Biomedicine & Pharmacotherapy 106 (2018) 68–76

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Fatty acid binding protein 5 promotes tumor angiogenesis and activates the IL6/STAT3/VEGFA pathway in hepatocellular carcinoma

Long Pan, Heng Xiao, Rui Liao, Qingsong Chen, Chong Peng, Yuchi Zhang, Tong Mu, Zhongjun Wu*

Department of Hepatobiliary Surgery, the First Affiliated Hospital of Chongqing Medical University, Chongqing, 400016, PR China

ARTICLE INFO

Keywords:
Fatty acid binding protein 5
Hepatocellular carcinoma
Angiogenesis
Vascular endothelial growth factor A

ABSTRACT

Background: Tumor angiogenesis is an essential process for facilitating tumor growth and metastasis. Fatty acid binding protein 5(FABP5)is highly expressed in hepatocellular carcinoma (HCC). Thus, we investigated the role of FABP5 in tumor angiogenesis during HCC development.

Methods: In this study, the protein and mRNA levels of FABP5 in matched HCC and adjacent noncancerous liver tissues from 43 patients were determined using immunohistochemistry and real-time quantitative PCR, respectively. Two HCC cell lines (Huh7 and SMMC-7721) and human umbilical vein endothelial cells (HUVECS) were used to investigate the pro-angiogenic effect of FABP5 by tube formation, CCK8 and Transwell migration assays. The expression levels of interleukin 6 (IL6) and vascular endothelial growth factor A (VEGFA) secreted from HCC cells were detected by enzyme-linked immunosorbent assay (ELISA).

Results: In 43 HCC patients, the expression of FABP5 mRNA was positively correlated with intratumoral VEGFA mRNA expression. FABP5 mRNA expression was also associated with adverse HCC characteristics. In vitro, cell viability, cell migration and tube formation in HUVECs were enhanced with increasing expression of FABP5 in HCC cells. Downregulation of FABP5 expression inhibited the IL6/STAT3/VEGFA pathway in HCC cells and inhibited tumor angiogenesis.

Conclusion: FABP5 was shown to promote angiogenesis and activate the IL6/STAT3/VEGFA pathway in HCC. FABP5 may be a potential antiangiogenic target in the treatment of HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is ranked third in mortality among the top cancers [1]. Currently, although multiple treatments are available for patients with HCC, the prognosis is still poor. Therefore, greater knowledge of the process of tumorigenesis in HCC could be indispensable in the prevention of metastasis and recurrence. Notably, blood supply is essential for tumor growth and spreading. Tumor cells can secrete cytokines to recruit endothelial cells and subsequently promote neovascularization [2,3]. Angiogenesis inhibitors such as sorafenib, bevacizumab and brivanib are becoming fundamental elements in the treatment of HCC patients [4]. To date, it has been found that not all antiangiogenic drugs are effective approaches when used in the general population due to both the heterogeneity of HCC and the process of angiogenesis, which consist of multiple, mutually dependent steps [4,5]. Consequently, an in-depth understanding of the angiogenesis cascade and associated mechanisms may provide useful therapeutic strategies against this disease.

Fatty acid binding protein 5 (FABP5), also known as epidermal fatty acid binding protein, is an isoform of the FABPs, which are small (~15 kDa), soluble intracellular lipid-binding proteins that bind a variety of retinoids and long-chain fatty acids (LCFAs) [6,7]. FABP5 can be found in a broad spectrum of tissue types, such as brain, kidney, liver, lung, and heart [6]. Notably, FABP5 is a pivotal factor in the promotion of tumor proliferation, invasion and metastasis in several tumors types, including prostate cancer, intrahepatic cholangiocarcinoma and colorectal cancer [8–10]. For example, in several types of human cancers, saturated LCFAs could suppress the oncogenic properties of FABP5-expressing carcinoma cells by promoting activation of retinoic acid receptors (RARs) and inhibiting the activation of PPARβ/δ [11]. Moreover, FABP5 ablation decreased the activation of EGFR downstream effector signals and expression of PPARδ target genes and subsequently suppressed mammary tumor development [12]. Interestingly, FABP5 has been reported to facilitate the malignant progression of prostate cancer cells via a FABP5-PPARγ-VEGF signal transduction axis that increases angiogenesis [13]. Recently, FABP5 was also shown

* Corresponding author at: Department of Hepatobiliary Surgery, the First Affiliated Hospital of Chongqing Medical University, Chongqing, 40016, PR China.
E-mail address: wzjtcy@126.com (Z. Wu).

https://doi.org/10.1016/j.biopha.2018.06.040
Received 23 April 2018; Received in revised form 10 June 2018; Accepted 11 June 2018

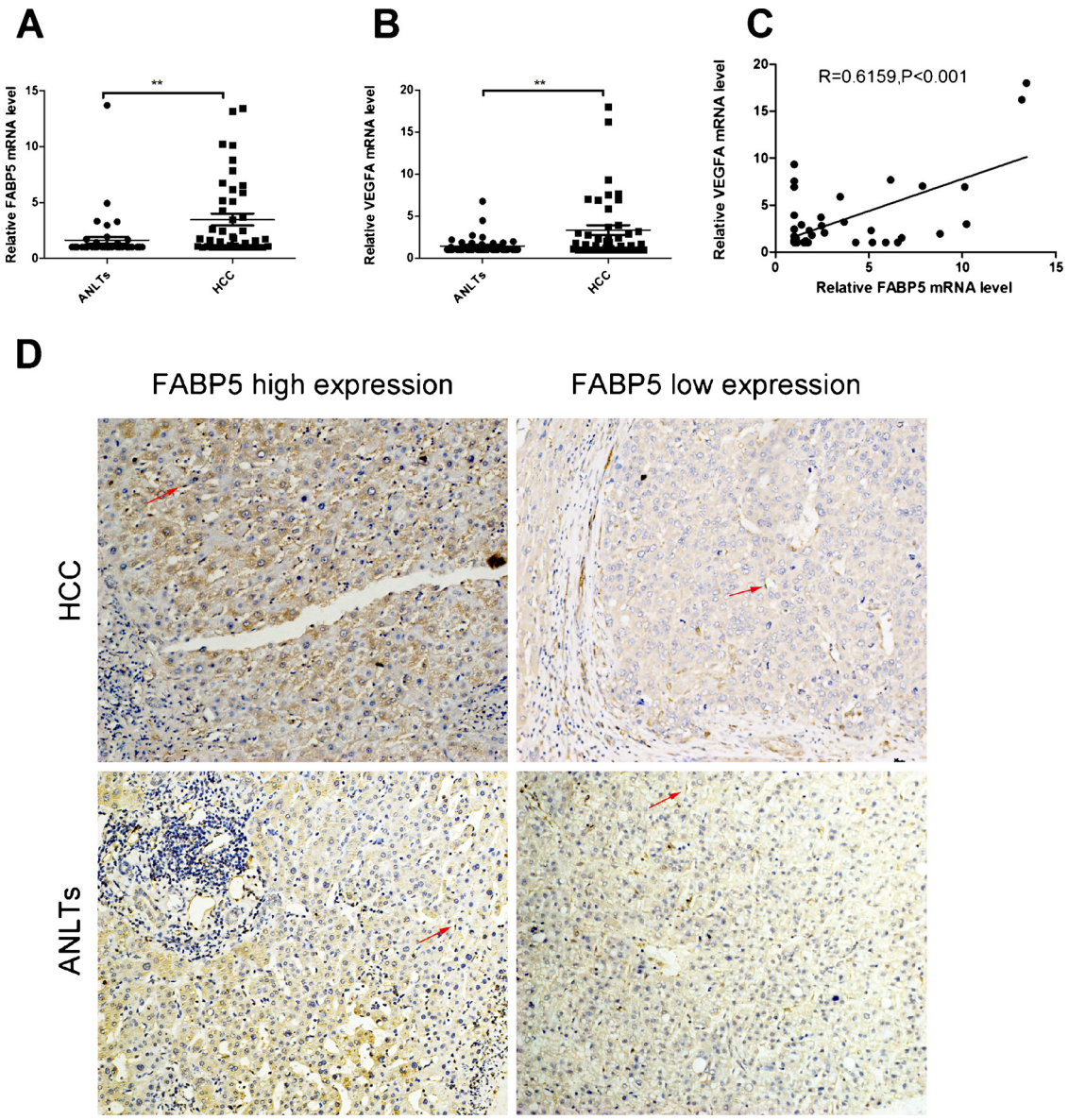

Fig. 1. The expression of FABP5 and VEGFA in HCC. The mRNA levels of FABP5 (**A**) and VEGFA (**B**) in ANLTs were lower than in cancerous tissues (*$p < 0.05$, **$p < 0.001$). A positive correlation between mRNA levels was found between FABP5 and VEGF in HCC tissues (**C**, $R = 0.6198$, $P < 0.001$). Immunohistochemical staining showed high and low expression levels of FABP5 in HCC and adjacent nontumorous tissues, respectively (**D**, x200). The red arrow indicates FABP5 expression in Kuppfer cells.

to be overexpressed in HCC and to promote tumor development through induction of epithelial-mesenchymal transition [7]. Additionally, given the important roles of both angiogenesis and FABP5 in HCC, it is necessary to have a better understanding of tumor angiogenesis as induced by FABP5 in HCC.

In this study, we evaluated the correlation between FABP5 and tumor angiogenesis in human HCC tissues and HCC cell lines. Moreover, we also evaluated the function of endothelial cells stimulated in FABP5-conditioned medium (CM) obtained from HCC cells. These findings have strongly suggested that FABP5 could promote tumor angiogenesis.

2. Materials and methods

2.1. Patients and specimens

Specimens were obtained from a total of 43 HCC patients who underwent surgical liver resection between May 2016 and August 2017 at the First Affiliated Hospital of Chongqing Medical University. All HCC specimens were verified by pathological examination. Clinical data from these patients was also collected. This study was approved by the Ethics Review Committee of the First Affiliated Hospital of Chongqing Medical University. Informed consent was obtained from each patient.

2.2. Immunochemistry

HCC tissues and adjacent, noncancerous liver tissues were fixed in formalin and embedded in paraffin for sectioning. Rabbit anti-human FABP5 antibody (1:100, ABclonal Bio, Wuhan, Hubei, China) was used for staining. Standard immunochemistry(IHC) protocols were used according to the manufacturers' protocols. Under high-power magnification (×400), images of three representative fields were captured using the Leica QWin Plus v3 software(Leica Microsystems Inc, Buffalo Grove, USA); identical settings were used for each photograph.