Journal of Proteomics 182 (2018) 65–72

Contents lists available at ScienceDirect

Journal of Proteomics

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

Curcumin exerts its antitumor effects in a context dependent fashion

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ARTICLE INFO

Keywords:
Cancer-associated fibroblasts
Breast cancer co-culture
Curcumin
Mass spectrometry
Proteome response profiling

ABSTRACT

Proteome profiling profoundly contributes to the understanding of cell response mechanisms to drug actions. Such knowledge may become a key to improve personalized medicine. In the present study, the effects of the natural remedy curcumin on breast cancer model systems were investigated. MCF-7, ZR-75-1 and TGF-β1 pretreated fibroblasts, mimicking cancer-associated fibroblasts (CAFs), were treated independently as well as in tumor cell/CAF co-cultures. Remarkably, co-culturing with CAF-like cells (CLCs) induced different proteome alterations in MCF-7 and ZR-75-1 cells, respectively. Curcumin significantly induced HMOX1 in single cell type models and co-cultures. However, other curcumin effects differed. In the MCF-7/CLC co-culture, curcumin significantly down-regulated RC3H1, a repressor of inflammatory signaling. In the ZR-75-1/CLC co-culture, curcumin significantly down-regulated PEG10, an anti-apoptotic protein, and induced RRAGA, a pro-apoptotic protein involved in TNF-alpha signaling. Furthermore, curcumin induced AKR1C2, an important enzyme for progesterone metabolism. None of these specific curcumin effects were observed in single cell type cultures. All high-resolution mass spectrometry data are available via ProteomeXchange with the identifier PXD008719. The present data demonstrate that curcumin induces proteome alterations, potentially accounting for its known antitumor effects, in a strongly context-dependent fashion.

Biological significance: Better means to understand and potentially predict individual variations of drug effects are urgently required. The present proteome profiling study of curcumin effects demonstrates the massive impact of the cell microenvironment on cell responses to drug action. Co-culture models apparently provide more biologically relevant information regarding curcumin effects than single cell type cultures.

1. Introduction

Curcumin is a naturally occurring polyphenol present in the Indian spice turmeric, exhibiting strong antioxidative activity which manifests itself, for example, in the up-regulation of heme oxygenase 1 (HMOX1) [1]. Furthermore, this compound has anti-inflammatory effects and displays promising chemo-preventive and antitumor activity; it can affect cancer cell proliferation and induce apoptosis [2], while being pharmacologically safe [3]. These features make curcumin a valuable drug candidate for the treatment of cancer, worthy for detailed examinations.

Accounting for 30% of all female cancer cases, breast cancer is one of the most common causes of cancer death for women worldwide [4]. Many studies concerning the potential of curcumin have concentrated on the antitumor effects of this compound on breast cancer cells. In this way, the modulation of the NF-κB signaling pathway by curcumin in MCF-7 cells has been observed [5]. Furthermore, fatty acid synthase inhibition [6] and modulation of the estrogen receptor and the p53 signaling pathway [7] were described as consequences of curcumin application on breast cancer cells. Similarly, changes in the mTORC1 pathway were observed in colon cancer cells [8]. A potential activity of curcumin on the focal adhesion kinase pathway influencing proliferation and migration was observed by Sathe et al. in head and neck cancer [9]. Moreover, a cancer-preventive role of the compound has been described as well, as mammary stem cells, which are considered as the source of breast cancer cells, were selectively affected by curcumin which altered their lipid metabolism and consequently disturbed their self-renewal potential [10]. Finally, curcumin is apparently able to inhibit epithelial-to-mesenchymal transition (EMT) in breast cancer cell lines [11]. Buhrmann et al. [12] observed similar effects in a co-culture model system of colorectal cancer cells and fibroblasts, where curcumin was impeding EMT by suppressing the crosstalk between these cells.

Abbreviations: CAFs, cancer-associated fibroblasts; CLCs, CAF-like cells; EMT, epithelial-to-mesenchymal transition; FDR, false discovery rate; IRDS, interferon-related DNA damage resistance signature; LC, liquid chromatography; LFQ, label free quantification; MRM, multiple reaction monitoring; MS, mass spectrometry; MS/MS, tandem mass spectrometry

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https://doi.org/10.1016/j.jprot.2018.05.007
Received 29 January 2018; Received in revised form 29 April 2018; Accepted 4 May 2018
Available online 09 May 2018
1874-3919/

Tumor-stroma interactions have a strong influence on tumor development, progression [13] and drug response [14]. While healthy stromal cells are supposed to prevent tumor development, emerging cancer cells can initiate their transformation into tumor-supporting cells [15]. Cancer-associated fibroblasts (CAFs), an important part of the tumor micro-environment, appear to be more aggressive than naive fibroblasts, having the ability to initiate angiogenesis, promote tumor progression and support invasiveness [15,16]. These cells are rather predominant in the tumor microenvironment and, in case of breast cancer, typically display a wound healing signature [17]. Based on the interplay between tumor cells and CAFs in vivo, co-culture models of cancer and stromal cells may hold great potentials to investigate drugs potentially affecting both normal and tumor cells [18]. As demonstrated recently by us, proteome profiling represents a qualified approach to investigate tumor-CAFs interactions, despite the complexity and challenging interpretation of the resulting data [19–22].

Differing individual responses to anti-cancer drugs may represent one of the most urgent scientific challenges of our modern society, as also successfully designed targeted drugs may exert unexpected responses in a large number of patients. The establishment of resistance mechanisms may be considered to be a typical response pattern which we need to better understand. Response profiling by proteomics actually represents a very powerful method to investigate host responses and mechanisms of drug action [23,24]. Several proteomic studies on curcumin effects have already been conducted on various types of cancer including neuroblastoma [25], gastric [26], prostate [27], colorectal [28,29] and breast cancer [30]. In the present study, we applied proteome profiling to investigate the effects of the natural compound curcumin on breast cancer cells MCF-7 or ZR-75-1 and appropriate co-culture models mimicking in vivo tumor-stroma interactions with mammary fibroblasts. Here we describe that curcumin displayed pronounced and previously unrecognized antitumor effects in a surprisingly strong context-dependent fashion.

2. Materials and methods

2.1. Cell culture

The investigated human breast carcinoma cell lines MCF-7 and ZR-75-1 were kindly provided by Dr. Walter Jaeger (Department of Clinical Pharmacy and Diagnostics, University of Vienna). Cells were cultured in phenol red free DMEM and RPMI (LifeTech, Austria), respectively, containing 10% FCS (ATCC, USA) and 100 U/mL penicillin/streptomycin (ATCC, USA). Human mammary fibroblasts (HMF, ScienCell Research Laboratories, Carlsbad, CA) were cultured in FBM (Lonza, Switzerland) supplemented with the FGM Bullet Kit, including 10% FCS, and 100 U/mL penicillin/streptomycin. The cells were stimulated with TGF-β1 (2 ng/mL; Sigma-Aldrich, Austria) for seven days in order to obtain cancer-associated fibroblast-like cells (CLCs) [17] and used up to passage 5. Experiments were conducted at 37 °C and 5% CO_2 in 6-well-plates in triplicates.

For co-culture models representing the in vivo situation the cancer cells – 1×10^6 and 3×10^5 cells per well of ZR-75-1 and MCF-7, respectively – were incubated with 10% of CLCs with a 1:1 mixture of the respective media. After 24 h of incubation, co-cultures as well as MCF-7, ZR-75-1 and CLCs alone were treated with 30 μM curcumin (Sigma-Aldrich, Austria) for 24 h. Medium was changed in control cells. The treatment concentration was chosen as two third of the IC_{50}-value of fibroblasts (data not shown) which were the most sensitive cells of the investigated ones. After 24 h treatment, cells were controlled for cell death using the Trypan blue exclusion protocol (Thermo Scientific, Austria) with consistently > 95% viable cells, washed with cold PBS and 200 μL sample buffer (7.5 M urea, 1.5 M thiourea, 4% CHAPS, 0.05% SDS, 100 mM dithiothreitol) were added. Cells were scraped off and lysed by means of an ultrasonic stick. Bradford assay (Bio-Rad-Laboratories, Germany) was employed to determine protein concentrations.

2.2. Sample preparation

Whole cell lysates were digested in solution as described previously [31]. Briefly, 20 μg proteins were concentrated on a 10 kDa cut-off filter (Pall Austria Filter GmbH, Austria), reduced with dithiothreitol, carbamidomethylated with iodoacetamide and finally digested with a trypsin/lys-c mixture (Promega, Germany) overnight at 37 °C. This was followed by a clean-up on C-18 spin columns (Pierce,Thermo Scientific). Therefor peptide samples were acidified with trifluoroacetic acid (TFA) (1% final concentration) and transferred on the prewashed and equilibrated spin columns. The column bound peptides were washed (5% acetonitrile ACN, 0.5% TFA) and finally eluted (50% ACN, 0.1% TFA). Dried eluates were stored at −20 °C until analysis.

2.3. LC-MS/MS analysis

As described previously [23] for shotgun proteomics analysis a Dionex Ultimate 3000 nano-HPLC system (Thermo Scientific, Austria) was coupled to a high resolution QExactive orbitrap mass spectrometer (Thermo Scientific, Austria). Samples were dissolved in 5 μL 30% formic acid (FA) containing 10 fmol each of four synthetic peptides as internal standard for LC-MS quality control. After dilution with 40 μL mobile phase A (98% H_2O, 2% ACN, 0.1% FA), 5 μL of the sample were injected. At a flow rate of 10 μL/min of mobile phase A the samples were loaded on a 2 cm × 75 μM C-18 Pepmap100 pre-column (Thermo Scientific, Austria). Peptides were separated over a 75 μm × 50 cm C-18 separation column (Thermo Scientific, Austria) with a 90 min gradient from 8 to 40% solvent B (80% ACN, 20% H_2O, 0.1% FA) at a flow rate of 300 nL/min. The MS parameters were set to resolution 70′000 and 17′500 at 200 *m/z*, AGC targets to 3e6 and 2e4, maximum ion filling time to 50 ms and 100 ms for MS^1 and MS^2, respectively. The *m/z* range was set to 400–1400 in this 115 min long top 8 method with a dynamic exclusion window of 45 s and an isolation window of 2 *m/z*. Peptide match and isotope exclusion were turned on. For MS^2 analysis, HCD fragmentation with 30 eV was applied. Only peptides with charges of +2, +3 and + 4 were considered. Samples were measured in replicates. Lock mass was chosen at 445.12003 m/z.

Multiple reaction monitoring (MRM) method was developed based on shotgun data and using Skyline software (v. 4.1) [32], as described previously [33,34] (Supplementary Table S4). Targeted MRM analysis was conducted on an Agilent 6490 triple quadrupole mass spectrometer coupled with a nano-Chip-LC Agilent Infinity Series HPLC1290 system. Digested sample were reconstituted in 25 μL of 30% formic acid solution containing equimolar concentration of 4 standard peptides (10 fmol/μL). Each time 2 μL of sample were injected and peptides were separated by applying 19 min gradient from 8% to 30% acetonitrile. Skyline software [32] was used for data evaluation and total peak area of each peptide was normalized to standard peptides (global standards).

2.4. Data analysis

The freely available MaxQuant software [35] version 1.6.0.1 was employed for protein identification and label-free quantification (LFQ). Protein identification was achieved searching against the UniProt Database [36] (organism: *Homo sapiens*, reviewed: yes, version 11/2015 with 20,193 entries) Peptide tolerances for first and main search were set to 50 and 25 ppm, respectively. Two peptides with at least one unique peptide were the minimum requirements for a positive identification. Match and alignment time windows were set to 10 and 20 min, respectively, false discovery rates (FDR) to 0.01 for peptides as well as for proteins.

Perseus [37], freely available software for data analysis of MaxQuant outputs, was used for statistical evaluations. Protein groups only identified by site as well as reverse and potential contaminants were