Effects of Genetic Manipulation of FASTKD1 and FASTKD4 on Mitochondrial Protein Expression and Function

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BACKGROUND

- Mitochondria play an important role in cell homeostasis and dysfunction of mitochondria can cause several diseases in multiple organs.
- Mitochondria have their own unique genome (mtDNA), which encodes for 13 mRNAs and their proteins, all involved in the electron transport chain (ETC) that is responsible for ATP production (**Figure 1**).

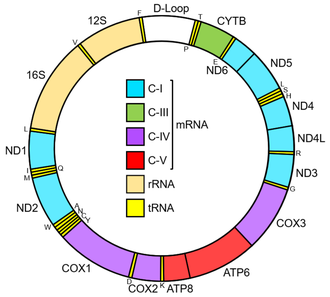

Figure 1. The mitochondrial genome and its genes. Schematic depicting the structure and composition of mtDNA. The 13 mRNA/protein-encoding genes are all subunits in Complexes-I, -III, -IV, and –V of the ETC.

- Consequently, regulation of this genome can play a huge part in how the organelle functions. However, attention has primarily focused on mtDNA and less is known about mtRNA and its regulation.

FASTKD PROTEINS

- Recently, a family of proteins called FASTKDs has been proposed to regulate mitochondrial mRNAs and therefore ETC protein expression (**Figure 2**).

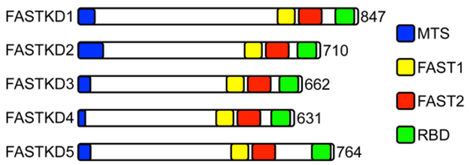

Figure 2. The FASTKD protein family. The 5 FASTKD isoforms and their domains. MTS, mitochondrial targeting sequence; FAST, fast homology domain; RBD, RNA-binding domain.

- However, the specific function of each of the FASTKD isoforms remains mostly unknown.

HYPOTHESIS

- We hypothesize that FASTKD1 and FASTKD4 regulate the expression of mtRNA-encoded ETC proteins, ATP levels, and mitochondrial potential.

OBJECTIVES

- Overexpress or deplete FASTKD1 and FASTKD4 in cultured mouse fibroblasts using adenoviruses or siRNAs, respectively.
- Measure ETC protein expression, ATP levels, and mitochondrial potential.

METHODS

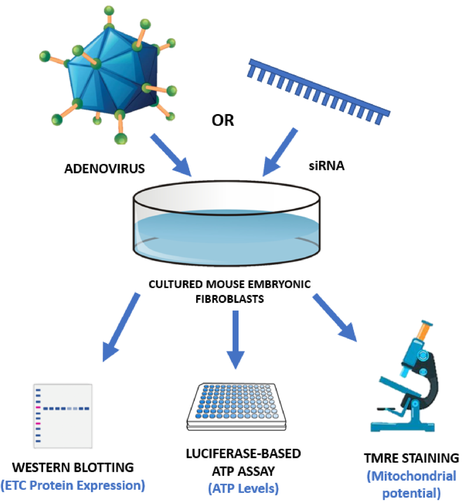

Figure 3. Schematic of methods. Mouse embryonic fibroblasts were cultured and then infected with adenovirus or transfected with siRNA for 48hrs to either over-express or deplete, respectively, FASTKD1 or FASTKD4. A β-galactosidase adenovirus or non-targeting siRNA were used as controls. Western blotting, luciferase-based ATP assays, and TMRE staining were used to measure ETC protein levels, ATP levels, and mitochondrial potential, respectively.

Localization of The Exogenous FASTKDs

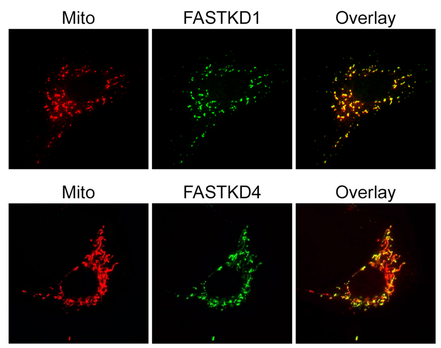

Figure 4. Mitochondrial localization of exogenous FASTKD1 and FASTKD4. We wanted to confirm that the overexpressed FASTKD isoforms still correctly localized to the mitochondria. Mouse embryonic fibroblasts were infected with adenoviruses encoding either Flag-tagged FASTKD1 (upper panels) or FASTKD4 (lower panels) for 48hrs and then stained for mitochondria (red) and Flag (green). The overlay indicated that both proteins did indeed localize correctly.

Effects of FASTKD1 and FASTKD4 Depletion on ETC Proteins

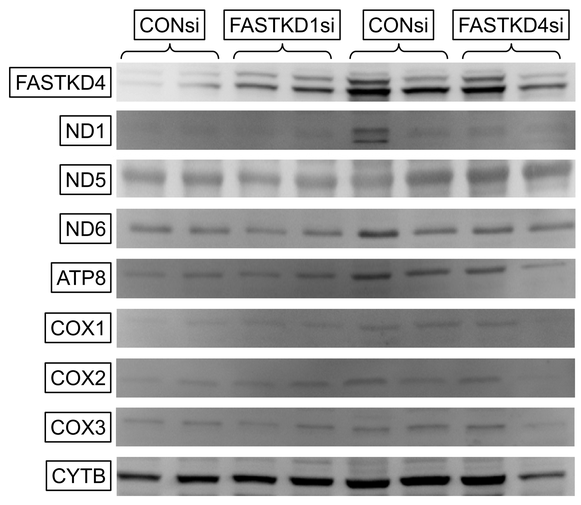

Figure 5. Effects of FASTKD1 and FASTKD4 depletion on ETC protein levels. Mouse embryonic fibroblasts were transfected with control, FASTKD1, or FASTKD4 siRNAs for 48hrs. Cells were then harvested, lysed, and blotted for FASTKD4, ND1, ND5, ND6, ATP8, COX1, COX2, COX3, and CYTB (n=2/group).

Effects of FASTKD4 Overexpression on ETC Proteins

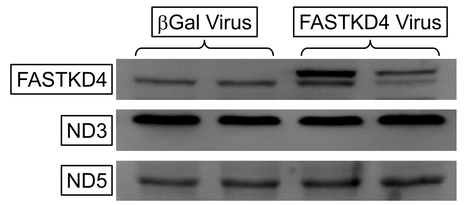

Figure 6. Effects of FASTKD4 overexpression on ETC protein levels. Mouse embryonic fibroblasts were infected with adenoviruses encoding β-galactosidase (βGal) or FASTKD4 for 48hrs. Cells were then harvested, lysed, and blotted for FASTKD4, ND3, and ND5 (n=2/group).

Effects of FASTKD Manipulation on ATP

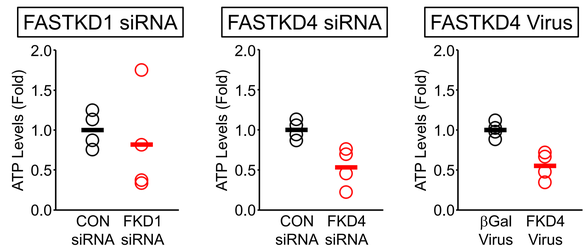

Figure 7. Effects of FASTKD manipulation on ATP levels. Mouse embryonic fibroblasts were transfected with control, FASTKD1, or FASTKD4 siRNAs or infected with adenoviruses encoding β-galactosidase (βGal) or FASTKD4 for 48hrs. Cells were then harvested, lysed, and ATP measured (n=4/group).

Effects of *Fastkd1* Deletion on ETC Proteins and ATP in Mouse Hearts

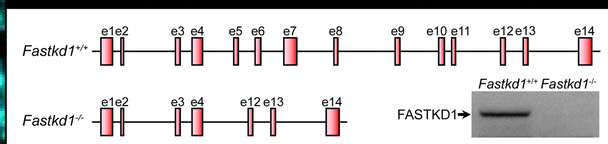

Figure 8. *Fastkd1* gene-targeting. Targeting strategy for CRISPR/Cas9-mediated deletion of exons 5-11 of the *Fastkd1* gene in mice. Western blot for FASTKD1 in cardiac lysates from *Fastkd1*$^{+/+}$ and *Fastkd1*$^{-/-}$ mice showing loss of the protein.

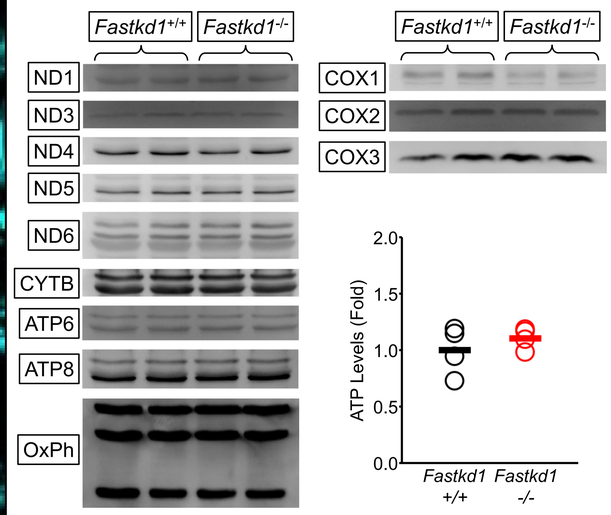

Figure 9. Effects of *Fastkd1* deletion on cardiac ETC protein and ATP levels. Hearts from 3mth-old *Fastkd1*$^{+/+}$ and *Fastkd1*$^{-/-}$ mice were homogenized and subjected to blotting for ND1, ND3, ND4, ND5, ND6, CYTB, ATP6, ATP8, OxPhos, COX1, COX2, and COX3, (n=2/group) as well as ATP measurements (n=4/group).

SUMMARY & FUTURE DIRECTIONS

- Preliminary results did not show overt changes in most mtDNA-derived ETC proteins, although COX1 and COX3 may be altered in the *Fastkd1*$^{-/-}$ mice.
- ATP levels decreased with both depletion and overexpression of FASTKD4 in the cultured cells.
- We will increase our replicates to quantify ETC protein changes and implement the TMRE staining to measure mitochondrial potential.
- In the future, we will also expand our ATP analyses to measure the *rate* of ATP synthesis.

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