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Original Article

Assoication of XRCC1 gene polymorphisms with risk of non-small cell lung cancer

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Abstract: DNA repair genes is a key factor for cancer susceptibility, and we conducted a case-control study to investigate the association of XRCC1 codons 194 (Arg to Trp), 280 (Arg to His) and 399 (Arg to Gln) with risk of NSCLC. 210 NSCLC patients and 210 health control subjects were randomly selected from Huaihe Hospital between January 2012 and June 2014. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was taken to assess the genotyping of XRCC1 Arg194Trp, Arg280His and Arg399Gln. By multivariate logistic regression analysis, we found individuals carrying with Trp/Trp and Arg/Trp + Trp/Trp genotypes were associated with a significantly increased risk of NSCLC compared with Arg/Arg genotype, and the OR (95% CI) were 3.15 (1.32-8.09) and 1.52 (1.02-2.28), respectively. The potential association of Arg/Trp+ Trp/Trp genotype of XRCC1 Arg194Trp with the risk of NSCLC is more evidence in smokers, and the OR (95% CI) was 1.78 (1.01-3.24). In conclusion, we found that XRCC1 Arg194Trp polymorphism may be associated with NSCLC risk, especially in smokers.

Keywords: XRCC1, polymorphism, non-small cell lung cancer

Introduction

Lung cancer is one of the most significant worldwide health problems [1]. Up to 80% of lung cancer is non-small cell lung cancer (NSCLC), and NSCLC patients are usually diagnosed at the advanced stages of disease, which leaves curable surgery impossible [2]. It is well known that NSCLC is caused by complex, multistep, and multifactorial process. The real etiology of NSCLC is not well understood, and exposure to cigarette smoking is a well-known risk factor for NSCLC [1, 3, 4]. However, although many people expose to cigarette smoking, only a few smokers develop lung cancer, which suggests that other factors, such as genetic factors, may play an important role in the development of NSCLC.

Previous study has shown that polymorphisms of DNA repair genes is an key factor for cancer susceptibility, since DNA-repair activities play a critical role in protection of the genome and the prevention of cancer [5]. Moreover, function of DNA repair is critical for protecting the cellular genome from environmental hazards, including cigarette smoking [6]. Previous studies reported that DNA repair gene polymorphisms are associated with increased risk of several cancer risks, since the gene polymorphisms contribute to modify gene function and alter DNA repair capacity [7-9].

There were several DNA repair pathway in repairing the DNA damage caused by chemical alterations of methylated, oxidized or reduced bases, such as nucleotide excision repair (NER), base excision repair (BER) and double-strand break repair (DSBR) [10]. DNA repair enzymes XRCC1 is an important component of the BER pathway. There were three important polymorphisms in the XRCC1 gene, including codons 194 (Arg to Trp), 280 (Arg to His) and 399 (Arg to Gln).

Molecular epidemiological studies have reported the association between XRCC1 codons 194 (Arg to Trp), 280 (Arg to His) and 399 (Arg to Gln) and risk of Non-small cell lung cancer, but the results remain inconsistent [11-15]. Therefore, we conducted a case-control study to investigate the association of XRCC1 codons 194 (Arg to Trp), 280 (Arg to His) and 399 (Arg to Gln) with risk of NSCLC.

Materials and methods

Subjects

This is a hospital-based case-control study. 210 NSCLC patients were randomly selected from the Affiliated Hospital of Inner Mongolia Medical College between January 2012 and June 2014. All the NSCLC patients were confirmed by computed tomography (CT) or pathological examination through bronchoscopy, and all patients were confirmed to be without treatment of preoperative chemotherapy or radiotherapy and were not secondary or recurrent tumors.

The control subjects were collected from healthy individuals who underwent routine physical examination health examination clinics at the Affiliated Hospital of Inner Mongolia Medical College during the same period. The inclusion criteria for controls were absence of cancer. The control subjects were matched with cases by age (±5 years).

Data on all NSCLC patients and controls were obtained from face-to-face interviewers, medical records and pathology reports. All patients and controls were informed about this study and their will to participate in this study on predesigned questionnaire. The collection and use of blood samples for this study were previously approved by the Ethics Committee of the the Affiliated Hospital of Inner Mongolia Medical College.

DNA extraction and genotype analysis

All the patients and control subjects were asked to provide 5 ml blood sample, and kept in -20ºC until use. Genomic DNA was isolated from a peripheral blood with TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was taken to assess the genotyping of XRCC1 Arg194Trp, Arg280His and Arg399Gln. The primers and probes of XRCC1 Arg194Trp, Arg280His and Arg399Gln were designed by Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA). For XRCC1 Arg194Trp, the forward and reverse primer sequences were 5'-GCCAGGG-CCCCTCCTTCAA-3' and 5'-TACCCTCAGACCCAC-GAGT-3', respectively; For XRCC1 Arg280His, the forward and reverse primer sequences were 5'-CAGTGGTGCTAACCTAATC-3' and 5'-AG-TAGTCTGCTGGCTCTGG-3', respectively; For XR-CC1 Arg399Gln, the forward and reverse primer sequences were 5'-CAGTGGTGCTAACCTAA-TC-3' and 5'-AGTAGTCTGCTGGCTCTGGG-3', respectively. The PCR reaction was conducted in a 25 μl reaction solution with 1.8 μl ddH2O, 0.5 μl 10 × buffer, 0.4 μl $MgCl^{2+}$, 0.1 μl dNTP, 0.2 μl Hotstar and 1 μl primer as well as 20 ng-50 ng DNA samples. The DNA was amplified as follows: one initial denaturation step at 95°C for 1 min, then 40 cycles of 95°C for 20 sec, 60°C for 1 min and 72°C for 1 min, and a final extension step at 72°C for 10 min.

Statistical analysis

The statistical difference in demographic and clinical characteristics between cases and controls was analyzed by A Chi-squared test and t test. The Hardy-Weinberg equilibrium (HWE) was tested by Fisher's exact test for XRCC1 Arg194Trp, Arg280His and Arg399Gln in controls. The OR and 95% confidence interval (CI) were calculated using logistic regression models adjusted for potential risk factors of NSCLC for genotype analysis. Interaction between genotypes of XRCC1 Arg194Trp, Arg280His and Arg399Gln gene polymorphisms and demographic characteristics were assessed by logistic regression analysis. *P* value less than 0.05 was considered as significant association, and all statistical tests were conducted using SPSS software for Windows version 16.0.

Results

Demographic and clinical characteristics

The distributions of demographic and clinical characteristics were summarized in **Table 1**. Of 210 confirmed cases of NSCLC, there were 58 males and 152 females. Of 210 control subjects, there were 64 males and 146 females. No significant differences were observed in sex, age and drinking status between the NSCLC cases and control subjects. NSCLC cases were more likely to be smokers when compared with control subjects.

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

Characteristics	Patients	%	Controls	%	χ^2 or t test	*P* value
Age, years						
< 55	96	45.71	99	47.14		
≥ 55	114	54.29	111	52.86	0.09	0.77
Sex						
Female	58	27.62	64	30.48		
Male	152	72.38	146	69.52	0.42	0.52
Cigarette smoking						
Never	68	32.38	131	62.38		
Current or former	142	67.62	79	37.62	37.90	< 0.05
Tobacco smoking						
Never	133	63.33	139	66.19		
Current or former	77	36.67	71	33.81	0.38	0.54
Histology						
Squamous cell carcinoma	60	28.57				
Adenocarcinoma	150	71.43				

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

XRCC1	Patients	%	Controls	%	OR (95% CI)[1]	*P* value
Arg194Trp						
Arg/Arg	99	47.14	106	50.48	1.0 (Ref.)	-
Arg/Trp	90	42.86	87	41.43	1.35 (0.89-2.06)	0.14
Trp/Trp	21	10.00	17	8.10	3.15 (1.32-8.09)	0.004
Arg/Trp + Trp/Trp	111	52.86	104	49.52	1.52 (1.02-2.28)	0.03
Arg280His						
Arg/Arg	100	47.62	109	51.90	1.0 (Ref.)	-
Arg/His	87	41.43	82	39.05	1.16 (0.76-1.77)	0.48
His/His	23	10.95	19	9.05	1.32 (0.64-2.73)	0.41
Arg/His + His/His	110	52.38	101	48.10	1.19 (0.79-1.77)	0.38
Arg399Gln						
Arg/Arg	156	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.

Genotype distributions of XRCC1 gene polymorphisms and NSCLC risk

The genotype frequencies of XRCC1 Arg194Trp, Arg280His and Arg399Gln were shown in **Table 2**. The observed genotype frequencies of XRCC1 Arg194Trp and Arg280His in controls were agreed with Hardy-Weinberg equilibrium (*P* = 0.89 and 0.54, respectively), while genotype distributions of Arg399Gln were not (*P* < 0.05). The genotype frequencies were not significantly different in frequencies of the three gene polymorphisms between the cases and controls. By multivariate logistic regression analysis, we found individuals carrying with Trp/Trp and Arg/Trp + Trp/Trp genotypes of XRCC1 Arg194Trp were associated with a significantly increased risk of NSCLC compared with Arg/Arg genotype, and the OR (95% CI) were 3.15 (1.32-8.09) and 1.52 (1.02-2.28), respectively.

Stratification analysis of NSCLC risk

We further analyzed the association between XRCC1 Arg194Trp polymorphism and risk of NSCLC stratified by variables including age, sex and cigarette smoking (**Table 3**). By multivariate logistic regression analysis, the potential association of Arg/Trp + Trp/Trp genotype of XRCC1 Arg194Trp with the risk of NSCLC is more evidence in smokers, and the OR (95% CI) was 1.78 (1.01-3.24).

Discussion

In this hospital-based case-control study, we investigated the role of three important polymorphisms of the XRCC1 gene, including codons Arg194Trp, Arg280His and Arg399Gln, in the risk of NSCLC, and their interaction with environmental factors in the development of NSCLC. In our study, we found that XRCC1 Arg194Trp polymorphism was associated with

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

Characteristics	Arg194Trp				OR (95% CI)[1]	*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

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

increased risk of NSCLC, and had interaction with tobacco smoking in the cancer risk.

Increasing evidences have been reported that genetic variation could influence the DNA repair capacities in the human, and thus the common polymorphisms of DNA repaired genes can result in cell death, genetic instability, mutagenesis or cancer [16]. DNA repair mechanisms play an important role in maintaining genome integrity and preventing carcinogenesis. BER pathway is an important mechanism in repairing small base lesions in DNA that are the results of oxidation and alkylation damage, and this pathway is correlated with risk of lung cancer [6, 17, 18]. XRCC1 is located on chromosome 19q13.2-13.3, and plays gene product is implicated in single-strand break repair and BER mechanisms [16]. Epidemiological studies have reported that XRCC1 gene polymorphisms may influence the development of several kinds of cancers, such as endometrial cancer, breast cancer, gastric cancer, glioma and colorectal cancer [19-23]. However, several meta-analysis showed that no association between XRCC1 gene polymorphisms and risk of bladder cancer, hepatocellular carcinoma and gastric cancer [24-26].

Several previous studies have investigated the association between XRCC1 gene polymorphisms and susceptibility to NSCLC [11-15]. However, the results of these studies are inconsistent. Du et al. conducted a case-control study to investigate the role of XRCC1 genes in the risk of NSCLC, and they found genetic variations in XRCC1 Arg194Trp and Arg399Gln were related to the risk of NSCLC [11]. Natukula et al. reported that Gln/Gln and Arg/Gln of XRCC1 Arg399Gln may influence the cancer susceptibility in NSCLC patients, especially in smokers [13]. Zienolddiny et al. found that XRCC1 Arg280His and Arg399Gln gene polymorphisms were correlated with risk of NSCLC [15]. However, Sun et al. did not find significant association between XRCC1 gene polymorphisms and risk of NSCLC [12]. One previous meta-analysis did not find that genetic variation of XRCC1 Arg399Gln could not affect lung cancer risk [14]. Another meta-analysis found that Trp/Trp of XRCC1 Arg194Trp could increase lung cancer risk, especially in Asians [27].

Our study found a significant interaction between XRCC1 Arg194Trp polymorphism and cigarette smoking. Cigarette smoking may induce various types of DNA damage including benzopyrene diol epoxide adduct, strand breaks, cross-links, and recombination, which are repaired through different DNA repair pathways, including NER [28]. Our study reported a significant gene-smoking interaction in the risk of NSCLC, suggesting that cigarette smoking has a synergistic effect with XRCC1 Arg194Trp polymorphism in cancer risk.

In conclusion, we found that XRCC1 Arg194Trp polymorphism may be associated with NSCLC risk, especially in smokers. However, no association was found between polymorphisms in XRCC1 Arg194Trp and NSCLC risk. Further large sample studies are needed to confirm the

role of XRCC1 polymorphisms in the development of NSCLC.

Disclosure of conflict of interest

None.

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