主論文の要約

Histological analysis of the skin of *Abca1*-deleted mice: A potential model for dry skin

（Abca1欠損マウスの皮膚の組織学的分析：乾皮症モデルとしての可能性）

名古屋大学大学院医学系研究科　総合医学専攻
社会生命科学講座　環境労働衛生学分野
（指導：加藤 昌志　教授）
邓 雨奇

【Introduction】

ATP-binding cassette transporter A1 (ABCA1/Abca1) mediates the release of cellular phospholipids and cholesterol as well as various precursor sterols in generation of nascent high-density lipoprotein (HDL) particles. Dysfunction of ABCA1 molecules by genetic mutation results in the development of Tangier disease (TD), which is known to have a severe HDL deficiency in plasma and excessive cholesterol accumulation in tissues. In previous studies, four of five patients with TD were found to have dermal deposition of lipids in their skin, and dry skin developed in 30%-79% of patients with TD caused by dysfunction of ABCA1. However, there is very limited information about the effect of dysfunction of ABCA1 in cutaneous tissue.

【Objects】

Due to the function of ABCA1 as a regulator of lipids, this study focused on the sebaceous glands (SGs), which are adnexa rich in lipids. SGs in the skin generate sebum, which consists of various lipids including cholesterol, triglycerides, fatty acids and squalene, and contribute to skin moisture by maintaining the skin barrier.

【Methods】

Male *Abca1*-KO(-/-) mice and littermate wild-type (WT) mice at 7 weeks of age were used in this study after approval of Nagoya University, Japan (approval number: 30258 and 17-65). The serum level of HDL in *Abca1*-KO(-/-) mice was about 3% of that in WT mice, corresponding to the characteristics of TD in humans.

Back skins from *Abca1*-KO(-/-) mice and WT mice were dissected and fixed in 4% paraformaldehyde (PFA) overnight at 4˚C, immersed in 1% PFA for 24 hours, and then transferred to 10% sucrose overnight, after which the solution was changed to 20% sucrose for 2 hours. The specimens were embedded in OCT compound and cut into 4-μm-thick sections, followed by H&E staining. Back skins from *Abca1*-KO(-/-) mice and WT mice were dissected without fixation, followed by immediate freezing with liquid nitrogen. Frozen sections were then used for immunofluorescent staining (primary antibody: mouse monoclonal antibody against *Abca1* 1:50, Santa Cruz, sc-53482; secondary antibody: Alexa594-anti-mouse IgG 1:3000, Invitrogen and DAPI for counterstaining) and Filipin staining (Sigma).

The number of hair follicles per mm, ratio of expanded follicular canals per total number of follicles and areas of sebaceous glands in WT mice (n = 3) and *Abca1*-KO(-/-) mice (n = 3) were determined by the software program WinROOF (Mitani Corp.). Fluorescent signals were observed under a fluorescent microscope (Leica DMI6000B). Mean pixel intensities were evaluated by the software program WinROOF (Mitani Corp.). Three serial sections from each mouse were used for analysis.

Stratum corneum (SC) hydration was measured by a Corneometer® CM825 (Courage &

Khazawa, Germany). The amount of hydration was detected as electrical capacitance using anesthetized animals at 1-4 days after hair removal with a hair remover (Veet, Reckitt Benckiser). Epidermal hydration was measured in an environment with room temperature of 23 ± 2˚C and humidity of 55 ± 10%. The measurement started on the second day after hair removal (23 h later) and was conducted 4 consecutive days. The parameter of hydration was obtained by triplicated measurements of 6 spots from WT mice and *Abca1*-KO(-/-) mice and the mean value was expressed for each group.

【Results】

To investigate the effect of ABCA1 on the epidermis and SGs, we examined the expression levels of ABCA1 in WT mice and *Abca1* knockout mice [*Abca1*-KO(-/-) mice]. ABCA1 was detected in SGs in WT mice, but ABCA1 expression was undetectably low in *Abca1*-KO(-/-) mice (Fig. 1A, B). Further histological analysis showed an increased number of hair follicles and expanded follicular canals in *Abca1*-KO(-/-) mice compared to those in WT mice (Fig. 1C-E). Quantitative analysis showed increased sizes of SGs in *Abca1*-KO(-/-) mice compared to those in WT mice (Fig. 1F). Next, we biochemically measured total cholesterol (TC), free cholesterol (FC) and cholesteryl esters (CE) in the skin of *Abca1*-KO(-/-) mice. *Abca1*-KO(-/-) mice had significantly increased levels of TC and FC, but not CE level, in skin compared to those in WT mice (Fig. 1G). The difference of FC levels in the liver between WT mice and *Abca1*-KO(-/-) mice was also significant but limited (Fig. 1H), as previously reported. To further characterize the alteration of sebum composition, we determined the level of FC by performing filipin staining. Increased levels of FC in the epidermis and sebocytes were found in *Abca1*-KO(-/-) mice compared with WT mice (Fig. 1I). After identifying the location of SGs by phase contrast images, quantitative analysis showed increased intensity of fluorescence in *Abca1*-KO(-/-) mice compared with that in WT mice (Fig. 1J). We then measured the skin moisture level in *Abca1*-KO(-/-)-mice. The skin moisture level in *Abca1*-KO(-/-)-mice after hair removal was significantly lower than that in WT mice (Fig. 1K).

【Discussion】

Our results show that deletion of Abca1 expression promotes sebaceous hyperplasia. The increases accumulation of FC in the epidermis and SGs possibly resulted from suppression of lipid release by deleted ABCA1 molecules. Abca1 was shown to function not only in sterol release but also in cellular cholesterol homeostasis. ABCA1 is required for sterol transport from the plasma membrane to the endoplasmic reticulum (ER) for sterol sensing since its deficiency causes cholesterol accumulation in the plasma membrane in mouse embryo fibroblasts (MEFs). It is therefore possible that accumulation of cholesterol at the plasma membrane causes the increased number of hair follicles and enlarged sebaceous glands. In previous studies, covalent modification of signaling proteins in the Wnt and Shh pathways

with cholesterol was also shown to be essential for the morphogenesis of hair follicles. Therefore, it is possible that accumulation of cholesterol in the epidermis and sebaceous glands modulates the Wnt- and Shh-mediated signaling pathways.

Dry skin is characterized by decreased SC hydration and histological abnormalities of the epidermis and dermis including expanded follicular canals, being similar to the histological impairment in *Abca1*-KO(-/-) mice in this study. In addition, this study showed that the skin moisture level in *Abca1*-KO(-/-) mice after hair removal was significantly lower than that in WT mice. Considering the histological phenotypes and lower moisture level in *Abca1*-KO(-/-) mice, it is possible that *Abca1*-KO(-/-) mice have the phenotype of dry skin. The mechanism of the low moisture level in the skin of *Abca1*-KO(-/-) mice is unclear. While further investigation using a tissue-specific gene knockout mouse is required to fully elucidate the molecular mechanism of ABCA1-mediated dry skin, this study showed morphological impairments of the skin in *Abca1*-deleted mice and the results of this study provide valuable information for the development of a drug to treat TD patients with dry skin.

【Conclusion】

The histological phenotypes and lower moisture level in *Abca1*-KO(-/-) mice found in this pilot study suggest that these mice are potential model mice for dry skin.