Role of pink1 in α-synuclein aggregation

The role of PINK1 in α-synuclein aggregation and toxicity in Parkinson's disease

State-of-the-art

Misfolding and aggregation of α-synuclein (ASYN) is the major component of Lewy bodies (LBs) [1], the pathological hallmark of Parkinson's disease (PD) [2, 3]. Although clinical and experimental studies suggest the involvement of protein misfolding, oxidative stress and mitochondrial dysfunction [4, 5], the fundamental cause of the disease, its underlying mechanism remains elusive. The normal function of ASYN is still unclear, but it is thought to be involved in the vesicular trafficking, regulation of dopamine neurotransmission and in synaptic function and plasticity [6, 7]. PTEN-induced kinase 1 (PINK1) was identified as an interactor of ASYN. In worms, mitochondrial fragmentation induced by expression of ASYN is rescued by co-expression of PINK1 [8]. Also, in flies, overexpression of PINK1 rescues motor and developmental defects induced by ASYN [9]. More than 70 mutations in the PINK1 gene were identified in familial PD in an autosomal recessive manner [10, 11]. Previous studies suggests that PINK1 plays a critical role in PD pathogenesis and dysregulation of PINK1 may contribute to the development of PD [12, 13]. However, whether PINK1 also plays a role in the ASYN pathology has not been addressed so far, and will be one of the central goals of this proposal. In this context, we pretend investigate that whether ASYN pathology differs in the absence of PINK1 in cell culture and *in vivo* . Thus, we hypothesize that the possible interaction between PINK1 and ASYN may be favorable to the cells through the decrease of ASYN aggregation and cell death.

Project aims

The central aims of this project are to study the modulatory effect of PINK1 on ASYN aggregation and toxicity, *in vitro* and *in vivo* , which would be important for therapeutic intervention in synucleinopathies, particularly in PD.

Experimental design

1. To assess the interaction between PINK1 andASYN

Over the past decade, mutations in several genes, among which ASYN and PINK1, have been identified in inherited PD [14, 15] and whether there is a direct interaction of PINK1 and ASYN awaits further research. In order to study if there is a direct interaction between PINK1 and ASYN *in vivo* , we will perform a co-immunoprecipitation analysis of ASYN and PINK1 proteins from brain lysate of wild type Sprague-Dowley adult female rats. Following the immunoprecipitation of endogenous ASYN, endogenous PINK1 will be detected using a PINK1 specific antibody.

2. To investigate the effect of PINK1 in ASYN aggregation

Although the process of ASYN aggregation has been extensively studied *in vitro* , it is still unclear which cellular pathways are involved. To investigate the effect of PINK1 on ASYN aggregation, recombinant ASYN will be incubated in the presence of total protein lysates from cells overexpressing either EGFP-PINK1 or EGFP, as a control. ASYN fibrillization will be followed by monitoring ThT fluorescence at 482 nm. ThT is an amyloid-specific dye

whose fluorescence dramatically increases upon binding to cross-β sheet structures such as those formed during ASYN self-assembly.

3. To assess the interaction between PINK1 and ASYN and the effect in mitochondrial morphology and membrane potential

Mitochondrial dysfunction has long been implicated in PD pathogenesis due to reduced activity in complex I [16, 17]. PINK1 contains a putative N-terminal mitochondrial targeting sequence, and evidence exists that PINK1 is targeted to mitochondria [18]. However, the influence of ASYN in mitochondrial targeting of PINK1 is not entirely settled. To assess putative mitochondrial defects caused by a loss of PINK1 function, we will verify mitochondrial morphology by fluorescence microscopy in SH-SY5Y cells expressing wild-type (WT) ASYN under control of the Tet-off regulatory expression system before and after siRNA mediated downregulation of PINK1.

To determine whether possible mitochondrial morphology alterations are associated with functional impairments, we will measure the mitochondrial membrane potential by TMRM fluorescence activated cell sorting (FACS).

4. To determine the effects of PINK1 in ASYN aggregation and toxicity *in vivo* .

The increase of ASYN aggregation was reported in synucleinophaties cell culture models in which PINK1 was silenced via RNA interference [19]. However, a detailed research of the interaction between PINK1 and ASYN toxicity *in vivo* is still missing.

Several different genetic mouse models have been generated based on the expression of ASYN driven by a diversity of promoters. To assess the influence of PINK1 in ASYN aggregation we will use the animal model which express full-length human WT ASYN under the Thy-1 promoter [20, 21]. Firstly, we will determine whether overexpression of PINK1 influences ASYN aggregation in the brain of transgenic mice. For this propose, we will inject adeno-associated viruses (AAV) encoding PINK1 in SN of transgenic or WT littermate control mice. Next, we will execute behavioral analysis and then we will sacrifice the animals to determine whether PINK1 increases ASYN aggregation and controls neuronal loss. Thus, we will perform immunohistochemistry and Thioflavin S staining, to assess whether inclusions display amyloid-like properties.

Interestingly, although fly models lacking PINK1 expression display a drastic phenotype, ranging from mitochondrial dysfunction to significant dopaminergic neurodegeneration and motor deficits [22] the PINK1 knockout and knockdown mouse models developed so far display mild neurodegenerative changes [23]. To investigate whether ASYN pathology differs in the absence of PINK1 in vivo, we will generate PINK1 knockdown mice by local delivery of a rAAV2/7-mediated, microRNA-based, short-hairpin RNA against PINK1 [24, 25]. We will analyze the vulnerability of the nigral dopaminergic neurons to a-synuclein toxicity when the PINK1 levels are reduced or absent. For this approach, we will deliver a rAAV2/7-a-synuclein WT vector to the SN of both PINK1 knockdown and PINK1 knockout mice.

References

1. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, et al. (1997) Alpha-synuclein in Lewy bodies. Nature 388: 839-840.

2. de Lau LM, Breteler MM (2006) Epidemiology of Parkinson’s disease. Lancet Neurol 5: 525-535.

3. Goedert M (2001) Alpha-synuclein and neurodegenerative diseases. Nat Rev Neurosci 2: 492-501.

4. Fasano M, Lopiano L (2008) Alpha-synuclein and Parkinson’s disease: a proteomic view. Expert Rev Proteomics 5: 239-248.

5. Outeiro TF, Lindquist S (2003) Yeast cells provide insight into alpha-synuclein biology and pathobiology. Science 302: 1772-1775.

6. Schneider BL, Seehus CR, Capowski EE, Aebischer P, Zhang SC, et al. (2007) Over-expression of alpha-synuclein in human neural progenitors leads to specific changes in fate and differentiation. Hum Mol Genet 16: 651-666.

7. Crews L, Mizuno H, Desplats P, Rockenstein E, Adame A, et al. (2008) Alpha-synuclein alters Notch-1 expression and neurogenesis in mouse embryonic stem cells and in the hippocampus of transgenic mice. J Neurosci 28: 4250-4260.

8. Kamp F, Exner N, Lutz AK, Wender N, Hegermann J, et al. (2010) Inhibition of mitochondrial fusion by alpha-synuclein is rescued by PINK1, Parkin and DJ-1. Embo j 29: 3571-3589.

9. Todd AM, Staveley BE (2012) Expression of Pink1 with alpha-synuclein in the dopaminergic neurons of Drosophila leads to increases in both lifespan and healthspan. Genet Mol Res 11: 1497-1502.

10. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, et al. (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 304: 1158-1160.

11. Valente EM, Bentivoglio AR, Dixon PH, Ferraris A, Ialongo T, et al. (2001) Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36. Am J Hum Genet 68: 895-900.

12. Exner N, Treske B, Paquet D, Holmstrom K, Schiesling C, et al. (2007) Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. J Neurosci 27: 12413-12418.

13. Oliveras-Salva M, Macchi F, Coessens V, Deleersnijder A, Gerard M, et al. (2014) Alpha-synuclein-induced neurodegeneration is exacerbated in PINK1 knockout mice. Neurobiol Aging 35: 2625-2636.

14. Farrer MJ (2006) Genetics of Parkinson disease: paradigm shifts and future prospects. Nat Rev Genet 7: 306-318.

15. Gasser T (2009) Mendelian forms of Parkinson's disease. Biochim Biophys Acta 1792: 587-596.

16. Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443: 787-795.

17. Olanow CW, Tatton WG (1999) Etiology and pathogenesis of Parkinson’s disease. Annu Rev Neurosci 22: 123-144.

18. Gandhi S, Muqit MM, Stanyer L, Healy DG, Abou-Sleiman PM, et al. (2006) PINK1 protein in normal human brain and Parkinson’s disease. Brain 129: 1720-1731.

19. Liu W, Vives-Bauza C, Acin-Perez R, Yamamoto A, Tan Y, et al. (2009) PINK1 defect causes mitochondrial dysfunction, proteasomal deficit and alpha-synuclein aggregation in cell culture models of Parkinson’s disease. PLoS One 4: e4597.

20. Rockenstein E, Mallory M, Hashimoto M, Song D, Shults CW, et al. (2002) Differential neuropathological alterations in transgenic mice expressing alpha-synuclein from the platelet-derived growth factor and Thy-1 promoters. J Neurosci Res 68: 568-578.

21. Chesselet MF, Richter F, Zhu C, Magen I, Watson MB, et al. (2012) A progressive mouse model of Parkinson’s disease: the Thy1-aSyn (“ Line 61”) mice. Neurotherapeutics 9: 297-314.

22. Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, et al. (2006) Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. Nature 441: 1162-1166.

23. Oliveras-Salva M, Van Rompuy AS, Heeman B, Van den Haute C, Baekelandt V (2011) Loss-of-function rodent models for parkin and PINK1. J Parkinsons Dis 1: 229-251.

24. Heeman B, Van den Haute C, Aelvoet SA, Valsecchi F, Rodenburg RJ, et al. (2011) Depletion of PINK1 affects mitochondrial metabolism, calcium homeostasis and energy maintenance. J Cell Sci 124: 1115-1125.

25. Osorio L, Gijsbers R, Oliveras-Salva M, Michiels A, Debyser Z, et al. (2014) Viral vectors expressing a single microRNA-based short-hairpin RNA result in potent gene silencing in vitro and in vivo. J Biotechnol 169: 71-81.