P3.36

Investigating the molecular and cellular basis of epsilon-sarcoglycan-related myoclonus-dystonia in an iPSC-derived neuronal model

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Abstract

Introduction: *SGCE*-related myoclonus-dystonia (M-D) underlies the epigenetic process of imprinting. This leads to the phenomenon of reduced penetrance upon maternal transmission of a pathogenic variant. We previously demonstrated that induced pluripotent stem cell (iPSC)-derived cortical neurons of carriers of pathogenic *SGCE* variants can serve as an adequate disease model for *SGCE*-related M-D[1], enabling the investigation of functional properties, such as the cellular localization of epsilon-sarcoglycan (encoded by *SGCE*). Interestingly, epsilon-sarcoglycan has been linked to the dystrophin-associated glycoprotein complex (DGC) which is located at the plasma membrane and varies in its composition.

Materials and Methods: iPSC lines of two M-D patients with pathogenic variants in *SGCE* (c.298T>G, p.Trp100Gly and c.304C>T, p.Arg102Ter) and two control iPSC lines were differentiated into mature cortical neurons. Localization of the brain-specific epsilon-sarcoglycan was investigated by cell-surface biotinylation and Western blotting in controls and the missense variant line with and without proteasomal inhibition (MG132). The nonsense variant was excluded since no protein was detectable in previous analyses. RNA samples of all four iPSC-derived cortical neuron lines were subjected to transcriptome analysis. Candidate transcripts were validated by quantitative real-time PCR (qPCR).

Results: Upon biotin treatment, brain-specific epsilon-sarcoglycan was detected in the membrane fraction of the controls. The protein with the missense variant was detected in whole-cell lysates, but not located at the cell surface. Incubation with MG132 increased levels of whole-cell epsilon-sarcoglycan but the location at the plasma membrane could not be restored. Transcriptome analysis revealed that of the DGC components *SGCA*, *SGCB*, *SGCG*, *SGCD*, and *SGCZ*, only *SGCD* and *SGCZ* (encoding delta- and zeta-sarcoglycan) were upregulated in neurons with *SGCE* variants with foldchanges and p-values of 20.96; 7.64*10-6 and 14.67; 5.05*10-4, respectively. Validation by qPCR indicated only small expression changes of these two genes. Six further differentially expressed candidate genes are under investigation.

Discussion: The endogenous M-D model studied here indicates that the brain-specific isoform of epsilon-sarcoglycan is indeed localized at the cell surface in control neurons but not in patient-derived cells. Proteasomal inhibition increases the amount of epsilon-sarcoglycan but has no effect on the cellular localization of the missense variant. mRNA expression analyses revealed that *SGCD* and *SGCZ* are upregulated in both lines with pathogenic *SGCE* variants, indicating a possible compensation in the composition of the DGC. Further analyses are warranted to expand our preliminary findings and understand the changes on the protein complex level.

Reference:

1. Grütz, K. et al. Sci. Rep. 7,41156 (2017).