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

Formation of biological barriers by diverse ceramides

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Purpose and Background of the Research

●Outline of the Research

The lipids ceramides are composed of a diverse group of molecules consisting of 30 classes and over 1,000 molecular species (Figure 1). The significance of the presence of a variety of ceramide classes or the lipids containing them (sphingolipids) is that each class has a distinctive role. For example, the stratum corneum (SC), the outermost layer of skin, contains acylceramides and protein-bound ceramides, which play an essential role in the formation of the permeability barrier (Figure 2). In the nervous system, sphingolipids (galactosylceramides) containing α-hydroxy ceramides are present in the myelin, which acts as an insulator for nerve impulses produced by neurons, forming an insulating barrier (Figure 2). The purpose of this study is to elucidate the detailed structure and composition of the various ceramides present in our body, and to clarify how they are synthesized and degraded, what physiological functions they have, and how they are related to diseases.

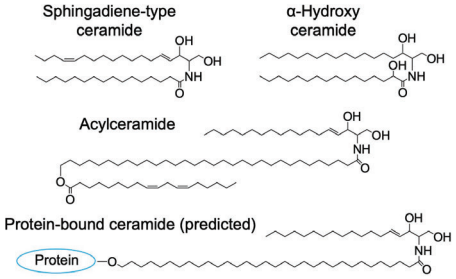

Figure 1. Ceramide Classes

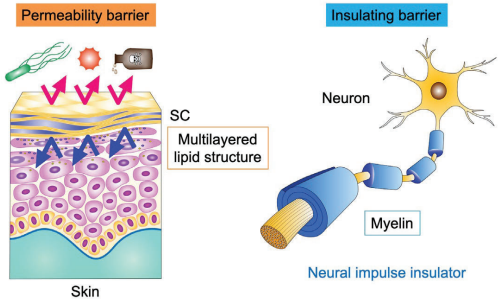

Figure 2. Barrier Formation by Ceramides

●Ceramides as a Permeable Barrier

The SC has a multilayered lipid structure that prevents the invasion of pathogens and allergens from the outside and the loss of water from the body (Figure 2). One of the most abundant lipids in this structure is ceramides. Abnormalities in the skin's permeability barrier increase the risk of infection and atopic dermatitis, and congenital defects in the barrier formation lead to the skin disease ichthyosis. The SC contains a variety of ceramide classes, including acylceramides and protein-bound ceramides. However, the method that separates and quantifies all ceramide species has not been established, leaving the overall picture of SC ceramides being unclear. In addition, despite its importance, the exact structure of protein-bound ceramides has not even been clarified.

●Ceramides as an Insulating Barrier

Myelin is a lipid-rich structure that wraps around the axon of a neuron by multiple layers and serves as an insulator (Figure 2). Galactosylceramides containing an α-hydroxy ceramide are abundant in myelin and play an important role in myelin formation and maintenance. Sphingadiene-type ceramides are also abundant in the nervous system. We recently identified a gene involved in its production (*FADS3*), but its role in the insulation barrier formation still remains unknown.

Expected Research Achievements

●Establishment of Ceramide Analysis Method

This study aims to establish a method for comprehensive ceramide measurement by combining liquid chromatography (LC) and tandem mass spectrometry (MS/MS) (Figure 3). LC separates ceramides by their hydrophobicity, while MS/MS separates ceramides by their mass and the mass of ceramide fragments cleaved by collision of inactive gas. These three steps of separation are expected to enable specific detection and quantification of all ceramide species. The established ceramide analysis method can be used for profiling SC ceramides from patients with skin diseases, leading to the identification of the ceramide species responsible for the pathogenesis and the usage in diagnosis of the diseases.

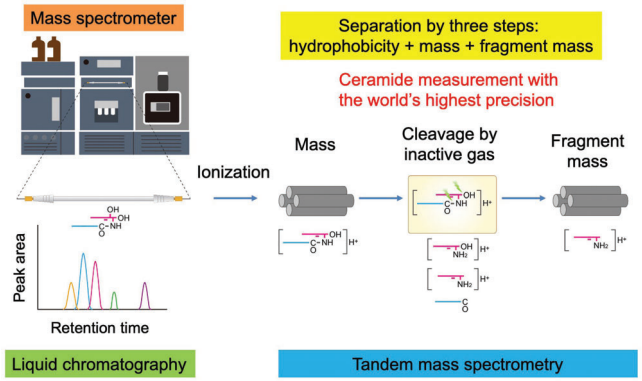

Figure 3. Ceramide Analysis Method

●Production Mechanism and New Functions in Permeability Barrier of Ceramides

The structure of acylceramides has already been determined, and the production pathway and synthesis genes have been mostly elucidated, mainly by us. On the other hand, the exact structure of protein-bound ceramides has not yet been clarified. Therefore, by clarifying it, this study will elucidate its role in the formation of multilayered lipid structure in SC and reveal the molecular mechanism of ichthyosis pathogenesis. We have recently found that acylceramides and protein-bound ceramides, which had been thought to exist only in the skin, are present in the oral cavity and esophagus. This suggests that these ceramides are also involved in the formation of permeability barriers in the oral cavity and esophagus, and their elucidation will provide insight into the infection defense mechanisms in these tissues.

●Ceramide Homeostasis and Insulating Barrier

Ceramide levels are maintained nearly constant by a balance between synthesis and degradation, and abnormalities in either lead to disorder. We will elucidate the role of sphingadiene-type ceramides in the insulation barrier formtation by analyzing the *FADS3* gene knockout mice. Regarding ceramide degradation, we have identified the *HACL2* gene specifically involved in the degradation of α-hydroxy ceramides and the *ALDH3A2* gene involved in the degradation of all types of ceramides. In this study, we will analyze model mice deficient in these genes to elucidate the molecular mechanisms by which impairment of ceramide homeostasis causes diseases.

Homepage Address, etc.	http://www.pharm.hokudai.ac.jp/seika/index.html