Genetic polymorphisms of XPD and CDA and lung cancer risk

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Abstract. To determine the susceptibility genes of lung cancer, we investigated the frequency distributions of the xeroderma pigmentosum complementary group D (XPD) and cytidine deaminase (CDA) genes in patients. A case-control study was conducted involving lung cancer patients and healthy controls. The genotypic distributions of XPD exon 10 G→A (Asp312Asn) and 23 T→G (Lys751Gln), and CDA 79 A→C (Lys27Gln) and 208 G→A (Ala70Thr), were determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The results demonstrated that the XPD Asp312Asn genotype distribution was G/G (82.52%) and A/G (17.48%) in the lung cancer patients, and G/G (82.52%), A/G (16.50%) and A/A (10.98%) in the controls. The genotypes of Lys751Gln were T/T (83.49%) and T/G (16.50%) in the lung cancer patients, and T/T (84.47%) and T/G (15.53%) in the controls. Mutations in the XPD single nucleotide polymorphism loci did not demonstrate a significant difference between the two groups (P>0.05). The risk of lung cancer in individuals with mutations at positions 312 and 751 increased 6.13-fold (P=0.047). The CDA Lys27Gln genotype distribution was A/A (78.65%), A/C (20.39%) and C/C (0.98%) in the lung cancer patients, and A/A (79.61%), A/C (19.42%) and C/C (0.98%) in the controls (P=0.985). The CDA Ala70Thr genotype distribution was G/G (98.06%) and A/G (1.94%) in the controls, while all the genotypes were wild-type in the lung cancer patients. The difference between the lung cancer patients and the controls was not statistically significant (P=0.155). There was also no significant difference in the frequency distribution of XPD or CDA between the different pathological types (P>0.05). Our findings demonstrate that the mutation of XPD codons 312 and 751 increases the risk of lung cancer. By contrast, polymorphisms of CDA appear to have little association with lung cancer.

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Introduction

Lung cancer is one of the most common types of malignancy worldwide, which has a complex pathogenesis. Epidemiological and etiological studies have revealed that risk of lung cancer can be affected by a complex interaction between a number of genetic and environmental factors (e.g., tobacco smoke, radiation and infectious agents) (1). Further research has identified that there are polymorphisms in susceptibility genes, including those coding for DNA repair enzymes.

The incidence of lung cancer is associated with the alteration of DNA by external environmental factors and endogenous carcinogens (2). If the alteration cannot be repaired, it may cause genetic instability, mutation and cell death. There are at least four pathways involved in DNA repair, including base excision repair, DNA double-strand break repair, mismatch repair and nucleotide excision repair (NER). The expression of genes involved in the different DNA repair pathways varies greatly with the age of the individual. The NER system is one of the most significant repair pathways. A number of enzymes are involved in the NER pathway, including xeroderma pigmentosum complementary group A (XPA), replication protein A (RPA), xeroderma pigmentosum complementary group C (XPC), xeroderma pigmentosum complementary group D (XPD), excision repair cross-complementing 1 (ERCC1) and xeroderma pigmentosum complementary group F (XPF) (3). A number of studies have examined the role of ERCC1 (4). However, whether XPD polymorphisms correlate with different lung cancer pathologies remains to be determined.

Cytidine deaminase (CDA) is one of the enzymes involved in the pyrimidine rescue pathway, which catalyzes the deamination of cytidine and deoxycytidine to form uracil derivatives (5). Recent studies on CDA have focused on the association between cytarabine (Ara-c) and CDA in leukemia patients, while other studies have investigated the effect of gemcitabine, one of the first-line treatments for non-small cell lung cancer (NSCLC), on CDA polymorphisms in lung cancer (14). However, there are few studies that have examined the correlation between CDA polymorphisms and lung cancer risk and pathogenesis.

To determine the susceptibility genes which are most relevant for screening the Chinese population for lung cancer, we investigated the frequency distributions of the XPD and CDA genes in lung cancer patients compared to healthy controls.

Patients and methods

Patients. The study included 103 patients with lung cancer and 103 healthy control subjects. All patients were diagnosed with NSCLC or small-cell lung carcinoma at the Department of Respiratory Medicine Ruijin Hospital School of Medicine, JiaoTong University, Shanghai, China, between January 2006 and August 2010. Each diagnosis was confirmed by histopathology or cytology. Individuals in the control group were selected following a medical examination at our hospital. All subjects lived in South East China. The recommendations of the Declaration of Helsinki for biomedical research involving human subjects were followed.

Clinical data on age, gender, smoking and medical history, and data from physical examinations were systematically recorded at the start of the study. Performance status, pathological type and clinical stage were recorded for the lung cancer patients.

Methods. Blood samples (3 ml) were collected for genotyping at the start of the study, and genomic DNA was isolated from blood by DNA extraction. Genotype distributions, including XPD Asp312Asn and Lys751Gln, and CDA Lys27Gln and Ala70Thr, were determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primers for Asp312Asn were 5'-gcggtcccaaaagggtcagttacc ggcgtctggtgga-3' and 5'-gcggtcccaaaagggtcagtgctcaccctgca gcacttctt-3'. The PCR product was 143 bp, and was digested with *Mbo*II. The A/A genotype was 143 bp, the G/G genotype was 114 bp and 29 bp, and the A/G genotype was 114, 29 and 143 bp. The primers for Lys751Gln were 5'-cttcataagacctt ctagcaccac-3' and 5'-ctccctcagccccatctta-3', and the *Eam1104*-restricted product of the G/G genotype was 368 bp, the G/T genotype was 130, 238 and 368 bp and the T/T genotype was 130 and 238 bp. The primers for CDA Lys27Gln were 5'-gcggtcccaaaagggtcagtttgctcccaggaggcgaag-3' and 5'-gcggtcccaaaagggtcagtagattctcccctcctgggt-3', which yielded a 129 bp product. The *Mbo*II-restricted products were C/C (129 bp), A/A (48 and 81 bp) and A/C (48, 81 and 129 bp). The primers for Ala70Thr were 5'-tgtccttctccccaccttg-3' and 5'-ggaagatgttggctaaagagatg-3', which yielded a 300 bp product. The *Cpo*I-restricted products were A/A (300 bp), G/G (119 and 181 bp) and A/G (119, 181 and 300 bp). PCR reactions (10 μl) contained 0.3 μl template DNA, 1 μl 10X Taq buffer, 0.5 μl dNTP, 0.5 pmol/μl primer, and 0.3 units of Taq polymerase (Bio Basic Inc., Canada). The PCR reaction conditions were as follows: initial denaturation for 5 min at 95˚C, then 40 cycles of 30 sec at 95˚C, annealing for 45 sec, extension for 60 sec at 72˚C, followed by 6 min at 72˚C. The annealing temperature for the XPD Asp312Asn and CDA Ala70Thr primers was 60˚C, and the temperature for the XPD Lys751Gln and CDA Asp312Asn primers was 58˚C. PCR products were evaluated by overnight restriction enzyme digestion at 37˚C, followed by 2% agarose gel electrophoresis (Fig. 1). Mutations were identified by DNA sequencing (Fig. 2).

Statistical analysis. Clinical information and genotype frequencies were compared between the lung cancer patients and controls using the Chi-square test. Analysis of genotypes and their interaction with smoking was evaluated using logistic regression. Data were analyzed using SPSS 13.0 (IBM) software. P<0.05 was considered to indicate a statistically significant difference.

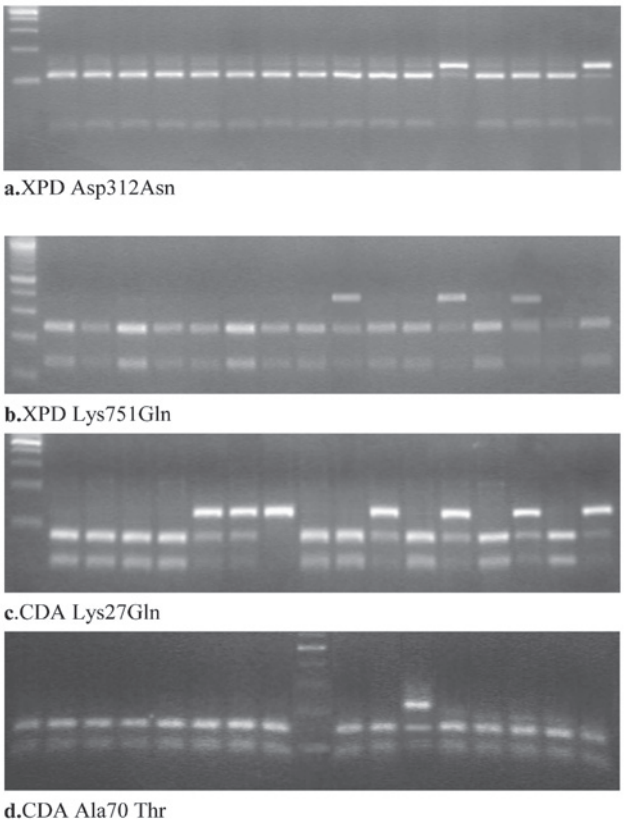

Figure 1. Resolution of PCR-RFLP products by agarose gel electrophoresis. The relevant codon is shown below each panel. XPD, xeroderma pigmentosum complementary group D; CDA, cytidine deaminase; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

Results

Demographic characteristics and genotype distributions for the lung cancer patients and the controls. No significant difference was found in the average age or gender ratio between the two groups (Table I). The XPD Asp312Asn genotypes in the lung cancer patients were G/G 85 (82.52%), A/G 18 (17.48%) and G/G 85 (82.52%), and in the controls the genotypes were A/G 17 (16.50%) and A/A 1 (0.98%). The XPD Lys751Gln genotypes were A/A 86 (83.49%) and A/C 17 (16.50%) in the lung cancer patients and A/A 87 (84.47%) and A/C 16 (15.53%) in the controls. No significant difference was evident between the two groups in the two single-nucleotide polymorphism (SNP) loci (P=0.598, 0.849). The CDA Lys27Gln genotypes were A/A 82 (79.61%), A/C 20 (19.42%) and C/C 1 (0.98%) in the lung cancer patients, and A/A 81 (78.65%), A/C 21 (20.39%) and C/C 1 (0.98%) in the controls. No significant differences were detected between the two groups (P=0.985). The CDA Ala70Thr genotypes were G/G 101 (98.06%) and A/G 2 (1.94%) in the controls. However, CDA Ala70Thr genotypes were all wild-type in the lung cancer patients. There were no significant differences in CDA Ala70Thr between the two groups (P=0.155).

Table I. Demographic characteristics and genotype distributions for lung cancer patients and controls.

Characteristic	Genotype	Healthy controls	Lung cancer patients	P-value
Number of cases		103	103	
Age (years)		60.79±1.16	61.53±1.21	0.912
Gender				
Male		65	66	1.000
Female		38	37	
Smoking history				
Smokers		74 (71.8%)	81 (78.6%)	0.462
XPD Asp312Asn	G/G	85 (82.52%)	85 (82.52%)	0.598
	A/G	17 (16.50%)	18 (17.48%)	
	A/A	1 (0.98%)	0	
XPD Lys751Gln	A/A	87 (84.47)	86 (83.49)	0.849
	A/C	16 (15.53)	17 (16.50%)	
CDA Lys27Gln	A/A	81 (78.65)	82 (79.61)	0.985
	A/C	21 (20.39)	20 (19.42)	
	C/C	1 (0.98%)	1 (0.98%)	
CDA Ala70Thr	G/G	101 (98.06%)	103 (100%)	0.155
	A/G		2 (1.94%)	0

XPD, xeroderma pigmentosum complementary group D; CDA, cytidine deaminase.

Table II. Genotypic differences between patients with various lung cancer pathologies.

Pathology	Genotype	Large cell lung cancer	Squamous cell carcinoma	Adenocarcinoma	Small cell lung cancer	P-value
XPD Asp312Asn	G/G	2 (1.94%)	16 (15.53%)	59 (57.28%)	8 (7.77%)	0.854
	A/G		4 (3.88%)	13 (12.62%)	1 (0.98%)	
XPD Lys751Gln	A/A	2 (1.94%)	16 (15.53)	60 (58.25%)	8 (7.77%)	0.858
	A/C		4 (3.88%)	12 (11.65%)	1 (0.98%)	
CDA Lys27Gln	A/A	2 (1.94%)	15 (14.6%)	58 (56.31%)	7 (6.79%)	0.070
	A/C		5 (4.85%)	14 (13.55%)	1 (0.98%)	
	C/C		0 (0%)	0 (0%)	1 (0.98%)	
CDA Ala70Thr	G/G	2 (1.94%)	20 (19.42%)	72 (69.90%)	9 (8.74%)	0.896

XPD, xeroderma pigmentosum complementary group D; CDA, cytidine deaminase.

Different frequency distributions of XPD and CDA genotypes in patients with different lung cancer pathologies. The XPD genotypes Asp312Asn and XPD Lys751Gln, and CDA Lys27Gln and CDA Ala70Thr were not significantly different between the large cell lung cancer, squamous cell carcinoma, adenocarcinoma or small cell lung cancer (Table II) (P>0.05).

Using logistic regression analysis, it was revealed that individuals with a smoking history combined with XPD Lys751Gln, had a 4.04-fold increased risk of lung cancer (P=0.044). Patients with XPD Lys751Gln and XPD Asp312Asn mutations had a 6.13-fold increase risk of lung cancer (P=0.047).

Discussion

Cancer is caused by a series of DNA alterations in a single cell or a clone of a cell, which leads to loss of normal cell function. At least four DNA repair pathways operate on specific types of damaged DNA. The NER pathway is one of the most significant and versatile repair systems that removes a wide range of DNA lesions, including UV-induced ones. A number of studies have examined the polymorphisms of genes within the NER pathway and their correlation to lung cancer susceptibility (2).

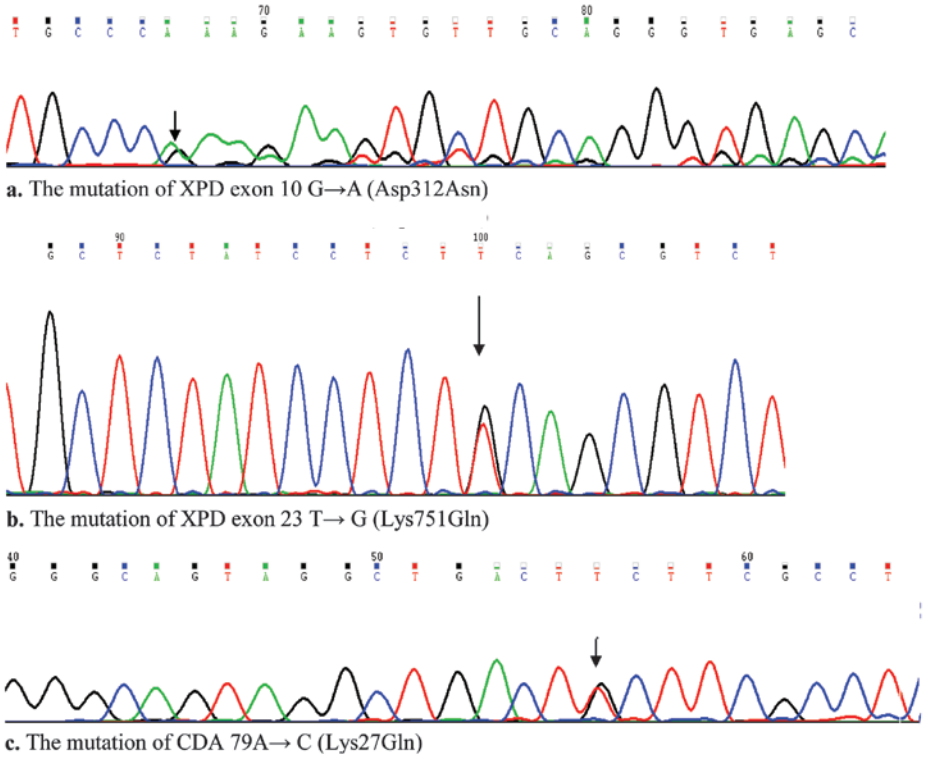

Figure 2. DNA sequencing of XPD and CDA SNPs. The codons from which the sequence data were obtained are shown in each panel. XPD, xeroderma pigmentosum complementary group D; CDA, cytidine deaminase; SNP, single-nucleotide polymorphism.

XPD is an ATP-dependent DNA helicase, which is involved in DNA repair in the NER pathway. XPD is a multifunctional gene that encodes a component of the TFIIH transcription factor and is involved in p53-mediated apoptotic responses (6). It is located on human chromosome 19 q13.3 and contains 23 exons. There are six identified SNPs in the XPD gene, located at codons 199, 201, 312, 711 and 751. The polymorphisms in codons 199, 201, 312 and 751 may result in amino acid changes. Mutation of the XPD gene may affect activity of the XPD protein. The mutation frequency of the 199 and 201 alleles is only 4%, whereas the mutation of codons 312 and 751 is more common, thus prompting our focus on Lys751G1n and Asp312Asn. The XPD mutated phenotype is closely correlated to diminished DNA repair capacity, which could increase the risk of cancer. Sequence alignment studies have demonstrated that XPD codon 312 is highly conserved, indicating that it may have an effect on preserving XPD protein function, whereas codon 751 on the N-terminus is poorly conserved. However, XPD 312 and 751 SNPs may lead to changes in DNA repair capacity (7).

Epidemiological studies revealed that the two polymorphisms were associated with certain malignancies, including lung cancer, head and neck squamous cell carcinoma and basal cell carcinoma (8-10). Hemminki *et al* (7) reported that 312 Asn/Asn and 715 Gln/Gln carriers were 50% less efficient in DNA repair compared to carriers of the wild-type. Our results demonstrated no significant difference in the mutation of XPD Asp312Asn and XPD LyS751Gln between the lung cancer patients and the controls. However, the risk of cancer may be increased in individuals carrying the Lys751Gln mutation and with a history of smoking. Individuals with XPD mutations are more likely to develop lung cancer, inferring an interaction between these two sites.

CDA is a key enzyme in pyrimidine metabolism (11), which is significant in the metabolism of deoxycytidine anti-neoplatic drugs (including Ara-C), which has a role in leukemia (12). CDA is also the key enzyme that determines gemcitabine clearance, since it catalyzes its degradation. Thus, CDA activity determines the transformation efficiency of gemcitabine to difluorodeoxyuridine and the length of exposure to gemcitabine. The majority of studies have focused on the correlation between CDA polymorphism or enzyme activity with gemcitabine efficacy and toxicity (13,14). However, little is known regarding the correlation between CDA polymorphisms and lung cancer risk. CDA contains three nucleotide mutations; 79 A→C, 208 G→A and 435 T→C. As the CDA 435 SNP is a nonsense mutation, this study focused on SNPs at codons 79 and 208.

Yue *et al* (15) observed that CDA polymorphisms differed between children with acute leukemia and healthy children. Results of that study showed that the mutation rate of codon 79 was approximately 12%, whereas that of codon 208 in the leukemia patients and healthy children studied was 0 and 0.94%, respectively. Our results demonstrated that the mutation rate of CDA 79 was 20.39% in the controls, compared with 19.42% in the lung cancer patients, indicating no significant difference between the two groups. The mutation rate of CDA 208 in the controls was 1.94%, while no mutations in the lung cancer group were observed; this difference was not statistically significant. This suggests that the mutation rate of CDA 208 is extremely low among this Chinese population. Previous studies have suggested that the CDA 208 mutation

rate varied according to ethnicity. No mutations were observed in Europeans, while there was a mutation rate of 12.5% in Africans. No significant difference in the mutation rate of CDA 79 and CDA 208 was observed between the lung cancer patients and the controls. This suggests that these CDA mutations do not increase the risk of lung cancer. CDA enzyme activity can be affected by base mutations, thus a change in a more critical position may be required to influence sensitivity to gemcitabine in lung cancer.

The association between SNPs and lung cancer susceptibility has examined, with varying results being obtained. These differences may stem from a limited number of samples. There may also be a range of susceptibility factors, which are difficult to fully investigate. In addition, gene polymorphism is correlated to ethnicity of the individual. Although there are certain limitations in SNP detection technology, susceptibility gene testing is important in providing early detection. Individuals who carry certain susceptibility mutations and smoke, or those who carry more than one mutation are increasingly susceptible to lung cancer. Thus, screening of susceptibility genes holds promise for the prevention and early diagnosis of lung cancer.

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