Local Redox Imbalance Induced by Intraorganellar Accumulation of Misfolded Proteins
オルガネラ内に蓄積した凝集タンパク質が引き起こす局所的なレドックス破綻

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学位論文内容要旨

Proper folding, maturation, and stabilization of newly synthesized proteins are vital for protein function. These include complicated processes composed of multiple reactions and steps. Although proteins are thermodynamically stable when they have reached the lowest free energy conformation, some of the newly synthesized proteins cannot achieve the correct structure. Even after proteins attain a mature conformation, cellular stresses such as heat shock can cause proteins to misfold, exposing the hydrophobic regions on the molecular surface. These misfolded or unfolded proteins in cells should be cleared by protein quality control systems because their existence trigger cellular toxicity. However, cellular stresses beyond the capacity of protein degradation system causes the accumulation of aberrant proteins in cells, which cause cellular dysfunction and eventual cell death. It is well known that such accumulation of abnormal or aggregated proteins is implicated with the pathogenesis of various diseases such as neurodegenerative diseases and diabetes.

Maintaining the homeostasis of redox state in cells is essential in cell survival. Cells are often exposed to various external stresses and are known to produce harmful reactive oxygen species (ROS) in response to such stresses. Not only the external stresses but also internal stresses such as misfolded proteins do cause intracellular ROS production. To deal with such oxidative stresses, cells have antioxidant mechanisms such as glutathione, catalase and peroxiredoxin but continuous ROS production and imbalance of the redox state in cells are known to cause damage to cells and eventual cell death. However, it is not clear how the misfolded proteins accumulated in a specific organelle affect the local redox state and how such a local redox imbalance in organelles affects cells.

In this study, I focused on three cases with abnormal protein accumulation inducing cellular toxicity via intraorganellar redox imbalance. In the first chapter, the mechanism underlying neuronal cell death caused by the intracellular accumulation of extracellularly added amyloid beta peptide was analyzed. Amyloid beta (Aβ42), the main cause of Alzheimer’s disease, is prone to form aggregate and thought to induce cell death by accumulating in cells. In Chapter 1, I elucidated the mechanism by which Aβ42 incorporated into cells leads to cell death by using chemically synthesized Aβ42 variants. The Aβ42 variant Aβ42 (E22P) accumulated in lysosomes at an earlier stage than wild-type Aβ42. On the other hand, Aβ42 (E22V) did not accumulate in cells or affect the cell viability. Intracellular localization of EGFP-Galectin-3 showed that accumulation of oligomerized Aβ42 in lysosomes caused lysosomal membrane permeabilization (LMP). Overexpression of lysosome-localized LAMP1-fused peroxiredoxin 1 and treatment with U18866A attenuated Aβ42-mediated LMP and cell death. These findings showed that lysosomal ROS generation by toxic conformer of incorporated Aβ led to cell death via LMP.

The second chapter shows ER redox dynamics following proteasome inhibition. Proteasome and ER are closely related in terms of ER associated（protein）degradation, however, there are few studies on how the redox state in the ER changes as the activity of the proteasome decreases. In Chapter 2, I revealed an ER reductive shift under proteasome inhibition and the underlying mechanism. Pre-treatment of BSO, an inhibitor of glutathione synthesis system in cells, and knockdown of ATF4 suppressed the reductive shift caused by proteasome inhibition. The amount of reduced glutathione in microsome fraction increased, although that of total glutathione content in whole cells decreased. The amount of secreted mIns2 and hα1AT proteins decreased under proteasome inhibition. The reductive shift might delay protein maturation in the ER by preventing disulfide bond formation. Taken together, I concluded that proteasome inhibition activated ATF4 through PERK arm in UPS and induced the influx of reductive glutathione into the ER and, by doing so, suppressed the secretion capacity for the proteins.

In Chapter 3, I newly established a high-throughput screening system to identify “ER-redox modulators” under the accumulation of misfolded proteins in the ER. When ER-stress was induced by the accumulation of mIns2 (C96Y), ER redox state became more oxidative. I established a high-

throughput screening system of “ER-redox modulator” based on the ER redox state using ERroGFP S4. As a result, two “ER-redox modulators” capable of attenuating ER redox imbalance were identified.

（注）字数は 2000 字程度（英語の場合は 800 語程度）とし、明朝体（英語の場合は Times New Roman）、11 ポイントで作成すること。続紙可。

（学位論文内容要旨の続紙）（氏名　　奥　勇紀　　）