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**Araştırma Makalesi /Research Article**

# Could FABP1 be Associated with Advanced Stages of Tumorigenesis, as Lymph Node Metastasis in Colorectal Tumors?

# FABP1 Kolorektal Tümörlerde Lenf Nodu Metastazı Gibi Tümörigenezin İleri Aşamaları ile İlişkili Olabilir mi?

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**Öz**

**Amaç:** Serbest yağ asidi bağlayıcı proteinler 1 (FABP1) çeşitli metabolik aktivitelerde önemli bir rol oynar ve başta kolorektal kanser olmak üzere birçok kanserde eksprese edilir. Bu çalışmada, tümörün histopatolojik evrelerine göre FABP1'in tümör ve tümör mikroçevresindeki ekspresyon düzeylerini değerlendirmeyi amaçladık.

**Gereç ve Yöntem:** İstanbul Eğitim ve Araştırma Hastanesi Genel Cerrahi Kliniğinden toplanan toplam 42 doku (21 kolorektal tümör ve 21 tümör mikroçevresi (TMÇ) dokusu) RNA izolasyonuna kadar -80 °C'de saklandı. RNA izolasyonundan sonra konsantrasyon ve saflık belirlendi. Daha sonra RNA'lardan cDNA sentezi gerçekleştirildi. FABP1 ekspresyon düzeyi qPCR yöntemi ile belirlendi ve elde edilen sonuçlar istatistiksel olarak değerlendirildi.

**Bulgular:** FABP1 ekspresyonu hem tümörde hem de TMÇ'de gözlenmiştir. Tümörde TMÇ'ye kıyasla biraz daha yüksek FABP1 ekspresyonu gözlenmiştir (p>0.07). FABP1 ekspresyonu, erken tümör evresine kıyasla ileri tümör evresinde 3.76 kat ve uzak organ metastazı olan hastalarda olmayanlara kıyasla 10.26 kat daha yüksek gözlenmiştir (sırasıyla, p=0.09; p=0.06). FABP1 ekspresyonunun lenf nodu metastazı olan hastalarda olmayanlara kıyasla yaklaşık 6.43 kat daha yüksek olduğu istatistiksel olarak belirlenmiştir (p=0.04).

**Sonuç:** FABP1, kolorektal kanserin ileri evrelerinde aşırı ekspresyonu nedeniyle metastaz ve invazyon süreçleriyle ilişkili olabilir. Bu artan ekspresyon, ileri evredeki tümörün bağışıklık profiliyle yakından ilişkili olabilir ve tümöre metastatik yetenek kazandıran süreçlere katkıda bulunabilir.

**Anahtar Kelimeler:** Kolorektal Kanser, Tümör mikroçevresi, Histopatolojik evre, FABP1, Metastaz

**Abstract**

**Background:** Free fatty acids binding protein 1 (FABP1) plays an important role in various metabolic activities and is expressed in many cancers, especially colorectal cancer. In this study, we aimed to evaluate the expression levels of FABP1 in the tumor and tumor microenvironment according to the histopathological stages of the tumour.

**Materials-Methods:** A total of 42 tissues (21 colorectal tumours and 21 tumour microenvironment (TME) tissues) collected from the General Surgery Clinic of Istanbul Training and Research Hospital were stored at -80 °C until RNA isolation. After RNA isolation, concentration and purity were determined. Then, cDNA synthesis was performed from RNAs. The FABP1 expression level was determined by the qPCR method, and the results obtained were statistically evaluated.

**Result:** FABP1 expression was observed in both tumor and TME. Slightly higher FABP1 expression was observed in tumor compared to TME (p>0.07). FABP1 expression was observed to be 3.76 times higher in the advanced tumor stage compared to the early tumor stage and 10.26 times higher in patients with distant organ metastasis compared to those without (Respectively, p=0.09; P=0.06). It was statistically determined that FABP1 expression was approximately 6.43 times higher in patients with lymph node metastasis than those without (p=0.04).

**Conclusion:** FABP1 may be associated with metastasis and invasion processes due to its overexpression in advanced stages of colorectal cancer. This increased expression is closely related to the immune profile of the tumor in the advanced stage and may contribute to the processes that give the cancer metastatic ability.

**Keywords:** Colorectal Cancer, TME, Histopathological stage, FABP1, Metastasis

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## INTRODUCTION

Free fatty acids (FAs) are molecules with both hydrophobic and hydrophilic properties and can have cytotoxic effects. They mostly show these effects on membranes such as mitochondria and lysosomes. They can inhibit the activities of systems such as cell surface receptors and enzyme-dependent kinases[1]. Free fatty acids are secreted from the liver and adipose tissue during the mobilization processes of fatty acids. The liver contains specific binding proteins to limit esterified and free fatty acids. FABPs have broad specificity, including the ability to bind long-chain (C16-C20) fatty acids, eicosanoids, bile salts, and peroxisome proliferators. Nine FABP genes have been identified in the human genome, including liver (L-FABP), intestine (I-FABP), heart (H-FABP), the fat cell (A-FABP), epidermal (E-FABP), ileal (I-FABP), brain (B-FABP), myelin (M-FABP) and testis-FABP (T-FABP). These different isoforms are named according to the organ in which they were first identified or predominant, but they generally show widespread expression in all tissues[2,3].

FABP1 is the first discovered member of the FABP family[4]. The L-FABP gene is located on chromosome 2p11.2. FABPs play a key role in some important mechanisms in the liver, such as the binding, transport, and processing of long-chain fatty acids, which are essential for various metabolic activities[5].

Dysregulation of L-FABP expression has begun to be associated with different tumor types. FABP1 binds to long-chain fatty acids, fatty acyl-CoA[6,7]. Expression levels of FABP3, another member of the FABP family, have been shown to correlate with tumor size, and lower FABP3 levels have a better prognosis in prostate tumors[8]. FABP5 has been found to promote angiogenesis in prostate cancer[9], and FABP9 is highly expressed in prostate cancer cell lines, and this increase is associated with survival[10]. Similarly, FABP7 expression was correlated with prostate cancer, while suppression of FABP7 resulted in growth inhibition in SK-RC-7/10 cell lines[11]. When different experimental and clinical studies were examined for FABP1 in solid organ cancers, quite strong associations were found. FABP1 positivity was detected at different levels in hepatocellular carcinoma[12], lung cancer[13], colorectal cancer[14], gastric adenocarcinoma[15], various kidney cancer subtypes[16] and pancreatic cancer[17]. Downregulation of FABP1 expression levels may lead to changes in tumor progression, and there are results that it is associated with poor differentiation and high expression of β-catenin and glutamine synthase in hepatocellular carcinoma[18].

Colorectal cancer is the third most common cancer worldwide[19]. It's more common in men and developed countries[20]. Hereditary and environmental factors play an important role in colorectal cancer development[21]. This cancer is mostly sporadic[22]. Environmental risk factors include smoking[23], excessive alcohol intake[24], increased bodyweight[25], processed meat intake[26] and some bacterial species (*Fusobacterium nucleatum and Bacteroides fragilis*)[27]. There are two main distinct precursor lesion pathways for colorectal cancer: the traditional adenoma–carcinoma and serrated neoplasia pathways. 70-90% of colorectal cancers develop through the traditional pathway[28]. Molecular biomarkers are an important part of the diagnosis and treatment process in sporadic colorectal cancer. In this context, since FABP1 is positive in many solid organ cancers, especially colorectal cancer, in the literature, the evaluation of FABP1 expression in tumor and tumor microenvironment samples of colorectal cancer cases according to histopathological stages constitutes the primary purpose of this study.

## MATERIALS AND METHODS

### Patient and Sample Collections

This study was approved by the Istanbul Faculty of Medicine Clinical Research Ethics Committee (Approval code: 1015). The General Surgery Clinic of Istanbul Training and Research Hospital collected 42 tissue samples, including 21 colorectal tumors and 21 tumor microenvironment tissues. The collected tissues were stored at -80 until RNA isolation. Tissues were subjected to histological evaluation after hematoxylin-eosin staining according to AJCC 8th Edition and TNM evaluation with other macroscopic findings.

### Molecular Analysis

A tissue homogenate was created using ceramic bead tubes (Lysing Matrix D 2 ml MP BIO) for 50 mg tissue according to the manufacturer's instructions. RNA was obtained after alcohol-based washing and elution via a spin column filter according to the kit instructions (GeneAll

Cat No./ID: 305101). Measurements were performed using a spectrophotometer (NanoDrop 8000, DE 19810, Thermo Fisher, USA). cDNA synthesis was performed following the kit's instructions containing the Reverse Transcription enzyme (A.B.T.™ GenEx SYBR Assay, South Korea). Expression of the detected FABP1 gene was performed using quantitative real-time PCR (RT-PCR) with Sybr Green PCR Master mix (A.B.T.™) with qRT–PCR Rotor-Gene Q (Qiagen). According to the assay protocol, the primer annealing temperature was set to 62°C, and 40 qRT–PCR cycles were performed. Ct values were evaluated using formula $2^{-\Delta\Delta Ct}$ to calculate the relative fold change. This calculation used glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control in all tissues.

### Statistical Analysis

In the statistical analysis of FABP1 gene expression levels of tumor and TME, the Mann-Whitney U test was applied using the GraphPadPrism 5 program. The distribution and statistical comparison of FABP1 according to histopathological parameters were evaluated with the SPSS 22nd Version using the Mann-Whitney U test due to the low sample size and non-normal distribution. The significance limit was determined to be 0.05 in all statistical evaluations.

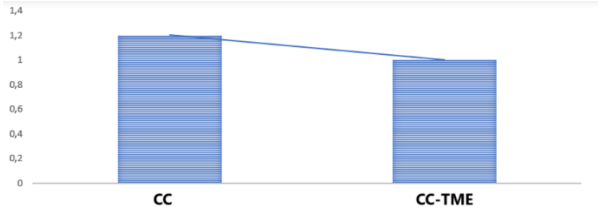
## RESULT

High FABP1 expression was observed in the tumor focus compared to TME, and the difference was statistically insignificant (p=0.07). There were 12 patients with early tumor stage (T1+T2), and FABP1 expression change was observed 1.70 times compared to the reference gene. There were 19 patients with advanced tumor stage (T3+T4), and the expression difference was 6.40 times. Although approximately 3.76 times difference was observed in the advanced tumor stage compared to the early tumor stage, it was not statistically significant (p=0.09). It was followed in 7 cases without lymph node metastasis, and FABP1 expression was detected 1.30 times compared to the reference gene. In 14 cases with lymph node metastasis, expression was detected as 8.36 times higher. FABP1 expression was detected as approximately 6.43 times higher in

The difference was statistically significant for those with lymph node metastasis compared to those without (p=0.04). While the expression change was 2.03 in 11 cases without distant organ metastasis, it was determined as 10.26 in those with distant organ metastasis. Although approximately a 5-fold FABP1 expression increase was observed in those with distant organ metastasis compared to those without, the difference could not be confirmed statistically (p=0.06). All these evaluations are shown in Table 1 and Figure 1.

**Table 1.** Relative FABP1 expression levels in colorectal patient samples. Comparison of FABP1 levels between early and late-stage tumor level, lymph node metastasis and distant metastasis

| Histopathological parameters | N (Number of Patients) | Relative FABP1 Gene Expression Level ±SE | Fold Change | P Value |
|---|---|---|---|---|
| TUMOR STAGE | | | | |
| Early Tumor Stage (T1+T2) | 2 | 1.70 ±0.16 | 3.76 | 0.091 |
| Advanced Tumor Stage (T3+T4) | 19 | 6.40 ±0.34 | | |
| LYMPH NODE METASTASIS | | | | |
| Met Absence (NO) | 7 | 1.30 ±0.54 | 6.43 | 0.043 |
| Met Presence (N1+N2+N3) | 14 | 8.36 ±0.45 | | |
| DISTANT METASTASIS | | | | |
| Distal Met Absence (MO) | 11 | 2.03 ±0.10 | 5.05 | 0.067 |
| Distal Met Presence (M1) | 10 | 10.26 ±0.60 | | |




***Abbreviations:*** *CC; Colorectal Cancer, TME; Tumor microenvironment*

**Figure 1:** Comparison of FABP1 expression between tumor and TME

## DISCUSSION

A study evaluating FABP1 positivity in 76 different tissues and 150 tumor types using immunohistochemistry found 86% positivity in colorectal adenocarcinomas and 65.3% in adenomas. At the same time, strong FABP1 positivity is also striking in healthy colon tissues. Decreased FABP1 expression in colorectal cancer was associated with low differentiation, right-sided tumor localization, microsatellite instability, and the absence of BRAF V600E mutation. However, no association was found between tumor stage and lymph node metastasis in this study[29].

In our results, FABP1 expression was detected in both colorectal tumors and TMEs of all cases. Contrary to this study, FABP1 expression was approximately 6.4 times higher in patients with lymph node metastasis than those without. This suggests that FABP1 should be expressed in both the tumor and the cells that form the TME as part of the physiological process, and that there should be a balance between this and increased FABP1 in the tumor, which may provide the cell with metastatic ability. At this point, it suggests that FABP1 may be a biomarker that can be used for metastatic colorectal cancer after further studies.

In parallel with the increase in FABP1 expression in colorectal cancer cases that we found to be associated with lymph node metastasis, it was found that FABP1 expression intensity was critically increased in gastric cancer cases with lymph node metastasis[30]. While Lawrie et al. did not find a significant correlation between FABP1 levels in tumor stages[31], our study supports this state. Lawrie et al. reported the presence of FABP1 expression in colorectal tumors, but no expression in TME members such as stroma, endothelial, and immune cells[31]. According to the results of our study, the presence of FABP1 expression in colorectal TME and tumors and the fact that it is generally dominant in tumors do not support Lawrie et al.'s study.

Epigenetic regulators were examined in the transformation of colorectal serrated lesions into microsatellite unstable colorectal cancer, and results were obtained indicating that the miRNA-6753-3p/FABP1 axis contributes to this process[32]. In single-cell transcriptomic analyses performed on GEO datasets, 342 distinctive transcripts were identified between normal tissue and colorectal tissue, and one of these was FABP1, which ranked 10th among the top 20 transcripts[33]. These results suggest that FABP1 is present in colorectal tissue as part of the biological process and is dysregulated during carcinogenesis. This regulation is often thought to be in the form of overexpression of FABP1. In contrast, Wood et al.'s study found that loss of FABP1 in colorectal tissue increased the malignant character of the cell. At the same time, a higher rate of tumor-infiltrating lymphocytes was detected in tumors with FABP1 loss compared to those without[34]. A preliminary hypothesis from these studies is that loss of FABP1 initiates the process by disrupting TCR. signalling and intracellular metabolism in the early stages of carcinogenesis, and then overexpresses again in the later stages, managing the metastatic processes of cancer cells.

The proliferative effects of FABP family members have been shown in many cancer types by both experimental and clinical studies. A-FABP is associated with breast cancer[35], FABP4 with angiogenesis promotion in glioblastoma[36], and FABP5 with prostate cancer[37]. In our study, increased expression of FABP1 was observed in advanced histopathological stages of cancer and was seen to be at the limit of statistical significance in lymph node metastasis. In our results, the expression we detected from both tumor and TME is consistent with the data in the literature. On the other hand, we disagree with the findings of some studies that FABP1, which is in the TME, is not expressed. The main reason is that the immune profiles of the selected tumor groups in the studies were not evaluated histopathologically. The critical decrease in FABP1 expression frequency in male mice with colorectal cancer compared to female mice has been associated with cancer cachexia[38]. This study suggests that FABP1 may show sexual variation, and similarly, gender distribution and ethnic factors in patients with colorectal cancer may be polymorphic elements that change FABP1 positivity. At this point, similar results can be obtained after standardizing various factors such as a higher number of cases, a colorectal cancer group standardized by gender, soluble lipid load and classification of tumors included in the study according to an immune fraction. We think that FABP1, especially in advanced stages of colorectal cancer, is a marker that can be used in metastasis determination, monitoring of relapse cases or response processes to antineoplastic agents.

**Etik Onay:** Bu çalışma, İstanbul Tıp Fakültesi Klinik Araştırmalar Etik Kurulu tarafından onaylanmıştır (Onay kodu: 1015).

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**Ethical Approval:** This study was approved by the Istanbul Faculty of Medicine Clinical Research Ethics Committee (Approval code: 1015).

**Conflict of Interest**: The authors declare that they have no competing interests.

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