Original Article

Molecular Characteristics of TYK2 Gene Expressions in Patients with Colorectal Cancer

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Abstract

Background: TYK2 is a member of the JAK family and is known to mediate signals of multiple cytokines that play a crucial role in immune and inflammatory signaling. Activation of TYK2 in tumor cells has been linked to promote cell survival, growth, and invasion. This study aimed to investigate the expression of tyrosine kinase 2 (TYK2) in colorectal cancer (CRC) and adjacent control tissues.

Materials and Methods: Quantitative Real-Time PCR (qRT-PCR) method was elaborated to examine the expression levels of TYK2 in 100 colorectal tumor tissues and adjacent tissues as a control. Furthermore, we analyzed the diagnostic power of the mentioned TYK2 by plotting the receiver operating characteristic (ROC) curve.

Results: Our results revealed that the expression level of TYK2 was significantly up-regulated in CRC patients sample compared to the adjacent sample of the control group. Analysis of patient's clinic pathological features shows that expressions TYK2 were differently associated with lymph vascular invasion and TMN stage ($P < 0.0001$, $P < 0.0006$).

Conclusion: These results indicated that TYK2 levels potential biomarkers for diagnosing colorectal cancer may be identified.

Keywords: Biomarker, colorectal cancer, cytokines, TYK2 kinase

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Introduction

Colorectal cancer (CRC) is the third most progressive cancer associated as fatal disease in the world; the incidence rates of this cancer are currently very high in most of the countries. Also, there are many risk factors such as genetic predisposition, diet rich in fat, and smoking reported.[1,2] Cancer is caused by the accumulation of somatic mutations in cells, as well as other genetic changes that can lead to abnormal cell growth and the formation of tumors. This carcinogenic process gives rise to cells with certain beneficial biological capabilities, known as cancer characteristics.[3,4] Colorectal cancer prognostic and gradual transition occur, that, associated with multistep tumor geneses and certain genetic changes in tumor suppressors or oncogenes are related with every stages.[5-7] Moreover, colorectal cancer is induced by a multi-stage genetic disorder and many other etiologies that cause colorectal cancer disease.[1,8] In recent years, the researches on the genetic and epigenetic treatment options have been successful in reducing the incidence of colorectal cancer patient mortality over the past few decades.[9,10] Genomic, proteomic, and transcriptomic analysis revealed the presence of tyrosine kinase 2 (TYK2) compilation proteins and altered expression of this gene in various cancers.[11] TYK2 is an oncogene, which is often mutated

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or overexpressed in various sorts of cancer and metastases. It is a member of the Janus kinase (JAK) family; this mediates the signals of cytokines that are essential in the functioning of the immune and inflammatory systems. TYK2 can lead to reduced cell death, increased invasion, and proliferation of cells.[12] Actually, TYK2 was essentially diagnosed for its roles in the path of immunity and inflammation. As well as, in immune responses as a mediator, it has an effect on vital cytokine signaling pathways.[13] Further, overexpression of tyrosine kinases has been determined in different cancers.[14] However, few studies have shown the role of tyrosine kinase involved in the improvements of colorectal cancer. A recent study has indicated that TYK2 expression levels were significantly enhanced in relation to cancer stage, tumor grade, gender, and nodal metastasis status.[15] In the current study, we aimed to examine the expression of TYK2 in colorectal cancer and adjacent control tissues. We examined the expression of TYK2 in CRC and adjacent control tissues, and explored the correlation between TYK2 expression and clinicopathological characteristics of CRC. Additionally, we pursue to uncover the involvement of TYK2 in the improvements and malignancy of CRC cells and to compare its expression in normal tissue.

Materials and Methods

Patients and sample

This case-control study collected tissue samples from patients referred to endoscopy, oncology, or surgery clinics from July 2020 to December 2022 in different hospitals. In total, 50 patients and 50 control subjects were included in the study, with samples collected through biopsies or surgical resections. Additionally, normal specimens from colorectal cancer sample patients were collocated, containing no tumor cells and located at least 2 cm away from the tumor site. Inclusion criteria for this study are patients aged 18–60 years old with a histologically confirmed diagnosis of colon adenocarcinoma, whose tumor tissue samples have been verified by a board-certified pathologist, and who have not received any colorectal cancer-associated therapy before the biopsy. This study excluded subjects who had received colorectal cancer-related treatments, such as surgical resections, as well as any other malignancies. Additionally, lifestyle, demographic, and histopathological information related to the clinical TNM staging was documented.

RNA extraction and cDNA synthesis

The commercial kit was used for RNA extraction (Cinnacolon, Tehran, Iran) and isolated RNA was eluted in 40 µl of RNase/free water, its viscosity and integrity of the total RNA were assessed by measuring the A260/A280, using Nano Drop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). That each sample ratio was intended between 1.7 and 2.1, then RNA suspension was stored at −80°C for further analysis, and it was converted to cDNA. Reverse transcription reaction was using a cDNA kit (Cinnacolon, Tehran, Iran), cDNA was prepared from 2 µg of total RNA, with Oligo (dT) and random hexamer primers. Consistent with the manufacturer's instructions the kit mix was run on a PCR thermocycler gene as follows: 10 min at 25°C, 2 h at 37°C, 5 min at 85°C, and thereafter on a PCR thermocycler gene. cDNA was diluted to a total concentration of 5 ng/µl.

Real-Time PCR

Real-time PCR analysis was conducted in duplicates using 2.0X Real Q-PCR Master Mix with SYBR Green (Ampliqon, Odense, Denmark). The reaction of each sample involves 10 µl 2 × RealQ-PCR Master Mix, 1 µl cDNA, 1 µl of each primer (10 pmol/µl), and 8 µl of distilled water. Reactions were run on the Step One Plus Real-time PCR System (Applied Biosystems, USA) using the thermal cycling parameters 95°C for 2 min and 40 cycles of 95°C for 5 s, 60°C for 30 s, specificity of products was verified by melting curve analysis. Gene expression levels were used to normalize the expression level of beta-2 macroglobulin (β2 M) the housekeeping gene) within a given for each sample. The primers were designed and positioned in a variety of exon junctions of TYK*2* to avoid false-positive results following DNA contamination [Table 1].

Statistical analysis

Efficiency values and cycle threshold (Ct) for each sample [Figure 1], the amplification efficiency was determined using the Lin Reg software (version: 2017.1), and the expression ratio (Fold change $2^{-\Delta\Delta Ct}$) of the TYK2 was estimated using REST 2009 software. The means statistical differences of TYK2 levels between patients and control were subjects analyzed with the Graph Pad Prism software version 8.0 (La Jolla, CA). That, using the Mann–Whitney test and unpaired *t*-test to compare *TYK2* mRNA levels in two groups. *P* value of ≤0.05 was considered significant.

Result

Overall, we studied 50 colorectal cancer patients (26 females and 24 males) aged between 22 and 64 years (mean ± SD = 45.2 ± 12.419 years). In patients, tissue location of malignant, 21 (42%) were colon, and 29 (58%) were rectum.

Table 1: qRT-PCR primer sequences

Genes	Primers	Sequences	Amplicon size (bp)
TYK2	Forward Primer	AGATCTGGGCGAGGGTCACTTC	203
	Reverse primer	GGTCCTCGCAGCAGCCCTTG	
Beta-2-microglogulin	Forward Primer	TGTCTTTCAGCAAGGACTGGT	143
	Reverse primer	TGCTTACATGTCTCGATCCCAC	

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Furthermore, our result revealed that, patient records were retrospectively reviewed, we observed 4 (11.3%) colorectal cancer patients with IBD, 19 (54.3%) colorectal cancer patients with polyp, and 12 (34.3) colorectal patients with colitis. Also, of these patients' clinical TNM stage, 12 (24%) were stage II, 14 (28%) were stage III, and 24 (48%) were stage IV. Our results clarified that the expression TYK2 was differently related to the TNM stage ($P < 0.0001$).

The stage IV group possessed expression TYK2 was up-regulated compared with stage II and III groups (stage II: 6.14% vs. 10.14%, stage III: 8.1% vs. 10. 14%) [Figure 2]. In this study, the selected clinical characteristics of the subjects below the study are shown in Table 2. We measured the TYK2 expression levels using qRT-PCR in CRC tissue. Since we observed up-regulated gene colorectal cancer tissues compared with controls [$P < 0.0001$; Figure 1]. Analysis of the ROC curve showed that the tissue could be some valuable biomarkers for distinguishing colorectal cancer patients from healthy individuals. The larger the area under the ROC curve (AUC), the more reliable the diagnostic value. The AUC for TYK2 has shown in Figure 3. In the present study, LVI+ was observed in 28 (56%) of 50 CRC, results revealed, that expression TYK2 was differently associated to lymph vascular invasion ($P < 0.0001$), the LVI+ group possessed expression was up-regulated compared with LVI− group (9.3% vs. 6.37%, $P < 0.0001$).

Moreover, our result revealed that the expression level of TYK2 were variously associated with the CRC patient with a history of polyps and showed a reduction in expression level than the IBD and colitis groups, but not significantly ($P < 0.5$). There is evidence that the regulatory role of an oncogene is linked to cancer which acts as tumor progression.

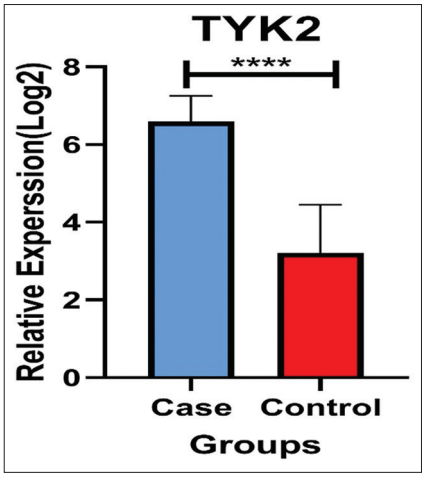

Figure 1: The relative expression levels (−ΔCt) of TYK2 and B2M in colorectal cancer

Discussion

Evidence from multiple lines has demonstrated that JAKs are essential for regulating cytokine signaling, which can have a profound impact on basic cellular functions, such as invasion, proliferation, apoptosis, and immunity.[15] Current studies have demonstrated that TYK2, a member of the Janus kinase (JAK) family, is in many types of cancer and metastases often display frequent mutations or overexpression. This suggests that TYK2 may act as an oncogene, mediating the signals of multiple cytokines implicated in immune and inflammatory signaling. Activation of TYK2 in tumor cells has been linked to reduction cell death, boost cell growth, and invasion. Despite this, the role of TYK2 in solid tumors remains largely undefined.[12] In this study, we understood that the expression levels of TYK2 in CRC patients were significantly enhanced than in the control group ($P < 0.0001$). Using 50 fresh CRC specimen and normal tissues by qRT-PCR, the results indicated that the expression level of TYK2 was up-regulated in the tissue samples. Moritsch *et al*.[10] examined the functions of TYK2 in colorectal cancer; they used three various mouse models with TYK2 removal and the AOM-DSS protocol for colitis-CRC, and they demonstrated that TYK2 plays a tumor suppressor role in cancer cell. Our data also suggest that the expression level of TYK2, with IBD groups, was more up-regulated compared with polyp and colitis groups, but not significantly ($P < 0.5$). The role

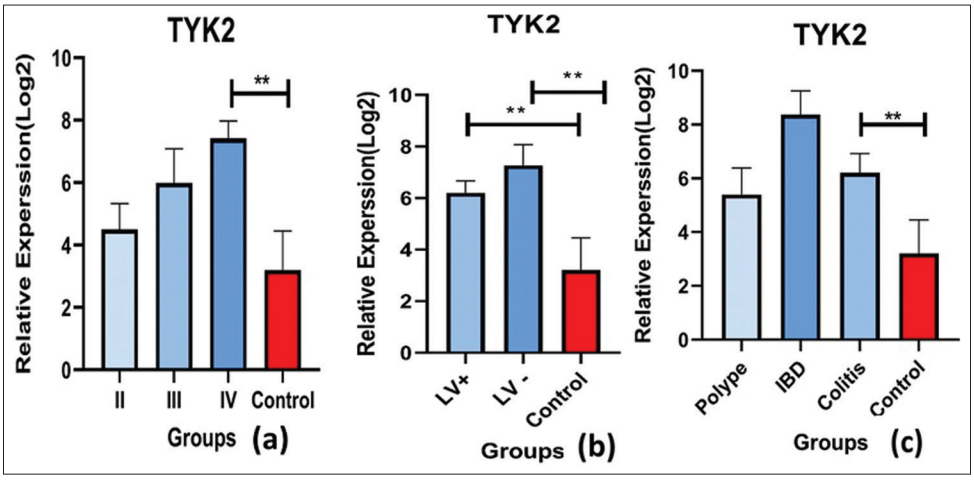

Figure 2: The relative expression levels (−ΔCt) of TYK2 and the relationship between clinicopathological characteristics in patients with CRC

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Table 2: Patients' clinic pathologic characteristics TYK2

Variable	Clinic pathological parameter	Number of samples (n=50)	Mean±SD	P
Age	≥45	28	15.67±10.02	P=0.887
	45<	22	15.98±4.37	
Gender	Male	24	15.65±3.34	P=0.687
	Female	26	15.47±4.72	
TNM stage	II	12	6.52±2.63	P<0. 0001
	III	14	10.79±2.48	
	IIV	24	12.32±3.36	
Tumor size	< 2	12	11.84±3.46	P=0.0001
	2–3.5	14	15.03±3.42	
	3.5–5	14	16.17±3.24	
	>5	10	19.09±3.38	
Localization	Colon	21	15.37±3.90	P=0.695
	Rectum	29	15.69±4.29	
Lymphatic invasion	Positive	28	17.47±3.06	P<0.0001
	Negative	22	10.82±1.74	

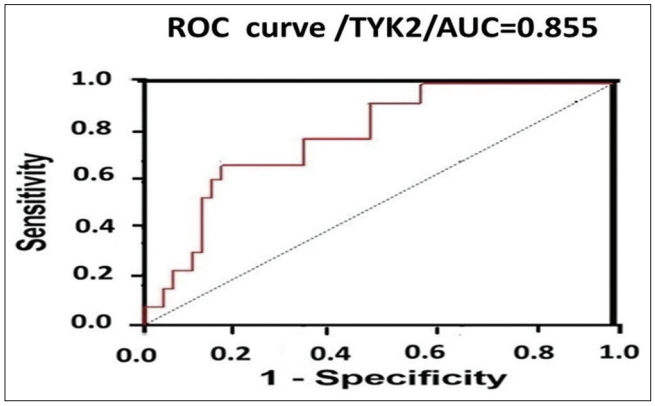

Figure 3: ROC curve analysis of the diagnostic value of CRC-related genes to distinguish between CRC patients and healthy individuals

of TYK2 in cancer immune surveillance is well-known, but there have been few studies on its functions in CRC cells. Recent research has revealed that TYK2 is implicated in the invasion and metastasis of cancer cells.[16,17] Meng *et al*.[15] discovered that the expression levels of JAK3 and TYK2 were significantly higher in tumor tissues than in normal tissues in STAD. Furthermore, their analysis indicated that JAK3 and TYK2 could serve as prognostic biomarkers in STAD and were related with improvements, tumor genesis, and metastasis of STAD. Fang *et al*.,[18] in 2021 showed that the expression level of TYK2 was significantly reduction with the progression of the tumor. Moreover, the investigation of TYK2 expression and correlation of clinical in LC was significantly associated with gender, lymph node status, and metastasis. Our result revealed that the expression level of TYK2 was differently associated to lymph vascular invasion, the LVI+ group possessed expression was up-regulated compared with LVI− group ($P < 0.0001$). Findings from Iranian scientists have indicated that colorectal cancer is on the rise in Iran, likely due to changes in lifestyle such as smoking more, exercising less, and having an unhealthy diet.[19] This study had some limitations, such as a small sample size, and we only examined the analysis at an mRNA level; it will be more beneficial to further diagnose the procedure with protein issues.

Conclusion

The current study proposed that TYK2 is probably a useful biomarker for diagnosing colorectal cancer. However, further research is needed to confirm these findings.

Author contributions

ZM and NM conducted the experiments and wrote the manuscript; BF contributed in laboratory works; AAK performed statistical analysis and data interpretation; AM supervised the project and revised the manuscript. All of the authors reviewed and approved the final manuscript HRM provided sample.

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Ethics approval and consent to participate

Current study was compiled following the requirements verified by the Ethics Committee of the Shahid Behest University of Medical Sciences, as well as, informed written consent was obtained from all subjects before joining the study (Code No: IR.SBMU.RETECH.REC.1401.451).

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Conflicts of interest

There are no conflicts of interest.

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