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Energy Metabolism and Mitochondrial Defects in Patient iPSC-derived Motor Neurons With the CMTX6-causative PDK3 Mutation.

Poster No

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Introduction:

The p.R158H missense mutation in the pyruvate dehydrogenase kinase 3 gene (PDK3) causes an X-linked form of CMT (CMTX6). Phosphorylation of the pyruvate dehydrogenase complex (PDC) by the PDK isoenzymes determines the extent of oxidative decarboxylation of pyruvate to generate acetyl CoA, linking glycolysis and the energy producing Krebs cycle.

Methods:

To model CMTX6 in vitro, we generated a line of induced pluripotent stem cells (iPSCs) by re-programming CMTX6 patient fibroblasts (iPSCCMTX6). We also engineered an isogenic control by correcting iPSCCMTX6 using the CRISPR/Cas9 system (iPSCisogenic). The iPSCCMTX6 and iPSCisogenic lines were differentiated into spinal cord motor neurons.

Results:

We have generated an in vitro human neuronal model for CMTX6 using iPSC-derived motor neurons from a recently identified CMTX6 patient carrying the p.R158H PDK3 mutation. As previously described in CMTX6 fibroblasts, patient-derived motor neurons (MNCMTX6) showed increased phosphorylation of the E1 subunit of PDC in the mitochondria of the diseased axons. Treatment of MNCMTX6 with a pan

PDK inhibitor, dichloroacetic acid (DCA), reversed E1 hyperphosphorylation to levels found in the MNisogenic control motor neurons. In this study we have analysed distribution of axonal mitochondria and shown that a statistically significant proportion of the mitochondria in MNCMTX6 were 1.5-fold smaller when compared to the MNisogenic cells. Functionally, the Seahorse Extracellular Flux Analyzer demonstrated that the p.R158H PDK3 mutation affects the ATP-linked respiration of MNCMTX6, indicating a general mitochondrial dysfunction in the patient-derived motor neurons.

Conclusions:

We have established a CMTX6 iPSC motor neuron cell line. The MNCMTX6 and MNisogenic motor neurons provide an excellent neuronal cell system for compound screening approaches using the PDC hyperphosphorylation phenotypic signature to identify effective drugs for the treatment of CMTX6 neuropathy.

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Reference 1:

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Perez-Siles, G., et al., Pathogenic mechanisms underlying X-linked Charcot-Marie-Tooth neuropathy (CMTX6) in patients with a pyruvate dehydrogenase kinase 3 mutation. *Neurobiol Dis*, 2016. 94: p. 237-44.

Keywords: Charcot-Marie-Tooth neuropathy, Induced pluripotent stem cells, Mitochondria, Disease modelling, Drug screening

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