The International Journal of Biochemistry & Cell Biology 45 (2013) 2512–2518

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

Short communication

Interference with the mitochondrial bioenergetics fuels reprogramming to pluripotency *via* facilitation of the glycolytic transition

Myung Jin Son[a,b], Bo Ram Jeong[a], Youjeong Kwon[a,b], Yee Sook Cho[a,b,*]

[a] Stem Cell Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon 305-806, Republic of Korea
[b] Department of Functional Genomics, University of Science & Technology, 217 Gajungro, Yuseong-gu, Daejeon 305-333, Republic of Korea

ARTICLE INFO

Article history:
Received 10 May 2013
Received in revised form 12 July 2013
Accepted 29 July 2013
Available online xxx

Keywords:
Reprogramming
iPSCs
Mitochondria
Bioenergetics
Glycolysis

ABSTRACT

The switch in cell metabolism from oxidative phosphorylation to glycolysis is critical for the reprogramming of cells to pluripotency. Here, we demonstrate that the disturbance of mitochondrial metabolism by canonical mitochondrial inhibitors enhances metabolic reprogramming toward a glycolytic state, enabling the highly efficient generation of induced pluripotent stem cells. This interference with mitochondrial bioenergetics resulted in enriched reprogrammable subpopulations and accelerated the conversion of refractory intermediates to pluripotent states without requiring additional genetic or epigenetic modifications. Conversely, the reprogramming efficiency and accelerated reprogramming kinetics promoted by mitochondrial inhibition were obstructed by glycolysis inhibitors. We suggest that changes in mitochondrial bioenergetics are a novel mechanism involved in the regulation of cell fate and, more importantly, in the reprogramming of cells to pluripotency.

1. Introduction

Recent advances in induced pluripotent stem cell (iPSC) technology (Takahashi et al., 2007; Takahashi and Yamanaka, 2006), which enables the conversion of adult somatic cells to embryonic stem cell (ESC)-like pluripotent cells, provide a useful tool for the study of processes involving changes in cell fate, such as aging/rejuvenation, differentiation, de-differentiation, and tumor initiation, as well as a valuable source of cells for regenerative medicine (Hanna et al., 2010; Stadtfeld and Hochedlinger, 2010).

Cellular reprogramming resets the genetic and epigenetic landscapes of somatic cells to recreate the pluripotent state (Li et al., 2012). Recent reports have also emphasized the importance of metabolic reprogramming in iPSC generation; this type of reprogramming is closely correlated with a bioenergetic transition from somatic oxidative phosphorylation (OXPHOS) to glycolysis (Folmes et al., 2011; Panopoulos et al., 2012; Varum et al., 2011; Zhang et al., 2011; Zhou et al., 2012). Such metabolic reprogramming occurs prior to epigenetic modifications and facilitates nuclear reprogramming (Folmes et al., 2011). Although glycolysis is inefficient in terms of energy production, highly proliferative cells, such as ESCs and iPSCs, preferentially use glycolysis because this process provides building blocks for cell replication and minimizes mitochondrial oxidative stress (Kondoh et al., 2007; Prigione et al., 2010; Vander Heiden et al., 2009). Small molecules that promote glycolytic metabolism, such as the activator of 3'-phosphoinositide-dependent kinase-1 and 2,4-dinitrophenol, have been reported to facilitate iPSCs generation (Zhu et al., 2010).

These reports have led to the intriguing hypothesis that the inhibition of mitochondrial function during iPSC generation could accelerate the glycolytic metabolic transition to meet the energy demands of these cells and promote the induction of pluripotency. Notably, cells with more glycolytic phenotypes exhibit lower mitochondrial complex IV activity (epiblast stem cells (EpiSCs) < embryonic stem cells (ESCs) < mouse embryonic fibroblasts (MEFs)) (Zhang et al., 2011; Zhou et al., 2012). Mitochondrial complex I and II subunits are also down-regulated in iPSCs compared with their levels in MEFs (Folmes et al., 2011). Here, we demonstrate that interference with mitochondrial function by electron transport chain (ETC) complex inhibitors, a direct ATP synthase inhibitor, or an uncoupler, stimulates a glycolytic bioenergetic transition that provides a novel strategy for efficiently reprogramming somatic cells to pluripotency.

* Corresponding author at: Stem Cell Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon 305-806, Republic of Korea. Tel.: +82 42 860 4479; fax: +82 42 860 4608.
E-mail address: june@kribb.re.kr (Y.S. Cho).

1357-2725/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biocel.2013.07.023

2. Materials and methods

2.1. 2. 1. Reagents

Rotenone, 2-thenoyltrifluoroacetone (TTFA), and sodium oxamate were purchased from Santa Cruz Biotechnology. Antimycin A, potassium cyanide (KCN), oligomycin A, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), and 2-deoxy-D-glucose (2-DG) were purchased from Sigma (St. Louis, MO, USA).

2.2. iPSC generation

To generate iPSCs, MEFs were seeded at 1×10^5 cells *per* well in 6-well plates the day before transduction and subsequently transduced with concentrated virus at a multiplicity of infection (MOI) of 1 in the presence of polybrene (8 μg/ml). Four days after transduction, the MEFs were trypsinized and replated at 6×10^4 cells per well in Matrigel-coated 12-well plates. On the next day, the medium was replaced with ESC medium, and the medium was changed every other day thereafter.

2.3. Oxygen consumption measurement

MitoXpress Xtra HS (Luxcel Biosciences, Cork, Ireland) was used as the oxygen probe. Briefly, reprogramming cultures (day 7) were dissociated into single-cell suspensions, and equal numbers of cells per well were placed into black wells of 96-well plates (4.5×10^5 cells/well) (Thermo Scientific, Waltham, MA, USA). One micromole of MitoXpress was added to the medium, and the wells were sealed with mineral oil at 30 °C; fluorescence was then measured at 30 °C using a SpectraMax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.4. Lactate assay

The intra- and extra-cellular lactate contents were measured from 10 μg of protein using a Lactate Assay Kit (BioVision, Milpitas, CA, USA) according to the manufacturer's protocols.

2.5. Glucose consumption assay

The glucose content in culture media harvested at the indicated time points was measured using a Glucose (GO) Assay Kit (Sigma). Glucose consumption was calculated by subtraction from the measured glucose concentrations in the fresh medium, as previously described (Mauro et al., 2011).

2.6. ATP assay

ATP was quantified from 1 μg of protein using an ATP bioluminescent assay kit (Sigma). Luminescence intensity was measured using a SpectraMax microplate reader (Molecular Devices).

2.7. Cell sorting

For flow cytometric cell sorting, reprogramming cultures (day 11) were dissociated into single-cell suspensions and labeled with anti-SSEA1 (R&D Systems, Minneapolis, MN, USA) and anti-Thy1-PE antibodies (BD, San Jose, CA, USA). The labeled cells were then physically sorted using a FACSAria flow cytometer (BD). Cell Quest Acquisition and Analysis software (BD) was used to acquire and quantify the fluorescence signal distributions and intensities of individual cells.

2.8. Statistical analysis

The data are presented as the mean ± SE ($n = 3$). Student's t test was used to evaluate between-group comparisons. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Inhibition of mitochondrial metabolism accelerates the glycolytic metabolic transition

During reprogramming, the energetic infrastructure changes effected by mitochondrial respiratory chain inhibition were examined (Fig. 1). Mouse embryonic fibroblasts (MEFs) were reprogrammed into iPSCs with or without exposure to the following specific inhibitors of the electron transport chain (ETC): rotenone (a complex I inhibitor), antimycin A (a complex III inhibitor), and KCN (a complex IV inhibitor), as depicted in Fig. 1A and B. Rotenone, antimycin A, and KCN significantly reduced oxygen consumption by 32.1%, 35.9%, and 70.8%, respectively, compared with control cells at day 7 (Fig. 1B). In cells treated with mitochondrial inhibitors, the cellular ATP content was also reduced compared to that of untreated controls at day 7, but it gradually recovered, and very little or no difference in cellular ATP content was observed on day 11 (Fig. 1C). iPSCs exhibited lower rates of mitochondrial respiration (Supplementary Fig. 1A) and a higher production of lactate, the end product of glycolysis, (Supplementary Fig. 1B) than MEFs. Similarly, during reprogramming, transduction of OCT4, SOX2, KLF4, and c-MYC (OSKM) gradually elevated the lactate production to twice the value observed in MEFs at day 7 (Fig. 1D). Notably, mitochondrial inhibitors accelerated the intracellular lactate production (Fig. 1D) and lactate secretion into the medium compared with untreated controls (Fig. 1E). Glucose consumption was also increased in cells treated with mitochondrial inhibitors compared to untreated controls and MEFs (Fig. 1F). These data demonstrate that the inhibition of mitochondrial metabolism by mitochondrial complex enzyme inhibitors accelerates the metabolic shift from somatic OXPHOS to glycolysis (Fig. 1G).

3.2. Interference with mitochondrial bioenergetics enhances somatic cell reprogramming

To examine whether interference with mitochondrial energy metabolism affects cell fate transition, we used multiple approaches to inhibit mitochondrial functions on reprogramming, including ETC complex inhibitors (rotenone, a complex I inhibitor; TTFA, a complex II inhibitor; antimycin A, a complex III inhibitor; and KCN, a complex IV inhibitor), a direct ATP synthase inhibitor (oligomycin A, a complex V inhibitor), and an uncoupler (FCCP) (Fig. 2). The application of these compounds in the micromolar concentration range commonly used in mitochondrial inhibition studies was toxic to the reprogramming cultures, as shown by alkaline phosphatase $(AP)^+$ colonies (Supplementary Fig. 2A). However, reprogramming efficiency was substantially increased by rotenone (10 nM), TTFA (1 nM), antimycin A (1 pM), KCN (10 nM), oligomycin A (1 pM), and FCCP (10 nM) at picomolar to nanomolar concentrations (Supplementary Fig. 3). Reprogramming efficiency was increased 10.2-fold (by 10 nM FCCP) to 21.4-fold (by 10 nM rotenone) over that of untreated controls, as revealed by the examination of AP^+ and Oct4-GFP^+ colonies (Fig. 2A and Supplementary Fig. 3). The time required for reprogramming was markedly reduced by up to 4–5 days by the addition of rotenone rather than simply occurring as a consequence of reprogramming (Fig. 2B and C). In addition to enhancing reprogramming efficiency and kinetics, mitochondrial inhibitors were effective in