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KIAA0101 inhibition suppresses cell proliferation and cell cycle progression by promoting the interaction between p53 and Sp1 in breast cancer

Wei Lv [a], Benhua Su [b], Yuyang Li [a], Chong Geng [a], Na chen [c, *]

[a] Department of Breast and Thyroid Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, 250021, PR China
[b] Department of Medical Engineering, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, 250021, PR China
[c] Department of Hematology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, 250021, PR China

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ABSTRACT

KIAA0101 functions as a regulator of centrosome number in breast cancer. Here, we identify the role of KIAA0101 in breast cancer cell proliferation and cell cycle progression. KIAA0101 knockdown significantly inhibited cell growth, colony formation and G1/S phase transition. Further investigation indicated that KIAA0101 silencing suppressed the expression of CCNE2, CDK6 and CDKN1A. Luciferase reporter assay and ChIP assay demonstrated that Sp1 positively regulated the transcription of CCNE2, CDK6 and CDKN1A. KIAA0101 knockdown promoted the interaction between p53 and Sp1, inhibiting the transcriptional activation of Sp1 on CCNE2, CDK6 and CDKN1A. Knockdown of p53 counteracted the inhibitory effect of KIAA0101 knockdown on breast cancer cells proliferation and cell cycle progression while Sp1 knockdown mimicked the effect of KIAA0101 knockdown. These results suggested that KIAA0101 knockdown suppressed the cell proliferation and cell cycle progression by promoting the formation of p53/Sp1 complex in breast cancer.

1. Introduction

Breast cancer is one of the most common malignancies, and is the leading cause of cancer-related death in women worldwide [1]. As a complex multigenic disease, breast cancer deregulates the expression of oncogenes implicating in cell proliferation, differentiation and apoptosis. Sustaining proliferation has been demonstrated as the most common characteristics of breast cancer. The activation of proliferative signals guides tumor cells into cell cycle progression, during which G1/S phase transition is the most essential step [2]. In this progression, cyclin E1 and E2 initiates DNA replication by activating CDK2, which in turn phosphorylates retinoblastoma (Rb) to promote the progression into S phase [3]. Inhibition of cyclin E-CDK2 activity notably suppresses tumor proliferation [4,5]. Therefore, it is necessary to clarify the precise mechanism of cell cycle transition in the progression of breast cancer.

KIAA0101 is a conserved proliferating cell nuclear antigen (PCNA)-binding motif, and also named as p15 PAF (PCNA-associated factor) or OEATC-1 (overexpressed in anaplastic thyroid carcinoma-1) [6]. KIAA0101 has been involved in translesion DNA synthesis, a DNA-repair process, by mediating the interaction with DNA synthesis polymerase [7]. KIAA0101 functions as an oncogene and is up-regulated in breast cancer, lung cancer, and colon cancer [8–10]. Overexpression of KIAA0101 in breast cancer is associated with significantly decreased survival time by controlling centrosome number [11]. Our results revealed that inhibition of KIAA0101 suppressed breast cancer cells proliferation and cell cycle progression by regulating the interaction between p53 and Sp1.

2. Materials and methods

2.1. Antibodies and reagents

The antibodies against KIAA0101 (pAb, ab226255), CCNE2 (mAb, ab32103), CDK6 (pAb, ab151247), CDKN1A (mAb, ab109520), CDKN1B (mAb, ab32034), BRCA1 (pAb, ab9141), p53 (mAb, ab26), and Sp1 (pAb, ab59257) were purchased from Abcam (Cambridge, MA). The antibody against GAPDH was obtained from Anbo Biotechnology Company (San Francisco, CA, USA). Lipofectamine 3000 and pcDNA3.1 plasmid were purchased from Invitrogen Life

* Corresponding author.
E-mail address: nachen1982@126.com (N. chen).

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Technologies (Carlsbad, CA). The shRNA of KIAA0101 was synthesized by GenePharma (Shanghai, China). The siRNAs targeting Sp1 and p53 were synthesized by Biosune biotechnology (Shanghai, China).

2.2. Cell culture

Human breast cancer MDA-MB-231 and T47D cell lines were cultured in DMEM/F12 medium (Hyclone) containing 10% fetal bovine serum (Gibco), 100 U/mL penicillin, and 100 U/mL streptomycin in 5% CO_2 at 37 °C.

2.3. Quantitative RT-PCR

Total RNA was isolated using Trizol reagent (Invitrogen). The cDNA was generated by reverse transcription using the RevertAid First Strand cDNA Synthesis Kit (Fermentas). The mRNA expression of KIAA0101 and GAPDH were determined using the specific primers (KIAA0101-forward: 5′-CTCTGCCACTAATTCGACATCA-3′, KIAA0101-reverse: 5′-TTCAGAATCTTTAGGGGACAAC-3'; GAPDH-forward: 5′-TGACTTCAACAGCGACACCCA-3′, GAPDH-reverse: 5′-CACCCTGTTGCTGTAGCCAAA-3′). The PCR reactions were performed in triplicate using a SYBR Premix ExTaq kit (Takara) in a LightCycler480 System.

2.4. Gene transfection

The MDA-MB-231 and T47D cells were plated at a density of 2×10^5 cells/well in 6-well plates, and were cultured overnight. The cells were transfected with lentivirus carrying small interfering RNA (si-RNA) targeting KIAA0101 (5′-GCTTTGTTGAACAGGCATTTA-3′), Sp1 (5′-ATCACTCCATGGATGAAATGA-3′) [12] or p53 (5′-GACUCCAGUGGUAAUCUAC-3′) [13].

2.5. Cell proliferation analysis

After transfection, the MDA-MB-231 and T47D cells were seeded at a density of 2×10^3 cells/well in 96-well plates, and were cultured overnight. Subsequently, cells carrying GFP were detected at indicated time using Cellomics ArrayScan VT1.

The transfected cells were seeded at a density of 1×10^4 in a 96 well-plates. After cultured for indicated time, cells were incubated with MTT solution (5 mg/ml) for 4 h. The produced formazan was dissolved in DMSO and the optical density (OD) value was measured at 490 nm using Biotek Elx800.

2.6. Clonogenic assay

After transfection, cells were plated at a density of 800 cells/well in 6-well plates and cultured for 14 days. Subsequently, the colonies were fixed with paraformaldehyde and stained with 1% crystal violet (Sigma) for 20 min. After washed with water, the images of colonies were captured by microscope.

2.7. Western blot analysis

The total protein was isolated using RIPA lysis buffer. The concentration of protein was determined using the bicinchoninic acid method. Equal amounts of proteins were subjected to SDS-PAGE prior to electrotransfer onto PVDF membranes. After blocked using 5% non-fat milk for 1 h at room temperature, the membranes were incubated with the indicated primary antibodies including anti-KIAA0101 (1:2000), anti-CCNE2 (1:500), anti-CDK6 (1:1000), anti-CDKN1A (1:1000), anti-CDKN1B (1:1000), anti-BRCA1 (1:500), anti-p53 (1:1000), anti-Sp1 (1:1000), and anti-GAPDH (1:2000) antibodies overnight at 4 °C, followed by incubation with horseradish peroxidase-conjugated secondary antibodies. The protein signals were detected using the enhanced chemiluminescence method and quantified by Scion Image 4.03 software.

2.8. Microarray analysis

Total RNA was isolated from the transfected cells using Trizol reagent (Invitrogen), followed by analysis by Agilent 2100. An aRNA (amplified RNA) was prepared using the GeneChip 3′ IVT Express Kit according to the manufacturer's instructions. The aRNA is purified, fragmented and hybridized with the chip probe. After the chip is washed and dyed, the image and data are obtained by GeneChip Scanner 3000.

2.9. Luciferase reporter assay

The promoter of CCNE2, CDK6 or CDKN1A was amplified and inserted into pLightSwitch promoter reporter vector to construct luciferase plasmids. HEK-293 cells were plated in 96 well-plates at a density of 1.0×10^4 cells/ml. After cultured overnight, cells were co-transfected with 1 μg pcDNA3.1-p53 or pcDNA3-Sp1 and 0.4 μg luciferase plasmids using Lipofectamine 3000; the pRL-SV40 plasmid (Promega) was used for a normalizing control. 48 h after transfection, luciferase activities were measured using the Dual-Luciferase reporter Assay kit (Promega) according to the manufacturer's instructions.

2.10. Immunoprecipitation

Cells were incubated in a lysis buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1%Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM b-glycerophosphate, 1 mM Na3VO4, 1 mM PMSF, 1 mg/ml Leupeptin. The cell lysates were incubated with 2 μg of anti-BRCA1, anti-p53 or a rabbit IgG at 4 °C for 2 h, followed by incubation with protein G-Sepharose (Life Technologies, CA, USA). Western blot analysis was performed as described above.

2.11. ChIP assay

Cells were crosslinked with 1% fresh formaldehyde for 15 min, and were quenched by adding glycine, followed by incubation in SDS lysis buffer containing 1% protease inhibitors. The cell lysates were sonicated to share DNA to 200−1000 base pairs in length. Protein/DNA complexes were precipitated using 2 μg of anti-Sp1 or control IgG overnight at 4 °C, and were pulled down using protein A/G agarose beads. After reverse crosslink using NaCl, PCR was performed using specific primers for CCNE2, CDK6 or CDKN1A. Primer sequences are listed in Supplementary Table 1.

2.12. Flow cytometric analysis

To test the cell apoptosis, after 120 h of transfection, the cells were harvested using trypsin and were rinsed using PBS. Subsequently, the cells were fixed with 4% paraformaldehyde and incubated with annexinV-FITC (5 μl, 20 μg/ml) in binding buffer at room temperature for 10 min in the dark, followed by flow cytometric analysis.

To determine the cell cycle progression, after grown to 80% confluence in 6 well-plates, cells were harvested and fixed in the 70% pre-cold ethanol for 1 h. After washed with PBS, cells were incubated in PBS containing 50 μg/ml propidium iodide and 100 μg/ml RNase at room temperature for 30 min prior to flow cytometric analysis.

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