The Relationship between *MDR1* Polymorphisms and the Response to Etoposide/Cisplatin Combination Chemotherapy in Small Cell Lung Cancer

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소세포폐암에서 *Multidrug Resistance-1* 유전자의 다형성과 Etoposide-cisplatin 항암화학요법 반응의 관계

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배경 및 목적 : *Multidrug Resistance-1* (*MDR1*) 유전자는 다약제내성에 관여하는 P-glycoprotein을 암호화한다. *MDR1* 유전자의 다형성은 P-glycoprotein의 발현과 기능의 차이를 일으켜 항암화학요법 반응에 영향을 미칠 수 있을 것이다. 저자들은 소세포폐암 환자에서 *MDR1* 유전자의 다형성과 일배체형에 따른 항암화학요법에 대한 반응을 조사하였다.
대상 및 방법 : 경북대학병원에서 병리적으로 소세포폐암으로 진단받고 etoposide-cisplatin 항암화학요법을 받은 54명을 대상으로 하였다. 전혈 5cc에서 DNA를 추출하고 PCR-RFLP법을 통해 *MDR1* 유전자 엑손 21의 2677G>T 다형성과, 엑손 26의 3435C>T 다형성을 조사하고 다형성과 일배체형에 따른 항암화학요법의 반응을 조사하였다.
결 과 : 2677G>T 유전자형에 따른 항암화학요법의 반응은 유의한 차이가 없었다. 3435 CC 유전자형은 3435 CT+TT 형에 비해 치료 반응율이 유의하게 높았다 (P = 0.025). 유전자형 분석 결과와 일치되게 2677G/3435C 일배체형은 다른 일배체형에 비해 치료반응을 보이는 경우가 유의하게 많았다 (P = 0.015).
결 론 : 소세포폐암에서 *MDR1* 유전자의 2677G>T와 3435C>T 다형성 및 이들 다형성의 일배체형은 etoposide-cisplatin 항암화학요법의 반응을 예측할 수 있는 지표로 사용될 수 있을 것으로 생각된다.
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Key words : *MDR1*, Polymorphisms, Chemotherapy Response, Small Cell Lung Cancer

Introduction

Lung cancer is one of the major causes of cancer-related deaths worldwide. Small cell lung cancer (SCLC) represents approximately 20% of primary lung cancers, and it is characterized by rapid doubling time, high growth fraction and the early development of widespread metastasis[1,2]. Although chemotherapy is the primary treatment for SCLC, intrinsic or acquired drug resistance is the major limiting factor for the effectiveness of chemotherapy. Resistance to anticancer drugs happens through several mechanisms: decreased drug accumulation, drug inactivation, or enhanced DNA repair[3].

The human *multidrug-resistance (MDR)-1* gene encodes P-glycoprotein (PGP), which functions as an energy-dependent membrane efflux pump for a wide variety of lipophilic compounds. The PGP protein plays an important role in multidrug resistance by impairing the intracellular retention of anticancer drugs such as *Vinca* alkaloids, taxanes, anthracyclines and topoisomerase inhibitors[4-6]. There have been several studies showing that chemotherapy response

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is inversely related with the level of PGP expression in various human cancers including SCLC 7-11, suggesting that variations in the PGP expression level or activity contribute to the therapeutic efficacy of chemotherapy.

Although the mechanism for altered *MDR1* expression has not been clearly elucidated, hypomethylation of the *MDR1* promoter, altered activity of transcription factors, or gene rearrangements have been implicated in *MDR1* regulation[12-14]. Several polymorphisms have been recently reported in the *MDR-1* gene [15], and some of these variants [2677G>T (Ala893Ser) at exon 21 and 3435C>T at exon 26) have been shown to affect the expression and function of PGP [16-18]. Therefore, we have hypothesized that these two variants of *MDR1* gene, and particularly their haplotypes, could influence the response to chemotherapy. To test this hypothesis, we evaluated the association of 2677G>T and 3435C>T polymorphisms and their haplotypes with the response to chemotherapy for SCLC patients treated with a combination chemotherapy of etoposide and cisplatin (EP).

Materials and methods

1. Study population

In the present study, we included 54 SCLC patients who were histologically diagnosed at Kyungpook National University Hospital, Daegu, Korea from January 2002 to June 2003. All these patients underwent complete staging procedures including chest radiograph, CT scan of the thorax and upper abdomen, brain MRI and bone scan. The clinical data for smoking habits, weight loss and Eastern Cooperative Oncology Group performance status (ECOG PS) were collected prospectively. All the patients were received EP combination chemotherapy for more than two cycles as a first therapy. After two or three cycles of chemotherapy, the response to chemotherapy was assessed according the WHO criteria[19]. Patients with a complete response or a partial response were defined as responders, and the patients having stable disease or progressive disease were defined as non-responders.

2. *MDR1* genotyping

Genomic DNA was extracted from peripheral blood lymphocytes by proteinase K digestion and phenol/chloroform extraction. The *MDR1* 2677G>T (Ala893Ser) and 3435C>T (Ile1145Ile) genotypes were determined by PCR-RFLP assay. PCR primers were designed based on the GenBank reference sequence (accession no. M29440). The PCR primers for 2677G>T and 3435C>T polymorphisms were 5'-GGTTCCAGGCTTGCTGTAAT-3' (forward) and 5'-TCACCTTCCCG(mutated A→G)G-3' (reverse); and 5'-GCTGCTTGATGGCAAAGA AA-3' (forward) and 5'-ATTAGGCAGTGACTCG ATGATGA-3' (reverse), respectively. PCR reactions were performed in a 20 $\mu\ell$ reaction volume containing 100 ng of genomic DNA, 10 pM of each primer, 0.2 mM dNTPs, 10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5mM $MgCl_2$, and 1 unit of Taq polymerase (Takara Shuzo Co., Otsu, Shiga, Japan). The PCR cycle conditions consisted of an initial denaturation step at 94℃ for 5 min followed by 35 cycles of 30 s at 94℃; 30 s at 58℃ for 2677G>T and at 56℃ for 3435C>T; 30 s at 72℃; and a final elongation step at 72℃ for 10 min. The PCR products were digested overnight with the appropriate restriction enzymes (New England Biolabs, Beverly, MA, USA; *Ban*I for 2677G>T and *Dpn*II for 3435C>T) at 37℃. The digested PCR products were resolved on 6% acylamide gel. For quality control, the genotyping analysis was repeated twice for all the subjects. To confirm the genotyping results, selected PCR-amplified

DNA samples (n = 2, respectively, for each genotype) were examined by DNA sequencing.

3. Statistical analysis

Chi-square test was used to evaluate the association between clinical variables and chemotherapy response. Hardy-Weinberg equilibrium of alleles at individual loci was tested with a goodness-of-fit x^2 test with one degree of freedom to compare the observed genotype frequencies with the expected genotype frequencies among the subjects. Haplotypes and their frequencies were estimated based on the Bayesian algorithm using the Phase program[20], which is available at http:// www.stat.washington.edu/stephens/phase.html. Logistic regression analysis was performed to examine the association between genotypes/haplotypes and chemotherapy response with adjustment for possible confounders [age as a continuous variable, and sex, staging (limited vs extensive stage) and PS (ECOG 0-1 vs ECOG 2) as nominal variables]. Referent and 3 alternative models (codominant, dominant and recessive for the minor allele) were applied in the analyses. When multiple comparisons are made, the corrected *P*-values (*Pc*-values) were also calculated for multiple testing using Bonferroni's inequality method. All analyses were performed using Statistical Analysis Software for Windows, version 6.12 (SAS institute, Gary, NC, USA).

Results

1. Patient characteristics

The Patients consisted of 46 men and 8 women, and their average age was 61.6 ± 7.9 years. The clinical staging was limited disease (LD) in 28 patients and extensive disease (ED) in 26 patients. The ECOG PS was 0-1 in 35 patients and 2 in 19 patients. The overall response rate was 61% (complete response in 20% and partial response in 41%); 28% of patients had stable disease; and 11% of patients had progressive disease. The overall response rate in the LD group tended to be higher than that in the ED group (75.0% vs 50.0%, P = 0.06), but age, sex and ECOG PS did not affect the response to chemotherapy.

2. *MDR1* genotypes/haplotypes and chemotherapy response

The frequencies of *MDR1* 2677 GG, GT and TT genotypes among the overall cases were 40.7%, 44.4% and 14.8%, respectively. The frequencies of the *MDR1* 3435 CC, CT and TT genotypes among the overall cases were 38.9%, 44.4% and 16.7%, respectively. The genotype distributions of both polymorphisms among the overall cases were in Hardy-Weinberg equilibrium. No significant difference was observed in the genotype distributions of both polymorphisms between the patients with LD and with ED (data not shown).

The distributions of *MDR1* 2677G>T and 3435C>T genotypes among the responders and nonresponders are shown in Table 1. The 2677 GG genotype was more frequent in the responders (47.1%) than in the nonresponders (30.0%), and the 2677 GT and TT genotypes were less frequent in the responders (41.2% and 11.8%, respectively) than in the nonresponders (50.0% and 20.0%, respectively), but these differences were not statistically significant. For the 3435C>T polymorphism, the 3435 CC genotype was associated with a significantly better chemotherapy response compared with the combined 3435 CT and TT genotype (P = 0.025).

The 2677G>T and 3435C>T polymorphisms were in linkage disequilibrium. The frequencies of the four haplotypes (G-C, G-T, T-C and T-T) among

Table 1. *MDR1* 2677GT and 3435CT genotypes and chemotherapy response

		Responders	Non-responders	Adjusteda OR (95% CI)
2677GT	GG	16 (47.1)[b]	6 (30.0)	1.0
	GT	14 (41.2)	10 (50.0)	0.55 (0.15-2.02)
	TT	4 (11.8)	4 (20.0)	0.27 (0.04-2.05)
	GT + TT	18 (52.9)	14 (70.0)	0.43 (0.12-1.51)
3435CT	CC	17 (50.0)	4 (20.0)	1.0
	CT	13 (38.2)	11 (55.0)	0.23 (0.05-1.07)
	TT	4 (11.8)	5 (25.0)	0.04 (0.001-1.14)
	CT + TT	17 (50.0)	16 (80.0)	0.19 (0.04-0.81)[c]

[a] Adjusted for age, sex, stage and performance status.
[b] Numbers in parenthesis is percentage.
[c] P = 0.025.

Table 2. *MDR1* haplotypes and chemotherapy response

Haplotypes	Responders	Non-responders	Adjusteda OR (95% CI)
2677G-3435C	44 (64.7)[b]	16 (40.0)	1.0
2677G-3435T	2 (2.9)	6 (15.0)	0.15 (0.02-0.97)[c]
2677T-3435C	3 (4.4)	3 (7.5)	0.30 (0.04-2.30)
2677T-3435T	19 (27.9)	15 (37.5)	0.44 (0.17-1.11)
2677G-3435C	44 (64.7)	16 (40.0)	1.0
Other haplotypes	24 (35.3)	24 (60.0)	0.34 (0.15-0.82)[d]

[a] Adjusted for age, sex, stage and performance status.
[b] Numbers in parenthesis is percentage.
[c] P = 0.047 and Bonferroni corrected P value = 0.188
[d] P = 0.015.

the overall cases were 55.6%, 7.4%, 5.6% and 31.5%, respectively. The haplotype distribution among the responders was significantly different from the haplotype distribution among the nonresponders (Table 2; G-C, G-T, T-C and T-T haplotype; 64.7%, 2.9%, 4.4% and 27.9% vs 40.0%, 15.0%, 7.5% and 37.5%, respectively; P = 0.03). Patients harboring the 2677G-3435C haplotype had a significantly better response to chemotherapy compared with the chemotherapy response of the other haplotypes combined (P = 0.015).

Discussion

We investigated the association of *MDR1* 2677G>T and 3435C>T polymorphisms and their haplotypes with the chemotherapy response in SCLC patients treated with a combination chemotherapy of EP. In the present study, the patients harboring the 2677G-3435C haplotype responded to chemotherapy significantly better than those patients with other haplotypes. These results suggest that the *MDR1* 2677G>T and 3435C>T polymorphisms and their haplotype could be used to predict treatment response to etoposide-based chemotherapy.

As was stated in previous studies[21,22], we also found that the 2677G>T and 3435C>T polymorphisms were in linkage disequilibrium. However, the allele frequencies of both polymorphisms among healthy Koreans (n = 371, the same subjects used as healthy controls in our previous study:Ref 23) differed from other ethnic populations. The frequency of the 2677T allele among healthy Koreans was 0.34, which was lower than the allele frequency in Chinese and Caucasians (0.44-0.50; and 0.38-0.46, respectively; Ref. 15). The frequency of 3435T allele among healthy Koreans was 0.36, which was also lower than those in Chinese and Caucasians (0.40-0.54; and 0.46-0.57, respectively; Ref. 15) but higher than

that in Africans (0.10-0.26; Ref. 15).

Several studies have reported that the *MDR1* 2677G>T and 3435C>T polymorphisms are associated with gene expression and function, but the results are inconsistent. Hoffmeyer *et al.*[16]reported that the 3435C>T polymorphism was associated with duodenal PGP levels in Caucasians. Individuals with the 3435 TT genotype had significantly lower duodenal *MDR1* expression and higher plasma digoxin levels in comparison to individuals with the 3435 CC genotype. Johne *et al.*[24]also reported that the 3435 TT genotype was associated with higher digoxin levels. In contrast to these studies, Gerloff *et al.*[25] reported no differences in digoxin levels among healthy Caucasian subjects carrying the 3435T allele or the 3435C allele. In addition, in a study that quantified *MDR1* mRNA in duodenum, Nakamura *et al.*[26] showed higher MDR1 mRNA levels in healthy Japanese subjects carrying the 3435T allele as compared to subjects with the 3435C allele. The controversy is not just limited to Asian populations: a study performed by Illmer *et al.*[27] found that the 3435 CC genotype was associated with lower *MDR1* expression in acute myeloid leukemia blast samples. Conflicting data of a similar nature have also been reported for the 2677G>T polymorphism[17,28]. Kim *et al.*[17] reported that the 2677T allele was associated with a 2-fold enhanced efflux of digoxin compared to the 2677C allele. In contrast, Tanabe *et al.*[28] reported a non-significant opposite trend for PGP expression in placenta in relation to the 2677G >T polymorphism (GG > GT > TT). In the present study, the 2677 GG genotype and 3435 CC genotype were associated with a better chemotherapy response. In consistent with genotyping analyses, the 2677G-3435C haplotype was significantly associated with a better chemotherapy response. These findings are in agreement with some reports[17,26,27], but are disagree with others[16,24,28]. Although the reason for all these discrepant results is currently unclear, it may be due to either that the regulation of PGP expression may be significantly different in different body tissues or that the methods used to measure PGP expression differed among different studies[15,27]. The variations in genetic backgrounds of the study subjects should also be taken into consideration.

MDR1 polymorphism may have an influence on disease risk, and/or disease progression[29,30]. In the present study, however, the frequencies of the 2677T and 3435T alleles among the SCLC cases were not significantly different from those among healthy Koreans (0.37 vs 0.34, and 0.39 vs 0.36, respectively). Moreover, no significant difference was observed in the genotype distributions of both polymorphisms between the patients with LD and with ED.

In conclusion, we found that the MDR1 2677G>T and 3435C>T polymorphisms and their haplotypes are associated with the response to EP combination chemotherapy in SCLC patients. Our finding suggests that these polymorphisms could be used as genetic markers for predicting treatment response to etoposide-based combination chemotherapy in SCLC patients, although additional studies with a larger sample size are required to confirm our results. Future studies for other *MDR1* sequence variants and their biologic function are also needed to understand the role of *MDR1* polymorphisms in determining the response to chemotherapy. Moreover, since genetic polymorphisms often vary significantly between different ethnic groups, further studies are warranted to clarify the association of *MDR1* polymorphisms with chemotherapy response in diverse ethnic populations.

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