Understanding protein homeostasis mechanisms to reduce the risk of Alzheimer's disease

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This project aims to identify important chaperones to combat Alzheimer's disease (AD), which is facing a super-aging society. The chaperone group Protein Disulfide Isomerase (PDI) family is also known as an endoplasmic reticulum (ER)-localized enzyme, and abnormal chemical modifications and mutation insertions have been found in AD patients. There are more than 20 members of the PDI family in the ER, but their functions is still unknown. Therefore, this project aims to find factors that reduce the risk of the accumulation of protein aggregation from the PDI family, the ER-resident chapeorne.

To this end, our group established an *in vitro* screening method to search for factors that reduce the risk of the accumulation of protein aggregation. In addition, our group tackled to elucidate the mechanism by the structure of the complex between amyloidogenic proteins and the PDI family is determined.

From the *in vitro* screening method established in this project, we discovered that the phase separation of PDIA6, a member of the PDI family, strongly inhibits amyloid fibril formation. This is a newly discovered new function of chaperones. To confirm the specifics of substrate uptake into PDIA6, we next used insulin and detected the substrate uptake by fluorescence microscopy. This results revealed that the dye-labeled insulin is incorporated into the phase separation of PDIA6. We also found that the phase separation of PDIA6 strongly inhibited amyloid fibril formation of insulin.

To interpret the phase separation mechanism of PDIA6 from the viewpoint of structural biology, we further carried out NMR analysis. The phase separation of PDIA6 is driven by calcium, which present in the ER at ~mM order. We identified the calcium binding site, and observed chemical perturbation in the first and last domains of the three domains within PDIA6, and identified the calcium binding site. Furthermore, in order to clarify the phase separation mechanism of PDIA6, which does not have an intrinsically disordered region, we identified the minimum unit domain required for the droplet formation through biochemical experiments. Transient but specific electrostatic interactions occur between the first and last domains to form the droplet. Here, we used a holotomography microscope that takes advantage of the refractive index to obtain structural information inside of the droplet. By separating each domain and evaluating the phase separation ability using a phase contrast microscope and a holographic microscope, we were able to identify the region essential for the phase separation.

The inhibition of amyloid fibril formation by chaperone phase separation needs to be verified intracellularly in the future. It will also need to be verified in model mice of various neurodegenerative diseases.

The results obtained through this project are basic research findings that target chaperone phase separation, which inhibits amyloid fibrils, and lead to elucidation of the causes of neurodegenerative diseases. A deeper understanding of the mechanisms of neurodegenerative diseases will lead to the establishment of treatments.