XPC gene intron 11 C/A polymorphism is a predictive biomarker for the sensitivity to NP chemotherapy in patients with non-small cell lung cancer

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The fact that intron single nucleotide polymorphisms could regulate gene expression or even alter gene expression levels has been the focus of attention. To study the relationship between the intron 11 C/A single nucleotide polymorphism of XPC gene and the efficacy of vinorelbine and cisplatin (NP) chemotherapy, 164 patients with non-small cell lung cancer (NSCLC) taking NP chemotherapy drugs were evaluated according to the efficacy of the treatment. We used polymerase chain reaction restriction fragment length polymorphism to examine the C/A polymorphism in the XPC gene intron 11 of the DNA samples extracted from peripheral blood. It was found that the frequency of patients in the effective group with C/C + C/A genotype (37.6%) had significant difference to chemotherapy than that of patients with A/A homozygotes (27.7%) in the same group ($P = 0.043$, odds ratio = 2.366, 95% confidence interval = 1.026–5.457). Therefore, NSCLC patients with the C/C + C/A genotype are more sensitive to NP treatment than those with the A/A genotype. The XPC gene intron 11 C/A polymorphism may be a predictive biomarker for sensitivity to NP chemotherapy in patients with NSCLC. *Anti-Cancer Drugs* 21:669–673 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

It has long been the golden rule to select chemotherapeutic agents based on anatomic sites of tumors. On account of intertumor and interindividual heterogeneity, however, patients with anatomically identical tumors can respond very differently to the same chemotherapy in terms of sensitivity and toxicity [1]. Armed with the recent achievements of the Human Genome Project, attempts have been made to predict chemotherapy efficacy based on patients' genetic profile [2–4]. It would be desirable to select chemotherapy drugs based on the molecular subcategories of malignant tumors, so as to achieve individualized treatments, rather than encountering unpredictable outcomes with the same traditional therapy. Single nucleotide polymorphisms (SNPs; i.e. the subtle variation of nucleotides in human genome) were shown to be highly correlated with the patients' sensitivity to the drug [5]. Therefore, it should be feasible to predict individual sensitivity to chemotherapy based on their SNP profile.

Lung cancer is one of the most common malignant neoplasms, with its incident rate gradually increasing over the past years. Non-small cell lung cancer (NSCLC) accounts for nearly 80% of all lung cancer cases, and the majority of them are diagnosed at advanced stages (stages IIIb–IV). Surgery is no longer the best option for these patients, whereas chemotherapeutic treatment is usually recommended, which is mainly aimed at prolonging life and relieving symptoms rather than curing the disease. Chemotherapies currently in use for NSCLC include combinations of cisplatin or carboplatin with some of the new drugs (gemcitabine, vinorelbine, paclitaxel, docetaxel), with vinorelbine and cisplatin (NP) as the primary choice. Vinorelbine (NVB; Pierre Fabre Medicament Production) is a cell cycle-specific agent that blocks cells in the metaphase of mitosis. It binds to tubulin monomers, thus inhibiting the formation and inducing the disassembly of microtubules. Cisplatin (DDP; Pharmacuetical Co., Ltd., Lianyungang hausen) induces its cytotoxic properties through binding to nuclear DNA and subsequently forming cisplatin–DNA adducts. The interchain or intrachain cross-linking leads to DNA damage that can cause tumor cell death. Clinical studies have shown that NSCLC patients respond differently to the NP regimen. It has also been reported that the difference is associated with an individual's DNA repair capacity [6].

A variety of DNA repair mechanisms exists, such as base excision repair, DNA double strand break repair, mismatch repair, and nucleotide excision repair. Despras *et al.*

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[7] knocked down various genes involved in nucleotide excision repair and found that only the xeroderma pigmentosum group C (XPC) gene knockdown impaired DNA damage repair, pointing to the role of XPC in DNA repair. XPC is located on chromosome 3p25. It has 16 exons and 15 introns, encoding a protein of 940 amino acids. Polymorphisms have been found in XPC, including intron 9 PAT polymorphisms, exon 15 A/C SNP, and intron 11 C/A SNP [8–10]. The above three polymorphisms show linkage disequilibrium. It has been reported that the XPC gene polymorphisms exon 15 A/C and intron 9 PAT were associated with cancer development [10,11]. However, it is still unknown whether intron 11 A/C polymorphism plays a role in tumor sensitivity to chemotherapy.

In our study, patients with NSCLC were treated with the same chemotherapy (NP-based drug), and were grouped according to their response to the therapy. Variables such as sex, age, smoking history, histopathology, and stage of tumors were strictly controlled. XPC intron 11 C/A polymorphisms from different responding groups are compared to show its correlation with patients' sensitivity to chemotherapy, in the hope of providing experimental evidence for individualized treatment.

Materials and methods

Patients

This research project was approved by the Ethics Committee of the China Medical University. One hundred and sixty-four cases of NSCLC patients were diagnosed by histopathology at the oncology department of China Medical University, the First Affiliated Hospital, and Liaoning Cancer Hospital and Institute, which belong to a multicentral research group, using the standard NP chemotherapy, from October 2006 to May 2008. They all accepted more than two courses of chemotherapy treatment, with 98 men and 66 women, aged 27–84 years (Table 1). Age, sex, and related clinical data of all the patients were collected using the methods of medical record and/or questionnaire survey and all gave informed consent. All the patients were confirmed by measurable tumor lesions, which were diagnosed by a computed tomography scan. Their blood, liver, and kidney functions were all in normal conditions, and the electrocardiogram was normal, and functional status before chemotherapy (Karnofsky Performance Status, KPS) was above 60.

Table 1 The primary characteristics of non-small cell lung cancer patients

Variable	Cases (%)
Sex	
Male	98 (59.8)
Female	66 (40.2)
Age (years)	
Average	59.58 ± 10.39
Range	27–84
Pathologic type	
Adenocarcinoma	93 (56.7)
Squamous carcinoma	70 (42.7)
Clinical stages	
III	100 (61.0)
IV	64 (39.0)
Smoking or not	
Yes	72 (43.9)
No	89 (54.3)
Others	3 (1.8)

The chemotherapy method and efficacy evaluation in NSCLC

The chemotherapy method: One hundred and sixty-four cases of NSCLC patients were all treated with the chemotherapy method of NP: vinorelbine of 25 mg/m^2 and 0.9% saline of 100 ml, intravenous injection, on the first and the eighth days; cisplatin of 40 mg/m^{2}and 0.9% saline of 500 ml, on the second, third, and fourth days, intravenous injection.

The determination standard of chemotherapy efficacy: According to the WHO standard (1981), the efficacy determination was divided into complete remission (CR), partial remission (PR), no change (NC) and progress degree (PD), and effective = CR + PR, ineffective = NC + PD. The evaluation standard was based on computed tomography imaging in which the measured tumor lesion was measured again after two cycles of treatment with NP chemotherapy. The chemotherapy patients were divided into two groups according to the evaluation standard: (i) effective group (chemotherapy sensitive group) and (ii) ineffective group (chemotherapy non-sensitive group).

The detection of XPC gene C/A polymorphism

Genomic DNA from the peripheral blood was extracted by the routine method [12–14]. In brief, each frozen clot (500 µl) was transferred to a centrifuge tube with 800 µl of TE buffer (triethanolamide), mixed well and centrifuged at 10 000*g* for 5 min, 400 µl of TE, 25 µl of 10% SDS, and 5 µl of 20 mg/ml proteinase K were added to the residual clot material and incubated overnight. The supernatant was collected and an equal volume of phenol was added and then placed on a rotator for 15 min. The supernatant was removed and an equal volume of a mixture of phenol and chloroform (1 : 1) was added. After centrifugation, the supernatant was removed and an equal volume of chloroform was added. After centrifugation, the supernatant was absorbed and two volumes of protein precipitation solution (two volumes of absolute ethanol containing 10% 3 mol/l sodium acetate) were added and incubated for 1 h at −20°C. Each sample was centrifuged at 10 000*g* for 10 min. Next, the resulting DNA pellet was rinsed with 75% ethanol and centrifuged at 10 000*g* for 5 min. The 75% ethanol was decanted and the tube inverted on a clean absorbent paper. The resulting DNA was reconstituted in a TE buffer and stored at −20°C

until use. The detection of XPC gene polymorphism was performed according to the literature [9] using polymerase chain reaction restriction fragment length polymorphism, and the forward primer 5′-AAATGACCTGGGCC TGTTTG-3′ is used in combination with the reverse primer 5′-GGCAGGAAGAGGTACACATTC-3′. The PCR system was in a total reaction volume of 50 μl containing: 5 μl of 10 × PCR buffer, 4 μl of 2.5 mmol/l dNTP mixture, 1 μl of upstream and downstream primers (10 pmol/μl), respectively, 2.5 U of rTaq DNA polymerase, 10 ng of template DNA, and appropriate amount of purified water. PCR conditions were as follows: predenaturing at 95°C for 5 min, 30 cycles with 95°C for 30 s, 65°C for 30 s, and 72°C for 30 s. Then the expected amplified band was digested by the restriction enzyme Smu I, the total reaction volume of 20 μl containing: 2 μl of 10 × NET buffer, 0.5 μl of the restriction enzyme Smu I (MBI Fermentas, Burlington, Ontario, Canada), 12 μl of PCR products, and appropriate amount of purified water. The A/A homozygous genotype did not contain the Smu I restriction enzyme site, resulting in complete 203 fragments whereas the C/C homozygous genotype contained a restriction enzyme site, resulting in two fragments of 160 and 43 bp; and the C/A heterozygous genotype resulted in three fragments of 203, 160, and 43 bp. All the 20 μl products were electrophoresed in 2% agarose gel at 150 V for 30 min, then stained by ethidium bromide for 5 min and observed. All the primers and reagents of the PCR reaction were purchased from the TaKaRa Company (Dalian, Liaoning, China). PCR products of the three different genotypes (C/C, C/A, and A/A) of XPC were purified and commercially sequenced by an ABI377 sequencer.

Statistical methods

Nonconditional logistic regression was used to determinate the difference in genotype distribution between different groups; the odds ratios and 95% confidence intervals were used to estimate the differences of chemotherapy sensitivity to the NP chemotherapy in different genotypes. The confounding factors, such as sex and age, were adjusted using the χ^2 and *t*-test. Nonconditional logistic regression, adjusted for age and sex, was used to estimate the odds ratios. A *P* value of less than 0.05 was considered significant. All statistical tests were two-sided, and carried out using the SPSS 13.0 software (SPSS Inc., Chicago, Illinois, USA).

Results

The effective rate of NSCLC patients treated with the NP chemotherapy

According to the efficacy evaluation, among the 164 cases of NSCLC patients, there were two CR cases, 55 PR cases, 88 NC cases, and 19 PD cases, whereas the effective group contained 57 cases and the ineffective group contained 107 cases. The total effective rate was 34.8%. As confounding factors, sex and age had no statistical difference to the sensitivity of chemotherapy ($\chi^2 = 1.048$, $P = 0.306$, $t = 0.084$, $P = 0.933$, respectively) (Table 2).

The frequency distribution of XPC intron 11 genotypes in NSCLC

The amplified products of the XPC gene were discerned as the C/C genotype, C/A genotype, and A/A genotype through electrophoresis in 2% agarose gel (Fig. 1a); a C or A variation was further confirmed by the sequencing of PCR products (Fig. 1b). The frequency distribution of the C/C genotype in patients with NSCLC was 31.1% (51/164), C/A was 40.2% (66/164), and A/A was 28.7% (47/164); the frequency distribution of the C allele was 51.2% and A allele was 48.8%.

The XPC gene C/A polymorphism in intron 11 and its association with the chemotherapy sensitivity in NSCLC

The frequency distribution of XPC gene intron 11 polymorphism C/C, C/A, and A/A genotypes in the effective group were 29.8, 47.4, and 22.8%, respectively, whereas that in the ineffective group were 31.8, 36.4, and 31.8%, respectively. For the statistical analysis, we combined the C/A and C/C genotypes into one group (C/C + C/A). We controlled the factors of age, sex, smoking, or pathologic type, and clinical stages for equilibrium. These factors were all matched to avoid the effect of the factors mentioned above to the XPC genotypes. The frequency of patients in the effective group with the C/C + C/A genotype (37.6%) had significant difference to chemotherapy than that of the patients with the A/A homozygotes (27.7%) in the same group ($P = 0.043$). Therefore, the chemotherapy effective rate of the C/C + C/A genotype was 2.366-fold higher compared with that of the A/A genotype (95% confidence interval = 1.026–5.457) (Table 2).

Discussion

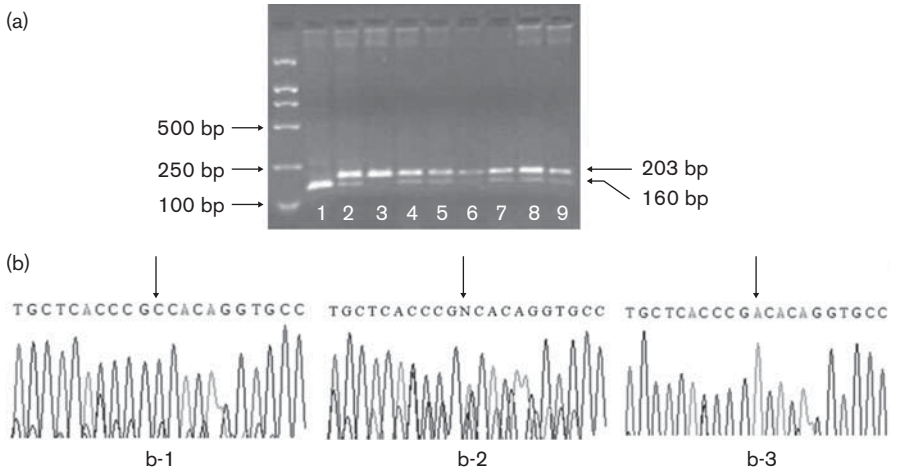
Major determinants of chemotherapeutic agents are drug metabolism and DNA repair capacity of target cells, which vary because of an individual's different genetic background. SNPs at genes involved in DNA repair can lead to amino acid substitution, thus, altering the activity of DNA repair enzymes. These changes are the main reason and molecular basis for interindividual differences in DNA repair capabilities [15]. Polymorphisms identified for XPC gene include intron 9 PAT polymorphism, intron 11 C/A SNP, and exon 15 A/C SNP. According to the Human Genome Project database (*http://www.ncbi.nlm.nih.gov*/SNP/snp_ref.cgi?rs = 2228001), the frequency of A:C allele in exon 15 is approximately 6 : 4 (62.2 vs. 37.8%). There is no information on the frequency of the C and A alleles in intron 11. We found that the distribution of the C and A alleles in intron 11 among patients with NSCLC is approximately 5 : 5 (51.2 vs. 48.8%).

Table 2 The association of XPC genotype and chemotherapy sensitivity in non-small cell lung cancer

XPC genotype	Cases	The chemotherapy sensitivity		P^a	OR	95% CI
		Effective group (%)	Ineffective group (%)			
C/C	51	17 (33.3)	34 (66.7)			
C/A	66	27 (40.9)	39 (59.1)			
A/A	47	13 (27.7)	34 (72.3)		1	
C/C + C/A	117	44 (37.6)	73 (62.4)	0.043[b]	2.366	1.026–5.457
Pathologic type						
Adenocarcinoma	93	33 (35.5)	60 (64.5)	0.581	0.811	0.386–1.706
Squamous carcinoma	70	24 (34.3)	46 (65.7)		1	
Clinical stages						
III	100	33 (33.0)	67 (67.0)	0.626	1.194	0.585–2.439
IV	64	24 (37.5)	40 (62.5)		1	
Smoking or not						
Yes	72	21 (29.2)	51 (70.8)	0.168	0.603	0.293–1.238
No	89	34 (38.2)	55 (61.8)		1	

CI, confidence interval; OR, odds ratio; XPC, xeroderma pigmentosum group C.
[a]Using non-conditional logistic regression.
[b]Individuals carrying C/C + C/A genotype were compared with that of A/A genotype.

Fig. 1



(a) Identification of XPC genotypes using polymerase chain reaction restriction fragment length polymorphism. A 2% agarose gel was electrophoresized and stained by ethidium bromide then observed by ultraviolet. DNA marker is DL2000; 1: C/C homozygous; 2, 4–9: C/A heterozygote; 3: A/A homozygous. (b) The sequencing results of the PCR products of XPC gene C/A polymorphism. The arrow in (b) referred to as the base of XPC gene C/A polymorphism, (b-1) identified C/C genotype, (b-2) identified C/A genotype, and (b-3) identified A/A genotype.

The relationship between XPC polymorphism and disease has mainly been focused on exon 15 A/C SNP and its hereditary susceptibility [10,11], whereas few studies have been carried out on the intron SNPs. So far, only Laczmanska *et al.* [15] have found that intron 11 C/A SNP is associated with bleomycin-induced chromosomal aberrations; however, there is no report about its correlation with chemotherapy sensitivity. Although polymorphisms located in introns do not directly affect the function of gene products, some introns can regulate gene expression [16,17], and therefore alter gene expression levels. It has been proposed that allele A in intron 11 can lead to an alternatively spliced transcript that skips exon 12, producing a product lacking 125 amino acids [15], which presumably impairs DNA repair capacity. Accordingly, individuals with allele A should be compromised in their ability to repair damaged DNA in target cells caused by chemotherapy, and thus respond better to chemotherapy.

In our study, the C/A SNP in XPC intron 11 of 164 NSCLC patients were typed, and its role in chemotherapy sensitivity explored. It was found that patients with C/C + C/A were 2.366 times more sensitive to chemotherapy than the A/A homozygotes ($P < 0.05$), which suggested a decrease in the sensitivity for allele A carriers. Our results did not support the deduction of the paper [9]. About the relationship between the C/A SNP in intron 11 and diseases, only Laczmanska *et al.* [15] found that it is related to bleomycin-induced chromosomal aberrations; however, there is no report about this SNP and sensitivity to chemotherapy. Therefore, we should objectively evaluate whether the DNA repair

capacity of the A allele in intron 11 decreased, and whether it is related to the decreased sensitivity to chemotherapy in patients with NSCLC of the A allele and its DNA repair capacity. It should be further studied because there is no direct evidence about whether the 125 amino acids in exon 12 play a crucial role on the DNA repair capacity of XPC protein. At the same time, which allele could meet the general laws of the XPC protein in the DNA repair process, the C or A allele needs to be examined. There is rarely a report about the relationship between the DNA repair capacity of XPC and sensitivity to chemotherapeutic drugs. Let us assume that the DNA repair capacity of the allele A carriers is impaired because of the lack of the 125 amino acids encoded by exon 12, then errors in DNA replication might not be successfully restored, resulting in apoptosis or unlimited proliferation [11]. This could explain their increased susceptibility to tumors. Besides, the unlimited proliferation caused by mutation may render these cells insensitive to the apoptosis [18] induced by chemotherapeutic drugs, such as cisplatin, which supports the findings of this study that allele A carriers were not sensitive to the NP treatment. In contrast, allele C carriers possess normal DNA repair capacity, so the cells will go into apoptosis on application of chemotherapy, and are thereby sensitive to NP treatment. Further studies are needed to clarify the following research questions: whether the 125 amino acids encoded by exon 12 play a role in DNA repair; whether allele C carriers or allele A carriers have the normal function of XPC protein in DNA repair; and what is the relationship between DNA repair capacity of XPC and sensitivity to chemotherapeutic drugs.

In this study, only patients receiving NP treatment were included, so as to rule out the variation that might be introduced by different treatments. Besides, other factors were strictly controlled and balanced among the groups. This is the first report on the association of the XPC gene intron 11 C/A polymorphism with NP treatment responsiveness in NSCLC patients. We found that NSCLC patients with the C/C + C/A genotype are more sensitive to NP treatment than those with the A/A genotype. Therefore, intron 11 C/A polymorphism of XPC gene can be used to help predict NSCLC patients' sensitivity to NP treatment, thus guiding individualized treatment decisions.

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