RESEARCH ARTICLE

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Expression Level of Caspase Genes in Colorectal Cancer

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Abstract

Background: Caspases proteins are protease enzymes involved in the initiation and execution of apoptosis process. Regulation of apoptosis process plays an important role in the normal biological events and development. In addition to developmental abnormalities, dysregulated apoptosis system may lead to tumorigenesis, autoimmunity, and other serious health problems. Aberrant regulation of apoptosis may also be the paramount cause of chemoresistance during cancer therapy. It is aimed through this study to evaluate the transcript levels of Caspase 3, 8, and 9 in tumoral tissues from patients with colorectal cancer (CRC) and compare it with normal marginal tissues. **Methods:** Fifty tumor tissues and their matched marginal tissues, as control group, were obtained from CRC patients. Total mRNA of all tissue samples was extracted and cDNA was synthesized. Using SYBR Green PCR master mix and Real-time gene expression technique, the transcript level of target genes was quantified. **Results:** Experiments indicated that mRNA expressions of caspase 9 and 3 were downregulated in tumoral tissues from CRC patients in comparison to marginal tissues. In contrast, tumoral tissues expressed mRNA of caspase 8 higher than normal marginal tissues. Modified transcript levels of caspase 3, 8, and 9 were correlated with the clinical manifestations of the patients. **Conclusions:** Alteration in the mRNA level of caspase genes may be involved in the development of CRC.

Keywords: Colorectal cancer- caspase- gene expression- mRNA level

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Introduction

Colorectal cancer (CRC) is one of the most prevalent and lethal cancers throughout the world. Similar to other cancers, it appears that both environmental factors such as obesity, physical activity, smoking and inflammation, and genetics play important roles in initiation and development of CRC. Alongside with environmental contributing factors, genetic variations also participate in CRC development, therefore approximately 10% of CRC cases originate from various hereditary impairments (Eberhart et al., 1994; Suzuki et al., 2004; Lanza et al., 2007; Haggar and Boushey, 2009; Asadi et al., 2017).

Apoptosis is one of the most important players, which is involved in etiopathology of several malignancies and most therapeutic ways of cancer like chemotherapy and radiotherapy are trying to induce apoptosis in cancer cells in order to kill them (Herr and Debatin, 2001; Johnstone et al., 2002). There are two pathways of apoptosis in cells, namely intrinsic and extrinsic pathways (Walczak and Krammer, 2000). Caspase 8 is an important molecule in extrinsic pathway. Activation of caspase 8, which is induced by external signals, leads to apoptosis (Eberhart et al., 1994; Lanza et al., 2007). However in intrinsic pathway, releasing of cytochrome c from mitochondria, causes cell death via activation of caspase 9 and some other apoptosis mediators (Suzuki et al., 2004). Both apoptotic pathways are merged in one point at the end in an apoptotic cell and both caspase 9 and caspase 8 activate caspase 3 at the end (Kumar, 1997; Sternberg et al., 1999; Zheng et al., 2003). In this point, cleavage of inactive caspase 3 is occurred by other apoptosis mediators, resulting in activation of caspase 3, which is a critical event in apoptosis initiation (Hsia et al., 2003; Kim et al., 2011).

Previous studies have shown that caspase 3, 8 and 9 expression levels are useful prognostic factors in digestive system related cancers, especially in CRC (de Heer et al., 2007; de Oca et al., 2008; Koelink et al., 2009; Sträter et al., 2010; Kim et al., 2011; Hector et al., 2012; Noble et al., 2013). However, most of previous studies have evaluated these genes separately and did not reach a clear conclusion. In this study, we try to fill this gap by evaluation of caspase 3, 8 and 9 expression levels together in order to better identify the caspase family's role in CRC.

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Materials and Methods

Sampling

This study was designed and performed according to the institutional bioethical guidelines by the Ethical Committee of Tabriz University of medical sciences. Fifty tumor tissues and their matched marginal tissues as control group were gathered from patients referred to Imam Reza Hospital of Tabriz University of Medical Sciences during surgery. Written informed consent was obtained from all the patients. Clinical and pathological characteristics of patients are summarized in table 1. All tissue samples were immediately transferred to RNAase inhibitor solation (Qiagen, Cat No. 76104) and stored in -80 ºC till RNA extraction procedures.

RNA isolation and cDNA synthesis

Tripure isolation reagent (Roche, Cat No.11667165001) was used in order to isolate total RNA from tissue samples, considering the company's manual. To determine the quality and quantity of the extracted RNAs, optical density (OD) of all the samples were measured by Nanodrop device. Afterwards, complementary DNA (cDNA) synthesis was done by TAKARA cDNA syntheses kit (TAKARA Cat No. 6130) according to the manufacture's protocol.

Quantitative real time PCR

Quantitative analysis was carried out by StepOne Plus Real-time PCR system (Applied Biosystems, Foster City, CA, USA). Relative quantification of mRNA expression level of caspase 3, 8, and 9 from tumoral and marginal tissue samples was performed using gene-specific primers and SYBR Green master mix (TAKARA Cat No. RR820W). Expression level of GAPDH (housekeeping gene) was used in order to normalize expression level of target genes. Primers (Table 2) were designed using Oligo 7 software, and for spasticity and accuracy all primers were blasted in NCBI website. Average score of duplicated Ct values was measured for each sample and comparative Ct method was used to determine the relative expression level of target genes (Pfaffl, 2001).

Statistical analysis

Statistical analysis was performed using the Graph Pad Prism 6 (Graph Pad Software Inc. San Diego, CA, USA). Kolmogorov-Smirnov's normality test was applied for evaluating the normal distribution of data. Independent sample t-test was conducted to compare target gene expression level between CRC tissues and their paired marginal tissues. Cross tab (Eta) analysis was conducted to evaluate relationship between clinical features of the patients with relative mRNA expression of caspase genes. All results were expressed as mean ± standard deviation (SD). Statistical significance level for all P value was less than 0.05.

Table 1. Clinicopathological Data of the CRC Patients

Feature	Grouping	Score
Sex	Male	32
	Female	18
Age	>60	26
	<60	24
Distant metastasis	pM0	42
	pM1	8
Tumor stage	1	4
	2	18
	3	22
	4	6
smoking	Yes	31
	No	19
Tumor location	Rectum	13
	Right colon	21
	Left colon	16
Differentiation	Poor	11
	Moderate	26
	Well	13

Results

Our experiments confirmed revealed downregulation of mRNA expression level of caspase 3 in tumoral samples in comparison to normal marginal colon tissues (Fold change = 0.32, P = 0.021; Figure 1). Tumor stage (Eta = 0.453, P = 0.037) and differentiation (Eta = 0.621, P = 0.038) were the clinical manifestations of the CRC patients with statistically significant relation with the transcript level of caspase 3. Table 3 summarizes the relation between caspase 3 mRNA expression level and clinicopathological manifestations of the patients.

On the other hand, it was found that mRNA expression level of caspase 8 was upregulated significantly in tumoral samples compared with normal marginal colon tissues (Fold change = 2.11, P = 0.0240; Figure 1). Lack of expression of caspase 8 mRNA was observed in just one sample. Considering the clinical data, caspase 8

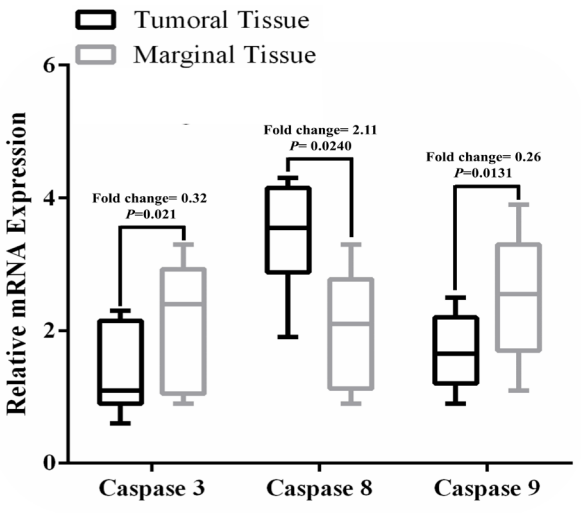

Figure 1. Box and Whisker Plot Demonstrates the Relative mRNA Expression Levels of Caspase 3, 8, and 9 in Tumoral Tissues of the CRC Patients in Comparison to Healthy Marginal Tissues.

Table 2. Primer Sequence and Characteristics Used in the Quantification of the Target Genes

Gene	Forward primer	Annealing temp (°C)
Caspase 3-Forward	5′-ATGGTTTGAGCCTGAGCAGA-3′	59.5
Caspase 3-Reverse	5′-GGCAGCATCATCCACACATAC-3′	58.5
Caspase 8- Forward	5′-ACCTTGTGTCTGAGCTGGTCT-3′	59.5
Caspase 8- Reverse	5′-GCCCACTGGTATTCCTCAGGC-3′	59
Caspase 9- Forward	5′-GCAGGCTCTGGATCTCGGC-3′	60.5
Caspase 9- Reverse	5′-GCTGCTTGCCTGTTAGTTCGC-3′	59.5
GAPDH- Forward	5′-CAAGATCATCAGCAATGCCTCC-3′	59
GAPDH- Reverse	5′-GCCATCACGCCACAGTTTCC-3′	59

Table 3. Association of Demographic and Clinical Characteristics of the Studied CRC Population with the Transcript Levels of Caspase 3, 8, and 9 (Data are shown as *P* values).

Characteristic	Caspase 3		Caspase 8		Caspase 9	
	Eta	P value	Eta	P value	Eta	P value
Sex	0.211	0.397	0.081	0.451	0.32	0.351
Age	0.321	0.346	0.225	0.371	0.287	0.246
Smoking	0.021	0.764	0.201	0.453	0.168	0.841
Tumor stage	**0.453**	**0.037**	0.01	0.121	**0.461**	**0.041**
Distant metastasis	0.354	0.078	**0.551**	**0.043**	**0.601**	**0.034**
Tumor location	0.178	0.341	0.022	0.578	0.208	0.436
Differentiation	**0.621**	**0.038**	**0.538**	**0.037**	**0.722**	**0.021**

*, Values in bold demonstrate significant p values.

expression level was associated with distant metastases (Eta = 0.551, P = 0.043) and differentiation stage (Eta = 0.538, P = 0.037) of the CRC patients (Table 3).

It was observed that caspase 9 expression level was downregulated significantly (Fold change = 0.26, P = 0.0131; Figure 1) in comparison to normal marginal colon tissues. However, in 4 samples we did not identify expression of caspase 9. Expression level of caspase 9 was associated significantly with some of the clinicopathological features of the patients, including tumor stage (Eta = 0.461, P = 0.041), distant metastases (Eta = 0.601, P = 0.034), and differentiation stage (Eta = 0.722, P = 0.021). But no significant association was seen between expression level of caspase 9 and age, sex, smoking, tumor location, and differentiation (Table 3).

Discussion

Achieving approaches toward prevention and early diagnosis of CRC requires better understanding of genetic and molecular pathways involved in the disease etiopathogenesis (Bodmer et al., 1994). Caspase 3, 8 and 9 are important molecules in apoptotic pathways which play key roles in cancer development and progression (Fearnhead et al., 1998). Due to important functions of caspase proteins in cancers, we evaluated expression levels of them in CRC tissues and compared them with normal marginal tissues. It was also aimed to reveal if there was any relation between mRNA expression level of these molecules and clinical features of the patients.

Caspase 9 expression level was downmodulated in tumoral tissues compared with normal marginal colon samples, which had previously been shown by other studies (Fearnhead et al., 1998; Shen et al., 2010). Moreover, association of mRNA expression level of caspase 9 and clinical manifestations of CRC patients was found in this study, as it was in accordance with another study (Shen et al., 2010). A number of studies demonstrated that caspase 8 had upregulation in tumoral tissues of CRC patients (Heijink et al., 2007; Xu et al., 2008; Chen et al., 2015). In the present study, there was also an upregulation of caspase 8 mRNA expression level in CRC patients. This upregulation may be related to other roles of caspase 8, especially in cell migration and cell-cell interaction (Helfer et al., 2006; Finlay and Vuori, 2007; Heijink et al., 2007; Senft et al., 2007; Barbero et al., 2009). In the present study, caspase 3 expression level was downregulated in tumoral tissues of CRC patients. Previous studies have also indicated downregulation of this gene in CRC patients (de Oca et al., 2008; Koelink et al., 2009; Meyer et al., 2009; Guan et al., 2012).

In conclusion, modifications in molecular markers throughout the onset and progression of malignancies can underpin the designing of much effective therapeutics and diagnostic tools and may prevent cancer development in early diagnosed cases. These molecular signatures can then be applied as a target of novel therapeutic strategies. In the ongoing investigation, we observed an aberrant mRNA expression level of caspase 3, 8, and 9 in tumoral tissues of CRC patients in relation to normal marginal tissues. Furthermore, the altered expression level of these genes was related to some of clinicopathological specifications of the patients. It is essential to perform further studies to confirm caspase molecules as therapeutic

or diagnostic tool in CRC patients.

Disclosure of conflict of interests
None.

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