RESEARCH COMMUNICATION

MDR1 Gene C3435T Polymorphism is Associated with Clinical Outcomes in Gastric Cancer Patients Treated with Postoperative Adjuvant Chemotherapy

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Abstract

Objective: To evaluate the impact of the multi-drug resistance 1(MDR1) C3435T polymorphism on clinical outcomes in gastric cancer patients treated with postoperative adjuvant chemotherapy. Methods: From January 2005 to December 2008, 102 patients with surgically resected gastric cancers were enrolled into this study in the Affiliated Jiangsu Cancer Hospital of Nanjing Medical University. The polymorphism was tested using real time polymerase chain reaction (RT-PCR) cycling probes and the relationship with clinical outcomes after postoperative adjuvant chemotherapy was analyzed by SPSS 17.0. Results: The CT/TT genotype of C3435T was significantly associated with a shorter progression-free survival (PFS) and overall survival (OS) compared with the CC genotype [PFS: adjusted hazard ratio(HR)= 2.01, 95% confidence intervals(CI): 1.17-3.45, P = 0.012; OS: adjusted HR = 2.37, 95% CI: 1.31-4.28, P=0.004]. TNM stage was also associated with PFS (adjusted HR = 2.33, 95% CI: 1.34-4.05, P = 0.003) and OS (adjusted HR = 2.62, 95% CI: 1.44-4.76, P = 0.002) in gastric cancer patients treated with postoperative adjuvant chemotherapy. Conclusion: Our results suggest that the MDR1 gene C3435T polymorphism is associated with clinical outcomes in gastric cancer patients treated with postoperative adjuvant chemotherapy. This now needs to be confirmed by a randomized prospectively controlled study.

Keywords: Multi-drug resistance - gastric cancer - adjuvant chemotherapy - genetic polymorphisms - clinical outcome

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Introduction

Gastric cancer is one of the most prevalent malignancies and the second leading cause of cancer-related death in the world (Kim et al., 1993; Parkin, 2001). Surgery is a curative treatment for early gastric cancer. However, even after gastrectomy, the majority of patients develop local or distant recurrence (Macdonald et al., 2004). Postoperative adjuvant chemotherapy has been demonstrated to associate with a significantly improved overall and disease-free survival (OS and DFS). However, the prognosis of the disease remains poor.

One of the main reasons that accounts for resistance to cancer chemotherapy is assumed due to factors that affect the efflux and influx of drugs across cell membrane (Terek et al., 2003; Leonessa and Clarke, 2003). Amongst these, one important factor is P-glycoprotein (P-gp) (Juliano et al., 1976), encoded by the human multi-drug resistance 1 (MDR1) gene and functioning as an energy-dependent membrane efflux pump for a wide range of anti-cancer agents (Ambdukar et al., 1999). P-gp plays an important role in multidrug resistance by impairing the intracellular retention of vinca alkaloids, taxanes, anthracyclines, and topoisomerase inhibitors, etc (Chen et al., 1986; Dean et al., 2001; Stouch et al., 2002). More than 50 single-nucleotide polymorphisms (SNPs) have been reported in MDR1 gene and some of these polymorphisms affect the expression and function of P-gp (Salama et al., 2006; Fromm et al., 2002). Among these SNPs, most studies focused on the C3435T polymorphism that is located in exon 26 (Brinkmann and Eichelbaum, 2001).

However, there is a controversy as to whether MDR1 gene polymorphisms correlate with survival and response in cancer patients treated with chemotherapy. The goal of the present study was to demonstrate the correlation between SNP C3435T of MDR1 gene and clinical outcomes of gastic cancer patients treated with postoperative adjuvant chemotherapy.

Materials and Methods

Patient eligibility

Patients with histologically confirmed gastric cancer were enrolled in this study at the Affiliated Jiangsu Cancer Hospital of Nanjing Medical University. Inclusion criteria included: (1) age ranges from 20 to 79 years old; (2) Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 and a life expectancy over 3

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months; (3) patients without clinically or radiographically measurable disease; (4) Adequate organ function, defined as absolute neutrophil count > 1500/ μL, platelet count >100,000/μ1, and levels of creatinine, liver enzymes and alanine aminotransferase (ALT) less than two times the upper limits of normal (ULN). Patients were ineligible if they had inadequate organ function, were pregnant or breast-feeding. All patients provided written informed consent prior to chemotherapy.

DNA extraction and genotyping

2mL peripheral venous blood sample was collected from all eligible patients with an EDTA tube before chemotherapy. Genomic DNA was extracted from blood lymphocytes. DNA samples were stored at -30°C.

MDR1 gene C3435T polymorphisms were analyzed using real time polymerase chain reaction (RT-PCR) and TaqMan Probe method. The primers were designed on the basis of target gene sequences obtained from GenBank: Forward: 5'-AGAGAGACTTACATTAGGCAG-3' and Reverse: 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 μ1 reaction mixture containing approximately 1μl DNA template, 5 pmol each primer, 0.3 mM each dNTP, 5 mM MgCl2, 1 x Cycleave PCR Buffer, 100 U Tli RNase HII, 1.25U TaKaRa Ex Taq HS, 5 pmol cycling probe and 11.25ul sterile deionized water, using TaKaRa Code DCY501 Cycleave PCR Core Kit. PCR cycling was done with an initial denaturation at 95°C for 20 seconds, followed by 45 cycles of denaturation at 95°C for 5 seconds, annealing at 55°C for 20 seconds, and extension at 72°C for 25 seconds, using a Roche Light cycler 1.5.

Statistical methods

Data analysis was carried out using the Statistical Software Package SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA) to compute all descriptive statistics. The relationship between the genotype frequencies and clinical characteristics were assessed by chi-square test. Progression-free survival (PFS) was defined as the time interval between the date of surgery and the date of disease progression or death. For PFS analyses, patients were censored at the time of clinical contact that disease progression was observed, or died before the cut-off date of follow up.

Overall survival (OS) was calculated from the date of surgery to the date of death or the cut-off date of follow up. The log-rank test and Kaplan-Meier plots were used to evaluate PFS and OS of MDR1 polymorphic genotypes. Cox proportional regression model was used to determine the association of MDR1 genetic polymorphisms and other factors with survival function. The following variates were included in the Cox regression model to conduct multivariate analysis: age, sex, differentiation, TNM stage, and chemotherapy regimens. Hazard ratio (HR) and 95% confidence intervals (95% CI) were calculated to demonstrate the magnitude of risk. For all analyses, p-values at the level of 0.05 were considered statistically significant.

Table 1. Demographic, Clinical, and Pathological Characteristics and MDR1 C3435T Polymorphism in 102 Patients with Gastric Cancer

Patients characteristics	n	MDR1 C3435T polymorphism C/C	C/T+T/T	χ2	P
Age(yr)					
≤55	51	21	30	0.165	0.685
>55	51	19	32		
Gender					
Male	74	28	46	0.215	0.643
Female	28	12	16		
Differentiation					
Well/Mod	47	18	29	0.031	0.861
Poor	55	22	33		
Lymphonode status					
Negative	30	16	14	3.554	0.059
Positive	72	24	48		
TNM stage					
I-II	42	15	27	0.367	0.545
III	60	25	35		
Regimen					
5-FU/LV/OXA	24	9	15	0.062	0.970
PTX/5-FU/OXA	47	19	28		
TXT/5-FU/OXA	31	12	19		

5-FU/LV/OXA, 5-fluorouracil/leucovorin/oxaliplatin; PTX/5-FU/OXA, paclitaxel/5-fluorouracil/oxaliplatin; TXT/5-FU/OXA, docetaxol/5-fluorouracil/ oxaliplatin

Results

Patients and treatment

From January 2005 to December 2008, 102 patients were enrolled in this study. The 102 patients consisted of 28 females (27%) and 74 males (73%), with a median age of 56 years old (range 20 years old to 79 years old). All the patients received radical surgery, and then were treated with at least four cycles of 5-fluorouracil(5-Fu)/ oxaliplatin-based adjuvant treatment, including 24 with 5-FU/leucovorin/oxaliplatin (FOLFOX4: oxaliplatin 85 mg/m2 on day 1 and leucovorin 200 mg/m2 followed on days 1 and 2 by 5-FU 400 mg/m2 intravenous (IV) bolus, then 600 mg/m2 IV over 22-h continuous infusion, and repeated every 2 weeks), 47 with 5-FU/paclitaxel/ oxaliplatin (5-FU 500mg/m2 on days 1-5, paclitaxel 175 mg/m2 on day 1 and oxaliplatin 130 mg/m2 on day 1 and repeated every 3-4 weeks), and 31 with 5-FU/docetaxol/ oxaliplatin (5-FU 500mg/ m2 on days 1-5, docetaxol 75 mg/m2 on day 1 and oxaliplatin 130 mg/m2 on day 1 and repeated every 3-4 weeks). If patients had hematologic toxic effects of grade 3 or grade 4 or nonhematologic toxic effects of grades 2-4 [as defined by the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 2.0], their daily dose was reduced properly.

MDR1 polymorphism

MDR1 genotype was analyzed for all the patients. Their demographic and disease characteristics are shown in Table 1. Of the 102 patients, the frequencies of MDR1 C3435T C/C, C/T and T/T were 39.2% (40/102), 43.1% (44/102) and 17.7% (18/102); and the allele frequencies of C and T were 60.8% and 39.2%, respectively. No significant associations were found between age,

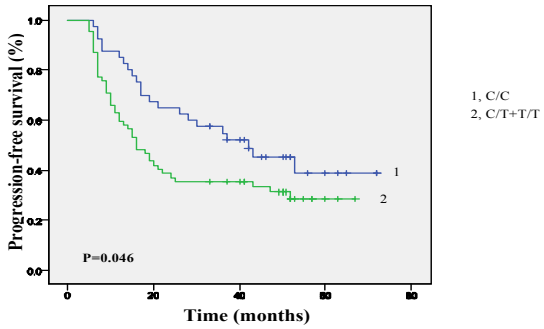

Figure 1. Progression-free Survival Curves for Gastric Cancer Patients According to MDR1 C3435T Polymorphisms

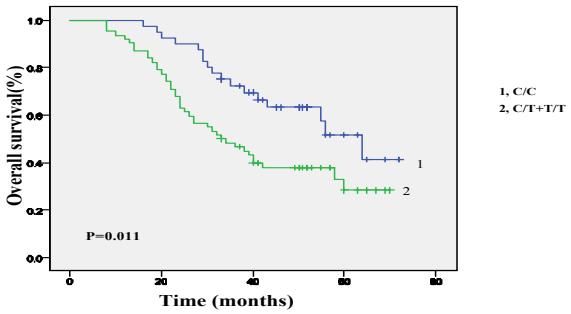

Figure 2. Overall Survival Curves for Gastric Cancer Patients According to MDR1 C3435T Polymorphisms

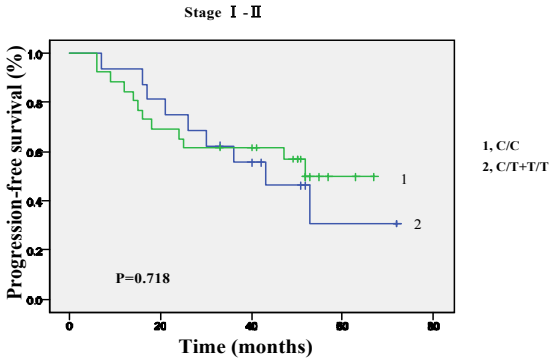

Figure 3. Progression-free Survival Curves for Gastric Cancer Patients of Stage I-II According to MDR1 C3435T Polymorphisms

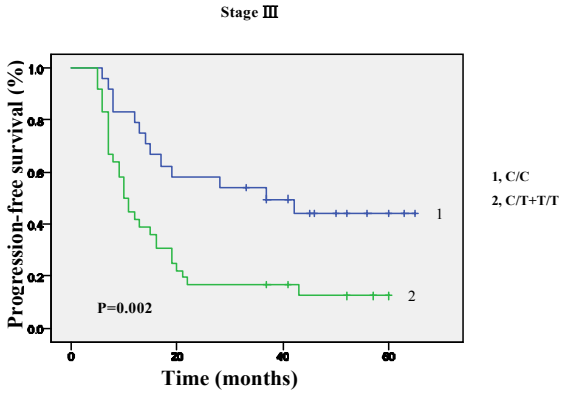

Figure 4. Progression-free Survival Curves for Gastric Cancer Patients of Stage III According to MDR1 C3435T Polymorphisms

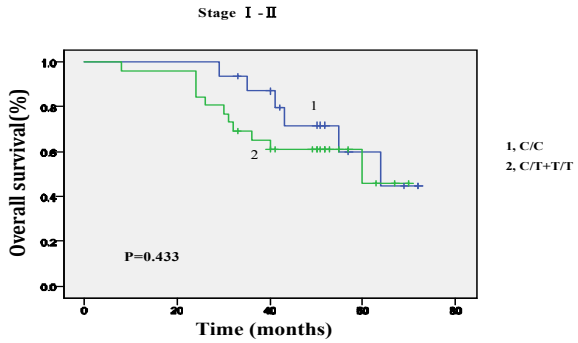

Figure 5. Overall Survival Curves for Gastric Cancer Patients of Stage I-II According to MDR1 C3435T Polymorphisms

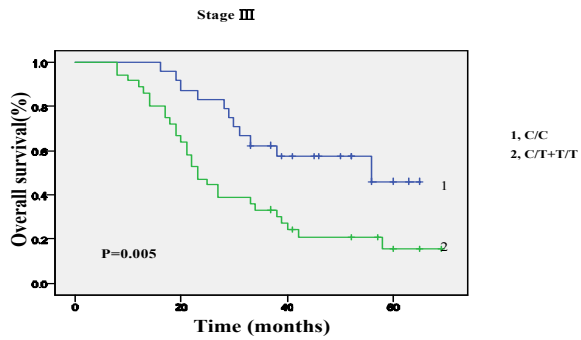

Figure 6. Overall Survival Curves for Gastric Cancer Patients of Stage v According to MDR1 C3435T Polymorphisms

gender, differentiation, lymphonode status, TNM stage or chemotherapy regimens and MDR1 C3435T polymorphisms (Table 1).

Associations between MDR1 C3435T polymorphisms and clinical outcomes

In this study, the median PFS for patients with C/C genotype was 42 months (95% CI = 22.4-61.6), whereas that for patients with C/T + T/T genotypes was 16 months (95% CI =11.2-20.8; $\chi 2$= 3.967, P = 0.046, Figure 1). The median OS was 64 months (95% CI = 50.7-77.3) for the patients with C/C genotype, and 33 months (95% CI = 21.1-44.9) in those with C/T or T/T genotype ($\chi 2$ = 6.407, P = 0.011) (Figure 2).

Stratified by TNM stage, we found that the survival advantage existed in stage III patients (for PFS, P=0.002; for OS, P=0.005) (Figure 4, Figure 6), but not in stage I-II patients (for PFS, P=0.718; for OS, P=0.433) (Figure 3, Figure 5).

Cox multivariate analysis showed that, after adjustment for age, gender, differentiation, TNM stage and chemotherapy regimens, patients with one or two T alleles appeared to be an independent risk factor for PFS (adjusted HR = 2.01, 95% CI: 1.17-3.45, P = 0.012) and OS (adjusted HR = 2.37, 95% CI: 1.31-4.28, P = 0.004), compared to those with C allele (Table 2).

TNM stage was also an independent risk factor for PFS (adjusted HR = 2.33, 95% CI: 1.34-4.05, P = 0.003) and OS (adjusted HR = 2.62, 95% CI: 1.44-4.76, P = 0.002). Age, sex, differentiation and chemotherapy regimens were not associated with PFS and OS in this cohort of patients (Table 2).

Table 2. Cox multivariate Analyses for Prognostic Variables in Patients with Gastric Cancer

Variables	PFS		OS	
	HR(95%CI)	P	HR(95%CI)	P
Age	0.99(0.96-1.02)	0.401	0.98(0.96-1.01)	0.177
Gender	1.19(0.64-2.20)	0.584	1.10(0.57-2.13)	0.776
TNM stage	2.33(1.34-4.05)	0.003	2.62(1.44-4.76)	0.002
Regimen	1.17(0.86-1.59)	0.306	1.14(0.83-1.57)	0.421
MDR1 C3435T	2.01(1.17-3.45)	0.012	2.37(1.31-4.28)	0.004

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; 95%CI, 95% confidence intervals

Discussion

In the present study, we investigated for the first time the association of MDR1 C3435T polymorphisms with the clinical outcome in gastric cancer patients treated with postoperative adjuvant chemotherapy. Our results demonstrated that SNPs in MDR1 C3435T was related to the progression-free survival and overall survival in this cohort of patients, especially those stage III.

Polymorphisms of MDR1 may increase the efflux of chemotherapeutic agents from tumor cells or increase their elimination from the body, resulting in lower plasma concentrations, thereby influencing their therapeutic efficacy. It has been reported that the MDR1 C3435T polymorphisms are associated with gene expression and function, but the results are inconsistent (Hoffmeyer et al., 2000; Johne et al., 2002; Nakamura et al., 2002; Illmer et al., 2002; Gerloff et al., 2002). Hoffmeyer et al (2000) reported that the C3435T polymorphisms were associated with duodenal P-gp levels in Caucasians. Individuals with 3435 TT genotype had significantly lower duodenal MDR1 expression and higher plasma digoxin levels than those with CC genotype. Johne et al (2002) also reported that TT genotype was associated with higher digoxin levels. In contrast to these studies, Nakamura et al (2002) found higher MDR1 mRNA levels in duodenum of healthy Japanese subjects with TT genotype as compared with those with CC genotype. Illmer et al (2002) also reported that CC genotype was associated with lower MDR1 expression in acute myeloid leukemia blast samples. In addition, Gerloff et al (2002) reported no differences in digoxin levels among healthy Caucasian subjects with TT genotype or CC genotype. The reason for all these discrepant results is currently unclear. The possible reasons may include: the different regulation of P-gp expression in different body tissues, the different methods used to measure P-gp expression among different studies (Marzolini et al., 2004; Illmer et al., 2002), the different ethnic backgrounds and geographic pattern of the study subjects, or other unknown factors.

Several studies have shown the effect of MDR1 C3435T polymorphisms on disease outcome, but the results are also inconsistent (Chang et al., 2010; Pan et al., 2009; Pan et al., 2008; Sohn et al., 2006; Illmer et al., 2002; Buda et al., 2007; Jamroziak et al., 2004; Johnatty et al., 2008). In the current study, individuals with 3435CC genotype had a longer PFS and OS compared to those with CT or CC genotype. In agreement with our results, Chang et al (2010) reported that individuals with 3435CC genotype had a longer PFS after paclitaxel-based combined chemotherapy in advanced gastric cancer patients. Similarly, the CC genotype was associated with better chemotherapy response in patients with NSCLC (Pan et al., 2009; Pan et al., 2008) and in patients with SCLC (Sohn et al., 2006). In contrast, the CC genotype was linked significantly with increased risk of relapse in AML patients (Illmer et al., 2002), and the CC genotype was associated with a shorter overall survival in patients with multiple myloma (Buda et al., 2007) and in patients with acute lymphoblastic leukemia (Jamroziak et al., 2004) compared to both CT and TT genotypes. Moreover , Johnatty et al. (2008) found no association between MDR1 C3435T polymorphisms and progression-free or overall survival in ovarian cancer patients treated with taxane/carboplatin. The possible reasons for these controversial results may include variations in the genetic background of the studied population, variable chemotherapy regimens, tumor sites and stage, and the small sample size, or due to other unknown factors. Therefore, at present, it is necessary to confirm whether MDR1 C3435T polymorphism has any prognostic value that could be useful for individualization of cancer chemotherapy.

Our study also showed that TNM stage was associated with PFS and OS in gastric cancer patients treated with postoperative adjuvant chemotherapy. Results of our study showed no significant association between the clinical outcome and patients characteristics such as age, gender, differentiation and chemotherapy regimen. In addition, the distribution of C3435T genotypes was not associated with patients characteristics.

In conclusion, we found that MDR1 C3435T polymorphism is associated with the clinical outcome in gastric cancer patients treated with postoperative adjuvant chemotherapy. Our finding suggests that this polymorphism could be used as genetic markers for predicting clinical outcome of gastric cancer patients treated with postoperative adjuvant chemotherapy, though additional randomized prospectively controlled studies with a larger sample size are required to confirm our results.

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