XPA A23G polymorphism is associated with the elevated response to platinum-based chemotherapy in advanced non-small cell lung cancer

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DNA repair capacity (DRC) is correlated with sensitivity of cancer cells toward platinum-based chemotherapy. We hypothesize that genetic polymorphisms in DNA repair gene XPA (xeroderma pigmentosum group A) and XPG (xeroderma pigmentosum group G) (ERCC5, excision repair cross-complementation group 5), which result in inter-individual differences in DNA repair efficiency, may predict clinical response to platinum agents in advanced non-small cell lung cancer (NSCLC) patients. In this study, we find that the A → G change of XPA A23G polymorphism significantly increased response to platinum-based chemotherapy. Polymorphism in XPG His46His was associated with a decreased treatment response, but was not statistically significant.

Keywords single nucleotide polymorphism; gene-chip; XPA; XPG; nucleotide excision repair; non-small cell lung cancer; chemotherapy

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Introduction

Lung cancer is the most common cause of cancer death in many countries; more than a million people in the world die from the disease each year [1]. Eighty percent of lung cancer patients are diagnosed with non-small cell lung cancer (NSCLC), of which nearly two-thirds are detected at advanced stages [2]. At present, platinum-based therapies are the major remedial measure of the advanced NSCLC. Cisplatin is still the scaffolding of combination chemotherapy. Results tend to be similar whether the partner drug is paclitaxel, docetaxel, or gemcitabine. Similar results are generally obtained with carboplatin [3]. Resistance to chemotherapy is also a challenge. As drug resistance varies from person to person, genetic factors are believed to influence the effectiveness of lung cancer treatment [4].

The Human Genome Project indicated that 99% of DNA within different individuals is identical and only 1% is variant, of which the major type is single nucleotide polymorphism (SNP). SNP is a point mutation carried by some individuals of a population. The pharmacogenetical study indicated that such a tiny diversity in the sequence of the genome significantly influenced individual treatment response, toxicity, and survival in cancer patients. SNP has greater clinical significance in terms of its ease of clinical application, rather than its mRNA, which present some clinical difficulties in terms of obtaining tissue samples from lung cancer patients.

A variety of distorting lesions, notably platinum-induced DNA adducts, is mainly repaired by the nucleotide excision repair (NER) pathway, one of the four DNA repair mechanisms [5]. NER is a complex process that involves more than 16 proteins [6,7], in which proteins of the xeroderma pigmentosum group A (XPA) and group G (XPG/ERCC5) are involved in the damage recognition-complex of NER. Although the detailed steps involved in NER are still not completely clear, several studies have described the approximate
assessment of how the NER systems appear to act, by maintaining an intricate network of contacts with some repair factors. In the global genome repair pathway, the protein complex XPC–HHR23B binds to damaged DNA [8]. Then, the multicomponent transcription repair factor TFIIH and other repair factors may be recruited to the damaged DNA site [9,10]. Next, XPG nuclease cleaves the DNA on the 3'-end [11]. XPA is capable of interacting with TFIIH. Following cleavage of the DNA, XPA/RPA proteins join the complex and recruit the ERCC1–XPF (excision repair cross-complementing group 1-xeroderma pigmentosum group F) complex with very high affinity, which cleaves the 5' end [12,13]. Finally, most of the complexes unbind, leaving only the RPA complex and making room for DNA polymerase δ and its cofactors to patch the gap left in the DNA. Because some polymorphisms in NER genes may predict repair phenotype, any variations that may occur in the XPA and XPG gene might have the potential to affect protein function and subsequently DNA repair capacity (DRC).

It has been speculated that SNP in DNA repair genes may alter their expression or activity, affect the functions of DNA repair, and in turn influence the effects of cancer treatment [14]. Therefore, we could expect that the lower the levels of expression of XPA and XPG, the more susceptible the tumors to platinum therapies. In this study, we investigated if polymorphisms in XPA A23G and XPG His46His can predict response in NSCLC patients treated with cisplatin/carboplatin combination chemotherapy.

### Patients and Methods

#### Study subjects

All patients for the study were recruited between March 2006 and September 2007 from several hospitals in Nanjing of China, which include Jiangsu Cancer Hospital, Zhongda Hospital, and Nanjing General Hospital of Nanjing Military Command. The 115 eligible patients were diagnosed with histologically confirmed advanced NSCLC (stages III–IV) and had a measurable lesion by CT scan, a Karnofsky performance status of no less than 60, and were normal in blood chemistries, hepatic and renal function, and electrocardiogram at the beginning of treatment. The study was approved by an Ethical Review Committee at the hospital and patients gave consent to participate. The information of patients was listed in Table 1.

#### Chemotherapy regimens and therapeutic effect evaluation

All patients had received platinum-based chemotherapy: 5 (4.3%) received NP/NC regimens (vinorelbine plus cisplatin/carboplatin), 60 (52.2%) had GP/GC regimens (gemcitabine plus cisplatin/carboplatin), and 50 (43.5%) were given TP/TC regimens (taxol/docetaxel plus cisplatin/carboplatin) (Table 1). Dosage regimen: cisplatin 30 mg/m² on day 2–4; carboplatin AUC = 4–5 g on day 1; vinorelbine 25 mg/m² on day 1 and day 8; gemcitabine 1 g/m² on day 1 and day 8; taxol 175 mg/m² on day 1 (kept for 3 h), docetaxel 60 mg/m² on day 1 (kept for 1 h). All chemotherapeutic drugs were administered intravenously, and treatment cycles were repeated every 3–4 weeks. Patient responses to treatment were determined after four cycles by the WHO criteria [15], which classify the response into four categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). CR was defined as complete disappearance of all measurable lesions. PR required at least 50% reduction in measurable lesions. Patients with SD had less than a 50% decrease or no more than a 25% increase in the size of measurable lesions. PD was assigned to patients when measurable lesions increased by more than 25% or new lesions appeared. For data analysis, CR and PR were combined.

### Table 1 Characteristics of the patients and of the chemotherapy regimens

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Numbers of patients</th>
<th>Year (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34–84</td>
<td>59.6</td>
</tr>
<tr>
<td>Gender</td>
<td>Female 37</td>
<td>32.2</td>
</tr>
<tr>
<td></td>
<td>Male 78</td>
<td>67.8</td>
</tr>
<tr>
<td>Histology</td>
<td>Squamous cell</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>carcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>71.3</td>
</tr>
<tr>
<td></td>
<td>Large cell and</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>undifferentiated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>carcinoma</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy regimens</td>
<td>Platin + taxane</td>
<td>43.5</td>
</tr>
<tr>
<td></td>
<td>Platin + GEM</td>
<td>52.2</td>
</tr>
<tr>
<td></td>
<td>Platin + NVB</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Note: platin, cisplatin/carboplatin; taxane, paclitaxel/docetaxel; GEM, gemcitabine; NVB, vinorelbine

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as responders, and SD and PD were grouped as non-responders.

**DNA collection and genotyping**
Each patient provided 2 ml pre-treatment blood for the study. The blood samples were collected in EDTA citric acid anticoagulation tubes and stored at \(-80^\circ C\) until analysis. Genomic DNA was isolated from the blood samples using the DNA purification kit or phenol-chloroform extract, and stored at \(4^\circ C\) until use.

SNPs in XPA (A23G, a A to G transition at 4 nucleotides upstream from the ATG start codon of 5'-UTR, rs1800975 (reference SNP ID) and XPG (C581T, a C to T transition at codon 46 of exon 2, without amino acid change – His/His, rs1047768) were analyzed by a gel-based DNA microarray genotyping method. Probes and primers were designed by Primerpremier 5.0 software from CLC bio (Aarhus, Denmark). The sequences of primers and probes are shown in Table 2. One of each pair of primers was modified with acrylamide phosphoramidite (Acrydite\textsuperscript{TM}; Matrix Technologies, New Hampshire, USA) at its 5'-terminal. Each couple of probes was labeled with Cy3 and Cy5 fluorescent dyes at its 5'-terminal, respectively. The PCR reactions were performed in 30 \(\mu l\) reaction solution containing 10 pmol primer and 50 ng genomic DNA. The PCR reaction consisted of an initial step at 95\(^\circ C\) for 5 min, then 35 cycles of denaturing at 94\(^\circ C\) for 30 s, annealing at 60\(^\circ C\) for 30 s, extension at 72\(^\circ C\) for 40 s, and a last extension at 72\(^\circ C\) for 5 min.

After PCR amplification and gel electrophoresis, PCR products were processed by ethanol precipitation and evaporation or left untreated. Solutions containing acrylamide-modified PCR products, glycerol, ammonium persulfate (APS), and acrylamide monomers were prepared, spotted, and polymerized onto the acryl-modified slide. In the process, TEMED (tetramethylethylenediamine) is introduced onto the spotted microarray to immobilize the modified nucleic acids [16,17]. Following the attachment, to obtain ssDNA for hybridization analysis, dsDNA on the slides was denatured in 0.1 M NaOH for 10 min. After hybridization, the slide was subjected to electrophoresis under 5–30 V/cm for 5–20 min in 1 \(\times\) ris-borate-EDTA (TBE) buffer at 4\(^\circ C\). Images of the slides were captured by a scanner (LuxScan\textsuperscript{TM}-10K Confocal Scanner, Packard BioScience, Billerica, USA) and were analyzed with Genepix Pro 3.0 software provided by Axon Instruments (Burlingame, USA). Sequencing of 10% samples were performed to validate the results.

**Statistical analysis**
Statistical analysis was performed using SPSS software package Version 13.0 (SPSS Inc., Chicago, USA). The significance of differences in frequencies and genotypes between good and poor responders was calculated using the \(\chi^2\) test. Fisher’s exact test was performed when the theoretical frequency had an expected count less than 1 or \(n<40\). The logistic regression model was used to calculate the odds ratios (OR) and their 95% confidence intervals (CI). In the regression analysis, the outcome variable was patient response to treatment; patients who had poor response to treatment (SD + PD) were compared with good responders (CR + PR). All \(P\) values reported were two-sided, and \(P<0.05\) was considered statistically significant.

**Results**

**Images of DNA-microarray hybridization for SNP genotyping**
Based on the immobilization efficiency, acryl-modified glass slides were selected to fabricate the DNA microarrays. By allele-specific oligonucleotide dual-color fluorescence hybridization, homozygous wild type, homozygous mutant type, and heterozygote type yielded green, red, and yellow fluorescence, respectively. Fig. 1 shows the microarray images.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>XPA (A23G, rs1800975)</td>
<td>Forward primer: 5'-ACGCGGCCCCAAAAACCTCCAGT-3'; reverse primer: 5'-Acrydite\textsuperscript{TM}-TCTCAGCACGGACCCCTTCTC-3'; probe: 5'-Cy3-CATCTCCTGGCCCA-3'; 5'-Cy5-CATCTCCGGCCCA-3'</td>
</tr>
<tr>
<td>XPG (C581T, rs1047768)</td>
<td>Forward primer: 5'-Acrydite\textsuperscript{TM}-CAGTATGTGAATAGGGGTAAC-3'; reverse primer: 5'-TTCTTCAATAGTAGCTGGAAC-3'; TCTTTCAATATGAGAGCCTC-3'; probe: 5'-Cy3-GTTCCCGTGGCGA-3'; 5'-Cy5-GTTCCCATGGCGA-3'</td>
</tr>
</tbody>
</table>
Treatment response and genotype

Of 115 patients, 30 (26.1%) had some response (CR + PR) and 85 (73.9%) showed no response (SD + PD). Table 3 shows the allele frequencies of SNPs locus in patients with different responses, and the association of genotypes with treatment response. Genotype frequencies for both XPA (A23G) and XPG (His46His) polymorphisms were found to be in Hardy–Weinberg equilibrium, which states that both allele and genotype frequencies remain constant in a population. No associations were detected between genotype and age, sex, or histological type. The polymorphic genotypes of XPG (His46His) were not significantly different between patients who responded and those who did not respond to the platinum-based treatment. For XPA (A23G), however, the genotypes were substantially different between the groups. Among responders, 4 (9.5%) were homozygous wild type (A/A), 15 (45.5%) were heterozygous (A/G), and 11 (27.5%) were homozygous variant (G/G). The corresponding genotypes in the non-responders were 38 (90.5%), 18 (54.5%), and 29 (72.5%), respectively. The difference between the two groups was statistically significant ($P = 0.002$). After combining the A/G and G/G genotypes, the difference remained statistically significant, suggesting that the XPA (A23G) genotype differed between patients who responded and those who did not respond to chemotherapy. Patients carrying at least one T allele (A/G + G/G) were more likely to be responders compared with those who did not carry the G allele; the OR for response was 0.190 and the 95% CI was between 0.061 and 0.593 ($P = 0.004$).

DISCUSSION

DRC may play paradoxical roles in carcinogenesis, response to cancer treatment, and toxicity. Reduced
DRC, however, may lead to increased response to therapies that act by damaging the DNA of cancer cells. Owing to its possible effect on gene expression, we anticipated that polymorphisms of DNA repair genes may influence tumor response to platinum-based chemotherapies. The identification of molecular variables that predict either sensitivity or resistance to chemotherapy is of major interest in selecting the most likely effective first-line treatment.

Platinum-based drugs inhibit tumor growth by the formation of bulky DNA adducts. The latter are mainly removed by the NER mechanism that is involved in removing a wide range of lesions. Low activity of NER in tumors makes them highly sensitive to platinum therapy [18]. Therefore the relationship between NER polymorphisms and the efficacy of platinum-based therapy remains a subject of investigation.

The XPA gene product is a zinc-finger DNA-binding protein. The XPA protein is also involved in both global genome and transcription-coupled repair pathways [10]. Considering its affinity for damaged DNA and its ability to interact with many (core) repair factors, XPA is likely to play a central role in NER. Wu et al. [19] showed that the specific inhibition of XPA mRNA by antisense strategy could decrease NER capacity, increase cisplatin IC50 value 10 fold and hence sensitize human lung adenocarcinoma cells to cisplatin, and might be able to sensitize other types of tumor cells to other drugs known to cause bulky lesions repaired by NER.

A common single-nucleotide polymorphism (A23G) in the 5’ non-coding region (at position −4 from the ATG start codon) of the gene was identified [18] and its functional relevance remains to be determined, although a decreased risk for lung cancer has been mentioned to be associated with this polymorphism [20–22]. This polymorphism is located in the vicinity of the translation initiation codon, and hence may alter translation efficiency. The proximal nearby nucleotides to the AUG initiation codon are important for the initiation of translation because the 40S ribosomal subunit binds initially at the 5’-end of mRNA [23]. It is possible that a nucleotide substitution of adenine to guanine at position −4 preceding the AUG codon (CCAGAGAUGG) [24] may affect ribosomal binding and thus alter the efficiency of XPA protein synthesis. An alternative explanation could be that the protective XPA allele is in linkage disequilibrium with an allele from an adjacent gene, which is the true susceptible gene.

Although the functional consequences of the XPA A23G polymorphism has not been known, it is possible that this polymorphism could have an effect on host capacity for removing bulky adducts and thus modulate the sensibility to platinum-based chemotherapy. In previous studies, the 23G allele was associated with a decreased risk of cancer, suggesting that the 23A>G may lead to increased XPA expression (and increased DRC). Based on these studies, it is conceivable that the 23A>G may be associated with poor response rate. Furthermore, we found a higher proportion of non-responders among the homozygous wild-type genotype patients than among the other patients. Since this polymorphism leads to a less active protein, this result seems to be consistent with the presumption that XPA polymorphism could increase response to platinum chemotherapy. This data may provide a means of identifying a subgroup of NSCLC patients likely to benefit from individualized chemoprevention or treatment strategies.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Numbers of cases</th>
<th>Response to chemotherapy</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Responders (%)</td>
<td>Non-responders (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XPA (A23G)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>42</td>
<td>4 (9.5)</td>
<td>38 (90.5)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A/G</td>
<td>33</td>
<td>15 (45.5)</td>
<td>18 (54.5)</td>
<td>0.126</td>
<td>0.037–0.435</td>
</tr>
<tr>
<td>G/G</td>
<td>40</td>
<td>11 (27.5)</td>
<td>29 (72.5)</td>
<td>0.278</td>
<td>0.080–0.961</td>
</tr>
<tr>
<td>A/G + G/G</td>
<td>73</td>
<td>26 (35.6)</td>
<td>47 (64.4)</td>
<td>0.190</td>
<td>0.061–0.593</td>
</tr>
<tr>
<td>XPG (His46His)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>16</td>
<td>7 (43.8)</td>
<td>9 (56.2)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>43</td>
<td>11 (25.6)</td>
<td>32 (74.4)</td>
<td>2.263</td>
<td>0.680–7.527</td>
</tr>
<tr>
<td>T/T</td>
<td>56</td>
<td>12 (21.4)</td>
<td>44 (78.6)</td>
<td>2.852</td>
<td>0.880–9.244</td>
</tr>
<tr>
<td>C/T + T/T</td>
<td>99</td>
<td>23 (23.2)</td>
<td>76 (76.8)</td>
<td>2.570</td>
<td>0.862–7.662</td>
</tr>
</tbody>
</table>
The XPG (ERCC5) protein, coded by XPG gene mapping on chromosome 13q32-33, is a structure-specific endonuclease that cleaves the damaged DNA ~5 m 3' to the site of the lesion [25]. XPG has a structural function independent of its cleavage activity in the assembly of the NER DNA-protein complex [26]. ERCC5/XPG polymorphism (His1104Asp) has been associated with reduced risk of lung cancer [27], but His46His polymorphism has seldom been evaluated in relation to lung cancer or the treatment response previously. Zienolddiny et al. [28] found the homozygote XPG (His46His) variant was associated with a significantly decreased risk of lung cancer. In relation to PAH-DNA adducts, His46His variant alleles were less frequent in the cases with higher adduct levels. The functions of these polymorphisms have not been measured and it might be speculated that cases with these alleles may have suboptimal DRC. Vila et al. [29] analyzed SNPs in several genes involved with DNA repair and correlated the results with toxicity and response to oxaliplatin in 33 cases of colorectal cancer (CRC). They found patients with C/C of the ERCC5/XPG (His46His) polymorphism showed better objective response, longer time to progression, and better median survival than those with C/T and T/T, suggesting that XPG C/C may be a predictive marker of response, time to progression, and survival in oxaliplatin-treated CRC patients, although the number of samples was small. Similarly, although it is statistically impossible to find the differences of the chemotherapeutic efficacy among XPG (His46His) variant genotype in this study, the presence of the C/C genotype in codon 46 showed a similar trend of better response. These observations were just like that a cancer cell line selected for resistance to ET743, a DNA minor groove binder alkylating guanine N2 position, were deficient in XPG protein [30], while cisplatin resistance was rather associated with increased NER activity. This illustrates the complex interplay between drug cytotoxicity and DNA repair alteration.

Our study suggests that the polymorphic status of XPA A23G might predict the treatment response of advanced stage NSCLC patients. Strengths of this study include the enrollment of a large number of patients without knowledge of their polymorphism status, the independent collection of data of clinical outcomes, and genotyping.

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**References**


