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Research Report

Mitochondrial complex I defects increase ubiquitin in substantia nigra

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ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the developed world, and is characterized by the loss of dopaminergic (DA) neurons in the substantia nigra (SN) of midbrain. Mitochondrial complex I dysfunction has been implicated in PD pathophysiology, yet the molecular mechanism by which complex I defects may cause DA neurodegeneration remain unclear. Using Ndufs4 mouse model of mitochondrial complex I deficiency, we observed a remarkable ubiquitin protein increase in SN of Ndufs4-/- (KO) mice. By contrast, neurofilaments were significantly decreased in SN of KO mice. Furthermore, mass spectrometry and co-immunoprecipitation (Co-IP) analysis indicated an increase in ubiquitinated neurofilaments in midbrain of KO mice, whereas 20 S proteasome activities were decreased, which could potentially explain the buildup of ubiquitin protein. Collectively, these data suggest that mitochondrial complex I defects cause proteasome inhibition, a consequent increase in ubiquitinated neurofilaments and other proteins, and decrease the expression of neurofilaments that could be relevant to the mechanism of DA neuronal death in PD.

1. Background

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder. The pathological hallmark of PD is the accumulation of Lewy bodies and the degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNC) of midbrain, yet the mechanism of selective DA neuron loss is still unclear. A growing number of clinical and experimental studies has indicated the involvement of mitochondrial complex I (CI) dysfunction in the pathogenesis of PD (Dauer and Przedborski, 2003; Mizuno et al., 1989; Pan-Montojo et al., 2010; Pickrell et al., 2013). CI is the largest respiratory chain complex, and deficiency of CI has been reported in PD patients (Pan-Montojo et al., 2010). The exposure of humans and animals to CI inhibitors causes parkinsonism and is a major model for studying parkinsonism (Dauer and Przedborski, 2003; Mizuno et al., 1989). CI inhibitor rotenone causes DA neurodegeneration and formation of Lewy body-like inclusions (Pickrell et al., 2013). However, despite our wealth of knowledge regarding its activities at the molecular level, the roles of CI and its potential contributions to DA neurodegeneration remain elusive.

Ndufs4 is an important subunit of complex I, and its mutation causes deficiencies in complex I activity (Petruzzella and Papa, 2002; Scacco et al., 2003). Monomer CI activities were undetectable in Ndufs4 knockout (Ndufs4-/-, KO) mice (Kruse et al., 2008). Recent studies indicated that Ndufs4 inactivation results in microtubule dysfunction of DA neurons, potentiates

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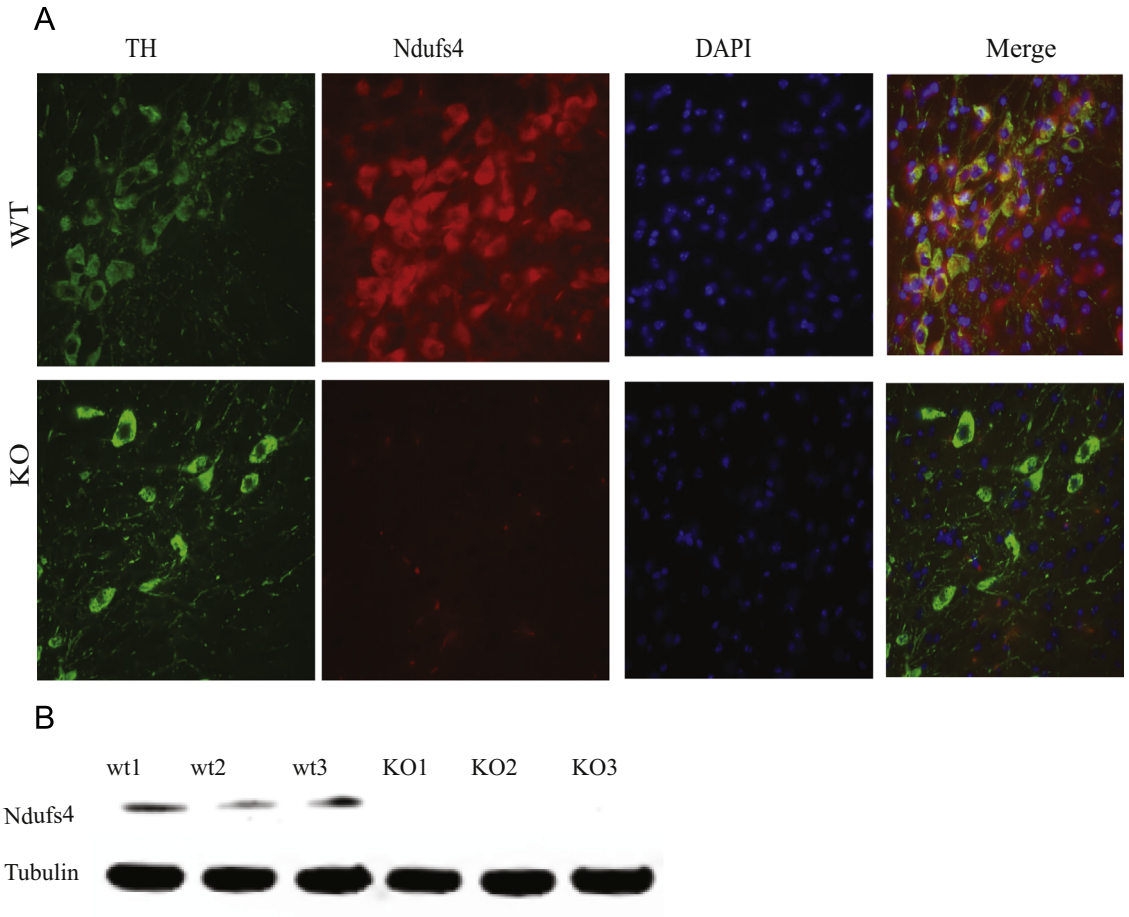

Fig. 1 – Ndufs4 expression in SNC. (A) Immunostaining in midbrain of Ndufs4 mice using Ndufs4 antibody (Green: TH positive neurons; Red: Ndufs4 signal). (B) The western-blot results of Ndufs4 in mouse SN.

rotenone and MPTP toxicity to DA neurons and impairs DA release (Choi et al., 2011; Sterky et al., 2012). This potentiated neurotoxicity is likely a result of inhibition of residual complex I+III activity in supercomplexes (Calvaruso et al., 2012). However, overt loss of DA neurons in SNC of Ndufs4 KO mice has not been observed (Choi et al., 2011; Sterky et al., 2012). Here, we investigated DA neuron metabolism and morphology using Ndufs4-/- mice. We found that complex I deficiency causes a remarkable increase of ubiquitin protein and the reduction of neurofilaments in SN. Utilizing mass spectrometry and co-immunoprecipitation (Co-IP) analysis we indicated an increase in ubiquitinated neurofilaments in midbrain of Ndufs4-/- mice. These results support a role for complex I dysfunction as a contributing factor in PD pathophysiology.

2. Results

2.1. *Loss of Ndufs4 decreases expression of tyrosine hydroxylase in SN*

To study possible biochemical defects in DA neurons caused by the loss of Ndufs4, we crossed Ndufs4 heterozygous (Ndufs4+/-, HET) male mice with Ndufs4+/- female mice to acquire Ndufs4 KO mice (Ndufs4-/-, KO) and littermate controls (Ndufs4+/+, WT). The efficiency of Ndufs4 deletion was observed in SN by immunostaining and Western-blot using Ndufs4 antibody (Fig. 1A and B). KO mice are almost the same size as the WT mice one week after birth, but progressively becomes smaller and weaker. KO mice stop gaining weight at p30 and die at around p50. As previously reported, at around p40, KO mice developed severe ataxia, and lose their balance(Kruse et al., 2008). Moreover, we found that KO mice initiate circling unidirectionally at about p45 as previously reported in Parkinson patients (Bracha, 1987).

Next we undertook a detailed analysis of the midbrain DA system, although several articles reported lack of TH neuron reduction in SNC of Ndufs4-/- mice (Choi et al., 2011; Sterky et al., 2012) we found that tyrosine hydroxylase (TH) expression was decreased in SNC of KO mice at P43-p48 using DAB immunohistochemistry (Fig. 2A and B). Furthermore, we found that both TH and catechol-O-methyltransferase (COMT) were reduced in the midbrain containing whole SN except cortex and hippocampus using Western-blot (Fig. 2C and F). Consistent with this, there was also a significant TH reduction in striatum of KO mice at around p45 (Fig. 2G and H), we confirmed this result in striatum using QRT-PCR (Fig. 2I). These results show that defects in complex I decrease the expression of enzymes involved in dopamine metabolism.