Mitochondrial Dysfunction Associated with TANGO2 Deficiency

Paige Heiman1, Al-Walid Mohsen1,2, Erik Koppes1, Anuradha Karunanidhi2, Jerry Vockley1,2, Lina Ghaloul-Gonzalez1,2
1Division of Genetic and Genomic Medicine, Department of Pediatrics, University of Pittsburgh, Pittsburgh, PA, USA,
2Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA.

BACKGROUND

• TANGO2-related disease is an autosomal recessive disorder caused by mutations in the TANGO2 gene.
• Function of the TANGO2-protein is not known; previous studies suggest that it is involved in redistributing Golgi membranes into the endoplasmic reticulum (ER) [1].
• Symptoms manifest in early childhood and are characterized by developmental delay, stress-induced rhabdomyolysis, cardiac arrhythmias, and severe metabolic crises.
• Clinical symptoms and metabolic abnormalities are consistent with a broader dysfunction of mitochondrial energy metabolism.
• Previous localization studies suggest that TANGO2 is localized to the cytosol and Golgi membranes [1,4]. Mitochondrial localization studies have been inconclusive, but the protein products of the mouse ortholog of TANGO2, T10, are mitochondrially localized [2,3].
• We sought to characterize mitochondrial function in patients with TANGO2-related disorders to explore its role in the pathophysiology of the disease and resolve the contradictions present in the current literature.

METHODS

Studies were performed on TANGO2-patient-derived fibroblasts from three patients and a control. Studies included western blotting of cell lysates and/or mitochondrial extracts for TANGO2 protein and mitochondrial proteins involved in β-oxidation and mitochondrial fission and fusion, including very long chain acyl-coA dehydrogenase (VLCAD) and medium chain acyl-coA dehydrogenase (MCAD). Mitochondrial dysfunction was measured by superoxide production after staining with MitoSox Red; oxygen consumption rate (OCR) via a Seahorse Bioanalyzer; ATP content via a bioluminescence assay; and immunofluorescent staining (IF) for mitochondrial proteins including TOMM20. Additionally, flux through the β-oxidation was quantified by tritium release from [3H]oleic acids. mRNA expression of mitochondrial proteins was assessed via qT-PCR and measurement of mitochondrial DNA (mtDNA) copy number was achieved with droplet-digital PCR.

RESULTS

• TANGO2 protein was present in mitochondrial extracts of control cells but not TANGO2 patient cells.
• Superoxide production was increased in patient cells.
• OCR, particularly under stress, relative ATP levels and β-oxidation of oleate was reduced.
• Studies of mitochondrial dynamics in these patients show increased mitochondrial fission and mitochondrial number, smaller mitochondrial size, and increased mitochondrial DNA copy number.
• Reduced mRNA and protein expression of numerous mitochondrial proteins was also identified.

A

Whole Cell

B

Mitochondrial Extract

Fig.1: (A) TANGO2 protein expression in whole cell lysate. (B) TANGO2 protein expression in mitochondrial extracts from patient and control fibroblasts.

A

VLCAD

MCAD

B

VLCAD

MCAD

GAPDH

Fig.2: mRNA and protein expression of various mitochondrial proteins. (A) Relative mRNA expression of VLCAD and MCAD. (B) Western blotting of VLCAD and MCAD on whole cell lysates.
RESULTS

Fig. 3. Mitochondrial functional studies in control and patients’ fibroblasts. (A) OCR measured in cells grown with and without glucose (B) Superoxide production with and without glucose measured with MitoSOX Red and normalized to mitochondrial mass (Mitotracker green). (C) ATP production using ATPlite bioluminescence assay. (D) Tritium release flux assay using oleic acids.

Fig. 4: (A) IF of fibroblasts stained with TOMM20 marker of mitochondrial content and analyzed for mitochondrial volume and number using Nikon NIS Elements software. (B) Droplet digital PCR analyzing mtDNA copy number normalized to nuclear DNA copy number. (C) Western blotting of mitochondrial fission and fusion proteins performed on whole cell lysates.
CONCLUSIONS

- Patient cells with TANGO2-related disease exhibit both physical and functional mitochondrial energy dysfunction in multiple pathways.
- Western blotting of mitochondrial extract lysates show that TANGO2 protein is at least partially localized to mitochondria. The intensity of the mitochondrial TANGO2 band is less than that of the whole cell lysate TANGO2 band, suggesting that TANGO2 may also be in other cellular compartments, and that its absence in patients may contribute to symptoms.
- Patient cells contain a higher number of mitochondria that are smaller in size, and each mitochondrion contains fewer copies of the mtDNA compared to control cells, indicative of mitochondria that are more fragmented, undergoing more fission, and replicating mitochondrial DNA at a slower rate.
- Mitochondrial function should be assessed and monitored in all patients with TANGO2 mutation as targeted treatment of the energy dysfunction could improve outcome in this condition.

REFERENCES


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