Mitochondrial dysfunctions caused by neuron-related RNA binding protein misexpression

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Abstract

RNA-binding proteins (RBPs) are proteins that bind to double- or single- stranded RNA molecules using conserved RNA recognition motifs (RRM). They perform post-transcriptional control of RNA molecules affecting their splicing, transport, localization and translation among others, thus controlling directly mRNA and protein homeostasis in cells. Existing reports highlight the potential pathogenic role of RBPs in neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), with mutant forms of Fused in Sarcoma (FUS) and TAR DNA-binding protein 43 (TDP-43) deregulating cellular processes and forming distinct cytoplasmic inclusions. Among the perturbations induced by these RBPs, mitochondrial impairments have been described. Furthermore, a plethora of mutated genetic loci related to Parkinson's disease (PD) are directly associated with mitochondria, such as mutations in PINK1 and Parkin. This feature implicates mitochondrial dysfunction as an integral disease component. Hereby, we aimed at characterizing the systemic effects of nine neuron-associated RBPs (ELAVL1-4, TIA-1, FUS, TDP-43, RBFOX2-3) in modulating mitochondrial functions following overexpression in neuroblastoma cell lines expressing dopaminergic markers, such as SK-N-SH and SH-SY5Y cells. Towards this we employed fluorophore-based assays to detect the generation of reactive oxygen species (ROS), mitochondrial membrane potential depolarization ($\Delta\Psi$m), alterations in mitochondrial mass, induction of mitochondrial oxidation and energy status perturbations. We observed that the majority of RBPs tested affected mitochondrial functions. Almost all RBPs deregulated $\Delta\Psi$m thus depolarizing mitochondrial membrane integrity. Five RBPs induced a change in mitochondrial size similar to what has been described for FUS and TDP-43, thus indicating that RBPs deregulate fission/fusion cycle. Perturbation in energy status of the cells was evaluated using ATP to ADP ratio measurement. Almost all RBPs affected ATP generation providing clues that cell metabolism processes may be altered. Of interest was that all RBPs contributed to enhanced ROS generation indicative of oxidative stress conditions similar to the ones presented in PD. Further, mild but significant increase in oxidative stress localized in mitochondria as observed with a molecular tool monitoring mitochondrial dynamics, further confirmed the generation of oxidative conditions in the case of six of the RBPs. Summarizing, all nine RBPs differentially regulated mitochondrial functions implying that they possess a variety of mechanisms on modifying mitochondrial dynamics. These findings reveal new candidates possibly implicated in PD pathogenesis and novel targets to reverse the neuronal death resulted by mitochondrial malfunctions.

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Keywords: RNA binding proteins, neurodegeneration, mitochondrion, post, transcriptional modifications, oxidative stress