EXTENDED ABSTRACT

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Does polyribonucleotide nucleotidyltransferase (PNPase) mutation affect the energetic metabolism and the microRNA profile in cell and mitochondria?

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Abstract

We hypothesized that the PNPase (gene PNPT1), a protein of the intermembrane space involved in RNA mitochondrial import, could play a role in miRNA import. PNPT1 mutations have been described in patients with a severe neurological disease and combined respiratory chain defect. The objective was to compare the miRNA and metabolomic profile from control and PNPT1 mutant fibroblasts. Fibroblasts from a patient with PNPT1 mutation (c.1160A>G, p.Gln387Arg) and from a control (C) were grown. Comparison of the metabolomic NMR spectra of the culture media samples indicated that PNPT1 cells had a lower glycolysis, TCA and protein synthesis activity than C cells. The number of miRNA detected in PNPT1 cells was 6 times lower than in C cells (47 vs 310 miRNAs) but their average level of expression is higher ($p<10^{-6}$). Their distribution among mitochondria, mitoplasts and cells is highly significantly different ($p<10^{-6}$): in C cells, there was a gradient between mitoplasts (153) > mitochondria (94); in PNPT1 cells, there is no gradient (130 vs 126). These preliminary results showed a high deficiency in the number of miRNAs detected in PNPT1 and the absence of miRNA gradient between the mitoplasts and mitochondria.

Introduction

Numerous microRNAs (miRNA) encoded by the nuclear genome have been identified in isolated mitochondria from different types of cells and species (Barrey et al., 2011). For examples, the miR-181c and miR-1 altered the transcriptions of COX1 and ND1, respectively (Das et al., 2014; Zhang et al., 2014). However, the import and RNA interference activity of miRNA in the mitochondria remain unknown.

Polynucleotide phosphorylase (PNPase) is a protein of the intermembrane space, encoded by the gene polyribonucleotide nucleotidyltransferase (PNPT1) and involved in RNA mitochondrial import (Wang et al., 2010). PNPT1 mutations have been described in patients suffering from a severe neurological disease and combined respiratory chain defect (Vedrenne et al., 2012).

Objective: We hypothesized that PNPase could play a role in miRNA import (Wang et al. 2010). The objective was to compare the miRNA and metabolomic profiles from control and PNPT1 mutant fibroblasts.

Materials & Methods

Cell culture and mitochondria isolation

Fibroblasts from a patient with PNPT1 mutation (c.1160A>G, p..Gln387Arg) and

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from a control (C) were grown in a classical medium enriched with uridine. Total RNA was extracted either from whole cells or after mitochondria isolation or finally from mitoplasts obtained by digitonine treatment.

MicroRNA profiling by RT-qPCR

The total RNA concentrations of the 6 samples were comprised between 7.52 to 12.42 ng/µL. Quanlity control tests were performed using 5 ubiquitous miRNA assays + 1 spike-in. No reverse transcription inhibitor was observed and significant miRNA expressions were recorded for the spike-in and 4 miRNAs (miR-451, 23a, 30c, 103a, not miR-142). The miRNA profile was performed by RT-qPCR multiplex analysis of 742 human miRNAs and the raw data were normalized by the average of 9 stable miRNAs identified by Normfinder among the miRNAs detected in all the samples.

Metabolomic analysis by nuclear magnetic resonance (NMR)

Proton NMR spectra were acquired at 500 MHz using a NOESY1D sequence. After data processing (phase and baseline correction, calibration), the spectra were divided into 0.001 ppm bins and normalized by the probalistic quotient method taking into account the cell number to generate the matrix for statistical analysis.

The multivariate analysis was the orthogonal projection on latent structure (OPLS) which provides a prediction axis (horizontal axis in the score plot) for discrimination of samples into two groups (controls and PPT1). The loading plot build with the covariance (y axis) and the spectral bins (x axis) is colored according to the correlation between the bin intensities and the model discrimination. Therefore, the spectral regions with a positive covariance and a high correlation (red) are increased in PPT1 culture medium and negative covariance is found for region decreased in PNPT1 medium.

Statistics

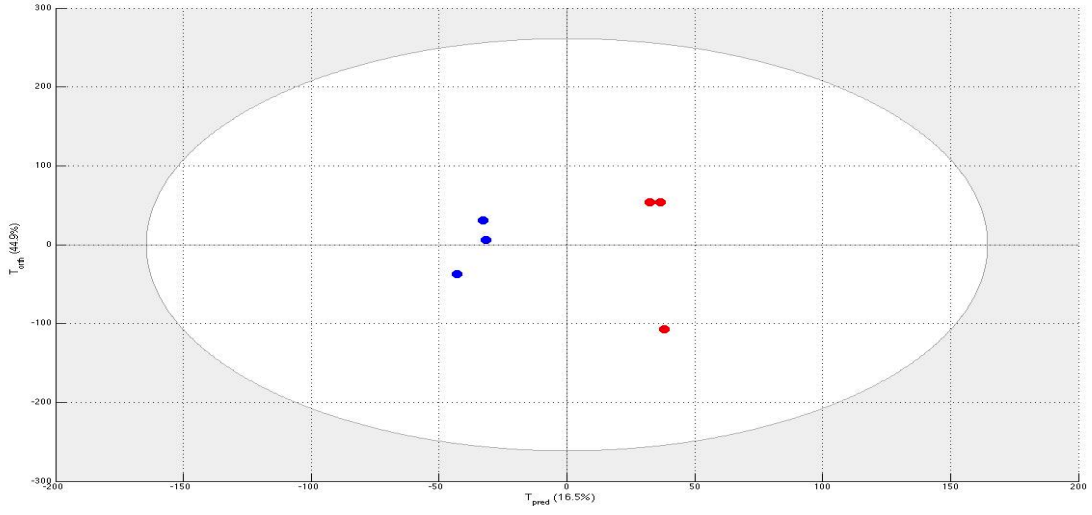
The counting of the unique miRNA detected in each type of cell and their respective mitochondria and mitoplasts were testes by a Chi^2 test. Analysis of variance and mean LSD test (GLM, NCSS) were performed to compare the miRNA expression level.

Results

Defect of the metabolomic profile of the PNPT1 fibroblasts

The multivariate analysis (Orthogonal projection to latent structure) could correctly discriminate (Q2=0.517) the two types of fibroblasts by taking into account all the metabolomic information included in the spectra (Figure 1).

Figure 1: Orthogonal projection to latent structure (OPLS) score-plot showing an acceptable discrimination (Q2=0.517; R2Y = 0.987) of the 6 samples of culture medium according to their metabolomic profile (3 culture medium samples of PNPT1 fibroblasts in red , and 3 samples of control fibroblasts in blue).



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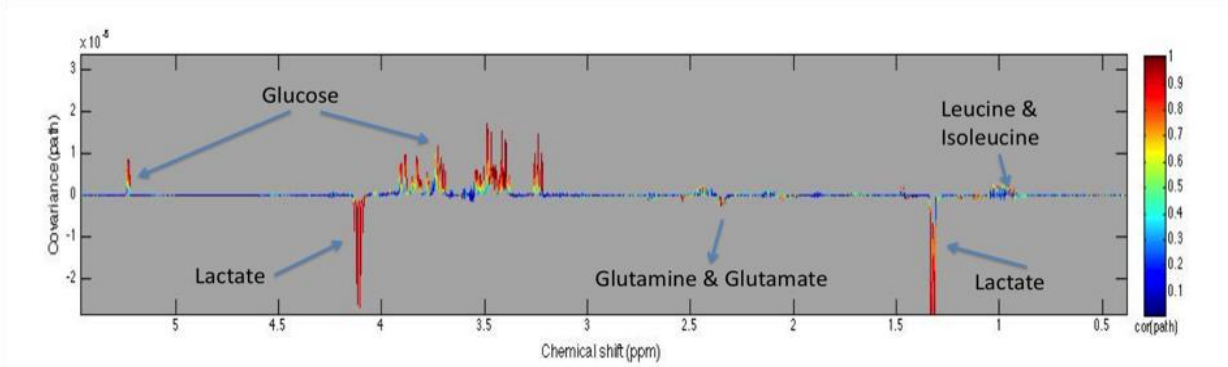

Figure 2: Loading plots showing the metabolomic profile discriminating the culture media of the PNPT1 and control fibroblasts (2 x 3 samples). Positive values indicated higher concentration in the culture medium of PNPT1. The color is related to the level of correlation between the metabolite levels and the group separation.

The comparison of the metabolomic profile of the two samples of culture medium showed that PNPT1 fibroblasts consumed (Figure 2):

- less glucose and produced less lactate in the culture medium;
- less glutamate and/or glutamine;
- less amino acids such as alanine, leucine and isoleucine .

Taken altogether these results suggested a lower glycolysis, Krebs cycle (TCA) and protein synthesis activity in PNPT1 than in control fibroblasts. This metabolomic profile is consistent with the respiratory chain defect known to be associated with PNPT1 mutation.

Comparison of the miRNA profiles between PNPT1 and control fibroblasts

The number of unique miRNAs detected either in PNPT1 or control fibroblasts, and in their respective mitochondria and mitoplasts is highly significantly different (Chi²-test $p<10^{-6}$; Figure 3). The number of miRNAs detected in PNPT1 fibroblasts was about 6 times lower than in control fibroblasts (47 vs 310 miRNAs). Ten miRNAs were only detected in PNPT1 and 272 only in control fibroblasts.

MiRNAs profiles in the mitochondria and mitoplasts

In the control fibroblasts, there was a gradient of miRNAs between mitoplasts (153) and mitochondria (94) (Figure 3) and their average expression was the opposite (Figure 4). In PNPT1 cells, there is no difference in the number or expression level between mitochondria and mitoplasts (Figures 3 & 4).

The average expression (normalized Cq) is higher in PNPT1 than in control fibroblasts (ddCq= 5.1; $p<10^{-6}$) (Figure 4). The miR-424-5p was more expressed in PNPT1 than in control fibroblasts (Fold change (FC)=2.4; $p<0.01$). Conversely, the miR-22-3p was more expressed in control than in PNPT1 fibroblasts (FC=-2.8; $p<0.05$). Two miRNAs were more expressed in the mitoplasts than the mitochondria fraction: miR-145-5p (FC=4.09), let-7b-5p (FC=2.29). Conversely, 5 miRNAs were more expressed in the mitochondria than in mitoplasts: miR-26a-5p, 361-5p, 29b-3p,193b-3p, 221-3p with FC=-2.06 to -4.43). Thus, they stayed out of the inner membrane and were poorly imported in the matrix in both types of fibroblasts.

Conclusion

The PNPT1 fibroblasts showed a deficient energetic metabolism according to NMR metabolomic profile. The miRNA profile showed a significant lower number of miRNAs detected in PNPT1 deficient fibroblasts than in control fibroblasts. In the control cells, a higher number of unique miRNAs were detected in the mitoplasts than in whole mitochondria which may suggest an active import by PNPase. In the PNPT1 fibroblasts, there is no gradient between mitochondria and mitoplasts and less miRNAs detected.

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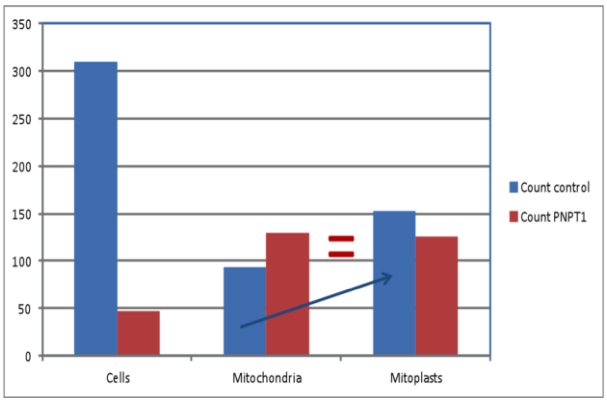

Figure 3: Counts of the unique miRNAs detected in the two types of fibroblast and their respective mitochondria and mitoplasts (Chi²-test $p<10^{-6}$).

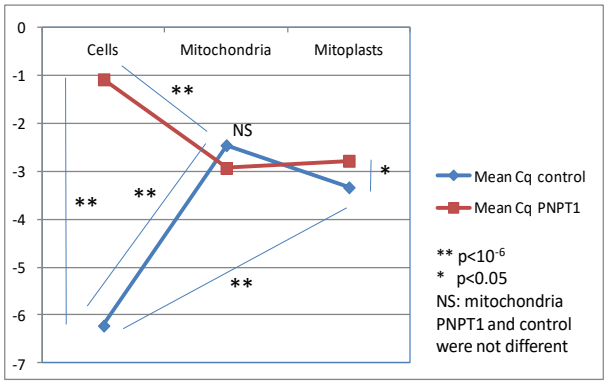

Figure 4: Average expression (normalized dCq) of the total miRNAs detected in the two types of fibroblasts and their respective mitochondria and mitoplasts.

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