Biochemical and Biophysical Research Communications xxx (2017) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

DOK1/PPARgamma pathway mediates anti-tumor ability of all-trans retinoic acid in breast cancer MCF-7 cells

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ARTICLE INFO

Article history:
Received 1 April 2017
Accepted 5 April 2017
Available online xxx

Keywords:
All-trans retinoic acid
Docking protein 1
Peroxisome proliferator activated receptor gamma
Proliferation
Apoptosis
Breast cancer cells

ABSTRACT

Previous studies have showed the anticancer effect of the all-trans retinoic acid (ATRA) in many tumors including breast cancer; however, the underlying molecular mechanism is still poorly understood. This study experimentally revealed that ATRA treatment inhibited MCF-7 cell proliferation and promoted its apoptosis, along with an enhanced expression of docking protein 1 (DOK1). ATRA's effects on cell proliferation and apoptosis were prevented by DOK1 knockdown. In addition, the genetic silence of DOK1 can inhibit PPARγ expression and its activity. Moreover, inactivation of PPARγ by its specific inhibitor GW9662 reversed the impacts of ATRA on cell proliferation and apoptosis. Taken together, these results indicate that ATRA-enhanced expression of DOK1 activates PPARγ leading to inhibition of cell proliferation and enhancement of cell apoptosis in MCF-7 cell.

1. Introduction

All *trans*-retinoic acid (ATRA) is a kind of vitamin A metabolic intermediate with extensive biological activities in vivo. Its antitumor effect has been widely studied in the field of basic and clinical medicine. Due to its characteristics such as inhibiting tumor cell differentiation, inducing tumor cell apoptosis, promoting antitumor effect of immune system, enhancing the sensitivity of tumor cells to radiotherapy and chemotherapy, ATRA has been used in clinical treatment of some types of tumors including acute promyelocytic leukemia (APL) [1–3]. Recent studies have shown that ATRA may also be used in all forms of breast cancer treatment [4]. However, the mechanism underlying anti-breast cancer ability of ATRA remains poorly understood.

Doking protein (DOK) family plays an important role in the regulatory of tumorigenesis, insulin resistance, immune regulation and etc [5–7]. Wherein, DOK1-6 mRNAs have been demonstrated to express in normal and breast cancer tissue [8,9]. Previous studies have shown that the molecular weight of 62 kDa protein DOK1 could bind with p120 rasGAP, thereby inhibiting the Ras-Raf-MEK-ERK signaling pathway [10,11]. Because of this feature, DOK1 is considered to be one of the major tumor suppressor gene in vivo [5,7,12]. Indeed, mice lacking of DOK1, DOK2, DOK3 predisposed to cancer [6]. Additionally, the decrease in the expression level of DOK2 and DOK6 is closely related to the progression of breast cancer [9]. However, the functional role of DOK1 in the development and treatment of breast cancer has not been reported.

PPARγ belongs to the family of nuclear hormone receptors (NHRs) which directly regulate transcription of target genes [13]. Accumulating evidence exists on the overexpression of PPARγ in many tumors including breast cancer. Yet, the biological significance of its role in cancer remains controversial. Previous studies have demonstrated that naturally-occurring and synthetic PPARγ agonists promote growth inhibition, apoptosis, and differentiation of tumor cells [13–16]. Moreover, PPARγ ligands can counteract leptin stimulatory effects on breast cancer growth in either in vivo or in vitro models [17]. However, Zhou et al. demonstrated that PPARγ ligands induced autophagy in MDA-MB-231 breast cancer cells by up-regulating the expression of HIF1α and BNIP3 leading to maintaining cell viability [18]. In short, PPARγ's specific role in breast cancer is uncertain.

We here reported that ATRA can activate PPARγ by increasing DOK1 expression, resulting in inhibition of cell proliferation and promotion of and promotion of cell apoptosis in human breast cancer MCF-7 cell. Our results reveal that DOK1-mediated activation of PPARγ contribute to anti-tumor ability of ARTA.

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http://dx.doi.org/10.1016/j.bbrc.2017.04.018
0006-291X/

2. Materials and methods

2.1. Reagents

All *trans*-retinoic acid (ATRA),3-(4,5)-dimethylthiahiazo (-2-y1)-2,5-diphenytetrazo-lium-bromide (MTT) and anti-tubulin antibody were purchased from Sigma-Aldrich (St Louis, MO, USA); Anti-Caspase 3 and anti-PPARγ antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA); Anti-DOK1 antibody and anti−antibody phospha-tase labered secondary antibody were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). DMEM (Dulbeccos Modified Eagles Medium) and fetal bovine serum (FBS) were purchased from HyClone (Logan, UT, USA).

2.2. Cell culture and virus infection

Human breast cancer MCF-7 cells were obtained from American Type Culture Collec-tion (ATCC, Rockville, MD, USA) and cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO2.

Lentivirus carrying human DOK1/shRNA and its control sequences was purchased from Santa Cruz Biotechnology. Lentivirus transduction was performed according to manufacturer's instructions.

2.3. Cell proliferation assay

Cell proliferation was determined by MTT and BrdU assays, respectively. MTT was performed substantially as follows: cells were seeded into 96 well plate at 1×10^3 cells/well and cultured in 100 μl of DMEM containing 2% FBS. After treatment, culture medium was removed, and 150 μl of fresh culture medium without phenol red was added with 50 μl of 0.5 mg/ml MTT, followed by incubation at 37 °C for 3 h. Thereafter, the medium was carefully removed and each well was added 150 μl MTT solvent. Plates covered with aluminum foil dark were orbitally shaked for 15 min. Absorbance at 590 nm with a reference filter of 620 nm was read by an ELISA plate reader (Biotek, Winooski, VT, USA). BrdU assay was carried out using BrdU Cell Proliferation ELISA Kit (colorimetric) (ab126556, Cambridge, MA, USA) according to manufacturer's protocol.

2.4. Western blot

Extraction and quantification of proteins was performed by the methods reported previously in our laboratory [19]. Cell lysate was mixed with an equal volume of 2× SDS-PAGE sample loading buffer, boiledat 100 °C for 10 min, and then centrifugated at 14,000*g* for 1 min. The supernatant was used to run SDS-PAGE electrophoresis. The separated proteins were transferred to polyvinylidene fluoride (PVDF) membrane, incubated with primary antibody for overnight at 4 °C and then secondary antibody at room temperature for 1 h. Protein levels were detected by NBT/BCIP alkaline phosphatase (AP) chromogenic reagent and quanificated by Image-Pro Plus software.

2.5. TUNEL assay

Apoptosis was determined by TUNEL assay kit purchased from Roche (Roche, Basel, Switzerland) according to manufacturer's instructions. To calculate the rate of apoptosis, the total number of intact cells and apoptotic cells (TUNEL positive) were counted in a random collection of 20% of the captured images from repeated experiments.

2.6. PPARγ activity assays

PPARγ activity was measured by PPARγ Transcription Factor Assay Kit (ab133101, Abcam, Cambridge, MA, USA) according to manufacturer's protocol.

2.7. Statistical analysis

The data were presented as the mean ± SEM. Differences between the groups were examined for statistical significance using analysis of variance (ANOVA) and independent samples *t*-test. $p < 0.05$ was considered as statistically significant. All experiment was repeated at least three times with similar results.

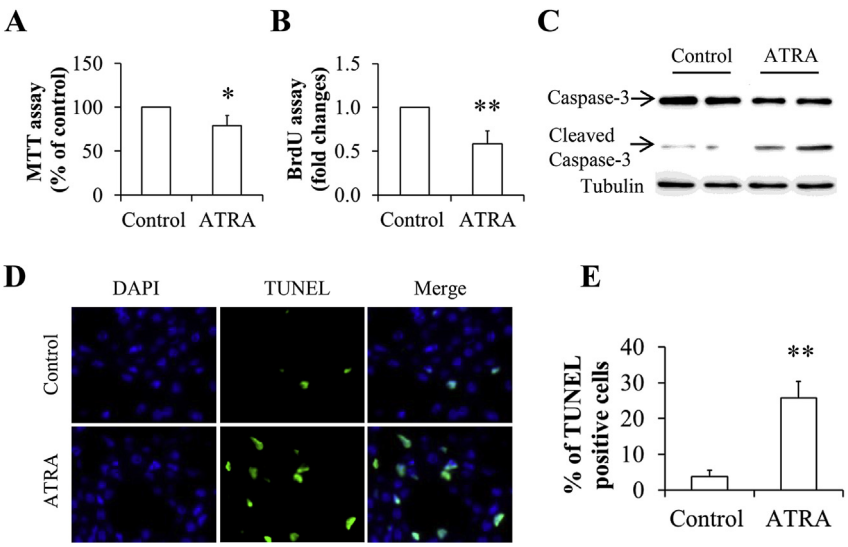

Fig. 1. All-trans retinoic acid (ATRA) suppressed proliferation and enhanced apoptosis in MCF-7 cells. (A, B) Effect of ATRA on proliferation. (C) Effect of ATRA on expression of cleaved caspase 3. (D, E) Effect of ATRA on apoptosis. n = 6. *P < 0.05, **P < 0.01 vs control groups.

Please cite this article in press as: X. Ding, et al., DOK1/PPARgamma pathway mediates anti-tumor ability of all-trans retinoic acid in breast cancer MCF-7 cells, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.04.018