USE OF GFP FUSIONS TO ANALYZE THE MECHANISMS OF TOXICITY OF HUMAN VAPB EXPRESSED IN \textit{Saccharomyces cerevisiae}.

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that affects motor neurons. Two mutations in the VAPB gene are associated with familial ALS type 8 (FALS8). The VAPB protein is located in the endoplasmic reticulum (ER) membrane, with a MSP domain facing to the cytosol. The mechanisms by which the mutated forms of VAPB underlie ALS disease are still poorly understood. Therefore, \textit{S. cerevisiae} was employed here as a model to study FALS8. SCS2 protein from \textit{Saccharomyces cerevisiae} is orthologous to human VAPB and is involved in different aspects of cell biology of the ER, such as lipid and inositol metabolism and in unfolded protein response (UPR). In previous studies in our group, we expressed wild type and P56S mutant forms of VAPB in \textit{Saccharomyces cerevisiae} under control of GAL1 promoter (pYES 2.1 plasmid). In both cases, growth of transgenic yeast was strongly inhibited. Expression of VAPB P56S was more toxic than expression of wild type allele. These results are consistent with a gain of toxic function with the formation of protein aggregates and ER stress, with accumulation of unfolded and/or misfolded proteins in this organelle. Therefore, to locate wild-type and mutant forms of VAPB, to visualize the formation of aggregates and their possible degradation pathway in yeast cells, we construct plasmids to express VAPB in fusion with GFP. BY4741 strain was transformed with PYES expression vector containing alleles for wild-type and mutant (P56S) VAPB fused or not with GFP. Cells carrying the VAPB\(^{P56S}\) allele display lower viability when compared to cells carrying the VAPB\(^{WT}\) and the control strain, independently of having fused GFP, as assessed by growth curves (OD\(_{600nm}\)) and serial dilutions. We are currently in the process of obtaining images of cells expressing VAPB-GFP fusions.

Key words: VAPB, protein aggregation, endoplasmic reticulum stress.

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