【Grant-in-Aid for Scientific Research (S)】

A Study on stress responses by co-creation of liquid-liquid phase separation and autophagy

Principal Investigator	Juntendo University, Graduate School of Medicine, Professor KOMATSU Masaaki	Researcher Number : 90356254
Project Information	Project Number : 24H00060	Project Period (FY) : 2024-2028
	Keywords : Phase separation, Autophagy, p62 body, Proteostasis, Stress response	

Purpose and Background of the Research

●Outline of the Research

We have been promoting pathophysiological analysis of autophagy using genetically engineered mice for a quarter of a century. As a result, it has been clarified that the suppression of autophagy causes various diseases (tumorigenesis and neurodegeneration) accompanied by the accumulation of protein aggregates and degenerative organelles, and that the accumulation of substrates that should be "selectively" degraded by autophagy is involved in the pathogenesis of these diseases. Degenerated and ubiquitinated proteins, which can be substrates for selective autophagy, need to be "concentrated in the cytoplasm" and "converted from a liquid to a gel-like state". The representative has identified p62 as a key protein for this concentration and gelation and found that p62 droplets (called p62 bodies) are produced in response to a wide range of stresses. Furthermore, we have shown that p62 body, which contains a large amount of degenerated and ubiquitinated proteins, is finally degraded in a selective autophagy-dependent manner and also activates stress response pathway with changes in physical properties . In other words, p62 bodies formed by liquid-liquid phase separation were found to be key players in "co-creating" proteostasis and cellular homeostasis in response to stress by "cooperating" with autophagy (Figs. 1 and 2).

Fig. 1 Proteostasis through phase-separated p62 body

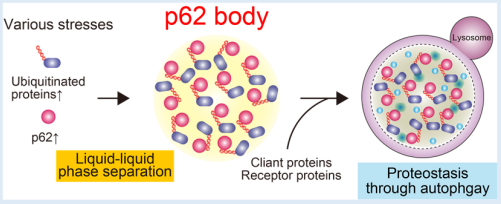
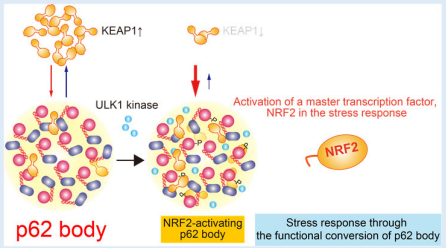


Fig. 2 Stress response through phase-separated p62 body



However, the molecular basis and pathophysiological significance of p62 body formation and regulation in response to various stresses have not been systematically analyzed. At this point, the academic "questions" at the core of this research project are the following three points:

1. What molecules localize to the p62 body in response to various stresses and become selective substrates for autophagy?
2. What is the molecular basis of biological responses and selective autophagic degradation of p62 bodies containing these selective substrates?
3. What is the pathophysiological significance of their biological response and degradation?

These are the main questions we would like to answer in this research project (Fig. 3).

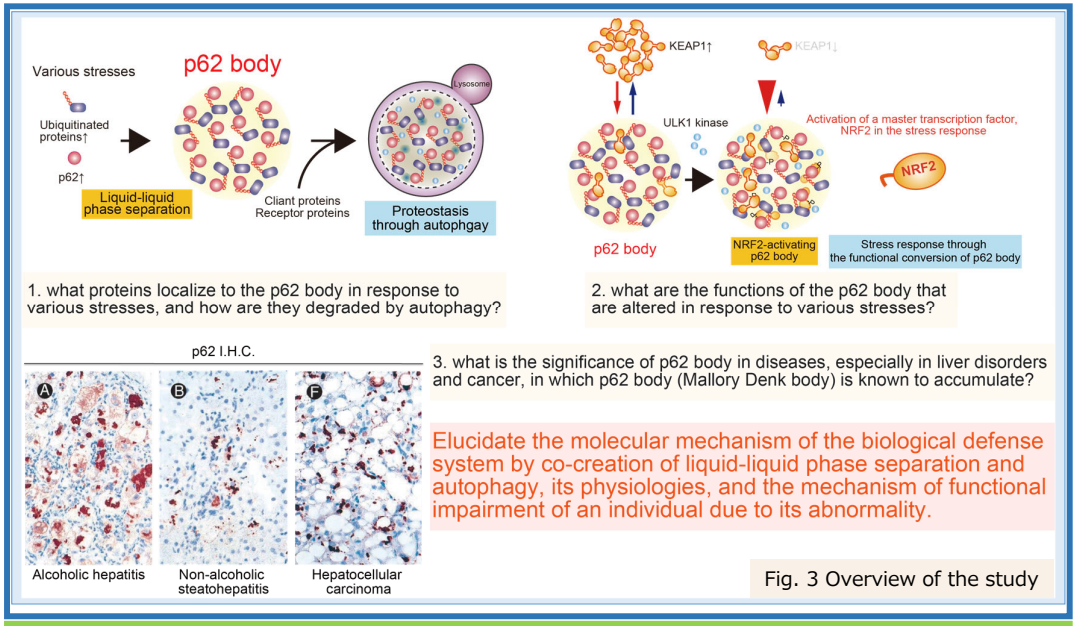


Fig. 3 Overview of the study

Expected Research Achievements

In this study, we will conduct comprehensively screening for novel components and regulators of the p62 body in response to various stresses by making full use of an innovative methods for purification of the p62 body and a selective autophagy inhibition, which were originally developed by the principal investigator. We will examine these novel p62 components and regulatory factors using pioneering protein analysis methods such as AlphaFold-Multimer, a highly accurate protein binding prediction method, and high-speed atomic force microscopy analysis and elucidate the molecular mechanism(s) of p62 body-dependent defense mechanism. We will also generate and analyze mice genetically engineered for p62 itself or novel p62 body components and regulators to elucidate the physiological functions of p62 body-dependent defense *in vivo*. At the same time, together with the project members, we will focus on liver disorders, in which abnormal p62 body formation and degradation are thought to be involved in the pathogenesis and examine the dynamics of p62 body using multiple liver disease model mice, human three-dimensional liver germ organoids, and human liver disease specimens. Furthermore, we will develop Nanobody, which can artificially manipulate p62 body.

The expected ripple effects from this study are

1. Elucidation of the formation and regulation mechanisms of cytoplasmic droplet p62 bodies will provide a new basis for liquid-liquid phase separation in cell biology.
2. The elucidation of the overall picture of autophagic degradation of ubiquitinated proteins via p62 phase separation will lead to the understanding of the whole picture of intracellular degradation integrated with other degradation systems such as the ubiquitin-proteasome system.
3. The biological defense by p62 body is directly involved in the pathogenesis of various pathological conditions, especially in metabolic diseases and cancer. It is expected that promotion of this research will lead to improvement of human health and prevention of diseases.

Homepage Address, etc.