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弘前大学文部科学研究科 カレッジの学術情報開示システム
THE ROLE OF NEDD8 ULTIMATE BUSTER 1, NUB1, IN NEURODEGENERATIVE α-SYNUCLEINOPATHIES

Kunikazu Tanji\(^1\), Fumiaki Mori\(^1\), Hitoshi Takahashi\(^2\), Tetsu Kamitani\(^3\) and Koichi Wakabayashi\(^1\)

**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.

**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.\(^2\) 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.\(^8\) 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.\(^1\)\(^12\) Moreover, synphilin-1 is a component of Lewy bodies (LB)

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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-synucleinopathies.\textsuperscript{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.\textsuperscript{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.
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