Original Article

Anacardic acid (6-pentadecylsalicylic acid) induces apoptosis of prostate cancer cells through inhibition of androgen receptor and activation of p53 signaling

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Abstract: Anacardic acid (AA) is a mixture of 2-hydroxy-6-alkylbenzoic acid homologs. It is widely regarded as a non-specific histone acetyltransferase inhibitor of p300. The effects and the mechanisms of AA in LNCaP cells (prostate cancer cells) remain unknown. To investigate the effect of AA on LNCaP cells, we had carried out several experiments and found that AA inhibits LNCaP cell proliferation, induces G1/S cell cycle arrest and apoptosis of LNCaP cell. The mechanisms via which AA acts on LNCaP cells may be due to the following aspects. First, AA can regulate p300 transcription and protein level except for its mechanisms regulating function of p300 through post-translational modification in LNCaP cells. Second, AA can activate p53 through increasing the phosphorylation of p53 on Ser15 in LNCaP cells. AA can selectively activate p21 (target genes of p53). Third, AA can down-regulates androgen receptor (AR) through supressing p300. Our study suggests that AA has multiple anti-tumor activities in LNCaP cells and warrants further investigation.

Key Words: Prostate cancer; anacardic acid; apoptosis; LNCaP; p300; p53

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Introduction

Many plants have been applied for the treatments of cancers (1). Tand have shown certain therapeutic effects, both *in vitro* or *in vivo* (2,3). Anacardic acid (AA) is a mixture of 2-hydroxy-6-alkylbenzoic acid homologs and is commonly found in Anacardiaceae family (4,5). It has been widely recosnized as a non-specific histone acetyltransferase inhibitor of p300 (6). Meanwhile, it has shown certain anti-tumor activities (7,8) by inhibiting histone acetyltransferase (9-13) and transcriptional factor nuclear factor-κB (14) in cell and animal models. In addition, it may efficiently inhibit the proliferation of breast carcinoma cells (15).

Prostate cancer (Pca) has a high mortality, ranking second only next to lung cancer (16). Androgen is an important biological effector of prostate cancer progression (17). Many studies have demonstrated that phytochemicals can effectively inhibit the apoptosis and proliferation of prostate cancer cells (18-22). Chun *et al.* reported inhibition of cell viability and apoptosis of Pca cells treated by andrographolide, which is extracted from a medicinal plant (23). Although the anti-tumor activity of AA has been confirmed in numerous cancers including prostate cancer, the mechanisms remain unknown.

In this study, we explored the role of AA for LNCaP cells. In the investigation of the mechanism, we used siRNA against the p300 to inhibit p300 gene in LNCaP cells, and to determine the effects of p300 on other genes closely related to prostate cancer such as AR and p53.

Materials and methods

Reagents

A 50-mmol/L solution of AA (Merck, Germany) was added in dimethylsulfoxide. AA was prepared in dilution with

culture medium when necessary. DMEM, growth factor-reduced matrigel and fetalbovine serum (FBS) were bought from BD Corporation (San Jose, CA). The Lipofectamine™ 2000 and the Total RNA Extraction kit were purchased from Invitrogen (Carlsbad, CA). The MTT Cell Proliferation and Cytotoxicity Assay kit and the Annexin V-FITC & PI Apoptosis Detection kit were purchased from KeyGen Biotech (Nanjing, China). SYBR Green PCR Master Mix were purchased from Fermentas (Burlington, Ontario, Canada). The Total Protein Extraction kit was purchased from ProMab (Changsha, China). Primary antibodies for p300, p21, p53, AR, cyclin D1 and siRNA specifically for p300 were bought from Santa Cruz Biotechnology (Santa Cruz, CA). Primary antibody for phospho-p53 (Ser 15) was purchased from Cell Signaling Technology. RPMI 1640 was purchased from GIBCO.

Cell lines and cell culture

LNCaP was purchased from Yinrun (Changsha, China). LNCaP is a classical metastatic prostate adenocarcinoma cell line, derived from metastatic lymph nodes. LNCaP was cultured with both RPMI 1640 and 10% FBS, and subcultured weekly (37 ℃, 5% CO_2).

p300 siRNA transfection

The Lncap cells were seeded into 6-well plates (4×10^4-5×10^4 cells/well) and cultured in 2 mL basic culture medium containing 10% FBS overnight until the cells were 70% confluent. Cells were transiently transfected with a validated scrambled control siRNA, or p300 siRNA by using InterferinTM transfection reagent. The mixture of siRNA and InterferinTM transfection reagent was incubated for 10 min, added to each well of the 6-well plates and incubated at 37 ℃ for 24 h before drug treatment.

Detecting the inhibition of cell growth using MTT assays

Lncap cells were treated with AA or transfected with p300 siRNA before drug treatment. Cells were incubated at various concentrations (0, 5, 25, and 125 μmol/L) at a series of time points (0, 4, 24 and 28 h). Then, 5 mg/mL of MTT solution (10 μL, Yinrun, Changsha, China) was carefully added to each plate well. Then, the LNCaP was cultured for another 4 hours. All media were subsequently discarded, and plates were read at 492 nm (A value) with the addition of 150 μL of DMSO in each well. All tests and determinations were repeated in triplicate. The survival rate was calculated by subtracting the background OD value (complete culture medium without cells) from the OD value from each test well.

Detection of cell cycles using flow cytometry

LNCaP was incubated in dishes with a density of 1×10^5/mL. It was incubated with AA at different concentrations (0, 5, 25 and 125 μmol/L) at 30 ℃ and 5% CO_2 for 24 hours. The cells were digested with 2.5 g/L trypsase in a shaking bath. Then LNCaP cells were collected by centrifuge (1,500 rpm, 5 min), and re-suspended in 70% ethanol in PBS for 24 h at 4 ℃ for fixation. Afterward they were harvested and ready for analysis of cell cycle by flow cytometer (Becton-Dickinson, Franklin Lakes, NJ).

Detection of cell apoptosis using flow cytometry

A detection of apoptosis of LNCaP cell treated by AA was analyzed by determining the ratio of cells with nucleus concentration and fragment. Cells were collected at 24 h following AA treatment at different concentrations (0, 5, 25 and 125 μmol/L), and then suspended in the buffer. During the apoptosis assay, the cells were stained with PI and annexin V-FITC (Invitrogen, Carlsbad, CA) and determined by flow cytometer.

Detection of mRNA expression using real-time PCR

Total RNA of Lncap was extracted using Trizol (Invitrogen), according to the manufacturer's instructions. cDNA synthesis was performed with 2 μg of total RNA using RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas, Burlington, Ontario, Canada). The primers (ProMab) were designed by using Primer Express 3.0 software (Applied Biosystems), and their sequences were as follows: p300(275bp), forward 5-CAGCGACTCCTTCAGCAA-3; reverse 5-GCTACCAGTCCAGGATGTG-3; p53(302bp), forward 5-TTGAGGTGCGTGTTTGTG-3, reverse 5-CTTCAGGTGGCTGGAGTG-3; p21(334bp), forward 5-CCCGTGAGCGATGGAACT-3, reverse 5-CGGCGTTTGGAGTGGTAG-3; AR(131bp), forward 5-CGGAAGCTGAAGAAACTTGG-3, reverse 5-ATGGGCTGACATTCATAGCC-3; cyclin D1(283bp), forward 5-GGATGCTGGAGGTCTGCGAGGAAC-3, reverse 5-GAGAGGAAGCGTGTGAGGCGGTAG-3;

