Biochemical and Biophysical Research Communications 417 (2012) 1052–1057

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Reduction of nuclear encoded enzymes of mitochondrial energy metabolism in cells devoid of mitochondrial DNA

Edith E. Mueller [a], Johannes A. Mayr [a], Franz A. Zimmermann [a], René G. Feichtinger [a], Olaf Stanger [b,1], Wolfgang Sperl [a], Barbara Kofler [a,*]

[a] Research Program for Receptor Biochemistry and Tumor Metabolism, Department of Pediatrics, Muellner Hauptstraße 48, 5020 Salzburg, Austria
[b] Department of Cardiac Surgery, Paracelsus Medical University, Muellner Hauptstraße 48, 5020 Salzburg, Austria

ARTICLE INFO

Article history:
Received 12 December 2011
Available online 26 December 2011

Keywords:
Mitochondrial DNA
ρ^0 Cells
Complex II
Citrate synthase
Depletion syndromes

ABSTRACT

Mitochondrial DNA (mtDNA) depletion syndromes are generally associated with reduced activities of oxidative phosphorylation (OXPHOS) enzymes that contain subunits encoded by mtDNA. Conversely, entirely nuclear encoded mitochondrial enzymes in these syndromes, such as the tricarboxylic acid cycle enzyme citrate synthase (CS) and OXPHOS complex II, usually exhibit normal or compensatory enhanced activities. Here we report that a human cell line devoid of mtDNA (HEK293 ρ^0 cells) has diminished activities of both complex II and CS. This finding indicates the existence of a feedback mechanism in ρ^0 cells that downregulates the expression of entirely nuclear encoded components of mitochondrial energy metabolism.

1. Introduction

Mitochondria generate most of the cellular adenosine-tri-phosphate (ATP) from carbohydrates and fat. The reduced redox carriers NADH and $FADH_2$, which are generated by numerous mitochondrial redox reactions, are oxidated by the electron transport chain of the inner mitochondrial membrane. In a process called oxidative phosphorylation (OXPHOS) a proton gradient across the inner membrane is generated by complex I, III and IV of the respiratory chain, which is subsequently used to produce ATP by complex V of the OXPHOS system [1].

The vast majority of OXPHOS subunits are encoded by nuclear genes. The extra-chromosomal mitochondrial DNA (mtDNA) encodes for thirteen subunits of OXPHOS complexes I, III, IV and V. The only OXPHOS enzyme that is entirely encoded by nuclear DNA is complex II (succinate dehydrogenase (SDH)) [2].

In mtDNA depletion syndromes, the activities of the OXPHOS complexes I, III, IV and V are usually reduced, as a consequence of the reduced amount of the mtDNA-encoded subunits. In contrast, normal or even upregulated complex II activities and/or protein levels are measured in patients with mtDNA depletion syndromes [3–5]. Citrate synthase (CS), an enzyme of the tricarboxylic acid (TCA) cycle, is also found to be normal or upregulated in these patients [3,5,6]. Therefore, reduced complex I, III, IV, and V and normal or elevated complex II and CS activities are characteristic biochemical parameters for the diagnosis of mtDNA depletion syndromes.

Contrary to this expectation, here we report finding diminished activity of complex II and CS in HEK293 (human embryonic kidney 293) ρ^0 cells devoid of mtDNA.

2. Materials and methods

2.1. Cell lines and culture conditions

The parental HEK293 and HEK293 cybrid cell lines were maintained in Dulbecco's modified Eagles's medium (DMEM) high glucose

Abbreviations: ATP, adenosine-tri-phosphate; BN PAGE, Blue Native polyacrylamide gel electrophoresis; CS, citrate synthase; DAPI, diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagles's medium; EDTA, ethylene-diamine-tetra-acetic acid; EtBr, ethidium bromide; HEK293, human embryonic kidney 293; hHPRT, human hypoxanthine phosphoribosyl transferase; MtDNA, mitochondrial DNA; MtTFA, mitochondrial transcription factor A; NDNA, nuclear DNA; Nrf, nuclear respiratory factor; OXPHOS, oxidative phosphorylation; PBS, phosphate-buffered saline; QPCR, quantitative real-time PCR; QRT-PCR, quantitative real-time reverse-transcription PCR; ROS, reactive oxygen species; RT, room temperature; SDH, succinate dehydrogenase; Sp1, specificity protein 1; TCA cycle, tricarboxylic acid cycle; VDAC1, voltage-dependent anion channel 1.

* Corresponding author. Address: Research Program for Receptor Biochemistry and Tumor Metabolism, Department of Pediatrics, Paracelsus Medical University, Muellner Hauptstraße 48, 5020 Salzburg, Austria. Fax: +43 662 4482 4765.

E-mail addresses: ed.mueller@salk.at (E.E. Mueller), h.mayr@salk.at (J.A. Mayr), f.zimmermann@salk.at (F.A. Zimmermann), r.feichtinger@salk.at (R.G. Feichtinger), o.stanger@rbht.nhs.uk (O. Stanger), w.sperl@salk.at (W. Sperl), b.kofler@salk.at (B. Kofler).

[1] Current address: Royal Brompton and Harefield NHS Trust, Sydney Street, London SW3 6NP, United Kingdom.

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.12.093

(4.5 g/l) (Sigma–Aldrich) supplemented with 10% FBS Mycoplex (PAA Laboratories), 44 mM sodium bicarbonate (Sigma–Aldrich), 1% Penicillin–Streptomycin–Amphotericin B mixture (Lonza), 2.5 mM pyruvate (Boehringer Mannheim) and 1% MEM non-essential amino acid solution (Sigma–Aldrich).

According to the method of King and Attardi [7], HEK293 ρ^0 cells were obtained by cultivation of HEK293 wild-type cells in uridine-supplemented medium (100 μg/ml, Sigma–Aldrich) containing 500 ng/ml ethidium bromide (EtBr) for 18 weeks. ρ^0 Cells were further cultivated in the uridine-supplemented medium without EtBr.

Cybrids were generated, as previously described [8], by fusion of HEK293 ρ^0 cells with isolated platelets of individuals carrying either mitochondrial haplogroup H or T. Individual clones were isolated after growth of cybrid cells in selective medium (without uridine) and analyzed, to control for their source of mtDNA and nuclear DNA.

2.2. Isolation of mitochondria and enzyme measurements

Confluent cells were harvested, washed with phosphate-buffered saline (PBS), and mitochondria were isolated according to Bentlage et al. [9]. Isolated mitochondria were used for enzymatic measurements and Blue Native polyacrylamide gel electrophoresis (BN PAGE). Enzyme activity measurements were performed as previously described [10,11]. The protein content of isolated mitochondria was determined by BCA assay (Pierce).

2.3. Western blot analysis and BN PAGE

Western blot analysis was performed as described by Feichtinger et al. [11], with the following modifications: crude cell lysates were separated on a 15% acrylamide/bisacrylamide gel; the nitrocellulose membrane was blocked in 2% blocking solution (Lumi-Light^{PLUS} Western Blotting Kit, Roche) overnight at 4 °C; the following primary and secondary antibody dilutions and incubation times were used: Nrf1 (Nuclear respiratory factor 1) mouse monoclonal antibody (1:100, 120 min, room temperature (RT), Santa Cruz Biotechnology); beta tubulin rabbit polyclonal antibody (1:1000, 60 min, RT, Abcam); mtTFA (mitochondrial transcription factor A) mouse polyclonal antibody (1:500, overnight at 4 °C, Abcam); labeled polymer-HRP anti-mouse or anti-rabbit (1:1000, 60 min to 120 min, Dako); after detection of Nrf1 and beta tubulin, the nitrocellulose membrane was washed in stripping buffer (25 mM glycine–HCl, pH 2.0, 2% sodium dodecyl sulfate) and a subsequent immunodetection with mtTFA antibody was performed as described above.

BN PAGE was performed as previously described [12], with the following modifications: isolated mitochondria were used for solubilization (in 1.5% laurylmaltoside) and loaded at 16 μg protein/slot on a 5% to 16% polyacrylamide gradient gel; Western Blocking Reagent (Roche) in TBST (50 mM Tris, 150 mM NaCl, 0.5% Tween 20, pH 7.5) was used for blocking of the membrane (overnight at 4 °C) and for antibody dilutions; the following primary and secondary antibody dilutions and incubation times were applied: complex II subunit 70 kDa Fp mouse monoclonal antibody (1:30,000, 90 min, MitoSciences); Porin mouse monoclonal antibody (1:1000, 60 min, MitoSciences); labeled polymer-HRP anti-mouse (1:1000, 60 min, Dako). All antibody incubations were carried out at RT.

2.4. Immunofluorescence staining

Cells were plated on coated slides and grown to 70% confluence. The slides were washed in PBS, fixed for 20 min in 4% formalin in PBS, washed in PBS and incubated in antigen retrieval buffer (1 mM ethylene-diamine-tetra-acetic acid (EDTA), 0.05% Tween-20, pH 8.0) for 20 min at 95 °C. After washing in PBS, slides were incubated for 15 min in 0.1% Triton-X 100, washed once in PBS and once in PBST (PBS, 0.5% Tween-20) and blocked with 10% normal goat serum in PBST for 30 min. The following primary antibody dilutions (diluted in DAKO antibody diluent with background reducing components (DAKO)) and incubation times were applied: Complex IV subunit 1 mouse monoclonal antibody (1:200, 60 min, MitoSciences); voltage-dependent anion channel 1 (VDAC1)/Porin rabbit polyclonal antibody (1:500, 60 min, Abcam); after washing three times in PBST the following secondary antibodies (diluted in PBST) were incubated for 60 min in the dark: Alexa Fluor 488 goat anti-mouse IgG (1:500, Invitrogen) and Alexa Fluor 594 goat

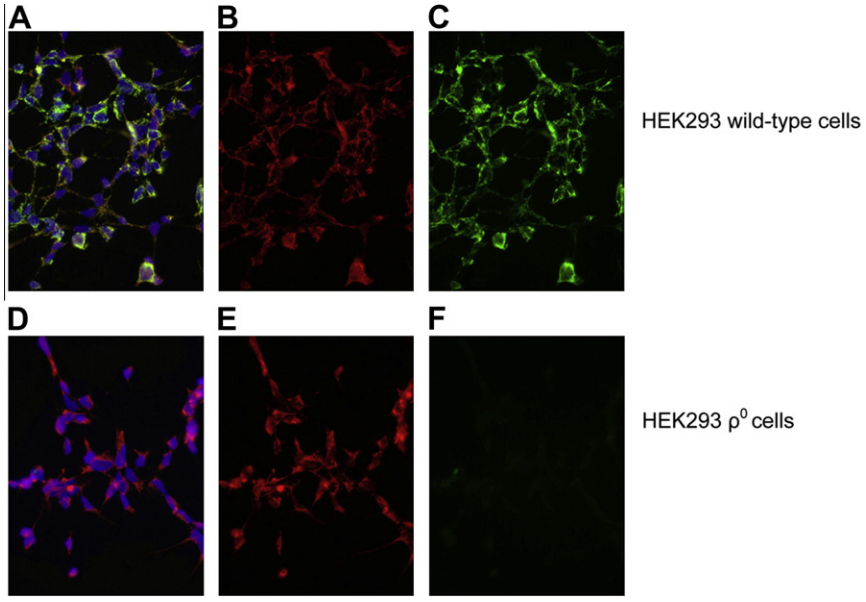

Fig. 1. Immunofluorescence staining of wild-type and ρ^0 HEK293 cells. (A, B and C) HEK293 wild-type cells. (D, E and F) HEK293 ρ^0 cells. (A) and (D) represent an overlay of the nuclear stain DAPI in blue, an antibody against the mitochondrial membrane protein porin in red ((B) and (E)) and an antibody against the mitochondrial encoded complex IV subunit 1 in green ((C) and (F)). Images were taken at 40× magnification. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)