Life Sciences 197 (2018) 122–129

Contents lists available at ScienceDirect

Life Sciences

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

LncRNA FAL1 promotes cell proliferation and migration by acting as a CeRNA of miR-1236 in hepatocellular carcinoma cells

Baoguo Li*,[1], Rui Mao[1], Changfu Liu, Weihao Zhang, Yong Tang, Zhi Guo*

Department of Interventional Therapy, Tianjin Medical University Cancer Institute and Hospital, Chinese National Clinical Research Center of Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin 300060, China

ARTICLE INFO

Keywords:
LncRNA FAL1
HCC
miR-1236
ceRNA mechanism
Exosome

ABSTRACT

Background: Long non-coding RNAs (LncRNAs) have been demonstrated to play crucial role in tumor growth and metastasis for hepatocellular carcinoma (HCC). LncRNA FAL1 has been indicated to promote the progression of various cancers. However, the role of lncRNA FAL1 in HCC was poorly understood.

Methods: The expression levels of lncRNA FAL1 in HCC tissues and cells were determined by RT-qPCR. The roles of lncRNA FAL1 on HCC cells were investigated by MTT, colony formation, transwell, RT-qPCR, and Western blotting. The miRNA binding sites of lncRNA FAL1 was predicted using RegRNA 2.0 and miR-1236 was validated to target lncRNA FAL1 by luciferase reporter assays and RT-qPCR. Finally, the expression levels of lncRNA FAL1 in serum exosome of HCC patients was also investigated and the role of exosome-mediated lncRNA FAL1 was further investigated by co-culturing with HCC cells.

Results: This study first showed that lncRNA FAL1 was up-regulated in HCC tissues and functioned as an oncogene in HCC. LncRNA FAL1 could accelerate cell proliferation and metastasis as a ceRNA mechanism by competitively binding to miR-1236. Moreover, lncRNA FAL1 was also up-regulated in serum exosome of HCC patients and could transfer lncRNA FAL1 to HCC cells to increase their abilities of cell proliferation and migration.

Conclusions: Taken together, this study indicated that lncRNA FAL1 functions as an oncogenic in HCC and may be a novel diagnostic biomarker or a novel target for the treatment of HCC in future.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most types of malignant tumor with high mortality and prevalence rates among males and females worldwide [1]. Despite advancements in the early detection of HCC, there are still many HCC patients initially diagnosed at an advanced stage, which is usually accompanied by the distant metastasis [2]. In addition to the limited and ineffective options for HCC treatment, the prevalence of HCC is still on the rise [3]. Therefore, it is urgent to exploit novel diagnostic targets and therapeutic strategies for HCC.

Long non-coding RNAs (LncRNAs), longer than 200 nucleotides (nt) in length, are a subtype of non-protein-coding transcripts and previously regarded as the "transcription noise" of genome because they do not contain an open reading frame for protein coding [4]. However, accumulating evidences have shown that lncRNAs can regulate gene expression in a variety of cellular processes [5–8]. Recent studies revealed that dysregulation of lncRNAs was associated with various human diseases, including cancers [9–11]. Dysregulation of lncRNAs can play important role in cell proliferation, apoptosis, migration and invasion in many types of cancers [12,13]. Of note, lncRNAs have been demonstrated to regulate gene expression through functioning as microRNA (miRNA) sponge or competing endogenous RNA (ceRNA) [14,15]. Recent studies have shown that lncRNA FAL1 functions as an oncogene in thyroid cancer [16], non-small cell lung cancer [17], and ovarian cancer [18]. However, the detailed function and mechanism of FAL1 in HCC remains poorly understood.

Exosomes are a class of extracellular vesicle of about 30–100 nm derived from endosomes by many types of cells, including cancer cells [19,20]. It is reported that exosomes can enrich proteins, mRNAs, miRNAs and lncRNAs, which may horizontally transfer to recipient cells and result in a phenotypic effect [21,22]. Many studies showed that exosomes secreted by cancer cells can play important role in cell survival, apoptosis, migration and invasion [23–25]. More recently,

* Corresponding authors at: Department of Interventional Therapy, Tianjin Medical University Cancer Institute and Hospital, Chinese National Clinical Research Center of Cancer, Key Laboratory of Cancer Prevention and Therapy, Huan-Hu-Xi Road, Tianjin 300060, China.
E-mail addresses: libao_gr6688@163.com (B. Li), guozhi_dr@163.com (Z. Guo).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.lfs.2018.02.006
Received 11 January 2018; Received in revised form 2 February 2018; Accepted 4 February 2018
Available online 06 February 2018
0024-3205/

Table 1
Primers and oligonucleotides used in this study. Forward and reverse primers are listed for relevant real time PCR experiments and oligonucleotides are listed for plasmid constructs.

Name	Sequence (5′-3′)
lncRNA FAL1 shRNA-sense	CCGGAGCGGAGACTTGTCTTTAAAGCTCGAGCTTTAAAG ACAAGTCTCCGCTTTTTTG
lncRNA FAL1 shRNA- antisense	AATTCAAAAAAGCGGAGACTTGTCTTTAAAGCTCGAGCTTTAAAGACAAGTCTCCGCT
qPCR-FAL1-forward	CCTGGCCAAGAAGCTCATAC
qPCR-FAL1-reverse	TGAGGACACCGACTACTGAGAA
lncRNA FAL1-exp-F	CCCAAGCTTGCGCATCTCCTACGGCCTCCAGGACAGAG
lncRNA FAL1-exp-R	CCGGAATTCAGACATCCAAGTGTCCTGTGTAATAGGC
qPCR-Zeb1-forward	AGAGATGCAATTGGTTCTCC
qPCR-Zeb1-reverse	GAATACAGGAACAAATTGGC
qPCR-AFP-forward	CACGGATCCAACTTGAGGCTGTCATTGC
qPCR-AFP-reverse	CGGAATTCGATAAGGAAATCTCACATAAAAGTC
qPCR-Vimentin-forward	GCTGAATGACCGCTTCGCCAACT
qPCR-Vimentin-reverse	GCTCCCGCATCTCCTCCTCGTA
qPCR-E-cadherin-forward	ATTGCTCACATTTCCCAACTCC
qPCR-E-cadherin-reverse	CTCTGTCACCTTCAGCCATCCT
qPCR-β-actin-forward	CGTGACATTAAGGAGAAGCTG
qPCR-β-actin-reverse	CTAGAAGCATTTGCGGTGGAC

exosomes have been found in many body fluids, including, amniotic fluid, urine, blood, malignant ascites, etc. [26,27]. LncRNAs from circulating exosomes are considered to be potential biomarkers in many cancers. However, whether potential biomarkers can horizontally transfer lncRNAs to cancer cells remain unclear.

In this study, we showed that lncRNA FAL1 was up-regulated in HCC tissues and functions as an oncogene. LncRNA FAL1 promoted cell viability, proliferation, migration, invasion and epithelial-mesenchymal transition (EMT) as a ceRNA mechanism by regulating the expression level of AFP and ZEB1 in a miR-1236-dependent manner. Moreover, we showed that circulating exosome lncRNA FAL1 was also up-regulated in HCC patients. We further showed that circulating exosome could transfer lncRNA FAL1 to HCC cells to promote cell growth, proliferation, migration and invasion. Our findings may provide novel insights into the mechanisms underlying carcinogenesis and potential biomarkers for HCC.

2. Materials and methods

2.1. Tissue samples and serum collection

Thirty pairs of HCC tissues and their adjacent normal tissues were collected from Tianjin Medical University Cancer Institute and Hospital (Tianjin, China). All the HCC patients have been pathologically and histologically confirmed and immediately stored in liquid nitrogen before use. A total of thirty blood samples from HCC patients and thirty blood samples from healthy people were collected and centrifuged at 3000 ×*g* for 10 min, then transferred the supernatant to RNase-free tubes and stored at −80 °C before use.

2.2. Cell culture

Human HCC cell lines (LO2, SMMC-7721, Huh7, HepG2 and HepG2.2.15) were cultured in DMEM (Invitrogen, Carlsbad, CA, USA) medium that contained with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin. Humidified air atmosphere at 37 °C with 5% (v/v) CO_2 was used to culture the above cells.

2.3. Exosomes isolation

ExoQuick TC (EXOQ20A-1, System Biosciences, USA) was used to precipitate the serum-exosomes according to the manufacturer's instruction. Briefly, 1 ml ExoQuick solution was added into 4 ml serum sample to mix well and incubated for 30 min at 4 °C. Then, centrifuge the mixture at 1500 ×*g* for 30 min at room temperature to precipitate exosomes pellets. The supernatant was removed carefully and the precipitate was centrifuged at 1500 ×*g* for 5 min for the purpose of removing extra fluids. After that, exosomes pellets were resuspended in 50 ml cold 1 × PBS and stored at −80 °C before use. Exosomal protein content was measured using BCA protein assay kit (Pierce).

2.4. Transmission electron microscopy (TEM)

10 ml of exosomes pellet was placed on formvar carbon-coated 200-mesh copper electron microscopy grids for incubating 5 min at room temperature. Then, the exosomes were subjected to standard 1% uranyl acetate to stain for 1 min at room temperature, followed by washing with 1 × PBS for three times. Before observation in transmission electron microscope (Hitachi H7500 TEM, Japan), the exosomes were allowed to semi-dry at room temperature.

2.5. Real-time quantitative PCR (RT-qPCR)

Isolation of total RNA and miRNA were carried out by using QIAGEN Rneasy Mini kit (Qiagen, Hilden, Germany) and QIAGEN miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Quantitative PCR analysis of lncRNA FAL1, AFP, ZEB1, E-cadherin, Vimentin, and GAPDH was carried out by using an ABI 7500 thermocycler (Applied Biosystems) with SYBR-Green PCR Master Mix (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Quantitative PCR analysis of miR-1236 was performed using commercial TaqMan microRNA assays (Thermo Fisher Scientific; miR-1236 (ID 002761)), and U6 snRNA (ID 001973)) according to the manufacturer's protocol. The primers for RT-qPCR were used in the present study are listed in Table 1.

2.6. MTT assays and colony formation

MTT assay was used to detect cell viabilities. 4×10^3 cells were seeded into 96-well plates for culturing 18–24 h before transfection with vectors, lncRNA or exosomes. Then, the cells were detected by MTT assay using a Quant Universal Microplate Spectrophotometer (BioTek, Winooski, VT, USA) at 570 nm for 24 h, 48 h and 72 h.

To measure the colony formation abilities, Huh7 and HepG2 cells (300 cells per well) transfected with vectors, lncRNA or exosomes were seeded into 12-well plates to grow 14 days. After that, crystal violet was used for cell staining, and only colonies > 50 cells were counted (colony formation rate = (colony number) / (seeded cell number)).