Genetic Variation in the ABCB1 Gene May Lead to mRNA Level Change: Application in Gastric Cancer Cases

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Abstract

Background: One of the major mechanisms for drug resistance is associated with altered anticancer drug transport, mediated by the human-adenosine triphosphate binding cassette (ABC) transporter superfamily proteins. The overexpression of adenosine triphosphate binding cassette, sub-family B, member 1 (ABCB1) by multidrug-resistant cancer cells is a serious impediment to chemotherapy. In our study we have studied the possibility that structural single-nucleotide polymorphisms (SNP) are the mechanism of ABCB1 overexpression.

Materials and Methods: A total of 101 gastric cancer multidrug resistant cases and 100 controls were genotyped with sequence-specific primed PCR (SSP-PCR). Gene expression was evaluated for 70 multidrug resistant cases and 54 controls by real time PCR. The correlation between the two groups was based on secondary structures of RNA predicted by bioinformatics tool. Results: The results of genotyping showed that among 3 studied SNPs, rs28381943 and rs2032586 had significant differences between patient and control groups but there were no differences in the two groups for C3435T. The results of real time PCR showed over-expression of ABCB1 when we compared our data with each of the genotypes in average mode. Prediction of secondary structures in the existence of 2 related SNPs (rs28381943 and rs2032586) showed that the amount of ∆G for original mRNA is higher than the amount of ∆G for the two mentioned SNPs. Conclusions: We have observed that 2 of our studied SNPs (rs283821943 and rs2032586) may elevate the expression of ABCB1 gene, through increase in mRNA stability, while this was not the case for C3435T.

Keywords: Gastric cancer - variation - ABCB1 gene - real time PCR - multidrug resistance (MDR) - over-expression

Introduction

The development of multidrug resistance (MDR) to chemotherapy remains a major challenge in the treatment of cancer (Ullah, 2008; Ren et al., 2012; Zhu et al., 2013). Resistance exists against every effective anticancer drug and can develop by numerous mechanisms including decreased drug uptake, increased drug efflux, activation of detoxifying systems, activation of DNA repair mechanisms, evasion of drug-induced apoptosis, etc. (Sonneveld, 2000; Wu et al., 2014). One of the major mechanisms for drug resistance is associated with altered anticancer drug transport, mediated by members of the ABC transporter superfamily proteins including ABCB1 gene (Golalipour et al., 2007; Oliveira et al., 2014).

ABCB1, the first discovered ABC transporter, is expressed in various tissues to protect them from the adverse effect of toxins (Fung and Gottesman, 2009). The properties ABCB1 expression have been extensively studied in cancer (Juliano and Ling, 1976; Walhner et al., 1993; Sonneveld, 2000; Felipe et al., 2014). Patients with high expression of ABCB1 at diagnosis may be predisposed to mutation development. Furthermore, increasing expression of ABCB1 over time may be contributing to acquiring drug resistance. ABCB1 activation may be an important prognostic marker, and potential target for pharmacological manipulation (Deborah et al., 2006). Different mechanisms proposed for ABCB1 over-expression including Mutation, Aneuploidy (rearrangements and gene amplification) and SNPs (including SNPs in promoter and structural SNPs in RNA) (Baker and El-Osta, 2003; Wang et al., 2006; Mahjoubi et al., 2008; Fung and Gottesman, 2009; Wang et al., 2009). Gene amplification is also described in some cellular models but it seems to be uncommon in clinical species (Roninson et al., 1991; Golalipour et al., 2007).

In the present study we have investigated the possible role of structural SNP in the over-expression of the ABCB1 gene. There is only one report of applying SNPs with the structural view for the ABCB1 gene that potentially have
an effect on performance (Fung and Gottesman, 2009).
As a result of the paucity of SNP polymorphism in the
processing locations in literature, we have assessed SNPs
polymorphism in splicing site, including rs2032586 and
rs2032586. We also included C3435T, located in exon
26, which is commonly used in different studies and has
shown different frequencies of polymorphisms that vary
significantly in different ethnicities (Gow et al., 2008;
Andersen et al., 2009; Fung and Gottesman, 2009).

Materials and Methods

Samples
Seventy fresh peripheral blood samples were collected
from patients with gastric cancers (including esophageal,
colon, stomach and rectum) treated with 5FU and as a
control 100 blood samples were collected from healthy
controls that did not have a history of cancers (10 ml from
each person which, collected in falcon tubes that contained
EDTA). In addition 31 tissue samples were collected
from paraffin embedded tissue blocks of patients with the
mentioned cancers (gastric cancers).

DNA and RNA extraction.
DNA is extracted using the phenol-chloroform method.
The PMBC (Peripheral blood mononuclear cell) was
separated using ficole. The PMBC was then cultured
in an RPMI-1640 medium and cells were stimulated
to divide with PMA and LPS. After 72 hours RNA was
extracted from cultured cells by TRIZOL based method.
The isolated RNA was used to synthesize cDNA using
Fermentase kit of first strand cDNA synthesis.

Genotyping
Genotyping was done with SSP-PCR (Sequence
Specific Primer) for 101 cancer cases and the 100 control
group. Primers for SSP-PCR were designed with primer3
software (Table 1). Human Growth Hormone (HGH)
primers were used as a control to check the results of
SSP-PCR.

ABCB1 mRNA quantification
The amount of gene expression was measured
using Relative Quantitative Real Time PCR (ABI
7300) for all 101 cancer cases and 100 controls. These
primers were used for Real Time PCR: Forward: 5’
GAGCCCATCCTGGTCTACTG 3’ and Reverse: 5’
ACTATAGGCGAGGCTGC 3’ (Song et al., 2002).
18srRNA was used as an internal control.

mRNA level increased in mutated genotype
The results of Real Time PCR showed an over-
expression of ABCB1 when we compared our data with
each genotypes in average mode. The results of the
expression of ABCB1 when we compared our data with
differences between groups and the control
subjects and the control group. For rs2032586 there is
no significant difference in allelic frequencies while
there existed a significant difference in the genotypic
frequencies between two groups.

For the C3435T position we have not seen any
differences between the cancer subjects and the control
group either in the allelic or genotypic frequencies. The
cancer subjects showed significant genotypic frequency
differences for both A/G and G/G genotypes for
rs2032586. Also, for rs28381943 a significant difference
in the G/G genotype is presented. The differences which
were observed in genotypes between tumoral and normal
tissue samples of a person may be because of lack of
control on DNA replication or mutations which occurs in
tumoral development.

Mutated mRNA have lower ∆G compared to original
mRNA

Table 1. Primer Sequences used for the ABCB1 Gene
in Positions C3435T, rs28381943 and rs2032586 for
Sequence-Specific Primer Genotyping Method

| Primer Sequence | Primer Position |
|-----------------|-----------------|
| 5’GTGGTGTGCAGGAAAGAGGTT3’ | C3435T T |
| 5’GTGGTGTGCAGGAAAGAGGTC3’ | C3435T C |
| 5’ACTATAGGCCAGAGGGCTGC3’ | Generic |
| 5’TGATCCATGCTACAGGCTGA3’ | rs28381943 A |
| 5’TGATCCATGCTACAGGCTG3’ | rs28381943 G |
| 5’TTCACCCGCTCTCTTCACGT3’ | Generic |
| 5’GGCCTACCGAGCCTACAGTACCA3’ | rs2032586 A |
| 5’GGCTACCGAGCCTACAGTACCA3’ | rs2032586 G |
| 5’ACTCTAGCTGCTACCCCTGA3’ | Generic |
| 5’GGGCCTCCACACATACCCCTTA3’ | HGH (sense) |
| 5’TCACGGATTCTGTGTTGTCTC-3’ | HGH (antisense) |
ABCB1 mRNA structure $\Delta G$ is -1586.68 kcal/mol. The amount of $\Delta G$ in rs28381943 is -2482.30 kcal/mol. rs28381943 may cause disruption in the splicing process, resulting in the inclusion of intron 19 that is usually deleted through normal splicing. Also rs2032586 may cause disruption in the splicing process, resulting in the inclusion of intron 11 that is usually deleted through normal splicing. The amount of $\Delta G$ is -1634.12 kcal/mol.

| Table 2. The Genotypic and Allelic Frequency Distribution for rs28381943 in ABCB1 Gene in Blood Samples |
|-----------------------------------------------|
| Genotype | Subjects (%) | Control (%) | P   | OR  | 95 CI |%
| A/A      | 35(42.7%)    | 47(57.3%)   | 1   | -   | -    |
| A/G      | 46(49.5%)    | 47(50.5%)   | 0.014 | 3.4057 | 0.089-0.854 |
| G/G      | 20(76.9%)    | 62(23.1%)   | 0.003 | 4.474 | 0.067-0.662 |
| Allele"A" | 116(57.42%) | 141(70.5%)  | 1   | -   | -    |
| Allele"G" | 86(42.57%)  | 59(29.5%)   | 0.007 | 1.7717 | 0.365-0.870 |

OR= odds ratio. CI= 95% Confidence Interval of the odds ratio. P= p-value

| Table 3. The Genotypic and Allelic Frequency Distribution for rs2032586 in ABCB1 Gene in Blood Samples |
|-----------------------------------------------|
| Genotype | Subjects (%) | Control (%) | P   | OR  | 95 CI |%
| A/A      | 61(53%)      | 54(47%)     | 1   | -   | -    |
| A/G      | 22(31%)      | 49(69%)     | 0.001 | 11.878 | 0.015-0.346 |
| G/G      | 16(84.2%)    | 3(15.8%)    | 0.01 | 4.721 | 0.038-0.806 |
| Allele"A" | 144(72.73%) | 157(74.06%) | 1   | -   | -    |
| Allele"G" | 54(27.27%)  | 55(25.94%)  | 0.82 | 0.934 | 0.588-1.484 |

OR= odds ratio. CI= 95% Confidence Interval of the odds ratio. P= p-value

| Table 4. The Genotypic and Allelic Frequency Distribution for C3435T in ABCB1 Gene in Blood Samples |
|-----------------------------------------------|
| Genotype | Subjects (%) | Control (%) | P   | OR  | 95 CI |%
| C/C      | 23(63.9%)    | 13(36.1%)   | 1   | -   | -    |
| C/T      | 56(44.8%)    | 69(55.2%)   | 0.475 | 0.738 | 0.344-1.58 |
| T/T      | 22(52.4%)    | 20(47.6%)   | 0.3618 | 1.608 | 0.038-0.806 |
| Allele"C" | 144(74.67%) | 157(74.06%) | 1   | -   | -    |
| Allele"T" | 54(25.33%)  | 55(25.94%)  | 0.82 | 0.934 | 0.588-1.484 |

OR= odds ratio. CI= 95% Confidence Interval of the odds ratio. P= p-value

| Table 5. The Genotypic and Allelic Frequency Distribution for C3435T in ABCB1 Gene in Blood Samples |
|-----------------------------------------------|
| Genotype | Subjects (%) | Control (%) | P   | OR  | 95 CI |%
| C/C      | 4(12.9%)     | 5(15.6%)    | 1   | -   | -    |
| C/T      | 19(61.3%)    | 19(59.4%)   | 0.5288 | 0.8 | 0.344-1.58 |
| T/T      | 8(25.8%)     | 8(25.0%)    | 0.6168 | 1 | 0.264-3.782 |
| Allele"C" | 27(43.55%)   | 29(45.31%)  | 1   | -   | -    |
| Allele"T" | 35(56.45%)   | 35(54.69%)  | 0.492 | 0.931 | 0.434-1.995 |

OR= odds ratio. CI= 95% Confidence Interval of the odds ratio. P= p-value

| Table 6. The Genotypic and Allelic Frequency Distribution for rs2032586 in ABCB1 Gene in Blood Samples |
|-----------------------------------------------|
| Genotype | Subjects (%) | Control (%) | P   | OR  | 95 CI |%
| A/A      | 4(13.8%)     | 5(16.7%)    | 1   | -   | -    |
| A/G      | 15(51.7%)    | 18(60.0%)   | 0.6286 | 0.96 | 0.159-5.395 |
| G/G      | 10(34.5%)    | 7(23.3%)    | 0.2756 | 0.583 | 0.149-2.216 |
| Allele"A" | 23(38.0%)    | 28(46.66%)  | 1   | -   | -    |
| Allele"G" | 35(62.0%)    | 32(53.34%)  | 0.28 | 0.751 | 0.338-1.660 |

OR= odds ratio. CI= 95% Confidence Interval of the odds ratio. P= p-value

| Table 7. The Genotypic and Allelic Frequency Distribution for rs28381943 in ABCB1 Gene in Blood Samples |
|-----------------------------------------------|
| Genotype | Subjects (%) | Control (%) | P   | OR  | 95 CI |%
| A/A/A    | 11(35.5%)    | 11(34.4%)   | 1   | -   | -    |
| A/A/G    | 14(45.2%)    | 11(34.4%)   | 0.4528 | 0.785 | 0.213-2.882 |
| G/G/G    | 6(19.4%)     | 10(31.2%)   | 0.202 | 2.121 | 0.496-9.410 |
| Allele"A" | 36(58.06%)   | 33(51.56%)  | 1   | -   | -    |
| Allele"G" | 26(41.94%)   | 31(48.44%)  | 0.289 | 1.3 | 0.607-2.791 |

OR= odds ratio. CI= 95% Confidence Interval of the odds ratio. P= p-value
We would like to thank Research Council of Golestan University of Medical Sciences for funding and a great thank from Dr. Habib Fakhrai the Chief Scientific Officer of NOVARX corporation- USA, Oncology and Shafa department of 5 Azar hospital-Golestan Province in Iran.

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