Alpha-synuclein interaction with UBL3 is upregulated by microsomal glutathione S-transferase 3, leading to increased extracellular transport of the alpha-synuclein under oxidative stress

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論文題目

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（α-シヌクレインと UBL3 の相互作用はミクロソームグルタチオン S-トランスフェラーゼ 3 によってアップレギュレートされ、酸化ストレス下で α-シヌクレインの細胞外輸送が増加する）

論文の内容の要旨

[Introduction]

Aberrant aggregation of misfolded alpha-synuclein (α-SYN), a major pathological hallmark of related neurodegenerative diseases such as Parkinson's disease, can translocate between cells. Ubiquitin-like 3 (UBL3) is a membrane-anchored ubiquitin-fold protein and post-translational modifier. UBL3 promotes protein sorting into small extracellular vesicles (sEVs) and thereby mediates intercellular communication. Our recent studies have shown that α-SYN interacts with UBL3 and that this interaction is downregulated by about half after silencing microsomal glutathione S-transferase 3 (MGST3). However, how MGST3 regulates the interaction of α-SYN and UBL3 remains unclear. In the present study, we further explored this by overexpressing MGST3. We hypothesize that MGST3 acts as a modulator to regulate the interaction of α-SYN with UBL3 and mediates the sorting of α-SYN into sEVs, which would provide a new idea to remove the intracellular α-SYN accumulation and thus provide a treatment for synucleinopathies. In the present study, we aimed to investigate whether MGST3 could act as a regulator of α-SYN-UBL3 interaction and attempted to explore the function of MGST3 in regulating this interaction.

[Materials and Methods]

The split Gaussia luciferase (Gluc) complementation assay was used to analyze of the effect of MGST3 on the interaction between the N-terminal end of Gluc of UBL3 (NGluc-UBL3) and α-SYN connecting the C-terminal end of Gluc (α-SYN-CGluc). Western Blotting (WB) and RT-PCR were used to analyze the effect of MGST3 on the expression of α-SYN and UBL3. UBL3 plasmid containing the stable fluorescent tag mStayGold was constructed, and then immunocytochemistry was used to observe the effect of MGST3 on the co-localization of α-SYN and UBL3. Hydrogen peroxide was used to induce oxidative stress assays to detect the effect of the function present in MGST3 on the interaction between α-SYN and UBL3. The HiBiT bioluminescence assay to explore the intracellular and extracellular distribution of α-SYN. This study

was approved by the Recombinant DNA Experiment Safety Committee of Hamamatsu University School of Medicine (approval number: 2-20).

[Results]

In the split *Gaussia* luciferase complementation assay, we found that the interaction between α-SYN and UBL3 was upregulated by MGST3. While WB and RT-qPCR analyses showed that silencing or overexpression of MGST3 did not significantly alter the expression of α-SYN and UBL3, the immunocytochemistry staining analysis indicated that MGST3 increased the co-localization of α-SYN and UBL3. We found that under oxidative stress conditions, MGST3 was able to rescue the inhibition of α-SYN-UBL3 interaction by oxidative stress and promote intracellular α-SYN translocation to the outside of the cell.

[Discussion]

Proteomic analysis revealed that in a protein interactome that relies on the binding of the two cysteine residues at the C-terminus of UBL3 via disulfide bonds to modify its protein interactome, we found that MGST3 was able to influence the interaction between α-SYN and UBL3, while MGST3 did not significantly alter the expression of α-SYN and UBL3. We hypothesize that MGST3 regulates UBL3 by covalently modifying thiols on UBL3 cysteine residues, thereby affecting the interaction between α-SYN and UBL3, which remains to be elucidated by more studies in the future.

Overexpression of MGST3 enhanced the co-localization of α-SYN with UBL3 and in combination with the previous results that silencing MGST3 downregulated this co-localization, this cellular staining further demonstrates that MGST3 serves as a regulator that affects the interaction of α-SYN with UBL3.

It is noteworthy that neither silencing nor overexpression of MGST3 affected cell viability. However, after oxidative stress treatment, the overexpression of MGST3 reversed to an extent the down-regulation of this impact by oxidative stress. Our results demonstrate that MGST3 stabilizes protein-protein interactions under oxidative stress conditions. Furthermore, we found that silencing MGST3 hindered α-SYN transport to the extracellular, whereas overexpression of MGST3 did not significantly alter intracellular α-SYN. Overexpression of MGST3 promoted intracellular α-SYN transport to the extracellular compartment when exposed to oxidative stress conditions.

When neurons are subjected to cellular stress or pathological injury, α-SYN can self-protect through small extracellular vesicle secretion in neurons. UBL3 acts as a post-translational modifier to promote protein sorting into sEVs, thereby reducing the aggregation of α-SYN in the cell. These evidences provide a new therapeutic target for MGST3 as a molecule that regulates the interaction of α-SYN with UBL3 in neurodegenerative diseases.

[Conclusion]

Our results show that silencing of MGST3 inhibits α-SYN and UBL3 interaction, in contrast, this interaction was enhanced by overexpression of MGST3. Under oxidative stress conditions, MGST3 was able to rescue the inhibition of α-SYN-UBL3 interaction by oxidative stress and promoted intracellular α-SYN translocation to the outside of the cell. Overall, our findings provide new insights and ideas for promoting the modulation of UBL3 as a therapeutic agent for the treatment of synucleinopathy-associated neurodegenerative diseases.