Biochimica et Biophysica Acta 1863 (2016) 2245–2254

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Effects of G6PD activity inhibition on the viability, ROS generation and mechanical properties of cervical cancer cells

Zishui Fang [1], Chengrui Jiang [1], Yi Feng, Rixin Chen, Xiaoying Lin, Zhiqiang Zhang, Luhao Han, Xiaodan Chen, Hongyi Li, Yibin Guo, Weiying Jiang *

Department of Medical Genetics, Zhongshan School of Medicine, Sun Yat-sen University, University and Key Laboratory of Tropical Disease Control (Sun Yat-sen University), Ministry of Education Guangzhou, 510080, China

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form 17 May 2016
Accepted 19 May 2016
Available online 20 May 2016

Keywords:
Cervical cancer cell
G6PD deficiency
Mechanical properties
Cytoskeleton
Anti-tumor

ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) deficiency has been revealed to be involved in the efficacy to anti-cancer therapy but the mechanism remains unclear. We aimed to investigate the anti-cancer mechanism of G6PD deficiency. In our study, dehydroepiandrosterone (DHEA) and shRNA technology were used for inhibiting the activity of G6PD of cervical cancer cells. Peak Force QNM Atomic Force Microscopy was used to assess the changes of topography and biomechanical properties of cells and detect the effects on living cells in a natural aqueous environment. Flow cytometry was used to detect the apoptosis and reactive oxygen species (ROS) generation. Scanning electron microscopy was used to observe cell morphology. Moreover, a laser scanning confocal microscope was used to observe the alterations in cytoskeleton to explore the involved mechanism. When G6PD was inhibited by DHEA or RNA interference, the abnormal Young's modulus and increased roughness of cell membrane were observed in HeLa cells, as well as the idioblasts. Simultaneously, G6PD deficiency resulted in decreased HeLa cells migration and proliferation ability but increased ROS generation inducing apoptosis. What's more, the inhibition of G6PD activity caused the disorganization of microfilaments and microtubules of cytoskeletons and cell shrinkage. Our results indicated the anti-cervix cancer mechanism of G6PD deficiency may be involved with the decreased cancer cells migration and proliferation ability as a result of abnormal reorganization of cell cytoskeleton and abnormal biomechanical properties caused by the increased ROS. Suppression of G6PD may be a promising strategy in developing novel therapeutic methods for cervical cancer.

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD), an X-linked enzyme that catalyses the first rate-limiting step in the pentose phosphate pathway(PPP), has been revealed to be involved in apoptosis, angiogenesis, tumor occurrence and efficacy to anti-cancer therapy [1,2]. Accumulated evidences have shown that G6PD elevated in various tumors, including leukemia [3], melanoma cancer [4], endometrial carcinomas [5], breast cancers [6], and colon cancers [7]. G6PD provides ribose and nicotinamide adenine dinucleotide phosphate (NADPH) that support biosynthesis and antioxidant defense through PPP [8]. Due to the large biosynthetic demands of a rapidly growing cancer and an adaptation to stressful environments, the PPP has been suggested to promote cancer progression and therapy resistance [9]. Given that G6PD plays a critical role in survival, migration, proliferation, and metastasis of cancer cells, development of potent and selective G6PD inhibitors and elucidation of its underlying anti-tumor mechanism would open up a novel way for cancer therapy.

In our study, two kinds of G6PD inhibitors were used to investigate the underlying anti-cancer mechanism. Dehydroepiandrosterone (DHEA),the non-competitive inhibitor of G6PD, is an adrenal steroid hormone with a wide variety of biological effects both in vivo and in vitro [10–13], showing promising potential in the treatment of different types of cancer cells, such as in breast, liver, and cervix [14–16]. The decline of G6PD activity seen with DHEA treatment is not the result of decreased protein expression but caused by binding to the enzyme–coenzyme substrate ternary complexes in mammalian cells [13,17]. In addition, the inhibition of G6PD activity by shRNA technology was also performed to research the effects of G6PD activity on cervical cancer cells.

Abbreviations: G6PD, Glucose-6-phosphate dehydrogenase; PPP, pentose phosphate pathway; AFM, atomic force microscopy; SEM, scanning electron microscopy; LSCM, laser scanning confocal microscope; RNAi, RNA interference; Ra, arithmetic average roughness; Rq, root-mean-square roughness; NADPH, nicotinamide adenine dinucleotide phosphate; DHEA, dehydroepiandrosterone; ROS, reactive oxygen species; RT, room temperature; MFs, microfilaments; MTs, microtubules; DCFDA, 6-carboxy-2′,7′-dichlorofluorescin diacetate.

* Corresponding author.
E-mail address: jiangwy@mail.sysu.edu.cn (W. Jiang).

[1] Zishui Fang and Chengrui Jiang are equally contributed to this work.

http://dx.doi.org/10.1016/j.bbamcr.2016.05.016
0167-4889/

Cellular mechanical properties have been proved to be effective label-free biomarkers for indicating cell states [18–20]. A change in cell biophysical property may disturb normal mechanisms and result in a pathophysiological state. Elucidating the mechanical properties of a living cell at quantization can provide information about the actual condition of the cell and the functional characterization of the cytoskeleton [21–23]. Atomic force microscopy (AFM) has provided a new opportunity to investigate single cell in aqueous environment, although it remains to be a challenge [24]. As a high-resolution type scanning probe microscopy, AFM can be applied to measure the changes in the biophysical properties of cells, such as stiffness, elasticity, adhesion, and roughness [25–27]. In the study, the advanced technology named peak force QNM atomic force microscopy was used to quantitative imaging including stiffness and roughness of living biological samples simultaneously. Except that, we evaluated the apoptosis and reactive oxygen species (ROS) generation of cervical cancer cells after treatment by G6PD inhibitors by flow cytometry. The morphology of cells was also observed by scanning electron microscopy (SEM). Cytoskeleton plays an important role in maintaining cellular architecture and internal organization, cell morphology, motility, cell division, and many other processes [28,29]. In our study, a laser scanning confocal microscope (LSCM) was used to observe the changes of microfilaments (MFs) and microtubules (MTs) of cytoskeleton in cells to explore the involved mechanism.

The aim of this work was to evaluate the effects of G6PD activity inhibition caused by DHEA or RNA interference on human cervical cancer cells and to explore the underlying anti-cancer mechanism. We expect that this method will be valuable for screening anti-cancer therapeutic agents and investigating potential targets and deepening the understanding of the anti-tumor mechanism of agents.

2. Materials and methods

2.1. Reagents and materials

Dulbecco's modified Eagle's medium (DMEM, Gibco), trypsin and Opti-MEM® as well as 6-carboxy-2′,7′-dichlorofluorescin diacetate (DCFH-DA, Molecular Probes) were purchased from Life Technologies Company. Fetal bovine serum was purchased from GEMINI Company USA. Flow cytometry reagents were purchased from Becton, Dickinson and Company. All sterile plastic materials for cell culture were purchased from Corning Incorporated. Lipofectamine® 2000 was purchased from Invitrogen Company. Cell Counting Kit-8(CCK-8) was purchased from Dojindo Company. DHEA and all other chemicals were purchased from Sigma Aldrich. All antibodies were purchased from Abcom Company.

2.2. Cell culture and pretreatment

The human cervical carcinoma cell line (HeLa cells) were used in this study and cultured according to the protocols provided by the American Type Culture Collection. Shortly, cells were maintained in DMEM supplemented with 10% fetal calf serum, 100 IU/mL penicillin, and 100 mg/mL streptomycin at 37 °C and 5% CO_2 in a humidified incubator. DHEA was diluted in DMSO and the final concentration of DMSO in the medium did not exceed 0.1%, showing no detectable effect on cell growth. The recombination eukaryotic plasmid for G6PD gene silence was employed and silencing efficiency was evaluated by RT-PCR and western blot. The target transformants with the best silencing effect were chosen and then transfected into cells. Cells were seeded with suitable density and cultured 24 h for adherent growth. One group cells were cultured for another 24 h, 48 h and 72 h in different DHEA concentrations (0, 25, 50, 100, 200 μM). Another group cells were transfected with plasmids for the inhibition of G6PD activity and empty vector plasmids for control, subsequently transfected cells were cultured for 48 h for further procedures.

2.3. Cell viability assay

Cell viability was measured by CCK-8 according to the manufacturer's instructions. In brief, cells were seeded in 96-well plates at the density of $4.0 * 10^3$ cells per well with 100 μL culture medium for 24 h. For one group, DHEA was added to the medium to the final concentrations (0, 25, 50, 100 and 200 μM).The cells treated by DHEA were then cultured for another 24 h, 48 h and 72 h. For another group, the cells were treated with shRNA for 48 h. Then, 10 μL of CCK-8 solution was added to each well, and the culture was incubated for another 2 h at 37 °C. The optical density (OD) values were read at 450 nm with a reference wavelength at 600 nm by a microplate reader (Tecan Sunrise Reader, Switzerland).

2.4. Flow cytometry assay for apoptosis

Harvested cells were washed with cold PBS and re-suspended in 100 μL 1× binding buffer, followed by incubation with 5 μL Annexin V-APC and 5 μL propidium iodide (PI). After 15 min of incubation at room temperature (RT) in the dark, samples were diluted to 500 μL at a cell density of $1 * 10^6$/mL with 1× binding buffer for final measurements. Then cells were analyzed by flow cytometry (Beckman CytoFLEX, Germany).

2.5. Measurement of intracellular ROS by flow cytometry

For the measurement of ROS, we harvested the cells which had been treated with either DHEA (0, 25, 50, 100 μM) or shRNA for 48 h, and then washed the treated cells twice with PBS. $1 * 10^6$ cells were suspended in 1 mL PBS and incubated with 10 μM DCFH-DA, a cell membrane permeable fluorescence probe, for 20 min at 37 °C. Then, the cells were washed and suspended in 1 mL of PBS for measurements. Fluorescence levels were measured using flow cytometry (Beckman CytoFLEX) with excitation and emission wavelengths set at 480 nm and 530 nm, respectively.

2.6. Hela cell morphology study by SEM

For this aim, cells were seeded on poly-L-lysine coated slides. After treatment, cells were washed twice with PBS then fixed in 2.5% glutaraldehyde-4% paraformaldehyde in 0.1 M PBS (pH 7.4) for 0.5 h at RT, rinsed twice for 5 min with PBS. Specimens were then dehydrated through a graded ethanol series (50%, 70%, 85%, 100%), dried in a CO_2 critical point dryer, mounted on specimens tub, sputter coated with gold, and examined in a FEI QUANTA 200 SEM.

2.7. The migration of cells study

HeLa cells were seeded into 24-well plates to yield confluent monolayer for wounding. A straight scratch line (about 1 mm width) was made on cell monolayer with a pipette tip then cells were washed twice by PBS to remove the cell debris. Subsequently different concentrations of DHEA (0, 25, 50, 100, 200 μM) or shRNA were added into 24-well plates. Photographs were taken at 0 h, 24 h, 48 h and 72 h using the imaging system of inverted living cell microscope (ZEISS AXIO OBSERVER Z1, Germany), respectively. The distance migrated by the cell monolayer to close the wounded area during the time periods was measured using the Image J software. Migration formula is following: migration rate (MR) $= (S_{0h} - S_{xh}) / S_{0h} * 100\%$, where S_{0h} represented the initial wound area at 0 h and S_{xh} represented the wound area at different time points(24 h, 48 h, 72 h) respectively.

2.8. Measurements of mechanical properties by AFM

Measurements of mechanical properties were performed in PBS buffer using a Dimension Fastscan with ScanAsyst™ AFM (Bruker,