Biomedicine & Pharmacotherapy 72 (2015) 58–65

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Involvement of miR-485-5p in hepatocellular carcinoma progression targeting EMMPRIN

Xiangjun Sun [a], Yonglei Liu [b,c,*], Ming Li [b], Mingchun Wang [d], Yutong Wang [c]

[a] Department of Hepatobiliary Surgery, Linyi People's Hospital, Linyi, Shangdong, China
[b] Research Center, Linyi People's Hospital, Linyi, Shangdong, China
[c] Liver Cancer Center, Zhongshan Hospital, Fudan University, Shanghai, China
[d] Department of Surgery, Linyi People's Hospital, Linyi, Shangdong, China

ARTICLE INFO

Article history:
Received 21 March 2015
Accepted 3 April 2015

Keywords:
EMMPRIN
miR-485-5p
Hepatocellular carcinoma
Metastasis

ABSTRACT

EMMPRIN plays important roles in cancer development, which includes EMMPRIN 1, 2, 3, and 4 isoforms. EMMPRIN2 is the main component in human cancers, but its regulation by miRNAs is still unclear. In this study, we will investigate the mechanism of EMMPRIN regulation in hepatocellular carcinoma (HCC) by miRNAs. Through RT-PCR, we found that EMMPRIN2 was the main isoform in HCC cells. EMMPRIN2 was down-regulated significantly by predicted miRNAs and miR-485-5p was one of the miRNA that regulated EMMPRIN in HCC cell lines. It was verified that EMMPRIN was a target gene of miR-485-5p by using luciferase analysis assay. We found that miR-485-5p was significantly downregulated in HCC tissues and that its expression was inversely correlated with the TNM stage and metastasis in HCC samples. Results of cellular functions in HCC showed that miR-485-5p could inhibit cell proliferation and metastasis. Additionally, miR-485-5p overexpression suppressed HCC growth in vivo by down-regulation of EMMPRIN. Our study for the first time demonstrated that miR-485-5p represses HCC invasive and metastatic capacities by targeting EMMPRIN expression.

1. Introduction

Hepatocellular carcinoma (HCC) is the third cause for cancer-related death in the world [1]. The incidence of HCC is much higher in China. Despite advances in HCC treatment, the 5-year overall survival rate remains very poor [2]. The molecular mechanism of HCC is very complicated and remains unclear [3,4]. It is needed to elucidate the mechanism of HCC carcinogenesis to find new targets or methods for developing effective strategies of HCC.

Extracellular matrix metalloproteinase inducer (EMMPRIN) is a widely expressed transmembrane glycoprotein which belongs to the immunoglobulin superfamily including four isoforms: EMMPRIN1, EMMPRIN2, EMMPRIN3 and EMMPRIN4 [5,6]. EMMPRIN involves in various physiological processes such as fetal development, reproduction, T cell differentiation, and neural and retinal functions [7–10]. EMMPRIN1 is a tissue specific protein expressed on retina tissue; EMMPRIN2 is the most predominant isoform, encoding two Ig-like extracellular domains and being well-known as basigin/CD147/EMMPRIN; the other two isoforms were rarely reported which showed that EMMPRIN3 and EMMPRIN4 were increased in HCC tissues and EMMPRIN3 acted as an inhibitor of proliferation and invasion of HCC [11–13].

EMMPRIN expresses widely in many tumors, including liver cancer, lung cancer, breast cancer, bladder cancer, lymphoma, oral squamous cell carcinoma, glioma, melanoma, and others [14–16]. Accordingly, EMMPRIN expression has been associated with known risk factors for breast cancer and with poor prognosis of breast cancer patients [14–16]. These suggest that EMMPRIN plays important roles in carcinogenesis. Moreover, it has been shown that higher expression of EMMPRIN was associated with poor prognosis of cancer patients and could serve as an independent predictor of poor survival in cancer. The most life-threatening aspects of the oncogenic process are invasion and metastasis.

MicroRNAs (miRNAs) are small RNAs and its noncoding mRNA sequences containing about 22–29 nucleotides that act as important regulators of gene expression [17,18]. miRNAs inhibit gene expression on transcriptional or post-transcriptional levels by specifically binding the seed sequence of target genes, cleaving mRNAs or inhibiting their translation [19,20]. Most of human miRNAs are located in cancer-associated genomic regions in the

* Corresponding author at: 27 Jiefang Road, Linyi People's Hospital, Linyi 276000, Shangdong, China. Tel.: +86 05398226999.
E-mail address: lylsdys@163.com (Y. Liu).

http://dx.doi.org/10.1016/j.biopha.2015.04.008
0753-3322/

chromosome, which function as inhibitor or onco-miRNAs depending on their targets in tumor. miRNAs have also been associated with HCC formation and growth [21,22].

In this study, we predicted that miR-485-5p was a regulator of EMMPRIN. Then we investigated the potential involvement of miR-485-5p in HCC. We examined the expression of miR-485-5p in human HCC cells and tissues and tested its effects on cell growth, cell-cycle distribution and metastasis. We also investigated a potential role of miR-485-5p on tumorigenesis in a murine model. We identified that miR-485-5p was a tumor suppressor and negatively regulated EMMPRIN in progression of HCC.

2. Material and methods

2.1. Cell culture

BEL-7405, SMMC7721, HepG2 and Huh7 human liver cancer cell lines were primary purchased from ATCC (Manassas, VA, USA). BEL-7404 cells were stored in our lab. The cells were maintained in the Dulbecco's Modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fatal bovine serum (Gibco, USA) in a humidified incubator at 37 °C and 5% CO_2.

2.2. Tissue samples

Primary HCC and their adjacent tissues were obtained from the patients with HCC of our hospital (Linyi People's Hospital, Shandong, China). Both tumor and normal tissues were histologically confirmed by H&E (hematoxylin and eosin) staining. The study was approved by the ethics committee of the hospital.

2.3. Antibodies and reagents

EMMPRIN, MMP9, E-cadherin, Vimentin, FSP1 and α-SMA primary antibody were ordered from Santa Cruz (Dallas, TX, USA). Anti-GAPDH antibody and secondary antibodies conjugated with HRP were purchased from Kang-Chen Biotech (Kangcheng, Shanghai, China). EMMPRIN plasmid was ordered from Origene (Rockville, MD, USA).

2.4. Plasmid vector construction and lentivirus transduction

Lentivirus vector expressing miR-485-5p was constructed using the BLOCK-iT pol II miR RNAi Expression Vector Kit with EmGFP (Invitrogen, Carlsbad, CA, USA). The primary miRNA sequence of miR-485-5p with flanking regions was obtained by PCR and inserted into the Block-iT Pol II miR RNAi Expression Vector, pcDNA6.2-GW/EmGFP-miR. For transfection, cells were seeded in antibiotic-free medium at a density of 30–40% with lipofectamine-2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

2.5. Transfection and dual luciferase assay

The pGL3 plasmid was modified by adding the human 3′-UTR or the 3′-UTR with mutations in regions complementary to miR-485-5p seed regions behind the firefly luciferase gene. Cells were plated on 24-well Plates 24 h before transfection. Transient transfection was carried out in Opti-MEM medium using lipofectamine-2000 according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). The medium was changed after transfection for 5 h, and the cells incubated at 37 °C for the indicated time. Cells were washed once with phosphate-buffered saline and luciferase assays were performed using the Dual Luciferase Assay System (Promega, WI, USA) according to the supplier's protocol.

2.6. RNA isolation and real-time RT-PCR

Total RNA, following the manufacturer's instructions, was isolated from the cells using Trizol reagent (Invitrogen). Briefly, the cells were lysed in TRIzol and then mixed with chloroform. The lysate was centrifuged to separate RNA, DNA and protein, total RNA recovered, precipitated with isopropanol, washed in 75% ethanol to remove impurities before dissolved in water. After that, 2 μg of RNA was taken and treated with DNase to remove contaminating DNA prior to the reverse transcription to cDNA using SYBR® PCR Kit (Takara, Japan). To measure mRNA expression, real-time RT-PCR was performed using a sequence detector (ABI-Prism, Applied Biosystems). The primers were ordered from Kang-Chen Biotech (Kangcheng, Shanghai, China).

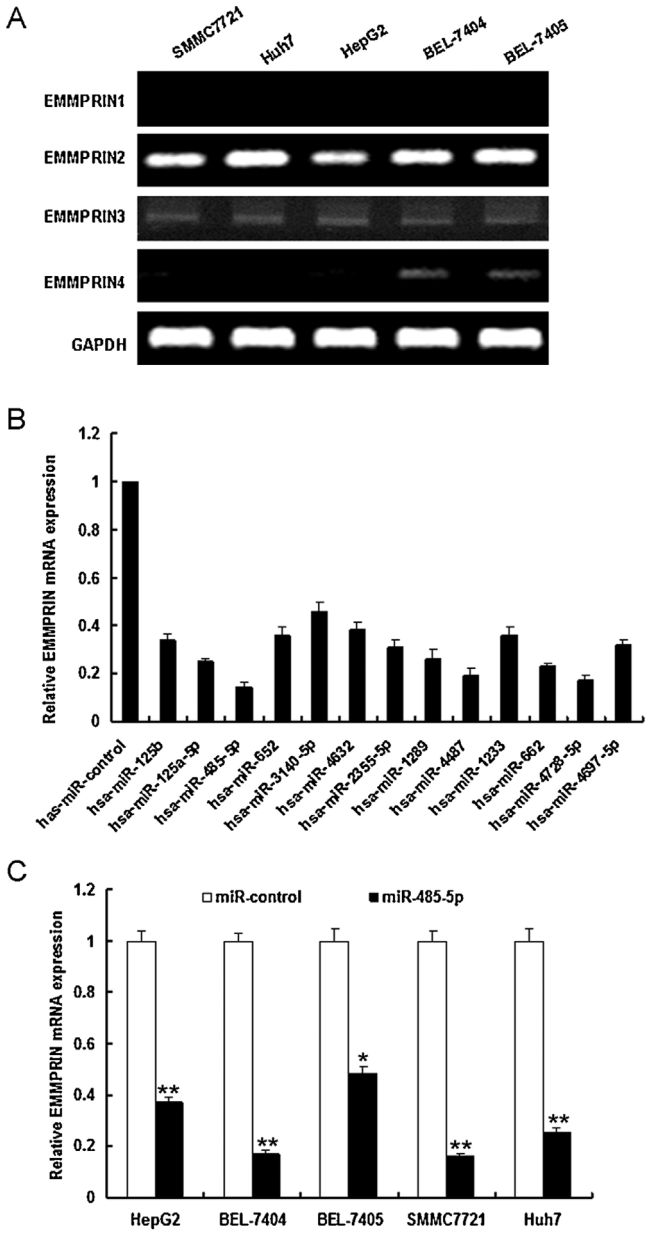

Fig. 1. miR-485-5p down-regulated EMMPRN expression in HCC cells. (A) The expression of EMMPRIN mRNA in five HCC cell lines. EMMPRIN 1 (NM_001728), 2 (NM_198589), 3 (NM_198590) and 4 (NM_198591) mRNA were examined by RT-PCR. (B) The most potential miRNAs targeted EMMPRIN2 in SMMC771 cells. The predicted miRNAs with high scores were verified by real time RT-PCR. (C) miR-485-5p inhibited EMMPRIN2 mRNA expression in HCC cell lines. The data presented are shown as means ± SD collected from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.