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Down-regulation of SIRT3 promotes ovarian carcinoma metastasis

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ABSTRACT

Distant metastasis and local recurrence are still the major causes for failure of treatment in patients with ovarian carcinoma (OC), making it urgent to further elicit the molecular mechanisms of OC metastasis. Sirtuin-3 (SIRT3), a member of the NAD^+-dependent Class III histone deacetylases, may function as different role depending on the cell-type and tumor-type. However, the function and mechanism of SIRT3 has been not explored in OC metastasis. Here, we found that SIRT3 was significantly down-regulated in the metastatic tissues and highly metastatic cell line of ovarian cancer. In addition, knockdown of SIRT3 enhanced the migration and invasion in vitro and the liver metastasis in vivo of ovarian cancer cell. By contrast, ectopic overexpression of SIRT3 dramatically suppressed cancer cell metastatic capability. Mechanistically, SIRT3 inhibits epithelial-to-mesenchymal transition (EMT) by down-regulating Twist in ovarian cancer cells. Furthermore, an interaction between SIRT3 and Twist was detected. In conclusion, our results demonstrated that SIRT3 plays a crucial suppressive role in the metastasis of ovarian cancer by down-regulating Twist, and that this novel SIRT3/Twist axis may be valuable to develop new strategies for treating OC patients with metastasis.

1. Introduction

As the leading cause of death from gynecological cancers in developed countries, ovarian carcinoma (OC) is one of the most common reproductive system malignant cancers [1,2]. Despite considerable efforts to improve early detection, and advances in treatment, metastasis remains a major challenge in the management of ovarian cancer [3]. Therefore, understanding the molecular mechanisms underlying the invasion and dissemination of ovarian cancer are needed.

Sirtuins (SIRT1–7) are proteins that possess either mono-ADP-ribosyltransferase, or deacylase activity, including deacetylase, desuccinylase, demalonylase, demyristoylase and depalmitoylase activity [4–6]. Many studies have demonstrated that these proteins are involved in a wide range of cellular functions, including the regulation of oxidative stress, maintaining genomic stability, apoptosis, protein and fatty acid metabolism [7,8]. SIRT3, a full-length 44-kD protein, has been shown to activate amino-acid metabolism and deacetylate a lot of mitochondrial proteins [9,10]. There is a strong association between SIRT3 alleles and longevity in males [11]. Notably, SIRT3 acts either as a tumor promoter or as a tumor suppressor depending on the cell- and tumor-type, and the presence of different stress or cell death stimuli [12–14]. However, SIRT3's function in the metastasis of ovarian carcinoma remains unknown.

In the present study, reduced expression of SIRT3 was detected in metastatic tissues and highly metastatic cell line of ovarian cancer. Functional studies demonstrated that SIRT3 repressed cell motility in vitro and tumor metastasis in vivo. We further found that SIRT3 down-regulate Twist in ovarian cancer, and the SIRT3/Twist axis may be valuable to develop new therapeutic strategies for OC patients with metastasis.

2. Materials and methods

2.1. Clinical samples and cell lines

All ovarian cancer samples and their matched metastatic tissues from surgical resection were obtained from Xinxiang Central Hospital (Xinxiang, China). Those patients recruited in our study haven't received radiotherapy or chemotherapy before surgery. All clinical samples used were approved by the committee for ethical review of research involving human subjects at Xinxiang Central

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Hospital. Two ovarian carcinoma cell lines (HO-8910, HO-8910PM) were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and maintained in RPMI-1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone). An embryonic kidney cell line 293T was obtained from the American Type Culture Collection (ATCC) and cultured according to its instructions. All cell lines have not been in culture for more than 2 months.

2.2. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from frozen clinical tissues and cultured cell lines using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. First-strand cDNA was synthesized using Revert AidTM First Strand cDNA Synthesis Kit (Fermentas). qRT-PCR was performed to detect the mRNA levels of SIRT3 and GAPDH (as an internal control). The primers used for amplifying SIRT3, Twist, E-Cadherin, N-Cadherin, Snail, Slug and GAPDH were as follows: SIRT3-F: 5′-GACATTCGGGCTGACGTGAT-3′; SIRT3-R: 5′-ACCACATGCAGCAAGAACCTC-3′; Twist-F: 5′-GTCCGCAGTCTTACGAGGAG-3′; Twist-R: 5′-GCTTGAGGGTCTGAATCTTGCT-3′; E-cadherin-F: 5′-ATTTTTCCCTCGACACCCGAT-3′; E-cadherin-R: 5′-TCCCAGGCGTAGACCAAGA-3′; N-cadherin-F: 5′-AGCCAACCTTAACTGAGGAGT-3′; N-cadherin-R: 5′-GGCAAGTTGATTGGAGGGATG-3′; Snail-F: 5′-ACTGCAACAAGGAATACCTCAG-3′; Snail -R: 5′-GCACTGGTACTTCTTGACATCTG-3′; Slug-F: 5′-CGAACTGGACACACATACAGTG-3′; Slug-R: 5′-CTGAGGATCTCTGGTTGTGGT-3′; GAPDH-F: 5′-ACAGTCAGCCGCATCTTCTT-3′; GAPDH-R: 5′-GACAAGCTTCCCGTTCTCAG-3′. Using the comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method, the relative expressions of the indicated genes were calculated and normalized relative to GAPDH. All experiments were repeated thrice.

2.3. Plasmids and stable cell lines

The full-length cDNA of human SIRT3 was cloned into pBabe-puro vector. Cell lines stably expressing Scramble and a short hairpin RNA targeting SIRT3 (shSIRT3) were established by the Sigma shRNA system according to the manufacturer's instructions. The oligonucleotide for human SIRT3 shRNA is 5′-CAACGTCACTCACTACTTT-3′.

2.4. Western blotting analysis

Total protein was extracted from clinic samples and cancer cells for western blot analysis (Beyotime Biotechnology). According to the standard protocol, 50 μg of harvested total protein were loaded, separated in 10% sodium dodecyl sulfate–polyacrylamide gradient gels and transferred onto PVDF membranes (Millipore) followed by blocking with 5% nonfat milk for 2 h at room temperature. Then, membranes were incubated with the indicated primary antibodies overnight at 4 °C. Membranes were washed thrice with PBS containing 0.1% Tween 20, incubated with horseradish peroxidase-conjugated secondary antibody, and then detected using the ECL chemiluminescence system (Pierce). Primary antibodies used: SIRT3, E-cadherin and N-cadherin from Cell Signaling Technology; Twist and Tubulin from Santa Cruz Biotechnology.

2.5. Immunoprecipitation

HO-8910PM cell was lysed in RIPA cell lysis buffer and then centrifuged at 12 000 rpm for 20 min. To investigate the interaction between endogenous SIRT3 and Twist, the supernatants were first incubated with an anti-SIRT3 antibody (Cell Signaling Technology) or an anti-Twist antibody (Santa Cruz Biotechnology) for 2 h at 4 °C. Protein A/G-agarose was then added for 2 h to overnight, and the precipitates were washed five times with RIPA lysis buffer and analyzed by western blotting.

2.6. Transwell assay

The transwell cell migration assay and invasion assay were performed using BD chambers containing polyethylene terephthalate membranes of 8 μm pore size and BD Matrigel Invasion Chambers with 8 μm porosity. Cells were suspended in serum-free medium onto the upper chambers and culture medium with 20% fetal bovine serum was added to the lower chambers. After 24 h of incubation, cells in the lower surface of the filter were fixed, stained, and examined using a Nikon Ti-Eclipse inverted microscope. The number of migrated or invaded cells in three random optical fields from triplicate filters was averaged.

2.7. In vivo tumor metastasis assay

Animal experiments were performed in accordance with established international guidelines. For a hepatic metastasis model, the athymic mice (Vital River Laboratories, China) were subjected to ventrotomy. Briefly, 3×10^6 cells for HO-8910 and 2×10^6 cells for HO-8910PM, were suspended in 40 μl of culture medium and injected into the spleen with an insulin syringe. After forty days, the mice were sacrificed and the intrahepatic metastatic nodules were carefully examined and counted.

2.8. Statistical analysis

Statistical significance was tested by Student's *t*-test. Differences were considered statistically significant when p values were <0.05.

3. Results

3.1. SIRT3 is down-regulated in the metastatic tissues and highly metastatic cell line of ovarian cancer

We first detected the mRNA level of SIRT3 in 30 pairs of ovarian cancer tissues and the corresponding metastatic tissues using qRT-PCR. The relative expression level of SIRT3 was significantly down-regulated in the tumor metastatic tissues compared with their primary counterparts (Fig. 1A). Western blot showed that the reduction of SIRT3 protein was detected in six randomly selected ovarian cancer metastatic tissues (Fig. 1B). Using HO-8910, HO-8910PM, two ovarian cancer cell lines with low and high metastatic ability, respectively, both the mRNA and the protein levels of SIRT3 were lower in the highly metastatic cell line HO-8910PM than that in HO-8910 (Fig. 1C, D). These results suggest that SIRT3 may play an important role in the ovarian cancer progression and metastasis.

3.2. SIRT3 suppresses the cell migration and invasion of ovarian cancer cells

In order to explore the function of SIRT3 in the metastasis of ovarian cancer, we generated stable transfectant of a specific shRNA targeting SIRT3 in HO-8910 cancer cell (Fig. 2A). As expected, the cell migration and invasion capabilities of HO-8910 were dramatically increased by knocking down SIRT3 (Fig. 2B). On the other hand, stably ectopic overexpression of SIRT3 in HO-8910PM substantially reduced cell migration and invasion (Fig. 2C, D). All these data demonstrate that SIRT3 plays a crucial role in regulation of cell migration and invasion of ovarian cancer cells in vitro.