Exploration of WDR45 function and contribution to BPAN

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Abstract

Beta propeller associated neurodegeneration (BPAN) is the most common neurodegeneration with iron accumulation (NDIA) disease. It is caused by mutations in the X-linked gene *WDR45* which encodes for a protein canonically associated with autophagy regulators, though the protein's function is largely unknown. BPAN is biphasic in its progression, with spontaneous epileptic seizures, intellectual disability, and autism features in infancy, followed by dystonia, parkinsonism, and dementia in early adulthood. In this study, we seek to explore the function of the WDR45 protein in a neuronal context and discover its role in the mechanisms which underlie the neurodegenerative disease BPAN.

Background

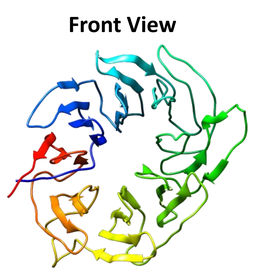

Fig. 1: predicted protein structure of WDR45. Much of the protein consists of beta sheets forming the shape of a propeller.

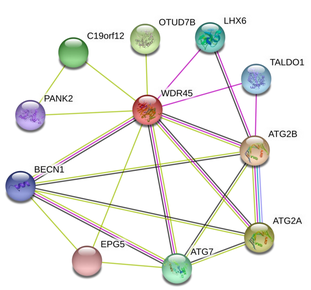

Fig. 2: WDR45 protein interactions. This network embodies the current understanding of WDR45 interactions though, to date much of the analysis of protein interaction has lacked a neuronal context. Ref: STRING consortium

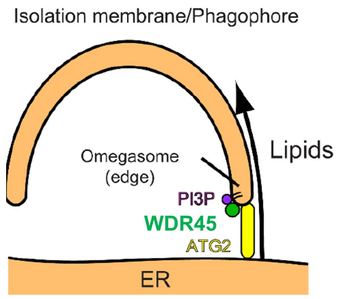

Fig. 3: Depiction of WDR45 performing its role in anchoring ATG2 for lipid transport to the developing autophagosome. Ref: Maeda et al. eLife, 2019

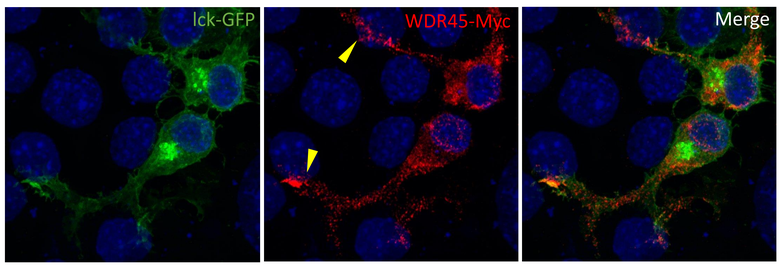

Fig. 4: Localization of Myc-tagged WDR45 in Neuro2A cells. Lck-GFP targets to the plasma membrane. WDR45 localizes throughout the cell even in the more distal protrusions.

What are the proteins interacting with WDR45?

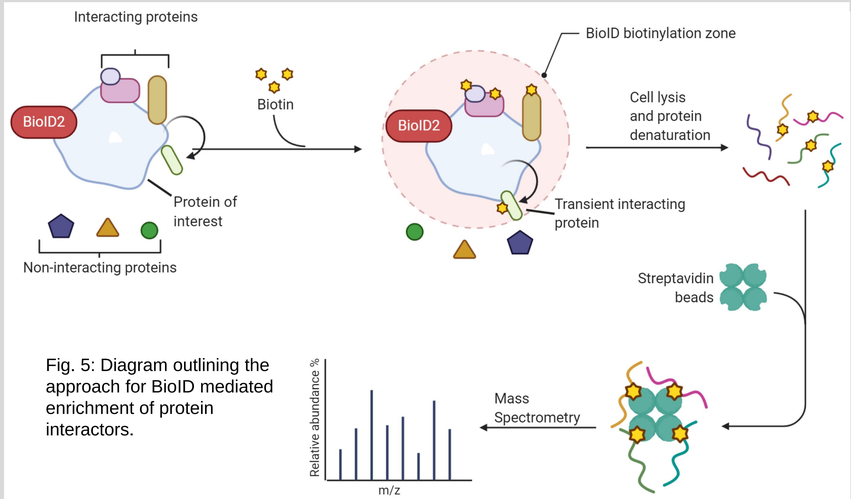

Fig. 5: Diagram outlining the approach for BioID mediated enrichment of protein interactors.

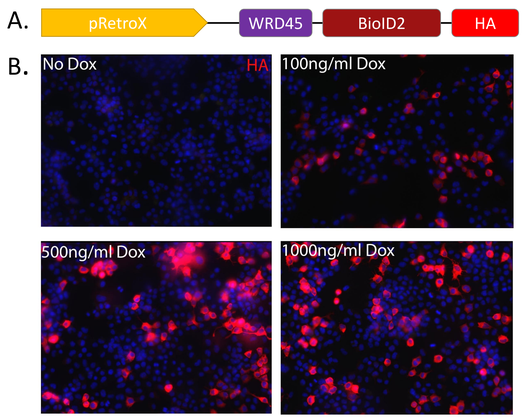

Fig. 6 (A) Schematic showing the construct used to stably induce expression of WDR45 protein with BioID2 fused to the C-terminal portion of the protein in Neruo2a mouse neuroblast cells. To control fusion protein expression we used a doxycycline inducible system. (B) Dose-dependent expression of fusion construct in generate stable Neuro2a cells which will be used for BioID protein screen.

How does WDR45 mutation affect cellular processes?

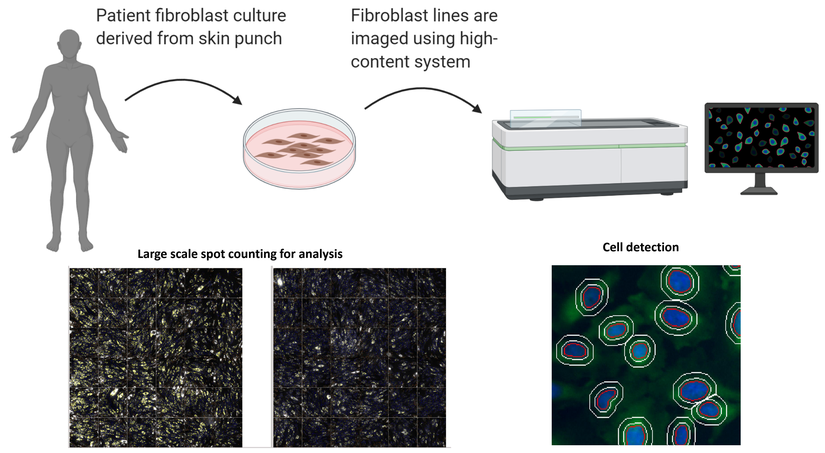

Fig. 7: Patient fibroblast lines will be subject to high-content imaging techniques to determine molecular and morphological phenotypes. This will allow us explore the mechanisms implicated in the BioID screen and serve as a platform for drug screening.

How do patient mutations of WDR45 affect mouse models?

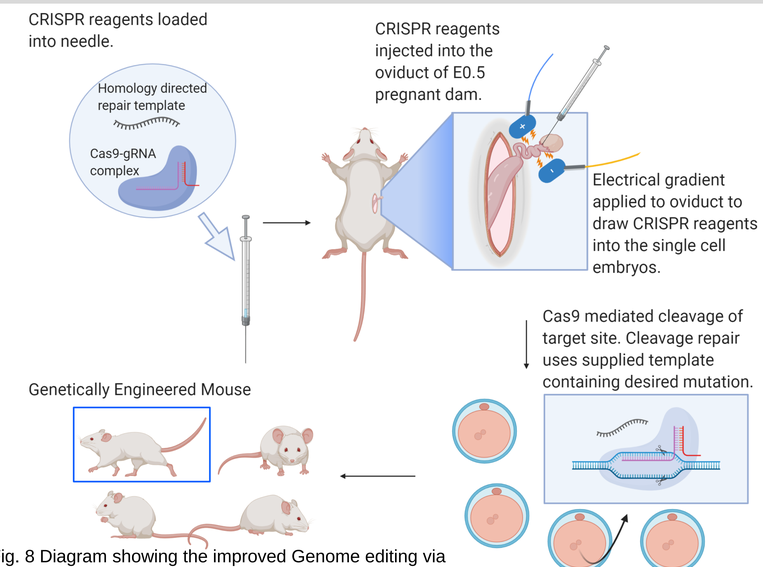

Fig. 8 Diagram showing the improved Genome editing via Oviductal Nucleic Acids Delivery (iGONAD) method used to induce directed germline mutation in mice.

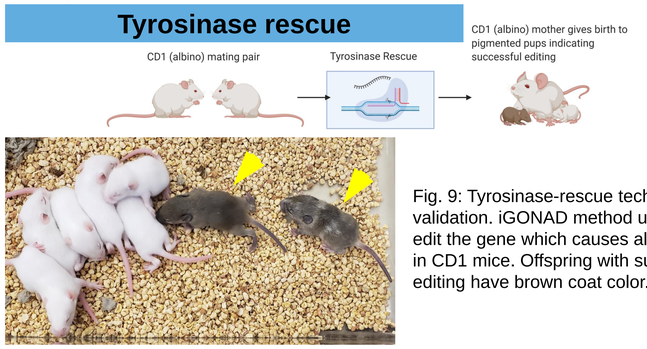

Fig. 9: Tyrosinase-rescue technique validation. iGONAD method used to edit the gene which causes albinism in CD1 mice. Offspring with successful editing have brown coat color.

Summary

- WDR45 likely plays a role in autophagy but alternative interactors are still being explored.
- BioID screen will give candidate interactors and highlight pathways that may be affected in BPAN.
- High content fibroblast imaging will explore cellular phenotypes of BPAN patients
- iGONAD allows for genetic engineering of mouse models. We will use this technique to make a new patient analogous mouse model that better mimics BPAN disease in humans

Acknowledgements

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