EXPERIMENTAL CELL RESEARCH 313 (2007) 357–368

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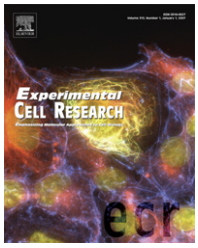

Research Article

Synergistic effects of retinoic acid and tamoxifen on human breast cancer cells: Proteomic characterization

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ARTICLE INFORMATION

Article Chronology:
Received 2 September 2006
Revised version received
12 October 2006
Accepted 19 October 2006
Available online 25 October 2006

Keywords:
Retinoic acid
Tamoxifen
Proteomics
Breast cancer
TGFβ
Apoptosis

ABSTRACT

The anti-estrogen tamoxifen and vitamin A-related compound, all-*trans* retinoic acid (RA), in combination act synergistically to inhibit the growth of MCF-7 human breast cancer cells. In the present study, we applied two-dimensional gel electrophoresis based proteomic approach to globally analyze this synergistic effect of RA and tamoxifen. Proteomic study revealed that multiple clusters of proteins were involved in RA and tamoxifen-induced apoptosis in MCF-7 breast cancer cells, including post-transcriptional and splicing factors, proteins related to cellular proliferation or differentiation, and proteins related to energy production and internal degradation systems. The negative growth factor-transforming growth factor β (TGFβ) was secreted by RA and/or tamoxifen treatment and was studies as a potential mediator of the synergistic effects of RA and tamoxifen in apoptosis. By comparing protein alterations in treatments of RA and tamoxifen alone or in combination to those of TGFβ treatment, or co-treatment with TGFβ inhibitor SB 431542, proteomic results showed that a number of proteins were involved in TGFβ signaling pathway. These results provide valuable insights into the mechanisms of RA and tamoxifen-induced TGFβ signaling pathway in breast cancer cells.

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doi:10.1016/j.yexcr.2006.10.016

Abbreviations:
DAPI, 4,6-diamidino-2-phenylindole
EF-Tu, translation elongation factor
EF-Tu like protein P43 precursor
ER, estrogen receptor
IC_{50}, half-maximal inhibitory concentration
hnRNP, heterogeneous nuclear ribonucleoprotein
HSP 27, heat shock protein 27 kDa
MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PI, propidium iodide
RA, retinoic acid
Rho GDI, Rho GDP dissociation inhibitor
SB 431542, 4-(5-benzo [1,3] dioxol-5-yl-4-pyridin-2-yl-1*H*-imidazol-2-yl)-benzamide
TGFβ, transforming growth factor β

Introduction

Retinoic acid (RA), a most potent biological active metabolite of vitamin A, has profound effects on regulating cellular growth and differentiation [1,2]. Acting in the same way as other hormones, RA exerts its effects by binding to its intracellular receptors (RARs α, β, γ and RXRs α, β, γ) that belong to the steroid/thyroid receptor superfamily [3]. Due to their antiproliferative and differentiating abilities, RA and related retinoids have been used clinically as an alternative differentiation therapy in combination with other chemotherapeutic or preventive agents, such as tamoxifen for breast cancer [4,5]. The mechanism of the synergistic action of antiestrogens and retinoids in combination on breast cancer cells has been intensively explored. Previous studies have implicated that the major benefits of tamoxifen stem from its competitive binding with ER and counteracting the biological functions of estradiol [6]. Others reported that combined treatment of RA and tamoxifen increased expression of preexisting E-cadherin along with a down-regulation of Bcl-2 and c-*myc* protein levels and a reduction of telomerase activity [7]. Some recent studies suggested that RA and tamoxifen induced synergistically secretion of the negative growth factor, transforming growth factor β (TGFβ) to stimulate apoptosis in MCF-7 breast cancer cells [4]. However, the detail mechanism of the synergistic action of RA and tamoxifen still remained unknown.

In the present study, we aim to explore the mechanisms of anticancer activity of RA, tamoxifen, and their synergistic effects in MCF-7 human breast cancer cells by proteomics. With a particular interest in elucidating the pathway(s) of eliminating cancer cells by artificially triggering cell death leading to tumor regression, we examined the response of MCF-7 human breast cancer cells to the combination treatment with RA and tamoxifen, and defined the possible involvement of TGFβ in mediating their anticancer actions by means of proteomic and biochemical approaches.

Proteome, the entire protein complement of the genome, determines cell phenotype and functions. With rapidly developed technologies, proteomics provides a systematic approach for the quantitative and qualitative mapping of the whole proteome under drug treatment of disease conditions [8,9]. The altered proteins identified by proteomic approach can be further characterized as potential drug targets and the global analysis of the protein alterations can result in valuable insights to understand the drug action mechanisms. By comparing protein profile alterations in treatments of RA and tamoxifen alone or in combination to those of TGFβ, or co-treatment with TGFβ inhibitor SB 431542, proteomic results showed that TGFβ is a potential mediator of RA and tamoxifen-induced apoptosis in MCF-7 breast cancer cells, and multiple clusters of proteins are involved in this process. These results provide valuable insights into the mechanisms of RA and tamoxifen-induced TGFβ signaling networks in MCF-7 breast cancer cells.

Materials and methods

Materials

Materials for cell culture were purchased from Gibco-BRL (Grand Island, NY). All-*trans* RA, tamoxifen, estradiol, 4,6-diamidino-2-phenylindole (DAPI), 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide (MTT), and SB 431542 were from Sigma-Aldrich Chemical Co. Purified porcine platelet TGFβ1 was obtained from Calbiochem. RA was prepared in dimethyl sulfoxide at 10^{-2} M and stored at −70°C; 10^{-3} M stock solution of tamoxifen in ethanol was stored at −20°C. MCF-7 cell line was purchased from the American Type Culture Collection. All other chemicals, except where specifically noted, were purchased from Sigma-Aldrich Chemical Co. and Amersham Biosciences.