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Investigation of the Genes MDR1/MRP1 and Their Relationship with Clinical and Para-Clinical Characteristics of Colorectal Cancer

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Abstract

***Background*:** Resistance to wide range of structurally and functionally different chemotherapy drugs acts as a major obstacle in the cancer therapy. There are different mechanisms that cause multi drug resistance (MDR) but the most important one is ATP Binding Cassette (ABC) transporters super-family that increases the ability of tumor cells to transport drugs out of the cells by using ATP molecule, over expression of these proteins such as MDR1 and MRP1 is responsible for MDR phenotype in cancerous cells.

***Materials and Methods*:** We analyzed the expression level of MDR1/MRP1genes by using Real time RT-PCR in 60 colorectal cancer patients also we investigate their relationship with clinical, Para-clinical characterizes of colorectal cancer patients and their responses to treatment.

***Results*:** The expression of the MDR1 gene showed a significant increase in cancerous regions compared to adjacent normal tissue. In addition the expression of MDR1 gene showed association with histological grade of tumors but expression level of MRP1 was not significantly important.

***Conclusion*:** Our study once more emphasizes the effects of MDR1 over expression which impact on response to drugs in Iranian colorectal cancer patients.

Introduction

Colorectal cancer, also called colon cancer or intestine cancer includes cancerous growths in the colon, rectum and appendix [1]. With 6-8 affected patients per 10,000 people it is the fourth most common form of cancer in Iranian population; it is the fifth most common form of cancer among men after skin, gastric, bladder and prostate cancers and the third one among women in the Iran, approximately 1/5 of colorectal cancers occurs in people below 40 years old in Iran [2].

Chemotherapy is one of the most effective ways of cancer treatment [3] but resistance to chemotherapy drugs acts as a major obstacle in the cancer therapy, Multi Drug Resistance (MDR) is a phenomenon in which cancerous cells become resistant to wide range of structurally and functionally different drugs [4]. Tumors usually consist of mixed populations of malignant cells, some of which are drug-sensitive while others are drug-resistant. Chemo-therapy kills drug-sensitive cells, but leaves behind a higher proportion of drug-resistant cells (MDR) [5].

The ability to predict response to chemotherapy and to modulate this response with targeted therapies will permit us not only to avoid useless treatments and side effects of them but also selection of the best treatment for individual patients [6].

A frustrating property of such acquired resistance is that the tumors become resistant to wide range of structurally and functionally different drugs so researchers focused on the mechanisms that cause MDR phenotype without considering the structure and function of the drugs.

One of the most important mechanisms responsible for MDR phenotype is ATP Binding Cassette (ABC) transporters super-family that is a class of transmembrane proteins that increase the ability of tumor cells to transport drugs out of the cells by using ATP molecule nonspecifically. Among them MDR1 and MRP1 were reported earlier than other members of ABC transporters and were investigated widely in resistant and sensitive to drugs cell lines, fresh and paraffin embedded normal and tumoral tissues by using different methods such as: immunohistochemistry, flocytometry, southern blotting and real time RT-PCR [7-9].

MDR1 gene is located on chromosome 7 and with 28 exons and 29 introns occupied more than 100 kb on its location and encodes a 170 kDa protein that is called pg-p this gene is first reported by Juliano and Ling [10]. MRP1 gene is located on chromosome 16 and encodes a 190 kDa protein; this gene is first reported by Cole. These proteins contain two ATP binding domains and two transmembrane domains that each one contains 6 membrane-spanning α-helices, trans membrane domain binds to cationic or neutral hydrophobic drugs and efflux them out of the cell [11]. The role of MDR1/MRP1 genes has been reported to be effective in causing MDR phenotype in in-vitro conditions but the results on patients

have been controversial [8, 12]. In this study we evaluate the expression level of MDR1 and MRP1 by using real time RT-PCR separately and simultaneously in Iranian colorectal cancer patients.

Materials and Methods

Sixty patients with colorectal cancer were enrolled in this study. The project was approved by the local ethics committee of the National Institute for Genetic Engineering and Biotechnology (NIGEB), and written informed consent was obtained in all cases. Tissue specimens were collected from the Hazrat rasool hospital during 2009-2011. Tumor and normal tissues adjacent to tumor were obtained from each patient.

The colorectal cancer patients had not yet received any treatment. Histologic diagnosis was confirmed for all samples. RNA extraction was carried out with the Tripure Isolation Reagent (Roche Applied Sciences). For cDNA synthesis, total RNA from each sample was used to synthesize first-strand cDNA according to the manufacturer's protocol (Fermentas) [13]. Evaluation of the expression level of MDR1 was performed by real-time quantitative PCR using the Lightcycler™ system (Roche Applied Sciences) and Fast-Start DNA Master SYBR-Green I kit (Roche Applied Sciences).

Data were analyzed using version 3.03 of the Lightcycler software. The software calculates the relative amount of the target gene and the reference gene (housekeeping gene) based on the crossing point which was defined as the cycle number at which the fitted line in the log-linear portion of the plot intersected the threshold level. An external standard curve for MDR1 and GAPDH was generated from a serial dilution of mRNA of each gene. For each sample, the amounts of MDR1 and the housekeeping gene were measured. Finally, the relative expression was calculated as the ratio of MDR1 to GAPDH in each sample [14].

Statistical analysis was performed using the SPSS-16. The sequences of the primers were used in this study is shown in table 1. Normal distribution of the data was analyzed by Kolmogorov-Smirnov test; Differences between groups were analyzed by independent sample *t*-test and chi-square test also for evaluating the expression correlation between genes regression curve was drawled. A *p*-value less than 0.05 was considered statistically significant.

Results

For assessments of expression level of MDR1 and MRP1 genes and its association with clinical and Para-clinical characterizes of colorectal cancer patients, we analyzed 60 patients with colorectal cancer. The patient and tumor characteristics, gathered from the pathology reports, are listed in table 2. Mean expression level of MDR1 in normal tissues adjacent to tumor was 0.7355±0.1154 and in tumoral tissues was 0.7994±0.1128 so it is apparent that expression level of MDR1 was significantly higher in tumoral tissue (p=0.03) (Fig. 1).

Mean expression level of MRP1 in normal tissues adjacent to tumor was 0.7424±0.1085 and in tumoral tissues was 0.7625±0.0705, however the expression level of MRP1 was higher in tumoral tissue but this difference was not significant (p>0.05). Concerning tumor size, 16 (53.3%) patients had tumor size <5 cm, 11 patients (36.7%) had tumor size between 5-8 cm, 2 (6.7%) between 8-10 cm and 1 (3.3%) over 10 cm, the expression level of MDR1 and MRP1 did not statistically correlate with tumor size in colorectal cancer patients (p>0.05). The expression level of MDR1 and not MRP1 was significantly different regarding stage of disease; grade 1 tumors had the lowest and grade 3 had the highest expression level of MDR1 (p=0.02).

In this study we observed no correlation between the expression level of MDR1/MRP1 genes and responses of colorectal cancer patients to chemotherapy, during 2 years of monitoring these patients there was no different in responses to chemotherapy drugs in patients with high expression level of MDR1 with the low ones, in fact in this period no resistant patient observed so we must admit that the monitoring time of patients was not enough in this study.

Analyzing the correlation between expressions of these two genres showed that there is a significant correlation between MDR1 and MRP1 expression (p<0.05) (Fig. 2).

Table 1. The sequences of the primers for evaluating the expression of genes

Gene	Forward primer	Reverse primer	Tm	Amplicon size
MDR1	TGACATTTATTCAA AGTTAAAAGC	TAGACACTTT ATGCAAACA TTTCAA	80	308
MRP1	AGTGGAACCCCTCT CTGTTTAAG	CCTGATACGT CTTGGTCTTC ATC	90	294
GAPDH	GCAGGGGGGAGCCA AAAGGGT	TGGGTGGCA GTGATGGCA TGG	87	219

Table 2. Patients and tumor characteristics

Characteristic		N (%)
Total patients		60 (100)
Gender	Female	32 (53.3)
	Male	28 (46.7)
Age average		65.10±20.57
Pathological grade	Grade 1	8 (13.3)
	Grade 2	6 (10)
	Grade 3	46 (76.7)
Tumor size	<5 cm	32 (53.3)
	5-8 cm	22 (36.7)
	8-10 cm	4 (6.7)
	>10 cm	2 (3.3)
Lymph node metastasis	Positive	28 (46.7)
	Negative	32 (53.3)
Metastasis		10 (16.7)

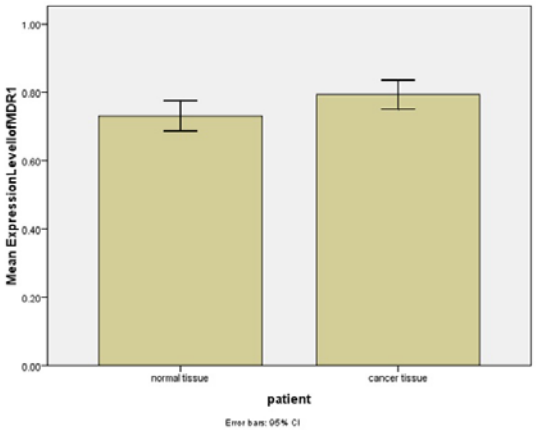

Figure 1. Expression level of MDR1 in normal tissues adjacent to tumor compared to cancer tissue

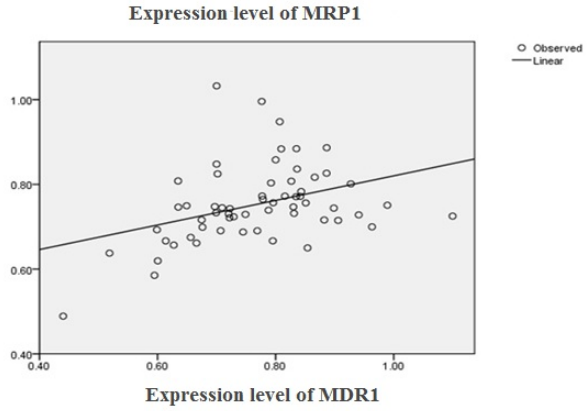

Figure 2. Regression curve for expression correlation between MDR1 and MRP1 expression.

Discussion

In this study we evaluate the expression level of MDR1 gene in 60 colorectal cancer patients; in 32 patients the expression level in tumoral tissue was higher than normal ones that this showed to be significantly important by independent sample *t*-test.

Goldstain et al., by evaluating the RNA level of MDR1 gene in colorectal cancer samples using northern blotting showed that the expression level of MDR1 had increased in tumoral tissues compared to normal tissues [15]. Cohen et al. reported that increase in MDR1 gene expression is a mechanism that is involved in multi-drug resistant phenomenon [16].

Although Mizoguchi et al. analyzed the expression level of MDR1 in 15 colorectal cancer patients and reported that there was no significant different between expression level of MDR1 in tumoral and normal tissues, many researchers reported the same results but others showed that the expression in normal tissues is higher than tumoral tissue in colorectal cancer patients [17]. In this study we observed no correlation between expression level of MDR1 gene and age, gender, tumor position, lymph node involvement and metastasis of patients but there was significant correlation between expression level of MDR1 gene and histological grade of colorectal tumors.

Yukihiko et al. conducted a study on 80 colorectal cancer patients and reported the same results [18], but Priker et al. showed that not only there is no correlation between expression level of MDR1 gene and age, gender, tumor position, lymph node involvement and metastasis of patients but also histological grade of colorectal tumors is not correlate with expression level of MDR1 gene [12].

In this study we showed that the expression level of MRP1 was not significantly different in tumoral tissues compared to normal tissues and also no correlation between expression level of MRP1 gene, age, gender, tumor position, lymph node involvement, metastasis and histological grade of patients was observed; Reyman et al. reported the same results [19]. In our research we showed that there is a significant correlation between MDR1 and MRP1 expression researchers such as Leith et al. and Fillpits et al. reported this correlation too [11, 20].

In conclusion, the results show that the expression level of MDR1 was significantly higher in tumoral tissue compared to normal tissue, but there was no association between MDR1 gene expression and responses to chemotherapy drugs. Although there was no association between MDR1 gene expression and clinicopathologic characteristics such as tumor size and position and lymph node involvement and metastasis, so we can say that pathologic information does not conduct us to find out if chemotherapy treatment will be useful for colorectal cancer patient or not for example characters of a large tumor that are invaded to inner lines of colon or invaded to lymph nodes are not suitable to predict if this tumor is resistant to chemotherapy drugs or not. Further studies aimed at determining the possible relation between MDR1 expression and treatment outcome in this group of patients would be of value.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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