RESEARCH COMMUNICATION

Lack of any Relationship between Chemotherapy Toxicity in Non-small Cell Lung Cancer Cases and Polymorphisms in XRCC1 Codon 399 or XPD Codon 751

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Abstract

Purpose: To examine the association between genetic polymorphisms (at XRCC1codon 399 or XPD codon 751) and chemotherapy related toxicities of non-small cell lung cancer. Methods: One hundred and fifteen patients with histologically or cytologically confirmed stage IIIB and IV NSCLC recruited from Department of Chemotherapy of Jiangsu Cancer Hospital and Research Institute from 2005 to 2008, to evaluated the occurrence of chemotherapy related toxicities and the association with single nucleotide polymorphisms in XRCC1codon 399 or XPD codon 751. Results: No significant association was observed between grade 0 or grade 1-4 overall toxicity and XRCC1 codon 399 (odds ratio=1.40, 95% confidence interval,0.73-2.66; adjusted odds ratio =1.43, 95% confidence interval,0.71-2.88), or XPD codon 751 genetic polymorphisms (odds ratio =0.87, 95% confidence interval,0.33-2.26; adjusted odds ratio=0.74, 95% confidence interval,0.26-2.13); similar results were found between hematologic, hepatic, gastrointestinal toxicities and XRCC1 399 or XPD 751 genetic polymorphisms. Conclusion: No statistically significant association was found between either XRCC1codon 399 or XPD codon 751 genetic polymorphisms and chemotherapy related toxicities.

Keywords: XRCC1 - XPD - non-small cell lung cancer - chemotherapy- toxicity

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Introduction

X-ray repair cross-complementing gene 1 (XRCC1) is one of the most important DNA repair genes (Cheng et al., 2009). XRCC1 is considered to be involved in the repair of DNA single-strand breaks after base excision repair of damage produced by ionizing radiation, alkylating agents, and reactive oxygen species (Norppa, 2004). XRCC1 acts as a scaffold protein by bringing the polymerase and ligase together at the site of repair and the consecutive ordered interactions may serve to protect reaction intermediates and ensure efficient completion of the correction process after the initial recognition of DNA damage (Lindahl and Wood, 1999). The XRCC1 399Gln allele may be protective against development of acute side effects after radiotherapy in patients with normal weight (Chang-Claude et al., 2005). Wang and his colleagues reported an association between DNA-repair gene XRCC1 single nucleotide polymorphisms (SNP) and increased cisplatin-induced-gastrointestinal toxicity in 139 lung cancer patients (Wang et al., 2008). Another study found that the interaction between XRCC1 Arg399Gln genotype and toxicity was not significant in platinum-treated lung cancer (Giachino et al., 2007). The xeroderma pigmentosum group D (XPD) is a helicase belonging to the TFIIH complex, which participates in DNA unwinding during both global genome and transcription-coupled nucleotide excision repair and transcription initiation (Benhamou and Sarasin, 2002). However, a study revealed that no significant differences in toxicities were found with respect to XPD751 polymorphisms (Tibaldi et al., 2008; Font et al., 2008). On the other hand, Richard Booton reported that grade of neutropenia demonstrated a significant correlation with XPD haplotype (Booton et al., 2006), and borderline reduction in risk of lung toxicity was noted for XPD Lys751Gln heterozygotes (Kuptsova et al., 2007).

Chemotherapy related toxicities remain a major consideration in cancer treatment, and it is not currently known how to predict severe dose-limiting toxicities during chemotherapy. To examine the association between the different genotypes (at XRCC1codon 399 or XPD codon 751) and toxicity, we conducted this study.

Materials and Methods

Study population

Patients hospitalized at Department of Chemotherapy of Jiangsu Cancer Hospital and Research Institute from 2005 to 2008 with histologically or cytologically confirmed non-small cell lung cancer (NSCLC) and clinical stage IIIB or stage IV patients with measurable

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diseases were recruited if they also met the following criteria: age >18 years, Karnofsky Performance Score (KPS)>60, life expectancy >3 months, adequate bone marrow reserve (leukocyte count $\geq 4.0\times10^9$/L, platelet count$\geq 100\times10^9$/L), adequate liver and renal function (bilirubin level ≤1.5 mg/dL, alanine aminotransferase/ aspartate aminotransferase < 2 times the upper limit of normal, creatinine level ≤1.5 mg/dL). A written informed consent should be obtained from each patient before chemotherapy.

Exclusion criteria for participation in the study included active infections, recent myocardial infarction, unstable angina, chronic conditions (such as autoimmune diseases) or symptomatic brain metastases, pregnant or breast-feeding.

The incidence of grade 3 or 4 toxicity was 58% in the Chinese lung cancer patients treated with platinum-based chemotherapy (Wang et al., 2008). The highest frequency of genotype (Lys/ Lys) in XPD Lys751Gln was 46.8% (Chen et al., 2002). Allowing for a permissible error of 20%, the minimum sample size required for this study was calculated to be 103 patients.

Chemotherapy

All patients received platinum-based chemotherapies, and the chemotherapy was repeated every 3 weeks. The chemotherapeutic regimen consisted of Gemcitabine / platinum (GP), docetaxel/ platinum (DP), novelbine/ platinum (NP) and paclitaxel/ platinum (TP) as reported elsewhere (Sun et al., 2009; Zhou et al.,2009).

Toxicity assessment

Hematologic and nonhematologic toxicities were recorded during every treatment course. The worst grade toxicity was recorded for each patient undergoing chemotherapy. Toxicities were assessed using National Cancer Institute common terminology criteria (version 3.0) (DCTD, 2006).

Sample collection and SNP genotyping

Genomic DNA was isolated from whole blood samples drawn from an antecubital vein before drug administration. PCR instrument was Model MJ2000, and the endonucleases were bought from New England BioLabs, Beverly, MA, USA.

The genotypes of XRCC1 Arg399Gln and XPD Lys751Gln sites were determined by polymerase chain reaction (PCR) based restriction fragment length polymorphism (RFLP) methods as described previously (Xing et al., 2002). Genotyping was done by laboratory personnel blinded to case status. The primers and reaction conditions were described before (Yao et al., 2009).

Statistical analysis

The objective of the analysis was to determine the association between genetic polymorphisms and chemotherapy related toxicities. Baseline characteristics of patients, toxicity, and genotypes were summarized by groups. Toxicities were grouped into hematologic toxicity, hepatic damage and gastrointestinal toxicity. The associations between toxicity and XRCC1 Arg399Gln or XPD Lys751Gln were estimated by odds ratios (OR), adjusted odds ratios (AOR) and 95% confidence interval (95% CI), which were calculated by unconditional logistic regression.

Factors included in analysis were genotypes, gender (man versus woman), performance status, age, clinical stage (IIIB versus IV), histology (adenocarcinoma, squamous and large cell cancer). All statistical tests were 2-sided and were performed by STATA 8.0 statistical software (Stata Corporation, 2003). Statistical significance was set at $P< 0.05$.

Results

Patient Characteristics

One hundred and fifteen advanced NSCLC patients treated with platinum-based chemotherapy with whole medical records available were recruited into study. The median age of 78 men was 62 years with a range from 42 to 80, and the median age of 37 women was 56 years with a range from 40 to 76. The KPS range was from 60 to 90. Forty patients had stage IIIB, and 75 had stage IV disease. Adenocarcinoma was the most common histology (n = 82, 71.3%), with squamous cell carcinoma (n =29, 25.2%) and large cell (n = 4, 3.5%) being less common. All patients received platinum-based chemotherapy. Seventy-four patients received GP regimen, 15 DP regimen, 18 TP regimen, and 8 patients received NP regimen.

Single nucleotide polymorphisms

Table 1 shows XRCC1 codon 399 and XPD codon 751 genetic polymorphisms in our patient cohort. For XRCC1 codon 399, 6 patients were homozygous allele (Arg/Arg), whereas 46 were heterozygous (Arg/Gln), 63 were homozygous Gln/Gln, and the Arg allelic frequency was 25.2%, Gln 74.8%. For the XPD codon 751 polymorphism, 94 (81.7%) patients had the Lys/Lys genotype, whereas 21(18.3%) were Lys/Gln, none was Gln /Gln, and Lys allelic frequency was 90.9%, Gln 9.1%.

Toxicity Outcomes

Sixty-nine patients developed at least one kind of toxicity during the administration of platinum-based chemotherapy, and none toxicity occurred in 46 patients. Some patients reported more than one toxicity: 6 patients experienced hematologic toxicity and hepatic damage, and 10 had hematologic and gastrointestinal toxicities.

SNPs and Toxicity

Table 1 describes the toxicities and clinical characteristics. Analysis of hepatic damage and ages

Table 1. XRCC1 Codon 399 and XPD Codon 751 Polymorphisms

Genotype		No.	%	Allelic Frequencies
XRCC1 codon 399	Gln/Gln	63	54.8	Gln 74.8%
	Arg/Gln	46	40.0	
	Arg/Arg	6	5.2	Arg 25.2%
XPD codon 751	Lys/Lys	94	81.7	Lys 90.9%
	Lys/Gln	21	18.3	Gln 9.1%

No, number of patients; XRCC1, X-ray cross-complementing group 1; XPD, xeroderma pigmentosum group D

Table 2. Toxicities and Clinical Characteristics

Clinical Finding	Hematologic Toxicity			Hepatic Damage			Gastrointestinal Toxicity		
	G0	G1-4	P	G0	G1-4	P	G0	G1-4	P
Age,year									
<60	23	30		45	8		44	9	
≥60	33	29	0.294	60	2	0.040	55	7	0.382
Gender									
man	42	36		73	5		72	6	
woman	14	23	0.111	32	5		27	10	0.008
KPS									
<80	18	22		34	6		36	4	
≥80	38	37	0.563	71	4	0.092	63	12	0.380
Histology									
AC	37	45		74	8		71	11	
SCC	17	12		27	2		24	5	
large	2	2	0.397	4	0	0.441	4	0	0.775
Clinical stage									
IIIB	19	21		36	4		33	7	
IV	37	38	0.851	69	6	0.717	66	9	0.419
Regimen									
GP	40	34		67	7		64	10	
DP	4	11		15	0		12	3	
TP	6	12		16	2		16	2	
NP	6	2	0.631	7	1	0.872	7	1	0.905

G0, grade 0 toxicity; G1-4, grade 1-4 toxicity; P, P value; KPS, Karnofsky Performance Score; AC, adenocarcinoma; SCC, squamous cell carcinoma; large, large cell; GP, Gemcitabine/ platinum; DP, docetaxel/ platinum; TP, paclitaxel/ platinum; NP, novelbine/ platinum

revealed statistically significant association (P = 0.04).The incidence of gastrointestinal toxicity was significantly different in men and women (P = 0.008). The incidence of the overall grade 0 or grade 1-4 toxicities was not significantly different in XRCC1 codon 399 polymorphisms (OR=1.40, 95% CI,0.73-2.66; AOR =1.43, 95% CI,0.71-2.88). There was no significant association between the XPD codon 751 polymorphism and the risk of the overall grade 0 or grade 1-4 toxicities (OR =0.87, 95% CI,0.33-2.26; AOR=0.74, 95% CI,0.26-2.13). No significant difference was found between XRCC1 codon 399 polymorphisms and grade 0 or grade 1-4 hematologic toxicities , hepatic damage or gastrointestinal toxicities. The findings were similar with respect to XPD codon 751 polymorphisms.

Discussion

SNPs of DNA repair genes related to metabolism may influence tumor response to chemotherapy or radiotherapy because of their possible effects on gene expression. The identification of SNPs that predict either toxicity or sensitivity to chemotherapy is of major interest in selecting patient who will be benefit from a chemotherapy regimen. Because XRCC1 and XPD are important DNA repair genes, we conducted this study to determine whether SNPs in XRCC1codon 399 or XPD codon 751 are associated with toxicity among advanced NSCLC patients treated with platinum-based chemotherapy. Some studies have assessed the association between SNPs in XRCC1codon 399 or XPD codon 751 and chemotherapy response in NSCLC patients treated with platinum-based chemotherapy, but chemotherapy toxicity was focused by only a few studies to date (Wang et al., 2008; Giachino et al., 2007; Tibaldi et al., 2008; Booton et al., 2006).

Chemotherapy toxicity is an important issue in the treatment of cancer patients. Individualized drug dosage to reduce unnecessary toxicity and improve therapeutic efficacy is critical. Oncologists could predict toxicity according to the pharmacological effects and drug dosages of regimen, patient's KPS, organ functions, and previous treatments, etc. But the toxicity may be quite different even though the patients with the similar body status received the same drug dosage. We believe that the toxicity could vary for patients with different genotypes.

Studies addressing the association of genotypes in XRCC1codon 399 or XPD codon 751 with chemotherapy toxicity in NSCLC patients have focused mainly on platinum-based chemotherapy. A study suggested that there was no statistically significant association between the XRCC1 Arg399Gln polymorphisms and hematologic grade 3 or 4 toxicity; but patients carrying at least one variant XRCC1 Arg399Gln allele have a 2.5-fold increased risk of grade 3 or 4 gastrointestinal toxicity when treated with first-line cisplatin-based chemotherapy in lung cancer (Wang et al., 2008). Another study showed that grade of neutropenia demonstrated a significant correlation with XPD haplotype, such that the XPD751 lysine allele was associated with greater Grade 4 neutropenia compared with XPD751 glutamine allele, but no significant association was demonstrated between XPD haplotype and anemia, thrombocytopenia and/or nonhematologic toxicity (Booton et al., 2006).

Arg /Arg genotype of XRCC1 codon 399 was 55.4% (77/139) (Wang et al, 2008), or 53.4% (55/109) (Chen et al., 2002), and the Lys/ Lys genotype of XPD codon 751 was 46.8% (51/109) (Chen et al., 2002). But in our patients the Arg/Arg genotype of XRCC1 codon 399 was only 5.2%, the Lys/Lys genotype of XPD codon 751 81.7% and none Gln/Gln genotype of XPD codon 751 was observed (Table 1). The significant difference in genotype frequency may account for the different results from previous studies.

This analysis was adjusted for potential confounding factors, including age, gender, KPS, clinical stages, histology and chemotherapy regimens, because these differences were reported to confound toxicity outcomes(Sloan et al.,2002; Schiller et al.,2002; Muss et al., 2007). Although SNPs in XRCC1codon 399 or XPD codon 751 and their relationships with the chemotherapy toxicity have not yet been thoroughly identified, more precise estimation of the clinical effects of platinum-based chemotherapy might be possible with improved genetic profiling. A better understanding of the roles of DNA repair genes will also provide further information on chemotherapy toxicity.

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