Biochimie 147 (2018) 98–104

Contents lists available at ScienceDirect

Biochimie

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

Research paper

MiR-143-3p inhibits the proliferation, cell migration and invasion of human breast cancer cells by modulating the expression of MAPK7

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ARTICLE INFO

Article history:
Received 28 November 2017
Accepted 11 January 2018

Keywords:
Breast cancer
Cell migration
Invasion
miR-143

ABSTRACT

Micro-RNAs have been reported to play crucial roles in a diversity of cellular processes such as cell proliferation, differentiation and development by regulating the expression of specific genes. They have also been shown to play vital roles in several diseases such as cancer. In the present study, we investigated the role of miR-143-3p in breast cancer. Our results showed that the expression of miR-143-3p is significantly downregulated in breast cancer cells. Upregulation of miR-143-3p inhibited the proliferation and migration of breast cancer cells. Conversely, inhibition of miR-143-3p promoted the proliferation of cancer cells. Bioinformatics analysis and other several experiments revealed MAPK7 as the potential target of miR-143-3p. Quantitative RT-PCR showed that the expression of MAPK7 correlated well with the expression of miR-143. Moreover, the inhibition of MAPK 7 in breast cancer cells abrogated the effects of miR-143 indicating that miR-143-3p-exerted effects on breast cancer are mediated by MAPK7. Takentogether, these results provide strong clues about the therapeutic potential of miR-143-3p in the treatment of breast cancer.

1. Introduction

Breast cancer is one of the frequently detected non-cutaneous cancers in woman around the world. In United States alone, one in every eight women is affected by breast cancer and it accounts for 26% of all female cancers [1]. In 2007 alone, around 17,840 women and 20,30 men were diagnosed with invasive breast cancers. Moreover, around 40,460 women and 480 men died of breast cancer in 2007 in United States [1,2]. Breast cancer still remains the most commonly detected cancerous in woman and its heterogeneity impedes its early diagnosis and subsequent treatment [3]. The breast cancers are mainly of epithelial origin and the different sub-types of breast cancers exhibit different microscopic and biological features. Treatment options for patients with breast cancer include surgery, radiotherapy, chemotherapy, endocrine therapy, biologic therapy and supportive care. However, despite the recent advancements in the field of pharmacology, the results are still far from descent [3,4]. Therefore, there is pressing need to develop more viable treatment strategies and/or to explore novel therapeutic targets to enable the management of breast cancer across the globe. MicroRNAs (miRNAs) include a large family of small non-coding RNAs that are involved in the inhibition of protein synthesis by binding to different sites on 3′-untranslated region (3′-UTR) of target genes. Therefore, microRNAs exhibit remarkable biological functions in diversity of cellular process [5,6]. Among MicroRNAs, miR-143-3p has been reported to play vital role in progression and tumorigenesis of cancers by acting as tumor suppressor. Its role has been studied in several cancers such as colon and breast cancer [7–10]. However, the role of miR-143-3p (now onwards referred to as miR-143 throughout the manuscript) on migration and invasion of breast cancer cells has not been investigated thoroughly. In the present study we investigated the function of miR-143 in different breast cancer cell lines. Our results showed that the expression of miR-143 is highly upregulated in all the breast cancer cell lines used in the present study. Suppression of miR-143 promoted proliferation, migration and invasion of breast cancer cells. Further, bioinformatic analysis revealed that miR-143 exerts its effects by targeting mitogen activated protein kinase 7 (MAPK7). MAPK7 is a member of the MAP kinase family that acts as a crucial point at which several of the signals are integrated. Studies carried out on MAPK7 have reported that it plays a role in diversity of processes, which include but are not limited to, cell differentiation and proliferation, regulation of gene expression and development [11–14]. The

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https://doi.org/10.1016/j.biochi.2018.01.003
0300-9084/

role of MAPK7 in miR-143 exerted effects on breast cancer was further confirmed by luciferase assays and the present study first time reports MAPK7 as the target of miR-143 in breast cancer cells. Takentogether, we propose that miR-143 could prove a potential therapeutic target for the treatment of breast cancers in general. Moreover, invasive breast cancers are difficult to treat; this study presents miR-143 as potential therapeutic target in invasive breast cancers given the fact that two aggressive and invasive breast cancer cell lines were used in this study.

2. Materials and methods

2.1. Cell lines, culture conditions and transfection assays

Four breast cancer cell lines (MDA-MB-231, SK-BR-3, CAMA-1 and MDA-MB-436), three normal breast cell lines (CMMT, CL-S1, MB 157) and human embryonic kidney HEK 293T cells were obtained from Type Culture Collection of Chinese Academy of Sciences, Shanghai, China. The cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/mL of each penicillin and streptomycin and maintained in a humidified atmosphere containing 5% CO_2. SK-BR-3 cells were cultured in DMEM medium supplemented with 10% FBS and used for dual-luciferase assay. MiR-143 mimics, miR-NC, miR-143 inhibitor and pcDNA/MAPK7 were all obtained from GenePharm (Shanghai, China). MiR-143 (Sequence 5′-UGAGAUGAAGCACUGUAGCUC-3′) mimics or miR-NC (5′-UUCUCCGAACGUGUCACGUTT-3′) was transfected in SK-BR-3 and MDA-MB-231 cells and miR-143 mimics, mimics NC were co-transfected with MAPK7- 30-UTR wt/MAPK7- 30-UTR mut in HEK 293T cells for dual-luciferase assay. SK-BR3 cells were transfected with miR-143 mimics or mimics NC or miR-143 inhibitor or inhibitor NC for MAPK protein expression assay. SK-BR-3 cells were transfected with mimics NC or miR-143 mimics and pcDNA/MAPK7 respectively for rescue assays. All transfection assays was performed using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instruction.

2.2. Isolation of RNA, cDNA synthesis and expression analysis

For isolation of RNA, RNeasy RNA isolation kit was used and the whole procedure was carried out as per the manufacturer's guidelines. Thereafter, cDNA was synthesized with the help of RevertAid cDNA synthesis kit (Fermentas) in accordance with the manufacturer's protocol. To carry out the RT-PCR, the cDNA was diluted 20 times and qRT-PCR was carried out in triplicates in ABI StepOne Real time using SYBR Green Master Mix (Fermentas). The relative quantification method ($^{\Delta\Delta-}$CT) was employed to determine quantitative variation between the replicates examined. The GAPDH was used as positive control.

2.3. CCK-8 assay

To estimate the cell viability of the breast cancer cell lines, CCK-8 assay was carried out. The cells were harvested at 24, 48, 72 and 96 h after transfection and subjected to CCK-8 assay. The cell viability was performed later by cell counting kit-8 (Dojindo, Japan) by following the manufacturer's instructions.

2.4. Colony formation assay

For colony formation, the cells were platted at 200 cells/well. After 6 days of incubation, the cells were washed with PBS and fixed with methanol. Afterwards, the cells were treated with crystal violet for 30 min and then counted under light microscope.

2.5. Wound healing assay

The cell migration potential of breast cancer cells was investigated by wound healing assay. Briefly 5×10^4 cells/well were seeded in 96-well plates. Afterwards the plates were incubated overnight at 37 $^{\circ}$C to allow the cells to adhere. Then a wound was scratched using a sterile pipette tip after the cells reached confluence. The cells were then washed with PBS to clear the detached cells. The cells were monitored after 20 h interval and photographed.

2.6. Boyden Chamber assay

Cell invasion was carried out by Boyden chamber assay with some modifications. The breast cancer cells at the density of 5×10^4 cells/well were suspended in 2% FBS medium and placed in the upper chamber of 8 μm pore size transwells with Matrigel. Afterwards, medium supplemented with 10% FBS was added to lower chamber. This was followed by an incubation of 24 h. On the upper surface of the membrane, unmigrated cells were removed while as on the lower surface of the membrane the migrated cells were fixed in methanol (100%) and giemsa stained. The cell migration was estimated by counting the number of the migrated cells under a microscope at 200× magnification.

2.7. Identification of target and dual-luciferase assay

To identify the target of miR-143, we used the online TargetScan software and MAPK7 was selected as the potential target. MAPK7-30-UTR-wt containing the putative miR-143 binding site and corresponding MAPK7-30-UTR-mut were cloned into pmirGLO Dual-Luciferase miRNA Target Expression vector (Promega, Madison, USA) respectively. This was followed by seeding of the cells in a 96-well plate and cotransfection with a recombinant plasmid and mimics NC or miR-143 mimics with the help of Lipofectamine 2000. Finally, the luciferase activity was checked with the help of a Dual-Luciferase Reporter Assay System (Promega) at 48 h post transfection.

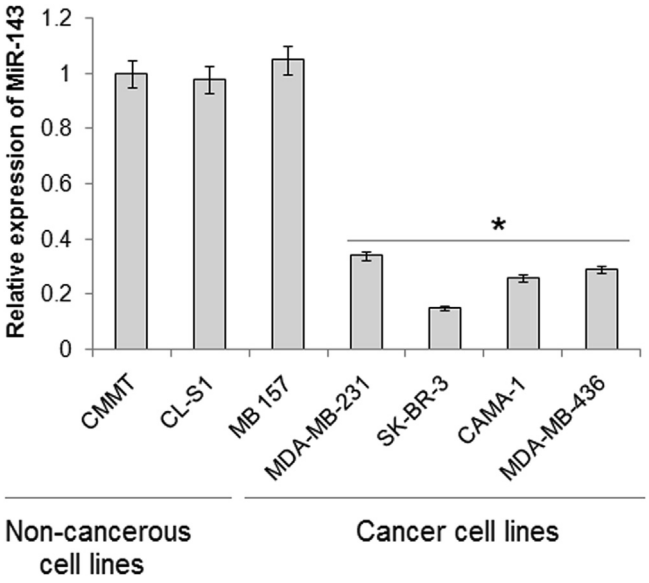

Fig. 1. Expression of miR-143 in cancerous and non-cancerous breast cancer cell lines as determined by quantitative RT-PCR analysis. The experiments were carried out in triplicates and expressed as mean ± SD. The values were considered significant at $^{*}p < 0.01$.