

RS28381943 SNP of ABCB1 gene may be the reason of mRNA stabilization which may lead to gene overexpression

Mansoori M.¹, Golalipour M., Alizadeh Sh., Jahangirerad A., Khanduzi S.R. and Shahbazi M.^{2 +}

¹ Student of Human Genetics MSc.

².PhD of Cellular and Molecular Biology,

Medical Cellular & Molecular Research Center, Talghani Children Hospital of Golestan University of Medical Sciences, Bolv Janbazan, 4916668197, Gorgan, Iran

ABSTRACT .Introduction. One of the major mechanisms for drug resistance is associated with altered anticancer drug transport, mediated by human-adenosine triphosphate binding cassette (ABC) transporter superfamily proteins. The overexpression of MDR1 by multidrug-resistant cancer cells is a serious impediment to chemotherapy. In our study we have searched if the mechanism, with which the overexpression of MDR1 gene occurs, may be because of the structural SNPs or not.

Materials and Methods. 101 cases and 100 controls have genotyped with SSP-PCR (Sequence Specific Primer) then the gene expression has evaluated for 70 cases and 54 controls by Real Time PCR, for the correlation between them we secondary structures of RNA have predicted by bioinformatics tool (Vienna RNA Fold Server).

Results. The frequency of ABCB1 G/G genotype in cases was 76.9%, significantly higher than 23.1% in healthy controls (p-value: 0.003). While the frequency of ABCB1 A/A and A/G genotypes in healthy controls were 57.3% and 50.5% respectively, which is higher than 42.7% and 49.5% in cases (p-value: 0.014). The results of Real Time PCR have shown overexpression of ABCB1 when our data has compared with each genotypes in average mode. The amount of ΔG for MDR1 mRNA is -1586.68 kcal/mol which is lower than -2482.30 kcal/mol for MDR1 mRNA in the case of rs28381943 SNP existence.

Discussion. We have observed significant differences in genotypes of SNP (rs28381943) that related to overexpression of MDR1 gene.

Keywords:Vienna RNA fold Web server, Secondary structures of RNA prediction, ABCB1, MDR, SNP, Cancer, RNA stability and Overexpression

1. Introduction

The development of multidrug resistance (MDR) to chemotherapy remains a major challenge in the treatment of cancer (1). Several mechanisms for MDR have been identified. One of the major mechanisms for drug resistance is associated with altered anticancer drug transport, mediated by members of the ABC transporter superfamily proteins (2). The overexpression of MDR1 by multidrug-resistant cancer cells is a serious impediment to chemotherapy. MDR1 is expressed in various tissues to protect them from the adverse effect of toxins (3). The precise mechanism of transcriptional regulation of MDR1 has been unclear due to the complex regulatory nature of the gene. There exist different mechanisms for MDR1 overexpression including Mutation, Aneuploidy (including rearrangements and gene amplification) and SNPs (including SNPs in gene promoter and structural SNPs in RNA) (4).

It has been proved that Mutations and Aneuploidy cannot be the reason of MDR1 overexpression in vivo (5). In our study we have searched if the mechanism, with which the overexpression of MDR1 gene occurs, may be because of the structural SNPs or not. If the reason of this overexpression would be because of

⁺ Corresponding author.

E-mail address: shahbazimajid@yahoo.uk.ac.ir.

structural SNPs it may affect the stability of mRNA. When MDR1 mRNA becomes more stable, in the unit of time, it may have more time to become expressed so it may be the reason of overexpression. We have assessed rs28381943 SNP which is located in splicing site.

2. Materials and Methods

101 patient cases and 100 healthy controls have genotyped with SSP-PCR (Sequence Specific Primer) then the gene expression has evaluated for 70 cases and 54 controls by Real Time PCR, for the correlation between them, secondary structures of RNA have predicted by bioinformatics tool (Vienna RNA Fold Server).

DNA and RNA have extracted from blood samples then cDNA has synthesized from RNA. Genotyping have done with SSP-PCR and the amount of gene expression has measured with Relative Quantitative Real Time PCR for 70 blood cases and 55 numbers of controls. To check the effect of mentioned SNP on mRNA structure, the Vienna RNA fold web servers (<http://rna.tbi.univie.ac.at/>) has used to predict the secondary structures of RNA and its folding based on MFE (Minimum Free Energy) and this database has predicted the amount of ΔG for the requested sequence. As we know the lower the amount of ΔG is the higher the amount of RNA stability would be.

Genotyping analysis has done by SPSS software and for expression the analysis has done by REST software.

3. Results

A total number of 201 Iranian people were classified in two different groups; Cancer patients cases (n=101), and healthy controls (n=100). ABCB1 polymorphism at position rs28381943 was determined and the distribution of genotypes in cases and healthy controls were compared. The frequency of ABCB1 G/G genotype in cases was 76.9%, significantly higher than 23.1% in healthy controls (p-value: 0.003). While the frequency of ABCB1 A/A and A/G genotypes in healthy controls were 57.3% and 50.5% respectively, which is higher than 42.7% and 49.5% in cases (p-value: 0.014).

The results of genotyping showed that there is a significant difference in genotypes of this SNP between case and control group so it can be the reason for overexpression.

The results of Real Time PCR showed overexpression of ABCB1 when we compared our data with each genotypes in average mode (Table 1).

The secondary structures of MDR1 mRNA and also the structures including our mentioned SNP, which may cause problems in splicing, are as followed (Shape 1 and 2).

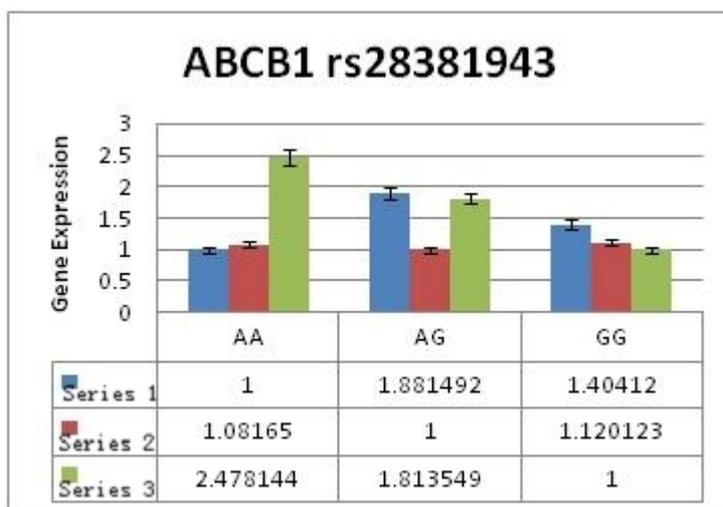
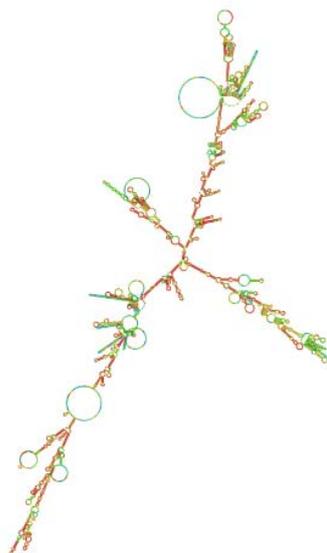
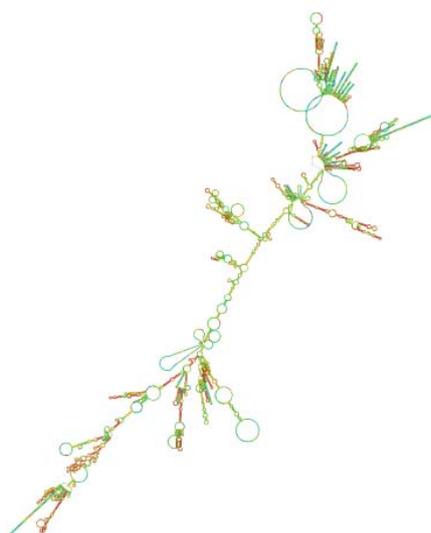


Table1. The results of Real Time PCR. The amount of fold (multiplication of the amount of ABCB1 gene expression).



Shape1. MDR1 mRNA structure based on MFE. The amount of ΔG is -1586.68 kcal/mol.



Shape2. MDR1 mRNA including rs28381943 SNP, which may cause a disruption in splicing process then may lead to existence of intron 19 that must have been omitted through normal splicing. The amount of ΔG is -2482.30 kcal/mol.

We have observed that the amount of ΔG for the original mRNA of ABCB1 is higher than ABCB1 mRNA including our SNP, so it can be concluded that ABCB1 mRNA is more stable when rs28381943 is exists.

4. Discussion

This study is the first report on this SNP, since there were no other similar previous studies. We have observed significant differences in genotypes of SNP (rs283821943) that related to overexpression of MDR1 gene which can be concluded by analyzing the Real Time PCR data. In the other hand we have proved that the existence of this SNP may lead to stabilization of MDR1 mRNA consequently it can be the reason the MDR1 gene to become overexpressed.

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6. References

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