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Distinct nuclear gene expression profiles in cells with mtDNA depletion and homoplasmic A3243G mutation

Roshan S. Jahangir Tafrechi [a], Peter J. Svensson [b,c], George M.C. Janssen [a], Karoly Szuhai [a], J. Antonie Maassen [a], Anton K. Raap [a,*]

[a] *Department of Molecular Cell Biology, Leiden University Medical Center, P.O. Box 9503, 2300 RA Leiden, The Netherlands*
[b] *Department of Toxicogenetics, Leiden University Medical Center, P.O. Box 9503, 2300 RA Leiden, The Netherlands*
[c] *Department of Oncology, Radiology and Clinical Immunology, University Hospital, 75185 Uppsala, Sweden*

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Abstract

The pathobiochemical pathways determining the wide variability in phenotypic expression of mitochondrial DNA (mtDNA) mutations are not well understood. Most pathogenic mtDNA mutations induce a general defect in mitochondrial respiration and thereby ATP synthesis. Yet phenotypic expression of the different mtDNA mutations shows large variations that are difficult to reconcile with ATP depletion as sole pathogenic factor, implying that additional mechanisms contribute to the phenotype. Here, we use DNA microarrays to identify changes in nuclear gene expression resulting from the presence of the A3243G diabetogenic mutation and from a depletion of mtDNA (ρ^0 cells). We find that cells respond mildly to these mitochondrial states with both general and specific changes in nuclear gene expression. This observation indicates that cells can sense the status of mtDNA. A number of genes show divergence in expression in ρ^0 cells compared to cells with the A3243G mutation, such as genes involved in oxidative phosphorylation. As a common response in A3243G and ρ^0 cells, mRNA levels for extracellular matrix genes are up-regulated, while the mRNA levels of genes involved in ubiquitin-mediated protein degradation and in ribosomal protein synthesis is down-regulated. This reduced expression is reflected at the level of cytosolic protein synthesis in both A3243G and ρ^0 cells.

Our finding that mitochondrial dysfunction caused by different mutations affects nuclear gene expression in partially distinct ways suggests that multiple pathways link mitochondrial function to nuclear gene expression and contribute to the development of the different phenotypes in mitochondrial disease.

Keywords: Mitochondria; mtDNA; Expression; Diabetes

Abbreviations: ES, enrichment score; GO, gene ontology; GSEA, gene set enrichment analysis; MIDD, maternally inherited diabetes and deafness; mtDNA, mitochondrial DNA; OXPHOS, oxidative phosporylation; PLA, probe level analysis

* Corresponding author. Tel.: +31 71 5276187; fax: +31 71 5276180.
E-mail address: A.K.Raap@lumc.nl (A.K. Raap).

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1. Introduction

Mitochondrial dysfunction caused by mutations in the mitochondrial genome is related to a variety of diseases such as type two diabetes mellitus (DM2), cancer and neuro-muscular diseases [1]. Mitochondria are involved in multiple cellular processes of which ATP production by oxidative phosphorylation (OXPHOS) is the most prominent one. However, mitochondria also accommodate other processes, such as the tricarboxylic acid cycle and fatty acid oxidation. In addition, mitochondrial function is linked to calcium, iron and ROS signalling and apoptotic pathways. The variation in clinical phenotype of mitochondrial diseases is difficult to explain by merely a reduced respiration rate [2]. Rather the consequences of additional mitochondrial dysfunction on retrograde signalling pathways may determine the distinct nature of the clinical manifestation [3]. A genome-wide differential gene transcription profile of normal cells and cells with dysfunctioning mitochondria is expected to give insight in the pathobiochemical pathways affected in mitochondrial disease [4,5].

In order to investigate how mitochondrial mutations affect the nuclear gene expression profile we created 143B cybrid cells with mitochondrial DNA being the only variable [6]. The first state of respiratory dysfunction is induced by an A–G conversion at location 3243 in the $tRNA^{leu}$ gene of the mitochondrial DNA. This mutation causes maternally inherited diabetes and deafness (MIDD) [7] in most carriers and associates also with the neuromuscular MELAS syndrome [8]. Another state of mitochondrial dysfunction is induced by a depletion of mtDNA (ρ^0 cells). Using cybrid cells with 100% wild-type mitochondrial DNA, cybrid cells with 100% A3243G mutant mitochondrial DNA of the same haplotype and ρ^0 cells, we found both common mitochondrial-defect and MIDD-specific responses in nuclear gene expression.

2. Materials and methods

2.1. Cell culture, cell characteristics and GeneChip hybridisation

The cybrid cells used in this report have been previously described [2,9]. In short, 143B osteosarcoma cells were treated with ethidium bromide to create ρ^0 cells devoid of mitochondrial DNA. Next, ρ^0 cells were fused with enucleated cells from a MIDD patient, generating clones with different but stable heteroplasmy levels for the 3243 mutation. Two apparently homoplasmic mutants (VM48 and VM50) and two apparently homoplasmic wild-type (VW6 and VW7) cybrid clones were selected and used as biological replicates in this study. The cells were grown on Dulbecco's modified eagle's medium containing 4.5 mg/ml glucose and 110 μg/ml pyruvate (DMEM) supplemented with 50 μg/ml uridine and 10% fetal bovine serum. Heteroplasmy levels were monitored by use of PCR-RFLP and ApaI, which cleaves the mutated PCR product.

The oxygen consumption of the cells was measured as described previously [6]. Mitochondrial copy numbers were determined by comparing the amount of mitochondrial DNA with the amount of β-globin DNA, in a SybrGreen real time PCR reaction with the primers described in Szuhai et al. [10] and using the ABi Prism 7700 spectrofluormetric thermal cycler (Applied Biosystems, USA). The same system was used for validation of the mRNA concentration data obtained by chip hybridisation analysis. Primer sets for 10 different genes were used, including 3 that were found to be differentially and consistently expressed: NADH dehydrogenase 1β8 (5′-ACGAACCTTACCCGGATGATG + 5′-CATGGATCTCTCTCATGCTGTGAG), ubiquitin-conjugating enzyme E2D3 (5′-ATCACAGTGGTCGCCTGCTT + 5′-ATAGATCCGTGCAATCTCTGGC) and collagen VIα2 (5′-CATCGATGACATGGAGGACGT + 5′-CAGCTCTGTTTGGCAGGGAA). The primers were all manufactured by Eurogentec, Belgium.

Cytoplasmic protein synthesis rate was estimated from the incorporation of L-[4,5-^{3}H]-leucine into trichloroacetic acid precipitable material essentially as described [2]. In brief, series of cells at different densities in 6-well plates were washed with phosphate-buffered saline and incubated for 60 min at 37 °C in 0.75 ml of leucine-free complete medium containing 10 μCi of L-[4,5-^{3}H]-leucine and 10 μM unlabeled leucine. Cells were then again washed three times with phosphate-buffered saline and thoroughly dissolved in 1 ml 0.2 M NaOH. One hundred microlitres aliquots were precipitated by the addition of 100 μl 20% trichloroacetic acid, and assayed for total protein