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Autophagy-related proteins (p62, NBR1 and LC3) in intranuclear inclusions in neurodegenerative diseases

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HIGHLIGHTS

- Intranuclear inclusions (INIs) are recognized as a hallmark of certain neurodegenerative diseases.
- We determined whether autophagy-specific proteins (NBR1 and LC3) are involved in INI formation.
- A significant proportion of INIs were positive for NBR1 and negative for LC3.
- NBR1 may have other functions than autophagy.

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ABSTRACT

Incorporation of ubiquitin and ubiquitin-related proteins including p62 into neuronal intranuclear inclusions (NIIs) has been reported in a variety of neurodegenerative diseases. However, involvement of autophagy-specific proteins (NBR1 and LC3) in NIIs has not been mentioned. We immunohistochemically examined the brain of patients with Machado–Joseph disease (MJD; $n=5$), dentatorubral-pallidoluysian atrophy (DRPLA; $n=5$) and intranuclear inclusion body disease (INIBD; $n=5$), using antibodies against ubiquitin, p62, NBR1 and LC3. The proportion of p62-, NBR1- and LC3-positive inclusions relative to the number of ubiquitin-positive inclusions was calculated in each case. NIIs were positive for p62 in MJD (19.3%), DRPLA (49.7%) and INIBD (99.8%). As for autophagy-specific proteins, NIIs were positive for NBR1 in MJD (4.2%), DRPLA (5.5%) and INIBD (13.2%) and negative for LC3 in MJD, DRPLA and INIBD, except for one case of INIBD. These findings suggest that autophagy–lysosome pathway is not involved in the formation/degradation of NIIs.

1. Introduction

NBR1 (neighbor of BRCA1 gene 1) is ubiquitously expressed and highly conserved protein in the eukaryotic kingdom [3,4,20]. Recently, NBR1 was identified as a binding partner of ATG8 family proteins including microtubule-associated protein light chain 3 (LC3), γ-aminobutyric-acid type A receptor associated protein (GABARAP), GABARAP-like 1 (GABARAPL1), and Golgi-associated ATPase enhancer of 16 kDa (GATE-16 or GABARAPL2) in mammalian cells [10]. The mammalian ATG8 family is known to function in an autophagosome formation, similarly to yeast ATG8 [9]. Importantly, NBR1 interacts with ubiquitin via the ubiquitin associated domain [23]. Based on these properties, it has been proposed that NBR1 functions as a cargo adapter for autophagic degradation of ubiquitinated substrates such as p62, which also has the ability to bind to ATG8 family proteins [8]. p62 is incorporated into a wide spectrum of ubiquitin-positive inclusions both in the cytoplasm and nucleus in various neurodegenerative diseases [1,11,12,16]. p62 is also a component of Marinesco bodies, ubiquitinated intranuclear inclusions in the substantia nigra pigmented neurons in aged population [17]. Indeed, both NBR1 and p62 localize in autophagosomal formation site on the endoplasmic reticulum from the early stage of this process [7]. Recently, it has been reported that LC3, GABARAP and GATE-16 are present in Lewy bodies (LBs) in Parkinson's disease (PD) and dementia with Lewy bodies (DLB) [5,6,22]. Odagiri et al. [18] have shown that

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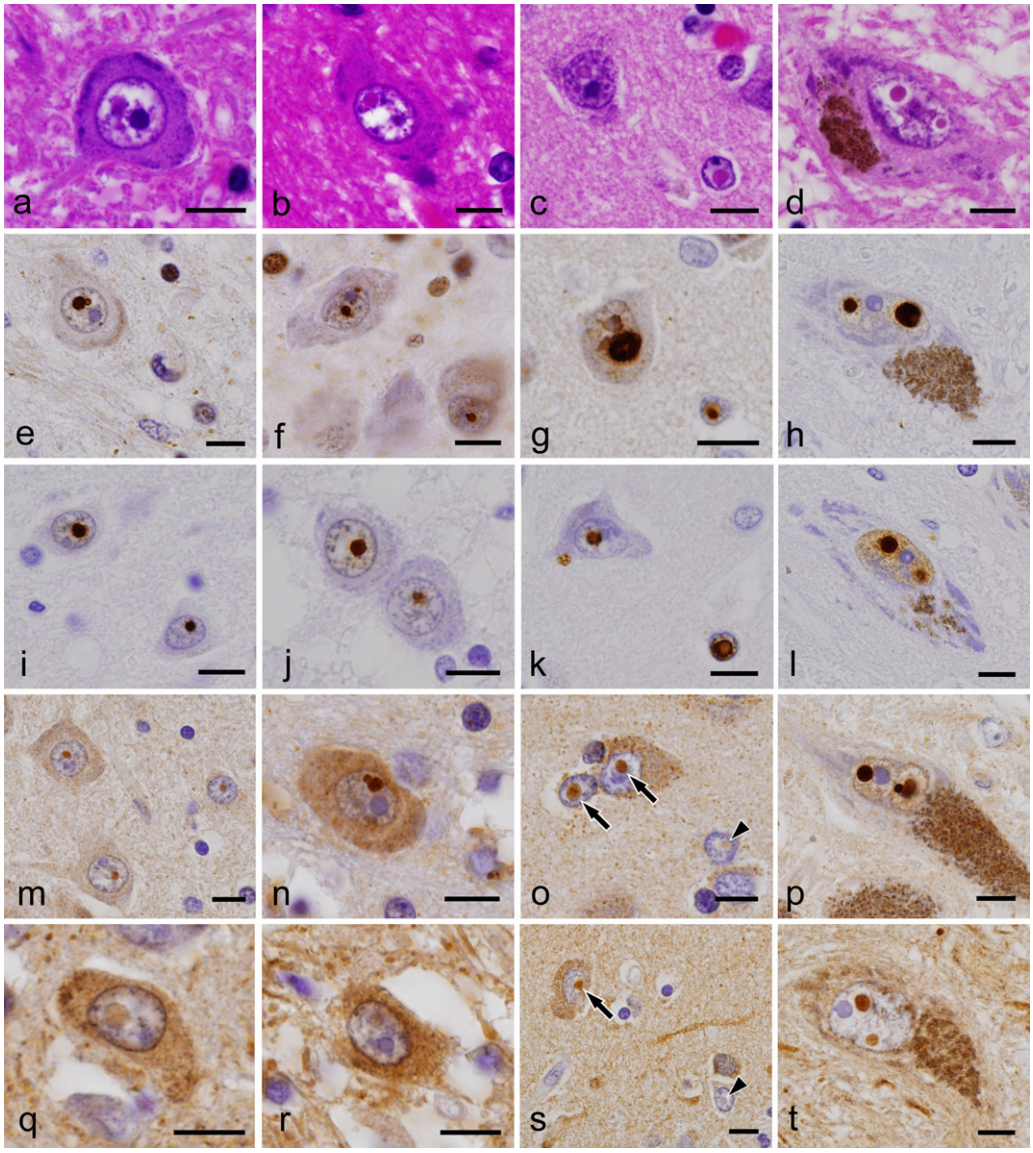

Fig. 1. Light micrographs of neuronal intranuclear inclusions (NIIs) in the pontine nucleus of Machado–Joseph disease (MJD; a, e, i, m, q) and dentatorubral-pallidoluysian atrophy (DRPLA; b, f, j, n, r), the frontal cortex of intranuclear inclusion body disease (INIBD; c, g, k, o, s), and Marinesco bodies in the substantia nigra of control subjects (d, h, l, p, t) stained with hematoxylin and eosin (a–d) or immunostained with antibodies against ubiquitin (e–h), p62 (i–l), NBR1 (m–p) and LC3 (q–t). In INIBD, NIIs (arrows) are positive for NBR1 (o) and LC3 (s) and glial intranuclear inclusions (arrowheads) are immunonegative. Bars = 10 μm.

NBR1 is localized in LBs in PD and DLB as well as in glial cytoplasmic inclusions (GCIs) in multiple system atrophy (MSA), suggesting that NBR1 is involved in the formation of cytoplasmic inclusions in α-synucleinopathy. However, it is uncertain whether NBR1 and LC3 are associated with neuronal intranuclear inclusions (NIIs), a histopathological hallmark of polyglutamine diseases such as Machado–Joseph disease (MJD) and dentatorubral-pallidoluysian atrophy (DRPLA). The NIIs in MJD and DRPLA consist of expanded polyglutamine, which could affect nuclear function and recruit other proteins, resulting in loss of physiological function of recruiting proteins followed by dysfunctions in neurons [21]. Similar mechanisms may exist in the pathogenesis of intranuclear inclusion body disease (INIBD), although the major component of nuclear inclusions in this disease is uncertain [14,15]. In order to investigate whether autophagy-related proteins are involved in the formation of NIIs in neurodegenerative diseases, we immunohistochemically examined the brain of patients with MJD, DRPLA, INIBD and control subjects, using antibodies against ubiquitin, p62, NBR1 and LC3.

2. Materials and methods

2.1. Subjects

Twenty post-mortem cases were utilized in the present study: these included cases of MJD (aged 29–80 years, average 59.6 years, $n = 5$), DRPLA (aged 32–72 years, average 57.8 years, $n = 5$), INIBD (aged 53–78 years, average 68.8 years, $n = 5$) and control subjects (aged 53–84 years, average 67.0 years, $n = 5$). The diagnoses were confirmed histopathologically. The brain of each subject was fixed with 10% buffered formalin and then embedded in paraffin. Four-micrometer-thick sections were cut and stained with hematoxylin and eosin (H&E) and by the Klüver–Barrera method. This study