

## Research Article | Araştırma Makalesi

# EXPLORING THE ROLE OF sFRP-4, TFF-3, NF-κB AND ROMO1 LEVELS IN COLORECTAL CANCER: IMPLICATIONS FOR PATHOPHYSIOLOGY AND DISEASE PROGRESSION

## KOLOREKTAL KANSERDE sFRP-4, TFF-3, NF-κB ve ROMO1 DÜZEYLERİNİN ROLÜNÜN ARAŞTIRILMASI: HASTALIĞIN PATOFİZYOLOJİSİ VE PROGRESYONUNA ETKİLERİ

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### ABSTRACT

**Objective:** Colorectal cancer (CRC) is a significant global health concern with high morbidity and mortality rates. Early detection and accurate diagnostic tools are critical for managing the clinical course. This research explores the molecular landscape of CRC, aiming to provide valuable insights beyond traditional diagnostic approaches. The main aim of this study was to investigate the potential contribution of specific biomarkers, such as secreted frizzled associated protein-4 (sFRP-4), trefoil factor-3 (TFF-3), nuclear factor-kappa-B (NF-κB) and reactive oxygen species modulator-1 (Romo1), to understanding the pathophysiology and determining the progression of CRC.

**Methods:** This study analyzed plasma levels of sFRP-4, TFF-3, NF-κB and Romo1 in a cohort of patients with CRC (n=50) and age- and gender-matched control group (n=40), utilizing ELISA. The diagnostic performance of these biomarkers was assessed through Receiver Operating Characteristic (ROC) analysis.

**Results:** Our research revealed a significant increase in the levels of NF-κB, TFF-3 and Romo1 in patients with a diagnosis of CRC. Furthermore, these parameters were found to maintain elevated levels in patients with tumors larger than 4 cm as opposed to those with smaller tumors. Patients with metastases also had elevated levels of the three parameters compared with patients without metastases. The ROC analysis revealed that NF-κB showed the most promise as a parameter for distinguishing patients from control subjects, whereas TFF-3 displayed the most potential in identifying tumor size and the presence of metastasis.

**Conclusion:** This research contributes valuable insights into understanding the pathophysiology and progression of CRC. The potential roles of NF-κB, TFF-3, and Romo1 as biomarkers, as revealed in our study, offer a promising avenue for early detection and improved management of CRC. Further validation and prospective studies are necessary to clarify the roles of these biomarkers in the pathophysiological mechanism of CRC and to establish their clinical utility.

**Keywords:** Colorectal cancer, NF-κB, Romo1, sFRP-4, TFF-3

### Öz

**Amaç:** Kolorektal kanser (KRK), yüksek morbidite ve mortalite oranları ile önemli bir küresel sağlık sorunudur. Erken teşhis ve doğru tanı araçları klinik seyirin yönetimi için kritik öneme sahiptir. Bu araştırma, geleneksel tanı yaklaşımlarının ötesinde bilgiler sağlamayı amaçlayarak KRK'nin moleküler manzarasını araştırmaktadır. Bu çalışmanın temel amacı, salgılanan frizzled ilişkili protein-4 (sFRP-4), trefoil faktör-3 (TFF-3), nükleer faktör-kappa-B (NF-κB) ve reaktif oksijen türleri modülatörü-1 (Romo1) gibi spesifik biyobelirteçlerin, KRK'nin patofizyolojisini anlamaya ve progresyonunu belirlemeye potansiyel katkılarını araştırmaktır.

**Yöntem:** Bu çalışmada, KRK'li hastalar (n=50) ile yaş ve cinsiyet açısından eşleştirilmiş kontrol grubunda (n=40) sFRP-4, TFF-3, NF-κB ve Romo1'in plazma düzeyleri ELISA yöntemi ile analiz edilmiştir. Bu biyobelirteçlerin tanısal performansı ROC analizi ile değerlendirilmiştir.

**Bulgular:** Araştırmamız, KRK tanısı alan hastalarda NF-κB, TFF-3 ve Romo1 seviyelerinde önemli bir artış olduğunu ortaya koymuştur. Ayrıca, bu parametrelerin 4 cm'den büyük tümörü olan hastalarda daha küçük tümörü olanlara kıyasla daha yüksek seviyelerde olduğu tespit edilmiştir. Metastazı olan hastalarda da metastazı olmayan hastalara kıyasla üç parametrenin seviyeleri de yüksek bulunmuştur. ROC analizi, NF-κB'nin hastaları kontrol bireylerinden ayırt etmek için potansiyeli en iyi biyobelirteç olabileceğini, TFF-3'ün ise tümör boyutunu ve metastaz varlığını belirlemede en fazla potansiyel gösterdiğini ortaya koymuştur.

**Sonuç:** Bu araştırma, KRK'nin patofizyolojisinin ve progresyonunun anlaşılmasına katkı sağlamaktadır. Çalışmamızda ortaya konduğu üzere NF-κB, TFF-3 ve Romo1'in biyobelirteç olarak potansiyel rolleri, KRK'nin erken teşhisi ve yönetimi için umut verici bir yaklaşım sunmaktadır. Bu biyobelirteçlerin KRK'nin patofizyolojik mekanizmasındaki rollerini netleştirmek ve klinik faydalarını belirlemek için daha fazla doğrulama ve ileriye dönük çalışmalar gereklidir.

**Anahtar Kelimeler:** Kolorektal kanser, NF-κB, Romo1, sFRP-4, TFF-3

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## Introduction

Colorectal cancer (CRC) stands as a significant global health challenge, with an escalating incidence and a substantial disease burden. CRC, which encompasses malignancies of the colon and rectum, is known for its silent progression, often remaining asymptomatic until the disease reaches advanced stages.<sup>1,2</sup> Early detection and precise diagnostic methods are pivotal in improving patient outcomes and reducing the impact of this disease.<sup>3</sup> Colonoscopy is recognized as the gold standard for the diagnosis of CRC, but its use is restricted by its invasiveness, the necessary bowel preparation, and the high costs. Although fecal immunochemical testing (FIT) is noninvasive and more affordable, it still lacks the accuracy required to effectively detect advanced adenoma. Despite the availability of novel therapies for CRC and enhanced survival rates, screening rates have not notably increased, highlighting the necessity for more accurate, non-invasive and tolerable screening tests.<sup>4,5</sup>

Secreted Frizzled Associated Protein-4 (sFRP-4), a member of the secreted frizzled-related protein family, has been implicated in Wnt signaling pathway modulation and is expressed in various tissues.<sup>6</sup> Since the cysteine-rich domain of sFRP-4 resembles the Wnt-binding site, it is predicted to be a tumor suppressor.<sup>7</sup> Studies have shown that sFRP-4 expression is generally down-regulated in CRC tissues and cell lines, supporting the view that it may function as a tumor suppressor.<sup>8,9</sup> However, Huang et al.<sup>10</sup> showed that sFRP-4 was overexpressed in CRC tissues compared to adjacent non-cancerous tissues. Moreover, sFRP-4 has been linked to the inhibition of epithelial-to-mesenchymal transition (EMT), a process where epithelial cells lose their adhesion properties and acquire invasive characteristics.<sup>11</sup> EMT is involved in cancer metastasis and the role of sFRP-4 in inhibiting this process suggests that it should be investigated as a CRC metastasis biomarker.

Trefoil Factor-3 (TFF-3), associated with mucosal protection and repair in the gastrointestinal tract, has displayed altered expression in CRC, hinting at its potential role as a diagnostic marker.<sup>12,13</sup> It has been suggested that elevated TFF3 tumor levels could be linked to reduced survival rates, lymph node metastasis, more advanced tumor stage, poor tumor differentiation, and increased clinical TNM stage.<sup>13,14</sup>

Nuclear Factor-Kappa-B (NF- $\kappa$ B), a transcription factor central to inflammatory processes and cancer, has been linked to the inflammatory microenvironment characteristic of CRC. NF- $\kappa$ B also can upregulate the expression of anti-apoptotic genes, enabling cancer cells to evade programmed cell death. This resistance to apoptosis contributes to tumor survival and growth.<sup>15,16</sup> In addition, it has been associated with the induction of epithelial-to-mesenchymal transition (EMT), a process that enhances the invasive properties of cancer cells and their ability to metastasize.<sup>17</sup>

Reactive Oxygen Species Modulator-1 (Romo1), is a mitochondrial membrane protein and associated with oxidative stress and cell proliferation, holds promise as a marker due to the oxidative stress commonly observed in CRC.<sup>18</sup> ROMO1 has also been reported to have a regulatory effect on NF- $\kappa$ B-dependent activation of EMT factors.<sup>19</sup>

In recent years, biomarker research has garnered significant attention, offering a promising avenue for the detection of CRC. Such markers can be detected through blood-based tests, thereby providing an easily accessible, non-invasive, and cost-effective approach to diagnosis and screening. This research explores the molecular landscape of CRC, aiming to provide valuable insights beyond traditional diagnostic approaches. The objective of this study was to examine the potential roles of sFRP-4, TFF-3, NF- $\kappa$ B, and Romo1 in gaining insight into the molecular mechanisms linked to CRC pathophysiology and progression.

## Methods

### Study Groups

The study protocol was approved by the local Ethics Committee of the Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa (No: 20502, date:04/07/2018), and it was conducted in accordance with the Declaration of Helsinki. Fifty individuals diagnosed with CRC (25 female (50%), 25 males (50%), mean age: 51.74 $\pm$ 7.19) and forty age- and gender-matched healthy individuals (20 females (50%), 20 males (50%), mean age: 49.93 $\pm$ 5.15) without a history of cancer were enrolled in the study. Patients were selected from Department of Internal Medicine, Division of Oncology, and Department of General Surgery, Faculty of Cerrahpasa Medicine, Istanbul University Cerrahpasa. Informed written consent was obtained from all participants prior to sample collection. Patients with primary CRC were included in the study. All patients were aged 25-70 years, had not received chemotherapy or radiotherapy, and had no secondary malignancies. Patients who underwent surgery, received chemotherapy and radiotherapy were not included in the study.

Venous blood samples were collected from each participant into EDTA-coated vacutainer tubes using aseptic techniques. Blood samples were obtained in the morning after an overnight fast. Blood samples were immediately centrifuged for 15 min at 3000 RCF at 4°C to separate plasma. The resulting plasma samples were carefully collected and stored at -80°C until further analysis.

Plasma TFF-3, Romo-1, NF- $\kappa$ B and sFRP-4 levels were measured by commercial ELISA kits (Human TFF3 ELISA kit, Cat. No: E-EL-H1108; Human ROMO1 ELISA kit, Cat. No: E-EL-H5430; Human NF- $\kappa$ B-p65 kit, Cat. No. E-EL-H1388; Human sFRP4 ELISA kit, Cat. No: -EL-H5447, respectively, Elabscience Biotechnology Inc., USA),

based on sandwich principle, according to the manufacturer's instructions.

The sensitivity of commercial assay kits for Romo-1, NF-κB and SFRP-4 were 0.10 ng/mL. The sensitivity of commercial assay kit for TFF-3 was 46.88 pg/mL. The intra- and inter-assay variation coefficients for all parameters were less than 10.

**Statistical Analysis**

All statistical analysis was performed with SPSS version 29.0 (IBM SPSS Statistics for MacOs, IBM Corp. USA). Categorical variables were presented as percentages or frequencies and were compared using Pearson's χ<sup>2</sup> tests. Continuous variables were described as mean values along with their corresponding standard deviations. The normal distributions of the variables were determined by histogram and/or by Kolmogorov–Smirnov/Shapiro–Wilk's test. The mean differences between the parameters among groups was determined by student t-test. The univariate associations were evaluated by Spearman's rank correlation analysis. The study assessed the sensitivity and specificity of the measured variables as biomarkers through a receiver operating characteristic (ROC) curve analysis. Cut-off values were determined by using the Youden's J Index. A two-sided p-value less than 0.05 was considered significant. In our study, the post-hoc power analysis for NF-κB, Romo-1 and TFF-3 shows that it reached a power of over 95%, indicating that the study has sufficient power to detect the observed effect size at an alpha level of 0.05.

**Results**

Clinicopathological features of patients with CRC are shown in Table 1. Tumor, lymph node, metastasis (TNM) stages of all patients were determined according to the American Joint Committee on Cancer (AJCC) TNM staging system.<sup>20</sup> In this study, 10 patients were

identified with TNM stage I, 21 with TNM stage II, 10 with TNM stage III, and 9 with TNM stage IV. In 28 patients, the tumor size exceeded 4 cm, and 17 of them had metastasis.

**Table 1.** Clinicopathological features of patients with CRC (n=50)

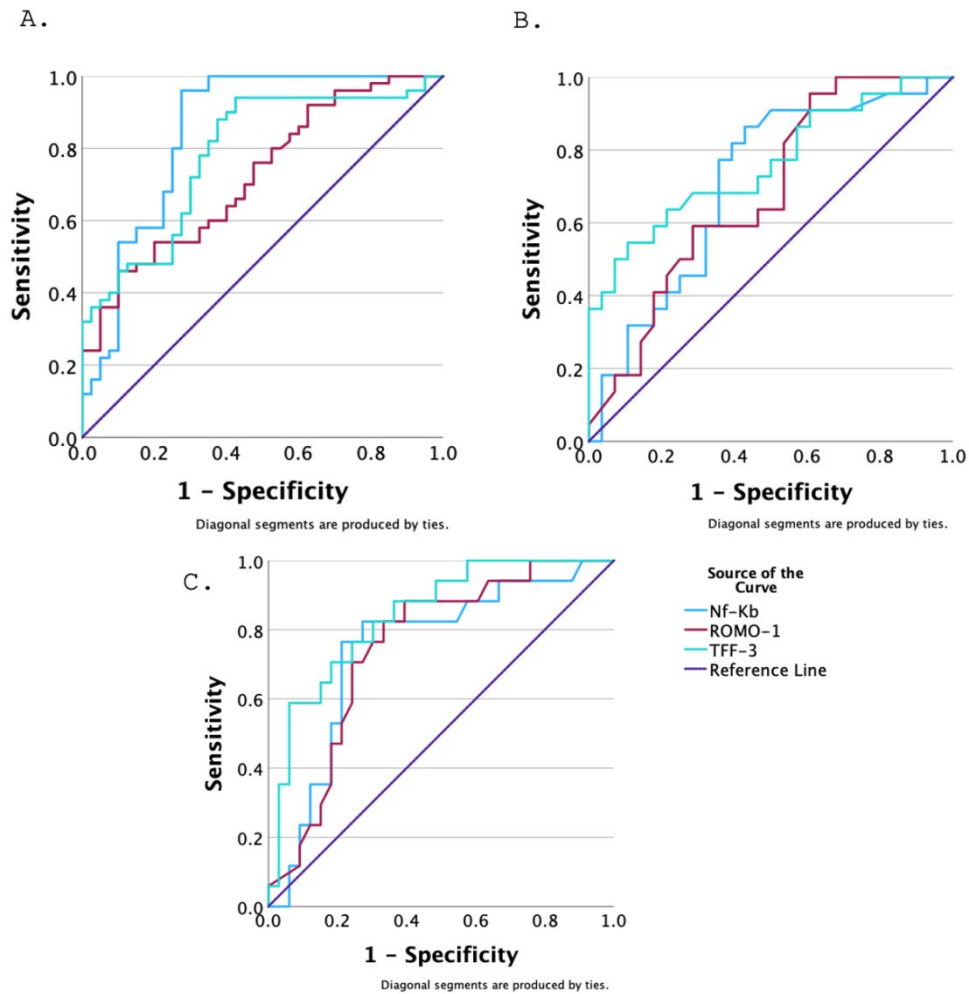
| Variables         | n (%)  |
|-------------------|--|
| TNM Stage         |  |
| I/II/III/IV       | 10 (20.0%) / 21 (42.0%) / 10 (20.0%) / 9 (18.0%) |
| Tumor Size        |  |
| ≤4 cm             | 22 (44.0%)                                       |
| >4 cm             | 28 (56.0%)                                       |
| Metastasis Status |  |
| Yes               | 17 (34.0%)                                       |
| No                | 33 (66.0%)                                       |

Demographic characteristics, tumor markers and biochemical parameters levels in patients with CRC and controls are summarized in Table 2. The mean age of CRC patients 51.74±7.19 years, and of controls 49.93±5.15 years. The ratio of female to male was the same in both the CRC patients and the controls. All three tumor marker levels (CA-15.3, CA-19.9 and CEA) were significantly elevated among patients with CRC. Our results reveal a significant increase in the plasma levels of NF-κB, Romo-1, and TFF-3 in CRC patients in comparison to healthy controls (all p<0.001, Table 2). There was no significant difference observed in the levels of sFRP-4 between the patients with CRC and the control subjects. ROC analysis demonstrated that all three parameters have the potential to serve as biomarkers for distinguishing CRC patients from control subjects. Notably, NF-κB was identified as the most significant parameter in terms of AUC values for distinguishing CRC patients from healthy controls (Figure 1-A and Table 5-A).

**Table 2.** Demographic characteristics, tumor markers and biochemical parameters levels in patients with CRC and controls

|  | CRC patients (n=50) | Controls (n=40)     | p      |
|--|---------------------|---------------------|--------|
| <b>Demographic characteristics</b>             |                     |                     |        |
| Mean age (mean±SD) (years)                     | 51.74±7.19          | 49.93±5.15          | 0.167  |
| Female/male ratio (% ,n)                       | 1 / 1 (25 F / 25 M) | 1 / 1 (20 F / 20 M) | 1.00   |
| <b>Tumor marker levels (mean±SD)</b>           |                     |                     |        |
| CA-15.3 (U/mL)                                 | 14.73±4.78          | 11.04±3.07          | <0.001 |
| CA-19-9 (U/mL)                                 | 26.34±9.06          | 4.44±2.12           | <0.001 |
| CEA (ng/mL)                                    | 6.84±8.77           | 1.52±0.59           | <0.001 |
| <b>Biochemical parameters levels (mean±SD)</b> |                     |                     |        |
| NF-κB (ng/mL)                                  | 4.75±2.13           | 2.29±1.88           | <0.001 |
| sFRP-4 (ng/mL)                                 | 1.68±1.13           | 1.90±0.74           | 0.137  |
| Romo-1 (ng/mL)                                 | 2.69 ±1.27          | 1.68±1.02           | <0.001 |
| TFF-3 (pg/mL)                                  | 3379.53±864.54      | 2319.96±916.17      | <0.001 |

CA-15.3: Cancer antigen 15.3, CA-19.9: Cancer antigen 19.9, CEA: Carcinoembryonic antigen, NF-κB: Nuclear Factor kappa B, SFRP-4: Secreted frizzled-related protein 4, Romo-1: Reactive oxygen species modulator 1, TFF-3: Trefoil Factor 3



**Figure 1.** Comparisons of the sensitivity and specificity of the diagnosis by biochemical parameters (NF-κB, Romo-1, TFF-3) and tumour markers (CA-15.3, CA-19.9 and CEA)

The ROC curve of A. CRC versus healthy controls; B. Tumor size > 4 cm versus tumor size ≤ 4 cm; C. Patients with metastasis versus patients without metastasis.

When patients with CRC were classified according to tumor size, significant increases in plasma NF-κB, Romo-1 and TFF-3 levels were observed in patients with tumors larger than 4 cm compared to those with tumors smaller than 4 cm ( $p=0.025$ ,  $p=0.017$  and  $p<0.001$ , respectively; Table 3). We also found that plasma levels of all three parameters were higher in CRC patients with

metastases compared to CRC patients without metastases ( $p=0.027$ ,  $p=0.004$  and  $p<0.001$ , respectively; Table 4). The ROC analysis revealed that TFF-3 displayed the most potential in identifying tumor size and the presence of metastasis (Figure 1-B, -C and Table 5-B, -C).

**Table 3.** Biochemical parameter levels in subgroups formed according to tumor size in CRC patients

| Parameters     | Tumor size ≤ 4 cm (n=22) | Tumor size > 4 cm (n=28) | p      |
|----------------|--------------------------|--------------------------|--------|
| NF-κB (ng/mL)  | 4.23±1.87                | 5.41±2.29                | 0.025  |
| sFRP-4 (ng/mL) | 1.78±1.15                | 1.57±1.11                | 0.260  |
| Romo-1 (ng/mL) | 2.35 ±1.27               | 3.11±1.16                | 0.017  |
| TFF-3 (pg/mL)  | 3032.68±825.79           | 3820.97±708.99           | <0.001 |

**Table 4.** Biochemical parameter levels in subgroups formed according to metastasis status in CRC patients

| Parameters     | Patients with metastasis (n=17) | Patients without metastasis (n=33) | p      |
|----------------|---------------------------------|------------------------------------|--------|
| NF-κB (ng/mL)  | 5.66±2.18                       | 4.28±1.97                          | 0.027  |
| sFRP-4 (ng/mL) | 1.40±1.23                       | 1.83±1.06                          | 0.103  |
| Romo-1 (ng/mL) | 3.39 ±1.04                      | 2.32±1.24                          | 0.004  |
| TFF-3 (pg/mL)  | 3988.07±539.80                  | 3066.04±837.09                     | <0.001 |

**Table 5.** AUC, specificity, sensitivity and cut-off values of NF-κB, Romo-1 and TFF-3

| Variables   | AUC   | p      | 95% CI (LB-UB) | Cut-off value | Sensitivity | Specificity |
|---|-------|--------|----------------|---------------|-------------|-------------|
| <b>A. CRC versus healthy controls</b>                                 |       |        |                |               |             |             |
| NF-κB   | 0.848 | <0.001 | 0.760-0.936    | 2,88          | %96         | %72.5       |
| Romo-1  | 0.724 | <0.001 | 0.621-0.827    | 2.99          | %46         | %90         |
| TFF-3   | 0.785 | <0.001 | 0.689-0.880    | 2195.40       | %94         | %57.5       |
| <b>B. Tumor size &gt; 4 cm versus tumor size ≤ 4 cm</b>               |       |        |                |               |             |             |
| NF-κB   | 0.705 | 0.014  | 0.558-0.851    | 3.40          | %86.4       | %57.1       |
| Romo-1  | 0.682 | 0.029  | 0.535-0.829    | 1.70          | %95.5       | %39.3       |
| TFF-3   | 0.761 | 0.002  | 0.624-0.897    | 3938.45       | %54.5       | %89.3       |
| <b>C. Patients with metastasis versus patients without metastasis</b> |       |        |                |               |             |             |
| NF-κB   | 0.745 | 0.005  | 0.598-0.892    | 4.41          | %76.5       | %78.8       |
| Romo-1  | 0.750 | 0.004  | 0.613-0.888    | 2.55          | %82.4       | %66.7       |
| TFF-3   | 0.841 | 0.000  | 0.730-0.953    | 3971.46       | %58.8       | %93.9       |

CI: Confidence Interval; LB: Lower Bound; UB: Upper Bound

Tumor size was positively correlated with Nf-κB, Romo-1 and TFF-3 ( $r=0.352$ ,  $p=0.012$ ;  $r=0.313$ ,  $p=0.027$  and  $r=0.448$ ,  $p<0.001$ , respectively). Among these parameters, the strongest positive correlation was found between tumor size and TFF-3. Also, NF-κB levels were positively correlated with TFF-3 levels ( $r=0.503$ ,  $p<0.001$ ).

**Discussion**

The exploration of biomarkers for colorectal cancer (CRC) diagnosis is paramount in advancing our understanding of this prevalent malignancy and improving clinical outcomes. In this study, the investigation into specific biomarkers, including secreted frizzled associated protein-4 (sFRP-4), trefoil factor-3 (TFF-3), nuclear factor-kappa-B (NF-κB), and reactive oxygen species modulator-1 (Romo1), has provided valuable insights into their potential roles in the pathophysiological mechanisms of CRC. The observed elevation of NF-κB, TFF-3, and Romo1 levels in CRC patients, particularly in those with larger tumors and metastases, underscores their relevance in understanding disease progression. These findings appear to be consistent with the literature and suggest their potential as contributors to the broader understanding of CRC pathophysiology.

There is growing evidence supporting the significance of NF-κB as a major mediator between inflammation and cancer as well as regulation of cell differentiation, proliferation, and apoptosis.<sup>21,22</sup> The activation of NF-κB signaling can occur through canonical or non-canonical pathways which have distinct roles in tumor progression. In particular, the canonical pathway has been extensively studied and found to have anti-apoptotic and immunomodulatory functions in response to the tumor microenvironment, whereas the non-canonical pathway plays a critical role in the maintenance of cancer stem cells and tumor re-initiation.<sup>23</sup> Supporting the results of our study,

researchers have reported that activated NF-κB is associated with metastasis and poor clinical outcome in CRC patients.<sup>24,25</sup>

The deregulation of the NF-κB signaling pathway has led to Romo1 being implicated in the invasion of cancer cells. Inhibition of the non-canonical NF-κB pathway, notably with the inhibitor of κB kinase (IKK), was demonstrated to counteract the invasive effects triggered by Romo1.<sup>18</sup> Romo1 is a crucial protein situated in the mitochondrial membrane that regulates ROS levels by altering the inner membrane potential and thereby impacting its permeability.<sup>26-28</sup> Overexpression of this protein has been reported in various cancers, including CRC, and has been associated with lymphatic metastasis and poorer prognosis.<sup>18,26,27,29,30</sup> In our study, we have shown that Romo1 is overexpressed in CRC patients and the elevation increases with increasing tumour size and is associated with metastasis in CRC patients. Studies have reported elevated levels of Romo1 in CRC patients, consistent with our findings and supporting the investigation of Romo1's potential role in the disease.<sup>18,26</sup> To the best of our knowledge, data on Romo1 levels in CRC are very limited in the literature. And yet, on the other hand, a study suggests that targeting Romo1 could have significant anti-cancer effects and help develop new drugs for CRC.<sup>31</sup> We found that plasma Romo-1 levels were not correlated with NF-κB levels. Therefore, we propose that the mechanism may operate via an independent signalling pathway to NF-κB in patients with CRC.

The correlation between elevated levels of plasma TFF-3 and NF-κB in individuals with CRC implies that TFF-3 has the ability to initiate the NF-κB signalling pathway in CRC. Consistent with our hypothesis, Chen et al.<sup>32</sup> found that TFF3 can induce NF-κB activation to inhibit apoptosis of gastric cancer cells. Another study reported a positive correlation between abnormal expression of TFF3 in gastric cancer and NF-κB p65 expression.<sup>33</sup> In accordance with our findings, recent research has revealed that elevated TFF-3 expression levels are

related to metastasis, depth of invasion, TNM stage, poor prognosis and recurrence.<sup>13,34</sup> It has been suggested that the possible role of TFF3 in CRC in carcinogenesis, tumor proliferation, infiltration, metastasis and angiogenesis is mediated by inducing PTGS2 expression mainly by activating STAT3 signalling.<sup>35</sup> In another study, it was reported that TFF3 is a novel regulator of EP4 expression, which plays the most important role in tumorigenic mechanisms, and STAT3 signaling is responsible for TFF3-induced EP4 expression.<sup>36</sup> However, in contrast to our results and these studies in the literature, Kondo et al.<sup>37</sup> found that low TFF3 expression in rectal mucosa was associated with ulcerative colitis-related cancer development. These conflicting results may have occurred due to potential bias arising from the small sample size of patients included.

Some research indicates that sFRP-4, which mainly inhibits Wnt signalling, may also enhance of NF-κB activity.<sup>38</sup> On the other hand, it has been reported that sFRP-4 may also inhibit cancer metastasis by inhibiting EMT.<sup>11</sup> While there is evidence for an increase in sFRP4 expression in various cancers, some studies have shown a decrease.<sup>39–41</sup> In our study, sFRP4 levels did not differ in CRC patients compared to healthy individuals, this may be due to the limited number of patients. It is important to note the discrepancies in findings and further research is required to fully understand the role of sFRP4 in cancer.

Our findings not only contribute to the elucidation of the pathophysiology and progression of CRC, but also hold promise for the development of effective non-invasive diagnostic approaches. The ROC analysis revealed the promising diagnostic performance of NF-κB, particularly in distinguishing CRC patients from the control group, emphasizing its potential as a robust biomarker. Furthermore, TFF-3 emerged as a noteworthy indicator, displaying efficacy in identifying both tumor size and the presence of metastasis. The consistent elevation of these biomarkers in larger tumors and metastatic cases reinforces their potential utility in aiding prognostic assessments, guiding treatment decisions and predicting disease progression. Since metastasis is the primary cause of cancer-related deaths, the survival rate of patients can be enhanced by targeting TFF-3.

NF-κB, TFF-3 and Romo1, which are implicated in CRC pathophysiology and progression, offer a promising avenue for early detection, a critical factor in improving patient outcomes. The potential clinical utility of these biomarkers is underscored by their ability to provide valuable information regarding tumor characteristics and metastatic status. However, further validation through large-scale and prospective studies is essential to establish their reliability and clinical applicability. Integration of these biomarkers into CRC diagnostic, screening and management of disease programs holds promise for advancing personalized medicine and enhancing the overall management of this global health concern.

### Compliance with Ethical Standards

The study protocol was approved by the local Ethics Committee of the Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa (No: 20502, date:04/07/2018)

### Conflict of Interest

The authors declare that they have no conflict of interest.

### Author Contribution

Concept: SD and HU, Design: SD and HU, Data Collection and Processing: SD, BPK, CP, Analysis and Interpretation: SD, HU, RG, BPK and CP, Literature Search: SD, HU and RG, Writing: SD, Reviewing and Editing: SD, BPK, CP, RG and HU. All the authors read and approved the final manuscript.

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