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Original Article

MDR1 polymorphisms are associated with sensitivity to platinum-based chemotherapy in gastric cancer

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Abstract: Objective: The study aimed to investigate the relationship of multidrug resistance gene 1 (*MDR1*) C1236T, G2677T/A and C3435T polymorphisms with sensitivity to platinum-based chemotherapy in gastric cancer. Methods: 96 patients who had experienced 6 months of platinum-based chemotherapy after the first operation were collected in the study. The genotypes of *MDR1* C1236T, G2677T/A and C3435T loci in peripheral blood from all subjects were tested by matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Haploview 4.2 software was utilized to analyze the linkage disequilibrium and haplotypes of *MDR1* polymorphisms. Odds ratio and 95% confidence interval (CI), calculated by χ^2 test, represent the relation of *MDR1* polymorphisms with sensitivity to chemotherapy. Results: The sensitivity to platinum-based therapy of each patient was evaluated, so the patients were divided into two groups: sensitivity and resistance groups. In the analysis, TT genotype of 1236C/T was significantly correlated with enhanced sensitivity to platinum-based therapy (OR: 0.21, 95% CI: 0.05-0.88). Among the genotypes of G2677T/A polymorphism, TA appeared to increase the sensitivity to the therapy (OR: 7.22, 95% CI: 1.08-48.48). As for C3435T, TT genotype carriers exhibited higher sensitivity to platinum therapy compared to CC (OR: 2.83, 95% CI: 1.05-7.66). Only the haplotypes with frequencies more than 5% were analyzed, in which TTT haplotype showed strong association with sensitivity to platinum therapy (OR: 3.52, 95% CI: 1.07-11.58). Conclusion: *MDR1* C1236T, G2677T/A and C3435T polymorphisms may be related to the sensitivity to platinum-based chemotherapy in gastric cancer.

Keywords: Gastric cancer, platinum, *MDR1*, polymorphisms

Introduction

Gastric cancer is one of the malignant tumors seriously threatening human health and lives [1]. The effect of surgery alone is limited because majority of patients are at the advanced stage when diagnosed. 70% of patients have received radical operation; however, 30%~40% of them will relapse. At the moment, therapeutic effects of chemotherapy are still unsatisfactory even though it becomes increasingly important in combined treatments for gastric cancer. In first-line chemotherapy for advanced gastric cancer, the efficiency of single drug is about 14%~44%, while that of combined treatments is only 30%~50% [2, 3]. Tumors with same histological type exhibit differently in response to the same drug, and multidrug resistance (MDR) is prevalent in tumors, which will impact the chemotherapy effect. To explore the roles of MDR-related genes in drugs response will help us better understand the underlying mechanism of drug resistance and improve the treatments efficiency of tumors.

Multiple drug resistance 1 (*MDR1*) gene is located on the long arm of human chromosome 7 (7q21.1). P-glycoprotein (P-gp), encoded by *MDR1* gene, can transport intracellular drugs outside the cell using energy released by ATP hydrolysis [4, 5]. P-gp-transported substrates include chemotherapy drugs, cardiovascular drugs, immune inhibitors and HIV protease inhibitors [6]. *MDR1* polymorphisms could influence pharmacokinetics of many drugs such as chemotherapy drugs and immune inhibitors [7-10]. The present study was designed to examine C1236T, G2677T/A and C3435T polymorphisms of *MDR1* and to analyze the correlation of them with the sensitivity to platinum-based chemotherapy in gastric cancer, which will contribute to exploring the strategy of per-

Table 1. Association of *MDR1* polymorphisms with sensitivity to platinum therapy

Site	Genotype	Sensitive group CR+PR (n=56)	Resistant group SD+PD (n=40)	X^2	*P*	OR (95% CI)
1236C/T	CC	12 (21.42)	3 (7.50)	-	-	1.00
	CT	27 (48.21)	17 (42.50)	1.734	0.188	0.40 (0.10-1.62)
	TT	17 (30.36)	20 (50.00)	5.018	0.025	0.21 (0.05-0.88)
G2677T/A	GG	3 (5.36)	5 (12.50)	-	-	1.00
	GT	16 (28.57)	14 (35.00)	0.633	0.426	1.90 (0.38-9.44)
	GA	11 (19.64)	8 (20.00)	0.938	0.333	2.29 (0.42-12.50)
	TT	7 (12.50)	5 (12.50)	0.833	0.361	2.33 (0.37-14.61)
	TA	13 (23.21)	3 (7.50)	4.594	0.032	7.22 (1.08-48.48)
	AA	6 (10.71)	5 (12.50)	0.540	0.463	2.00 (0.31-12.84)
C3435T	CC	12 (21.43)	17 (42.50)	-	-	1.00
	CT	18 (32.14)	10 (25.00)	2.998	0.083	2.55 (0.88-7.43)
	TT	26 (46.43)	13 (32.50)	4.314	0.038	2.83 (1.05-7.66)

Table 2. LD analysis between *MDR1* polymorphisms (r^2)

SNP	1236C/T	G2677T/A
1236C/T	1.00	0.42
G2677T/A	0.42	1.00
C3435T	0.67	0.73

sonalized medication, enhancing the specificity and sensitivity of treatments, and further providing new chemotherapy ideas for gastric cancer in clinic.

Materials and methods

Subjects

The subjects were diagnosed by pathology as diffuse-type and intestinal-type gastric cancer. They were all unrelated Han population of Shanghai region, hospitalized in Jinan Central Hospital. In diffuse-type gastric cancer, there were 51 cases, 31 males and 20 females, with a median age of 53.71±11.96, while 45 patients of intestinal-type gastric cancer contained 26 males and 19 females with an average age of 52.31±12.34. All these patients were included according to the following criteria: diagnosed as gastric cancer by pathology tests; experienced no chemotherapy or radiotherapy before surgery, but underwent systematically standard treatments after first surgery in the hospital; treated by platinum-based chemotherapy after surgery. The individuals were excluded if they suffered systemic multiple organ failure, gastric cancer relapse or other malignancies.

Chemotherapy regimens

Patients all received the chemotherapy regimen of FOLFOX4 (L-OHP+5-FU+CF). The specific performance was as follows: L-OHP 130 mg/m^2 was used once per day; 5-FU 750 mg/m^2 was given continuously by intravenous infusion for 48 h once to twice per day; CF 200 mg/m^2 was utilized one to three times per day and repeated every two weeks. The aforementioned process was kept for 4 weeks as one treatment cycle. Rene Titi and Tropisetron were used to symptomatically treat the side effects produced by chemotherapy.

Efficacy evaluation

Efficacy evaluation was conducted after 6 cycles of chemotherapy. According to WHO efficacy evaluation criterion, complete remission (CR) is defined as the complete disappearance of all target lesions along with no new lesion and serum CA125 is less than 35 U/ml; partial remission (PR) refers to the sum reduction of maximum diameter in lesions no less than 30% continuously for 4 weeks, and serum CA125>35 U/ml or less than 50% of original value in diameter; stable disease (SD) means that the sum of maximum diameter in lesions is not reduced to reach that of PR or not increased to that of progressive disease (PD), and serum CA125 expresses no significant increase; PD indicates that the increase in sum maximum diameter of

Table 3. Association of *MDR1* haplotypes with sensitivity to platinum drugs

Haplotype	Sensitive group CR+PR (2 n =112)	Resistant group SD+PD (2 n =80)	χ^2	*P*	OR (95% CI)
CAC	7 (6.25)	8 (10.00)	-	-	1.00
TTT	40 (35.71)	13 (16.25)	4.545	0.033	3.52 (1.07-11.58)
TGC	15 (13.39)	20 (25.00)	0.062	0.804	0.86 (0.25-2.89)
CGC	20 (17.86)	12 (15.00)	1.047	0.306	1.90 (0.55-6.59)

lesions is no less than 20% or new lesions appear, and serum level of CA125 is upregulated. According to the criterion, sensitivity to platinum drugs refers to CR and PR or no progress for more than 6 months after first chemotherapy, while drug resistance means SD and PD after the first chemotherapy or tumor relapses within 6 months.

MDR1 polymorphisms

96 DNA samples were extracted from the anticoagulant whole blood containing 0.4% ethylenediamine tetraacetic acid dipotassium (EDTAK2) using whole blood genome DNA extraction kit (Centrifugal columnar). DNA samples were tested and checked on quality control by agarose gel electrophoresis and gel imaging system. 3 chosen polymorphisms in *MDR1* gene were tested using matrix assisted laser desorption ionizing-time of flight mass spectrometry (MALDI-TOF).

Statistical analysis

Data processing was completed in SPSS18.0 software. Haplotype analysis of *MDR1* polymorphisms were conducted by Haploview 4.2 software. Odds ratio (OR) with 95% confidence interval (CI) represent the association between *MDR1* polymorphisms with sensitivity to platinum-based chemotherapy. x^2 test was adopted to calculate OR and 95% CI. All the tests were two-tailed and $P<0.05$ refers to significant differences.

Results

Correlation of MDR1 polymorphisms with sensitivity to platinum drugs

Table 1 listed the genotypes frequencies of *MDR1* polymorphisms. The frequency of *MDR1* 1236C/T TT genotype was lower in sensitivity group than that of resistant group (30.36% vs. 50.00%, $P<0.05$). OR and 95% CI provided the evidence that 1236C/T might decrease the sensitivity to platinum-based therapy (OR: 0.21, 95% CI: 0.05-0.88). The genotypes of G2677T/A all intended to increase the sensitivity to platinum therapy, while only TA genotype was significantly related with the sensitivity (OR: 7.22, 95% CI: 1.08-48.48). As for C3435T, TT genotype carriers were more likely to experience higher sensitivity to platinum therapy (OR: 2.83, 95% CI: 1.05-7.66).

Haplotype analysis of MDR1 polymorphisms

The haplotypes were analyzed by Haploview 4.2 software, and the outcome demonstrated that SNPs of 1236C/T, G2677T/A and C3435T were found in linkage disequilibrium (LD). The strength of pairwise LD between the SNPs was listed in **Table 2**. Among the 12 haplotypes in Han population, only the frequencies of four haplotypes (CAC, TTT, TGC and CGC) were more than 5%. So our study analyzed the possible relationship of the included haplotypes with sensitivity to platinum therapy (**Table 3**). It turned out that TTT haplotype could increase the sensitivity to platinum therapy (OR: 3.52, 95% CI: 1.07-11.58).

Discussion

Gastric cancer is the most common malignant tumor with high incidence in China. Both of its morbidity and mortality are at top of the list among the malignancies. Prevention and treatment of the cancer has taken priority in medical field. Compared with other malignant tumors, gastric cancer is affected by numerous factors featured by rapid development, so most patients are at advanced stage at the time of first diagnosis. Despite of the systematic treatments, the prognosis of patients in advanced stage is always poor [11-14].

Gastric cancer is regarded as one of the most sensitive tumors to cytotoxic chemotherapy

drugs in solid tumors. Majority of the patients have received standardized platinum-based combined chemotherapy. But more than 75% of patients resistance to platinum chemotherapy drugs experience tumor relapse. It is known that resistance to platinum drugs is caused by multiple factors, including tumor properties and the acquired factors during therapy course. Current studies have shown that drug resistance of tumors is correlated with many molecular pathways, such as strengthened DNA repair, increased drug efflux and inactivation of key molecules [15-17], which can act as relevant factors to predict prognosis of patients in clinic. Getting more knowledge about drug-resistant mechanism and risk factors for the resistance is one of the approaches to develop new drugs and mechanisms avoiding drug resistance.

Single nucleotide polymorphism (SNP) is a common polymorphism form in human inheritance. DNA sequence polymorphisms caused by SNP may bring about individual difference in susceptibility to malignancies. Some studies show that *MDR1* gene possesses several SNPs which are related to the development of malignant tumors, such as breast cancer, leukemia, colorectal cancer, and glioma [18-21]. C1236T, G2677T/A and C3435T are the mostly studied SNPs. Hemauer et al. found that C3435T and G2677T/A were associated with decreased level of P-gp protein, while C1236T is related with increase in P-gp transport activity [22]. Meanwhile, GG genotype of G2677T/A was observed to be related with highest level of *MDR1* and AT was for the lowest level [23]. Low level of *MDR1* was presented in gastric cancer cell lines [24]. In vitro experiment indicted that knockdown of *MDR1* could obviously increase the sensitivity to adriamycin treatment [25]. Further study suggested that P-gp protein behaved as an oncofetal protein in cells of gastric cancer, which could promote cell survival [26]. However, the roles of *MDR1* polymorphisms in the response process to platinum drugs of gastric cancer are still not investigated yet.

In the present study, the sensitivity of 96 gastric cancer patients to platinum-based chemotherapy were examined, and then the connections of *MDR1* C1236T, G2677T/A and C3435T polymorphisms with the sensitivity were analyzed. After the data processing, we found that TT genotype carriers were more likely to suffer drug resistance. On the contrary, G2677T/A TA and C3435T TT genotypes tended to increase the sensitivity to platium therapy, which means that they could relieve the resistance to platium drugs. The different results about the three SNPs may derive from their relationship with *MDR1* or P-gp expression. Further analysis on haplotypes suggested that TGC tended to decrease the sensitivity to platium drugs; however, the result was not statistically significant. TTT haplotype appeared to be an important regulator in drug response process. It could increase the sensitivity to platium therapy.

In conclusion, sensitivity to platinum-based chemotherapy in gastric cancer was found to be correlated not only with *MDR1* polymorphisms (C1236T, G2677T/A and C3435T), but also with the haplotypes formed by them. The research will contribute to rationalized treatments for each individual with specific therapy, thus promotes the treatment efficacy in clinic.

Disclosure of conflict of interest

None.

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References

[1] Murray CJ and Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet 1997; 349: 1269-1276.

[2] Liu QS, Zhang J, Liu M and Dong WG. Lentiviral-mediated miRNA against liver-intestine cadherin suppresses tumor growth and invasiveness of human gastric cancer. Cancer Sci 2010; 101: 1807-1812.

[3] Feng XY, Chen YB, Chen S, Li YF, Zhou ZW, Li W, Zhan YQ, Sun XW, Xu DZ and Guan YX. [Relationship between the number of lymph node detection and prognosis in stage II gastric cancer after D(2) dissection]. Zhonghua Wei Chang Wai Ke Za Zhi 2010; 13: 346-349.

[4] Zinzi L, Capparelli E, Cantore M, Contino M, Leopoldo M and Colabufo NA. Small and Innovative Molecules as New Strategy to Revert MDR. Front Oncol 2014; 4: 2.

[5] Riordan JR, Deuchars K, Kartner N, Alon N, Trent J and Ling V. Amplification of P-gly-

coprotein genes in multidrug-resistant mammalian cell lines. Nature 1985; 316: 817-819.
[6] Schwab M, Eichelbaum M and Fromm MF. Genetic polymorphisms of the human MDR1 drug transporter. Annu Rev Pharmacol Toxicol 2003; 43: 285-307.
[7] Woodahl EL, Crouthamel MH, Bui T, Shen DD and Ho RJ. MDR1 (ABCB1) G1199A (Ser-400Asn) polymorphism alters transepithelial permeability and sensitivity to anticancer agents. Cancer Chemother Pharmacol 2009; 64: 183-188.
[8] McBride BF, Yang T and Roden DM. Influence of the G2677T/C3435T haplotype of MDR1 on P-glycoprotein trafficking and ibutilide-induced block of HERG. Pharmacogenomics J 2009; 9: 194-201.
[9] Ameyaw MM, Regateiro F, Li T, Liu X, Tariq M, Mobarek A, Thornton N, Folayan GO, Githang'a J, Indalo A, Ofori-Adjei D, Price-Evans DA and McLeod HL. MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. Pharmacogenetics 2001; 11: 217-221.
[10] Kroetz DL, Pauli-Magnus C, Hodges LM, Huang CC, Kawamoto M, Johns SJ, Stryke D, Ferrin TE, DeYoung J, Taylor T, Carlson EJ, Herskowitz I, Giacomini KM and Clark AG. Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. Pharmacogenetics 2003; 13: 481-494.
[11] Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, Rodrigues A, Fodor M, Chao Y, Voznyi E, Risse ML and Ajani JA. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. J Clin Oncol 2006; 24: 4991-4997.
[12] Kang YK, Kang WK, Shin DB, Chen J, Xiong J, Wang J, Lichinitser M, Guan Z, Khasanov R, Zheng L, Philco-Salas M, Suarez T, Santamaria J, Forster G and McCloud PI. Capecitabine/cisplatin versus 5-fluorouracil/cisplatin as first-line therapy in patients with advanced gastric cancer: a randomised phase III noninferiority trial. Ann Oncol 2009; 20: 666-673.
[13] Boku N, Yamamoto S, Fukuda H, Shirao K, Doi T, Sawaki A, Koizumi W, Saito H, Yamaguchi K, Takiuchi H, Nasu J and Ohtsu A. Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: a randomised phase 3 study. Lancet Oncol 2009; 10: 1063-1069.
[14] Ohtsu A, Shah MA, Van Cutsem E, Rha SY, Sawaki A, Park SR, Lim HY, Yamada Y, Wu J, Langer B, Starnawski M and Kang YK. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. J Clin Oncol 2011; 29: 3968-3976.
[15] Ye XL, Zhao YR, Weng GB, Chen YC, Wei XN, Shao JP and Ji H. IL-33-induced JNK pathway activation confers gastric cancer chemotherapy resistance. Oncol Rep 2015; 33: 2746-52.
[16] Fu F, Nowak MA and Bonhoeffer S. Spatial heterogeneity in drug concentrations can facilitate the emergence of resistance to cancer therapy. PLoS Comput Biol 2015; 11: e1004142.
[17] Li X, Li Y, Qiu LH, Tong X, Wang QM and Li T. [Expression of excision repair cross-complementation gene in drug-resistant process of carboplatin administration in tongue squamous cell cancer (Tca8113)]. Zhonghua Kou Qiang Yi Xue Za Zhi 2011; 46: 437-441.
[18] Kafka A, Sauer G, Jaeger C, Grundmann R, Kreienberg R, Zeillinger R and Deissler H. Polymorphism C3435T of the MDR-1 gene predicts response to preoperative chemotherapy in locally advanced breast cancer. Int J Oncol 2003; 22: 1117-1121.
[19] Kaya P, Gunduz U, Arpaci F, Ural AU and Guran S. Identification of polymorphisms on the MDR1 gene among Turkish population and their effects on multidrug resistance in acute leukemia patients. Am J Hematol 2005; 80: 26-34.
[20] Humeny A, Rodel F, Rodel C, Sauer R, Fuzesi L, Becker C and Efferth T. MDR1 single nucleotide polymorphism C3435T in normal colorectal tissue and colorectal carcinomas detected by MALDI-TOF mass spectrometry. Anticancer Res 2003; 23: 2735-2740.
[21] Miller KL, Kelsey KT, Wiencke JK, Moghadassi M, Miike R, Liu M and Wrensch M. The C3435T polymorphism of MDR1 and susceptibility to adult glioma. Neuroepidemiology 2005; 25: 85-90.
[22] Hemauer SJ, Nanovskaya TN, Abdel-Rahman SZ, Patrikeeva SL, Hankins GD and Ahmed MS. Modulation of human placental P-glycoprotein expression and activity by MDR1 gene polymorphisms. Biochem Pharmacol 2010; 79: 921-925.
[23] Samanian S, Mahjoubi F, Mahjoubi B, Mirzaee R and Azizi R. MDR1 gene polymorphisms: possible association with its expression and clinicopathology characteristics in colorectal cancer patients. Asian Pac J Cancer Prev 2011; 12: 3141-3145.
[24] Park JG, Kramer BS, Lai SL, Goldstein LJ and Gazdar AF. Chemosensitivity patterns and expression of human multidrug resistance-asso-

ciated MDR1 gene by human gastric and colorectal carcinoma cell lines. J Natl Cancer Inst 1990; 82: 193-198.

[25] Zhu CY, Lv YP, Yan DF and Gao FL. Knockdown of MDR1 increases the sensitivity to adriamycin in drug resistant gastric cancer cells. Asian Pac J Cancer Prev 2013; 14: 6757-6760.

[26] Rocco A, Compare D, Liguori E, Cianflone A, Pirozzi G, Tirino V, Bertoni A, Santoriello M, Garbi C, D'Armiento M, Staibano S and Nardone G. MDR1-P-glycoprotein behaves as an oncofetal protein that promotes cell survival in gastric cancer cells. Lab Invest 2012; 92: 1407-1418.