UBL3 interacts with alpha-synuclein in cells and the interaction is downregulated by the EGFR pathway inhibitor osimertinib

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論文題目

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（UBL3 は細胞内で α-シヌクレインと相互作用し、その相互作用は EGFR 経路阻害剤オシメルチニブによってダウンレギュレーションされる）

論文の内容の要旨

[Introduction]

Ubiquitin-like 3 (UBL3) is characterized as a post-translational modification (PTM) factor regulating protein sorting to small extracellular vesicles (sEVs). UBL3 can interact with more than 22 disease-related proteins, including neurodegenerative disease-related molecules. Pathological α-syn can be packaged in sEVs for cell-to-cell transport in synucleinopathies. The formation of α-syn aggregation is influenced by various factors, including protein-protein interactions (PPI). PPI are promoted or inhibited by many drugs and compounds. However, it remains unknown whether α-syn interacts with UBL3 and whether the interaction is affected by clinical drugs. In this study, we aim to investigate whether UBL3 can interact with α-syn in cells, and be affected by clinical drugs.

[Materials and Methods]

The animal study protocol was approved by the Institutional Animal Care and Use Committees of Hamamatsu University School of Medicine (protocol code: 2022024). Firstly, we conducted immunohistochemistry (IHC) staining to detect phosphorylated serine-129 (p-S-129) α-syn in wild type (WT) and *Ubl3* knock-out (*Ubl3*$^{-/-}$) mice. Then, *Gaussia princeps* (Gluc) based split luciferase complementation assay, a powerful approach taking advantage of the reconstruction of the N-terminal and C-terminal fragments of Gluc (NGluc and CGluc), was used to detect protein-protein interactions. NGluc-UBL3, NGluc-UBL3Δ5, and α-syn-CGluc cDNAs were used to explore the interaction between UBL3 and α-syn. And co-immunoprecipitation (co-IP) was performed to confirm the interaction. HEK293 cells transfected with NGluc-UBL3 and α-syn-CGluc were used as the screen model. 32 candidate drugs or compounds were selected to screen the drugs that affect the interaction at a final concentration of 1 and 10 μM. Student’s t-test and one-way analysis of variance with Dunnett’s post hoc test were used for the comparison of the unpaired data and multiple groups, respectively.

[Results]

The IHC results showed that p-S-129 α-syn was significantly upregulated in the substantia nigra of *Ubl3*$^{-/-}$ mice. In the luminescence intensities result of cell culture

medium and cell lysate from transfected cells, strong luminescence intensities were detected in both fractions in the cells expressing NGluc-UBL3 + α-syn-CGluc, and NGluc-UBL3Δ5 + α-syn-CGluc. As a positive control, the samples from Gluc over-expressing cells showed markedly higher luminescence intensities. On the other hand, the luminescence intensities of samples from cells expressed signal cDNA, were as low as the background. The co-IP results confirmed that UBL3 interacted with α-syn in cells.

The interaction level of UBL3 and α-syn was significantly upregulated by 1-Methyl-4-phenylpyridinium (MPP^+) exposure at concentrations of 300 - 600 μM. Under the treatment of 1 μM, the interaction level was upregulated by gemcitabine (ratio = 1.37), while was significantly downregulated by erlotinib (ratio = 0.73). Under the treatment of 10 μM, the interaction level was significantly upregulated by gemcitabine (ratio = 1.53), while was significantly downregulated by erlotinib (ratio = 0.72), and osimertinib (ratio = 0.55).

[Discussion]

The expression level of p-S-129 α-syn was upregulated in the substantia nigra of *Ubl3*$^{-/-}$ mice. α-syn can be transferred cell-to-cell via sEVs. UBL3 may affect misfold α-syn. Ageta et al. reported that UBL3 can modify protein through disulfide binding depending on the cysteine residues at its C-terminal. Our results showed that the interaction between UBL3 and α-syn in HEK293 cells is not completely erased after the deleting mutation of the cysteine residues at its C-terminus. Taken together, UBL3 interacts with α-syn not only dependent on cysteine residues at its C-terminal.

The treatment of osimertinib, a third-generation epidermal growth factor receptor (EGFR)-(tyrosine kinase inhibitor) TKI, downregulated the interaction level of UBL3 and α-syn. EGFR-TKI reduces the p-S-129 α-syn pathology by reducing the seeding and propagation of pathological α-syn. And sEVs-associated α-syn can facilitate the propagation of α-syn through cell-to-cell transfer. These results indicated that interaction between UBL3 and α-syn may crosstalk with the EGFR pathway, associated with the α-syn pathology propagation via sEVs.

The treatment of MPP^+ and nucleoside analog anti-cancer drug gemcitabine upregulated the interaction level of UBL3 and α-syn. MPP^+ exposure and treatment of gemcitabine initiate mitochondrial dysfunction (MD), and MD can induce and promote α-syn accumulation. The interaction between UBL3 and α-syn was involved in the process of induction of MD. Whether the upregulated interaction affects the accumulation of α-syn will need to be investigated in future studies.

[Conclusion]

This study shows that UBL3 interacts with α-syn and the interaction level of UBL3 and

α-syn is upregulated in response to MPP^+ exposure and the treatment of gemcitabine, but downregulated by the treatment of EGFR inhibitor osimertinib. Our study provides the first evidence identifying UBL3 as an interactor of α-syn, and UBL3 may be a new therapeutic option for α-synucleinopathies in the future.