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Lack of Telomerase Reverse Transcriptase Promoter C228T and C250T Hotspot Mutations in Colorectal Cancer Patients in Türkiye

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ABSTRACT Objective: Telomerase reverse transcriptase *(TERT)* is one of the catalytic subunits of the telomerase enzyme involved in the lengthening of telomeres during cell division. Two hotspot mutations in the promoter region of the *TERT* gene, C228T and C250T have been observed in many different types of cancer. Besides, a limited number of available studies are related to colorectal cancer. However, no study to date has analyzed these mutations in the Turkish population. Hence, this study aimed to determine the frequency of C228T and C250T hotspot mutations in Turkish patients with colorectal cancer. **Material and Methods:** Tumors and adjacent healthy tissues of 43 colorectal cancer patients were analyzed in the study material. After genomic DNA extraction, 163 bp DNA fragment of the *TERT* promoter region was amplified by polymerase chain reaction (PCR) method. PCR products were sequenced using the bi-directional Sanger technique and a wild-type *TERT* promoter sequence obtained from the National Center for Biotechnology Information database was used for the comparison and detection of mutations. **Results:** Sequence analysis revealed no mutations in the promoter region of the *TERT* gene in colorectal cancer tissues or in healthy tissues. **Conclusion:** These findings of the study suggest that colorectal cancer in the Turkish population is not associated with the *TERT* promoter C228T and C250T hotspot mutations.

Keywords: Colorectal cancer; *TERT*; promoter mutation; C228T; C250T

Colorectal cancer (CRC) is the third most common cancer in 2020, constitutes 10% of the new cancer cases, and accounts for approximately 930,000 deaths.[1] CRC arises from multi-step carcinogenesis involving sequential accumulation of numerous genetic and epigenetic alterations.[2,3] The genetic mechanisms involved in the genesis of CRC have not been fully elucidated yet.

Telomeres are the long stretches of tandemly repeating short DNA sequence TTAGGG at the ends of linear eukaryotic chromosomes.[4-6] In the human somatic cells, the telomere length varies from 5-15 kilobases and reduces by an average of 30-200 base pairs during each cell division. This telomere shortening causes aging or senescence and eventually cell death.[7,8] There are different approaches being explored to prevent telomere shortening; one of them is the upregulation of the telomerase gene.[9,10] Telomerase, a ribonucleoprotein complex composed of telomerase reverse transcriptase (TERT) protein and telomerase RNA component, loses efficiency in many somatic cells but has been found to be active in almost 80-90% of human carcinomas.[11] The human *TERT* gene is silenced in the somatic cell by epigenetic mechanisms; thus, the life span of these cells is shortened.[12,13]

The *TERT* [National Center for Biotechnology Information (NCBI) Entrez Gene ID: 7015] locus on chromosome 5p15.33 contains 16 exons and is approximately 40 kb in length.[5,14] The promoter region of *TERT* (accession number: KJ442845.1) lacks a TATA or CAAT box; instead, it contains a GC box sequence rich in G and C bases.[6] Promoter sequence of the *TERT* gene contains binding sites for many

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transcription factors and 2 different hotspot mutations, namely C228T and C250T, have been identified in this region. These mutations are C>T transitions located at -124 bp and -146 bp upstream of the translation initiation codon.[15,16]

Recently, the presence of *TERT* promoter hotspot mutations in different types of cancer, including melanoma, glioblastoma, hepatocellular carcinoma, cutaneous squamous cell carcinoma, oligodendroglioma, thyroid cancer, bladder cancer, was observed. The study also reported that these mutations increase the transcription of the *TERT* gene and thereby affect the prognosis of cancer patients.[15-23] Contrary to these findings, it was reported that there was a very low frequency of these mutations in some types of tumors, such as gastric cancer and esophageal squamous cell carcinoma.[24,25] Although these mutations are frequently seen in many tumor types, it is not yet clear whether they are adequate for activating the *TERT* gene in cancer cells or not. The promoter hotspot mutations of the *TERT* gene were investigated in patients with colorectal precursor lesions and in a small number of CRC patients from a few different countries. However, to the best of our knowledge, no study has evaluated these mutations in Turkish patients with CRC. Therefore, the present study aimed to investigate the presence of these 2 mutations in the *TERT* promoter region in Turkish patients with CRC.

MATERIAL AND METHODS

SAMPLE COLLECTION

The study protocol was approved by the Local Ethics Committee of Gaziantep University, Türkiye (ethical approved number: 2017/192, data: 08.05.2017) and conducted in accordance with the Declaration of Helsinki. All patients read and signed informed consent forms before participating in the study. Tissue samples (tumor and adjacent healthy tissue) were collected from 43 patients who were diagnosed with CRC and underwent a surgical operation at the General Surgery Department of the Gaziantep University Hospital, Gaziantep, Türkiye, between 2017 and 2018. The tissue samples were kept at -80 °C until genomic DNA extraction.

GENOMIC DNA EXTRACTION

Genomic DNA extraction was performed using the PureLink Genomic DNA Mini Kit (Cat.no. ≠k1820-02) (Invitrogen, USA) as per the manufacturer's instructions. The purity and concentrations of the extracted DNA samples were measured by using a spectrophotometer (NanoDrop, Maestrogen). DNA samples were kept at -20 °C till further analysis.

FRAGMENT SEQUENCING AND MUTATION ANALYSIS

A 163 bp fragment of *TERT* promoter, including C228T and C250T hotspot mutation sites, was amplified by polymerase chain reaction (PCR) analysis using the forward primer 5'-CAGCGCTGCCTGAAACTC -3' and reverse primer 5'-GTCCTGCCCCTTCACCTT -3'.[26] PCR was performed in a total of 40 µL of the reaction mixture comprised of 2.4 µL of each primer (10 nmol/µL) (Cat.No ≠10336-022, ThermoFisher, USA), 20 µL of 2X PCR master mix (Cat.no ≠K0171, ThermoFisher, USA), 1 µL of formamide (Applied Biosystem, USA), 12.6 µL deionized water and 1.6 µL of genomic DNA (100 ng/µL). The reaction mixture was subjected to initial denaturation at 95 °C for 5 min, followed by 40 cycles of a denaturation step at 95 °C for 30 s, an annealing step at 61 °C for 40 s, and an extension step at 72 °C for 30 s, and subsequently a final extension step at 72 °C for 10 min. The PCR products were sequenced using the bi-directional Sanger technique. DNA sequences were then compared with the wild-type *TERT* promoter sequence obtained from NCBI database by using the CLC Main Workbench 8.0.1 program (Qiagen, Denmark), and the mutational screen was performed.

RESULTS

DEMOGRAPHICS AND CLINICAL CHARACTERISTICS OF PATIENTS WITH CRC

The mean age of the patients with CRC was 53.6 years (range 26-84), and 60.5% of the patients were >50 years old. Of 43 patients, 29 were males, and 14 were females. Overall, 60.5% of the tissues were collected from the colon and the rest from the rectum (39.5%). Metastasis was observed in 23.3% of all

cases. 53.5% of the patients were defined as Stage I and II, and 46.5% as Stage III and IV. The clinicopathologic features of the patients with CRC are presented in Table 1.

TERT PROMOTER HOTSPOT MUTATIONS IN CRC

In the present study, 2 hotspot mutations of the *TERT* promoter region, C228T and C250T were screened in the tumor and adjacent non-tumor tissues of Türkiye patients with CRC. No *TERT* promoter hotspot mutations were detected in tumor or non-tumor tissues (Figure 1A and Figure 1B).

DISCUSSION

According to Globocan 2020 data, after coronary heart diseases, cancer is the second leading cause of death worldwide.[1] Cancer that occurs because of the accumulation of various types of mutations in cells is an inherited disease and characterized by uncontrolled cell proliferation.[8,27,28]

TABLE 1: The clinicopathological characteristics of CRC patients.

Variables	Patients (%)
Age (years)	
Mean 53.6 (range 26-84)	43 (100)
≤50	17 (39.5)
>50	26 (60.5)
Gender	
Male	29 (67.4)
Female	14 (32.6)
Tissue type	
Colon	26 (60.5)
Rectum	17 (39.5)
Smoking habit	
Yes	16 (37.2)
No	27 (62.8)
Stage of tumor	
I-II	23 (53.5)
III-IV	20 (46.5)
Lymph node metastasis	
Yes	19 (44.2)
No	24 (55.8)
Distant metastasis	
Yes	10 (23.3)
No	33 (76.7)

CRC: Colorectal cancer.

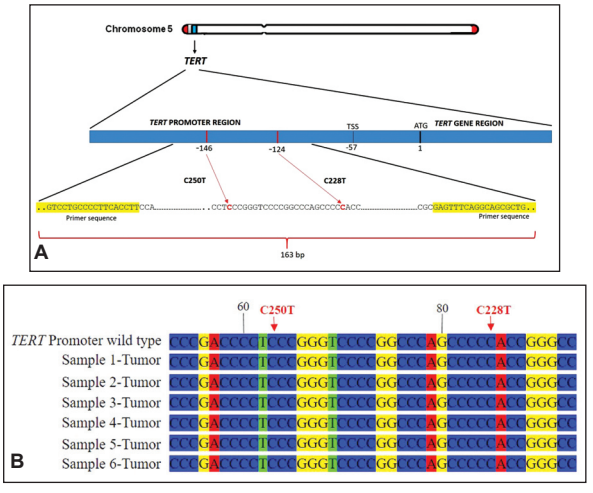

FIGURE 1: Schematic representation of C228T and C250T muations in *TERT* promoter region (TSS: Transcription start site) **(A)** Results of the sample sequences alignment with the wild-type sequence of the *TERT* promoter region **(B)** *TERT*: Telomerase reverse transcriptase.

The *TERT* plays an important role in tumorigenesis as well as in human diseases.[29] Approximately 80-90% of cancers are known to have enhanced telomerase activity.[30] The upregulation of the *TERT* gene is directly associated with telomerase activity and carcinogenesis. This upregulation may occur due to *TERT* promoter mutations, *TERT* promoter methylation, *TERT* gene amplification, epigenetic alterations, and alternative splicing of the *TERT* mRNA.[4,8] Previous studies have shown that the telomerase activity plays an important role in the prognosis of CRC.[7] Both C228T and C250T mutations, identified in the *TERT* promoter region, cause the formation of a new binding site for E-twenty-six transcription factors and thereby increases the expression level of the *TERT* gene.[5,31]

Hotspot mutations in the promoter region of the *TERT* gene were first identified in melanomas but were also frequently seen in various other tumors.[15,16,31] To the best of our knowledge, this is the first study investigating *TERT* promoter mutations in Turkish CRC patients. Two hotspot mutations of *TERT* promoter were not detected in the Turkish CRC patients enrolled in this study. Likewise, Killela et al. also did not find *TERT* promoter mutations in 22 colorectal adenocarcinoma samples.[18] Similarly, these mutations were also not found in Brazilian CRC patients.[32] Besides, *TERT* promoter mutations were

also not found in pheochromocytoma, gastrointestinal and kidney tumors, gastric cancer, colorectal precursor lesions, including tubular adenomas and serrated polyps.[7,33,34]

On the other hand, Siraj et al. reported *TERT* promoter mutations, -124C>T and -146C>T, in 13 different cancer types seen in Middle Eastern countries. They reported that *TERT* promoter mutations were most frequently detected in the 68.6% of bladder cancer, followed by 15.4% of thyroid cancer, 28.7% of nervous system tumors, 9.3% of prostate cancer, 3.7% of endometrial carcinoma, 1.4% of rhabdomyosarcoma, 1% of CRC, and 0.7% of breast cancer cases. However, in the same study, researchers did not observe *TERT* promoter mutations in acute lymphoblastic leukemia, diffuse large B cell lymphoma, gastric cancer, and lung cancer.[35] In addition, there are other studies reporting the presence of *TERT* promoter mutations in various types of cancer, including melanoma, glioblastoma, thyroid, head and neck, hepatocellular and bladder cancers.[15-20,23,30] Cevik et al. investigated *TERT* promoter mutations in patients with hepatocellular carcinomas living in different geographic regions, such as Asia, Africa found the highest mutation frequency in African patients.[36]

There are differences in the prevalence of *TERT* mutations observed in tissues of various origins, and the mechanisms that cause these differences have not been elucidated yet. Although no relationship was found between *TERT* promoter mutation and CRC in our study, numerous studies on different cancer types have shown that *TERT* promoter mutations can be useful in the disease prognosis.[15-23] However, the differences in the *TERT* promoter mutations were also observed in the same type of cancer across populations living in different geographies, which may be due to genetic predisposition and environmental conditions.

The primary limitation of this study was the small sample size. Further studies must be directed toward investigating the 2 hotspot mutations, along with other *TERT* promoter mutations in a larger patient population with CRC.

CONCLUSION

To date, multiple studies have been conducted to analyze *TERT* promoter mutations in different cancer types. For the first time, *TERT* promoter mutations were analyzed in Turkish CRC patients in this study, but *TERT* promoter C228T and C250T hotspot mutations were not observed. Consequently, it can be concluded that *TERT* mutations may not be associated with colorectal carcinogenesis.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Türkan Gürer;* ***Design:*** *Türkan Gürer;* ***Control/Supervision:*** *Türkan Gürer;* ***Data Collection and/or Processing:*** *Nisreen Al Doori* ***Analysis and/or Interpretation:*** *Türkan Gürer, Nisreen Al Doori;* ***Literature Review:*** *Türkan Gürer, Nisreen Al Doori;* ***Writing the Article:*** *Türkan Gürer;* ***Critical Review:*** *Türkan Gürer;* ***References and Fundings:*** *Gaziantep University;* ***Materials:*** *Alper Aytekin.*

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