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Proteomic analysis of changes in the protein composition of MCF-7 human breast cancer cells induced by all-*trans* retinoic acid, 9-*cis* retinoic acid, and their combination

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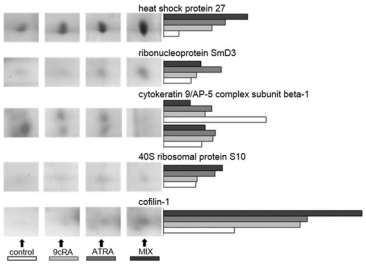
HIGHLIGHTS

- We used proteomic approach for monitoring selected protein induction or repression after treatment of MCF-7 human breast cancer with retinoic acid isomers.
- We found significant differences in occurrence of proteins probably affecting the cell migration process in tumour cells.
- The significant differences between individual experimental groups were observed in heat shock protein 27 and cofilin-1.
- Down-regulation of proteins induced by ATRA and/or 9cRA, even more pronounced by their combination, might affect cell migration process dependent on cytoskeletal remodeling in tumour cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Retinoic acid (all-*trans* and 9-*cis*) isomers represent important therapeutic agents for many types of cancers, including human breast cancer. Changes in protein composition of the MCF-7 human breast cancer cells were induced by all-*trans* retinoic acid, 9-*cis* retinoic acid, and their combination and subsequently proteomic strategies based on bottom-up method were applied. Proposed approach was used for the analysis of proteins extracted from MCF-7 human breast cancer cell line utilizing a commercially manufactured kit RIPA and separated on two dimensional (2D) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) after treatment with both retinoic acid isomers. We found significant differences in occurrence of proteins probably affecting the cell migration process in tumour cells. Heat shock protein 27, ribonucleoprotein SmD3, and cofilin-1 were significantly upregulated after treatment with combination of individual retinoic acid isomers. On the other hand, AP-5 complex subunit beta-1 shows the different response. Thus, the results might help to find the

Abbreviations: RA, retinoic acid; 9cRA, 9-*cis* retinoic acid; ATRA, all-*trans* retinoic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; MALDI, matrix-assisted laser desorption/ionization; TOF, time-of-flight; MS, mass spectrometry.

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answer to important medical questions on (i) the identification of signaling pathways affected by retinoic acid isomers or (ii) how the observed proteomic pattern might reflect the effectiveness of retinoic acids treatment.

1. Introduction

Retinoids and rexinoids comprise a family of polyisoprenoid compounds containing a cyclohexenyl ring which include retinol (vitamin A) and its natural metabolites or synthetic derivatives. They are involved in a multitude arrangement of physiological and developmental responses in many tissues of higher vertebrates that include embryonic development, vision, reproduction, immune responses, bone formation, hematopoesis, metabolism, growth, and differentiation of a variety of cell types, apoptosis and processes of carcinogenesis (Lotan, 1995; Sun and Lotan, 2002; Brtko and Thalhamer, 2003). The diversity of retinoic acid-induced signalling pathway is associated with existence of at least three isotypes of nuclear receptors for all-*trans* retinoic acid, RAR (alpha, beta, and gamma) and three isotypes of nuclear receptors for 9-*cis* retinoic acid, RXR (alpha, beta, and gamma) with distinct amino- and carboxy-terminal domains. The retinoids selective for specific binding to RXRs are called rexinoids (Germain et al., 2006; Brtko and Dvorak, 2011). Nuclear RARs upon all-*trans* retinoic acid binding act as all-*trans* retinoic acid-inducible transcription factors by directly interacting as heterodimers with 9-*cis* retinoic acid receptor. The RXR/RAR heterodimer interacts with specific DNA response elements of target genes and its effect on transcription is mediated also through recruitment of a number of coregulators (corepressors and coactivators) (Chambon, 1996; Mangelsdorf et al., 1995). Retinoid signalling is often compromised early in carcinogenesis suggesting that an impairment of retinoid signalling may be required for tumour development. Retinoids also interact with other signalling pathways, including estrogen signalling in breast cancer (Tang and Gudas, 2011). Thus, the nuclear retinoid receptors are considered to be ligand-activated, DNA-binding, *trans*-acting, transcription-modulating nuclear proteins involved in a general molecular mechanism responsible for transcriptional responses in target genes (Brtko and Dvorak, 2011). Retinoids acting through their cognate nuclear receptors exert both beneficial and detrimental activity. They are known to have tumour-suppressive activity but on the other hand, they are teratogenic (Hinds et al., 1997). A number of nuclear receptor selective retinoids and rexinoids, have been successfully tested using a variety of cell lines or animal models. Many data have clearly shown that retinoids inhibit carcinogenesis, suppress premalignant epithelial lesions and tumour growth and invasion in a variety of tissues (Clarke et al., 2004; Brtko, 2007).

The most direct implication of retinoic acids in human disease is given by acute promyelocytic leukaemia, which is characterized by selected expansion of immature myeloid precursors or malignant myeloid cells blocked at the promyelocytic stage of hemopoietic development (Lin et al., 1999). Breast cancer development is associated with deregulation of cell growth and cell death. It has been shown that retinoids are able to inhibit mammary gland cancer in animal models as well as in human breast cancer (Garattini et al., 2014).

In the present study, we aim to offer novel information on the proteomic pattern of proteins evaluated after the treatment of human breast carcinoma MCF-7 cells with either all-*trans* retinoic acid, 9-*cis* retinoic acid or their combination with respect to their role in the cancer treatment by comparative proteomics based on 2D-SDS-PAGE and tandem MS. At present, proteomics gives a powerful view of the proteome including the qualitative and quantitative changes of proteins. Furthermore, various proteomic tools allow functional investigation of proteome (Chandramouli and Qian, 2009). Proteomic analysis using 2D gel electrophoresis for identification of individual proteins by MS sequencing was used in analysis of ductal carcinoma in situ (DCIS) of the human breast (Wulfkuhle et al., 2002). This proteomic techniques offer also the advantage of detection of posttranslational modification of proteins or detection of changes in proteins expression (Wulfkuhle et al., 2001).

Since, the particular mechanism of the action of all-*trans* retinoic acid, 9-*cis* retinoic acid or their combination still remains unknown, these proteomic data can figure out direction for studying their mechanism at the cellular level.

2. Materials and methods

2.1. Chemicals and samples

All-*trans* retinoic acid (ATRA), 9-*cis* retinoic acid (9cR), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), radio-immunoprecipitation assay (RIPA) buffer, and other conventional chemicals were obtained from Sigma (Schnelldorf, Germany). Penicillin/streptomycin, gentamicin, and glutamine were from PAA Laboratories GmbH (Cölbe, Germany). Enzymes were obtained from Roche Diagnostics (Mannheim, Germany). ZipTip C18 pipette tips were purchased from Merck Millipore (Billerica, MA, USA) and α-cyano-4-hydroxycinnamic acid was purchased from LaserBio labs (Sophia–Antipolis Cedex, France).

2.2. Cell culture

The MCF-7 human breast cancer cell line was grown and passaged routinely as monolayer culturesin 75 cm^2 flasks (Sarstedt, Germany). The cells were used at passage 10–30. Cells were seeded in Petri dishes (Sarstedt, Germany) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), glutamine and antibiotics (penicillin/streptomycin, gentamicin), and cultured at 37 °C in humidified atmosphere of 5% CO_2 in the air. The cells were treated for 48 h either with 1 μmol/L 9-*cis* retinoic acid (9cRA) or 1 μmol/L all-*trans* retinoic acid (ATRA) or with their combination. Compounds at selected concentration were dissolved in ethanol and then added into medium. Control cells were incubated with particular concentration of ethanol. After incubation cells were washed twice with ice-cooled PBS. The cell lysis was made according to an instruction manual of the RIPA (radio-immunoprecipitation assay) buffer. The cell lysates were stored at −70 °C for further use.

2.3. Growth inhibition assay – incucyte

Growth characteristics of cells were measured using the INCUCYTE™ Kinetic Imaging System (Essen BioScience, UK) based on high-quality phase-contrast imaging confluence assessment. 10 000 MCF-7 cells per well were plated in 96-well Sarstedt plates and led to adhere for 16 h. Each dose of ATRA and 9cRA (added in the volume of 50 μL) in concentration ranging from 0.01 μmol/L to 25 μmol/L were tested in triplicates and confluence assessment was done 72 h after the addition of retinoic acids.