Biochemical and Biophysical Research Communications 469 (2016) 1090–1096

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Mitochondrial regulation of cell cycle progression through SLC25A43

Marike Gabrielson [a, 1], Edwin Reizer [a], Olle Stål [b, c], Elisabet Tina [d, *]

[a] School of Health and Medical Sciences, Faculty of Medicine and Health, Örebro University, SE 70182 Örebro, Sweden
[b] Department of Clinical and Experimental Medicine, Linköping University, SE 58185 Linköping, Sweden
[c] Department of Oncology, Linköping University, SE 58185 Linköping, Sweden
[d] Department of Clinical Research Laboratory, Faculty of Medicine and Health, Örebro University, SE 70182 Örebro, Sweden

A R T I C L E   I N F O

Article history:
Received 13 December 2015
Accepted 20 December 2015
Available online 23 December 2015

Keywords:
SLC25A43
Mitochondrial checkpoint
Proliferation
Cell cycle regulation
Ki-67

A B S T R A C T

An increasing body of evidence is pointing towards mitochondrial regulation of the cell cycle. In a previous study of HER2-positive tumours we could demonstrate a common loss in the gene encoding for the mitochondrial transporter SLC25A43 and also a significant relation between SLC25A43 protein expression and S-phase fraction. Here, we investigated the consequence of suppressed *SLC25A43* expression on cell cycle progression and proliferation in breast epithelial cells.

In the present study, we suppressed SLC25A43 using siRNA in immortalised non-cancerous breast epithelial MCF10A cells and HER2-positive breast cancer cells BT-474. Viability, apoptosis, cell proliferation rate, cell cycle phase distribution, and nuclear Ki-67 and p21, were assessed by flow cytometry. Cell cycle related gene expressions were analysed using real-time PCR.

We found that SLC25A43 knockdown in MCF10A cells significantly inhibited cell cycle progression during G_1-to-S transition, thus significantly reducing the proliferation rate and fraction of Ki-67 positive MCF10A cells. In contrast, suppressed SLC25A43 expression in BT-474 cells resulted in a significantly increased proliferation rate together with an enhanced G_1-to-S transition. This was reflected by an increased fraction of Ki-67 positive cells and reduced level of nuclear p21. In line with our previous results, we show a role for SLC25A43 as a regulator of cell cycle progression and proliferation through a putative mitochondrial checkpoint. These novel data further strengthen the connection between mitochondrial function and the cell cycle, both in non-malignant and in cancer cells.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Cell proliferation is an energy consuming process partly sustained by mitochondrial metabolism. The progression of cells through the cell growth-and-division cycle is stringently controlled by checkpoints and the coordination of cyclins and cyclin-dependent protein kinases (Cdks) [1]. Entry of the cell into the G_1-phase of the cell cycle has been associated with a burst of mitochondrial activity [2,3] and G_1-to-S transition increases mitochondrial oxygen consumption [4]. A blocked G_1-to-S transition induced by compromised cellular ATP levels [5] or by inhibited oxidative phosphorylation [3,6] support the presence of a mitochondrial checkpoint in late G_1-phase [5]. In concordance, progression through the G_1-phase has also been linked to mitochondrial function [4], cyclin E build-up and altered mitochondrial morphology [7]. However, it appears that further progression through the cell cycle is not supported by respiratory modes of energy generation [8,9]. Mitochondrial activity is inhibited by increased levels of cyclin D, which also inactivates the retinoblastoma protein and thereby allows entry the S-phase of the cell cycle [9]. Although new mechanisms of mitochondrial cell cycle regulation are being reviled, it remains in many parts elusive.

In a previous study on HER2-positive breast cancer, we demonstrated a common deletion in the gene encoding the solute carrier family 25, member 43 (SLC25A43) [10]. We also showed a significant relation between SLC25A43 protein expression and S-phase fraction in the breast tumours [10,11]. SLC25A43 is a member of SLC25 transporters localised in the mitochondrial inner membrane [12–14]. However, the biological function of SLC25A43 remains unknown, although the closest family

* Corresponding author.
E-mail address: elisabet.tina@regionorebrolan.se (E. Tina).
[1] Permanent address: Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, SE 17177 Stockholm, Sweden.

http://dx.doi.org/10.1016/j.bbrc.2015.12.088
0006-291X/© 2015 Elsevier Inc. All rights reserved.

members are transporters for coenzyme A and/or ADP [12,15]. Dysfunctional transport activity of SLC25 family members has been related to several diseases [16]. Alteration in SLC25A33 expression has for instance been shown to influence proliferation of breast cancer cells [17]. Cancer cells acquire altered mitochondrial function and reprogrammed energy metabolism to sustain uncontrolled cell proliferation [18]. This is enabled by genetic alterations and/or the inactivation of major regulators of the metabolic switch and the cell cycle [19]. Considering the limited knowledge about SLC25A43, the present study was undertaken to study the consequence of suppressed *SLC25A43* expression on cell cycle progression and proliferation in breast epithelial cells.

2. Material and methods

2.1. Cell lines and culture

The immortalised non-cancerous breast epithelial cell line MCF10A and the HER2-positive breast cancer cell line BT-474 were obtained from American Type Culture Collection (Manassas, VA, USA). MCF10A was cultured in D-MEM/F-12 supplemented with 10% FBS, 10 μg/mL insulin, 20 ng/mL H-EGF and 0.5 μg/mL hydrocortisone. BT-474 was cultured in RPMI-1640 supplemented with 10% FBS and 10 μg/mL insulin. Cells were cultured in a humidified atmosphere at 37 °C with 5% CO_2.

2.2. RNA interference

Four different siRNAs (Hs_LOC203427_2; Hs_LOC203427_3; Hs_LOC203427_4 and Hs_LOC203427_5) were tested over time before selecting the most effective and stable siRNA targeting *SLC25A43*. Cells were then transfected as previously described [11] using *SLC25A43*-specific siRNA (Hs_LOC203427_2), referred to in this study as siSLC, or scrambled siRNA referred to as siCtrl (Qiagen Sciences, Maryland, USA). siRNA transfections were carried out and scaled using Lipofectamine™2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. Cells were seeded at a concentration of 25 x 10^3 cells/cm^2 and transfected 24 h after seeding.

2.3. Gene expression

Total RNA was prepared using RNeasy Plus Micro Kit (Qiagen Sciences) according to manufacturer's description. cDNA was synthesised from equal amounts (0.1 μg) of total RNA using the High-Capacity cDNA Reverse-Transcription kit (Applied Biosystems, Stockholm, Sweden). Relative quantification was conducted using 7500 Fast Real-time PCR System and TaqMan® gene expression assay primers and probes (Applied Biosystems) for *SLC25A43* and cell cycle related genes (*CCNE1*, *CCNG2*, *CUL3*, *E2F4*, *HERC5*, *RBL1*). Fold expression change was determined by the formula $2^{-\Delta\Delta CT}$, using *ACTB* (Applied Biosystems) or *ABL1* as reference genes. Accession numbers for all TaqMan® Gene Expression primers used can be found in Table 1.

2.4. Cell cycle PCR array

To explore possible alterations in gene expression connected to cell cycle regulation the Cell Cycle RT^2 *Profiler*™ PCR array version 3.0 (SABiosciences, Qiagen Sciences) was used, according to manufactures description, using 7500 Fast RT-PCR System. The time points 24 and 48 h after transfection were explored and data analysis was performed on exported CT values using the free software RT2 Profiler™ PCR array Data Analysis Webportal (SABiosciences, Qiagen Sciences) and the $\Delta\Delta^{CT}$ method with normalisation of the raw data to housekeeping genes.

2.5. Flow cytometry assays

2.5.1. Viability and apoptosis assay

Both viability and apoptosis were measured as previously described [11]. In separate experiments, viability was determined with 0.25 μg 7-AAD (BD Biosciences, San Jose CA, USA), and apoptosis/necrosis was verified with APOTEST™-FITC (Dako, Glostrup, Denmark). Both assays were performed according to manufacturer's protocols.

2.5.2. Cell proliferation assay

Measurement of cell proliferation was performed as previously described using the green fluorochrome PKH67 Green Fluorescent Cell Linker (Sigma Aldrich, St Louis MO, USA) [11]. After cell division fluorescence intensity is decreased due to dilution of the fluorochrome. The cells were stained with PKH67 at time of seeding and analysed by flow cytometry after transfection.

2.5.3. Cell cycle phase analysis

Analysis of cell cycle phase distribution was performed as previously described [11,20] on isolated cell nuclei using 100 μg/mL propidium iodide (PI) (Sigma Aldrich) for DNA-staining.

2.5.4. Cell cycle regulation assay with Ki-67 and p21

The expression of Ki-67 and p21 was analysed on isolated nuclei as previously described [11]. In brief, pelleted cells were resuspended for 10 min with an ice cold lysing solution containing 0.1% Igepal CA-630 in wash buffer (1% FBS in PBS) to isolate cell nuclei. The nuclei were then washed once with ice cold wash buffer before adding antibodies against p21 Alexa Fluor® 488 (1:50, clone 12D1

Table 1
Descriptions of all genes analysed by Fast Real time PCR and the primers used.

Gene name	Function	TaqMan® gene expression assay
ABL1	Reference gene	Hs01104728_m1
ACTB	Reference gene	Hs99999903_m1
CCNE1	Cyclin E1 (G_1-to-S phase transition)	Hs01026536_m1
CCNG2	Cyclin G2 (G_0 phase)	Hs00171119_m1
CUL3	Ubiquinates Cyclin E1	Hs00180183_m1
E2F4	Transcription factor (regulates G_1-to-S phase transition)	Hs00608098_m1
HERC5	Ubiquinates Cyclin E1	Hs00180943_m1
RBL1	Block G_1-to-S phase transition via E2F	Hs00765700_m1
SLC25A43	Mitochondrial transporter	Hs00933775_m1

Abbreviations: ABL1, Abelson tyrosine-protein kinase 1 (p150); ACTB, actin beta; CCNE1, Cyclin E1; CCNG2, Cyclin G2; CUL3, cullin 3; DSB, double strand break; E2F4, transcription factor 4, p107/p130-binding; HERC5, HECT and RLD domain containing E3 ubiquitin protein ligase 5; RBL1, retinoblastoma-like 1 (p107); SLC25A43, solute carrier family 25, member 43.

Download English Version:

https://daneshyari.com/en/article/10749585

Download Persian Version:

https://daneshyari.com/article/10749585

Daneshyari.com