Association of Matrix Metalloproteinase-9 and p53 Gene Polymorphisms with Genetic Susceptibility to No-small-cell Lung Cancer

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Abstract Matrix metalloproteinase-9(MMP-9) and p53 genes play an essential role in the multi-step process of tumorigenesis in lung cancer. Single nucleotide polymorphisms(SNPs) of MMP-9 and p53 genes are associated with the risk and progression of many cancers. In this study, we evaluated the association of the R279Q polymorphism of MMP-9 or the A_1/A_2 polymorphism of p53 gene with the risk of no-small-cell lung cancer(NSCLC) in Han population of Northeast China. We examined the frequency of SNPs in the two kinds of genes of 50 patients with NSCLC and 50 cancer-free controls frequency-matched by age and sex. Polymerase chain reaction-restriction fragment length polymorphism(PCR-RFLP) technique was used to determine the genotypes. The results indicate that the 279RR genotype in MMP-9 gene and the A_1/A_2 genotype in p53 gene show a significantly increased risk of NSCLC. Therefore, the MMP-9 279RR and p53 A_1/A_2 genotypes may be used as markers for susceptibility to NSCLC in Han population of Northeast China.

Keywords Single nucleotide polymorphisms(SNPs); No-small-cell lung cancer(NSCLC); Matrix metalloproteinase-9(MMP-9); p53; Susceptibility

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1 Introduction

Lung cancer is one of the most common cancers worldwide and its mortality rate has increased year after year[1]. In China, there were about 0.54 million lung cancer cases and 0.48 million deaths in 2005[2]. The overall 5-year survival rate remained less than 15% in the United States and less than 9% in developing countries[1]. The two main types of lung cancer are small-cell-lung cancer(SCLC) and non-small-cell lung cancer(NSCLC). NSCLC accounts for approximately 85% of lung cancer. Epidemiological studies have identified many risk factors for lung cancer, including cigarette smoking, alcohol, air pollution and occupational exposure. Although smoking is unquestionably the leading cause of lung cancer, approximately 10% of cases occur in patients who have never smoked[3]. Environmental exposure and genetic susceptibility are also thought to contribute to lung cancer risk[4]. Since the last decade, single nucleotide polymorphisms(SNPs) have been identified in many diseases. Genome-wide association studies with phase 2 and phase 3 confirmations have now provided overwhelming evidence for the association of some common SNPs with a number of diseases including lung cancer[5].

It has been reported that matrix metallproteinase-9 (MMP-9) and p53 genes played an important role in the oncogenesis of NSCLC[6,7]. Both MMP-9 and p53 genes are highly polymorphic, but few SNPs are within the promoters or coded regions that may be potentially functional(http://www.ncbi.nlm.nih.gov/SNP/). Of these variants, the R279Q polymorphism in the exon 6 of MMP-9 gene and the A_1/A_2 polymorphism in the intron 6 of p53 gene have been described to be involved in the etiology of various cancers, including breast cancer, bladder cancer and lung cancer[8—10]. However, the results are sometimes contraversal. For example, Biros *et al.*[11] reported a higher frequency of the intron 6 polymorphism in Caucasian patients with lung cancer. However, Birgander *et al.*[12] found no association between the polymorphism and lung cancer risk.

The aim of the present study was to elucidate whether genetic variations of MMP-9 and p53 genes might be markers for NSCLC. We conducted a case-control study to evaluate the association of MMP-9 R279Q polymorphism or p53 A_1/A_2 polymorphism with NSCLC risk in Han population of Northeast China.

2 Materials and Methods

2.1 Patients Selection

The case-control blood samples stored at –20 °C were obtained from 50 patients with proven histopathological diagnosis of NSCLC at the Department of Thoracic Surgery, the Second Hospital of Jilin University(China) between March 2005 and July 2006. All the cases had a complete history and physical examination, blood chemistry analysis, chest

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radiography, chest tomography and abdominal ultrasonography. The 50 cancer-free control subjects genetically unrelated to the cancer cases were recruited from persons who were undergoing the routine body check in outpatient department. They were all Chinese Han people from Changchun City(China). The study protocol and informed consent form used in this study were approved by our institutional review board.

2.2 Polymerase Chain Reaction and Restriction Fragment Length Polymorphism

Genomic DNAs were extracted from 5 mL of blood sample. The following primer pairs were used to amplify the fragments from MMP-9 and p53 genomic DNA by PCR reaction: MMP-9 sense, 5′-GAGAGATGGGATAAGAGG-3′; antisense, 5′-GTGGTGGAAATGTGGTGT-3′; p53 sense, 5′-TCTGGCCCCTCCTCAGCATC-3′; antisense, 5′-AGTAGAGACGGGGTTTCACC-3′. PCR was carried out with a thermal cycler in 50 μL of PCR buffer($MgCl_2$ 5 μL, 10×buffer 5 μL, DNA 2 μL, DNTP 1 μL, Fprimer 2 μL, Rprimer 2 μL, Taq polymerase 0.4 μL and double distilled H_2O). Conditions for PCR were 30 cycles of 30 s at 94 °C(denaturing step), 30 s at 58 °C(annealing step), and 1 min at 72 °C(extension step). The PCR product of MMP-9 was 439 bp and that of p53 was 427 bp. The PCR products were digested with MspI at 37 °C for 3 h and separated on a 2% agarose gel.

2.3 Statistical Analysis

Data were presented as mean±SD. Comparison between results from different groups was performed with a two-sided χ^2 test. Statistical significance was defined as $P<0.05$. Unconditional logistical regression analysis was used to calculate odds ratios(ORs) and their 95% confidence intervals(CIs).

3 Results and Discussion

We analyzed 50 patients and 50 controls. Because of frequency-matching by sex and age, there were no significant differences in sex and age between the patients[male, 66%; mean age, (58±9) year] and controls[male, 60%; mean age, (54±14) year]($P>0.05$).

The PCR product of MMP-9 was cleaved into 123, 129, 187 and 252 bp with restriction endonuclease MspI. The 279R allele had two restriction sites and produced three fragments of 187, 129 and 123 bp, and the 279Q allele had only one restriction site, resulting in two fragments of 252 and 187 bp(Fig.1). Similarly, the PCR product of the p53 was cleaved into 173 and 254 bp with restriction endonuclease MspI. The A_1/A_1 allele had no restriction sites and A_2/A_2 allele had one restriction site, resulting in two fragments of 173 and 254 bp(Fig.2).

The genotype distributions of MMP-9 R279Q and p53 A_1/A_2 SNPs between the cases and controls are summarized in Table 1. In the NSCLC cases, the frequencies of MMP-9 R/R genotype(24/50 *vs.* 14/50 in control) and p53 A_1/A_2 genotype (22/50 *vs.* 12/50 in control) were significantly higher than those in the control cases($\chi^2=4.42$, $P<0.05$ and $\chi^2=4.46$, $P<0.05$, respectively). Further logistic regression analysis reveals that MMP-9 R/R homozygotes and p53 A_1/A_2 heterozygous genotypes were significantly higher in NSCLC patients. The odds ratio of the risk of R/R in NSCLC patients was 2.37 (1.04<OR<5.40). Similarly, the odds ratio of the risk of A_1/A_2 heterozygous in NSCLC patients was 2.49(1.06<OR<5.83). The results suggest the increased susceptibility of NSCLC with the two SNPs.

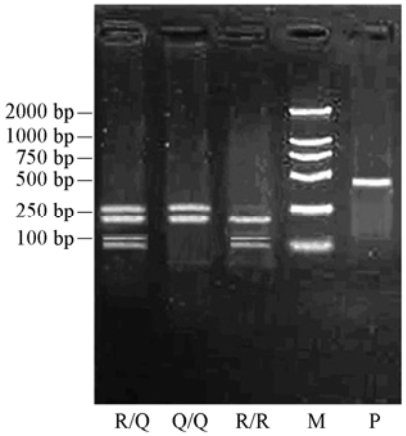

Fig.1 MMP-9 genotypes by PCR-RFLP

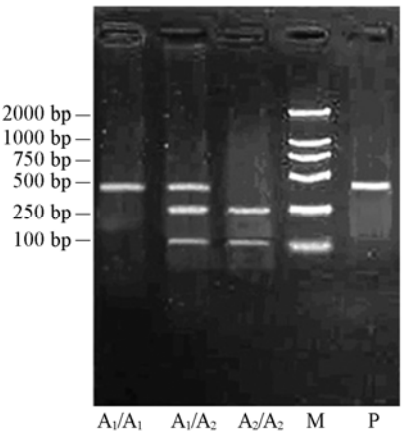

Fig.2 p53 genotypes by PCR-RFLP

Table 1 Genotype distributions of MMP-9 and p53 MspI SNPs in patients with NSCLC and controls

Gene	Genotype	Case		Control		χ^2
		n	(%)	*n*	(%)	
MMP-9(R279Q)	Q/Q	6	12.0	8	16.0	0.80
	R/Q	20	40.0	28	56.0	2.56
	R/R	24	48.0	14	28.0	4.24*
p53(A_1/A_2)	A_1/A_1	13	20.0	18	22.0	1.17
	A_1/A_2	22	38.0	12	18.0	4.46*
	A_2/A_2	15	42.0	20	60.0	1.10

*$P<0.05$.

The role of R279Q SNP in MMP-9 has been implicated in the etiology of various human cancers. Our result is similar to that in another previous study[13], where the 279R/R genotype for MMP-9 was reported to be associated with the metastasis of lung cancer, but different from that in other studies[14], where 279Q/Q(not 279R/R) was found to be associated with renal cell carcinoma. Despite those differences, the present study added to the evidence that genetic variations of MMP-9 may affect cancer progression.

Birgander *et al*.[12] found no association between the intron

6 polymorphism in p53 and lung cancer risk in Caucasian patients. However, in our current study, p53 A_1/A_2 genotype was found to be an increased risk of NSCLC cases(OR=2.49, 95% CI=1.06—5.83). A possible explanation is that there may be an ethnic difference in the etiology of lung cancer. Therefore, larger scale studies are warranted to further confirm the increased risk of NSCLC in Han population.

4 Conclusions

In summary, our study provided evidence that in Han population of Northeast China, the R/R genotype in MMP-9 gene and the A_1/A_2 genotype in p53 gene are closely associated with the susceptibility of NSCLC and may confer a potential biomarker in the occurrence of NSCLC. These results, once validated, may help to identify high-risk populations as well as determine an individual's risk of NSCLC.

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