RESEARCH COMMUNICATION

Influence of MDR1 Gene Codon 3435 Polymorphisms on Outcome of Platinum-based Chemotherapy for Advanced Non Small Cell Lung Cancer

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Abstract

Objective: To evaluate the influence of multi-drug resistance 1 (MDR1) gene codon 3435 polymorphisms on response to platinum-based chemotherapeutic regimens for advanced non small cell lung cancer (NSCLC). Methods: Responses and overall survival were evaluated in a series of patients presenting between March 1, 2005 and December 31, 2010. MDR1 gene C3435T polymorphisms were genotyped using peripheral blood with real time polymerase chain reaction (RT-PCR) and relationships between the MDR1 C3435T genetic polymorphismand response rate of chemotherapy were analyzed by SPSS 13.0. Results: Overall response to chemotherapy in the eligible 103 patients was 21.4%. Patients with C/C genotype in MDR1 codon 3435 had a significantly higher response rate (24.5%) than those for C/T(19.0%) and T/T(12.5%) (P<0.05). The overall median survival time (MST) of patients was 19 months, values with C/C, C/T and T/T genotype were 21, 15.5 and 17 months, respectively (P=0.487). Conclusion: Our research suggested that patents with the C/C genotype in MDR1 codon 3435 could be more sensitive to platinum-based chemotherapy than patients with C/T and T/T; however, no significant difference was found between overall survival and MDR1 codon 3435 genetic polymorphisms.

Keywords: Multi-drug resistance - platinum - non small cell lung cancer - chemotherapy - genetic polymorphisms

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Introduction

Mortality of lung cancer is higher than any other cancer type globally (Jemal et al., 2009). Approximately 85% of lung cancer patients would be reclassified as non small cell lung cancer (NSCLC), and 70% these patients could be further diagnosed with advanced NSCLC (Lustberg et al., 2007; Li et al., 2011). Platinum-based doublet chemotherapy was considered as the standard treatment for patients with advanced NSCLC based on several large clinical trials, with longer survival time and better quality of life comparing with other regimens (Grilli et al., 1993; Souquet et al, 1993; Marino et al, 1994; Obasaju et al., 2009).

However, according to previous report, among oriental patients with advanced NSCLC treated with platinum-based chemotherapy, the response rate is around 19%, and many of these patients would experience disease progression (Schiller et al, 2002; Yao et al, 2009; Li et al, 2011). Previous studies suggested that such chemotherapy failure was related to multi-drug resistance (MDR), which might be influenced by MDR1 gene single nucleotide polymorphisms (SNPs) (Gottesman et al, 2002; Illmer et al, 2002; Oselin et al, 2003; Woodahl et al, 2004; Sohn et al., 2006). Among a total of 50 SNPs and 3 insertion/deletion MDR1 gene polymorphisms have been reported in recent years (Ambudkar et al., 2003), codon 3435 genotypes in exon 26 (C3435T) appeared to be the most functional relative, which was the only silent polymorphism identified so far that might influence P-glycoprotein (P-gp) expression in human tissues (Schaeffeler et al., 2001; Jamroziak et al., 2002; Bernal et al., 2003; Wang et al., 2005; Li et al., 2006).

This study was aimed to evaluate response of chemotherapy between MDR1 codon 3435 SNPs and platinum-based chemotherapy for advanced NSCLC.

Materials and Methods

Patients

Eligible patients were aged 18 years or older, with histologically or cytologically confirmed, unresectable locally advanced (stage IIIB with pleural or pericardial effusion) or metastatic NSCLC (stage IV), and were hospitalized at the Department of Chemotherapy of Jiangsu Cancer Hospital and Research Institute. Patients were innocent to chemotherapy, containing any platinum agents (cisplatin or caboplatin). Patients had to have a life expectancy of more than 3 months and a Eastern Cooperative Oncology Group (ECOG) performance

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status (PS) of ≤ 2, and adequate organ function [serum bilirubin ≤ 1.5 times the upper normal limit (UNL); AST and ALT ≤ 2.5 UNL in the absence of perceptible liver metastases, or ≤ 5 UNL in the presence of liver metastases; serum creatinine ≤ 1.5 times the UNL; neutrophils ≥ 1.5 × 10^9/L, and platelets ≥ 100 × 10^9/L]. Patients with known, symptomatic central nervous system metastases were ineligible. Other eligibility criteria were: absence of active infection, history of significant cardiac disease(unstable angina, congestive heart failure, myocardial infarction within the previous 6 months, ventricular arrhythmias) or malnutrition (loss of ≥ 20% of the original body weight). All patients provided written informed consent to participate in the study.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood lymphocytes. The genotypes of MDR1 at codon 3435 were determined by real-time polymerase chain reaction (RT-PCR) based cycling probe method as described previously(Song et al, 2002). The primers were:5'-AGAGAGACTTACATTA GGCAG-3' and 5'-AGTGGCTCCGAGCACACC-3'.The probes were 5'-CCCTCACGATC-3' for allele C and 5'-CCCTCACAATC -3' for allele T.

PCR was performed in a 25 μl reaction mixture containing approximately 1μl DNA template, 5 pmol each primer, 3 mM each dNTP, 5 mM MgCl2, 1 x Cycleave PCR Buffer, 100 U Tli RNase HII,1.25U TaKaRa Ex Taq HS and 5 pmol cycling probe, using TaKaRa Code: DCY501 Cycleave PCR Core Kit. Amplification was carried out under the following conditions: an initial melting step of 20 seconds at 95°C, followed by 45 cycles of 5 seconds at 95°C, 20 seconds at 55°C and 25 seconds at 72 °C, using a Roche Light cycler 1.5.

Response and survival assessment

Response was evaluated according to Response Evaluation Criteria in Solid Tumors, the revised version of the International Union Against Cancer/WHO criteria(World Health Organization, Geneva). Our primary end point was overall survival from the time of histological or cytopathological diagnosis to December 2010, and the second end point was response to chemotherapy between genetic polymorphisms and the treatment regimens. Survival data were obtained from the hospital follow-up team. Records with no reply were followed by local Ministry of Public Security.

Statistical methods

Associations between overall survival and the genetic polymorphisms (or the total number of variant alleles from both polymorphisms) were estimated using method of Kaplan-Meierw with log-rank test. Logistic regression model was used to caculate the odds ratio (OR) of response to chemotherapy among patients with different genetypes, adjusted for gender, age, stage, histology, performance status, and chemotherapy regimens. All statistical analyses were performed using SPSS 13.0, under the condition that alpha=0.05 and statistical power=0.8.

Results

Patients and treatment

One hundred and three patients met the study eligibility and enrolled. Patient characteristics are displayed in Table 1, the majority having an ECOG performance status of 1 and stage IV disease. Of all the 103 patients assessable for chemotherapy response, 22(21.4%) patients had a partial response, 50 (48.5%) had stable, and 31 (30.1%) had progressive disease. The median survival time (MST) was 19 months, 16 months in stage IV patients and 20 months in those with IIIB, respectively.

Genetic polymorphisms and treatment response

Table 2 shows the association of genotypes with treatment response in patients receiving platinum-based chemotherapy. Patients with C/C genotype in MDR1 codon 3435 had a significantly higher response rate (24.5%) than those carried at least one allele T, with C/T(19.0%) and T/T(12.5%) (P<0.05).

Genetic polymorphisms and overall survival

Univariate analysis suggested no association of the MDR1 codon 3435 genotypes with overall survival: the median survival time of individuals with the C/C,

Table 1. Patient Characteristics, Treatment and Genotypes

Characteristics	No.	%
Age		
Median(min-max)	61(39-79)	
Sex		
Male	67	65.0
Female	36	34.9
Histological type		
Adenocarcinoma	72	69.9
squamous-cell	28	27.1
Large-cell	3	2.9
Chemotherapy regimens		
Platinum + Gemcitabine	68	66.0
Platinum + Docetaxel	13	12.6
Platinum + Paclitaxel	17	16.5
Platinum + Vinorelbine	5	4.9
MDR1 3435 genotype		
C/C	53	51.5
C/T	42	41.7
T/T	8	6.8

MDR, multi-drug resistance

Table 2. MDR1 Codon 3435 Polymorphisms and Chemotherapy Response in Advanced NSCLC Analyzed by Logistic Regression

Genotype	CR+PR	SD+PD	P value	OR	95%CI
C/C	13	40	-	1	-
C/T8	34	0.043	1.54	1.01-2.34	
T/T1	7	0.033	1.35	1.03-1.79	
C/T+T/T	9	41	0.013	1.62	1.11-2.37

*Adjusted by gender, age, disease stage, histological type, performance status, and chemotherapy regimen; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; OR, odds ratio; CI, confidence interval; MDR, multi-drug resistance; NSCLC, non small cell lung cancer.

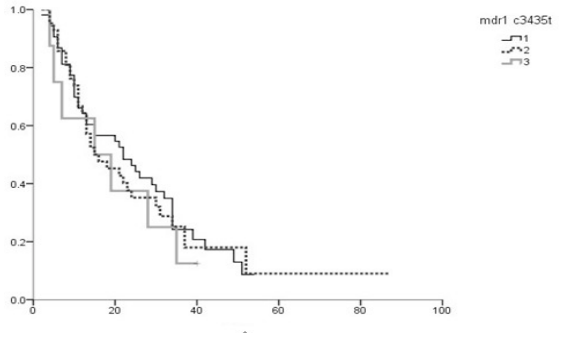

Figure 1. Kaplan-Meier Estimates of the Overall Survival (Months) for NSCLC According Genetic Polymorphisms of Multi-Drug Resistance 1 in Codon 3435

C/T and T/T genotypes were 21, 15.5 and 17 months, respectively. In the Cox proportional hazard model, after adjusted by gender, age, stage, histology performance status and chemotherapy regimen, the odds ratio (OR) for genetic polymorphisms of MDR1 in codon 3435 was 1.13 (95%CI, 0.80-1.59; P=0.487) (Figure 1).

Discussion

Cytotoxic chemotherapy, especially platinum-based doublet chemotherapy continues to be important treatment for advanced NSCLC (Lustberg et al., 2007; Obasaju et al., 2009). Yet individualizing chemotherapy could deliver the most active agent to properly selected patient, and some promising pharmacogenomic predictors have been identified for efficacy and survival in advanced cases treated with platinum-based chemotherapy(Rosell et al,2005). Somehow, from the present results, even though functional polymorphisms in the MDR1 codon 3435 might affect sensitivity to chemotherapy, genotypes itself seemed not play an important role in survival of advanced NSCLC patients.

MDR1, located on chromosome 7q21.1, is composed of 28 exons and encodes a protein of 1 280 amino acids (Klein et al., 1999; Dean et al., 2001; Licinio et al., 2002; Ambudkar et al., 2003). P-gp, the product of MDR1, was an energy-dependent efflux pump and a pivotal member of ATP-binding cassette (ABC) transporters (Sakaeda et al., 2003; Woodahl et al., 2004). P-gp could transport moleculars, from sugars, glycans, phospholipids, amino acids, peptides, proteins, drugs, to toxins out from cells (Licinio et al., 2002). Expression level and functional integrity of P-gp might affect pharmacogenetics and pharmacodynamics of drugs (Sakaeda et al., 2003; Woodahl et al., 2004). Therefore, it played an important role in drug efficacy and toxicity during treatment. High levels of P-gp expression may result in a decrease in intracellular drug concentration, which might lead to multi-drug resistance(Licinio et al., 2002;Sakaeda et al., 2003; Woodahl et al., 2004). It was suggested that individuals carrying T/T at MDR1 codon 3435 might have a significantly higher P-gp expression than those carrying C/C, so that T/T cancer patients might be less sensitive to chemotherapy than C/C patients. Several studies were in line with the conclusion, Pan et al. (2009) reporting that advanced NSCLC patients with C/C genotype areassociated with a better response to docetaxel-cisplatin chemotherapy compared with the combined C/T and T/T genotypes although the difference was not statistically significant. Vinolas et al. (2011) described genetic polymorphisms of MDR1 in codon 3435 to be an important prognostic factor, with C/C genotype associated with lower risk of progression in advanced NSCLC patients treated with cisplatin plus vinorelbine.

Our result also suggested advanced NSCLC patients with C/C to be more chemotherapy sensitive than those with C/T and T/T. However, this difference in chemotherapy sensitivity was not sufficient to change the long term survival. Mechanisms underline patients survival could include factors other than sensitivity to chemotherapy (Maione et al., 2010). The functional or biologic relevance of genetic polymorphisms deserve to be further elucidated.

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