RESEARCH ARTICLE

Common Variations of DNA Repair Genes are Associated with Response to Platinum-based Chemotherapy in NSCLCs

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Abstract

Aim: Individual differences in chemosensitivity and clinical outcome of non-small-cell lung cancer (NSCLC) patients may be induced by host inherited factors. We investigated the impact of XPD Arg156Arg, XPD Asp312Asn, XPD Asp711Asp and XPD Lys751Gln gene polymorphisms on the efficacy of platinum-based chemotherapy in NSCLC patients. Methods: A total of 496 were consecutively selected from the Affiliated Hospital of Nantong University between Jan. 2003 and Nov. 2006, and all patients were followed-up until Nov. 2011. The genotyping of XPD Arg156Arg, XPD Asp312Asn, XPD Asp711Asp and XPD Lys751Gln was conducted by duplex polymerase-chain-reaction with the confronting-two-pair primer methods. Results: Individuals with XPD 312 C/T+T/T and XPD 711 C/T+T/T exhibited poor responses to chemotherapy when compared with the wild-type genotype, with adjusted ORs(95% CI) of 0.67(0.38-0.97) and 0.54(0.35-0.96), respectively. Cox regression showed the median PFS and OS of patients of XPD 312 C/T+T/T genotype and XPD 711 C/T+T/T genotype to be significantly lower than those with wild-type homozygous genotype. Conclusion: We found polymorphisms in XPD to be associated with response to platinum-based chemotherapy in NSCLC, and our findings provide information for therapeutic decisions for individualized therapy.

Keywords: Xeroderma pigmentosum group D - polymorphism - non-small cell lung cancer - chemotherapy - response

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Introduction

Lung cancer is one of the most common cancers worldwide and the leading cause of cancer death in China and many other countries of the world, which is classified as small-cell lung cancer and non-small-cell lung cancer (NSCLC), with the latter accounting for 80% of primary lung cancers (IARC, 2008). Approximately more than 65% of NSCLC are diagnosed in advanced stages due to the asymptomatic nature of early disease and lack of effective screening modalities (William et al., 2009). Platinum-based regimens are regarded as standard first-line chemotherapy in NSCLC patients, and the positive response rate is about 40% with a one-year survival rate of 30%-40% (Schiller et al., 2002).

Platinum agents are used for chemotherapy cause DNA damage and cell death by activating the cell signaling pathways (Azuma et al., 2007), the host cellular DNA repair capacity may influence the outcome of NSCLC (Gurubhagavatula et al., 2004; Lord et al., 2002).

Efficiency of DNA damage repair systems is considered to be one of the most important mechanisms affecting interindividuals differences in response to chemotherapy and clinical outcome of patients. Nucleotide excision repair (NER) is the major pathway for repair of platinum-induced DNA cross-links in mammalian cells (Wu et al., 2005). The Xeroderma pigmentosum group D (XPD) gene encodes for an ATP-dependent helicase, which mediates DNA unwinding for the initiation of NER (Spitz et al., 2001). It is reported that XPD polymorphisms have an important role on DNA repair capacity possibly by altering the function of protein product (Lunn et al., 2000; Wolfe et al., 2007). Common variants in the XPD gene are found to be correlated with various cancer risk (Duan et al., 2012; Mi et al., 2012; Zhang et al., 2012), and it is also reported that the XPD gene polymorphisms are significantly associated with chemotherapy effect of cancer (Provencio et al., 2012; Zhang et al., 2012). However, few studies explored the association of polymorphisms of XPD Arg156Arg and XPD Asp711Asp with the clinical outcomes of NSCLC patients.

Based on these observations, we aimed to investigate the polymorphisms of four genes, XPD Arg156Arg, XPD Asp312Asn, XPD Asp711Asp and XPD Lys751Gln, on the prognosis of NSCLC. In our study, we used Sequenom MassARRAY platform to assess the association between polymorphisms in the four XPD genes and the response and survival of NSCLC patients.

Materials and Methods

Patient recruitment

A total of 496 cases with histologically confirmed stage III and IV NSCLC were consecutively selected from the Affiliated Hospital of Huaihe Hospital between Jan. 2003 to Nov. 2006. All hospital patients with newly

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diagnosed primary NSCLC were asked to participate within one month after diagnosis, and all cases were histopathologically confirmed. The eligible criteria of all patients were ECOG performance score 0~2, good renal, hepatic and cardiac function, as well as no other significant co-morbidities. Patients who had a prior history of cancer, and received previous chemotherapy, radiotherapy or surgery were excluded.

Before treatment, all patients were recorded the demographic and clinical characteristics, such as age, sex, and tumor histology by doctors and nurses. All patients were followed up to Jan. 2011. The survival data were collected by follow-up calls, and inpatient and outpatient clinical medical records.

All patients received the first-line platinum-based chemotherapy. The chemotherapeutic regimens were as follows: navelbine dosage 25 mg/m^2 on day 1 and 8 plus cisplatin dosage 75 mg/m^2 or carboplatin dosage AUC 5 on day 1; gemicitabine dosage 1,250 mg/m^2 on day 1 and 8 plus cisplatin dosage 75 mg/m^2 or carboplatin dosage AUC 5 on day 1; Taxol dosage 175 mg/m^2 plus cisplatin dosage 75 mg/m^2 or carboplatin dosage AUC 5 on day 1; docetaxel 75 mg/m^2 plus cisplatin dosage 75 mg/m^2 on day 1. If patients presented progressive disease or experienced unacceptable toxicity, the treatment would be stopped. If patients showed Grade 3 non-haematology toxicity and Grade 4 haematology toxicity, febrile neutropenia or infection and/or thrombocytopenia associated with bleeding, the dose of cytotoxic agents in the next cycle would be reduced by 25%.

Genotyping

Blood samples were collected from all cases. Genomic DNA was extracted using a Qiagen Blood Kit (Qiagen, Chastworth, CA) according to the manufacturer's protocol. XPD Arg156Arg, XPD Asp312Asn, XPD Asp711Asp and XPD Lys751Gln polymorphisms was determined in a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). Primers for polymerase chain reaction (PCR) amplification and single base extension (SBE) assays were designed by Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA) according to the manufacturer's instructions. The PCR was performed with 5 ng of genomic DNA in a reaction volume of 5 μL using GeneAmp® PCR System 9700 with Dual 384-Well Sample Block Module (Applied Biosystems, Carlsbad, CA, USA). For quality control, genotyping was performed without knowledge of the case/control status of the subjects, and a random sample of 5% of cases and controls was genotyped again by different researchers.

Statistical analysis

All statistical analyses were performed by Stata 8.0 (StataCorp, College Station, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were shown as frequencies and percentages. Patients achieving complete response (CR) or partial response (PR) were defined as ''responders'', and patients with stable disease (SD) or progressive disease were defined as ''non-responders''. We analyzed the association of polymorphisms of genotypes with response to chemotherapy by odds ratios (OR) and a corresponding 95% confidence interval (CI). Overall survival (OS) was evaluated from date of diagnosis to the time of death. Progression-free survival (PFS) was evaluated from the date of diagnosis to the time of progression or death without progression. Multivariate Cox proportional hazards models were used to estimated hazard ratios (HR) with 95% confidence intervals (95% CI) of XPD Arg156Arg, XPD Asp312Asn, XPD Asp711Asp and XPD Lys751Gln for NSCLC. The XPD Arg156Arg, XPD Asp312Asn, XPD Asp711Asp and XPD Lys751Gln polymorphisms were modeled in a dominant model due to the little number of homozygous variant genotypes. All comparisons were two-sided, and $p < 0.05$ was regarded as statistically significant.

Results

Patients' characteristics

The demographic and clinical features are presented in Table 1. The median age was 63.12±7.92 years old (33-79 years old), and 324(65.25%) patients were male. Considering the histopathological type, 304 patients (60.59%) were diagnosed with adenocarcinoma,

Table 1. Characteristics of NSCLC Patients

Patients characteristics	Number of Patients	%
Mean age (year)	63.12±7.92	
<60	86	17.32
60-75	309	62.34
>75	101	20.34
Gender	0	
Male	324	65.25
Female	172	34.75
Histology	0	
Adenocarcinoma	304	61.22
Squamous-cell carcinoma	139	28.08
Other	53	10.7
TMN stage	0	
IIIA	34	6.91
IIIB	161	32.5
IV	301	60.59
Smoking status	0	
<40 pack/year	234	47.12
≥40 pack/year	262	52.88

Table 2. XRCC1 and XPD Polymorphisms and Response to NSCLC

Genotypes	Responders		Non-responders		P value	OR(95% CI)[1]
	N=157	%	N=339	%		
XPD Arg156Arg						
C/C	66	41.89	144	42.58		-
C/A+A/A	91	58.11	195	57.42	0.93	1.02(0.68-1.52)
XPD Asp312Asn						
C/C	108	68.85	208	61.25		-
C/T+T/T	49	31.15	131	38.75	0.11	0.67(0.38-0.97)
XPD Asp711Asp						
C/C	130	82.65	250	73.84		-
C/T+T/T	27	17.35	89	26.16	<0.05	0.54(0.35-0.96)
XPD Lys751Gln						
C/C	134	85.32	272	80.35		-
C/G+G/G	23	14.68	67	19.65	0.17	0.67(0.35-1.09)

[1]Adjusted for sex, age, histological stage, TMN stage and smoking status

Table 3. Association Between XRCC1 and XPD Gene Polymorphisms and Survival of NSCLC

Polymorphisms	Patients	%	Progression-free survival			Overall survival		
			Median survival, mo(95% CI)	Log-rank *P*	HR (95% CI)[1]	Median survival, mo(95% CI)	Log-rank *P*	HR (95% CI)[1]
XPD Arg156Arg								
C/C	210	42.34%	8.82(3.74-16.75)		-	23.41(13.22-28.56)		-
C/A+A/A	286	57.66%	8.15(3.62-15.76)	0.76	1.32(0.64-1.92)	22.30(11.43-27.95)	0.63	1.24(0.82-1.89)
XPD Asp312Asn								
C/C	316	63.71%	9.54(4.13-16.46)		-	27.55(17.42-32.55)		-
C/T+T/T	180	36.29%	8.07(3.45-14.57)	0.32	1.27(0.68-1.84)	19.41(12.34-24.72)	<0.05	1.85(1.12-3.15)
XPD Asp711Asp								
C/C	380	76.61%	10.65(4.52-17.74)		-	26.89(15.87-33.21)		-
C/T+T/T	116	23.39%	7.67(3.06-15.34)	<0.05	2.06(1.15-3.16)	18.90(12.04-24.87)	<0.05	1.76(1.23-2.98)
XPD Lys751Gln								
C/C	406	81.85%	9.32(4.06-16.20)		-	23.65(18.75-26.54)		-
C/G+G/G	90	18.15%	8.15(3.15-15.23)	0.55	1.42(0.73-1.82)	20.54(18.85-24.50)	0.24	1.34(0.79-1.75)

[1]Adjusted for sex, age, histological stage, TMN stage and smoking status

139(28.08%) with squamous-cell carcinoma and the remaining 53(10.7%) with other histology. 34 patients (6.91%) had stage IIIA, 161(32.5%) had stage IIIB and 301(60.59%) had stage IV.

Association between XPD polymorphisms and clinical outcomes

In Table 2, we found XPD 312 C/T+T/T was significantly associated with poor response to chemotherapy (OR=0.67, 95%CI=0.38-0.97). Similarly, we found individuals carrying XPD 711 C/T+T/T genotype presented poor response to chemotherapy when compared with the C/C genotype, and the OR(95% CI) was 0.54(0.35-0.96).

Association between XRCC1 and XPD gene polymorphisms and survival of NSCLC

In Table 3, we found the overall median PFS and OS were 8.15(3.64-15.31) and 21.8(9.75-26.51), respectively. The XPD 711 C/T+T/T was significantly associated with a shorter PFS when compared with C/C genotype, and the HR (95% CI) was 2.06(1.15-3.16). However, we did not find the significant associated with PFS among advanced NSCLC patients who received chemotherapy.

For overall survival, XPD Asp312Asn and XPD Asp711Asp genotypes were associated with OS of advanced NSCLC patients. The median OS time for patients with XPD 312 C/T+T/T genotype was 19.41(12.34-24.72) months, which was significantly lower than those with C/C genotype [27.55(17.42-32.55)], and the HR(95% CI) was 1.85(1.12-3.15). Similarly, the median OS time of patients with XPD 711 C/T+T/T genotype was 18.90(12.04-24.87), which was significantly lower than those with C/C genotype [26.89(15.87-33.21)], and the HR (95% CI) was 1.76(1.23-2.98). However, we did not find the significant association of XPD Arg156Arg and XPD Lys751Gln with OS of advanced NSCLC patients.

Discussion

In the present study, we evaluated whether common XPD polymorphisms would influence clinical outcomes of advanced NSCLC patients treated with platinum-based chemotherapy. We found XPD gene polymorphisms would influence response to chemotherapy and the clinical outcomes of NSCLC patients, and XPD 312 C/T+T/T and XPD 711 C/T+T/T polymorphisms significant contribute to shorter survival time compared with wide-type homozygous genotype.

Currently, the identification of novel genetic variants for evaluating the prognosis of advanced NSCLC is attracting increasing in interest in researches on cancer risk worldwide (Provencio et al., 2012; Zhang et al., 2012). Based on the genetic information, we could determine the genetic factors in terms of prognosis of this disease to be used for identifying the sensitive individuals to chemotherapy and perform targeting therapy to the individual's genetic make-up.

Platinum-based chemotherapy is the first line treatment for NSCLC, and the growing evidences show the inherent factors have a role in modifying the drug response and toxicity of NSCLC patients by metabolism, signaling, DNA-repair and cellular response pathways (Ada et al., 2010; Butkiewicz et al., 2012). XPD is involved in DNA repair, and its expression has been linked to cisplatin resistance, however, the results of previous studies are inconclusive (Wei et al., 2011; Yin et al., 2011). A recent meta-analysis included 17 studies (2097 cancer patients) examining the predictive value of XPD Lys751Gln and XPD Asp312Asn polymorphisms for clinical outcome of NSCLC (Yin et al., 2011), and this meta-analysis indicated XPD Lys751Gln and XPD Asp312Asn was not statistically significantly associated with objective response, PFS and OS in NSCLC patients (Yin et al., 2011). Another meta-analysis included 12 studies indicated the pooled odds ratios were 1.33 (95% CI, 0.92-1.91) and 1.02 (95% CI, 0.72-1.45) for XPD Lys751Gln and XPD Asp312Asn, respectively (Wei et al., 2011), and it showed that the XPD could not be a predictive marker for platinum-based chemotherapy. However, our study suggests the XPD Asp312Asn and XPD Asp711Asp polymorphisms would influence response to chemotherapy and the clinical outcomes of NSCLC patients. Previous study indicated that lymphoblastoid cells carrying XPD 312A/A showed a higher apoptotic response to UV compared with those carrying the wild-type genotype. Moreover, several studies suggested that XPD 751Gln substitutions might produce significant conformational change to the protein (Monaco

et al., 2009), which might induce a small proportion of chromatid aberration and lower risk of suboptimal DNA repair, and thus was related to more efficient DNA repair capacity and poor effect of cytotoxic chemotherapy.

There are several limitations in our study. Firstly, our study was conducted in a single hospital in China, and the samples might not include all the characteristics of patients from other centers. Therefore, our sample may not well represent all the Chinese population in China. Secondly, prognosis of NSCLC might be induced by multiple genetic factors. There might be other genes involved in the prognosis of NSCLC, and thus other genetic factors may be considered in further study. Therefore, further multi-center prospective and large sample size study is strongly needed.

In conclusion, we found the polymorphisms in XPD Asp312Asn and XPD Asp711Asp are associated with prognosis of NSCLC, which suggested that the genes polymorphic status might be used for predictive markers for the prognosis of advanced NSCLC patients with platinum-based chemotherapy. Our find would provide information for therapeutic decisions for individualized therapy in advanced NSCLC patients.

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