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

Diversity of oxidized lipids and their functional understanding

Principal Investigator	Kyushu University, Faculty of Pharmaceutical Sciences, Professor YAMADA Ken-ichi　　Researcher Number:60346806
Project Information	Project Number : 23H05481　　Project Period (FY) : 2023-2027 Keywords : lipid, oxidation, function, cell death, protein

Purpose and Background of the Research

●Research Outline

Unsaturated fatty acids, which are cell membrane components, are readily oxidized to produce oxidized lipids. The oxidized lipids produced are likely oxidative damage byproducts. In the recent development of genetic modification and mass spectrometry technologies, various functions of oxidized lipids have been revealed, including their roles in cell death, inflammatory reactions, and disease induction, and they have attracted considerable attention. For example, ferroptosis induced by oxidized lipids was reported in 2012. Ferroptosis has been implicated in many diseases, including ischemia-reperfusion injury and neurodegenerative diseases, such as ALS and Parkinson's disease. In addition to ferroptosis, oxidized lipids have also been reported to have a variety of other functions, such as promoting inflammatory responses and allergies, and complexes of oxidized lipids and proteins are involved in angiogenesis. However, numerous studies to date have used commercially available oxidized lipids, and given the diverse functions of oxidized lipids, many oxidation products may exist. We detected several hundred types of oxidized lipids, which is sufficient to assume that there may be many more functions of oxidized lipids. In this study, we focus on the "diversity" of oxidized lipids and aim to analyze and identify the causative molecules and elucidate their functions.

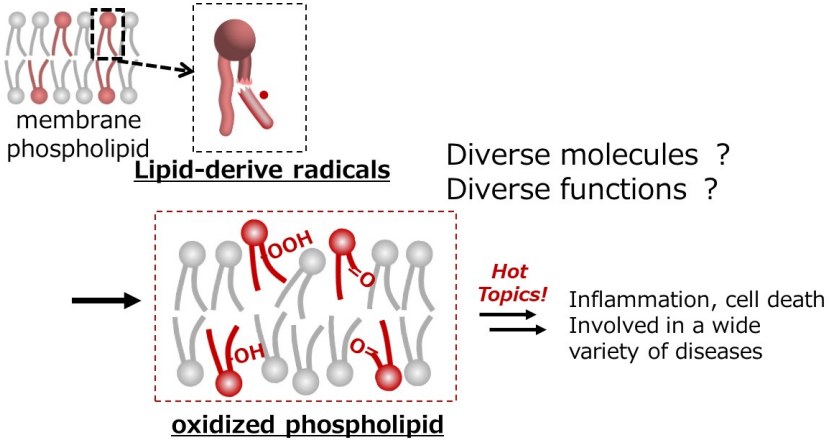
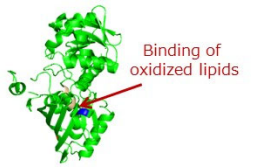


Figure 1. Do oxidized lipids have diverse molecules and functions?

●Research Methods

1) Structural analysis of oxidized lipids: We have successfully detected 465 types of oxidized phosphatidylcholines (oxPCs) (*Nat Commun*. 2021), including 309 new substances. As this technique advances, we will able to detect more molecules and oxidized lipid molecules produced in various diseases.

2) Oxidized lipids site of action analysis: Oxidized lipids contain highly reactive molecular species, such as aldehydes, which may readily modify proteins. Additionally, there is evidence that specific oxidized lipid metabolites with proteins can induce angiogenesis or accumulate in the retinas of patients with age-related macular degeneration. In this study, we conducted an adductome analysis of the interactions between proteins and the oxidized lipid species described above and analyzed their functions. Also, we evaluated the cellular responses. Although oxidized lipids-induced cell death has been investigated in several studies, little is known about oxidized lipid molecules, their downstream factors, and the signaling pathways involved in cell death. Hence, in this study, we analyzed three cell death processes: ferroptosis, lipoxytosis, and apoptosis. We will also elucidate where oxidized lipids are generated and how they induce cell death. Similarly, we will analyze and evaluate inflammatory reactions in animal diseases.



Analysis of modified adducts by LC-MSMS

Figure 2. Analysis of oxidized lipids modifies proteins and other substances to regulate their functions.

Expected Research Achievements

To clarify the site of action of oxidized lipids, it is essential to identify their molecular species. To address this issue, principal investigators have developed a detection probe for lipid-derived radicals (*Nat Chem Biol*, 2016; Anal Chem, 2023), which are the starting point of lipid peroxidation reactions, and found that 132 lipid-derived radicals can be generated (*Anal Chem*, 2020). In addition, we have been working on the structural analysis of 465 types of oxidized phospholipids as phosphatidylcholine-derived oxidized lipids (*Nat Commun*, 2021). In this study, we will (1) comprehensively analyze the structures of these oxidized lipid molecules, identify the molecules that increase during cell death induction, and identify the molecules by organic chemical synthesis. Next, (2) to elucidate the point of action, we will analyze protein modifiers (adductome) and identify proteins that can be modified during disease. Finally, using the above techniques, we will (3) clarify the induction mechanism of cell death (ferroptosis, lipoxytosis, and apoptosis) involving oxidized lipids, identify and functionally analyze oxidized lipid molecules that induce inflammatory effects, analyze and evaluate them in animal models of disease, and clarify the signaling pathways triggered by oxidized lipids. The above three points will allow us to better understand the diversity of oxidized lipids and elucidate their functions.

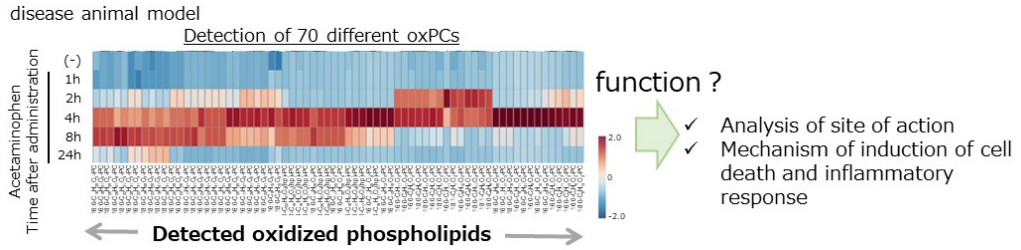


Figure 3. What are the functions of the various oxidized lipids?

This research will create and develop novel academic fields and basic drug discovery research.

Homepage Address, etc.	https://bukka.phar.kyushu-u.ac.jp/