RESEARCH ARTICLE

XPG is Predictive Gene of Clinical Outcome in Advanced Non-small-cell Lung Cancer with Platinum Drug Therapy

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Abstract

Polymorphisms in XPG are considered to contribute to the clinical outcome of patients receiving platinum drug chemotherapy. We aimed to investigate the role of five potential SNPs of XPG gene on the response to platinum-based chemotherapy in advanced Chinese NSCLC patients. A total of 451 patients with newly diagnosed and histopathologically confirmed primary NSCLC were consecutively collected. XPG rs2296147, rs4150261, rs17655, rs1047768 and rs2094258 were genotyped by the Taqman real-time polymerase chain reaction (PCR). In our study, we found patients carrying rs1057768 TT genotype had a significantly lower treatment response when compared with the CC genotype (OR=0.38, 95% CI=0.18-0.78). Patients carrying rs1047768 TT genotype showed a significantly short median PFS (11.2 months) and OS (13.6 months) than CC genotype, and the hazard ratios (HR) for PFS and OS were 2.06 (1.01-4.50) and 2.29 (1.21-2.49), respectively. Moreover, we found a significant decreased risk of death from NSCLC among patients carrying the rs2296147 TT genotype when compared with the CC genotype, the HR (95% CI) for OS being 0.50 (0.27-0.95). In conclusion, our study found that polymorphisms in rs1047768 C/T and rs2296147 C/T are associated with response to platinum-based chemotherapy in advanced NSCLC, and XPG polymorphisms could be predictive of prognosis.

Keywords: Non-small-cell lung cancer - xeroderma pigmentosum group G - response - chemotherapy

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Introduction

Lung cancer is the leading cause of cancer-related death worldwide (IARC, 2008). Among different histologies, non-small-cell lung cancer (NSCLC) has the highest incidence and almost accounted for 80% of all the lung cancer. Although advanced treatment, 70% of affected patients present metastatic disease and advanced cancer when they are diagnosed (Molina et al., 2008).

For advanced NSCLC patients, platinum-based doublets chemotherapy is used as the standard first-line chemotherapy. However, it is estimated that the response rate to platinum-based regimen of these patients is about 20%, and the median survival is about 7 to 9 months (Jemal et al., 2002; Tahara et al., 2009). Recently, many studies have shown that single nucleotide polymorphisms (SNPs) influence the mechanisms of DNA repair function, and have a role of removing DNA adducts. Therefore, the polymorphisms in SNPs might affect the response to chemotherapy. Repair of DNA damage is a complex process, which is conducted by a series of DNA repair pathways. In humans, more than 130 genes play a role in the DNA repaired pathway (Wood et al., 2001). Nucleotide excision repair (NER) is a key DNA repair mechanism, which has a role of removing DNA lesions caused by UV radiation or chemical agents (Friedberg, 2000). The defective NER could induce xeroderma pigmentosum (XP), such as xeroderma pigmentosum group A(XPA) to xeroderma pigmentosum group G(XPG), and this protein has function of extreme UV-sensitivity and a high genetic predisposition to sunlight-induced skin cancers following a recessive model (Cordonnier and Fuchs, 1999).

The XPG gene (ERCC5) encodes an 1186-amino acid protein as an endonuclease, and is an indispensable component of NER (Clarkson, 2003). This protein combined with ERCC1 is involved in a dual incision at both the 3' and 5' sites of the lesion, and produces the excision of damage-containing oligomers of 22-32 nucleotides in length (Friedberg, 2003). Currently, various epidemiological studies suggested that defective XPG gene polymorphisms have a vital role in the development of various cancers, such as lung, esophageal, colorectal cancer, bladder, ovarian and gastric cancer (Nouspikel et al., 1997; Chang et al., 2008; Bartolucci et al., 2009; Sun et al., 2009; Sakano et al., 2010; Fleming et al., 2012; He et al., 2012). However, only three studies with small sample

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Table 1. Sequences of Primers and Probes of XPG

SNP	Major/Minor allele	MAF	Primer sequence (5'-3')	Probe sequence (5'-3')
rs2296147	C/T	0.3045	AGCTGTCACCGCCTCCC	CGGCCATTCTCTGGACC
rs873601	A/G	0.4556	CTGGTATGAGCCCATCTA	AGTGACAAGCCTGTAGCC
rs17655	C/G	0.3768	TTACGTCTTTGCGACAAATTCATT	CATTAAAGATGAACTTTCAGCAT
rs1047768	C/T	0.4881	CAGTATGTGAATAGGGGTAAC	TTCTTCAATAGTGGAGCATC
rs2094258	A/G	0.2358	AGCCTCGCCTTTGCCGAT	CTTCTGACCCATGCCCACC

Table 2. Demographic and Clinical Characteristics among NSCLC Patients

Characteristics	Number of Patients (%)
Median age (years)	64.3(31.7-76.1)
Sex	
Male	306(67.8)
Female	145(32.2)
ECOG performance status	
0-1	412(91.3)
≥2	39(8.7)
Histology	
Adenocarcinoma	256(56.7)
Squamous cell carcinoma	169(37.4)
Other	27(5.9)
Disease stage	
III(A/B)	159(35.2)
IV	292(64.8)
Chemotherapy regimens	
NP and NC	234(51.8)
GP and GC	106(23.5)
TC	111(24.7)

NP or NC, Vinorelbine+Cisplatin/ carboplatin; GP or GC, Gemicitabine+Cisplatin/ carboplatin; TC, Docetaxel+cisplatin

size reported the association between XPG polymorphisms and response to platinum-based chemotherapy among advanced NSCLC (Walsh et al., 2008; Chen et al., 2009; Liu et al., 2012). Therefore, our study aimed to assess the role of five potential SNPs of XPG gene on the response to platinum-based chemotherapy in advanced Chinese NSCLC patients.

Materials and Methods

Subjects

This case-control study included 475 patients with newly diagnosed and histopathologically confirmed primary NSCLC from three hospitals: Tumor Hospital of Jilin Province, The China-Japan Union Hospital and the First Affiliated Hospital of Xinxiang Medical College between January 2006 and December 2007. Finally 451 patients were willing to participate into our study, with the participation rate of 94.9%. All the NSCLC were at the stage of IV or stage IIIB/A, and received platinum-based chemotherapy as the first line treatment. Patients who previously received radiotherapy or chemotherapy, and were diagnosed with symptomatic brain metastases, spinal cord compression and uncontrolled massive pleural effusion were excluded from our study. The demographic and clinical information were collected from medical record. All the patients were followed up every 4 weeks by telephone until death or the end of follow-up.

The platinum-based doublets chemotherapy regimens included 25 mg/m^2 vinorelbine on days 1 and day 8 plus 75 mg/m^2 cisplatin or AUC=5 carboplatin on day 1; 1,250 mg/m^2 gemicitabine on day 1 and 8 plus 75 mg/m^2 cisplatin or AUC=5 carboplatin on day 1; 75 mg/m^2 docetaxel plus 75 mg/m^2 cisplatin on day 1. The treatment was repeated every three weeks for a maximum of six cycles. The treatment would not be continued when patient presented progressive disease or experienced unacceptable toxicity. When patients developed serious toxicity, we would decrease the dose of cytotoxic agents in the next cycle by 25%.

The response to chemotherapy was evaluated in accordance with the response evaluation criteria from European Organization for Research and Treatment of Cancer. The response was assessed after 4 weeks of treatment. The response to chemotherapy was divided into good responders and poor responders. The good responders were defined as complete response or partial response, and the poor responders were defined as stable disease or progressive disease. Progression-free survival (PFS) was calculated from the date of the chemotherapy to progressive disease or death. Overall survival (OS) was calculated from the date of chemotherapy to the date of death or last clinical follow-up.

SNP selection and genotyping

Genomic DNA was obtained from peripheral blood using a Qiagen Blood Kit (Qiagen, Chastworth, CA). Five potentially SNPs of XPG were selected according to the following criteria: 1) SNPs were located at two ends of XPG gene; 2) the minor allele frequency (MAF) was more than 5%; 3) the SNPs influence the microRNA binding sites activity. Finally, five SNPs were included in the analysis, including rs2296147, rs4150261, rs17655, rs1047768 and rs2094258. Probes and primers were designed by Primer 5.0 software (Table 1). The genotyping of SNPs were conducted using the Taqman real-time polymerase chain reaction (PCR) method with a 7900 HT sequence detector system (Applied Biosystems, Foster City, CA). The PCR reactions were conducted in a reaction of 30μL solution of 10 pmol primer and 50ng genomic DNA. The PCR reaction started at 94°C for 5 minutes, denatured at 94°C for 30s, annealed at 64°C for 30s and extended at 72°C for 60s. A total of 35 cycles were performed. For quality control, a minimum of 10% of DNA samples were randomly selected and were genotyped again to confirm the results. The results confirmed 100% concordance.

Statistical analysis

Descriptive data in our study were expressed as mean and percent. The differences between the categorical

variables were analyzed by Pearson χ^2 test, and the continuous variables were assessed by student's t-test. Survival distributions were estimated by using the Kaplan-Meier method and assessed using the log-rank test. The association between genotype and survival was estimated by hazard ratios (HR) and confidence intervals (CI) from multivariate Cox proportional hazards model. Wild genotype was severed as a reference group. $P<0.05$ was considered to be significant. All tests were two-sided and analyzed by SPSS 11.0 software.

Results

Table 2 summarized the clinical characteristics of 451 patients in our study. The median age of included patients was 64.3(31.7-76.1) years, and 67.8% of the patients were males. Most of the patients presented disease stage of IV (64.8%). Patients with histological type of adenocarcinoma and squamous cell carcinoma were accounted for 56.7% and 37.4%, respectively. All patients received chemotherapy based on platinum agent combined with other chemotherapy agents.

Table 3. Response to Platinum-based Chemotherapy According to XPG Genotypes

Genotype	Cases		χ^2	*P* value
	Good responder (%)	Poor responder (%)		
rs2296147				
CC	71(51.7)	178(56.7)		
CT	45(32.8)	99(31.5)		
TT	21(15.5)	37(11.8)	0.58	0.50
rs873601				
AA	63(45.9)	140(44.6)		
AG	51(37.3)	120(38.2)		
GG	23(16.8)	54(17.2)	0.18	0.91
rs17655				
CC	76(55.5)	164(52.2)		
CG	45(32.8)	106(33.8)		
GG	16(11.7)	44(14.0)	3.15	0.75
rs1047768				
CC	75(55.1)	137(43.6)		
CT	50(36.5)	120(38.2)		
TT	12(8.4)	57(18.2)	8.08	0.018
rs2094258				
AA	84(61.4)	205(65.3)		
AG	36(26.4)	77(24.5)		
GG	17(12.2)	32(10.2)	0.78	0.676

Among all patients, 137 patients showed good response to chemotherapy, and the remained 314 showed poor response to chemotherapy. A significant association was found between polymorphisms of rs1047768 and treatment with platinum-based chemotherapy ($\chi^2=8.08$, $P=0.018$, Table 3). The rs1057768 TT genotype had higher proportion of poor response to chemotherapy. The logistic regression analysis showed a significantly higher treatment response with rs1057768 TT genotype when compared with the CC genotype (OR=0.38, 95% CI=0.18-0.78).

In our study, patients carrying rs1047768 TT genotype showed a significantly short median PFS (11.2 months) and OS (13.6 months) than CC genotype, and log-rank test showed significantly different in PFS and OS (Table 4). In the Cox proportional hazards model after adjusting for potential confounding factors, the hazard ratios (HR) for PFS and OS in patients carrying rs1047768 TT genotype were 2.06(1.01-4.50) and 2.29(1.21-2.49), respectively, when compare CC genotype as a reference variable. Moreover, we found a significant decreased risk of death from NSCLC among patients carrying rs2296147 TT genotype when compared with CC genotype, and HR (95% CI) of OS was 0.50(0.27-0.95). We did not find significant association of rs873601, rs17655 and rs2094258 with PFS and OS in advanced NSCLC patients.

Discussion

In this study, we investigated whether the XPG gene polymorphisms in NER pathway were associated with

Table 4. Univariate Analysis of XPG in Relation to PFS and OS

Gene	PFS				OS			
	event	Median (month)	Log-rank	HR(95%CI)[1]	event	Median (month)	Log-rank	HR(95%CI)[1]
rs2296147								
CC	193	11.7		1	175	15.2		1
CT	108	13.7		0.90(0.55-1.50)	90	19.4		0.72(0.46-1.16)
TT	40	17.5	0.11	0.75(0.38-1.55)	30	21.8	<0.05	0.50(0.27-0.95)
rs873601								
AA	155	14.6		1	135	18.8		1
AG	128	14.9		0.92(0.56-1.52)	110	19.3		1.10(0.70-1.73)
GG	58	15.7	0.53	0.94(0.49-1.85)	50	20.7	0.35	0.93(0.52-1.69)
rs17655								
CC	179	15.7		1	155	20.9		1
CG	115	15.3		1.08(0.66-1.81)	99	18.7		1.04(0.67-1.64)
GG	47	14.8	0.61	1.23(0.60-2.65)	42	18.5	0.38	1.28(0.67-2.511)
rs1047768								
CC	148	18.5		1	123	22.4		1
CT	131	16.7		1.34(0.82-2.20)	115	19.1		1.43(0.91-2.24)
TT	62	11.2	<0.05	2.06(1.01-4.50)	57	13.6	<0.05	2.29(1.21-2.49)
rs2094258								
AA	220	14.1		1	193	18.1		1
AG	86	15.7		1.0(0.59-1.74)	74	19.6		0.94(0.58-1.54)
GG	34	16	0.25	0.71(0.35-1.49)	28	20.2	0.49	0.66(0.34-1.30)

[1]Adjusting sex, age, histology, ECOG performance status, and disease stage

the clinical outcome of advanced NSCLC treated with platinum-based chemotherapy. We found that XPG rs1047768 in the NER pathway genes were associated with response to platinum-based chemotherapy, and rs1047768 TT genotype was correlated with poor PFS and OS of NSCLC. Our study indicated the down regulation of XPG rs1047768 activity leads to decreased PFS and OS of advanced NSCLC with platinum-based chemotherapy, and down regulation of XPG rs2296147 activity was correlated with increased OS. This is possible the function of XPG in the NER pathway which repairs DNA damage caused by platinum-based chemotherapy.

The XPG gene has been mapped to chromosome 13q33 and consisted of 15 exons spanning ~30kb of genomic DNA, and this kind of gene participates in two incision steps to correct the excision repair deficiency (Mudgett and MacInnes, 1990; Takahashi et al., 1992). During the NER pathway, the XPG has a role of making one of the incisions required to excise a damaged oligonucleotide through cleaving 3' to DNA damaged site, and it also stabilizes the DNA repair complex to damaged DNA (Friedberg, 2003). Previous studies showed enhanced NER activity to the resistance of platinum agents and diminished NER activity to the sensitivity of platinum agents. Previous studies have indicated XPG may have a role on the response to chemotherapy in various cancers, such as colorectal cancer, lung cancer and ovarian cancer (Walsh et al., 2008; Chen et al., 2009; Liu et al., 2012). A previous study has shown that those carrying XPG rs1047768TT are more likely to have poor responder than CC genotype (Liu et al., 2012). Our study also found XPG rs1057768 TT had a significantly poor treatment response when compared with the CC genotype.

Up to now, only three studies have reported that XPG promoter polymorphisms contribute to clinical outcome of NSCLC patients receiving platinum-based chemotherapy (He et al., 2012; Liu et al., 2012; Zhang et al., 2012). However, the results are inconsistence. He reported that XPG rs751402 AA genotype increased the chemotherapy response to advanced NSCLC (He et al., 2012). Liu reported polymorphism in XPG rs1047768 C/T was associated susceptibility of chemotherapy (Liu et al., 2012). Zhang reported that XPG rs17655 C/G in the NER pathway was correlated with toxicity treated with chemotherapy (Zhang et al., 2012). However, our study showed XPG rs1047768 C/T and rs2296147 T/T could be a predictive marker of chemotherapy treatment for advanced NSCLC patients. The possible inconsistence of these results may be explained by different backgrounds of cases, controls selection, sample size and etc. Therefore, large sample size studies are greatly needed to validate their association.

In conclusion, we found that the polymorphism in XPG rs1047768 C/T and rs2296147 C/T are associated with response to platinum-based chemotherapy in NSCLC, and XPG polymorphisms could be a predictive marker for the prognosis of advanced NSCLC. Our study provides significant information on prognostic value of XPG SNPs, and detecting of polymorphisms in XPG could be used as predictive markers toward individualizing NSCLC treatment strategies.

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