UBL3 interaction with α-synuclein is downregulated by silencing MGST3

博士（医学）Zhang Hengsen

論文題目

UBL3 interaction with α-synuclein is downregulated by silencing MGST3

（UBL3 とα-シヌクレインの相互作用は、MGST3 のサイレンシング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), a membrane-anchored ubiquitin-fold protein, serves as a post-translational modifier of proteins, tagging them for sorting into small extracellular vesicles (sEVs). sEVs, with a diameter of 30-150 nm, play crucial roles in mediating cell-to-cell communication and are implicated in neurodegenerative diseases. Alpha-synuclein (α-syn) is a protein which is highly expressed in the central nervous system and the aggregations of α-syn is a major feature of Lewy bodies in neurodegenerative diseases like Parkinson's disease (PD). Interactions between α-syn with specific proteins can influence α-syn aggregation and toxicity. Therefore, regulation of the interaction between α-syn with specific proteins is emerging as a promising therapeutic strategy for neurodegenerative diseases. Recently, UBL3's interaction with α-syn has been discovered, but the regulators remain unknown. In my degree research, I screened the regulators using split gaussian luciferase (Gluc) complementation assay and RNA interference.

[Materials and Methods]

For split Gluc complementation assay, the N-terminal of Gluc connecting with UBL3 (NGluc-UBL3) and α-syn connecting with C-terminal of Gluc (α-syn-CGluc) carried by the pCI vector, and Gluc plasmid were used in the previous study of our laboratory (Chen B et al, 2023). The siRNAs used for silencing target candidate proteins were based on Silencer Select system (ThemoFisher). The plasmids and siRNA were transfected into the human embryonic kidney cells (HEK293). Cell culture medium and cell lysate were collected for the split Gluc complementation assay and the Western Blot using anti-MGST3 antibody, respectively. Hydrogen peroxide (H_2O_2) was used to induce oxidative stress. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide (MTT) cell growth kit was used for measuring cell viability and the following interaction evaluation formula: degree of interaction = luminescence intensity/cell viability. Statistical significance was assessed by two-tailed Student's t-test for two groups. $p<0.05$ was considered statistically significant.

[Results]

Among the tested ten targets, the knockdown of MGST3, well-known to play a

protective role against oxidative stress, most strongly reduced the interaction between UBL3 and α-synuclein (to a half degree). Western blot analysis confirmed successful knockdown of MGST3 expression. The interaction between UBL3 and α-syn was more reduced by 52% at 800 μM H_2O_2 even under the condition of MGST3 knockdown. And the results showed there was no difference of between the groups treated with 800μM H_2O_2 in the presence or absence of silent MGST3.

[Discussion]

MGST3, an enzyme belonging to the MAPEG family, plays a crucial role in cellular protection against oxidative stress by facilitating the removal of electrophilic compounds and reducing H_2O_2 and lipid peroxides. It is widely expressed in various tissues and has been associated with brain structure. The MGST3 was thought to be related to the pathology of neurodegenerative diseases, while the specific influence of MGST3 on α-syn aggregation is yet to be elucidated. Our study highlights its potential involvement and provides a clue for further investigation into the relationship between MGST3 and α-syn aggregation in neurodegenerative diseases.

Based on our results, we hypothesized that MGST3 can form a complex with UBL3 and α-syn. The presence of MGST3 can use glutathione to break down H_2O_2 in the vicinity of the complex, thereby protecting the UBL3-α-syn interaction from being downregulated by H_2O_2. In the future, it would be interesting to investigate whether overexpression of MGST3 can prevent the downregulation of the UBL3-α-syn interaction by H_2O_2.

It is still unclear how H_2O_2 alters the interaction of UBL3 with α-syn. The C-terminal cysteine residues of UBL3 are essential for the post-translational modification of the target protein with UBL3, but that modification seemed not to occur to the interaction of UBL3 with α-syn. It is possible that H_2O_2 affect the intracellular interaction of UBL3 with α-syn by altering the covalent modification of UBL3 with other unknown factors mediated by the C-terminal cysteine residues.

Oxidative stress, resulting from excessive production of reactive oxygen species (ROS), plays a significant role in neurodegenerative diseases. Patients with PD exhibit ROS overproduction, and oxidative damage. ROS can trigger the formation of Lewy bodies, a characteristic feature of PD. Our finding that the interaction between UBL3 and α-syn was downregulated by oxidative stress, prompted inquiries into its potential contribution to neurodegenerative processes. The downregulation of UBL3's interaction with α-syn by oxidative stress promotes the α-syn free from the UBL3-α-syn complex disrupting the transfer of α-syn to the extracellular compartment by UBL3. This interferes with the potential role of UBL3 in preventing α-syn aggregation within the cell, leading to the accumulation of α-syn and the formation of aggregates.

[Conclusion]
MGST3 plays a role in regulating the interaction between UBL3 and α-syn.