Presence of Progesterone Receptors in the Granular Cell Layer of Epidermis : Immunohistochemical Localization of Estrogen and Progesterone Receptors

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Background **: Hormones influence various normal biological processes in the skin and hairs.**

Objective **: This study was undertaken to investigate the presence of estrogen receptors(ER) and progesterone receptors (PR) in the skin and to assess differences in sex and age.**

Methods **: We examined seven normal volunteers' skin. The mouse monoclonal antibodies against human ER and PR were used to identify the localization of ER and PR in the frozen tissue sections by using a standard two stage indirect immunoperoxidase technique.**

Results **: The granular layer of epidermis and infundibulum of hair follicle in all the samples showed strong positivity of PR. Although each skin section did not contain all skin appendages, most of the samples showed that eccrine gland duct, inner root sheath of hair follicle stained weakly positive of PR. ER was not demonstrate in all samples epidermis.**

Conclusion **: PR was presented in the granular layer of epidermis, infundibulum of hair follicle, eccrine gland duct, and inner root sheath of hair follicle. Therefore, we might suspect that the progesterone probably contributes to the keratinization of the skin because these positively staining sites are prior to complete keratinization layers.**

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Key Words : Progesterone receptors, Granular cell layer of epidermis.

Various changes of the skin and hairs are attributed to hormone-dependent[1]. Recently, hormonal involvement in aging processes of the skin has attracted increasing interest[2,3]. The rapid onset of aging and thin dryness of the skin resulting from the climacterium seem to be correlated with the decrease of estrogens[4].

Sex hormones, such as estrogen, progesterone and androgen, exert their function on target organs, such as uterus, breast, pituitary gland etc. However, steroid receptors are more widely distributed in the "nontarget" tissues, such as liver, bone, and thyroid[5]. Also, the presence of steroid receptors has been demonstrated in a lot of neoplastic tissues, such as hepatocellular carcinoma, colon cancer, osteosarcoma, Hodgkin's disease, and myelogenous leukemia[5]. Clinical significance of these steroid receptors in the nontarget tissue is not fully understood. Early reports[6,7] quantitatively demonstrated the presence of estrogen receptors(ER) and progesterone receptors(PR) in the skin by dextran-coated charcol method; whereas, by immunohistochemical method, only oestradiol receptor-related protein(P29) and nuclear ER in epidermal keratinocytes were demonstrated[8-10].

However, to the best of our knowledge, the presence of PR in human skin was not reported by a morphological method. Now herein, we demonstrate the presence of PR in the granular cell layer of epidermis in the normal skin of males and females by immunohistochemical study. It may indicate that the progesterone contributes to the keratinization or barrier function of the skin because the granular

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cell layer represents the keratogenous zone of the epidermis, in which the dissolution of the nucleus and other cell organelles is prepared.

MATERIALS AND METHODS

Seven normal healthy Koreans were enrolled in the study (Table 1). The methods were performed under the conditions recommended by supplier (Novocastra Laboratories, Newcastle, UK). Briefly, the skin samples were snap frozen, and 4-μm sections were cut and fixed immediately in Zambonis fixative[11] for 10 minutes. And they were washed 2 times for 10 minutes in Tris buffered saline(TBS) at pH 7.6, and then covered with normal goat serum diluted 1 in 5 with TBS. After removing the excess serum, they were covered with primary antibody(A mouse monoclonal antibodies against human ER, diluted with 1:20 with TBS ; and human PR, supplied by prediluted, diluted 1:3 with distilled water) and incubated for 50 minutes at room temperature. After washing in TBS 3 times for 3 minutes, they were covered with secondary antibody(Goat antimouse peroxidase conjugated immunoglobulins, diluted 1:40 with TBS) and incubated for 30 minutes at room temperature. Thereafter, the skin sections were washed in TBS for 3 times for 3 minutes, and developed with diaminobenzidine(DAB) for 3 minutes. Finally the sections were washed with distilled water and dehydrated with 95% and 100% alcohol and cleared with xylene and mounted with malinol. Breast carcinomas were used as positive controls, and the blocking serum(normal goat serum) was applied in place of the primary antibody as a negative control. All skin samples were processed in an identical fashion throughout this study.

Table 1. Subject profiles

Patient's No	Sex	Age	Site
1	M	37	areola of nipple
2	M	23	occipital scalp
3	M	4	occipital scalp
4	M	19	pretibial skin
5	F	26	occipital scalp (menstrual cycle 26 day)
6	F	7	pretibial skin
7	F	24	sole(menstrual cycle 9 day)

RESULTS

Only clearly defined staining was considered indicative of the presence of ER and PR. ER showed positive staining only in the secretory portions of eccrine and sebaceous gland of areolar area of man. However, the granular layer of epidermis and infundibulum of hair follicle always showed PR strong positivity in all samples(Fig.1 a, c). Although every skin section did not contain all skin appendages, most of the samples showed that eccrine gland duct (intraepidermal and lumen of the secretory portion)(Fig.1, b) and inner root sheath of hair follicle were stained weakly positive of PR and negative of ER, and the lower portion of the hair follicle did not show any positivity. Staining was not observed in any of the negative control sections using the blocking serum(Fig. 1,d). Breast carcinomas as positive controls were stained positively.

DISCUSSION

Progesterone is a hormone of the corpus luteum and placenta. It is also formed in the adrenal cortex as a precursor of corticosteroids and found in all age groups of both sexes, even though its amount is variable.[12] Progesterone, although much less investigated than estrogen, has been shown to have vasoconstrictive effects on skin vessels, so that body temperature tends to increase during the luteal phase of the menstrual cycle. Clinically it is used as an indicator of ovulation[13].

Fraser et al8, using a monoclonal antibody technique, pointed out that epidermis, hair follicle, sebaceous gland and sweat duct contain an oestradiol receptor-related protein (P29) and must be considered as oestrogen target tissues. However, the content of this protein does not appear to change significantly during the normal menstrual cycle. Also, Viac et al[9] suggested, by indirect immunofluorescence method, that keratinocytes might be estrogen sensitive like other cells in which estrogen receptor-related protein(P29) has already been located. MacLean et al[9] proposed that estrogen receptor in females was demonstrated in epidermal keratinocytes, dermal fibroblasts of the vulva and perineum, hair and non-hair bearing, but at a much

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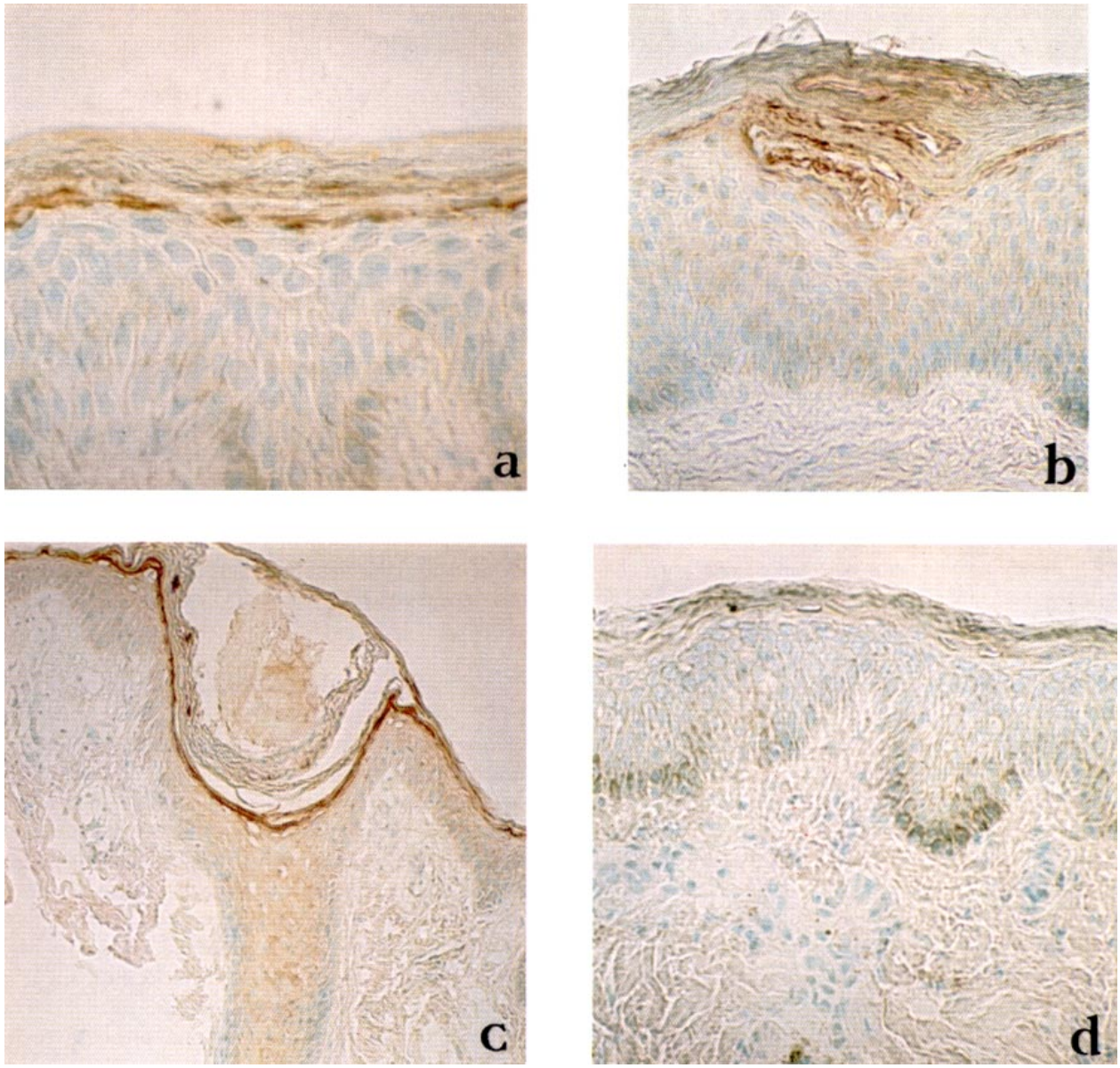

Fig. 1. Staining of epidermis with monoclonal antibody against progesterone receptor showed immunostaining of PR antigen in granular cell layers of epidermis (a), intraepidermal eccrine duct(b), infundibulum of hair follicle (c), and negative control (d). (original magnification; ×400, ×200, ×100, and ×200 respectively).

lower frequency than those of the vagina. Specific ER staining was not detected in the majority of skin specimens for extragenital sites. Also, we could not find the ER in normal skin, we suspect the cause is difference of a monoclonal antibody specificity or sensitivity to tissue.

In this study, regardless of the sex and age, the granular layer of epidermis and infundibulum of hair follicle, eccrine gland duct, and inner root sheath of hair follicle contain PR. These positive sites are prior to complete keratinization sites. Especially, granular cell layer represents keratogenous zone of the epidermis, in which the dissolution of the nucleus and other organelles is prepared. Normally the granular cell layer exists to be differentiated into horny layer. Therefore we suspect that the presence of PR on the granular cell layer may be related to horny layer differentiation

and/or function. Moreover, its presence in the stain indicates a possibility to use anti-progesterone or progesterone-analogue as an anti-aging remedy . However, the exact role of this PR in the granular layer remains to be elucidated.

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