Neurobiology of Disease 114 (2018) 140–152

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Lysosomal response in relation to α-synuclein pathology differs between Parkinson's disease and multiple system atrophy

Gina Puska[a], Mirjam I. Lutz[b], Kinga Molnar[a], Günther Regelsberger[b], Gerda Ricken[b], Walter Pirker[c], Lajos Laszlo[a], Gabor G. Kovacs[b,*]

[a] Department of Anatomy, Cell and Developmental Biology, Eötvös Lorand University, Budapest, Hungary
[b] Institute of Neurology, Medical University of Vienna, Vienna, Austria
[c] Department of Neurology, Wilhelminenspital, Vienna, Austria

ARTICLE INFO

Keywords:
α-Synuclein
Cathepsin D
Endosome
Lysosome
Multiple system atrophy
Neurodegeneration
Parkinson's disease
Prion-like spreading

ABSTRACT

Intracellular deposition of pathologically altered α-synuclein mostly in neurons characterises Parkinson's disease (PD), while its accumulation predominantly in oligodendrocytes is a feature of multiple system atrophy (MSA). Recently a prion-like spreading of pathologic α-synuclein has been suggested to play a role in the pathogenesis of PD and MSA. This implicates a role of protein processing systems, including lysosomes, supported also by genetic studies in PD. However, particularly for MSA, the mechanism of cell-to-cell propagation of α-synuclein is yet not fully understood. To evaluate the significance of lysosomal response, we systematically compared differently affected neuronal populations in PD, MSA, and non-diseased brains using morphometric immunohistochemistry (cathepsin D), double immunolabelling (cathepsin D/α-synuclein) laser confocal microscopy, and immunogold electron microscopy for the disease associated α-synuclein. We found that i) irrespective of the presence of neuronal inclusions, the volume density of cathepsin D immunoreactivity significantly increases in affected neurons of the pontine base in MSA brains; ii) volume density of cathepsin D immunoreactivity increases in nigral neurons in PD without inclusions and with non-ubiquitinated pre-aggregates of α-synuclein, but not in neurons with Lewy bodies; iii) cathepsin D immunoreactivity frequently colocalises with α-synuclein pre-aggregates in nigral neurons in PD; iv) ultrastructural observations confirm disease-associated α-synuclein in neuronal and astrocytic lysosomes in PD; v) lysosome-associated α-synuclein is observed in astroglia and rarely in oligodendroglia and in neurons in MSA. Our observations support a crucial role for the neuronal endosomal-lysosomal system in the processing of α-synuclein in PD. We suggest a distinct contribution of lysosomes to the pathogenesis of MSA, including the possibility of oligodendroglial and eventually neuronal uptake of exogenous α-synuclein in MSA.

1. Introduction

Parkinson's disease (PD) and multiple system atrophy (MSA) are progressive neurodegenerative disorders characterised by the accumulation of the misfolded protein α-synuclein, therefore also denoted α-synucleinopathies (Kovacs, 2016). The main difference between the two disorders is the distribution pattern of the intracellular α-synuclein inclusions: while neuronal Lewy bodies (LB-s) are the hallmark neuropathological features of PD, mostly oligodendroglial inclusions are found in MSA (glial cytoplasmic inclusion, GCI; glial nuclear inclusion, GNI) (Goedert et al., 2017). Emerging evidence supports the notion that various modifications including the oligomer form of α-synuclein contribute primarily to the neuronal cell death by inducing a cascade of pathological changes (Kayed et al., 2003; Rockenstein et al., 2014; Volles and Lansbury Jr, 2007).

The two cellular degrading systems including ubiquitin-proteasomal and lysosomal pathways are involved in this process not just in α-synucleinopathies but in other neurodegenerative disorders like Alzheimer's disease (AD) or Huntington's disease as well (Nixon et al., 2008; Rubinsztein, 2006). Several studies have described that both, the ubiquitin-proteasomal and lysosomal system play a crucial role in the degradation of α-synuclein (Cuervo et al., 2004; Webb et al., 2003). Dysfunction of the protein clearing machinery may lead to impaired α-synuclein turnover whereby the cellular homeostatic balance will be

Abbreviations: AD, Alzheimer's disease; CathD, cathepsin D; ELS, endosomal-lysosomal system; GCI, glial cytoplasmic inclusion; GNI, glial nuclear inclusion; IR, immunoreactivity; LB, Lewy body; MSA, multiple system atrophy; NCI, neuronal cytoplasmic inclusion; NNI, neuronal nuclear inclusion; PD, Parkinson's disease; SN, substantia nigra

* Corresponding author at: Institute of Neurology, Medical University of Vienna, AKH 4J, Währinger Gürtel 18-20, A-1090 Vienna, Austria.
E-mail address: gabor.kovacs@meduniwien.ac.at (G.G. Kovacs).

https://doi.org/10.1016/j.nbd.2018.02.019
Received 23 October 2017; Received in revised form 26 January 2018; Accepted 27 February 2018
Available online 02 March 2018
0969-9961/

injured, eventually resulting in neurodegeneration (Rubinsztein, 2006; Stefanis et al., 2001). A recent study revealed that rat primary neurons with deficiency of a lysosomal K^+ channel, referred to as transmembrane protein 175, are prone to increased α-synuclein deposition via the dysregulation of the catalytic lysosomal enzymes (Jinn et al., 2017). Reduced lysosomal activity as a consequence of the mutation in the aspartyl protease referred to as the cathepsin D (CathD) encoding gene results in neuronal ceroid lipofuscinosis in a mouse model (Koike et al., 2000). Importantly, internalized α-synuclein aggregation is noticeable after α-synuclein infection in a CathD deficient cell line (Bae et al., 2015). Depletion of the lysosomal membrane protein ATP13A2 mediates α-synuclein aggregation-related pathology and cell death caused by decreased lysosomal processing (Usenovic et al., 2012). In addition, there is a link between synucleinopathies and the lysosomal storage disorder Gaucher disease, as reduced β-glucocerebrosidase activity is associated with α-synuclein accumulation in PD and the mutation of the gene encoding glucocerebrosidase is a risk factor for parkinsonism (Siebert et al., 2014). Besides this, diminished CathD level has been described in nigral neurons in the prominently affected PD brain (Chu et al., 2009). These studies, together with further genetic observations, such as association of *VPS35* mutations with PD, suggest a causal relationship between the endosomal-lysosomal system (ELS) and the pathologic α-synuclein (Vilarino-Guell et al., 2011; Zimprich et al., 2011).

Moreover, neurons in cell culture can take up exogenously preformed α-synuclein filaments and partially break down the spawned intracellular diseased protein by lysosomes (Domert et al., 2016; Sacino et al., 2017). These mechanisms overload the protein clearing system and induce cytoplasmic inclusion formation emerging from endogenous α-synuclein (Sacino et al., 2017). It would be crucial to understand the uptake mechanisms of pathologic α-synuclein and the relation with the cellular degradation systems since recent studies seem to confirm that α-synucleinopathies show propagation properties in diseased brain reminiscent of prion disease (Angot et al., 2010; Brundin et al., 2016; Prusiner et al., 2015). Indeed, emerging evidence including human graft transplantation experiments (Kordower et al., 2008; Li et al., 2008) and human *post-mortem* examinations (Kovacs et al., 2014) underpins the role of the ELS in the prion-like cell-to-cell spreading of disease associated α-synuclein in human PD brains. The concept of hierarchical involvement of brain regions as reflected by the Braak stages of PD pathology also supports this (Braak et al., 2003). This latter concept allows the definition of a stage preceding the involvement of the substantia nigra (SN) (i.e., stage 2). Moreover, recent studies on cell culture and mouse models infected with human MSA diseased brain homogenates demonstrate propagation of pathologic α-synuclein related to MSA (Prusiner et al., 2015; Watts et al., 2013; Woerman et al., 2015; Woerman et al., 2017).

The role of the ELS and CathD has been described as one major pathogenetic aspect in experimental and human prion diseases (Arnold et al., 1995; Baron et al., 2002; Borchelt et al., 1992; Caughey et al., 1991; Kovacs et al., 2007; Laszlo et al., 1992; Magalhaes et al., 2002; Taraboulos et al., 1992). Therefore, evaluation of the ELS on a cellular level allows a comparison of neurodegenerative conditions with the model disorder prion disease. In spite accumulating experimental evidence supporting the hypothesis that the lysosomal degrading system is related to the prion-like spreading of α-synuclein, its precise role is not well understood in human diseases. Based on these observations, we aimed to investigate whether the lysosomal system and in particular the enzyme CathD is implicated in these mechanisms in human brains with the neuronal predominant α-synucleinopathy PD and the oligodendroglial predominant α-synucleinopathy MSA.

2. Materials and methods

2.1. Case selection

This study includes the examination of a total of 30 subjects including patients with neuropathologically confirmed MSA and PD, the latter staged following Braak et al. (Braak et al., 2003) and grouped as early (Braak 2; “pre-nigral”) and late (Braak 4; “post-nigral”) stage PD. As controls we selected cases lacking neurological symptoms and neuropathological evidence of neurodegenerative diseases including α-synucleinopathy. Accordingly, we included eight patients with MSA (female: 5, male: 3; mean ± SE age at death: 69.0 ± 3.05), six patients with early (female: 5, male: 1; mean ± SE age at death: 83.7 ± 0.33) and eight with late PD (female: 4, male: 4; mean ± SE age at death: 81.4 ± 1.60), and eight controls (female: 3, male: 5; mean ± SE age at death: 81.0 ± 0.46) (Table 1). We examined one case from all groups for Western blot analysis. One MSA (male, age at death: 57), one early (male, age at death: 69, Braak stage = 2) and one late PD (male, age at death: 67, Braak stage: 6) case were included in the study for electron microscopy. Samples were collected following local regulations and the study was approved by the Ethical Committee of the Medical University of Vienna (“Molecular neuropathologic investigation of neurodegenerative diseases”, Nr. 396/2011).

Table 1
Clinical and neuropathological data of cases involved in the study. Abbrerviations: NP: neuritic plaques score (CERAD), NFD-BB: Braak and Braak stages of neurofibrillary degeneration (Alafuzoff et al., 2008), LRP-B: Lewy-related pathology according to Braak staging (Braak et al., 2003),THAL: Aβ plaque score (Thal et al., 2002).

Nr	Age	Sex	Disease	THAL	NFD-BB	LRP-B	Other
1	78	f	–	0	I	–	–
2	81	m	–	0	I	–	–
3	81	m	–	0	III	–	–
4	81	m	–	0	II	–	–
5	81	f	–	0	II	–	–
6	82	m	–	0	I	–	–
7	82	m	–	0	I	–	–
8	82	f	–	0	I	–	–
9	57	m	MSA	3	I	–	–
10	57	m	MSA	1	II	–	–
11	65	f	MSA	0	II	–	–
12	70	m	MSA	0	0	–	–
13	72	m	MSA	1	III	–	–
14	75	f	MSA	0	0	–	–
15	76	m	MSA	0	II	–	–
16	80	f	MSA	3	III	–	–
17	83	m	PD	0	II	2	–
18	83	f	PD	2	III	2	–
19	83	f	PD	0	III	2	–
20	84	f	PD	0	III	2	–
21	84	f	PD	2	III	2	–
22	85	f	PD	3	IV	2	–
23	74	m	PD	0	II	4	–
24	78	f	PD	0	I	4	–
25	80	m	PD	4	V	4	–
26	80	f	PD	4	VI	4	phTDP-43 +
27	82	m	PD	1	II	4	–
28	83	f	PD	0	I	4	–
29	85	m	PD	0	II	4	–
30	89	f	PD	3	IV	4	–

2.2. Immunohistochemistry

For the confocal microscopic examination, we used formalin-fixed, paraffin-embedded tissue blocks of the pons and the mesencephalon containing the SN. We applied an accurate approach to evaluate colocalisation as immunofluorescence labelling is the most appropriate method to determine the presence of two proteins in the same cellular compartment (Lutz et al., 2017). Immunostaining was performed on 5 μm thin sections. The following antibodies were used: CathD (1:200, rabbit polyclonal, Biogenex, San Ramon, CA), LAMP-2 (lysosomal associated membrane protein 2; 1:50, mouse monoclonal, Santa Cruz Biotechnology, Inc., Dallas, TX), α-synuclein 5G4 (1:2000, mouse monoclonal, Roboscreen, Leipzig, Germany; detecting only the disease-associated forms but not the physiological monomer) (Kovacs et al.,