Gln does not cause an increased risk of lung cancer. The discrepancies of the reported results might be caused by differences in ethnicities, study design, or sample size.

In conclusion, the *XRCC1* Arg399Gln polymorphism was found to be associated with increased risk of NSCLC, especially in tobacco smokers. Further, studies with larger sample sets must be performed to confirm the role of *XRCC1* polymorphisms in the development of NSCLC.

Conflicts of interest

The authors declare no conflict of interest.

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