Application for a Research Grant on Angelman Syndrome

Molecular mechanisms of Angelman Syndrome

Ugo Mayor

University of the Basque Country UPV/EHU, Spain

WORKING HYPOTHESIS

Angelman Syndrome (AS) is caused by the loss of function of the ubiquitin ligase UBE3A in the brain. Ubiquitin ligases regulate cell function by attaching ubiquitin (a small protein) to their substrates; therefore, in AS patients, UBE3A substrates are expected to be less ubiquitinated. Using a number of cellular and model systems some progress has been achieved in identifying UBE3A substrates, but -so far- none of those putative substrates has been validated in human neurons. Similarly, the protein(s) that interact(s) with UBE3A to facilitate its function, as well as the deubiquitinating (DUB) enzyme(s) capable of reversing the function of UBE3A, remain elusive. As a result, past clinical trials lacking a sound biochemical understanding of their mode of action resulted unsuccessful. Given that symptoms presented in AS patients must result from the reduced ubiquitination on UBE3A substrates, we hypothesize that unequivocally identifying the mechanisms affected by the lack of UBE3A will contribute to understand and treat this disorder. Moreover, identifying the interactors of UBE3A might open yet unknown avenues to regulate those mechanisms. And finally, given that DUB enzymes can counteract the action of E3 ligases, our focus is now directed to identifying the DUB(s) removing ubiquitin from UBE3A substrates. We hypothesize that inhibition of such DUB(s) could result in a rescue of AS phenotypes. Based on those three lines of research, we hope to develop new therapeutic opportunities for AS treatment.

EXPERIMENTAL STRATEGY, METHODS AND OBJECTIVES

AS is a neurodevelopmental disorder caused by a loss of maternal contribution of the UBE3A gene, and results on lack of UBE3A activity in the brain. UBE3A is an E3 ubiquitin ligase belonging to the HECT family. HECT ligases are subject to strict regulation, and thus require activators and effectors in addition to binding substrates (Kuhnle, 2011; Mortensen, 2015). The molecular mechanisms regulating UBE3A activity remain poorly understood, and our understanding of the function and role of UBE3A loss in AS is still very limited. Identifying cofactors and/or other enzymes of the ubiquitin system –as DUBs- regulating UBE3A function in the human brain is not possible, and therefore we use the best lab models to address those questions: we extract proteins from mice brains, and we also use human neuronal-like cells in culture when the experiments require it. Which proteins are less ubiquitinated in AS patients is still unknown, but candidates have been proposed in cell-based studies (Sell, 2015). In our lab we have identified the proteasome as a substrate of UBE3A (Lee, 2014; Ramirez, 2018; Elu, 2019 *in press*), and we now aim to reach two further milestones: (i) to identify the cofactors UBE3A uses to ubiquitinate its substrates in mice brains, and (ii) to identify any DUB enzyme that counteracts the effect of UBE3A in neuroblastoma cells.

Main objectives

1. To identify and validate the substrates and cofactors (including DUB enzymes) of UBE3A.
2. To test the viability of targeting DUB enzymes as a therapeutic strategy for AS.

Research Plan and methodology

1. Elucidating the molecular targets and regulatory mechanisms of UBE3A in vivo

1.1. Validation of UBE3A substrates in mouse brain tissue.

1.2. Identification of UBE3A cofactors and interacting DUBs in mouse brain tissue.

2. Targeting DUB enzymes that oppose UBE3A function as a therapeutic strategy

PROJECT BUDGET

Total 175,000€ (3 years project)

1. Elucidating the molecular targets and regulatory mechanisms of UBE3A in vivo: 116,500€. With the following breakdown: -Personnel: 61,500€ (70% Postdoc salary for 2 years); -Minor equipment and reagents: 29,000 €; -Animal Housing: 26,000 €

2. Targeting DUB enzymes that oppose UBE3A function as a therapeutic strategy: 58,500€. With the following breakdown: -Personnel: 30,750€ (70% Postdoc salary for 1 year); -Cell culture media, reactives and plasticware: 14,750 €; -Animal Housing: 13,000 €

BRIEF INFORMATION ON THE RESEARCH INSTITUTION

The UPV/EHU (www.ehu.eus) is a teaching and research institution leading Research, Development and Innovation in the region of the Basque Country. It employs over 7.000 people throughout 31 faculties, and gathers 220 consolidated research groups, generating a good scientific output with international impact (61% in first quartile). The UPV/EHU is one of the Spanish seven best universities, and was the fifth Spanish university in creating spin offs during the 2007-2012 period. It holds 77 patent applications/year and 29 licensed patents. The advanced core facility Proteomics Service (with state-of-the-art proteomic equipment) and the Animal Housig facilities of the UPV/EHU (https://www.ehu.eus/en/web/sgiker/) are key for the research proposed by Dr Mayor. The Mayor lab counts will all the other required equipment to perform this research.

BRIEF INFORMATION ON COLLABORATION PARTNERS

Our lab collaborates locally with Jesus M Arizmendi, leader of the UPV/EHU Proteomics Unit; Mazahir T. Hasan, neurobiologist working in Memory Circuits at the UPV/EHU Achucarro Neuroscience; and with Rosa Barrio, who works on ubiquitin-likes and developmental biology at the CIC bioGUNE. We also collaborate internationally with Helen Walden, structural biologist in Dundee (UK); Andreas Frick, neurobiologist working in Cortical Plasticity in Burdeaux (France); Michael Clague and Sylvie Urbe who work on the Cellular and Molecular Physiology of DUB enzymes in Liverpool (UK); and Larry Reiter, who has been working on AS model systems for many years and is now based in Memphis (US).

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