Redox Biology 15 (2018) 253–265

Contents lists available at ScienceDirect

Redox Biology

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

Research paper

Impaired pentose phosphate pathway in the development of 3D MCF-7 cells mediated intracellular redox disturbance and multi-cellular resistance without drug induction

Wenjie Wang[a,1], Qingyun Cai[a,1], Fang Zhou[a], Jiali Liu[a], Xiaoliang Jin[a], Ping Ni[a], Meng Lu[b,*], Guangji Wang[a,*], Jingwei Zhang[a,*]

[a] Key Lab of Drug Metabolism and Pharmacokinetics, State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing, Jiangsu, China

[b] Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu, China

ARTICLE INFO

Chemical compounds studied in this article:
Myristic-1,2-$^{13}C_2$ acid (PubChem CID: 16213483)
Menadione (PubChem CID: 4055)
PFT-α (PubChem CID:9929138)
DMSO (PubChem CID: 679)
N-acetylcysteine (PubChem CID: 12035)

Keywords:
3D cell culture
Cellular metabolomics
Pentose phosphate pathway
Redox-status
P-gp regulation

ABSTRACT

Although metabolic reprogramming and redox imbalance are widely reported to be involved in chemo-resistance in cancer treatment, much more attention was paid to anti-cancer drug induced effect. Our previous studies showed that cancer cells can develop P-gp overexpression-mediated intrinsic drug resistance in the formation of 3D MCF-7 multi-cellular layers (MCLs) without any drug induction. However, whether metabolic reprogramming and redox imbalance functioned during this progress remained unrevealed. In our present study, LC-Q/TOF-MS and GC-MS were used in combination for analysing intracellular metabolites. The contribution of pentose phosphate pathway (PPP) and its related redox status were checked by chemical interfering and silencing/over-expression of glucose-6-phosphate dehydrogenase (G6PD). The downstream products of G6PD were assayed by quantitative real-time PCR, western blot and flow cytometry. Results showed that not only G6PD expression but also G6PD activity was significantly lowered along with 3D MCF-7 cells culture time. Impaired PPP disturbed redox-cycling, generated reactive oxygen species (ROS), which triggered cell cycle arrest and caused the switch to Chk2/p53/NF-κB pathway-mediated P-gp induction. Our results provided a new attempt to associate intrinsic small molecule metabolites (impaired PPP) communicating with cell signalling pathways through disturbed intracellular redox status to elucidate multi-cellular resistance (MCR) in 3D MCF-7 cells, which improved the understanding of the mechanisms of P-gp up-regulation in MCR with metabolomic and related redox status support.

1. Introduction

Solid tumours are more difficult to be cured than haematological tumours due to their three-dimension (3D) structure. Therefore, the conventional anti-tumour agents shown to be effective in a two-dimension (2D) cell culture system in vitro often become less or even not effective when applied to the tumour mass; this is called multi-cellular resistance (MCR) [1,2]. The most significant features that distinguish MCR from multi-drug resistance (MDR) could be attributed to the spatial structure and micro-environment of multi-cellular clusters rather than drug induction. For this reason, a large number of 3D cell culture models in vitro have been developed to mimic the tumour mass in vivo for more accurate evaluations of new drugs and mechanistic explorations of new drug targets [3,4]. Until recently, many factors have been identified as responsible for MCR, including poor penetration caused by ATP-binding cassette (ABC) transporters [5], anti-apoptosis gene expression [6], abnormal endogenous oxidative stress status [7], secretion of cytokines and proteins through tumour/stromal cell-cell interactions [8], etc.

However, above mentioned researches only dealt with MCR from individual perspective. It is still unknown whether these factors work separately or in cascade for MCR, and the relationships between these factors have not been elucidated clearly. Since MCR arises without exogenous drug induction, intrinsic alteration or disturbance caused by

Abbreviations: $NADP^+$, (Nicotinamide adenine dinucleotide phosphate); NADPH, (Reduced form of Nicotiamide adenine dinucleotide phosphate); G-6-P, (Glucose-6-phosphate); F-6-P, (Fructose-6-phosphate); 6-PG, (6-Phosphoglycerate); R-5-P, (Ribose-5-phosphate); Ribu-5-P, (Ribulose-5-phosphate); Xylu-5-P, (Xylulose-5-phosphate); S-7-P, (Sedoheptulose-7-phosphate); GSH, (Reduced Glutathione); GSSG, (Oxidized Glutathione); NAC, (N-acetyl cysteine); G6PD, (Glucose-6-phosphate dehydrogenase); Mena, (Menadione); DOX, (Doxorubicin)

* Corresponding authors.
E-mail addresses: ahwhlm@163.com (M. Lu), guangjiwang@hotmail.com (G. Wang), zhangjw_cnnj@sina.com (J. Zhang).

[1] W.J. Wang and Q.Y. Cai contributed equally to this work.

https://doi.org/10.1016/j.redox.2017.12.009
Received 15 November 2017; Received in revised form 6 December 2017; Accepted 20 December 2017
Available online 21 December 2017

spatial structure might be the headstream. Intracellular redox status has attracted much attention in cancer treatment. On one hand, many anticancer agents are designed to exert over production of reactive oxygen species (ROS) to disturb the redox homeostasis to kill cancer cells [9,10]. And any adaption of cancer cells to encounter ROS is believed to cause drug resistance. On the other hand, cancer cells themselves could rearrange intracellular metabolism (metabolic reprogramming) to satisfy their rapid proliferation, and they might gradually adapt to such disorder including high oxidative stress environments and exhibit drug resistance [11]. Therefore, the relationship between redox status and drug resistance in cancer cells was not in consistence, and most studies were performed at 2D cell level [12,13]. For MCR at 3D cell level, how endogenous oxidative stress status was disturbed, where intrinsic ROS was produced, and whether redox status triggered cell signalling pathway and conferred drug resistance, all these issues need to be addressed in the formation of 3D cancer cells.

In this study, we combined cellular metabolomics and 3D cell culture to answering why intracellular redox disturbance and MCR occur without drug induction and how to overcome it in cancer therapy with new potential target. Nevertheless, there were only a few papers describing this joint field, and most cellular metabolomic investigations of tumours still relied on 2D cell culture [14,15]. Recently, we have successfully developed a multi-cellular layer (MCL) 3D cell culture model for MCF-7 breast cancer cells and revealed the possible MCR mechanisms that up-regulate P-gp expression through the Chk2/p53/NF-κB pathway [16]. However, it is still unknown what occurs prior to cell cycle arrest and Chk2 activation, and the potential metabolomic mechanisms and related redox-status need further elucidation. Therefore, in our present study, cellular metabolomics and related redox-status in the formation of 3D cell culture (MCLs and spheroids) were analysed and were further correlated with P-gp expression.

2. Materials and methods

2.1. Materials

Myristic-1,2-$^{13}C_2$ acid, methoxamine hydrochloride and menadione (Mena) were purchased from Sigma-Aldrich (USA). 5-^{13}C-glutamine was purchased from Camabridge Isotope Laboratories, Inc. (USA). N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and chlorotrimethylsilane (TMCS) were purchased from Pierce Chemical Co. (USA). Pifithrin-α (PFT-α) was purchased from Selleckchem (USA). HPLC-grade acetonitrile, methanol and n-heptane were purchased from Merck (Germany). Deionized water was prepared by a Milli-Q system (Millipore, USA). N-acetyl cysteine (NAC), glutathione (GSH) and an oxidized glutathione (GSSG) Test Kit were purchased from Beyotime Institute of Biotechnology (Jiangsu, China). NADP/NADPH Quantitation Colorimetric Kit was purchased from BioVision, Inc. (USA). The primers for glucose-6-phosphate dehydrogenase (G6PD), mdr1, p53 and β-actin for qPCR analysis were synthesized by Invitrogen (Life Technologies, USA). Monoclonal antibodies against Chk2, p-Chk2, p53, p65, histone, and horseradish peroxidase-conjugated goat anti-mouse/rabbit IgG secondary antibodies were purchased from Cell Signalling Technology (Danvers, MA, USA). The antibody for GAPDH was purchased from Bioworld Technology (Dublin, Ohio, USA).

2.2. MCF-7 cell culture

MCF-7 human breast carcinoma cells were purchased from the American Type Culture Collection. The cells (passage 10–20) were cultured in RPMI 1640 medium supplemented with 10% foetal bovine serum, and 100 U ml^{-1} penicillin and streptomycin (Invitrogen, Carlsbad, CA) at 37 °C with 5% CO_2, and the medium was changed every other day.

2.3. Multi-cellular layer culture

The culture of MCF-7 MCLs was performed as we described previously [16]. Briefly, the MCF-7 cells were re-suspended with pre-chilled serum-free medium containing 6% matrigel and then seeded on a 6-well plate. After incubating at 37 °C for 1 h, culture medium was added to cells in each well. The cells were cultured for 3, 5, 8, 10 and 14 days, and the medium was changed every other day (n = 5).

2.4. Spheroid cell culture

MCF-7 cells were resuspended with 10% RPMI 1640 medium, and then seeded on a PrimeSurface 96U 3D cell culture plate (Sumitomo Bakelite, Japan). The cells were cultured for 3, 5, 8 days, and the medium was changed every other day (n = 5).

2.5. Cell viability assays

MCF-7 MCLs (3, 5, 8, 10 and 14 days) and spheroids (3, 5 and 8 days) were exposed to a series of concentrations of doxorubicin (DOX) for 72 h at 37 °C with 5% CO_2. After treatments, cells were incubated with 3-(4,5-dimethylthiazol-2-yl) − 2,5-diphenyltetrazolium bromide (MTT) solution (0.5 mg/ml) for 4 h, and then, precipitated MTT was dissolved with DMSO for 30 min. Absorbance was measured at 490 nm. The IC_{50} values were calculated from inhibition curves using Graphpad Prism 6.

2.6. Animal and Xenograft study

All animal care and experimental procedures were conducted according to the National Research Council's Guidelines for the care and use of laboratory animals and were approved by the SPF Animal Laboratory of China Pharmaceutical University (Animal authorization reference number: SYXK2016-0011). Every effort was made to minimize animal pain, suffering and distress and to reduce the number of animals used.

Healthy female BALB/c nude mice (18–22 g and 8–10 weeks old) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The mice were maintained in air-conditioned rooms under controlled light (12 h light: 12 h dark) and temperature (23 ± 2 °C, 55 ± 5% humidity), fed with standard laboratory food and water ad libitum. Before MCF-7 cells were transplanted into the animal, a 2-mg E_2 pellet was placed subcutaneously in the interscapular region of each mouse. Then, tumours were generated by subcutaneous injections of 5 × 10^6 exponentially growing MCF-7 cells into the right flank regions of the nude mice. Tumour volume (TV) was measured with micrometer calipers, and calculated using the following formula: TV (mm^3) = d^2 × D/2, where d and D means the shortest and the longest diameters, respectively. The maximum tumour size during the experiments was allowed by Institutional Animal Care and Use Committee. As designed, when the tumours grown to 500, 1000, and 2000 mm^3 (five mice per group), the mice were killed by CO_2 asphyxiation and cervical dislocation, and tumours were collected for further assessment.

2.7. GC-MS-based metabolomics assays

The samples of tumour tissues and 3D MCF-7 cells were processed as we described previously [17]. Briefly, the tissues and cells were washed and lysed by repeated freezing and thawing and homogenization, followed by adding methanol containing myristic-1,2-$^{13}C_2$ acid as an internal standard to extract intracellular metabolites. After centrifugation, the supernatant was evaporated to dryness, and the remaining residue underwent methoximation and trimethylsilylation subsequently. Finally, external standard methyl myristate was added before GC-MS analysis. GC-MS was performed with the SHIMADZU QP2010Ultra/SE system (Tokyo, Japan) with a 30 mm × 0.25 mm ID