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Research Article

ASSESSING THE DECREASED EXPRESSION OF TP53 AND P21 IN COLORECTAL CANCER

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ABSTRACT

Colorectal cancer is the second cancer related deaths in the world. Studying the molecular pathway of that can provide some useful information about therapeutic manners. Hyper activity of P21 and TP53 genes has been reported in recent years. The purpose of this study was assessing the expression of TP53 and P21 tumor suppressor genes in blood samples of 92 samples with colorectal cancer. In this study, 92 patients and 16 healthy samples were selected and after preparation of blood samples, RNA was extracted and then cDNA synthesis was performed by using MMULV enzyme, Oligo dt and random hexamer primers. Real Time PCR was performed for evaluating the decreased expression of mentioned gene. Real Time PCR confirmed the decreased expression of mentioned genes in cancer samples cancer samples. The results showed the decreased expression of mentioned genes in comparison with normal samples.

Keywords: *TP53, P21, Real Time PCR*

INTRODUCTION

Colorectal cancer is the third common cancer in Iranian men and the forth one in Iranian women (Yazdanbod *et al.,* 2004). Approximately 1.5 million people have been diagnosed with colorectal cancer and more than 600.000 people lose their lives annually (Haghighi *et al.*, 2009). Risk factors for colorectal cancer include lifestyle, older age, and inherited genetic disorders. A number of genetic syndromes are also associated with higher rates of colorectal cancer (Pinczowski *et al.*, 1998). Despite the advancements in field of preventive medicine (Marra *et al.,* 1995), colorectal cancer is the second most common cancer in western countries (Loupakis *et al.,* 2012). There were many studies on the molecular aspects of colorectal cancer and in this regard, the role of tumor suppressor genes is of much importance (Thiagalingam *et al.,* 1996). Assessing the expression and also the decreased expression of some key tumor suppressor genes like TP53 and P21 could be effective in monitoring the procedure of the disease and detection of different stages of colorectal cancer (Fu *et al.,* 1998). These genes can control the homeostasis by expressing their specific proteins. For example P21 is a potent cyclin-dependent kinase inhibitor (CKI). The p21 (CIP1/WAF1) protein inhibits the activity of cyclin-CDK2, -CDK1, and -CDK4/6 complexes and thus, it functions as a regulator of cell cycle progression at G_1 and S phases (de Nooij *et al.*, 1996). In addition to growth arrest, p21 can mediate cellular senescence. And also, Mutations on p53 gene are common in colorectal cancer and the Alterations in the p53 pathway contribute to colorectal tumor progression and are likely to provide relevant prognostic information to assist in the management of colorectal cancer patients (Rodrigues *et al.*, 1990). Utilization of Chemotherapy drugs is really common in treatment of cancers since they can affect the DNA of cancerous cells and prevent these

cells from their unregulated function (Van Cutsem *et al.*, 2009). According to the importance of this treatment, there were many studies in this case like Potter *et al.*, in 1999 published an article with the title of colorectal cancer: molecules and populations (Potter, 1999) and also Lengauer in 1997 studied the Genetic instability in colorectal cancers (Lengauer *et al.*, 1997). The purpose of this study was assessing the expression of TP53, P21 tumor suppressor genes in blood samples of 92 samples with colorectal cancer.

MATERIALS AND METHODS

RNA Extraction by RNX-PLUS Solution: RNA extraction was performed by the protocol of Sinaclon Company.

cDNA Synthesis

Vivantis kit (2 step RT-PCR kit) was used for cDNA synthesis. cDNA synthesis was performed according to kit instructions.

Optimization of Real Time PCR Essential Factors for B-ACTIN, P21 and Tp53:

B-actin gene was used as an internal control. After preparing the sequences of B-actin, P21 andtP53 on NCBI, gene specific primers were designed by primer express software. In order to verify the accuracy and specialization of primers, their sequences were blasted. Sequencing of primers is listed in table 1.

Table1: Forward and reverse primers of P21, B-Actin and TP53 genes

Name	Sequences	Molecular weight
P21 F	5′-GCAGACCAGCATGACAGATTT-3′	70bp
P21 R	5′-GGATTAGGGCTTCCTCTTGGA-3′	
B-ACTIN F	5′-CGTCTTCCCCTCCATCG-3′	94 bp
B-ACTIN R	5′-CTCGTTAATGTCACGCAC-3′	
TP53 F	5'-TTCGACATAGTGTGGTGGTGC-3'	
TP 53 R	5'-AGTCAGAGCCAACCTCAGGC-3'	98 bp

For this purpose, separated reactions were prepared for internal control genes and the primers were designed in final volume of 20 ml. reactions were performed in parallel on ABI 7500 instrument. Each reaction contained SYBR TM premix (1X), 0.4 mM of forward and reverse primers and 2 µg of CDNA template.

The temperature of reaction included 40 complete cycles in 95°c for 15 seconds and 60°c for 1 minute. Dissociation curve analysis was used in order to verify the amplified fragment and absence of non-specific amplification, primer-dimer and pollution.

After the reaction, raw data was obtained from the device as a CT. and the measurement of gene expression was performed by using △△Ct.

RESULTS AND DISCUSSION

Results

To determine the underlying mechanism of TP53, P21 tumor suppressor genes, we tested Tp53 and P21 expression in blood samples of 92 samples with colorectal cancer by Real-Time PCR.

Gene expression analysis revealed the decreased expression of mentioned genes in cancer samples. The results showed decreased expression of TP53 and P21 genes in comparison with normal samples (figure 1).

Table 1

Gene	Type	Reaction Efficiency	Expression	Std. Error	95% C.I.	P(H1)	Result
p53	TRG	1.0	0.500	0.250 - 1.000	0.125 - 2.000	0.013	DOWN
p21	TRG	1.0	0.500	0.250 - 1.000	0.125 - 2.250	0.047	DOWN

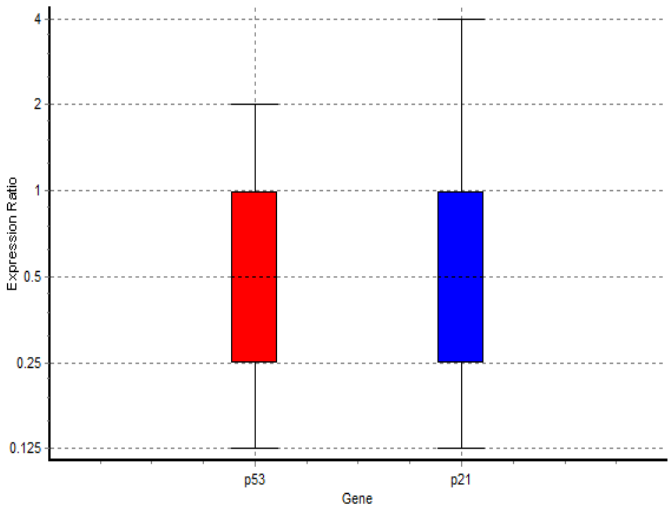

Figure 1:TP53 is DOWN-regulated in sample group (in comparison to control group) by a mean factor of 0.500, TP53 sample group is different to control group. P(H1)=0.013

P21 is DOWN-regulated in sample group (in comparison to control group) by a mean factor of 0.500, P21 sample group is different to control group. P(H1)=0.047

Discussion

Colorectal cancer is one of the most common cancers worldwide (Nkondjock, 2009). About 40-50% of newly diagnosed patients are affected with metastatic disease and the average survival of patients is between 18-21 months. Most colorectal cancers are adenocarcinomas (cancers that begin in cells that make and release mucus and other fluids) (Sano *et al.,* 1995). Colorectal cancer often begins as a growth called a polyp (Levin *et al.,* 2008) which may form on the inner wall of the colon or rectum. Some polyps become cancerous over time. Finding and removing polyps can prevent colorectal cancer. The transition from normal epithelium to adenoma to carcinoma is associated with acquired molecular events (Valtin, 2002). This tumor progression model was deduced from comparison of genetic alterations seen in normal colon epithelium, adenomas of progressively larger size, and malignancies (Vogelstein *et al.,* 1998). Mutant genes cause colon cancer. The human body is composed of trillions of cells (Tosun *et al.,* 2002). Inside each cell, there are two sets of 23 chromosomes, one set from each parent. Each chromosome contains long strands of DNA. The DNA damage can be repaired by the function of tumor suppressor genes like p21 and p53 (Smith *et al.,* 2000). P21 (WAF1), P53 and cyclin D1 belong to the cell cycle-regulating family of proteins, and the loss of activity of proteins P53 and P21 (WAF1) seems to be one of the most important regulatory mechanisms of carcinogenesis in colorectal cancer (Pasz-Walczak *et al.,* 2001). p21 (Cyclin-dependent kinase inhibitor-1A, CDKN1A or CIP1) plays a role in regulating cell cycle, and its expression is lost in most colorectal cancers (Harper *et al.,* 1993). P21 Is related with energy balance status, cellular senescence, and stem cell aging. Approximately half of all colorectal cancers show p53 (TP53) gene mutations, with higher frequencies observed in distal colon and rectal tumors and lower frequencies in proximal tumors and those with the microsatellite instability or methylator phenotypes (Rodrigues *et al.,* 1990). Alterations to this gene appear to have little or no prognostic value for colorectal cancer patients treated by surgery alone, but it could be associated with lower survival for patients treated with chemotherapy. The "p53 → p21 pathway" is activated in cells after DNA damage. Activation of this pathway temporarily arrests cells at the G_1 and G_2 checkpoints of the cell cycle, and terminates DNA replication and cell division (Rothenberg, 1997). These events provide the cells with enough time to repair damaged DNA and prevent accumulation of deleterious mutations in the genome that would otherwise be subsequently transferred to daughter cells (Hertzberg *et al.,* 1989). Activated p53, in turn, induces the expression of many proteins including p21, which is a universal inhibitor of the cyclin-

dependent kinases (Cdks) (Siegel *et al.,* 2000) and is required to arrest cells at the G_1 and G_2checkpoints of the cell cycle after DNA damage (Yamaoka *et al.,* 2004). But when the tumor suppressor genes get damaged, it can increase the probability of cancers. There were many scientists who studied the genetic aspects of colorectal cancer. In 1999 potter *et al.,* published a project with the title of colorectal cancer: molecules and populations. The review article was divided into some parts. Its primary purpose was to provide an overview of the epidemiology and molecular biology of colorectal cancer and to consider some of the links between them and there was a brief recap of the descriptive epidemiology which was followed by a more detailed consideration of major environmental risk factors. The third section outlined the inherited syndromes that carried a markedly elevated risk of colorectal cancer. The role of high-prevalence genetic polymorphisms in metabolizing enzymes and the way in which these may interact with environmental exposures were discussed in this article. Finally some of the somatic genetic changes in relation to the environmental, genetic, and other host influences were mentioned in this article (Pasz-Walczak *et al.,* 2001).

In 1997 Lengaur and his colleagues published an article about genetic instability in colorectal cancers and studied the role of chromosomal instability in creation of colorectal cancers (Harper *et al.,* 1993). In 1995, Waldman and his colleagues published an article which showed the role of p21 tumor suppressor gene in p53-mediated G1 arrest in human cancer cells and also its function in maintaining the stable condition of body (Waldman *et al.,* 1995).

In 1998, Bunz *et al.,* published an article which discussed the requirement for p53 and p21 to sustain G2 arrest after DNA damage (Bunz *et al.,* 1998). To test hypotheses about the importance of p21 in the p53-mediated growth suppression of tumor cells, they used homologous recombination to create a homozygous deletion of p21 in a colon cancer cell line.

In 1993, El-Deiry *et al.,* published an article about WAF1 as a potential mediator of p53 tumor suppression. These studies defined a gene whose expression was directly induced by p53 and that could be an important mediator of p53-dependent tumor growth suppression (El-Deiry *et al.,* 1993).

Conclusion

Finally it can be concluded that the expression of TP53 and P21 were decreased in colorectal cancer in comparison with healthy samples.

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