RESEARCH COMMUNICATION

Predictive Role of ERCC1 and XPD Genetic Polymorphisms in Survival of Chinese Non-small Cell Lung Cancer Patients Receiving Chemotherapy

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Abstract

Aim: There is increasing evidence that ERCC1 and XPD have roles in response to chemotherapy among patients with NSCLC, but the results are conflicting. Therefore, we conducted the present prospective study in a Chinese population. Methods: A total of 632 primary NSCLC patients were included, followed-up from May 2006 to May 2011. Polymorphisms were detected by real time PCR with TaqMan probse, using genomic DNA extracted from peripheral blood samples. The Cox regression model was used to analyze the hazard ratios (HR) for ERCC1 and XPD. Results: The median time of follow-up was 31.6 months. Our results showed the ERCC1 118 T/T(HR=1.65, 95% CI=1.17-2.43) and XPD 751 Gln/ Gln genotypes (HR=1.52, 95%CI=1.04-2.08) were associated with an increased risk of death from NSCLC. Moreover, the ERCC118 T allele and XPD 751 Gln allele genotypes had a more higher risk of death from NSCLC among both ex-smokers and current smokers. Conclusion: In summary, ERCC1 and XPD gene polymorphisms might provide better prognostic predictive information for NSCLC patients in Chinese populations, with smoking possibly interacting with the genotypes.

Keywords: ERCC1 - XPD - polymorphism - non-small cell lung cancer - prognosis - chemotherapy

Asian Pacific J Cancer Prev, **13**, 2583-2586

Introduction

Non-small cell Lung cancer (NSCLC) has been the most common cancer in the world for several decades, and by 2008, there were an estimated 1.61 million new cases, representing 12.7% of all new cancers. It was also the most common cause of death from cancer, with 1.38 million deaths (18.2% of the total) (IARC, 2008). The majority of NSCLC patients have reached the advanced stages of the disease by the time of diagnosis. Chemotherapy, including cisplatin- or platinum-based method, is associated with improvement of survival for advanced NSCLC patients (Bunn et al., 1998; NSCLCAG, 1995; Schille et al., 2002; Evans, 2005). However, the polymorphisms in DNA repair genes could be associated with chemotherapy sensitivity. The excision repair cross-complementing group 1 (ERCC1) and xeroderma pigmentosum group D proteins (XPD) are two main DNA repair genes. Single nucleotide polymorphisms (SNP) of the ERCC1 and XPD may modulate repair capacity and contribute to individual variations in chemotherapy sensitivity.

ERCC1 and XPD belong to the nucleotide excision repair pathway (NER). Preclinical data suggest that the ERCC1 C118T synonymous SNP could influence the mRNA and protein levels of ERCC1, and ERCC1 mRNA is known to be significantly associated with sensitivity to platinum and cisplatin (Olaussen et al., 2006). XPD is reported to have dual functions in cell, including nucleotide excision repair and cell cycle regulation (Chen et al., 2003). There are two non-synonymous SNPs occuring in the XPD gene, including codon 312 and codon 751, and the two mutations are reported to be related to reduce DNA repair capacity and enhance cisplatin sensitivity.

There are evidences about the predictor role of ERCC1 and XPD for response to chemotherapies among patients with NSCLC, however, the clinical data about SNPs and their predictive role in NSCLC are inconclusive. Moreover, although there are many studies regarding the gene polymorphism and prognosis of lung cancer (Kageyama et al., 2011; Yan et al., 2011), the role of the two gene polymorphisms in NSCLC in the Chinese population has not been established. Therefore, we conducted this prospective study in a Chinese population to detect the association between ERCC1 and XPD gene polymorphisms and survival of NSCLC patients treated with chemotherapy.

Materials and Methods

A total of 632 cases were selected from Shengjing Hospital Affiliated to China Medical University, and all patients included in this study had primary NSCLC and were treated with chemotherapy. The chemotherapy

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regimen incuded gemcitabine 1,200 mg/m^2 administered i.v. over 30 min on the first and eighth day, and cisplatin 80 mg/m^2 infused over 60 min given on the first day, with a maximum of six courses. Treatment was discontinued in case of disease progression, major toxicities, or according to the patient's or physician's decision.

The outcome for this study was overall survival, and death from NSCLC or other causes were the end point in the present study. Survival time was calculated from the date of diagnosis to the date of last follow-up from any causes. A total of 632 patients were followed-up May 2006 to May 2011.

Genotyping

The genomic DNA was extracted from blood samples (5 mL) drawn from an antecubital vein before drug administration, and using the QIAamp DNA mini Kit (Qiagen). The ERCC1 C118T, XPD Asp312Asn and XPD Lys751Gln polymorphisms were studies with Taqman probe–based assays using the ABI PRISM 7900HT instrument equipped with the Sequence Detection System version 2.0 software (Applied Biosystems). Forward and reverse primers and probes (Applied Biosystems SNP Genotyping Assays products) were obtained using the File Builder version 1.0 software, on the basis of Genbank database, and the sequences are available upon request. We also performed the genotyping of internal positive control samples, use of no template controls, and use of replicates for 20% samples for quality control.

Data collection

A uniform questionnaire was used for all subjects regarding socio-demographic characteristics, including alcohol consumption, smoking and other potential confounding factors. We will record all patients' telephone number or their relatives to enable our followed-up, and all patients were followed up every one month until death.

Statistical analysis

All analysis was performed by using the STATA statistical package (version 9, STATA, College Station, TX, USA). A univariate Cox's regression analysis was used to assess the association between ERCC1 and XPD gene polymorphism and survival. The primary death of NSCLC was defined as the failure events and the time of survival was the time between diagnosis and death. The cause of death was confirmed by clinical documents. If a patient died of other causes rather than NSCLC, and he would be censored at the date of death. All survival of patients were censored at the time of death. The relative risk [hazard ratio (HR) and 95% CI] was calculated from the Cox regression model for all significant predictors from cancer diagnosis to the endpoint of the study (event). All statistical tests were two sided and differences were taken as significant when the p value was less than 0.05.

Results

By the end of May 2011, a total of 632 consecutive patients were followed-up, and 432 patients were died during the following up period. The median time of follow-up was 31.6 months. The mean age of the enrolled patients were 62.6±3.7 years old. Majority of the patients were males (76.5%). Most of the patients were ever smokers(77.6%) and drinkers (91.2). Patients had stage I, II, III and IV accounted for 3.1%, 39.5%, 24.8% and 32.6%, respectively.

Genotype information

For the ERCC1 C118T polymorphism, the frequencies of T/T, C/T and T/T genotypes were 18.5%, 45.3% and 36.2%, respectively. The allele frequencies of C and T genotype were 41.2% and 58.8%, respectively. In terms of XPD Lys751Gln polymorphism, Lys/Lys had a frequency of 28.9%, whereas the heterozygous Lys/Gln and homozygous Gln/Gln variants had a frequency of 50.7% and 20.4, respectively. The wide-type XPD 312 Asp/Asp variant was found in 36.4% of cases, whereas the heterozygous Asp/Asn and Asn/ Asn variant were

Table 1. Clinical Characteristics of the NSCLC Patients

Characteristics	Patients %	N=632
Age		
Mean age	62.6±3.7	
<40	54	8.6
40-49	73	11.5
50-59	150	23.7
>60	355	56.2
Sex	0	
Male	483	76.5
Female	149	23.5
Smoking status	0	
Non-smokers	134	21.2
Ex-smokers	356	56.4
Current smokers	142	22.4
Alcohol use	0	
Non-drinkers	62	9.8
Ex-drinkers	514	81.4
Current drinkers	56	8.8
Histology of cancer	0	
Adenocarcinoma	463	73.2
Squmous-cell	137	21.6
Large-cell	33	5.2
Stage	0	
I	20	3.1
II	250	39.5
III	157	24.8
IV	206	32.6

Table 2. ERCC1 C118T, XPD Lys751Gln and XPD Asp312Asn Polymorphisms

Genotype	Number of Patients N=632, %	Allele frequencies
ERCC1 C118T		
C/C	117 (18.5)	C 41.2%
C/T	286 (45.3)	T 58.8%
T/T	229 (36.2)	
XPD Lys751Gln		
Lys/Lys	183 (28.9)	Lys 54.3%
Lys/Gln	320 (50.7)	Gln 45.7%
Gln/ Gln	129 (20.4)	
XPD Asp312Asn		
Asp/Asp	230 (36.4)	Asp 59.2%
Asp/Asn	288 (45.6)	Asn 40.8%
Asn/ Asn	114 (18.0)	

Table 3. Cox Proportional Hazard Model for ERCC1 C118T and XPD Lys751Gln Polymorphisms and Survival of NSCLC

Genotype	Number of death N=432	Five years survival(%)	Univariate analysis HR (95% CI)	P	Multivariate analysis HR (95% CI)	P
ERCC1 C118T						
C/C	45(11.0)	48.3	1.0(Ref.)	-	1.0(Ref.)	-
C/T	178(43.1)	33.5	1.30(0.89-1.91)	0.15	1.43(0.93-2.13)	0.11
T/T	189(45.9)	20.9	1.54(1.06-2.27)	<0.05	1.65(1.17-2.43)	<0.05
XPD Lys751Gln						
Lys/Lys	103(25.1)	40.6	1.0(Ref.)	-	1.0(Ref.)	-
Lys/Gln	211(51.2)	31	1.17(0.86-1.59)	0.29	1.25(0.92-1.74)	0.13
Gln/ Gln	98(23.7)	20.6	1.34(0.93-1.93)	0.1	1.52(1.04-2.08)	<0.05
XPD Asp312Asn						
Asp/Asp	139(33.7)	36.7	1.0(Ref.)	-	1.0(Ref.)	-
Asp/Asn	193(46.8)	29.8	1.11(0.83-1.47)	0.47	1.07(0.80-1.45)	0.35
Asn/ Asn	80(19.5)	25.9	1.16(0.81-1.67)	0.41	1.19(0.83-1.74)	0.37

Table 4. Cox Proportional Hazard Model for ERCC1 C118T and XPD Lys751Gln polymorphisms and Survival of NSCLC by Smoking Status and Histological Type

Genotype	Smoking status HR(95% CI)			Histology of cancer HR(95% CI)	
	Non-smokers	Ex-smokers	Current smokers	Adenocarcinoma	Squmous-cell
ERCC1 C118T					
C/C	1.0(Ref.)	1.76(1.14-2.14)	1.89(0.87-3.16)	1.0(Ref.)	0.89(0.74-1.54)
C/T+T/T	1.26(0.87-1.88)	2.17(1.36-3.98)	2.44(1.45-4.21)	1.15(0.81-1.28)	0.84(0.69-1.67)
XPD Lys751Gln					
Lys/Lys	1.0(Ref.)	1.56(1.02-1.87)	1.63(0.89-2.94)	1.0(Ref.)	0.80(0.62-1.33)
Lys/Gln +Gln/ Gln	1.18(0.79-1.89)	1.75(1.09-1.93)	1.85(1.12-3.05)	1.21(0.80-1.78)	1.13(0.77-1.43)

observed in 45.6% and 18.0% of cases, respectively. The allele frequencies of XPD codon 751 and 312 were showed in Table 2.

When the survival time of patients were compared among ERCC1 118, XPD 751 and XPD 321 genotypes, a significant difference was found in the five years survival of patients carrying the ERCC1 T/T(20.9%) and XPD Gln/ Gln genotypes(20.6%) when compared with ERCC1 T/T and XPD 751 Lys/Lys genotypes (Table 3). Individuals with ERCC1 T/T genotypes showed a significantly lower risk of death from NSCLC than C/C genotype (HR=1.65, 95% CI=1.17-2.43). Individuals carrying XPD 751 Gln/ Gln genotype showed significantly longer survival than Lys/Lys genotype and a higher significant hazard ratio (HR=1.52, 95%CI=1.04-2.08).

Further analysis was conducted on the interaction between ERCC1 C118T and XPD Lys751Gln polymorphism with the environmental risk factors, such as smoking status and histology of cancer. The results showed that ERCC1 118 T allele genotype had a higher risk of death from NSCLC among ex-smokers and current smokers, with the HRs (95% CI) of 2.17 (1.36-3.98) and 2.44 (1.45-4.21), respectively. In terms of XPD Lys751Gln polymorphism, we also found a higher risk of death among ex-smokers (HR=1.75, 95% CI=1.09-1.93) and current smokers (HR=1.85, 95% CI=1.12-3.05) with XPD 751 Gln allele genotype.

Discussion

There is increasing evidence that the SNPs in DNA repair genes could change the DNA repair capacity and the activity to chemotherapy, thereafter influence the survival of cancer patients with chemotherapy (Isla et al., 2004; Ryu et al., 2004; Stoehlmacher et al., 2004). Therefore, assessing genetic polymorphisms of DNA repair genes as either predictive or prognostic markers is increasing in current studies. Our study showed that polymorphisms of patients with ERCC1 118 T/T had a shorter survival time than C/C genotype, and patients with XPD 751 Gln/ Gln also had a shorter survival time than Lys/Lys genotype. Moreover, the ERCC118 T allele and XPD 751 Gln/ Gln genotypes had a higher risk of death from NSCLC in ex-smokers and current smokers.

Excision repair cross-complementary group (ERCC1) is the leading enzyme in the process of nucleotide excision repair, and previous study showed the raised ERCC1 mRNA level or protein expression may affect the gene transcription, translation, mRNA stability, protein activity, and the protein activity could play a important role in the toxicity to anticancer-drugs, and then affect the survival of cancer patients. Previous studies showed the polymorphism of ERCC1 is associated with the prognosis of patients receiving chemotherapy in human gastric, cervical, colorectal, non-small cell lung cancer and bone cancer (Metzger et al., 1998; Britten et al., 2000; Shirota et al., 2001; Lord et al., 2002; Rosell et al., 2002; Ren et al., 2010; Krivak et al., 2011; Zhang et al., 2012). In our study, we included 632 Chinese NSCLC patients treated with chemotherapy showed that ERCC1 118 T/T genotype had a significantly lower overall survival time when compared with CC genotype. Moreover, in the multivariable Cox regression, ERCC1 118 T/T genotype could be a predictor for the prognosis of NSCLC patients receiving chemotherapy (HR=1.65, P<0.05). Therefore, the decreased survival in patients with T/T genotype may be due to reduce the chemotherapy drug sensitivity from lower levels of ERCC1 transcription leading to

lower efficient repair of platinum induced DNA adducts. Moreover, we found a interaction between smoking and ERCC1 gene polymorphism. Patients with ERCC1 118 T/T genotype had lower survival time of NSCLC patients with ex-smoking and current smoking compared to non-smoking patients with C/C genotype. The smoking is related to reduce the function of DNA repair and protein activity of ERCC1, therefore, the smoking patients might have higher risk of death from NSCLC.

The XPD gene is absolutely necessary for NER, which is the major pathway for removal of bulky DNA lesions, particularly those induced by cigarette smoking (Chen et al., 2003). XPD plays an integral role in the NER pathway as a part of a transcription complex that mediates transcription of a gene that encodes an essential 5'--3' helicase, whose activity includes unwinding the DNA helix prior to incision and cleavage of platinum-damaged DNA (Evans et al., 1997). The polymorphisms of XPD codon XPD Lys751Gln is reported to be a risk a allele and that patients with Gln allele genotype had a significantly increased risk of developing lung cancer (Kiyohara et al., 2007). Previous studies on the association between XPD Lys751Gln and NSCLC are inconclusive (Lance et al., 2011). The differences in the results of XPD gene polymorphism might be due to country of origin, types of regimen used as first-line chemotherapy, sample sizes, study design and clinical management.

In summary, the present study based on the analysis of ERCC1 and XPD gene polymorphisms shows that ERCC1 118 T/T and XPD 751 Gln/ Gln genotypes might be association with lower survival of NSCLC patients, and smoking might be interactive with the two genes. Further studies are needed to validate the results of our study in Chinese population.

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