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Research report

Accumulation of beta-synuclein in cortical neurons is associated with autophagy attenuation in the brains of dementia with Lewy body patients

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ABSTRACT

Dementia with Lewy bodies (DLB) is the second most prevalent neurodegenerative dementia, where an accumulation of aggregated fibrillar alpha-synuclein in neurons of limbic and forebrain regions of the brain leads to visual hallucination, cognitive impairment of a fluctuating nature and extrapyramidal motor disturbances. Beta-synuclein counteracts aggregation of alpha-synuclein *in vitro* and in animal models, however it is not clear whether this effect occurs in human Lewy body dementia (LBD) diseases. Here we examine expression of alpha-, beta-synuclein and autophagy markers in the frontal cortex (BA9) and occipital cortex (BA18-19) of patients with neuropathologically confirmed DLB/LBD and age-matched controls. We provide evidence for neuronal upregulation of beta-synuclein within the frontal cortex and its decrease in occipital cortex of DLB patients. While beta-synuclein-containing neurons were consistently devoid of oligomeric alpha-synuclein in the frontal cortex, we did not observe an overall correlation between total beta-synuclein and 5G4 levels (marker of oligomeric alpha-synuclein). The autophagy markers LC3-II and p62 were increased in the areas of beta-synuclein upregulation in DLB brains, and we show attenuation of autophagy flux when beta-synuclein is overexpressed *in vitro*. Altogether, this data suggests that beta-synuclein changes in DLB may exacerbate neuronal dysfunction caused by accumulation of alpha-synuclein by influencing protein degradation pathways; this should be taken into consideration when designing therapeutic strategies aimed to decrease alpha-synuclein burden in Lewy body diseases.

1. Introduction

Dementia with Lewy bodies (DLB) is the second most common form of neurodegenerative dementia after Alzheimer's disease (Vann Jones and O'Brien, 2014) accounting for approximately 15% of all diagnosed dementias (McKeith et al., 2005). The clinical presentation of DLB is manifested by the cognitive impairment of a fluctuating nature, vivid visual hallucinations, and extrapyramidal motor disturbances, combined with a range of autonomic dysfunctions. Clinical diagnosis of DLB is supported by an onset of motor extrapyramidal dysfunction 12 months or later from the onset of the memory deficit (McKeith et al., 2005). DLB, among other alpha-synucleinopathies such as Parkinson's disease (PD) and multiple system atrophy (MSA), feature the abnormal fibrillization and aggregation of alpha-synuclein in the shape of Lewy bodies (LBs) and Lewy neurites (LNs), a defining neuropathological feature of synucleinopathies. These neuronal inclusions are composed largely of the fibrillar insoluble alpha-synuclein and seen in the frontal cortex, parietal cortex, parahippocampal and cingulate gyri, insula, basal nucleus of Meynert and diencephalon (Dickson et al., 2009). While LB pathology is considered to be the proximate cause for the development of cognitive decline in DLB, Alzheimer's-related pathology (amyloid-beta plaques and neurofibrillary tau tangles) has been also described in cortical and subcortical areas (Compta et al., 2011), and often there is a range of clinical DLB presentations due to the multiple pathology present. Cortical LBs are present in various neurons, including GABA-ergic interneurons and pyramidal neurons (Gomez-Tortosa et al., 2001; Wakabayashi et al., 1995), and display higher density in entorhinal and cingulate gyri than in the occipital cortex (Gomez-Tortosa et al., 2000).

Events occurring prior to alpha-synuclein aggregation in DLB, as well as modifiers of this process, are poorly understood; however existing evidence points towards early synaptic, mitochondrial and proteasome dysfunction. We have shown dramatic redistribution of synaptic proteins in early PD human post-mortem tissues

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(Garcia-Reitbock et al., 2010), while others reported oligomeric alpha-synuclein accumulates at the synapse in early DLB (Kramer and Schulz-Schaeffer, 2007), and alteration of the autophagy-lysosomal pathway (ALP) within the same brain regions (Crews et al., 2010; Miki et al., 2016). Moreover, a recent genome wide association study (Bras et al., 2014) has identified lysosomal pathways as crucially important in DLB.

Another member of the synuclein family – beta-synuclein is also a pre-synaptic protein; it shares 60% homology with alpha-synuclein and presents with a similar pattern of expression in the brain (Spillantini et al., 1995). Under normal conditions, beta-synuclein does not spontaneously aggregate (Clayton and George, 1998) and is not a constituent of LBs (Baba et al., 1998), but seems to be involved in the maintenance of vesicular membrane curvature (Westphal and Chandra, 2013). Beta-synuclein has unfolding chaperone properties, as it prevents aggregation of alpha - synuclein in *in vitro* models (Park and Lansbury, 2003; Wright et al., 2013). Changes in expression of the *SNCB* gene may be crucial for maintenance of the monomeric alpha-synuclein protein's balance in DLB (Beyer et al., 2010). It is not clear, however, if alpha-synuclein aggregation-caused neuronal dysfunction occurs when beta-synuclein expression or function is modulated in the DLB cortex.

In this work, we examined beta-synuclein expression and distribution in the cortical post-mortem tissues of patients with DLB and age-matched controls, and correlated its levels with oligomeric alpha-synuclein and markers of autophagy-lysosomal protein degradation pathways. Our data show that beta-synuclein is selectively upregulated in neurons of the frontal cortex of DLB patients, and that this upregulation is associated with an attenuation of autophagy flux. We suggest that beta-synuclein is an important component of pathological cascade in early DLB, and that cytoplasmic accumulation of beta-synuclein may exert negative influence on neuronal survival.

2. Results

2.1. Human tissues used for the study

AD-type of pathology is frequent in DLB post-mortem tissues (Compta et al., 2011; Pletnikova et al., 2005), where a range of senile plaques and neurofibrillary tangles is observed in cortical regions together with LBs and LNs. "Pure" DLB, without a concomitant AD pathology is relatively rare (Hyman et al., 2012). In order to minimise the influence of AD pathology, we have excluded patients with extensive NFT burden (Braak stage >3). Amyloid β immunoreactivity reports were not available for all cases, and the pathology was confined to mild/moderate diffuse plaques staining in the cortex for the cases where information was provided. We therefore conclude that our cohort had a mild AD associated pathology. The average disease duration is presented in Table 1. Most of the DLB patients had experienced visual hallucination throughout the course of the disease. We have further sub grouped DLB cases according to the level of LB burden in the cortex – low/very low LB burden, with Braak LB stage 2–4, and high LB burden, with Braak LB stage 6, while control patients did not have LB pathology. Higher LB stage represents an increased involvement of other than cortical brain regions and may suggest different degrees of temporal involvement in the pathological process.

2.2. Distribution of beta-synuclein in cortical areas of human brain

Beta-synuclein positive structures were present in both control and DLB brains, apparent as a fine granular staining distributed between neurons, representing the neuropil, as well as beta-synuclein – positive somata. In the cortex, beta-synuclein staining intensity varied between the layers, with layer II showing the highest levels of staining in the frontal cortex, while deeper layers displayed stronger staining in the occipital cortex. Beta-synuclein positive neuropil structures in the cortex had a speckled appearance, homogenously distributed throughout the entire cortical volume, with a higher intensity of staining along the surface of neuronal soma (Fig. 1A, B).

Table 1
Cumulative patient data used for the study.

Patient data	Control	Low LB DLB	High LB DLB
N	16	12	10
Gender (male/female)	11/5	11/1	10/0
Age at death (years ± Std. Error)	80.9 ± 2.42	83.2 ± 2.01	79.1 ± 1.47
Post-mortem interval (hours ± Std. Error)	34.6 ± 3.88	27.4 ± 6.81	38.7 ± 5.57
Disease duration (years ± Std. Error, (n of known cases)	N/A	4.55 ± 0.51 (11)	7.44 ± 0.85 (9)
Braak stage tau (stage/N)	1/4 2/9 3/3	1/0 2/3 3/9	1/2 2/2 3/6
Braak stage alpha-synuclein (stage/N)	0/16	1/0 2/1 3/6 4/5	6/10

While the general pattern of beta-synuclein-positive staining was not changed in DLB, immunohistochemistry revealed more abundant positive neuropil consisting of larger clumps of immunopositive signals, morphologically similar to the redistribution of alpha-synuclein and synaptic proteins we have previously observed (Garcia-Reitbock et al., 2010). Beta-synuclein positive staining was stronger in the frontal cortex of DLB patients and slightly weaker in the occipital cortex (Fig. 1C, D). Beta-synuclein positive cells (15–30 μm in diameter) were distributed throughout the grey matter, with often both cytoplasm and processes stained (Fig. 1B, E). While in the control brain we observed one-two cells per field of vision (objective x40), this was increased in DLB – both in the frontal and occipital cortex (control frontal cortex 0.42 ± 0.15, n = 6; DLB frontal cortex 8.58 ± 5.46, n = 6; control occipital cortex 0, n = 2; DLB occipital cortex 6.67 ± 1.67, n = 3).

2.3. Beta-synuclein upregulation in DLB frontal cortex, independent of alpha-synuclein aggregation

As beta-synuclein staining in the cortical areas of DLB patients was upregulated, we quantified levels of synucleins in brain lysates. Alpha-synuclein levels were decreased in the frontal cortex of DLB patients (Fig. 1 G, Table 2) in both low and high LB group of patients, but were unchanged in the occipital cortex. We detected a significant increase in beta-synuclein in the frontal cortex (in low LB group, with a similar degree of changes in the high LB group), and a decrease in occipital cortex in the low LB group (Fig. 1 H). qPCR analysis of *SNCA* and *SNCB* mRNA in frontal or occipital cortex revealed no significant changes in both alpha-synuclein and beta-synuclein mRNA levels (Fig. 1F). While mRNAs for synuclein were slightly higher in the frontal cortex compared to occipital cortex, the difference was not significant (SNCA, frontal cortex vs occipital cortex p = .14, n = 4; SNCB, frontal cortex vs occipital cortex p = .06, n = 3).

It has been suggested that beta-synuclein counteracts aggregation of alpha-synuclein (Park and Lansbury, 2003; Wright et al., 2013). Since oligomerisation of alpha-synuclein is crucial for the formation of insoluble aggregates, we examined whether alpha-synuclein oligomer-enriched cells in the DLB cortex would display