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Consequences of the natural retinoid/retinoid X receptor ligands action in human breast cancer MDA-MB-231 cell line: Focus on functional proteomics

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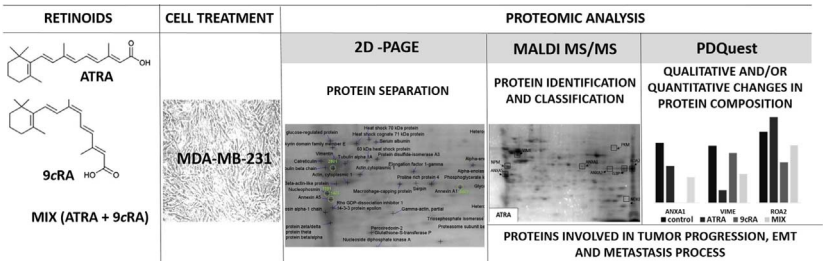
GRAPHICAL ABSTRACT



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ABSTRACT

The main intention of this study was the investigation of impact of natural biologically active ligands of nuclear retinoid/retinoid X receptors (all-*trans* and 9-*cis* retinoic acid) on proteomic pattern in human estrogen receptor negative breast cancer cell line MDA-MB-231. For this purpose, proteomic strategies based on bottom-up method were applied. The total cell proteins were extracted utilizing a commercially Radio-Immunoprecipitation Assay (RIPA) buffer and separated on 2D sodium dodecyl sulfate polyacrylamide gel electrophoresis (2D SDS-PAGE). The proteins were subsequently digested in-gel by trypsin and their characterization was achieved by MALDI-TOF/TOF. By employing PDQuest™ software, we identified more than 50 proteins affected by retinoic acid isomers. For more information, 9 proteins which are associated with tumor process were selected. We determined that derivatives of retinoic acid led to significantly reduced level of proteins belonging to metabolic pathway (e. g. glyceraldehyde-3-phosphate dehydrogenase or pyruvate kinase 2) or to other cellular processes as apoptosis, regulation of transcription process or epithelial–mesenchymal transition (e.g. annexins, nucleoside diphosphate kinase B, vimentin). On the other hand all-trans retinoic acid treatment indicates up-regulated effect for heterogeneous nuclear ribonucleoprotein A2/B1.

Abbreviations: ANXA 1, ANXA 2, ANXA5, annexins; ATRA, all-trans retinoic acid; EMT, epithelial-mesenchymal transition; FBS, fetal bovine serum; G3P, glyceraldehyde-3-phosphate dehydrogenase; NDKB, nucleoside diphosphate kinase B; NPM, nucleophosmin; NR, nuclear receptor; PKM, pyruvate kinase; PKM2, pyruvate kinase 2; 9cR A, 9-*cis* retinoic acid; RAR, retinoic acid receptor; RIPA, radio-immunoprecipitation assay; ROA2, heterogeneous nuclear ribonucleoprotein A2/B1; RXR, nuclear retinoid X receptors; TFA, trifluoroacetic acid; VIME, vimentin; ND, non-detectable

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1. Introduction

The application of modern "omic" approaches (genomics, transcriptomics, proteomics, and metabolomics) and technologies helps to better understand molecular events associated with cancer development (Kalita-de Croft et al., 2016). Analysis of biomarkers is crucial for the detection of initial stages of cancer diseases. The using of modern chromatographic or electrophoretic separation methods together with mass spectrometry (MS) is critical for analysis of cancer cells and especially for the identification of new diagnostic and prognostic biomarkers, which has mainly protein characters. Such studies, including the detection of qualitative and quantitative changes in cell proteome and analysis of posttranslational modification of proteins, enable to understand the molecular events associated with cancer development (Patwardhan et al., 2006; Rodland, 2004; Domon and Broder, 2004).

Significant part of breast carcinoma studies are based on analysis of breast cancer cell lines. The most usually used are namely e.g. MCF-7, T-47-D and MDA-MB-231 comprising more than 2/3 of all abstracts of published studies. The using of cancer cell lines is advantageous, because they provide an unlimited source of homogenous material, without contamination, easily cultured in standard media. Their using is convenient e.g. for studies where new treatments or potential drugs are tested (Lacroix and Leclercq, 2004).

Retinoids and rexinoids include polyisoprenoid compounds containing a cyclohexenyl ring, i.e., retinol (vitamin A) and its natural metabolites or synthetic derivatives. They regulate the expression of genes connected with cell proliferation, differentiation and apoptosis (Liu et al., 2005). The breakthrough in comprehension of the mechanism of retinoids action brought the discovery of the superfamily of nuclear receptors comprising nuclear retinoic acid (RAR) and nuclear retinoid X (RXR) receptors (Berbis, 2010; Uray et al., 2016). Ligand binding to heterodimeric nuclear RXR partnered with nuclear RAR and consequently regulation of their transcriptional activities is very complex. Some heterodimers are "permissive," whereby an RXR-selective ligand ("rexinoid") and a nuclear receptor (NR) partner ligand can independently or synergistically activate the transcriptional activity of the heterodimer. In contrast, "nonpermissive" heterodimers, e.g. RXR/dihydroxyvitamin D_3 receptor (VDR) are unresponsive to rexinoids alone, and can only be stimulated by ligands that bind to the RXR partner VDR. RXR/RAR heterodimer belongs rather to the group of "conditional heterodimers", which are unresponsive to rexinoids, but these (RXR) agonists superactivate transcription by synergizing with partner agonists (Brtko and Dvorak, 2015). In addition, an RXR homodimer antagonists function as an agonist when RXR is paired to specific partners, including RAR. Thus, the ability of a given receptor ligand to activate or repress gene expression can be influenced by other ligands bound to the dimer partner (Brtko and Dvorak, 2015; Perez et al., 2012).

Retinoids are known to inhibit carcinogenesis because they induce suspension of growth, differentiation and cause cell death in many types of cancer cells (e.g. mammary gland cancer, acute promyelocytic leukemia, neuroblastoma, gastric carcinoma or animal and human breast tumors) (Nguyen et al., 2016; Siddikuzzaman et al., 2011; Brtko, 2007). The inhibition of proliferation of breast cancer cell induced by retinoids is carried out by the blocking of G1-phase of cell cycle progression (Bardon and Razanamahefa, 1998; Teixeira and Pratt, 1997; Wilcken et al., 1997; Zhu et al., 1997). It has already been specified that the critical block occurs in early G_1. T-47D human breast cancer cell line treated by retinoid acid, were arrested in G_0 or early G_1 by measuring DNA content at different times after the addition of retinoid acid to the culture media or after reducing the serum content of the culture media (Tighea and Talmagea, 2004).

The fact that the use of retinoids in breast cancer is in a cynosure is reflected by increasing number of scientific articles and pre-clinical as well as clinical studies. Several reviews dealing with the potential of all-*trans* retinoic acid (ATRA) and their derivatives in the growth and progression of breast cancer were published (Shilkaitis et al., 2015; Garattini et al., 2014).

Here, our investigations were aimed to define the proteins pattern of the human breast carcinoma metastatic hormone-insensitive cells (MDA-MB-231) after application of ATRA, 9-*cis* retinoic acid (9cRA) or their combination. The main aim of present study was to use the combination of 2D-SDS-PAGE and MALDI-TOF mass spectrometry for comparative proteomics with the emphasis of analysis of proteins with proved or potential role in the cancer process.

2. Materials and methods

2.1. Chemicals and samples

All-*trans* retinoic acid, ≥98% HPLC (ATRA), 9-*cis* retinoic acid, ≥98% HPLC (9cRA), Dulbeccós modified Eaglés 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 alpha-cyano-4-hydroxycinnamic acid was purchased from LaserBio Labs (Sophia-Antipolis Cedex, France).

2.2. Cell culture

The estrogen receptor negative breast carcinoma cell line MDA-MB-231 was obtained from the HPACC (Health Protection Agency Culture Collections, Salisbury, U.K.), 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 Dulbeccós modified Eaglés medium (DMEM) supplemented with 10% 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 9cRA or 1 μmol/L 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 the Instruction manual of the RIPA buffer. The cell lysates were stored at −70 °C for further use. Protein concentrations were assessed using the Lowry assay.

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 MDA-MB-231 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 10 μmol/L were tested in triplicates and confluence assessment was done 72 h after the addition of retinoic acids.

2.4. 2D-PAGE

2D-PAGE was performed using ReadyPrep 2D Starter Kit, ReadyStrip IPG strips 7 cm, pH 3–10 nonlinear and 4–20% Mini-Protean TGX gel (all from Bio-Rad, Hercules, CA, USA). Samples were dialyzed against deionized water using Slide-A-Lyzer Dialysis Cassettes (Thermo Fisher Scientific, Waltham, MA, USA) with 2 kDa cut-off and lyophilized.

Purified samples were dissolved in 300 μL of rehydration/sample buffer and the IPG strip was passively rehydrated using 125 μL of reconstituted sample overnight at room temperature. Then, IEF was