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

Dissecting mechanisms of functional cell death "corneoptosis" and stratum corneum homeostasis regulated by pH and its clinical application

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Purpose and Background of the Research

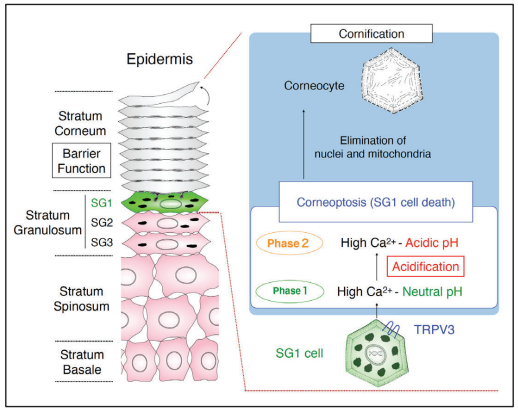
●Outline of the Research

The skin acts as a protective barrier by covering our delicate body. The stratum corneum (SC), the outermost layer of the skin, is composed of layers of dead cell bodies of keratinocytes, which underwent differentiation and a unique type of cell death. However, the SC maintains a constant thickness, i.e., a constant number of dead cells. In this study, we will determine the mechanism of unique cell death of keratinocytes (corneoptosis) and the SC homeostasis at the molecular level. Dead cells do not have an energy-consuming mechanism. Therefore, we will focus on pH and investigate the mechanism in detail from the perspective of pH regulation.

Specifically, we will focus on SG1 cells, the uppermost keratinocytes in the stratum granulosum, which are close to the cell death stage and will become the SC, in mice using a technique that enables simultaneous observation of calcium ion (Ca^{2+}) and pH in real time (live imaging method). Using this technique, we found that during the SG1 cell death (corneoptosis), intracellular Ca^{2+} is elevated for approximately 1 hour (phase 1), and subsequent acidification (phase 2) is necessary to eliminate nuclei and mitochondria without causing any inflammation (Figure 1). We will further analyze and elucidate the precise molecular mechanism of the corneoptosis and also perform live imaging methods of SC pH to determine how the SC maintains its homeostasis

This study has three specific aims (Figure 2). We aim to (1) determine the process of corneoptosis at the molecular level, (2) determine the molecular mechanisms how SG1 cells continue to differentiate after corneoptosis and how SC maintain thickness as well as barrier function from the perspective of pH regulation and, (3) develop a novel therapeutic strategy for atopic dermatitis (AD), which causes persistent inflammation of the skin, by controlling SC pH and skin microbiota.

Figure 1 SG1 cell death (Corneoptosis)



Expected Research Achievements

1. Determine the molecular mechanism of corneoptosis

To determine ion channels expressed in SG1 cells that allow Ca^{2+} and proton (H^+) transport, we will isolate SG1 cells and perform RNA sequencing (RNA-seq) analyses. Next, we will develop mice with SG1 cells that do not express the candidate ion channels that were detected by the analysis using genome editing technology (epidermal mosaic gene disruption method). Furthermore, we will perform live Ca^{2+} and pH imaging in SG1 cells of these mice and examine whether abnormalities have occurred in the process of intracellular Ca^{2+} elevation (phase 1) and/or acidification (phase 2) during corneoptosis, thereby clarify the molecular mechanisms of corneoptosis phases 1 and 2.

2. Determine the molecular mechanism of maintenance of SC homeostasis

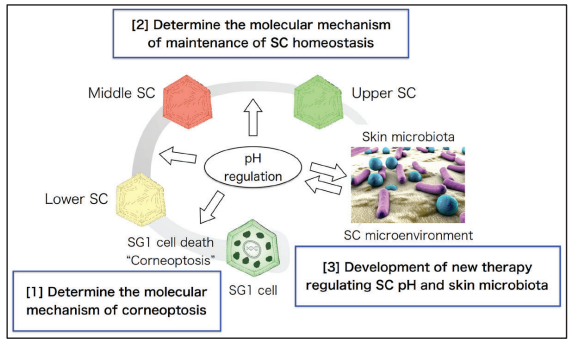
Inflammatory skin diseases, such as AD, exhibit keratinocytes with nuclei in the SC (called parakeratosis) and higher pH compared to normal healthy skin. These results suggest that corneoptosis is impaired and pH distribution of SC is abnormal. The SC is rich in lipids such as phospholipids and ceramides, and mice lacking certain phospholipase- and ceramide-degrading enzymes are known to exhibit skin higher pH than normal healthy skin. Therefore, we will perform nontargeted lipidomics analysis to determine the characteristic lipid components (phospholipids, ceramides, fatty acids, etc.) in SC. Next, we will perform live SC pH imaging in mice that are unable to produce the lipid components detected by the analysis as well as in AD mouse models. Subsequently, we will examine the pH value and pH distribution in SC, and how they differ from those of wild-type mice. Furthermore, we will determine molecules and mechanism that help maintain regular SC pH distribution.

3. Development of new therapy regulating SC pH and skin microbiota

Various skin microorganisms (skin microbiota) inhabit the SC, and an imbalance in the skin microbiota (dysbiosis) is associated with the development of AD. Therefore, it may be possible that the skin microbiota is involved in SC pH changes. Using a technique that allows for simultaneous live imaging of SC pH and fluorescently labeled *Staphylococcus aureus*, we will investigate the extent to which *S.aureus* invades and proliferates in the SC as disease progresses in AD mouse models. We will also investigate how the SC pH value and SC pH distribution change as *S.aureus* proliferates, and further elucidate the relationship between SC pH and skin microbiota.

Furthermore, we will perform RNA-seq analysis of the skin and its microbiota in AD mouse models to identify skin commensal bacteria that produce metabolites useful for maintaining a healthy SC pH. Using these bacteria and metabolites, we will develop novel therapeutic strategies for AD and evaluate their efficacy using AD mouse models.

Figure 2 Three specific aims in our study



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