Int J Clin Exp Pathol 2019;12(3):851-856
www.ijcep.com /ISSN:1936-2625/IJCEP0089571

Original Article

EFEMP2 promotes colon cancer cell invasion and growth through the ERK1/2 signaling pathway

Jianfeng Zhao[1], Jian Xu[2], Jian Zhao[2], Rui Zhang[2]

[1]Department of Colorectal Surgery, Dalian Medical University, Dalian, China; [2]Department of Colorectal Surgery, Liaoning Cancer Hospital and Institute, Cancer Hospital of China Medical University, Shenyang, Liaoning Province, China

Received December 8, 2018; Accepted January 13, 2019; Epub March 1, 2019; Published March 15, 2019

Abstract: EFEMP2 is an extracellular matrix (ECM) glycoprotein that is a pivotal oncogene in tumor progression and metastasis. However, the function of EFEMP2 in colon cancer remains unknown. In this study, we found that EFEMP2 was highly expressed in colon cancer cells. Knockdown of EFEMP2 suppressed the growth and invasion of colon cancer LoVo and SW620 cells. Moreover, knockdown of EFEMP2 attenuated ERK1/2 activation, as well as decreased MMP-3 expression. Taken together, our study demonstrates that EFEMP2 is significantly expressed in colon cancer cells. EFEMP2 can promote the growth and invasion of colon cancer cells, possibly by regulation of ERK1/2 activation and MMP-3 expression. Therapies targeting EFEMP2 may be an effective strategy for treatment of colon cancer.

Keywords: EFEMP2, colon cancer, invasion, growth, ERK1/2, MMP-3

Introduction

Colon cancer is one of the most common malignant tumors in the world [1, 2]. It is a heterogenous disease, and diverse molecules and signaling pathways are involved in its development and metastasis [3]. Tumor metastasis accounts for the major cause of cancer-related death [4]. Thus, it is important to reveal the molecular mechanisms involved in the metastasis of colon cancer.

EGF-containing fibulin-like extracellular matrix protein 2 (EFEMP2) is an extracellular matrix (ECM) glycoprotein, and acts as a candidate oncogene in tumor progression [5]. It is reported that EFEMP2 is upregulated in gliomas, and overexpression of EFEMP2 promotes glioma cell proliferation and invasion [6]. Using weighted correlation network analysis (WGCNA) co-expression network analysis, EFEMP2 was found to be a pivotal recurrence-associated molecular and prognostic indicators in colon cancer [7]. Moreover, it was reported that expression of EFEMP2 is greatly increased in colon cancer tissue and serum patients, and EFEMP2 was identified as a serum biomarker for the early detection of colon cancer [8]. However, the function of EFEMP2 in colon cancer has not been clarified.

In the current study, we found that EFEMP2 was highly expressed in colon cancer cells. Furthermore, cell invasion and growth were investigated, and we sought to explore the effect of EFEMP2 on colon cancer cell invasion and proliferation.

Materials and methods

Cell culture and reagents

The colon cancer HT-29, LoVo, SW480 and SW620 cells were purchased from ATCC. All cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS). Cells were incubated in a humidified incubator (37°C, 5% CO_2). Antibodies of EFEMP2, ERK1/2 and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), while anti-phosho-ERK1/2 was obtained from Cell Signaling Technology (Danvers, MA, USA).

Real-time PCR

Total RNA was extracted using Trizol reagent (Invitrogen), following the manufacturer's protocol. Reversed transcription PCR was performed with cDNA Synthesis Kit (Fermentas, Glen Burnie, MD, USA). Next, real-time PCR was carried out with cDNA, primers (EFEMP2: forward: 5'-ACTCAGCTGCCAGAGCAC-3'; reverse: 5'-TCAAAGCAAGACCTGAAGTTC-3'; MMP-3, forward: 5'-TGCGAGCCCTATACACAGC-3'; reverse: 5'-TCGCCGACAATTACCACATG-3'; β-actin forward: 5'-CATGTACGTTGCTATCCAGGC-3'; reverse: 5'-CTCCTTAATGTCACGCACG-3') and real-time PCR mix (Applied Biosystems, Foster City, CA, USA). β-actin expression was used as an internal control, and the data were calculated using the $2^{-\Delta\Delta CT}$ method [9].

Western blotting

Cells were lysed in RIPA lysis buffer, and protein concentration was determined using BCA Protein Assay Kit (Pierce Company, Rockford, IL, USA). The protein was added into 10% SDS-PAGE gel, and then transferred to PVDF membrane. After blocking in 5% BSA for 1 hour, the membrane was incubated in primary antibodies at 4°C overnight. Next, the membrane was washed with 5% BSA for three times, and then incubated with secondary antibodies. Finally, the bands were developed by Enhanced Chemiluminescence (ECL) Plus detection system (Pierce Company, Rockford, IL, USA).

siRNA transfection

EFEMP2 siRNA (siEFEMP2) was purchased from Genchem Biotechnology Company (Shanghai, China). A scramble siRNA was purchased as control siRNA (siNC). Cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The knockdown efficiency was determined by western blotting.

Invasion assay

Invasion assay was performed with a 24-well transwell plate (Corning Incorporated, Corning, NY, USA). Briefly, the upper chambers were coated with 30 μl Matrigel for invasion assay. Cells (200 μl) at a density of 3×10^5 cells/ml were added into the upper chambers, and 500 μl of DMEM medium containing 20% FBS was added into the lower chambers. 24 hours later, the invaded cells were fixed with methanol, and stained with crystal blue. Cell number was counted under a light microscope in five random fields.

CCK-8 assay

After transfection of siRNA, cells were seeded in a 96-well plate at the density of 1×10^3 cells/well. Then cells were further incubated in the medium for 36 h or 72 h. CCK-8 was added into the plate and incubated for 2 h. Optical density (OD) was measured by microplate reader (Bio-Rad Model 680) at 490 nm, and relative cell growth was obtained through (OD of siEFEMP2/OD of siCon) × 100%.

ELISA assay

After knockdown of EFEMP2 by siRNA, cells were further incubated for 60 h. Next, the supernatant was collected, MMP-3 ELISA kit (Invitrogen) were used to measure the protein level of MMP-3, according to the manufacturer's instruction.

Statistical analysis

The experiments were performed three times. Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL). Student's t test was used for comparison between two groups. *P* values <0.05 were considered significant.

Results

EFEMP2 expression is highly expressed in colon cancer cells

EFEMP2 expression was examined by real-time PCR and western blotting in the colon cancer HT-29, LoVo, SW480 and SW620 cells. As shown in **Figure 1**, the results showed that the mRNA and protein levels were significantly highly expressed in all detected colon cancer cells.

Knockdown of EFEMP2 suppresses colon cancer cell growth

To investigate the role of EFEMP2 in colon cancer, EFEMP2 expression was silenced by siRNA in LoVo and SW620 cells (**Figure 2A**). Fur-

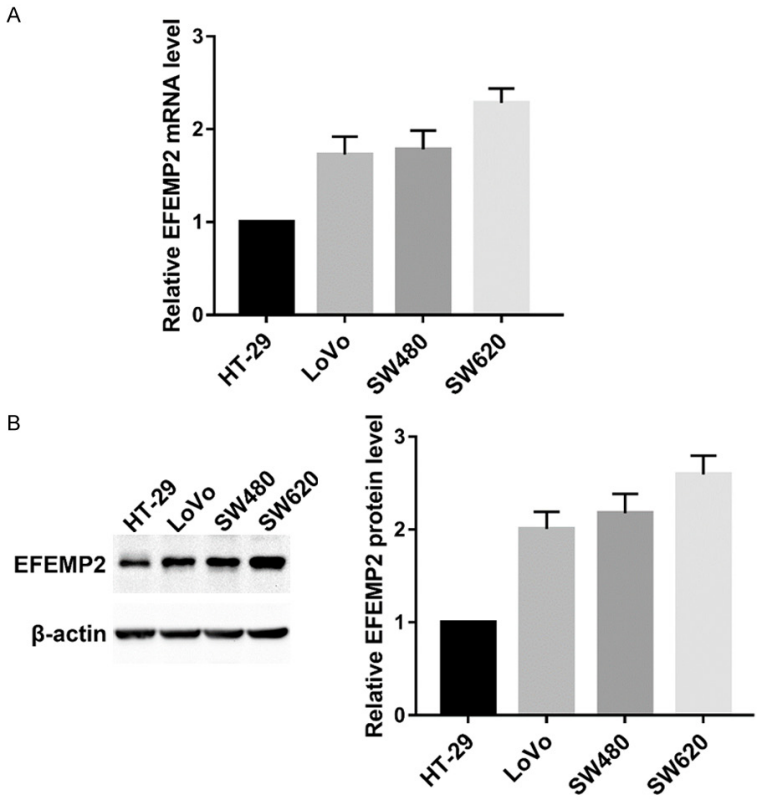

Figure 1. Expression of EFEMP2 in colon cancer cells. A. The mRNA level of EFEMP2 in HT-29, LoVo, SW480 and SW620 cells. B. The protein level of EFEMP2 in HT-29, LoVo, SW480 and SW620 cells.

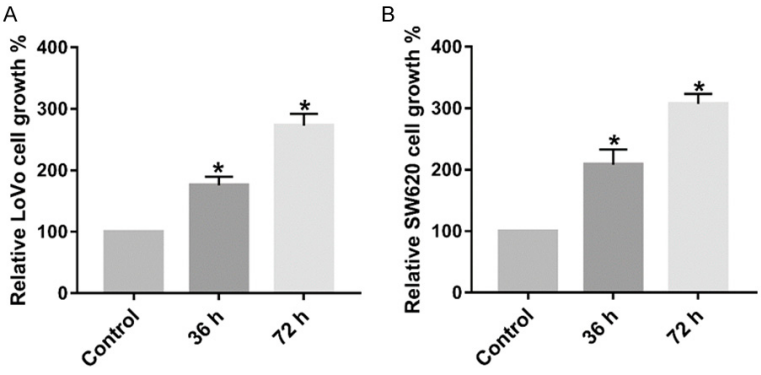

Figure 2. Cell growth was determined by CCK-8 assay. A. Effect of EFEMP2 knockdown on the growth of LoVo cells. B. Effect of EFEMP2 knockdown on the growth of SW620 cells. *P<0.05.

thermore, CCK-8 assay was performed to examine the role of EFEMP2 in cell growth in LoVo and SW620 cells. The results showed that knockdown of EFEMP2 greatly suppressed the growth of LoVo and SW620 cells (**Figure 2B** and **2C**), indicating that EFEMP2 is involved in colon cancer cell growth.

Knockdown of EFEMP2 suppresses colon cancer cell invasion

After knockdown of EFEMP2 by siRNA, invasion assay was carried out to examine the function of EFEMP2 in colon cancer cells. Using invasion assay, we found that knockdown of EFEMP2 inhibited the invasion of LoVo and SW620 cells (**Figure 3A** and **3B**). These data suggest that EFEMP2 contributes to colon cancer invasion.

Knockdown of EFEMP2 suppresses ERK1/2 pathway

ERK1/2 pathway plays an important role in the progression of colorectal cancer [10]. Using western blotting, we found that knockdown of EFEMP2 downregulated the activation of ERK1/2 in LoVo and SW620 cells (**Figure 4**). These findings suggest that EFEMP2 participates in the activation of the ERK1/2 pathway in colon cancer cells.

Knockdown of EFEMP2 suppresses MMP-3 expression

Matrix Metalloproteinases (MMPs) act as critical players in tumor invasion and metastasis [11, 12]. Here, we found that knockdown of EFEMP2 markedly decreased MMP-3 expression, at both the mRNA and protein level (**Figure 5A** and **5B**).

Discussion

EFEMP2, an extracellular matrix (ECM) glycoprotein, has been found to participate in tumor development. It is reported that EFEMP2 is a promising serum biomarker for early detection of colon cancer [8]. However, the molecular mechanisms of EFEMP2 in colon cancer have not been established. In this current study, we

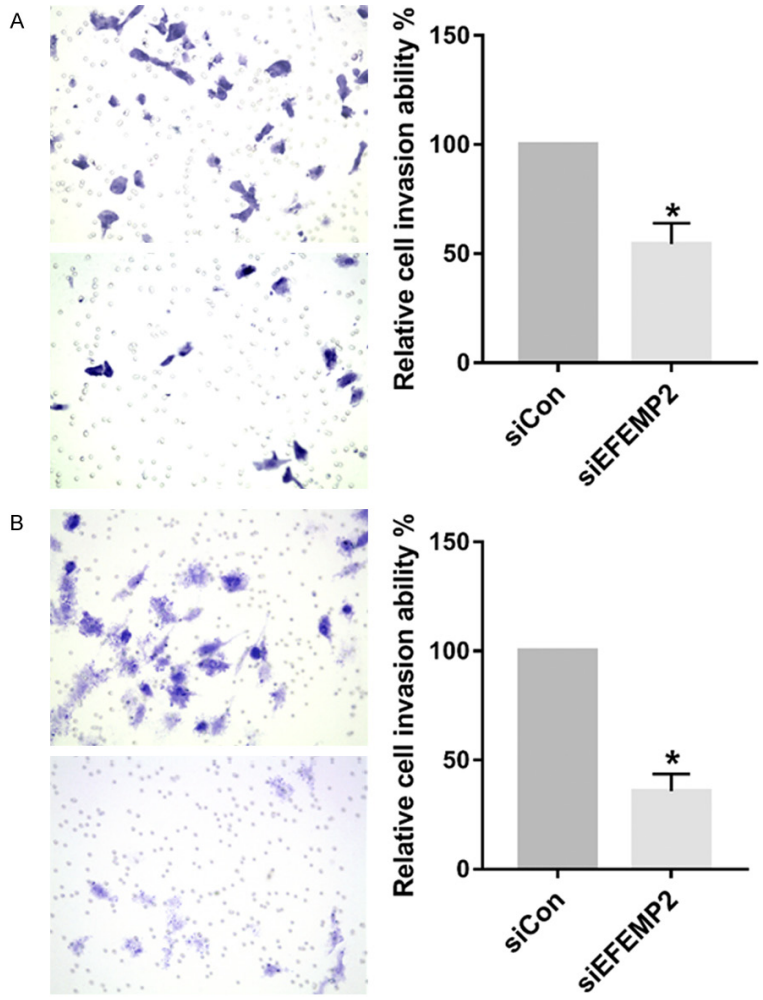

Figure 3. Cell invasion ability was detected by invasion assay. A. Effect of EFEMP2 knockdown on the invasion of LoVo cells. B. Effect of EFEMP2 knockdown on the invasion of SW620 cells. *P<0.05.

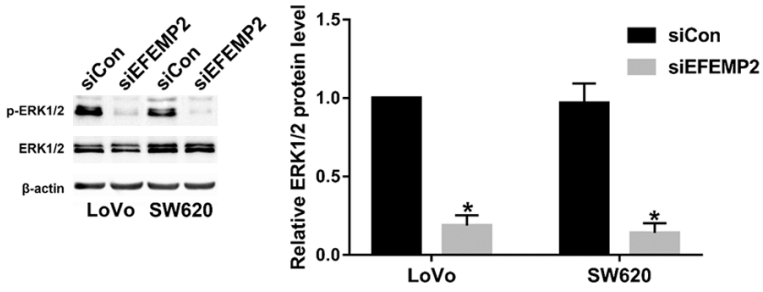

Figure 4. Knockdown of EFEMP2 expression decreased ERK1/2 activity. LoVo and SW620 cells were transfected with siEFEMP2 or siCon, respectively. ERK1/2 activation was determined by western blotting. *P<0.05.

found that EFEMP2 was highly expressed in colon cancer cells. Knockdown of EFEMP2 suppressed colon cancer cell growth and invasion. Moreover, knockdown of EFEMP2 attenuated ERK1/2 activation, and decreased MMP-3 expression.

Using weighted gene co-expression network analysis (WGCNA), Zhai et al. has observed that EFEMP2 is overexpressed in colon cancer tissues, and - is a molecular and prognostic indicator for recurrence in colon cancer [5]. In this study, we detected the mRNA and protein levels of EFEMP2 in colon cancer cells, including HT-29, LoVo, SW480 and SW620 cells. Our results showed that EFEMP2 was highly expressed in colon cancer cells, suggesting an important role of EFEMP2 in colon cancer.

Using gene expression-based classification, Lakes et al. have found that EFEMP2 can promote glioblastoma proliferation [13]. However, the function of EFEMP2 in colon cancer is not well characterized. In this study, we found that EFEMP2 could promote the growth of colon cancer LoVo and SW620 cells. Zuo et al. have found that EFEMP2 can greatly elevate the invasion ability of breast cancer MCF-7 and MBA-MD-231 cells [14]. Here, our study showed that EFEMP2 played a positive role in the invasion of colon cancer cells.

As one important member of the MAPK signaling pathways, ERK1/2 kinases are involved in regulating tumor proliferation and metastasis [15, 16]. In this study, we found that knockdown of EFEMP2 markedly suppressed ERK1/2 activation, indicating that EFEMP2 may exert its role in colon cancer cells by the ERK1/2 pathway. MMPs contribute to invasion and metastasis in tumor progression [17, 18]. It is reported that EFEMP2 significantly increases

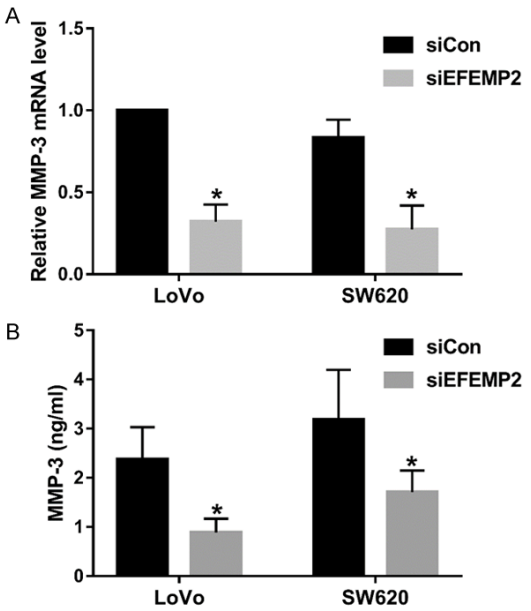

Figure 5. MMP-3 expression was examined by real-time PCR and ELISA. A. Effect of EFEMP2 knockdown on the MMP-3 mRNA expression. B. Effect of EFEMP2 knockdown on the MMP-3 protein expression. *P<0.05.

the invasive ability of glioma cells by MMP-2 and MMP-9 upregulation [6]. In our study, we found that knockdown of EFEMP2 significantly decreased MMP-3 expression, suggesting that EFEMP2 may enhance colon cancer cell invasion by upregulating MMP-3 expression.

In summary, our study demonstrated that EFEMP2 was highly expressed in colon cancer cells. EFEMP2 could enhance colon cancer cell growth and invasion, possibly by regulating ERK1/2 activation and MMP-3 expression.

Acknowledgements

This work was supported in part by the National Natural Science Foundation of China (No. 81672427), Liaoning BaiQianWan Talents Program ([2017] No. 45), the Project of Liaoning Clinical Research Center for Colorectal Cancer (No. 2015225005) and Clinical Capability Construction Project for Liaoning Provincial Hospitals (No. LNCCC-D42-2015).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Rui Zhang, Department of Colorectal Surgery, Liaoning Cancer Hospital and Institute, Cancer Hospital of China Medical University, 44 Xiaoheyan Road, Shenyang 110042, Liaoning Province, China. Tel: +86-24-31916293; Fax: +86-24-24315679; E-mail: zhangrui04004@163.com

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