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24-Methylenecycloartanyl ferulate, a major compound of γ-oryzanol, promotes parvin-beta expression through an interaction with peroxisome proliferator-activated receptor-gamma 2 in human breast cancer cells

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ABSTRACT

Parvin-β is an adaptor protein that binds to integrin-linked kinase (ILK) and is significantly down-regulated in breast tumors and breast cancer cell lines. We treated the breast cancer cell line MCF7 with 24-methylenecycloartanyl ferulate (24-MCF), a γ-oryzanol compound. We observed upregulation of parvin-β (GenBank Accession No. AF237769) and peroxisome proliferator-activated receptor (PPAR)-γ2 (GenBank Accession No. NM_015869). Among γ-oryzanol compounds, only treatment with 24-MCF led to the formation of reverse transcription-PCR products of parvin-β (650 and 500 bp) and PPAR-γ2 (580 bp) in MCF7 cells, but not in T47D, SK-BR-3, or MDA-MB-231 cells. 24-MCF treatment increased the mRNA and protein levels of parvin-β in MCF7 cells in a dose-dependent manner. We hypothesized that there is a correlation between parvin-β expression and induction of PPAR-γ2. This hypothesis was investigated by using a promoter-reporter assay, chromatin immunoprecipitation, and an electrophoretic mobility shift assay. 24-MCF treatment induced binding of PPAR-γ2 to a peroxisome proliferator response element-like *cis*-element (ACTAGGACAAAGGACA) in the parvin-β promoter in MCF7 cells in a dose-dependent manner. 24-MCF treatment significantly decreased anchorage-independent growth and inhibited cell movement in comparison to control treatment with dimethyl sulfoxide. 24-MCF treatment reduced the levels of GTP-bound Rac1 and Cdc42. Evaluation of Akt1 inhibition by 24-MCF revealed that the half maximal effective concentration was 33.3 μM. Docking evaluations revealed that 24-MCF binds to the ATP-binding site of Akt1(PDB ID: 3OCB) and the compound binding energy is -8.870 kcal/mol. Taken together, our results indicate that 24-MCF treatment increases parvin-β expression, which may inhibit ILK downstream signaling.

1. Introduction

Integrin-linked kinase (ILK) overexpression is a hallmark of several solid tumors [1–3]. Recent experiments suggest that deregulation of ILK signaling can affect anchorage-independent cell growth, cell survival, and oncogenic transformation [4,5]. Complexes such as that of the adaptor protein PINCH (Particularly Interesting Cys-His-rich protein) and parvin-β are important regulators of integrin-mediated signaling in breast cancer [1]. Parvin-β

Abbreviations: 24-MCF, 24-methylenecycloartanyl ferulate; CAF, cycloartenyl ferulate; CH, calponin homology; ChIP, chromatin immunoprecipitation; CSF, campesteryl ferulate; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, dimethyl sulfoxide; EGF, epidermal growth factor; EMSA, electrophoretic mobility shift assay; FBS, fetal bovine serum; FA, ferulic acid; GSK, glycogen synthase kinase; GST, glutathione S-transferase; ILK, integrin-linked kinase; PBS, phosphate-buffered saline; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; RT, reverse transcription.

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binds to ILK through one of two calponin homology (CH) domains and is widely expressed in human/mammalian tissues [6]. It was reported in recent studies that parvin-β expression is significantly downregulated in a number of breast cancer tumors [2], which correlates with the upregulation of ILK signaling [1], suggesting that parvin-β can suppress oncogenic ILK signaling [1]. However, the biochemical functions of parvin-β remain unclear.

In recent years, specific dietary compounds (dietary lipids, isoflavones, and other flavonoids) have been the focus of scientific interest because they transactivate peroxisome proliferator-activated receptors (PPARs) [7]. PPARs mainly function as heterodimers in association with the co-activator complex, which binds to peroxisome proliferator response elements (PPREs) [8], with a DNA recognition motif composed of a direct repeat separated by one nucleotide with a consensus sequence of RGGTGA-A-AGGTCA [9]. PPREs are present in the promoters of target genes and underlie the transactivation and transrepression of various genes [8].

24-methylenecycloartanyl ferulate (24-MCF), a gamma-oryzanol (γ-oryzanol) compound, is a non-toxic dietary compound that reduces the risks of cancer, hyperlipidemia, and plasma cholesterol [10]. However, its precise molecular mechanism has not been fully elucidated.

Using a cDNA microarray we found that parvin-β and PPAR-γ2 expression were increased by 24-MCF treatment in the breast cancer cell line MCF7 (data not shown). In the present study, we aimed to investigate whether a PPRE-like *cis*-element in the parvin-β promoter was involved in the upregulation of parvin-β and whether this upregulation can inhibit ILK downstream signaling.

2. Materials and methods

2.1. Materials

The rice bran compounds ferulic acid (FA), cycloartenyl ferulate (CAF), 24-methylenecycloartanyl ferulate (24-MCF), and campesteryl ferulate (CSF) were provided by the National Academy of Agricultural Science (Korea). Total RNA was extracted from breast cancer cell lines using TRI-RNA reagent (Favorgen, Taiwan) following the manufacturer's instructions. The human parvin-β promoter was amplified by PCR from MCF7 cell genomic DNA using Taq DNA polymerase (Bionner, Korea), with the forward primer 5′-CTCGAGAGACCAAAAATAAGGCACGTTCAT-3′, and the reverse primer 5′-AAGCTTGAGAAGTGAACCAGCCAGAGAAGC-3′. The 1.05 kb fragment with *Xho*I and *Hind*III site was ligated into the equivalent site of pGL3-basic, a promoter-less firefly luciferase reporter vector. Full-length human PPAR-γ1 (Accession No. NM_138712) and PPAR-γ2 (Accession No. NM_015869) were subcloned into the p3XFLAG-CMV 7.1 vector. Antibodies were purchased as indicated: vs parvin-β (Santa Cruz Biotechnology, Santa Cruz, CA); Rac1 and Cdc42 (Abcam, Cambridge, MA) and Flag M2 and Anti-Flag M2 affinity gel (Sigma, St. Louis, Mo).

2.2. Cell culture and cell viability

The human breast cancer cells MCF7, MDA-MB-231, T47D, SK-BR-3, and the non-cancerous human breast cell line MCF-10A, were cultured in Dulbecco's Modified Eagle's Medium (DMEM, HyClone, Logan, UT) supplemented with 10% heat-inactivated fetal bovine serum (FBS, HyClone, UT), 100 U/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2 at 37 °C. Cell viability was determined using an MTT kit (Sigma, St. Louis, Mo) according to the manufacturer's instructions. Briefly, 1×10^4 cells were seeded in triplicate in 96-well plates. MCF7, MDA-MB-231, SK-BR-3, and MCF-10A cells were treated with 24-MCF in a dose-dependent manner and incubated for 24 h. Cell viability was measured at 490 nm using a xMark Microplate Absorbance Spectrometer (Bio-Rad, Philadelphia, PA). All experiments were performed in triplicate.

2.3. Reverse transcription (RT)-PCR

Total RNA was isolated using the TRI-RNA reagent (Favorgen, Taiwan) according to the manufacturer's instructions. Thereafter, 1 μg of total RNA was converted to cDNA using an oligo-dT primer and AccurPower RT premix (Bioneer, Korea) in a 20 μl reaction. The PCR products were generated using AccurPower PCR Mix (Bioneer, Korea) and the following primers: parvin-β, 5′-TGTGAAGCAGCTG-GAGGAAG-3′ and 5′-AAGTGCAT GGCCAGAGAGAC-3′; PPAR-γ1, 5′-TCTCTCCGTAATGGAAGACC-3′ and 5′-GCATTAT GAGACATCCCCAC-3′; PPAR-γ2, 5′-GCGATTCCTTCACTGATAC-3′ and 5′-GCATTAT GAGACATCCCCAC-3′. The amplified PCR products were separated on a 2.0% agarose gel and visualized using ethidium bromide (Sigma, St. Louis, Mo).

2.4. Western blot analysis

Cells at 70–80% confluency were treated with 24-MCF for 24 h. Cells were washed twice with ice-cold phosphate-buffered saline (PBS) and lysed on ice for 30 min in RIPA buffer (Cell Signaling, Danvers, MA) containing 1 × protease inhibitor cocktail reagent (ThermoFisher, Waltham, MA) and 1 × phosphatase inhibitor (Sigma, St. Louis, Mo). The extract was centrifuged at 14,000 rpm for 15 min at 4 °C to remove cellular debris. Thereafter, 20 μg of protein was separated on a 4–12% NuPAGE gel (Invitrogen, San Diego, CA), transferred to a nitrocellulose membrane using standard procedures (Invitrogen, San Diego, CA), and probed by Western blot with the indicated antibodies.

2.5. Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was modified from a previously described strategy [11]. Chromatin was prepared from MCF7 cells that had been transiently transfected with 3XFLAG-vector, 3XFLAG-PPAR-γ1 and 3XFLAG-PPAR-γ2 using the Lipofectamine LTX Plus Reagent (Invitrogen, San Diego, CA). Immunoprecipitated samples were prepared for PCR and analyzed using the following primers targeting the parvin-β promoter: forward, 5′-GAGAGCTCAGA-GAGCAAAGA-3′ and reverse, 5′-GAGAGCACCGCAGATTCACG-3′. PCR was performed with 35 cycles (denaturation for 30 s at 94 °C, annealing for 30 s at 70 °C, and extension for 90 s at 72 °C) and a final extension at 72 °C for 7 min. PCR products were detected on a 2% agarose gel.

2.6. Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared from MCF7 cells using a NE-PER Nuclear and Cytoplasmic Extraction Reagents kit (ThermoFisher, Waltham, MA) according to the manufacturer's instructions. For the EMSA, 5 μg of nuclear extracts by 24-MCF treatment and biotin end-labeled PPRE-like *cis*-element (5′-ACTAGGACAAAGGACA-3′) probes were directly added to binding buffer (0.01 M phosphate buffer, pH 7.5, 0.15 M NaCl, and 2.7 mM KCl). The mixture was incubated on ice for 30 min and at room temperature for 30 min. For the competition assay, a 100-fold excess of unlabeled PPRE-like *cis*-element was used along with biotin end-labeled PPRE-like sequence probes. The DNA-protein complexes were resolved on 5% non-denaturing polyacrylamide gels in 0.5 × TBE buffer and detected using a chemiluminescence reagent (ThermoFisher, Waltham, MA) according to the manufacturer's instructions.