

Involvement of the epilepsy gene *TBC1D24* in resistance to oxidative stress

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Description du projet

Mutations in *TBC1D24* cause DOORS syndrome (Deafness, Onychodystrophy, Osteodystrophy, mental retardation and Seizures), myoclonic epilepsy and early infantile epilepsy. We have previously reported 11 patients from 9 unrelated families with DOORS syndrome¹. *TBC1D24* has been implicated in the regulation of synaptic vesicle trafficking and proper neurotransmitter release^{2,3}. Recent studies have also highlighted a role of *TBC1D24* in the oxidative stress response, resulting in neuroprotection⁴. To characterize the mechanisms that are responsible for the phenotype observed in patients harboring *TBC1D24* mutations, we identified proteins that

¹ Campeau PM et al. The genetic basis of DOORS syndrome: an exome-sequencing study. *Lancet Neurol.* 2014 Jan;13(1):44-58.

² Sheehan P and Waites CL. Coordination of synaptic vesicle trafficking and turnover by the Rab35 signaling network. *Small GTPases.* 2017 Jan 27:1-10.

³ Fischer B et al. Skywalker-*TBC1D24* has a lipid-binding pocket mutated in epilepsy and required for synaptic function. *Nat Struct Mol Biol.* 2016 Nov;23(11):965-973.

⁴ Finelli MJ et al. The Evolutionarily Conserved Tre2/Bub2/Cdc16 (TBC), Lysin Motif (LysM), Domain Catalytic (TLDC) Domain Is Neuroprotective against Oxidative Stress. *J Biol Chem.* 2016 Feb 5;291(6):2751-63.

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interact with TBC1D24 using mass spectrometry. We found many proteins implicated in the regulation of intracellular and mitochondrial calcium (Ca²⁺) homeostasis like SERCA2, LETM1, MAIP1 and FUNDC1^{5 6}. Another candidate, ARL6IP5, has been shown to increase the expression of Nrf2 upon neuronal damage⁷, a master regulator of the antioxidative response⁸.

Seizure activity is known to induce the production of reactive oxygen species (ROS), which eventually compromise cell survival⁹. Mitochondrial Ca²⁺ overload, as well as impaired oxidative stress response, can contribute to the accumulation of ROS, both of which could be regulated by TBC1D24. Sulforaphane, an activator of Nrf2, is in clinical trials for the treatment of Autism Spectrum Disorder¹⁰, and could be considered in treating TBC1D24 associated disorders as it has emerged as a potential therapeutic target for epilepsy¹¹. We hypothesize that TBC1D24 is implicated in calcium homeostasis and plays a role in the regulation of ROS accumulation, which could protect against seizure induced cell damage.

First, we will confirm the interaction of TBC1D24 and the identified interacting proteins by co-immunoprecipitation as well as their sub-cellular localization by confocal microscopy using specific markers for different organelles. Next, we will generate TBC1D24 knockout SH-SY5Y neuronal cell lines using Crispr-Cas9 and inducible cell lines expressing either WT TBC1D24 or the mutants found in DOORS patients, and also generate iPSCs-derived neurons from TBC1D24 patient cells. Using these models, we will measure changes in Ca²⁺ homeostasis in response to surges in cytoplasmic Ca²⁺ using fluorescence microscopy. Secondly, we will measure ROS by FACS upon acute and/or prolonged Ca²⁺ release. Thirdly, we will monitor the expression of select downstream target genes regulated by Nrf2, and also determine the effect on other genes by RNAseq. Finally, we will determine in vitro if Sulforaphane can protect against some the consequences of TBC1D24 mutations. This will open the door to therapeutic tests in mice, and perhaps one day in patients.

Mots clés

Épilepsie, stress oxydatif, génétique.

⁵ Huang E t al. PINK1-mediated phosphorylation of LETM1 regulates mitochondrial calcium transport and protects neurons against mitochondrial stress. *Nat Commun.* 2017 Nov 9;8(1):1399.

⁶ König T et al. The m-AAA Protease Associated with Neurodegeneration Limits MCU Activity in Mitochondria. *Mol Cell.* 2016 Oct;64(1):148-162.

⁷ Zhao X et al., JWA antagonizes paraquat-induced neurotoxicity via activation of Nrf2. *Toxicol Lett.* 2017 Aug 5;277:32-40.

⁸ Vomund S, Schäfer A, Parnham MJ, Brüne B, von Knethen A. Nrf2, the Master Regulator of Anti-Oxidative Responses. *Int J Mol Sci.* 2017 Dec 20;18(12).

⁹ Kovac S et al. The Role of Reactive Oxygen Species in Epilepsy. 2016. *Cell Med Press* ; 1(1):38–52, 2016

¹⁰ Singh K et al. Sulforaphane treatment of autism spectrum disorder (ASD). *Proc Natl Acad Sci U S A.* 2014 Oct 28;111(43):15550-5.

¹¹ Carmona-Aparicio L et al. Overview of Nrf2 as Therapeutic Target in Epilepsy. *Int J Mol Sci.* 2015 Aug 7;16(8):18348-67.