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FABP7 promotes cell proliferation and survival in colon cancer through MEK/ERK signaling pathway

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ABSTRACT

Colon cancer (CC), one of the most frequently diagnosed malignancies deriving from the digestive system, has greatly threatened human health and life. Fatty acid binding protein 7 (FABP7), an intracellular protein with the tissue-specific expression pattern, has been reported to be implicated in diverse types of human tumors. However, the biological role of FABP7 in CC is still poorly understood. The current study aimed to investigate the role of FABP7 in CC and illuminate the potential molecular mechanisms. In this present study, we found that FABP7 was highly expressed in CC tissues and cell lines, suggesting the possible involvement of FABP7 in CC tumorigenesis. Moreover, functional investigations showed that FABP7-overexpression promoted CC cell proliferation, colony formation, cell cycle progression and inhibited cell apoptosis; on the contrary, FABP7 knockdown produced an inhibitory effects on CC cell proliferation and survival. Notably, FABP7 knockdown inhibited colon tumor growth *in vivo*. In addition, mechanistic investigations demonstrated that FABP7 exerted its promoting effects on CC cell proliferation and survival through activation of the MEK/ERK signaling pathway. Collectively, our data indicate that FABP7 may be used as a novel diagnostic bio-marker and a potential therapeutic target for CC.

1. Introduction

Colon cancer, one of the most frequently diagnosed and aggressive carcinomas deriving from digestive system, is one of the major causes of cancer-related deaths around the world [1–3]. About 1.4 million new cases and over half a million deaths are estimated to occur from CC annually worldwide [4,5]. In the United States, it is estimated that around 147,000 CC cases are newly diagnosed annually, resulting in roughly 50,000 deaths [6,7]. In spite of recent advances in the diagnosis and treatment, the long-term prognosis remains unfavorable. Thus, there is a pressing need to develop novel and effective therapies for CC patients.

It is well known that documented that the ectopic expression of key cancer-related genes is involved in the tumor development and progression of numerous human cancers [8–11]. Furthermore, gene therapy is considered as a promising therapeutic option for human tumors. Fatty acid binding proteins (FABPs) are a class of intracellular structurally-related proteins with tissue-specific expression patterns [12–14]. It is well documented that FABPs plays vital roles in lipid metabolism, signaling transduction and gene expression regulation [15,16]. FABP7, also known as brain lipid binding protein (BLBP), belongs to the FABP super-family [17,18]. Previous studies have reported that the aberrant expression of FABP7 is involved in numerous types of human neoplasms, such as breast cancer [19], melanoma [20], glioblastoma [21], clear cell renal cell carcinoma [22], salivary adenoid cystic carcinoma [23], and tracheobronchial adenoid cystic carcinoma [24].

The present study aimed to investigate the biological role of FABP7 in CC and elucidate the potential molecular mechanisms. In the current study, we found that FABP7 was highly expressed in both CC tissues and cell lines, suggesting the potential involvement of FABP7 in colon tumor development and progression. Functional investigations showed that FABP7 promoted CC cell proliferation, cell cycle progression and inhibited cell apoptosis. Moreover, mechanistic investigations

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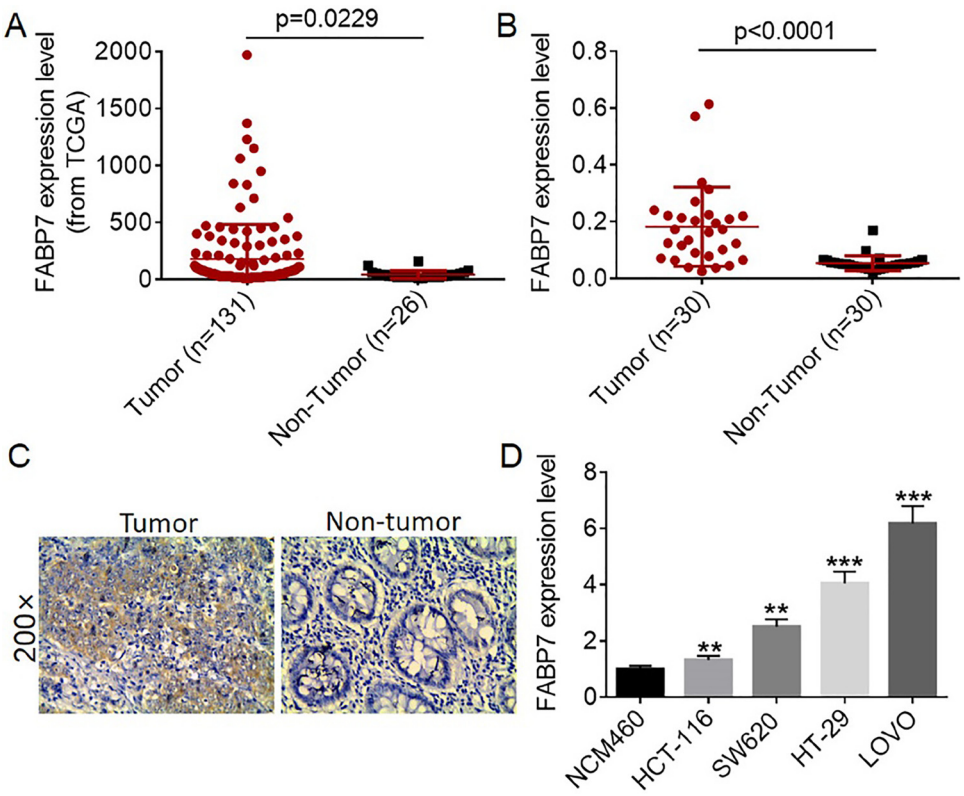

Fig. 1. FABP7 is significantly up-regulated in both CC tissues and cell lines. (A) Data of FABP7 mRNA expression levels in CC tissues and normal colon tissues were downloaded from TCGA database. (B) FABP7 mRNA expression levels in 30 pairs of CC tissues and adjacent non-tumorous tissues were determined by qRT-PCR analysis. (C) FABP7 protein expression in CC tissues and para-cancerous tissues were detected by IHC analysis. (D) FABP7 mRNA expression levels in one normal human colon mucosal epithelial cell line NCM460 and four CC cell lines (HCT-116, SW620, HT-29 and LOVO) were determined by qRT-PCR analysis. $^{**}P < 0.01$; $^{***}P < 0.001$. Difference between two groups was analyzed by Student two-tail t-test. Difference among three or more groups was compared by ANOVA with Turkey post-hoc analysis.

demonstrated that FABP7 exerted its promoting effects on cell proliferation and survival *via* activation of MEK/ERK signaling pathway.

2. Materials and methods

2.1. Patients and tissue samples

Paired primary colon cancer tissues and matched adjacent non-tumorous colon tissues were obtained from 30 patients who underwent tumor surgical resection between 2015 to 2017 in Jining No.1 People's Hospital (Jining, China). Samples were kept frozen at -80℃ for further studies. Patients received no systemic treatment or targeted therapy during the time of sample collection. This study was approved by the Clinical Research Ethics Committee of Jining No.1 People's Hospital (Jining, China). All the patients enrolled in the current study gave their written informed consent.

2.2. RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted using the TRIZOL reagent (Invitrogen, Foster City, CA, USA) in accordance with the manufacturer's protocol. The purity and concentration of total RNA were determined using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Total RNA (0.8 μg) was reverse-transcribed into cDNA in a 20 μl reaction mixture using the High Capability cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative real-time PCR was performed in a 7500 Fast Real Time PCR system (Applied Biosystems) with the following procedure: 95℃ for 10 min, followed by 40 cycles of 95℃ for 10 s and 60 ℃ for 30 s. The specific primers were as followed: FABP7 sense primer, 5'-CCAGCTGG GAGAAGAGTTTG-3' and anti-sense primer, 5'-CTCATAGTGGCGAACA GCAA-3' ; β-actin sense primer, GGACTTCGAGCAAGAGATGG-3' and anti-sense primer, 5'-AGCACTGTGTTGGCGTACAG-3'. The $2^{-\triangle\triangle Ct}$ method was applied to analyze the gene expression levels. β-actin served as an internal control to normalize the mRNA expression of FABP7. Results were the mean of three independent experiments.

2.3. Immunohistochemistry (IHC)

As previously described [25], sections of formalin-fixed, paraffin-embedded tissues were prepared for IHC analysis. In brief, deparaffinized sections were microwaved for antigen retrieval in low pH buffer (pH 6.0) for 20 min. After treatment with 1% hydrogen peroxide for 5 min to block endogenous peroxidase, the sections were incubated with corresponding primary antibodies at room temperature for 30 min, followed by incubation with horseradish peroxidase-labeled secondary antibodies for 30 min. Subsequently, the sections were developed using 3, 3'-diaminobenzidine (DAB) chromogen. All the sections were counter-stained using hematoxylin. Primary antibodies were purchased from Abcam (Cambridge, MA, USA) and used at the following dilutions: anti-FABP7 (ab27171, 1:200) and anti-Ki67 (ab833, 1:500).

2.4. Cell lines and cell culture

One normal human colon epithelial cell line NCM460 and four CC cell lines (HCT-116, SW620, HT-29 and LOVO) were obtained from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). These cells were cultured in DMEM medium (Gibco, Grand Island, New York, USA) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA). All the cells were maintained at 37℃ in a humidified incubator with 5% CO_2.

2.5. Cell transfection

Cell transfection was performed using Lipofectamine 2000 (Invitrogen, USA) in accordance with the manufacturer's instructions. FABP7 expression vector and short hairpin RNA (shRNA) specially targeting FABP7 were obtained from Shanghai GenePharma Co., Ltd. (Shanghai, China). Cell were incubated for 24 h and then collected for