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Immunohistochemical localization of fatty acid transporters and MCT1 in the sebaceous glands of mouse skin

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ABSTRACT
The sebaceous glands secrete sebum to protect the epidermis and hairs by the oily products. The glands express several transporters and binding proteins for the production of fatty acids and uptake of their sources. The present immunohistochemical study examined the expression and localization of CD36, MCT1, FATP4, and E-FABP in the sebaceous glands, including the meibomian and preputial glands of mice. CD36 and MCT1 in sebaceous glands were largely co-localized along the plasma membrane of secretory cells, while they were separately expressed in the glandular portion of meibomian and preputial glands. Immunoreactivities for FATP4 and E-FABP appeared diffusely in the cytoplasm of secretory cells. Genetic deletion of CD36 did not affect the immunolocalization of the three other molecules. The sebaceous glands were judged to be useful for analyzing the functions and relation of fatty acid transporters and binding proteins.
serve as materials for lipogenesis via acetyl-CoA. Thus, the sebaceous glands throughout the body contain an intense MCT1 immunoreactivity in the plasma membrane of secretory cells along their entire lengths (19). The sebaceous glands in hairless regions such as the eyelids, penis, and anus also show a similar staining pattern for MCT1. In the present paper, we examined the four fatty acid-related molecules in sebaceous glands, meibomian gland, and preputial glands by immunostaining and immunoelectron microscopy, focusing on their relationships.

Eight-week-old male mice of the ddY strain were supplied by Japan SLC (Hamamatsu, Japan). CD36-knockout mice were kindly provided by Dr. Mason W. Freeman of Harvard Medical School (12) and maintained on a C57BL6/J background. Wild-type and CD36-knockout littersmates were bred from the same cross and therefore were of identical genetic background. For immunohistochemistry at the light and electron microscopic levels, deeply anesthetized mice were perfused via the aorta with a physiological saline, followed with 4% formaldehyde plus 0.2% picric acid in 0.1 M phosphate buffer, pH 7.4. The fixed tissues were dipped in 30% sucrose solution overnight at 4°C, embedded in OCT compound (Sakura Finetek, Tokyo, Japan), and quick-frozen in liquid nitrogen. Frozen sections, about 14 μm in thickness, were mounted on poly-L-lysine-coated glass slides and stained by the immunofluorescence method. After immersion in 0.01 M phosphate buffered saline (PBS) containing 0.3% Triton-X100, the sections were pre-incubated with a normal donkey serum. They were stained overnight with a mixture containing two of either a rabbit or guinea pig anti-mouse MCT1 antibody (469–493 amino acids of mouse MCT1) (7), a rabbit anti-rat E-FABP (1 : 2000) (22), a rabbit monoclonal anti-human FATP4 (SLC27A4) antibody (1 : 2000; ab200353; abcam) or a goat polyclonal anti-mouse CD36/SR-B3 antibody (1 : 1000; AF2519; R&D Systems, Minnesota, MN, USA). The sites of antigen-antibody reactions were detected by using Cy3-labeled anti-goat or guinea pig IgG (1 : 400 in dilution; Jackson ImmunoResearch) and AlexaFluor 488-labeled anti-rabbit IgG (1 : 200 in dilution; Invitrogen, Carlsbad, CA). Some of the immunostained sections were counterstained with SyTO 13 (SYTOX, Invitrogen) for observation of the nuclei. Stained samples were mounted with glycerin-PBS and observed under a confocal laser scanning microscope (Fluoview; Olympus, Tokyo, Japan). The specificity of the immunoreactions on sections was confirmed according to a conventional procedure, including absorption tests. In the silver-intensified immunogold method for electron microscopy, frozen sections, 10–14 μm in thickness, were pretreated with normal donkey serum for 30 min, incubated with either the rabbit anti-FATP4 or anti-E-FABP antibody (1 μg/mL overnight, and subsequently reacted with goat anti-rabbit IgG covalently linked with 1-nm gold particles (1 : 200 in dilution; Nanoprobes, Yaphank, NY). Following silver enhancement using a kit (HQ silver; Nanoprobes), the sections were osmicated, dehydrated, and directly embedded in Epon. Ultrathin sections were prepared and stained with an aqueous solution of uranyl acetate and lead citrate for observation under an electron microscope (H-7100; Hitachi, Tokyo, Japan).

Intense immunoreactivities for CD36 and MCT1 were observed in the plasma membrane of glandular cells along their entire length in the sebaceous glands. The two transporter molecules appeared to be co-expressed in hair-associated sebaceous glands of the lips and eyelids (Fig. 1a–c). However, their cellular localization slightly differed in each gland: MCT1-expressing cells tended to occupy the basal (deeper) region of glandular acini, while CD36-expressing cells were located close to the excretory ducts (arrows in Fig. 1a, b). There appeared a clear discordance of the cellular localization in large-sized sebaceous glands, such as the preputial glands and meibomian glands. Meibomian glands in the eyelids, a representative of free sebaceous glands, demonstrated an intense immunoreactivity for MCT1 on whole cell membranes of secretory cells occupying the middle and peripheral regions of the glands, while the CD36-immunoreactive cells gathered in the center regions close to the excretory ducts. The preputial glands associated with the penis also displayed a similar staining pattern (Fig. 2). In the CD36-knockout mice, the expression of CD36 was entirely eliminated, but the MCT1 expression did not change in the immunoreactivity (data not shown).

In contrast to MCT1 and CD36, E-FABP and FATP4 were immunolocalized diffusely in the cytoplasm of secretory cells in sebaceous glands (Figs. 3–5). Immunoreactivity for FATP4 essentially occupied the ductal side of acini (Fig. 5), while E-FABP was more intense on the ductal side of acini (Figs. 3, 4). Therefore, E-FABP and CD36 tended to co-localize in the acini (Fig. 3). The absence of CD36 did not affect the staining results of E-FABP and FATP4 in the sebaceous glands. Electron microscopically, the cytoplasm among lipid droplets contained immunogolds showing the existence of E-FABP and FATP4 in the
Immunohistochemical localization of CD36, MCT1, E-FABP, and FATP4. Immunoreactivities for both CD36 and MCT1 are localized along the cell membrane of alveolar cells in cutaneous sebaceous glands of the lip (Fig. 1a–c). The CD36 immunoreactivity occupies the more apical portion of the alveoli as indicated by arrows. In the preputial gland (Fig. 2), the immunoreactivities of CD36 and MCT1 are separately localized. The antibody against E-FABP labels the cytoplasm in the sebaceous gland of the eyelid, and the immunoreactivity appears to be colocalized at a cellular level with that of membrane-bound CD36 (Fig. 3a, b). In a double staining of E-FABP and MCT1, the E-FABP immunolabeling occupies the apical portion of the alveoli in contrast to the immunoreactivity for MCT1, which is more intense in the basal portion (Fig. 4). Another double staining of CD36 and FATP4 shows a slight gap in the predominant localization within the alveoli (Fig. 5). Figs. 4 and 5 are the sebaceous gland of the eyelid. Bars 50 μm (Figs. 1, 2), 20 μm (Figs. 3–5).
sebaceous glands (Fig. 6). Although the immunogolds tended to gather around the lipid droplets, we could not obtain any clear evidence of a restricted localization to special organella.

Variable staining for CD36 has been reported in the normal epidermis, with some studies reporting no CD36 immunoreactivities in the human epidermis (1). In the mouse epidermis, CD36 is weakly expressed but upregulated in both dermal and epidermal layers after barrier disruption (5, 16). CD36 was localized as a membrane-associated protein in a human sebocyte cell line (13). An *in vitro* study using the same
cell line has demonstrated that the CD36-mediated uptake of free fatty acids facilitates the production of β-defensin, an anti-microbial peptide (13). Immunohistochemistry using tissue sections of rat sebaceous glands reported the localization of CD36 with heavy labeling on the cell membrane of glandular cells (24). We confirmed the membrane-bound expression in the mouse sebaceous glands and further clarified a unique localization of CD36 different from that of MCT1 in meibomian and preputial glands: CD36 was expressed in the more mature glandular cells, while MCT1 was weak in those cells. Analyses of CD36-deficient mice and humans did not show any apparent skin abnormalities (9). In accordance, our mouse model with genetic deletion of CD36 did not affect the essential expression of MCT1 or fatty acid binding proteins in the sebaceous glands.

The epidermis of the skin intensely expresses MCT1 in the basal layer, where GLUT1 with a sufficient expression is also provided (20). Cellular localization along the entire length of the plasma membrane is common between the two nutrient transporters. These transporters must supply nutrients derived from the circulation for cell division and proliferation in the epidermis, a representative tissue consistently renewing itself (20). Here, sebaceous glands were more intensely immunoreactive for MCT1 than GLUT1. The MCT1 immunoreactivity was very intense at the margin of glandular portion but gradually decreased in reactivity toward the excretory ducts (19). The active lipogenesis in the holocrine cells indicates a simple role for MCT1 in the uptake of monocarboxylates, possibly acetate, for the production of the oily secretions. The intense localization of MCT1 in interstitial cells of Leydig in the testis also suggests the involvement of MCT1 in the uptake of acetic acid for biosynthesis of the steroid hormone. Although the co-localization of CD36 and MCT1 is found in the brown adipose tissue and testis also suggests the involvement of MCT1 in the synthesis predominates in the sebaceous gland and fetal epidermis (16). FATP4 regulates the trafficking of very long-chain fatty acids necessary for the synthesis of sebum lipids. FATP4 may play crucial roles in the development and maturation of both sebaceous glands and meibomian glands as well as in the production of sebum (10). Although FATPs have been reported to be present in the plasma membrane (15), the present immunostaining at light and electron microscopic levels detected FATP4 mainly in the cytoplasm of sebaceous glands. The subcellular localization of FATPs in mammalian cells is known to vary from plasma membrane to distinct organelles (8). The transfected FATP4 suggests that FATP4 is an endoplasmic reticulum-localized membrane protein (11).

The present study is the first to demonstrate the localization of four fatty acid-related molecules in the sebaceous glands. As the preputial gland is huge and isolated from surrounding tissues, it is a suitable subject for further study regarding the functional analyses of fatty acid transporters and binding proteins.

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