Abstract

Mutations in the leucine-rich repeat kinase 2 (LRRK2) are the leading cause of genetically inherited Parkinson’s disease (PD). Two of the common found mutations namely R1441C and G2019S, mediate their toxic effects through their increased kinase activity. Furthermore, the kinase activity of LRRK2 is regulated through some essential serine and threonine autophosphorylation residues that are located in the LRR, ROC and kinase domains. In this study we identified Protein Phosphatase 2A (PP2A), as an interacting partner of LRRK2. Additionally we were able to demonstrate that only the ROC domain is needed to interact with the three subunits of PP2A in SH-SY5Y cells and in Hela cells. We have shown that the alpha subunit of PP2A (PP2Aa) is interacting with LRRK2 in the perinuclear region of Hela cells. We also investigated the physiological role of PP2A in SH-SY5Y cells transiently expressing R1441C-LRRK2, by silencing the catalytic subunit of PP2A (PP2Ac). As a result, cell death which was induced by R1441C-LRRK2 was significantly aggravated. Finally, pharmacological activation of PP2A by sodium selenate in SH-SY5Y in cells transiently transfected with R1441C-LRRK2, showed a partial neuroprotection from the mutation. All these data suggest that PP2A is a new interacting partner of LRRK2 and reveal the importance of PP2A as a potential therapeutic target in PD.

The second part of my PhD Thesis focuses in VDAC-1, which is a protein also correlated with PD mitochondrial induced cell death. Initially we found that the ROC domain of LRRK2 is the domain, through which the interaction with LRRK2 takes place. When comparing the binding of the ROC-WT to the VDAC versus the ROC-R1441C, we noticed that the ROC-WT protein binds in a much bigger extent to the total VDAC levels, compared to the ROC-R1441C protein. Additionally, using reductase assays, plasma membrane VDAC-1 seems to be accountable for the majority of the reductase activity in HEK293T cells. After expressing ROC-WT or R1441C proteins in HEK293T cells, the total reductase activity levels in ROC-WT transfected HEK293T cells was reduced, compared to ROC-R1441C transfected cells. These data suggest that the ROC/LRRK2 protein could possibly bind the plasma membrane-VDAC-1 as a chaperone and reduce its reductase enzyme properties. On the other hand, in the presence of R1441C mutation, ROC-R1441C cannot bind plasma membrane VDAC-1 so efficiently and this leads to elevated reductase activity levels of plasma membrane VDAC-1.