The role of JAK/STAT signaling pathway and TNF-α crosstalk in human colorectal cancer

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ABSTRACT

Colorectal cancer (CRC) constitutes a significant portion of mortality and morbidity due to cancer around the world. The present study was designed to evaluate the possible effects of the JAK/STAT, TNF-α, and NFκB pathways, and the caspase-3 apoptotic marker in human colorectal tissue. Data from tumor tissue and adjacent normal colon tissue (n:12) were obtained. Given the biological role of the JAK/STAT signaling pathway in human colorectal tissue, we evaluated interactions with TNFα, NFκB, and caspase-3. We measured the mRNA levels of STAT1, TNF-α, TNF-α R1A, NFκB, and caspase-3 by RT-PCR. The STAT1, TNF-α, TNF-α R1A, and NFκB mRNA levels were increased in colorectal cancer tissue compared to adjacent normal colon tissue ($P < 0.01$, $P < 0.05$, $P < 0.05$, and $P < 0.05$, respectively). There were no significant differences between colorectal cancer and adjacent normal colon tissue regarding the caspase-3 mRNA levels. STAT1 contributes to oncogenesis by promoting the progression of the cell cycle and preventing cells from undergoing apoptosis. In addition, TNF-α has proangiogenic activity and increases the neovascularization of tumors. According to our results, the mRNA levels of STAT1 and TNF-α were significantly higher in cancer tissues, but the mRNA levels of the apoptotic genes caspase-3 and 9 were similar to those of the normal colon tissue, suggesting that STAT1 and TNF-α are important components in the progression of colorectal cancer. These data support the importance of the JAK/STAT signaling pathway in colorectal cancer and suggest targets for intervention. We will be investigating potentially related genes, pathways, and interactions in our future studies.

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1. Introduction

Several other studies have reported that the JAK/STAT signaling pathway is more active in colorectal cancer tissues than in normal colorectal tissues (Uchiyama et al. 2011). The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway is important in cell growth and differentiation. Once activated by cytokines, JAKs serve as docking sites for signaling molecules, such as STATs. Among the members of the STAT family proteins, STAT1 plays a critical role in the regulation of primary inflammatory responses. Pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and its receptors, have been shown to up-regulate STAT proteins (Slattery et al. 2013). In addition to the regulatory effect of inflammatory responses, STAT-1 has several physiological roles depending on the cell type, and its overexpression plays a role in cancer cell survival (Forester et al. 2014).

The transcription factor, nuclear factor-κB (NFκB) plays a central role in inflammation and is constitutively expressed in cancers. NFκB is activated in response to various carcinogens, growth factors, and inflammatory stimuli. TNF-α, an important upstream activator of NFκB signaling, plays a major role in carcinogenesis. In the nucleus, NFκB binds DNA and enhances the transcription of various gene products that regulate proliferation, invasion, inflammation, apoptosis, and metastasis (Al-Halabi et al. 2011). TNF-α plays a dual role in activating both pro- and anti-apoptotic mechanisms (Benderska et al. 2014). In our study, we investigated the relationship between TNF-α, NFκB, and caspase-3 as an apoptotic marker.

Apoptosis, also known as programmed cell death, is a biological process that is a key feature of cancer development (Yun et al. 2013). The members of the caspase family are major regulators of the activation pathway of apoptosis. In this study, we focused on caspase-3, one of the most commonly involved mediators of apoptosis in human colorectal cancer development.

In this study, we aimed to determine the role of the JAK/STAT signaling pathway and TNF-α crosstalk in human colorectal cancer tissue and to compare the findings with those in other reports.

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2. Materials and methods

2.1. Study populations

The present study was approved by the Ethics Committee of Eskisehir Osmangazi University for Clinical Research (80558721/90) and was performed in accordance with the ethical standards of Helsinki Declaration. Tissue specimens from tumor and adjacent normal colon tissue (n:12) were collected within a 1-year period at the Department of General Surgery, Hospital of Eskisehir Osmangazi University, Eskisehir, Turkey.

2.2. Tissue samples

Tumor and adjacent normal colon tissue from patients with CRC used in our study. All specimens were obtained during routine surgery performed in patients with CRC. The study included 12 patients (6 men and 6 women) with mean age of 63.6 years (range: 34–79 years). We reported the clinical characteristics of patients such as age, gender, body weight, height, and body mass index differences (Table 1). Before use in the study, each specimen was verified by a pathologist. Immediately after excision, tissue samples were fixed in RNAlater® solution (Qiagen) for the isolation of STAT1, TNF-α, TNF-α R1A, NFκB, and caspase-3 mRNA expression and stored at −80°C.

2.3. RNA extraction and quantitative real-time PCR

Total RNA was extracted from approximately 100 mg of tissue using RNA later solution according to the manufacturer's instructions (Qiagen). RNA concentration and purity were analyzed by measuring the optical density at 260 nm (NanoDrop 1000, Thermo Scientific, USA). One microgram of RNA was converted to cDNA using cDNA Synthesis Kit (Roche Nano Lightcycler Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. The abundance of STAT1, TNF-α, TNF-α R1A, NFκB, and caspase-3 mRNA were analyzed using the beta-actin as a reference gene. FAM-labeled primers/probes of the target genes and the reference gene were amplified in a 20-μl reaction volume in 0.2 ml PCR tubes. Amplification was detected by using Dual Label Taqman probes, and the measurements were performed using a Roche Nano Lightcycler (Roche Diagnostics, Mannheim, Germany).

Table 1
Clinical characteristics of patients.

Variable	Patients
Number	12
Men/women	6/6
Mean age (range)	63.6
Mean weight (kg)	65.7
Mean height (cm)	166.6
BMI (kcal/day)	23.8
Tumor localization	
Left colon	11
Rectum	1
Pathological type	
Tubular and papillary adenocarcinoma	10
Mucinous and signet ring cell carcinoma	2
Tumor stage[a]	
T2	0
T3	11
T4	1
Lymph node metastases (N1/N2)	5/2
Distant metastases (M1)	3

[a] Tumor staging was done according to the TNM classification.

2.4. Statistical analysis

Transcript data were expressed relative to the control (set to 1) ± standard. Statistical analysis was performed by using the GraphPad software program. Following the determination of STAT1, TNF-α, TNF-α R1A, NFκB, and caspase-3 mRNA expression using beta-actin as a reference gene, the data obtained from RT-PCR were calculated using the formula $2^{-\Delta\Delta Ct}$.

3. Results

3.1. Patient characteristics

We categorized 12 patients with colorectal cancer by comparing gene expression patterns of tumor samples and adjacent normal mucosa after data trimming. For this purpose, we summarized clinicopathologic profile of patients such as age, gender, body weight, height, body mass index, tumor localization, tumor stage, and lymph node metastasis in Table 1.

3.2. Gene expression

STAT1 mRNA abundance was significantly higher in the CRC tissue compared with the adjacent normal colon tissue ($P < 0.01$ Fig. 1A). Similar to STAT1, TNF-α, TNF-α R1A, and NFκB mRNA abundance were significantly increased in the CRC tissue compared with the adjacent normal colon tissue ($P < 0.05$, Fig. 1B; $P < 0.05$, Fig. 1C; $P < 0.05$, Fig. 1D). In addition, there are no differences in caspase-3 gene expressions between the groups (Fig. 1E). There are also no differences for gene expressions between gender in human CRC tissue.

4. Discussion

The aim of this study was to evaluate the expression, significance, and interaction of JAK/STAT signaling pathways with the clinical characteristic of CRC patients. We analyzed the role of STAT1, TNF-α, NFκB, and an apoptosis marker in the tumorigenesis and development of human CRC. We found that the STAT1 mRNA abundance was significantly higher in the CRC tissue compared with the adjacent normal colon tissue. Similar to STAT1, the levels of TNF-α, TNF-α R1A, and NFκB mRNA were significantly increased in the CRC tissue compared with the adjacent normal colon tissue.

As in many malignant tumors, CRC has been associated with abnormalities at the level of signal transduction. The signal transducer and activator of transcription (STAT) proteins as well as cytokine-driven Janus kinase (JAK)/STAT pathways have important roles in these processes (Klampfer, 2008). Signal transducer and activator of transcription proteins are activated through tyrosine phosphorylation, mostly by cytokine receptor-associated JAKs. Taken together, numerous studies have addressed the role of aberrant STAT3 function in cancer and have not yet revealed a consistent picture. However, oncogenic STAT3 needs to be viewed in a wider context and while considering STAT1 expression and activity data (Gordziel et al. 2013). STAT1 has known antiproliferative and proapoptotic effects when it is expressed and activated in tumor cells (Kim and Lee, 2007). Slattery et al. (2013) reported that STAT1 was significantly associated with colon cancer. Similar to these reports, we also found that STAT1 mRNA levels increased in human CRC compared to adjacent normal colon tissue. Huang et al (2014) reported that the inhibition of STAT1 using the available small molecule inhibitors resulted in growth inhibition. Therefore, the inhibition of STAT1 may play an important role in human CRC patients. In our study, increased STAT1 levels correlated with lymph node metastasis and the tumor stage T3 in human CRC tissues. We found that increased STAT1 levels were associated with poor prognosis.

We tested the biological role of the JAK/STAT signaling pathway in cancer development and its involvement and interaction with target