Zinc-finger CCHC-domain containing protein 6 (Zcchc6) is translocated to mitochondria and modulates neuronal cells survival

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Objective

Mitochondria are the center of cellular energy production and also home for several pro-apoptotic proteins. Mitochondria express 13 protein coding genes, which are transcribed, translated and are degraded within the mitochondria. The regulation of mitochondrial gene expression is not very well understood. Zinc-finger CCHC-domain containing protein 6 (Zcchc6) is a member of the family of terminal uridylyltransferases (TUTs) and is known to Adenylate and Uridylate the 3' ends of mRNAs and miRNAs in a template independent fashion and has been implicated in mRNA degradation as well as miRNA-mediated regulation of gene expression. However, the role of ZCCHC6 in regulating mitochondrial gene function and apoptosis in the pathogenesis of Alzheimer's disease is unknown.

Methods

Neuroblastoma cells, Neuro-2a (N2a), were cultured in DMEM/F12 and were transformed into neuron-like cells by serum starvation. Oxidative stress was induced by treating N2a cells with hydrogen peroxide (H2O2) and the expression of Zcchc6 was determined by qPCR and cell death was determined by LDH release assay and caspase 3/7 activity assay. Mitochondrial translocation of Zcchc6 was determined by confocal microscopy. Mitochondrial depolarization and mitochondrial network fragmentation was determined by JC-1 and MitoTracker dye staining, respectively. The generation of mitochondrial ROS was determined by MitoSOX staining.

Results

Our data showed increased expression of Zcchc6 in N2a cells exposed to H2O2 in vitro which also induced significant cell death. H2O2-induced oxidative stress increased mitochondrial depolarization and fragmentation of mitochondrial network in N2a cells. Interestingly, we found that Zcchc6 was translocated to mitochondria in both the transformed and non-transformed N2a cells as determined by immunofluorescence staining of Zcchc6 and MitoTracker or SDHA (Succinate Dehydrogenase Complex Flavoprotein Subunit A). Overexpression of Zcchc6 in N2a cells altered mitochondrial membrane potential and correlated with the increased cell death.

Conclusion

We show here for the first time that Zcchc6 is translocated to mitochondria and play a role in mitochondrial dysfunction and cell death in N2a cells. However, the mechanism is yet to be understood but may involve Zcchc6-mediated mitochondrial gene regulation.