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THE ROLE OF NEDD8 ULTIMATE BUSTER 1, NUB1, IN NEURODEGENERATIVE α-SYNUCLEINOPATHIES

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Abstract NEDD8 is developmentally down-regulated ubiquitin-like protein, which can conjugate covalently to their target proteins. Recently, we identified NUB1 as a NEDD8-interacting protein, which is composed of 601 amino acid residues with a calculated molecular mass of 69.1 kDa. More recently, we showed that NUB1 is an interferon-induced protein and also recruits NEDD8 to the proteasome for degradation. Here, we performed a yeast two-hybrid screening using NUB1 as bait and isolated the cDNA of synphilin-1 from a human testis cDNA library. Synphilin-1 is a major component of inclusion bodies found in the brains of patients with α-synucleinopathies, including Parkinson's disease. Our biochemical study showed that NUB1 directly interacts with synphilin-1 through its NEDD8-binding site. In addition, our immunohistochemical study showed that NUB1, as well as synphilin-1, accumulates in the inclusion bodies found in the brains of patients with α-synucleinopathies. These findings imply that NUB1 plays a role in the formation of synphilin-1-positive inclusions.

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Key words: α-synucleinopathies; ubiquitin; NEDD8

Background

NEDD8 is a ubiquitin-like protein, that conjugates to a large number of target proteins in a manner analogous to ubiquitination.[1] These target proteins include cullin family members, the von Hippel-Lindau tumor suppressor gene product, and p53.[2-4] Because NEDD8 conjugation modifies the function of target proteins, the conjugation system appears to regulate many important biological events.[5-7]

Recently, we identified NUB1 as a NEDD8-interacting protein, which is composed of 601 amino acid residues with a calculated molecular mass of 69.1 kDa. It possesses a ubiquitin-like (UBL) domain at the N-terminal region and two ubiquitin-associated (UBA) domains at the C-terminal region. In a biochemical analysis, we found that NUB1 recruits NEDD8 and its conjugates to the proteasome for degradation, making NUB1 a downregulator in the NEDD8 conjugation system.[8,9] More recently, to elucidate the function of NUB1, we performed a yeast two-hybrid screening using NUB1 as bait and isolated the cDNA of synphilin-1 from a human cDNA library.[10] Synphilin-1 is thought to link to the pathogenesis of Parkinson's disease (PD) based on its identification as an α-synuclein- and a parkin-interacting protein.[11,12] Moreover, synphilin-1 is a component of Lewy bodies (LB)

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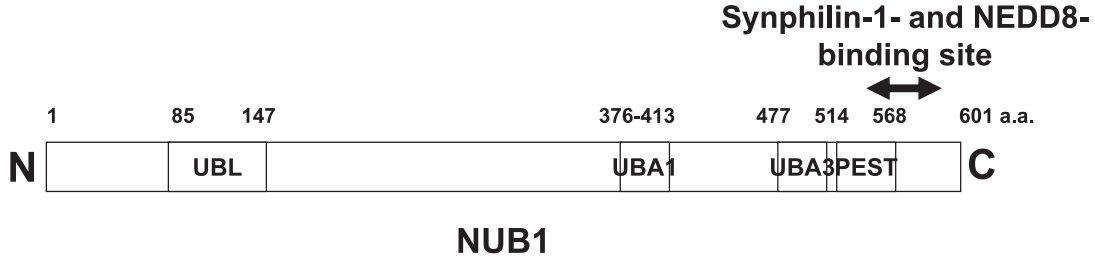

Figure 1 Mapping for the synphilin-1-binding site on NUB1, using yeast two-hybrid system. We prepared cDNAs of human synphilin-1 and the truncated NUB1 by using a polymerase chain reaction (PCR) with appropriate primers.[17] The cDNA of synphilin-1 was subcloned into pGADT7 (a Gal4 DNA-activating domain vector for Gal4-AD fusion), and the cDNA of each mutant of NUB1 was subcloned into pGBKT7 (a Gal4 DNA-binding domain vector for Gal4-BD fusion). The plasmids of the two fusion constructs were then cotransfected into AH109 yeast cells using the lithium acetate method.[18] Transformed yeast cells were grown on a His-/Trp-/Leu- synthetic agar plate for 3 days at 30℃. N; NH2-terminus, C; Carboxyl-terminus, UBL; Ubiquitin-like domain, UBA; Ubiquitin-associated domain, PEST; proline, glutamate, serine, and threonine-rich domain, a.a.; amino acid. Binding site are indicated in arrow.

in brains of PD, dementia with Lewy bodies (DLB), and glial cytoplasmic inclusions in the brains of patients with multiple system atrophy (MSA), collectively referred to as α-synucleinop athies.[13-16]

Here, we demonstrated the interaction between NUB1 and synphilin-1 and showed that NUB1, as well as synphilin-1, was localized in the inclusion bodies found in the brains of patients with α-synucleinopathies.

Interaction of synphilin-1 with NUB1

We used a yeast two-hybrid system to assess the interaction of synphilin-1 with NUB1. The specific protein-protein interaction was determined by the growth of the yeast on the selection plate. We summarized the interaction and its binding site on NUB1 in Figure 1. The schema shows that the C-terminus of NUB1 is crucial to bind to synphilin-1. Interestingly, this region corresponds to the NEDD8-binding site, suggesting that synphilin-1 can be degraded by NUB1, because NUB1 recruits NEDD8 to the proteasome for degradation.

The presence of NUB1 in the cytoplasmic inclusions in brain of patients with α-synucleinopathies

Immunohistochemical studies were performed to determine the presence of NUB1 in samples from the brains of patients with α-synucleinopathies, because synphilin-1 is shown to be localized in cytoplasmic inclusions of α-synucleinopathies.[13] As shown in Figure 2, we found that Lewy bodies are positive for anti-NUB1 antibody. In the brain of patients with MSA, glial cytoplasmic inclusions is also immunostained with anti-NUB1 antibody (data not shown). These observations suggest that NUB1 seems to play an important role in formation of cytoplasmic inclusions under pathological condition of α-synucleinopathies.

Conclusion

We demonstrated that NUB1 interacts with synphilin-1 through C-terminal region and also localizes to cytoplasmic inclusions. Further study is necessary to clarify NUB1 on cytoplasmic and/or intranuclear inclusions in brains of patients with other neurodegenerative diseases.

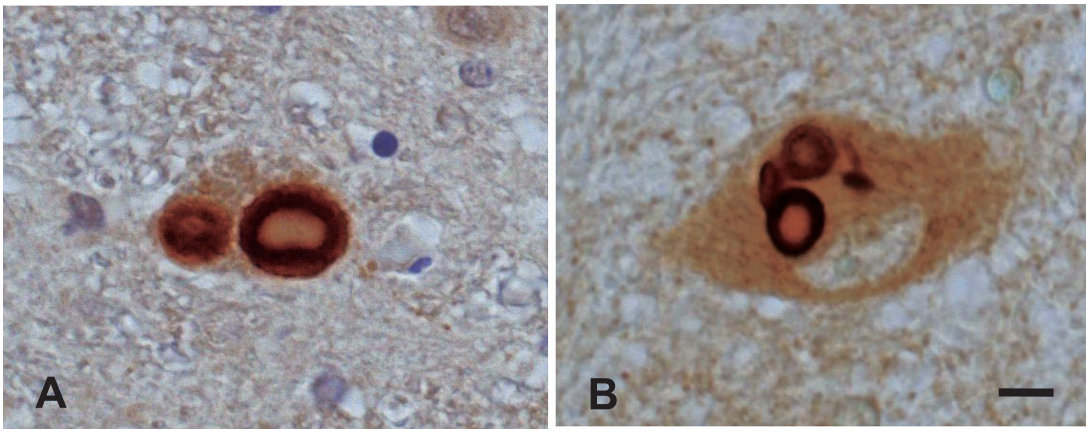

Figure 2 Immunohistochemical localization of NUB1 in the substatia nigra of patients with Parkinson's disease. Lewy bodies are immunostained with anti-NUB1 (A) and anti-synphilin-1 (B). The antibodies used for the immunostaining were polyclonal anti-NUB1 and polyclonal anti-synphilin-1. The sections were immunostained using the avidin-biotin-peroxidase complex method with diaminobenzidine as described previously. [19] Scale bar indicates 10 μm.

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