Immunohistological Localization of Peroxisome Proliferator-Activated Receptor α and γ in Human Sebaceous Glands

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Immunohistological Localization of Peroxisome Proliferator-Activated Receptor α and γ in Human Sebaceous Glands

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Abstract

The immunohistological localization of peroxisome proliferator-activated receptor α (PPARα) and PPAR γ was examined in 28 pilosebaceous units in 10 paraffin-embedded normal human skin specimens. Rabbit polyclonal antibody against human PPARα and monoclonal antibody against human PPARγ were used as specific primary antibodies. The nuclear and cytoplasmic expression of PPARα was detected in basal to differentiated sebocytes. In contrast, the expression of PPARγ was confined to nuclei of suprabasal to early-differentiated sebocytes. The nuclear PPARγ expression was present only occasionally in the basal sebocytes. These results suggest that PPARα and PPARγ are integral parts of sebocyte differentiation in human sebaceous glands.

Key words : sebaceous gland, peroxisome proliferator-activated receptor α, peroxisome proliferator-activated receptor γ, sebocytes

Peroxisome proliferator-activated receptors (PPARs), which are transcription factors activated by fatty acids and their derivatives, belong to the nuclear hormone receptor family[1)]. Three PPAR isotypes, PPAR α, PPAR β, and PPAR γ, have been shown to be present in the interfollicular epidermis of mouse and rat embryos, but to disappear progressively from this site except for pilosebaceous units after birth[1)2)]. Human keratinocytes express all three PPARs[3)]. In terms of the functions of these isotypes, PPARβ is known to increase keratinocyte survival by inhibiting apoptosis[4)], while PPARα and PPARγ are critical transcription factors for lipogenesis in sebaceous glands[5)~9)]. Moreover, PPARγ is essential for the development of sebaceous glands[10)]. However, conflicting findings have been reported regarding the immunohistological localization of PPARα and PPARγ in human pilosebaceous units[5)7)].

We examined formalin-fixed and paraffin-embedded tissues of 10 normal skin specimens (perilesional normal skin of non-inflammatory epidermoid cysts located in the head, neck, back, and upper extremities). Sections were deparaffinized with xylene for 10 min and rehydrated through a graded ethanol series. Antigen retrieval was performed using Heat Processor Solution pH6 (Nichirei Biosciences Inc., Tokyo, Japan) at 100 ℃ for 40 min, and endogenous peroxidase was blocked by incubating the sections with 3 % H_2O_2 (Nichirei Biosciences Inc., Tokyo, Japan). The sections were then incubated with rabbit polyclonal antibody against PPARα (aa153-181 ; Lifespan BioSciences, Seattle, WA, USA) and monoc-

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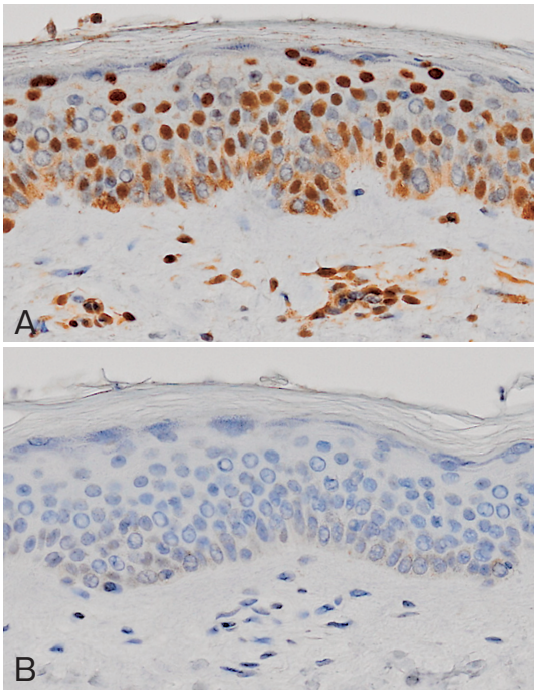

Fig. 1 **A** : Immunohistological localization of PPAR α in normal human epidermis. **B** : Immunohistological localization of PPARγ.

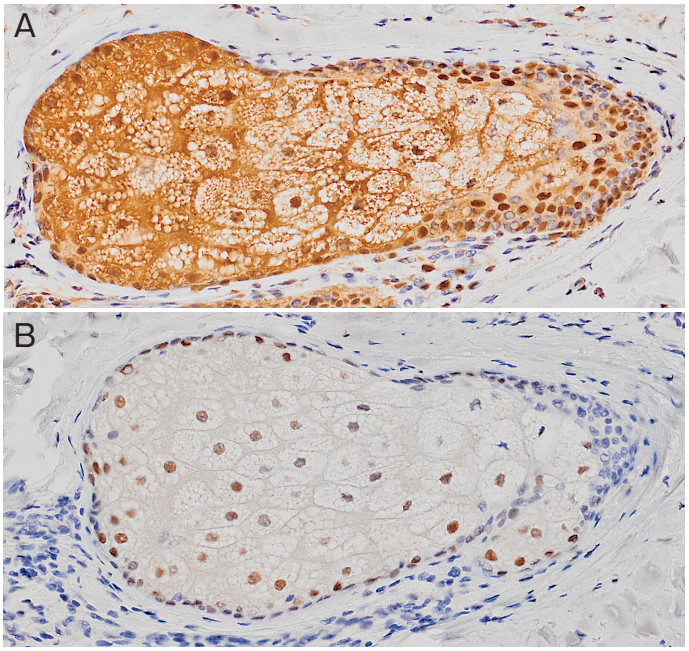

Fig. 3 **A** : Immunohistological localization of PPARα in normal sebaceous gland. The nuclear and cytoplasmic expression of PPARα was detected in basal to differentiated sebocytes. **B** : Immunohistological localization of PPARγ in normal sebaceous gland. The expression of PPARγ was confined to nuclei of suprabasal to early-differentiated sebocytes. The nuclear PPARγ expression was present only occasionally in basal sebocytes.

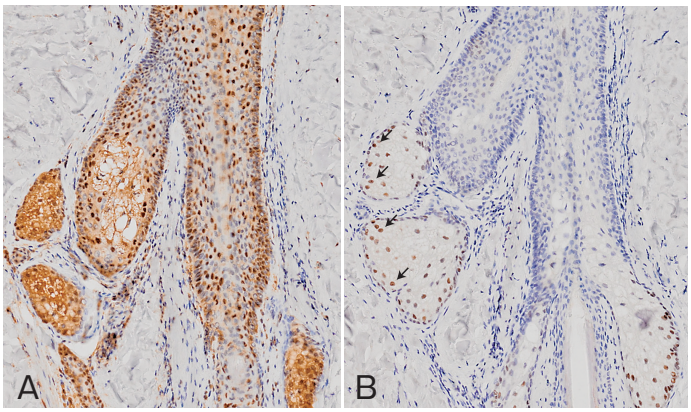

Fig. 2 **A** : Immunohistological localization of PPARα in normal pilosebaceous unit. **B** : Immunohistological localization of PPARγ in normal pilosebaceous unit. The expression of immunoreactive PPARγ was exclusively detected in the nuclei of sebocytes (arrows).

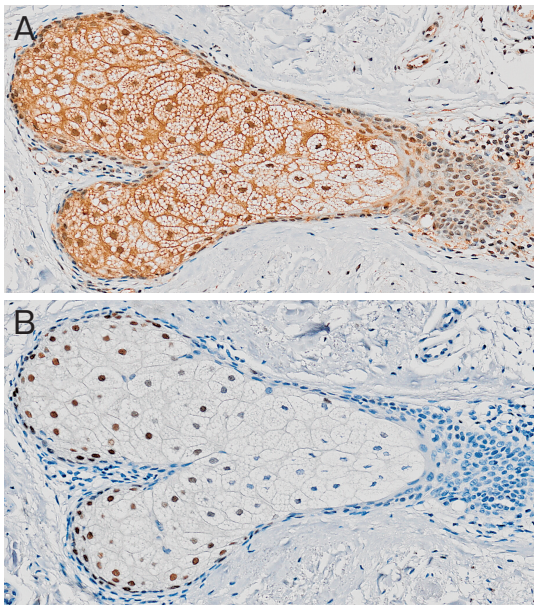

Fig. 4 **A** : Immunohistological localization of PPAR α in normal sebaceous gland. The nuclear and cytoplasmic expression of PPARα was detected in basal to differentiated sebocytes. **B** : Immunohistological localization of PPAR γ in normal sebaceous gland. The expression of PPARγ was confined to nuclei of suprabasal to early-differentiated sebocytes. The nuclear PPARγ expression was present only occasionally in basal sebocytes.

lonal antibody against PPARγ (A3409A ; Perseus Proteomics, Tokyo, Japan) at 4 ℃ overnight, followed by incubation with secondary antibody, N-Histofine® Simple Stain MAX-PO MULTI (Nichirei Biosciences Inc.). Immunodetection was conducted with 3,3-diaminobenzidine as a chromogen, followed by light counterstaining with hematoxylin. Sections stained without primary antibody served as a negative control.

Immunoreactive PPARα was readily detectable in the normal epidermal keratinocytes throughout the basal to granular layers (positivity rate : mean ± standard deviation, 56.5 ± 7.8 %) (Fig. 1A). The staining pattern was mainly nuclear, but cytoplasmic positivity was occasionally seen in the basal keratinocytes (Fig. 1A). Immunoreactive PPARγ was not detected in the normal epidermis (Fig. 1B). The intensity and pattern of PPARα staining of the follicular epithelium were similar to those of the normal epidermis (Fig. 2A). Immunoreactive PPARγ was also negative in the follicular epithelium (Fig. 2B). Notably, immunoreactive PPARγ was clearly detected in the nuclei of a certain population of sebocytes in the normal sebaceous glands (Fig. 2B). In a high-power view, the nuclear and cytoplasmic expression of PPARα was detected in the basal to differentiated sebocytes (Fig. 3A and 4A). In contrast, the expression of PPARγ was confined to the nuclei of suprabasal to early-differentiated sebocytes (Fig. 3B and 4B). The nuclear PPARγ expression was present only occasionally in the basal sebocytes (Fig. 3B and 4B). These staining results were similar in all 28 pilosebaceous units found in 10 normal skin specimens.

Trivedi et al. reported that both PPARα and PPARγ were expressed in basal sebocytes in human sebaceous glands ; however, the expression of PPARγ was also noted within differentiated sebocytes[5]. Dozsa et al. described that PPARγ was detectable in terminally differentiated mature sebocytes located in the central regions of normal sebaceous glands, but was hardly detectable in basal layer cells[7]. This discrepancy may have been due to the different source and specificity of the antibodies used. However, the clear nuclear localization of PPARα and PPARγ in sebocytes in our and previous studies indicates the importance of these isotypes in sebaceous differentiation. Notably, the distribution of PPARγ was more strictly confined to sebocytes than that of PPARα. This is supported by the finding that the PPARγ agonist rosiglitazone induced much higher lipid production than the PPARα agonist fenofibrate or GW7647 in cultured sebocytes[5]. Taking our findings together, our immunohistological examination has confirmed that PPARα and PPARγ are integral to sebocyte differentiation in human sebaceous glands.

Conflict of Interest

The authors have no conflicts of interest to declare.

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（和文抄録）

ヒト脂腺における Peroxisome Proliferator-Activated Receptor α (PPARα) と PPARγ の免疫組織学的発現

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ヒト脂腺における peroxisome proliferator-activated receptor α（PPARα）と PPARγの免疫組織学的発現を，パラフィン包埋した正常皮膚（N=10）中の 28 個の毛嚢脂腺系で検討した．ウサギポリクロナル抗ヒト PPARα 抗体とマウスモノクロナル抗ヒト PPARγ 抗体を一次抗体として用いた．PPARα は基底層および分化した脂腺細胞の核内および細胞質に一様に染色された．PPARγ は，基底層上層から分化初期の脂腺細胞の核内に染色された．また PPARγ は基底層のごく一部の脂腺細胞の核内にも認められた．これらの結果は，PPARα と PPARγ が脂腺分化に関与していることを示唆していると考えた．

キーワード：脂腺，PPARα，PPARγ，脂腺細胞