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

Broad Section E

Title of Project： Chemical Challenge for Elucidation of the Molecular Mechanism of Lipid Peroxidation-mediated Necrosis

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Research Project Number： 21H05029 Researcher Number： 60192142
Term of Project： FY2021-2025 Budget Allocation ： 141,600 Thousand Yen
Keyword： Cell Death, Necrosis, Lipid Peroxidation, Affinity Labeling

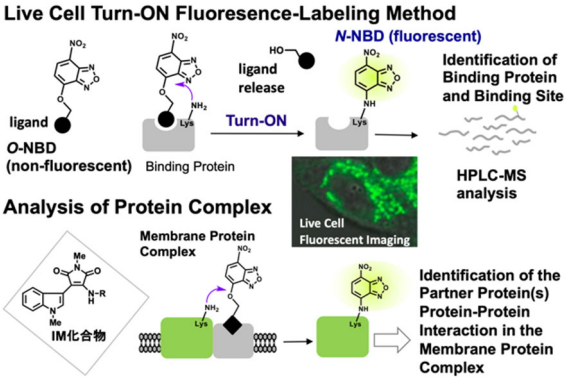
【Purpose and Background of the Research】

Cell death is one of the most fundamental phenomena, and elucidation of its mechanism is one of the most important propositions in life science. The dysregulation causes various diseases such as cancer, autoimmune disease, and neurodegenerative disease. Since the existence of a programmed cell death called apoptosis was revealed in 1980s, cell death research has become very active, and the regulatory mechanism of apoptosis has been elucidated. On the other hand, cell death called necrosis, which involves swelling and rupture of cells, was originally considered to be passive cell death caused by severe injury. However, in the 2000s, various types of necrosis with a regulatory mechanism were discovered, and among them, necrosis involving lipid peroxidation has attracted attentions.

We have developed IM compounds that selectively inhibit oxidative stress-induced necrosis (*Bioorg. Med. Chem. Lett.* **2005**, *15*, 3114) and have shown excellent protective effects in myocardial infarction models. (*ACS Med. Chem. Lett.* **2018**, *9*, 182). It was also found that IM compounds inhibit only two types of cell death, called ferroptosis and NETosis, which have the common feature that lipid peroxidation is involved, and do not suppress other cell deaths (*ACS Med. Chem. Lett.* **2019**, *10*, 1272). Ferroptosis is known as cell death in which iron-induced lipid peroxidation is involved, and NETosis is a characteristic cell death in which neutrophils release NETs (Neutrophil Extracellular Traps) containing their own DNA and other components to capture and kill bacteria. In this study, we aim to elucidate the regulatory mechanism of the lipid peroxidation-mediated necrosis by using small molecule cell death control compounds such as IM compounds as probes.

【Research Methods】

We have developed a unique method for target protein identification/fluorescence imaging of small molecules, called Turn-ON fluorescence labeling method using a relatively small fluorescent group, nitrobenzoxadiazole (NBD) (*Chem. Sci.* **2014**, *5*, 1021). When an *O*-NBD probe binds to a target protein, it undergoes a selective substitution reaction with a nearby lysine residue due to the proximity effect, becomes *N*-NBD, and fluorescence is turned on. Unlike photoreactive groups, *O*-NBDs do not degrade until they encounter a lysine residue. Therefore, *O*-NBDs can be used not only to identify target proteins and binding sites, but also to analyze protein complexes by selecting the appropriate introduction position and linker.



Previous studies suggest that the target of IM may be a membrane protein complex. Therefore, by analyzing the target protein complex of the IM compound using the NBD method, the regulatory mechanism common to ferroptosis and NETosis will be elucidated. In addition, we have already found compounds that induce ferroptosis, and compounds that promote or suppress the induction of NETosis. By using chemical methods such as the NBD method, we will elucidate the mechanism of action of these compounds and gain insight into the unique regulatory mechanisms of ferroptosis and NETosis.

【Expected Research Achievements and Scientific Significance】

Since ferroptosis plays an important role in ischemia-reperfusion injury such as myocardial and cerebral infarction, and NETosis plays an important role in immunity, the elucidation of the regulatory mechanism is expected to contribute to the development of new therapeutic agents for these diseases.

【Publications Relevant to the Project】

- T. Yamaguchi, M. Asanuma, K. Dodo, M. Sodeoka *et al.* Turn-ON Fluorescent Affinity Labeling Using a Small Bifunctional *O*-Nitrobenzoxadiazole Unit: Selective Labeling and Imaging of Target Protein. *Chem. Sci.* **2014**, *5*, 1021-1029.
- K. Dodo, Yotsumoto, K. Asano, T. Suda, M. Tanaka, and M. Sodeoka *et al.* Development of a water-soluble indolylmaleimide derivative IM-93 showing dual inhibition of ferroptosis and NETosis. *ACS Med. Chem. Lett*. **2019,** *10*, 1272-1278.

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