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miR-218 and miR-129 regulate breast cancer progression by targeting Lamins

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ABSTRACT

Breast cancer is the most frequently diagnosed life-threatening cancer in women. Triple-negative breast cancer (TNBC) has an aggressive clinical behavior, but the treatment of TNBC remains challenging. MicroRNAs (miRNAs) have emerged as a potential target for the diagnosis, therapy and prognosis of breast cancer. However, the precise role of miRNAs and their targets in breast cancer remain to be elucidated. Here we show that miR-218 is downregulated and miR-129 is upregulated in TNBC samples and their expressions confer prognosis to patients. Gain-of-function and loss-of-function analysis reveals that miR-218 has a tumor suppressive activity, while miR-129 acts as an oncomir in breast cancer. Notably, miR-218 and miR-129 directly target Lamin B1 and Lamin A, respectively, which are also found to be deregulated in human breast tumors. Finally, we demonstrate Lamins as the major factors in reliable miR-218 and miR-129 functions for breast cancer progression. Our findings uncover a new miRNA-mediated regulatory network for different Lamins and provide a potential therapeutic target for breast cancer.

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1. Introduction

Breast cancer is the most frequently diagnosed life-threatening cancer in women and the leading cause of cancer death among women [1]. Triple-negative breast cancers (TNBCs; clinically negative for expression of estrogen and progesterone receptors (ER/PR) and HER2 protein) comprise 15–20% of all breast cancers but are highly recurrent and metastatic, currently lack validated targeted therapies, and account for worse mortality [2]. Thus, there is an urgent need for new targets for the treatment of breast cancer, including TNBC.

MicroRNAs (miRNAs), a subset of small non-coding RNAs, have emerged as novel targets for cancer therapy [3]. MiRNAs negatively regulate expression of their cognate target genes by directly targeting the 3′-untranslated region (UTR) of mRNA, leading to degradation of mRNA molecules or inhibition of their translation [4]. Indeed, miRNAs are involved in regulating cancer cell proliferation, invasion and migration, implying that they can function either as oncogenes or tumor suppressors [3]. Several recent studies have proposed miRNAs as novel and potential biomarkers for the diagnosis, therapy and prognosis of breast cancer. However, the precise role of miRNAs and their targets in breast cancer remain to be elucidated.

Lamins, type V intermediate filaments, are the main components of the nuclear lamina. Lamins are divided into A and B types based on similarities in their primary sequence and biological properties. In mammals, two major A-type (Lamin A and C, which are all products of alternative splicing from the *LMNA* gene) and two major B-type (Lamin B1 and B2, which are encoded by two separate genes, *LMNB1* and *LMNB2*, respectively) Lamins have been characterized [5]. The well-established function of Lamins is to provide shape and mechanical stability to the nucleus. Lamins also regulate and support protein complexes involved in DNA replication, transcription, nuclear positioning and aging. Intriguingly, expression of Lamin A is often reduced or absent in human cancers such as breast, ovarian, colon and gastric cancers [6–8]. In contrast, Lamin B1 is upregulated in liver, pancreas and prostate cancers [9–11]. Nevertheless, however, the regulatory mechanism for expression of Lamins and their clinical significance in breast cancer still remain poorly understood.

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In this study, we identify novel Lamins-targeting miRNAs, miR-218 and miR-129, that can regulate breast cancer progression, and present an unexpected evidence for differential regulatory networks modulated by miRNAs for individual types of Lamins.

2. Materials and methods

2.1. Human tissue samples

The study cohort comprised triple-negative breast cancer (TNBC) and adjacent normal tissues consecutively ascertained at the MD Anderson Cancer Center (MDACC). All biopsies were evaluated at MDACC, and the histological diagnosis was based on established criteria. This work was performed in accordance with the Institutional Review Board (IRB) approval at MDACC.

2.2. Cells

MCF10A, MCF7, MDA-MB-231 and LM2, and HEK293T cells were obtained from ATCC. Cells were cultured in DMEM medium with 10% FBS and 1% P/S.

2.3. Oligonucleotides

Oligonucleotides were purchased from Dharmacon: hsa-miR-129−5p mimic (C-300539-03), 5′-CUUUUUGCGGUCUGGGCUUGC-3’; hsa-miR-129−5p inhibitor (IH-300539-05); hsa-miR-218 mimic (C-300574-03), 5′-UUGUGCUUGAUCUAACCAUGU-3’; hsa-miR-218−5p inhibitor (IH-300574-05); miRNA mimic negative control #1 (CN-001000-01), based on cel-miR-67 mature sequence: 5′-UCACAACCUCCUAGAAAGAGUAGA-3’; miRNA inhibitor negative control (IN-001005-01). The oligonucleotides were transfected into cells using DharmaFECT reagent (Dharmacon) with a final concentration at 100 nmol/L.

2.4. RT-qPCR analysis

Total RNAs and small RNAs from cells were extracted using the TRIzol reagent (Ambion) and the mirVana miRNA isolation kit (Invitrogen) according to the manufacturer's instructions and then reverse-transcribed into cDNAs with the high capacity cDNA reverse transcription kit (Applied Biosystems) or the TaqMan microRNA reverse transcription kit. Taqman probes included hsa-miR-129−5p (miRBase accession number: MIMAT0000242), hsa-miR-218−5p (miRBase accession number: MIMAT0000275), and U6 snRNA (NCBI accession number: NR_004394.1). Primers for human *LMNA*, 5′-TGGAGATGATCCCTTGCTGA-3’; 5′-GCATGGC-CACTTCTTCCCA-3′, human *LMNB1*, 5′-AAGCAGCTGGAGTGGTTGTT-3’; 5′- TTGGATGCTCTTGGGGTTC-3’. 18S rRNA (5′-GGCCCTGT-AATTGGAATGAGTC-3’; 5′- CCAAGATCCAACTACGAGCTT-3′) was used for normalization. Expressions of specific genes were determined using an ABI StepOnePlus System (Applied Biosystems).

2.5. Antibodies

Anti-Lamin A/C (1:2000; sc-20681, Santa Cruz Biotech or 1:5000; 2032, Cell Signaling), anti-Lamin B1 (1:500; sc-6216, Santa Cruz Biotech or 1:5000; ab16048, Abcam), anti-pRb (1:1000; 9313, Cell Signaling), anti-Cyclin D2 (1:1000; sc-376676, Santa Cruz Biotech) and mouse anti-β-Actin (1:10000; A1978, Sigma).

2.6. Lentiviral infection

To prepare lentiviral particles, 3×10^6 293T cells were plated and then pEZX-MR03 (Genecopeia) for scrambled control (CmiR0001), hsa-miR-129 (HmiR0114) or (HmiR0064), and/or pCDHblast MCSNard OST-LMNA (22661; Addgene) or CCSB-Broad LentiORF clone-LMNB1 (OHS6085-213579044; Dharmacon) were transfected with Lipofectamine 2000 (Invitrogen). For infection, 3×10^5 cells of MDA-MB-231 were plated and infected by virus from 293T cells 48 h after transfection. After selection in puromycin (2 μg/ml) or blasticidin (10 μg/ml), cells were used for growth curves, transwell, RT-qPCR and immunoblot analysis.

2.7. Luciferase reporter assay

293T cells were transfected with psiCHECK2 containing 3′UTR of *LMNA* or *LMNB1* together with miRNA mimics or inhibitors. Cells were harvested 48 h after transfection and subjected to the luciferase assay using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activity was determined with a GloMax Discover Multimode Detection System (Promega).

2.8. Cell proliferation analysis

Cell proliferation assays were performed as previously described [12]. Briefly, cells were plated at 1×10^4 per well of a 12-well plate in triplicate and fixed with 10% formalin followed by staining with crystal violet at the indicated time point. The dried dye was dissolved in 10% acetic acids followed by measuring the absorbance of each sample at 595 nm using a GloMax Discover Multimode Detection System.

2.9. Cell migration assay

Cell migration assays were performed as previously described [13]. To distinguish the effects of proliferation from migration, cells were pretreated with mitomycin C (5 μg/ml), a potent DNA crosslinker, in serum-free medium for overnight. Cells were then seeded at 1×10^4 per well of a 24-well plate onto 8 μm Transwell chamber (BD Biosciences), and the chambers were placed and incubated in complete medium with 10% serum for 12 or 24 h. Migrated cells were stained with crystal violet followed by quantification.

2.10. Cancer biostatistical analysis

For correlation analysis between miR-129/miR-218 or *LMNA*/*LMNB1* and prognosis in breast cancer patients, Kaplan-Meier plotting and log-rank test were done using a publicly accessible KM Plotter (http://kmplot.com/analysis/) [14,15]. Patients were divided into two classes based on miR-129/miR-218 or *LMNA*/*LMNB1* expression: the high- and low− miR-129/miR-218 or *LMNA*/*LMNB1* groups were split based on the median value calculated across the entire dataset to generate two groups of equal size. $n = 1262$ for miRNA prognosis; $n = 3951$ for Lamins prognosis.

2.11. Statistical analysis

Statistical analysis was performed with SPSS V.20.0 and GraphPad Prism 6. Two-tailed Student's t tests were used for single comparison, and analysis of variance (ANOVA) with Bonferroni post-hoc tests was used for multiple comparisons unless otherwise specified. The correlation coefficients were calculated by the PASS Pearson Chi-Square test. *p* values below 0.05 were considered statistically significant.

3. Results

miR-218 is downregulated and miR-129 is upregulated in triple-

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