



Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

SIRT1 promotes proliferation, migration, and invasion of breast cancer cell line MCF-7 by upregulating DNA polymerase delta1 (POLD1)

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ARTICLE INFO

Article history:

Received 18 May 2018

Accepted 24 May 2018

Available online xxx

Keywords:

SIRT1

POLD1

Breast cancer

Proliferation

Migration

Invasion

ABSTRACT

Sirtuin 1 (SIRT1), class III histone deacetylase, plays an important character in cell proliferation, cell cycle, apoptosis, energy metabolism and DNA repair. In recent years, researchers have attached increasing attention on the role of SIRT1 in tumorigenesis, development and drug resistance. The effect of SIRT1 on breast cancer is still controversial and its exact role remains to be elucidated. In the present study, we investigated the significant role of SIRT1 in breast cancer by exploring the effect of SIRT1 on DNA polymerase delta1 (POLD1), the gene coding for DNA polymerase δ catalytic subunit p125. Immunohistochemistry showed that the protein expression level of SIRT1 was higher in breast cancer tissues relative to adjacent normal tissues. Knockdown of SIRT1 by shRNA decreased the proliferation, migration, and invasion of human breast cancer cell line MCF-7, while the overexpression of SIRT1 promoted the proliferation, migration, and invasion of MCF-7 cells. Clinically, the immunohistochemistry results revealed that the expression of SIRT1 was positively correlated with p125. Further analysis demonstrated that silencing of SIRT1 increased the expression of p53, while the expression level of POLD1/p125 decreased, and the result by overexpressing SIRT1 was opposite. Collectively, these data suggest that SIRT1 is an oncogenic factor in breast cancer cells and can be involved in the progression of breast cancer by inhibiting p53 and activating POLD1. Our finding provides new insights into the mechanisms of breast cancer.

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1. Introduction

Breast cancer is currently ranked first in the incidence of cancer in women and mortality is second only to lung cancer. In the latest cancer statistics report of the United States in 2018, breast cancer accounted for 30% of new cancer cases among women, far more than other cancers [1]. The incidence of breast cancer in some developing countries is showing an increasing trend and the mortality rate is higher than that in developed countries [2,3]. With the in-depth study of the molecular mechanisms in breast cancer, some genes are thought to be associated with breast cancer, but the mechanisms are not completely clear [4]. Accordingly, the exploration of deeply pathogenesis in breast cancer is urgent.

Sirtuin 1 (SIRT1), a mammal homologue of the yeast protein silent information regulator 2 (Sir2), belongs to class III histone deacetylase and is a nicotinamide adenine dinucleotide (NAD⁺) dependent deacetylase [5]. SIRT1 can not only deacetylate histone, such as histone H3, H4, but also interact with a variety of transcription factors and their transcriptional cofactors including p53, NF- κ B, FOXO family, c-Myc, etc. to regulate cell proliferation, cell cycle, apoptosis, DNA repair and energy metabolism, thereby playing a critical role in tumor initiation, progression and drug resistance [5,6].

DNA polymerase δ is a member of the DNA polymerase family, consisting of four subunits: p125, p68, p50, and p12 [7]. It plays an important part in the stability of the genome by participating in DNA replication and various repair processes [8]. Human DNA polymerase delta1 (POLD1) acts as the gene coding for p125, which is a catalytic subunit of DNA polymerase δ [9]. POLD1 was reported to be involved in the regulation of cell proliferation and cell cycle through the combination with proliferating cell nuclear antigen (PCNA) [10]. Researchers have validated that POLD1 can help cancer cells to tolerate DNA damage [11,12]. Meanwhile, as a

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transcriptional target of p53, POLD1 was regulated by p53 to control cell proliferation, cell cycle, and DNA repair [13]. Our previous studies showed that POLD1 was closely related to the progression of breast cancer [14].

In recent years, most studies have demonstrated that SIRT1 played as an oncogene in tumors [15]. However, some reports suggested that the effect of SIRT1 on tumor was bidirectional; it can act as an oncogene or a tumor suppressor, depending on the study conditions and the context [16]. Therefore, the exact role and mechanism of SIRT1 in breast cancer remain to be verified. Behrouzfar et al. [17] showed that increased production of NAD⁺ in breast cancer cells provided a basis for SIRT1 to promote the proliferation of breast cancer cells by affecting multiple transcription factors or oncogenes, and the tumor suppressor gene p53 was a target of SIRT1. Meanwhile, as one of the target genes of p53, POLD1 can be involved in regulating the proliferation of cancer cells [13]. Therefore, whether SIRT1 can regulate the activity of POLD1 through influencing the function of p53 to affect the occurrence and progression of breast cancer will be analyzed in this experiment.

In the present study, we investigated the role of SIRT1 in breast cancer cell line MCF-7 and the correlation between SIRT1 and POLD1. Our finding is hoped to provide new insights into the roles and mechanisms of SIRT1 in breast cancer.

2. Materials and methods

2.1. Patient samples wax block

The pathological wax block was obtained from the cancerous tissues and adjacent normal tissues of 60 patients with breast invasive ductal carcinoma in Department of Breast Surgery, Affiliated Tumor Hospital of Guangxi Medical University. All patients underwent modified radical mastectomy or breast-conserving surgery. They did not receive any radiotherapy or chemotherapy before surgery, and had no serious or uncontrolled medical diseases or infections. Consents approving the use of their tissues for this study were obtained from the patients. The study protocol was approved by the Institute Research Ethics Committee at the Affiliated Tumor Hospital of Guangxi Medical University.

2.2. Cell lines and cell culture

MCF-10A (human normal breast epithelial cell line) was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany), and MCF-7 (human breast cancer cell line) was obtained from the Cell Culture Bank of Chinese Academy of Sciences (Shanghai, China). MCF-10A were cultured in Mammary Epithelial Cell Medium (ScienCell Research Laboratories, California, USA), MCF-7 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Gibco, New York, USA), both were maintained in a constant temperature incubator at 37 °C and 5% CO₂.

2.3. Immunohistochemistry

The wax blocks were routinely sectioned, dewaxed, and heat-treated with high pressure for 2 min, then incubated with primary antibodies and secondary antibody, followed by DAB staining (Meixin Reagent Co. Ltd., Fuzhou, China), Hematoxylin stained, dehydration, and fixed with neutral gum. PBS was used instead of rabbit anti-SIRT1 and rabbit anti-POLD1 as a negative control. Each section was independently evaluated by 2 pathologists without knowing the data of patients. The intensity was scored as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. The positive area was

defined as follows: 0, less than 5%; 1, 5%–25%; 2, 26%–50%; 3, 51%–75%; and 4, greater than 75%. The immunohistochemical staining score (values, 0–12) was determined by multiplying the score for staining intensity with the score for positive area. For statistical analysis, scores of 0–3 were considered negative expression and scores of 4–12 considered positive expression.

2.4. Cell transfection

The short hairpin-RNA (shRNA)-induced RNA interference (RNAi) was used to knock down or overexpress SIRT1 in MCF-7 cells. The recombinant lentivirus vectors including LV-shRNA-SIRT1-KD, LV-shRNA-SIRT1-OE and the corresponding negative control (LV-shRNA-NC-KD, LV-shRNA-NC-OE) were synthesized by Genechem Co. Ltd. (Shanghai, China). MCF-7 cells were used to transfect. The cells were divided into five groups including LV-shRNA-SIRT1-KD transfected cells (KD group), LV-shRNA-NC-KD transfected cells (NC-KD group), LV-shRNA-SIRT1-OE transfected cells (OE group), LV-shRNA-NC-OE transfected cells (NC-OE group) and non-transfected cells (Control group). Each group set 3 replicate wells. MCF-7 cells suspension (5×10^4 cells per well) was plated in a 6-well plate. Transfection was performed when the cell density reached approximately 70%. At the multiply of infection (MOI) = 30, the final volume of each well was 1 ml, containing the virus particles, polybrene (a final concentration of 5 g/ml), and the enhanced infection solution (ENi.s). The fluorescence intensity and area of cells were observed at 24 h, 48 h, and 72 h after infection by using the inverted fluorescence microscope, qRT-PCR and western blot were used to examine the efficiency of transfection.

2.5. Cell proliferation assay

Cell counting kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan) was used to detect cell proliferation. The cell suspension (2×10^3 cells per well) was seeded in a 96-well plate and cultured 6 h to allow attachment. At 1, 2, 3, 4 and 5 d, cells were incubated with 10 μ l CCK-8 solution for 1 h. The absorbance at 450 nm was analyzed using a microplate reader (Thermo Fisher Scientific, Waltham, USA) to plot the growth curve. Each group set 5 replicate wells.

2.6. Cell migration assay

Cell migration was conducted by using the 24-well transwell chamber. The cells were resuspended in serum-free medium at a cell density of 5×10^5 cells/ml. 100 μ l of the cell suspension (5×10^4 cells per well) was added in the upper chamber, and 10% FBS DMEM medium was added into the lower chamber. After placing the plate in a constant temperature atmosphere at 37 °C of 5% CO₂ for 24 h, the cells that did not pass through the polycarbonate membrane in the upper chamber were wiped off. Cells on the lower surface were fixed with 4% paraformaldehyde for 30 min, stained with 1% giemsa (Solarbio, Beijing, China) for 50 min, rinsed by PBS, dried in air and counted under an inverted microscope (OLYMPUS, Tokyo, Japan). Result was the average of cell numbers in five different views. Each group set 3 replicate wells.

2.7. Cell invasion assay

The cells were placed in a constant temperature incubator at 37 °C, 5% CO₂ to starve for 10 h in serum-free medium. The upper surface of the 24-well transwell chamber was spread evenly with a layer of matrigel (BD, New Jersey, USA). The cells were resuspended in serum-free medium at a cell density of 1×10^6 cells/ml. 100 μ l of the cells suspension (1×10^5 cells per well) was added in the upper

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