Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

miR-125b-5p inhibits breast cancer cell proliferation, migration and invasion by targeting KIAA1522

Yongzhen Li [a], Yongxia Wang [a], Hongzhe Fan [b], Zheying Zhang [a], Na Li [a, *]

[a] Department of Pathology, Xinxiang Medical University, No. 601 Jinsui Avenue, Hongqi District, Xinxiang, 453003, PR China
[b] Cardiothoracic Surgery, The Third Affiliated Hospital of Xinxiang Medical University, East Hualan Avenue, Hongqi District, Xinxiang, 453000, PR China

ARTICLE INFO

Article history:
Received 14 August 2018
Accepted 27 August 2018
Available online xxx

Keywords:
miR-125b-5p
KIAA1522
Breast cancer
Tumor suppressor

ABSTRACT

Abnormal gene expression due to the dysregulation of microRNAs (miRNAs) often occurred in the initiation or progression of cancers. The aim of this present study was to investigate the function role of miR-125b-5p in breast cancer (BC). Expression levels of miR-125b-5p were determined by quantitative Real-time PCR. Biological functions of miR-125b-5p in the progression of BC were investigated with a series of *in vitro* experiments including cell counting kit-8 assay, colony formation assay, wound-healing assay and transwell invasion assay. The target of miR-125b-5p in BC was validated by luciferase activity reporter assay and western blot assay. We found miR-125b-5p expression was significantly reduced in BC cell lines compared to the normal breast epithelial cell line. Functional assays showed that cell proliferation, colony formation ability, cell migration, and cell invasion can be suppressed by miR-125b-5p overexpression. Besides, KIAA1522 was validated as a direct target of miR-125b-5p in BC. Collectively, our study showed that miR-125b-5p functions as a tumor suppressor and regulates BC progression through targeting KIAA1522.

1. Introduction

4,292,000 new cancer cases and 2,814,000 cancer deaths were predicted to occur in China in 2015 [1]. The top 5 diagnosed cancers among female in China are: breast cancer (BC), lung and bronchus cancer, stomach cancer, colorectal cancer, and esophagus cancer [1]. Notably, BC is estimated to account for 15% of all new cancers and the leading cause of cancer death in female [1,2]. The initiation of BC is complex which contained both genetic and environment contributions [3]. Early BC can be effectively treated with surgical resection method combined with radiotherapy or chemotherapy [4]. However, treatment efficiency for advanced or metastatic cancer is still unsatisfactory and thus contributed to the largest proportional of cancer death [4]. The utilization of biomarkers such as estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 have already provided substantial prognostic value for BC patients [5], but it is still imperative to develop new biomarkers with the aim to provide more valuable information for disease diagnose and treatment.

KIAA1522 is identified at 2000 through sequencing the human tissues but its biological roles are largely undetermined [6]. Chen et al. revealed that *KIAA1522* gene promoter region was hypermethylated through screening genome-wide methylation status in esophageal squamous cell carcinoma (ESCC) tissues collected from Xinjiang Kazakh nationality patients but its biological roles were not fully determined [7]. Subsequently, Xie et al. revealed that KIAA1522 was overexpressed in ESCC tissues and correlated with tumor differentiation [8]. They also found KIAA1522 could promote ESCC cell growth and metastasis both *in vitro* and *in vivo* through regulating the activity of extracellular signal-regulated kinase [8]. Moreover, Liu et al. investigated KIAA1522 expression in 583 paired tissues and found it was overexpressed in non-small cell lung cancer (NSCLC) tissues [9]. They found KIAA1522 expression was a prognostic predictor for NSCLC patients [9]. These studies revealed that KIAA1522 functions as tumor promoter in multiple cancers but its role in BC was not investigated until now.

In our study, we examined KIAA1522 expression levels in BC cell lines and normal breast epithelial cell line by western blot. In vitro functional determination measures were conducted to investigate the roles of KIAA1522 in BC. microRNAs (miRNAs) have been previously demonstrated could regulate the expression of tumor-related genes to participate in cell behaviors regulation process [10,11]. Therefore, we also investigated the miRNA that can regulate

* Corresponding author.
E-mail address: xxmu_lina@163.com (N. Li).

https://doi.org/10.1016/j.bbrc.2018.08.172
0006-291X/

KIAA1522 expression to fully elucidate the mechanisms underlying BC progression.

2. Materials and methods

2.1. Cell lines and transfection

We purchased three BC cell lines (MCF-7, MDA-MB-231, T47D) and one normal breast epithelial cell line (MCF-10A) from American Type Culture Collection (Manassas, VA, USA). All BC cell lines were incubated in DMEM (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA); while MCF-10A cell line was incubated in RPMI-1640 (Invitrogen, Thermo Fisher Scientific) in humidified incubator containing 5% CO_2. 10% fetal bovine serum, 100U/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Thermo Fisher Scientific) were supplemented to all the cell culture. The cultivation temperature was maintained at 37 °C.

Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific) was employed to transfect the synthesized miRNAs (GenePharma, Shanghai, China) including miR-125b-5p mimic, miR-125b-5p inhibitor, and negative control miRNAs (NC-miRNA) or expression-ready KIAA1522 ORF construct and NC construct (Genecopeia, Guangzhou, China) into the investigated BC cell line according to the manufacturer's protocols. The cells were collected for the following experiments after 48 h of transfection.

2.2. Quantitative Real-time PCR (qRT-PCR)

We extracted the total RNA from the cultured cells using Trizol reagent (Invitrogen, Thermo Fisher Scientific) according to the provided protocols. The extracted RNA was quantified using NanoDrop-1000 equipment (Thermo Fisher Scientific). First strand cDNA was synthesized from the extracted RNA using BeyoRT™ cDNA synthesis kit (Beyotime, Haimen, Jiangsu, China). Expression levels of miR-125b-5p were calculated using BeyoFast™ SYBR Green qPCR Mix (Beyotime) at an ABI 7500 Real-time PCR system (Applied Biosystems, Thermo Fisher Scientific) with U6 snRNA as endogenous control and analyzed with the $2^{-\Delta\Delta Ct}$ method [12]. The primers used in this study were listed as follows: miR-125b-5p, forward: 5′-TCCCTGAGACCCTAACTTGTGA-3′ and reverse: 5′-AGTCTCAGGGTCCGAGGTATTC-3’; U6 snRNA, forward: 5′-TGCGGGTGCTCGCTTCGGCAGC-3′ and reverse: 5′−CCAGTGCA GGGTCCGAGGT-3’.

2.3. Western blot

We isolated the total protein samples from the cultured cells using RIPA lysis buffer supplemented with protease inhibitor (Beyotime). The concentration of the extracted protein was analyzed using BCA kit (Beyotime). 10% SDS-PAGE was employed to separate the protein samples. The separated protein samples were transferred to PVDF membrane (Beyotime) and incubated with fat-free milk. Subsequently, the membranes were incubated with primary antibodies (rabbit anti-KIAA1522: ab122203; rabbit anti-GAPDH: ab181602; Abcam, Cambridge, MA, USA). The membranes were washed with TBST, and incubated with horseradish peroxidase conjugated goat anti-rabbit secondary antibody (ab6721, Abcam). The bands were visualized using enhanced chemiluminescence kit (Beyotime) and analyzed using Image J 1.42 software (Bethesda, MD, USA).

2.4. Cell counting kit-8 (CCK-8) assay

Cell proliferation of the transfected cells was analyzed with CCK-8 assay. Cells were seeded at the density of 40,000 cells/well to 96-well plate and incubated for 0−3 days. The CCK-8 reagent (Beyotime) was added to the plate at indicated time and further cultured for 2 h. The optical density at 450 nm was measured with a microplate reader (Bio-Rad, Hercules, CA, USA).

2.5. Colony formation assay

Cells transfected with synthetic miRNAs were seeded at the density of 500 cells/well to 6-well plate. Cells were incubated in the aforementioned medium for 2 weeks. The cells were fixed with methanol, washed with PBS and then stained with 0.1% crystal violet solution (Beyotime). Colonies were observed under microscope.

2.6. Wound-healing assay

Cell migration of the transfected cells was analyzed using wound-healing assay. Cells were seeded onto 6-well plate and cultured to 90% confluence. Then, a wound at the cell surface was created using pipette tip. Cells at each well was observed at 0 and 24 h after wound creation under microscope.

2.7. Transwell invasion assay

Cell invasion of the transfected cells was analyzed using transwell invasion assay. The cells to be examined were seeded to the upper chamber and supplemented with DMEM. The lower chamber was supplemented with DMEM supplemented with FBS. The membrane was pre-coated with Matrigel (Franklin Lakes, NJ, USA). The cells were incubated for 48 h and the invasion cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet solution (Beyotime). Invasion numbers were counted under microscope.

2.8. Luciferase reporter assay

Bioinformatic analysis algorithm TargetScan (http://www.targetscan.org/vert_72/) was used to predict the targets of miR-125-5p. The binding site of miR-125-5p in the 3′-untranslated region (3′-UTR) of KIAA1522 (wild-type or mutant) was cloned into pMIRREPORT vector (Promega, Madison, WI, USA) and named as wt KIAA1522 or mut KIAA1522. Cells were co-transfected with miRNAs and luciferase vector contains wt or mut KIAA1522 using Lipofectamine 2000 (Invitrogen). Luciferase activity was analyzed using Dual-luciferase reporter system (Promega) after transfection for 48 h.

2.9. Statistical analysis

Statistical difference between two groups was analyzed using Student's *t*-test. Differences among three or above groups were analyzed using One-way analysis of variance and Tukey post hoc test. Data analysis was performed at GraphPad Prism 5 (Graph pad Software, La Jolla, CA, USA) and presented as means ± SD. Difference was considered as significant when $p < 0.05$.

3. Results

3.1. miR-125b-5p expression was significantly reduced in BC cell lines

The role of miR-125b-5p in BC was investigated by examining the levels of miR-125b-5p in BC cell lines (MCF-7, MDA-MB-231, T47D) and one normal breast epithelial cell line (MCF-10A) by qRT-PCR. The results showed expression level of miR-125b-5p in

Please cite this article in press as: Y. Li, et al., miR-125b-5p inhibits breast cancer cell proliferation, migration and invasion by targeting KIAA1522, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.08.172