The role of α-synuclein in the pathology of murine Mucopolysaccharidosis type IIIA

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List of abbreviations

Autophagy	macroautophagy
CD-MPR	cation-dependent M6P receptor
CI-MPR	cation-independent M6P receptor
D31N	aspartic acid to asparagine change at codon 31
ELISA	enzyme-linked immunosorbent assay
G_{M2}	monosialoganglioside 2
G_{M3}	monosialoganglioside 3
HS	heparan sulphate
hr	hour(s)
IGF-II	insulin-like growth factor II receptor
Kb	kilobase (1000 base pairs of DNA or RNA)
kDa	kilodalton (unified atomic mass unit)
LC-MS/MS	liquid chromatography- tandem mass spectrometry
LSDs	lysosomal storage disorders
min	minute(s)
MPS	mucopolysaccharidoses
MPS IIIA	mucopolysaccharidosis type IIIA
MPS IIIA-$SNCA^{+/+}$	mucopolysaccharidosis type IIIA with intact α-synuclein
MPS IIIA-$SNCA^{+/-}$	mucopolysaccharidosis type IIIA with heterozygous α-synuclein
MPS IIIA-$SNCA^{-/-}$	mucopolysaccharidosis type IIIA with an absence of α-synuclein
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NACP	precursor of non-Aβ component of Alzheimer disease amyloid
Normal mice	wild type mice ($SGSH^{+/+}$ $SNCA^{+/+}$)
PBS	phosphate buffered saline
PC12	pheochromocytoma cells of the rat adrenal medulla
sec	seconds
SGSH	sulphamidase
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SNCA	α-synuclein

Thesis abstract

Lysosomal storage disorders are a heterozygous group of inherited metabolic disorders caused by a deficiency in one or more lysosomal enzymes, resulting in the accumulation of undegraded substrates in cells. Although specific enzymes deficits are the primary cause of specific lysosomal storage disorders, the underlying pathological mechanisms responsible for subsequent clinical features are largely unknown. About two-thirds of affected cases are associated with neurodegeneration with no effective therapies available due to the lack of understanding of the pathological mechanisms in the brain.

One of the foremost pathological events in several lysosomal storage disorders is the progressive accumulation of α-synuclein in the affected brain (Hamano et al., 2008a). α-Synuclein has long been known for its pathological involvement in Parkinson's disease and other neurological disorders, collectively known as 'synucleopathies'. Moreover, wild-type α-synuclein at physiological levels can impair lysosomal autophagy both *in vitro* and *in vivo*, but deletion of α-synuclein improves autophagy and clinical features in Huntington's disease mice (Corrochano et al., 2012; Tomás-Zapico et al., 2012).

Few studies have investigated the pathophysiological role of endogenous α-synuclein in lysosomal storage disorders *in vivo*. To address this issue, this study utilised a naturally occurring murine model of the neurological lysosomal storage disorder, mucopolysaccharidosis type IIIA (MPS IIIA; Sanfilippo syndrome type A) to investigate the role of α-synuclein in disease pathogenesis. MPS IIIA is an autosomal recessive disorder caused by the lysosomal deficiency of sulphamidase and the subsequent accumulation of heparan sulphate and secondary ganglioside substrates. Due to an inefficient degradation process, accumulation of aggregate-prone proteins such as α-synuclein was observed in MPS IIIA mouse brain as early as three-weeks of age and in humans with the disorder (Beard et al., unpublished).

In this study, MPS IIIA mice were crossed with α-synuclein knockout mice to create a colony of MPS IIIA mice deficient in α-synuclein. The progeny of these MPS IIIA mice (MPS IIIA-SNCA$^{+/+}$, MPS IIIA-SNCA$^{+/-}$ and MPS IIIA-SNCA$^{-/-}$) showed a similar clinical phenotype, such as coarse, apathetic facial features, hunched posture and aggressiveness toward cage mates. The α-synuclein-deficient MPS IIIA mice showed significant hypoactivity, increased anxiety, motor gait impairment, and reduced learning and memory abilities compared to normal

littermates in a battery of behavioural tests. Histopathological investigations confirmed the deposition of both primary and secondary substrates in the brains of MPS IIIA mice with or without the deficiency of α-synuclein. Moreover, preliminary studies have shown increased amounts of α-synuclein in native forms, instead of toxic oligomers, in congenic MPS IIIA brains and skin fibroblasts compared to wild-type normal tissues, possibly a result of a defect in lysosomal autophagy.

All MPS IIIA mice showed more than 30% loss in dopamine levels compared to normal mice, regardless of α-synuclein *SNCA* gene composition. This shows a possible functional link between MPS IIIA and the abnormal motor phenotypes observed. The results suggest that lysosomal dysfunction in the MPS IIIA brain may lead to impaired synthesis or trafficking of dopamine, or may result in selective loss of dopaminergic neurons in the substantia nigra. Further studies are needed to determine which of these scenarios underlie the observations made here.

Modification of α-Synuclein expression did not change the progressive proteinaceous accumulation (e.g. ubiquitin, phosphorylated-tau) or that of endo/lysosomal proteins (lysosomal integral membrane protein II) and neuroinflammatory proteins (glial fibrillary acidic protein) in MPS IIIA mice. It was also demonstrated that failure of fusion between autophagosomes and lysosomes in MPS IIIA mice resulted in the accumulation of ubiquitin-positive inclusions and toxic substrates (Settembre et al., 2008). Consequently, impaired lysosomal autophagy can also disrupt the continuous clearance of cytosolic proteins by the ubiquitin-proteasome system (Hara et al., 2006; Komatsu et al., 2006).

The major conclusion from this study is that deletion or deficiency of α-synuclein made little or no contribution to the clinical and neuropathological disease progression in MPS IIIA mice. The data suggest that defects in autophagy and/or ubiquitin-proteasome system may play the main pathological mechanism, and α-synuclein accumulation is a secondary downstream event and of itself is unlikely to contribute significantly to the pathogenesis of MPS IIIA.

Declaration

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