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Correlation among MDR1, MRP and hTERT Genes Expression Level and Clinical Response in Colorectal Cancer Patients

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Abstract

Introduction: Colon cancer is one of the common cancers in the world. Despite current advances in the treatments of cancer, the clinical result is far away from expectation yet. Drug resistance is still a major obstacle in treatment of cancer .In this study, we attempted to investigate the possible correlation among MRP1, MRP and hTERT expression level and multidrug resistance in colon cancer patients.

Materials and Methods: Tumor and adjacent normal tissues from 35 colorectal cancer patients were assessed for the mRNA expression level of MDR1, MRP and hTERT by Real Time RT-PCR.

Results: A statistically significant increase in MDR1 and hTERT expression level was observed in tumoral tissues in comparison with normal tissues. However MRP expression level was not significantly increased in tumoral tissues. Furthermore, no correlation was seen among MDR1, MRP and hTERT expression level.

Conclusion: MDR1 and hTERT have no direct correlation, but mRNA expression of these two genes in addition to other factors indirectly helps to tumorgenesis and cancer progression.

Keywords: Correlation; Multidrug resistance; Real time PCR

Introduction

One of the major public health problems is cancer. Globally, colorectal cancer is the fourth common cancer in men and the third common cancer in women [1]. Resistance to chemotherapy limits the effectiveness of chemotherapeutic treatment, where most of tumoral cells become insensitive to many drugs with different structure and function, this phenomenon is recognized as multi drug resistance (MDR) [2]. Several mechanisms for drug resistance have been suggested including mutation and over expression of the drug's target, inactivation and efflux the drug from the cell by ATP Binding Cassette transporters [3]. ATP-binding cassette (ABC) transporters are a family of transporter proteins with ability to transport a range of substrate molecules across membranes [4]. Molecular investigations on drug resistance have led to the discovery of genes coding for Multidrug resistance1 (MDR1), multidrug resistance-associated protein 1 (MRP1) [5]. MDR1 protein or P-gp is a transmembrane glycoprotein with molecular weight of 170 kDa, encodes by MDR gene. It transports many hydrophobic substrates and anti-cancer drugs including etoposide, doxorubicin and vinblastine [6,7].

Also, The multidrug resistance related protein1 (MRP1) is a member of the ABC-transporter super families which located on chromosome 16p13 and transports a wide range of compounds including glutathione conjugates and cyclic nucleotides out of the cells [8,9].

Telomerase is a specific ribonucleoprotein DNA polymerase. This enzyme activity is quite high in the germ cells, stem cells and cancer cells [10]. Protein subunit of telomerase called as hTERT (human telomerase reverse transcriptase) and its activity helps to maintain the length of telomeres at the end of eukaryotic linear chromosome [11]. Overexpression of hTERT could help initiate and progress of the most of the cancers [12]. Furthermore, it seems hTERT plays a role in multi-drug resistance like MDR1.

Attending to maintenance of MDR1, MRP and hTERT in multi-drug resistance, evaluate of their correlation in different stages of cancers is inevitable.

Material and Methods

Patients

This project was accepted by the local ethics committee of National Institute of Genetic Engineering and Biotechnology (NIGEB) and written informed consent was obtained from each case. Thirty five patients with colon cancer admitted to Rasool-e Akram Hospital in Tehran were enrolled in this study. These patients had not yet received any chemotherapy treatment.

RNA extraction and cDNA synthesis

RNA extraction was performed by Tripure Isolation Reagent (Roch applied sciences). cDNA synthesis was carried out for each sample by using first-strand cDNA synthesis kit of Fermentase.

Real-time RT-PCR

The mRNA levels of MDR1, MRP and hTERT were measured by Real Time RT-PCR using a lightcycler TMsystem (Roche Applied Sciences) with Fast-Start DNA Master SYBR-Green I kit (Roche Applied Sciences). In addition, the mRNA level of GAPDH as internal control was measured and used to normalize the mRNA levels of these genes. The following primers were used for Real Time RT-PCR:

MDR1 forward-5'-TGACATTTATTCAAAGTTAAAAGCA- 3'

MDR1reversed 5'-TAGACACTTTATGCAAACATTTCAA3'

MRP forward5'AGTGGAACCCCTCTCTGTTTAAG-3'

MRP reverse5'-CCTGATACGTCTTGGTCTTCATC

hTERT forward5' AGTGTGTACGTGGTCGAGCTG 3'

hTERT Reversed 5' GGGGATGAAGCGGAGTCT 3'

GAPDH Forward 5' GCAGGGGGGAGCCAAAAGGG3'

GAPDH Reverse 5' TGGGTGGCAGTGATGGCATGG 3'

Real time RT-PCR was carried out on Corbett Real Time Thermal cycler with the following condition: initial denaturing step at 95°C for 5 minute, then 50 cycles of 95°C for 10 seconds, 59°C (annealing temperature) for 10 second and 72°C for 25 second. The expression level of **(MDR1,** MRP and hTERT) mRNAs was evaluated by each gene mRNA/GAPDH gene mRNA ratio.

Statistical analysis

The row data from Real time RT-PCR was analyzed by Linreg software. Statistical analysis for correlation investigation and differences between groups were performed using the SPSS software version 18 by one-way analysis of variance (ANOVA) and the Turkey multiple comparison and correlation tests. A p value less than 0.05 was considered statistically significant.

Results

MDR1 gene expression analysis in normal and tumoral tissues

The clincopathological information of patients was listed in Table 1. As we previously published, there was a significant difference between MDR1gene expression levels in tumoral tissue and adjacent normal tissues. This gene expression level increased significantly in tumoral tissues (P<0.03) (Figure1).

Characteristic		**No (%)**
Total Patient		35 (100)
Gender	Female	16 (45)
	Male	1 7 (55)
Average age	50	29-72
TNM staging	Stage 1	11 (31)
	Stage 2	8 (23)
	Stage 3	8 (23)
	Stage4	8 (23)
Tumor size	<5 cm	10 (27)
	5-8 cm	16 (46)
	8-10 cm	5 (14)
	>10 cm	4 (13)
Lymph node metastasis	Positive	18 (51)
	Negative	17 (49)
Resistance	Drug resistance	6 (17)
	Drug sensitive	29 (83)
Metastasis		6 (17)

Table 1: The clincopathological information of patients

No significant relation was observed between expression level of MDR1 and some clinopathology features such as gender, age, tumor size, lymph node involvement and resistance to chemotheraphy (Table2) [13] of patients (NS: no significantly important, S: significantly important). The correlation between MDR1 expression level and histological grade of patient tumors was significant (P<0.05).The expression level of MDR in stage 1 was higher than stage 2-4.

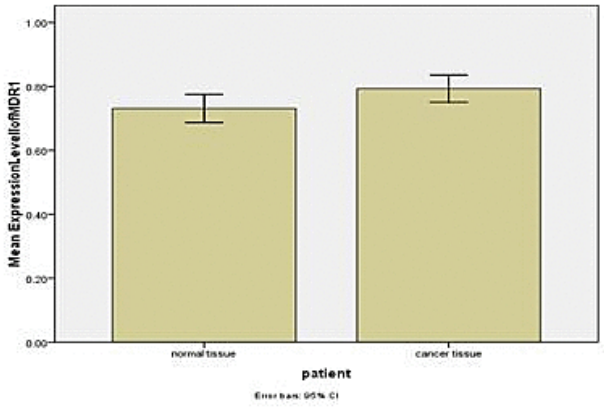

Figure 1: MDR1 expression levels in tumoral tissue and adjacent normal tissues

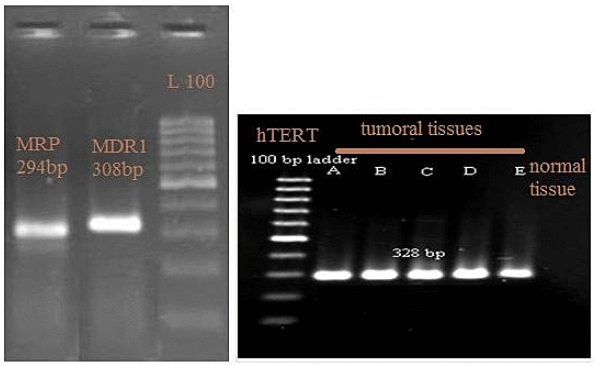

Figure 2: Gel Electrophoresis of MDR1 and MRP and Htert

	Expression of MDR1
Age	NS (P>0.05)
Gender	NS(P>0.05)
Size of tumor	NS(P>0.05)
Lymph node involvement	NS(P>0.05)
Histological grade	S(Pc0.05)
Drug resistant	NS (P>0.05)

Table 2: Relation between MDR1 gene expression and clinicopathology characteristics

MRP gene expression analysis in normal and tumoral tissues

Statistical analysis showed although MRP expression level increased in tuomral tissues in comparison to normal tissues, it was not significant (p=0.4) (Figure3) [13].

hTERT gene expression analysis in normal and tumoral tissues

Telomerase expression was observed in all of the tumoral tissues while it was not observed in all adjacent normal tissues. The hTERT expression level was different according to the stage of tumoral tissues; in a way that hTERT expressed more in stage 1 in comparison to stage 2, 3 and 4 (Figure 4) [14].

A significant correlation was observed between hTERT expression level and histological grade of patient tumors (P>0.05).

No significant relationship was seen between hTERT expression level pathologic features such as gender, age, tumor size, lymph node involvement and drug resistance (Table 3).

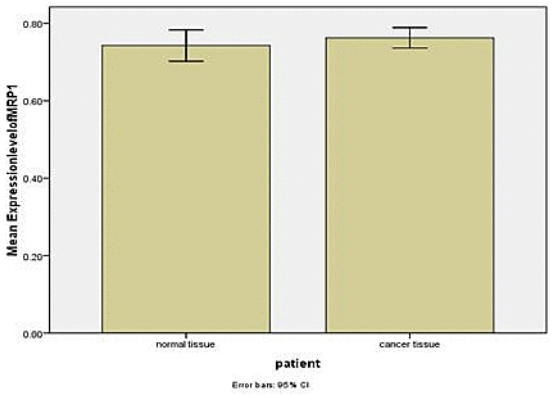

Figure 3: MRP expression level in tuomral and normal tissues

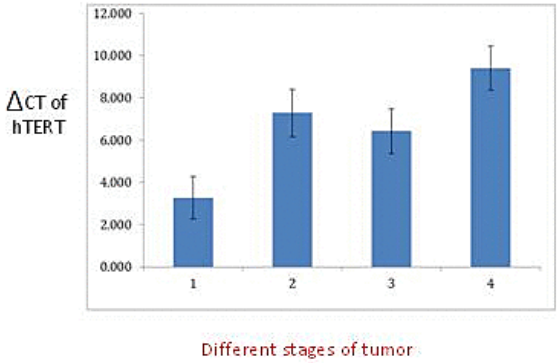

Figure 4: Different ΔCT in different stages (ΔCT of hTERT/ GAPDH mRNA levels in different stages)

	Expression of hTERT
Age	NS (P>0.05)
Gender	NS(P>0.05)
Size of tumor	NS(P>0.05)
Lymph node involvement	NS(P>0.05)
Histological grade	S(P<0.05)
Drug resistant	NS (P>0.05)

Table 3: Relation between hTERT gene expression and clinicopathology characteristics of patients (NS: no significantly important, S: significantly important)

Correlation among MDR1, MRP and hTERT expression level in colon cancer patients

The ΔCT results for hTERT/GAPDH, MDR1/GAPDH and MRP/GAPDH (mRNA levels in different stages) in tumoral tissues were statistically analyzed for each two gene (MDR1/hTERT, MRP/hTERT and MDR/MRP) by correlation test in SPSS sotware. The results indicated there was no correlation between MDR1/hTERT, MRP/hTERT and MDR/MRP expression levels.

Discussion

The cancer cell rapid reproduction and proliferation is one of the most troublesome problems in clinical cancer treatment [15]. On the other hand resistance to chemotherapeutic drugs limits the effectiveness of treatment [16].

Several studies have been investigate the role of multidrug resistance gene (MDR-1) and multidrug resistance-associated protein (MRP) in various human tissues [17,18]. Some of them have been proven the role of these genes in the progression of drug resistance in some cancers [19,20]. However, Samanian et al and other studies did not confirm the direct roles of these genes in multidrug resistance [15]. In this study observation indicated that correlation between MDR1 expression level and drug resistance is not significant, although the relation between MDR1 expression and tumor stages in patients with colon cancer was significant.MDR1expression level in stage 1 was higher than other stages. No correlation was found between MDR1expression level and pathologic features like age, gender, lymph node involvement and tumor size.

In this study also results showed MRP expression level increases in tuomral tissues in comparison to normal tissues, but it was not significant (p=0.4).

The relation between MRP expression levels and drug resistance, histological grade and other pathological characters also was not significant.

In addition, the role of telomerase mRNA levels in tumorgenesis and acquisition of multidrug resistance has been investigated in this paper. Several studies have been illustrated telomerase role in tumorgenesis and acquisition of multidrug resistance [21,22] Cerone et al. indicated that telomerase inhibition increases the response to chemotherapeutic drug treatment in human breast cancer cells [23]. On the other hand Sakin et al showed there were no significant differences in expression levels of hTERT in resistant and sensitive MCF-7 cells [24]. In this study Real Time PCR result indicated that there was not a significant relation between telomerase mRNA levels and sensitivity to chemotherapeutic drugs. It has been illustrated that the correlation between hTERT expression levels and clinicopathological features such as age, gender, tumor size and lymph involvement were not significant. However, the relation between telomerase mRNA levels and tumor histological grades were significant. Furthermore, telomerase has the highest expression levels in stage 1 in comparison with stage 2, 3 and 4.

At the end the correlation between MDR1/hTERT, MRP/MDR and MRP/hTERT mRNA levels were investigated. The statistical analysis showed there was no correlation between these gene expression levels. However, results indicated that both MDR1 and hTERT have a significant relation with histological grades of tumors. Both of them have the highest expression levels in stage 1 in comparison with other stages. These results shows although MDR1 and hTERT have no direct correlation, but expression of these two genes in addition to other factors indirectly helps to tumorgenesis and cancer progression especially in stage 1 where tumor is in early stages. Only one in vivo study have investigated the correlation between MDR/MRP mRNA level and hTERT expression levels , Kuranagu et al showed there is a correlation between MDR/MRP and hTERT expression levels in colorectal carcinoma cells [25].

Conclusion

According to the results, there was not a significant correlation among MDR1, MRP and hTERT expression level in colorectal cancer patients. MDR1 and hTERT have a significant relation with histological grades of tumors. Both of them have the highest expression levels in stage 1 in comparison with other stages. These results shows although MDR1 and hTERT have no direct correlation, but expression of these two genes in addition to other factors indirectly helps to tumorgenesis and cancer progression especially in stage 1 where tumor is in early stages Taken together, it seems complementary tests may help to conclude certainly.

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