RESEARCH ARTICLE

Prognostic Significance of GSTP1, XRCC1 and XRCC3 Polymorphisms in Non-small Cell Lung Cancer Patients

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Abstract

Aim: Individual differences in chemosensitivity and clinical outcome in non-small cell lung cancer (NSCLC) patients treatment with platinum-based chemotherapy may be due to genetic factors. Our study aimed to investigate the prognostic role of GSTP1, XRCC1 and XRCC3 in NSCLC patients treated with chemotherapy. Methods: A total of 460 cases were consecutively selected from The Affiliated Hospital of Nantong University between Jan. 2003 to Nov. 2006, and all were followed-up until Nov. 2011. Genotyping of GSTP1 Ile105Val, XRCC1 Arg194Trp, XRCC1 Arg399Gln and XRCC3 Thr241Met was conducted by duplex polymerase-chain-reaction with confronting-two-pair primer methods. Results: Patients with GSTP Val/Val exhibited a shorter survival time, and had a 1.89 fold greater risk of death than did those with the IIe/IIe genotype. For XRCC1 Arg194Trp, the variant genotype Trp/Trp was significantly associated with a decreased risk of death from NSCLC when compared with the Arg/Arg. Individuals carrying XRCC1 399Gln/Gln genotype had a longer survival time, with a lowered risk of death from NSCLC. Conclusion: This study indicated that GSTP1 Ile105Val, XRCC1 Arg194Trp and XRCC1Arg399Gln genes have a role in modifying the effect of platinum-based chemotherapy for NSCLC patients in a Chinese population. Our findings provide information for therapeutic decisions for individualized therapy in NSCLC cases.

Keywords: GST-pi - XRCC1 - XRCC3 - non-small cell lung cancer - prognosis - chemotherapy

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Introduction

Lung cancer is one of the most common cancer worldwide, and it is reported to the main cause of death from cancer worldwide and with 1.38 million deaths (IARC, 2008). In Chinese population, the lung cancer is the leading death cause in cities (IARC, 2008). It is estimated almost 80% of the lung cancer patients were non-small cell Lung cancer (NSCLC), of which approximately more than 65% are diagnosed in advanced stages due to the asymptomatic nature of early disease and lack of effective screening modalities (William et al., 2009).

Advanced NSCLC patients showed poor prognosis and few effective treatment option. Since curative surgery is not a better treatment for NSCLC, the chemotherapy has become the main treatment measure for advanced NSCLC patients. The first line chemotherapy regimen is platinum-based method with cisplatin or carboplatin (Azzoli et al., 2010). The chemotherapy could improve the survival of NSCLC. However, NSCLC patients with similar clinical characteristics would show different respond to standard platinum-based regimens, which indicated the genetic factors could influence the effect of chemotherapy.

Glutathione S-transferases (GSTs) are phase II metabolic enzymes that are involved in the detoxification of mutagenic and cytotoxic DNA-reactive molecules mediated by glutathione conjugation. It is well known that many drugs used in the chemotherapy are metabolized by the glutathione system (Fuertes et al., 2003). Glutathione S-transferase P1 (GSTP1) is a a subclass of GSTs, which plays a role in detoxification of platinum compounds. This enzyme is also an important mediator of both instrinsic and acquired resistance to platinum (Peklak-Scott et al., 2008). Previous studies show a single nucleotide substitution (A to G) at position 313 induces replacement of isoleucine (IIe) with valine (Val) at codon 105, has been found to modify enzyme activity and affinity for electrophilic substrates (Watson et al., 1998), and previous epidemiologic studies show polymorphism of GSTP1 has a profound impact on chemotherapy for NSCLC, especially for platinum (Yang et al., 2009; Zhou et al., 2011; Joerger et al., 2012).

It also demonstrates that enhanced DNA repair capacity is further critical mechanism of resistance to platinum-based chemotherapy that leads to the removal of cisplatin-DNA adducts. X-ray repair cross complementing protein 1 (XRCC1) and x-ray repair cross complementing

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Table 1. SNP Characteristics and Population

Gene	rs no.	SNP	Codon	Change in amino acid	Forward primer	Reverse primer
GSTP1	rs1695	A/G	105	Val>Isol	5'-ACCCCAGGGCTCTATGGGAA-3'	5'-TGAGGGCACAAGAAGCCCCT-3'
XRCC1	rs1799782	C/T	194	Arg>Trp	5'-GCCCCGTCCCAGGTA-3'	5'-AGCCCCAAGACCCTTTCACT-3'
XRCC1	rs25487	G/A	399	Arg>Gln	5'-TTGTGCTTTCTCTGTGTCCA-3'	5'-TCCTCCAGCCTTTTCTGATA-3'
XRCC3	rs861539	T/C	241	Thr>Met	5'-GGTCGAGTGACAGTCCAAAC-3'	5'-TGCAACGGCTGAGGGTCTT-3'

protein 3 (XRCC3) are two key DNA repaired genes, which could increase cancer risk and significantly alter patient responses to chemotherapy (Butkiewicz et al., 2012; Liao et al., 2012).

Therefore, only analysis of single polymorphism is difficult to explain its role in altering the extent of a physiologic or pathologic phenotype. Due to the limited evidence on DNA repaired genes and GSTP1 gene in NSCLC Chinese population, we conducted a prospective analysis on the role of these genes in the survival of NSCLC treated with platinum-based chemotherapy.

Materials and Methods

A prospective study was conducted. A total of 460 cases were consecutively selected from the Affiliated Hospital of Nantong University between Jan. 2003 to Nov. 2006. All hospital patients with newly diagnosed primary NSCLC were asked to participate within one month after diagnosis, and all cases were histopathologically confirmed. The inclusion criteria request ECOG performance score no greater than 2, good renal, hepatic and cardiac function, as well as absence of other significant co-morbidities.

Patients had a prior history of malignancy, or an already cured tumor, previous chemotherapy, radiotherapy or surgery were excluded.

All the patients received platinum-based chemotherapy as the first line treatment in Table 1. The chemotherapy regimen included cisplatin 75 mg/m^2 on the first day plus gemcitabine 1,250 mg/m^2 on the first and eighth day, docetaxel 75 mg/m^2 on the first day, vinorelbine 25 mg/m^2 on the first and eighth day or paclitaxel 150 mg/m^2 on the first day. All the chemotherapy treatment was intravenously given, and the treatment cycles were repeated every three or four weeks.

Clinical evaluation

All patients were followed every two months until death or the end of the study period. The overall survival was the end point. Survival time was calculated from the date of diagnosis to the date of last follow-up and death from any causes. All the patients were followed up until Nov. 2011. All patients were followed up by telephone.

We collected socio-demographic characteristics of included patients such as smoking and family history of cancer.

Genotyping

All participants provided 5ml blood, and the blood were stored at -20°C. Genomic DNA was extracted using a Qiagen Blood Kit (Qiagen, Chastworth, CA) according to the manufacturer's protocol. The GSTP1 Ile105Val (rs1695), XRCC1 Arg194Trp (rs1799782), XRCC1 Arg399Gln (rs25487) and XRCC3 Thr241Met (rs861539) genotyping was performed by duplex polymerase-chain-reaction with the confronting-two-pair primer (PCR-CTPP) method. The primers of the four SNPs were showed in Table 1. The PCR conditions included initial denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 30 s, 58.5°C for 25 s, and 72°C for 30 s. Final extension was done at 72°C for 5 min.

Statistical analysis

All analyses were performed with SPSS Version 16.0 software (SPSS Inc., Chicago, IL, USA). Demographic and clinical information of patients was compared across genotypes using a chi-square test. The Kaplan-Meier method was adopted to estimate survival curves, and the log-rank test was used to compare patients' survival time between genotype groups. Cox's proportional hazard model was used to assess the associations of GSTP1 Ile105Val, XRCC1 Arg194Trp, XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphisms with survival. Primary death from NSCLC was defined as the failure event, and the time of survival as the time between diagnosis and death. If a patient died of causes other than NSCLC, he was censored at the date of death. All surviving patients were censored at the date of last follow-up. Statistical significance was defined as a two-sided p value of less than 0.05.

Results

Among the 460 cases, 16 cases were lose to follow-up due to emigrating to other cities and changing the phone number. All patients were followed-up until their death and the end of Nov. 2011. The median follow-up of all patients were 28.7 months. During the follow-up, 267 patients died from NSCLC or other diseases.

The basic clinical characteristics of the NSCLC patients were showed in Table 2. The mean age of patients was 55(32-79) years, and almost of the patients were smokers. The stage III and IV accounted for 72.5% of all the patients.

Table 3 showed the association of four SNPs with NSCLC prognosis. Patients with GSTP Val/Val showed a shorter survival time, and had a 1.89 fold risk of death than did those with IIe/IIe genotype (HR=1.89, 95% CI=1.10-3.17) (Figure 1). For XRCC1 Arg194Trp, the variant genotype Trp/Trp was significantly associated with a decreased risk of death from NSCLC when compared with the Arg/Arg, with the HR (95% CI) of 0.45 (0.23-0.87) (Figure 2). Individuals carrying XRCC1 399Gln/Gln genotype had a longer survival time, and lowered the risk of death from NSCLC (HR=0.42, 95% CI=0.21-0.82) (Figure 3). Patients carrying XRCC3 241Met/Met

Table 2. Clinical Characteristics of the NSCLC Patients

Characteristics	Patients N=460	%	Patients death N=267	%	Five-year survival rate (%)	P value
Age						
Mean age(years)	59.5±3.5		64.5±6.7			
Sex						
Male	334	72.6	199	74.7	40.3	0.68
Female	126	27.4	68	25.3	46.4	
Smoking status						
Non-smokers	150	32.7	80	29.8	47.1	0.46
Current smokers	310	67.3	187	70.2	39.5	
Family history of cancer						
Yes	44	9.5	23	8.5	48.1	0.67
No	416	90.5	244	91.5	41.3	
Histology of cancer						
Adenocarcinoma	300	65.3	184	68.9	38.8	<0.05
Squmous-cell	109	23.7	55	20.5	49.8	
Large-cell	51	11	28	10.6	44.1	
Stage						
I	21	4.5	7	2.5	67.8	0.26
II	137	29.7	67	27	51.1	
III	123	26.7	77	27.9	37.2	
IV	180	39.1	116	42.6	35.3	

Table 3. Associations Between Four SNPs and NSCLC Prognosis

Genotype	No. of patients N=460	%	Patient deaths N=267	%	Five-year survival rate (%)	HR (95% CI)[1]
GSTP1 Ile105Val						
IIe/IIe	297	64.5	146	54.5	51	1.0 (Reference)
IIe/Val	121	26.3	83	31.2	31.2	1.39 (0.95-2.03)
Val/Val	42	9.2	38	14.3	9.1	1.89(1.10-3.17)
XRCC1 Arg194Trp						
Arg/Arg	300	65.3	196	73.4	34.7	1.0 (Reference)
Arg/Trp	97	21.1	52	19.4	46.6	1.23 (0.81-1.89)
Trp/Trp	63	13.6	19	7.2	69.5	0.45 (0.23-0.87)
XRCC1Arg399Gln						
Arg/Arg	232	50.4	160	59.9	31.1	1.0 (Reference)
Arg/Gln	177	38.4	92	34.3	48.3	0.76 (0.53-1.07)
Gln/Gln	52	11.2	15	5.8	70.2	0.42 (0.21-0.82)
XRCC3 Thr241Met						
Thr/Thr	196	42.7	127	47.5	35.3	1.0 (Reference)
Thr/Met	199	43.3	111	41.7	44.1	0.86 (0.61-1.22)
Met/Met	64	14	29	10.8	54.9	0.71 (0.40-1.21)

were more likely to have lower risk of death compared with those who carry the Thr/Thr genotype, however, no significant association was found for this genotype.

Discussion

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). In our study, we found the GSTP1 Ile105Val, XRCC1 Arg194Trp and XRCC1Arg399Gln genes were related to the metabolism of platinum-based chemotherapy, and these three SNPs could be used as predictors of clinical outcome of NSCLC patients with chemotherapy. Moreover, we found GSTP1 Val/Val genotype was strong association with increasing the risk of death from NSCLC, while decreased risk of death was found in XRCC1 194Trp/Trp and 399Gln/Gln genotypes.

Glutathione S-transferases (GSTs) are crucial to the cell defence system, and the phase II detoxification enzymes are reported to have a role of detoxification of a variety of chemotherapeutics. GSTP1, located on chronmosome 11q13 in humans, is expressed in many human epithelial tissues and is the most abundant GST isoform in the lung. Single nucleotide substitution (A to G) at position 313 is one of the common single nucleotide polymorphisms in GSTP1. Previous experimental studies states the variant polymorphisms of this gene contribute to alleviating enzymatic activity, and the heterozygote GSTP1 expression decreases the sensitivity to platinum agents and increases the risk of low effective chemotherapy (Oguri et al., 2000). The results of epidemiologic studies on the relationship between GSTP1 and NSCLC are conflicting. Several studies conducted in western countries showed the GSTP1 polymorphism was favorable associated with response to chemotherapy and survival of NSCLC patients (Kalikaki et al., 2009; Ada et al., 2010). In contrary, many studies conducted in Chinese population suggested GSTP1 were associated inversely with response to chemotherapy and survival of NSCLC patients (Sun et al., 2010; Xu et al., 2010; Zhou et al., 2011). Our results indicated that GSTP1 Val/Val genotype was significantly increased platinum-based chemotherapy response and survival of patients, which are consistent with previous reported in Chinese population and explain the alleviating enzymatic activity association with low effective chemotherapy. The inconsistency of these studies may be due to population background, source of patients, disease condition or by chance. Further large sample multi-center studies are needed.

There are increasing evidences that DNA repair genes polymorphisms induce inter-individual variability in platinum inactivation, and that reduced DNA capacity resulting from polymorphisms is associated with survival after treatment with platinum-based chemotherapy in human glioma, bladder and gastric cancer, etc. (Bianchino et al., 2011; Tahara et al., 2011; Zhou et al., 2011; Mittal et al., 2012). In our study, we found XRCC1 Arg399Gln and XRCC1 Arg194Trp influenced clinical outcome in NSCLC patients. The possible cause might be that XRCC1 is thought to be involved in DNA single-strand break repair, and also plays an important role in the BER pathway, the polymorphisms of the two genes might alleviate DNA repair capacity. Thus the capacity of repairing the damaged cancer cell would also be alleviated by the polymorphisms of XRCC1, and the cytotoxic effects of chemotherapy would be enhanced (Zhou et al., 2011). Our study results were in line with two previous studies in China (Xu et al., 2011; Zhou et al., 2011). However, the results from other epidemiologic studies are inconsistence, such as Yao et al. (2009) and Penas et al. (2006) reported no significant association was found between XRCC1 Arg399Gln and NSCLC survival. The possible reason might be that the small sample size of

previous studies limit the power to find their association.

In conclusion, this study conducted in a Chinese population indicated GSTP1 Ile105Val, XRCC1 Arg194Trp and XRCC1Arg399Gln genes have a role in modifying the effect of platinum-based chemotherapy for NSCLC patients. Our find would provide information for therapeutic decisions for individualized therapy in NSCLC.

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