RESEARCH ARTICLE

Association of the XRCC1 c.1178G>A Genetic Polymorphism with Lung Cancer Risk in Chinese

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Abstract

The X-ray repair cross-complementing group 1 protein (XRCC1) plays important roles in the DNA base excision repair pathway which may influence the development of lung cancer. This study aimed to evaluate the potential association of the XRCC1 c.1178G>A genetic polymorphism with lung cancer risk. The created restriction site-polymerase chain reaction (CRS-PCR) and DNA sequencing methods were utilized to evaluate the XRCC1 c.1178G>A genetic polymorphism among 376 lung cancer patients and 379 controls. Associations between the genetic polymorphism and lung cancer risk were determined with an unconditional logistic regression model. Our data suggested that the distribution of allele and genotype in lung cancer patients was significantly different from that of controls. The XRCC1 c.1178G>A genetic polymorphism was associated with an increased risk of lung cancer (AA *vs* GG: OR=2.91, 95% CI 1.70-4.98, *p*<0.001; A *vs* G: OR=1.52, 95% CI 1.22-1.90, *p*<0.001). The allele A and genotype AA may contribute to risk of lung cancer. These preliminary results suggested that the XRCC1 c.1178G>A genetic polymorphism is statistically associated with lung cancer risk in the Chinese population.

Keywords: Lung cancer - XRCC1 gene - genetic polymorphisms - susceptibility

Introduction

Lung cancer is one of the major causes of cancer mortality in the world, with more than a million deaths each year (Guilbert, 2003; Yin et al., 2009; Siegel et al., 2012; Yuan et al., 2013; Wang et al., 2014). However, the exact mechanism of lung cancer remains poorly understood. Recently, many studies have investigated the association of lung cancer with genetic polymorphisms of human X-ray repair cross-complementing group 1 (XRCC1) gene in different populations (Hao et al., 2006; Giachino et al., 2007; Lopez-Cima et al., 2007; Sreeja et al., 2008; Chang et al., 2009; Kalikaki et al., 2009; Wang et al., 2009; Yin et al., 2009; Yin et al., 2009; Butkiewicz et al., 2011; Huang et al., 2011; Li et al., 2011; Perez-Morales et al., 2011; Qian et al., 2011; Rybarova et al., 2011; Chen et al., 2012; Cui et al., 2012; Dai et al., 2012; Karkucak et al., 2012; Ke et al., 2012; Li et al., 2012; Guo et al., 2013; Guo et al., 2013; Huang et al., 2013; Letkova et al., 2013; Li et al., 2013; Li et al., 2013; Natukula et al., 2013; Ouyang et al., 2013; Sun et al., 2013; Wang et al., 2013; Wu et al., 2013; Wang et al., 2014; Wang et al., 2014; Zhang et al., 2014; Zhang et al., 2014). The XRCC1 proteins play important roles in DNA base excision repair (BER) pathway which may be influence the development of lung cancer. The XRCC1 genetic polymorphisms may affect the expression and function of XRCC1 proteins, which contributing to the susceptibility of lung cancer and

influencing the prognosis of patients (Viktorsson et al., 2005; Yin et al., 2009). Several XRCC1 genetic variants, such as Arg194Trp, Arg 280His and Arg399Gln (Giachino et al., 2007; Lopez-Cima et al., 2007; Sreeja et al., 2008; Kalikaki et al., 2009; Yin et al., 2009; Yin et al., 2009; Butkiewicz et al., 2011; Qian et al., 2011; Guo et al., 2013; Letkova et al., 2013; Li et al., 2013; Zhang et al., 2014), have been identified to play potentially influence on the risk of lung cancer in different populations. However, the potential association of XRCC1 c.1178G>A genetic polymorphism with lung cancer risk has not been reported. Thus, the purpose of this study is to evaluate the effects of this genetic polymorphism on influencing lung cancer.

Materials and Methods

Studied subjects

The studied subjects were enrolled from the Shandong Jining NO.1 People's Hospital between September 2008 and November 2012. All studied subjects were unrelated ethnic Han Chinese and lived in Jining, Shandong Province, China. Patients were diagnosed and histologically confirmed with lung cancers by doctors. Controls were free from lung cancer and other medical diseases. The controls were frequency-matched to lung cancer patients on sex and age. The general characteristics of studied subjects, including sex, age, smoking status, pack-years, histology type, and family history of lung

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cancer are summarized in Table 1. The study was approved by the Ethics Review Committee of the Shandong Jining NO.1 People's Hospital. All enrolled subjects signed an informed consent form.

DNA extraction and genotyping

The genomic DNA was extracted from each enrolled subjects' peripheral venous blood using the DNA Blood Mini kit (QIAGEN, Valencia, CA). The specific polymerase chain reaction (PCR) primers of XRCC1 c.1178G>A genetic polymorphism were designed by Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA). The primers sequences, amplification region, annealing temperature and fragment size are shown in Table 2. The PCR reaction was carried out in a total volume of 20 μL PCR solution which containing 50ng template DNA, 1×buffer (100 mmol Tris-HCl, pH 8.3; 500 mmol KCl), 0.25 μmol primers, 2.0 mmol $MgCl_2$, 0.25 mmol dNTPs (Bioteke Corporation, Beijing, China), and 0.5U Taq DNA polymerase (Promega, Madison, WI, USA). Thermal cycling conditions consisted of an initial denaturation at 94°C for 5 minutes, followed by 32 cycles at 94°C for 30 seconds, at 61.7°C for 30 seconds, at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. The XRCC1 c.1178G>A genetic polymorphisms were genotyped by the created restriction site-polymerase chain reaction (CRS-PCR) method with one of the primers containing a nucleotide mismatch, which enables the use of restriction enzymes for discriminating sequence variations (Haliassos et al., 1989; Zhao et al., 2003; Yuan et al., 2012; Yuan et al., 2013; Yuan et al., 2013). According to the manufacturer's instructions, the PCR amplified products were digested with MaeII restriction enzyme (MBI Fermentas, St. Leon-Rot, Germany) at 37°C for 10 hours. The digested products were separated on a 2.5% agarose gel, stanied with ethidiium bromide and observed under UV light. 10% of samples for each genotype were sequenced (ABI3730xl DNA Analyzer, Applied Biosystems, Foster City, CA) to verify the CRS-PCR results.

Statistical analyses

The chi-squared (χ^2) test was used to compare the frequency distributions of XRCC1 c.1178G>A genetic polymorphism, and the differences of general characteristics between lung cancer patients and controls. The Hardy–Weinberg equilibrium (HWE) of genotype distributions were assessed in both the case and control groups. Unconditional logistic regression analysis was utilized to examine the potential associations between the XRCC1 c.1178G>A genetic polymorphism and lung cancer risk by computing the odds ratios (ORs) with their 95% confidence intervals (CIs). The statistically significant was settled at p value<0.05. All statistical analyses were analyzed by the Statistical Package for Social Sciences software (Windows version release 14.0) (SPSS Inc.; Chicago, IL, USA).

Results

Subject characteristics

In this case-control study, a total of 755 subjects were recruited, including 376 lung cancer patients (Male: 289, Female: 87, Mean age±standard deviation (SD): 63.14±13.52) and 379 controls (Male: 271, Female: 108, Mean age±SD: 62.57±14.21). Table 1 summarizes the subject characteristics. There were no statistically significant differences between lung cancer patients and controls with regarded to sex, age, smoking status, pack-years, histology type, and family history of lung cancer (all p values >0.05).

Genotyping and distribution of XRCC1 genetic polymorphism

The XRCC1 c.1178G>A genetic polymorphism and the three possible genotypes (GG: 195 bp and 20

Table 1. Characteristics of Lung Cancer Cases and Controls

	Cases (n)	%	Controls (n)	%	χ^2 value	p value
Number	376	49.8	379	50.2		
Sex (n)					2.8282	0.0926
Male	289	76.86	271	71.5		
Female	87	23.14	108	28.5		
Age (years)					0.8177	0.3659
Mean ± SD	63.14 ± 13.52		62.57 ± 14.21			
< 58	213	56.65	227	59.89		
≥ 58	163	43.35	152	40.11		
Smoking status					0.5845	0.4446
Yes	236	62.77	248	65.44		
No	140	37.23	131	34.56		
Pack-years					3.096	0.0785
< 36	142	37.77	167	44.06		
≥ 36	234	62.23	212	55.94		
Family history of lung cancer (n)					3.6465	0.0562
Yes	49	13.03	33	8.71		
No	327	86.97	346	91.29		
Histology type (n)						
Squamous-cell carcinoma	186	49.47	-			
Adenocarcinoma	125	33.24	-			
Others	65	17.29	-			

Table 2. The CRS-PCR Analysis for XRCC1 c.1178G>A Genetic Polymorphism

Primer sequences	Annealing temperature (°C)	Amplification fragment (bp)	Region	Restriction enzyme	Genotype (bp)
Forward primer: 5'-TGCTGGACTGTCACCGCATAC-3'	61.6	216	Exon10	MaeII	GG:195,20
Reverse primer: 5'-CCTTCCCTCATCTGGAGTACCC-3'					GA:215,195,20
					AA:215

*PCR means polymerase chain reaction; CRS-PCR, created restriction site-polymerase chain reaction. Underlined nucleotides mark nucleotide mismatches enabling the use of the selected restriction enzymes for discriminating sequence variations

Table 3. The Genotype and Allele Frequencies of XRCC1 c.1178G>A Genetic Polymorphism in Cases and Controls

Groups	Genotype frequencies (%)			Allele frequencies (%)		χ^2	p
	GG	GA	AA	G	A		
Case group (n=376)	172(45.74)	151(40.16)	53(14.10)	495(65.82)	257(34.18)	4.3371	0.1143
Control group (n=379)	208(54.88)	149(39.32)	22(5.80)	565(74.54)	193(25.46)	0.484	0.785
Total (n=755)	380(50.33)	300(39.74)	75(9.93)	1060(70.20)	450(29.80)	1.9113	0.3846
	χ^2=16.2255, p=0.0003			χ^2=13.7012, p=0.0002			

Table 4. The Association of XRCC1 c.1178G>A Genetic Polymorphisms with Lung Cancer Risk

Comparisons	Test of association		
	OR (95% CI)	χ^2-value	p value
Homozygote comparison (AA *vs* GG)	2.91(1.70-4.98)	16.14	0
Heterozygote comparison (GA *vs* GG)	1.23(0.91-1.66)	1.73	0.189
Dominant model (AA/GA *vs* GG)	1.44(1.08-1.92)	6.29	0.012
Recessive model (AA *vs* GA/GG)	2.66(1.58-4.48)	14.48	< 0.001
Allele contrast (A *vs* G)	1.52(1.22-1.90)	13.69	< 0.001

*OR, odds ratio; CI, confidence interval; vs,versus

bp; GA: 215 bp, 195 bp and 20 bp; and AA: 215 bp, Table 2) were detected and confirmed by the CRS-PCR and DNA sequencing methods in the Chinese Han populations. According to the DNA sequences (GenBank ID: NC_000019.9), mRNA sequences (GenBank ID: NM_006297.2), and protein sequences (GenBank ID: NP_006288.2) of human XRCC1 gene, our sequence analysis indicate that this genetic polymorphism is a non-synonymous mutation. This genetic polymorphism causes from a G to A mutation in exon10 of XRCC1 gene, and leading to the arginine (Arg) to histidine (His) amino acid replacement (*p*.Arg393His). Table 3 shows the distributions of allele and genotypes and the χ^2 comparison for general characteristics between the cases and controls. The allele frequencies of lung cancer patients (G, 65.82%; A, 34.18%) were significantly different from those in controls (G, 74.54%; A, 25.46%; χ^2=13.7012, *p*=0.0002, Table 3). The genotype frequencies of lung cancer patients (GG, 45.74%; GA, 40.16%; AA, 14.10%) were not consistent with controls (GG, 54.88%; GA, 39.32%; AA, 5.80%), the differences being statistically significant (χ^2=16.2255, *p*=0.0003, Table 3). The distributions of allele and genotype in both cases and controls were found to be in HWE (all P values>0.05, Table 3).

Association of XRCC1 genetic polymorphism with lung cancer risk

Table 4 shows the potential association of XRCC1 c.1178G>A genetic polymorphism and lung cancer risk. Our data indicated that this genetic polymorphism was significantly increased risk of lung cancer in homozygote comparison (AA *vs* GG: OR=2.91, 95%CI 1.70-4.98, χ^2=16.14, *p*<0.001), dominant model (AA/GA *vs* GG: OR=1.44, 95%CI 1.08-1.92, χ^2=6.29, *p*=0.012), recessive model (AA *vs* GA/GG: OR=2.66, 95%CI 1.58-4.48, χ^2=14.48, *p*<0.001) and allele contrast (A *vs* G: OR=1.52, 95%CI 1.22-1.90, χ^2=13.69, *p*<0.001, Table 4).

Discussion

Lung cancer is nowadays a common public health problem in the world. It has a very high incidence and the overall survival rate has still an extremely poor. A large number of epidemical studies have suggested that genetic variants of candidate genes play key roles in the risk of lung cancer. It has been reported the XRCC1 gene is one of the most important candidate genes for influencing lung cancer risk. In this case-control study, we firstly investigated the effects of XRCC1 c.1178G>A genetic polymorphism on influencing lung cancer in 755 Chinese Han subjects by association analysis. Our data indicated that there were significant differences in the distribution of allele and genotype between lung cancer patients and controls (Table 3). Compared to allele G and genotype GG, the allele A and genotype AA were significantly associated with the increased risk of lung cancer (Table 4). Therefore, the allele G and genotype GG may be contribute to play a protection from lung cancer in Chinese, and could be a useful molecular biomarker for assessing the risk of lung cancer. The XRCC1 c.1178G>A genetic polymorphism might be linked to other non-synonymous polymorphisms, such as Arg194Trp, Arg280His, Arg399Gln in XRCC1 gene, which have been approved to associate with the risk of lung cancer (Giachino et al., 2007; Lopez-Cima et al., 2007; Sreeja et al., 2008; Chang et al., 2009; Kalikaki et al., 2009; Yin et al., 2009; Yin et al., 2009; Butkiewicz et al., 2011; Qian et al., 2011; Chen et al., 2012; Guo et al., 2013; Li et al., 2013; Zhang et al., 2014). These genetic variants might influence the expression and function of XRCC1 proteins in BER pathway, which has been approved to be associated with lung cancer risk (Hao et al., 2006; Giachino et al., 2007; Lopez-Cima et al., 2007; Sreeja et al., 2008; Kalikaki et al., 2009; Yin et al., 2009; Yin et al., 2009; Butkiewicz et al., 2011; Qian et al., 2011;

Li et al., 2013). Our findings provide more evidence for further analysis of the biological function role of XRCC1 genetic variants with the risk of lung cancer.

In conclusion, this is the first investigation about the potentially influence of XRCC1 c.1178G>A genetic polymorphism on the risk of lung cancer. Our findings indicated that this genetic polymorphism could be significantly associated with the increased risk of lung cancer in Chinese Han population, and might be a useful molecular biomarker for assessing the risk of lung cancer. These observations need to be confirmed in larger different ethnic populations, and the role of molecular mechanisms of XRCC1 genetic polymorphisms with the risk of lung cancer should be fully explained.

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