J Korean Med Sci 2000; 15: 647-54
ISSN 1011-8934

Expression of Progesterone Receptor in Human Keratinocytes

Despite the various responses of human skin to female sex hormones, cellular and subcellular targets and the mechanisms of action of estrogen and progesterone in human skin are not well understood. The detection of estrogen receptor (ER) and progesterone receptor (PR) in the skin is of great importance to understand the effect of estrogen and progesterone. In primary cultures of human keratinocytes, expression of ER and PR was monitored by immunocytochemistry and reverse transcriptase polymerase chain reaction (RT-PCR). Paraffin embedded skin tissues were stained with monoclonal antibodies to human ER and PR by immunohistochemistry. Cultured human keratinocytes expressed cytoplasmic PR protein and PR mRNA transcripts. By contrast, ER was detected only at the mRNA level. Suprabasal keratinocytes from samples of pruritic urticarial papules, plaques of pregnancy (PUPPP) and psoriasis were stained positively only for PR, while those from samples of erythema nodosum were negative for both ER and PR. Lesional epidermis of PUPPP showed positive PR immunoreactivity, while nonlesional epidermis did not. No other cells in the normal human skin were stained with ER and PR. The present study suggests that by expressing PR human keratinocytes act as targets for progesterone action.

Key Words: *Receptors, Progesterone; Receptors, Estrogen; Keratinocytes*

Sungbin Im, Eun-So Lee, Wankee Kim*, Jisub Song, Jaehyun Kim, Miok Lee, Won Hyoung Kang

Department of Dermatology and *Institute for Medical Science, Ajou University School of Medicine, Suwon, Korea

Received: 31 May 2000
Accepted: 31 August 2000

Address for correspondence
Won Hyoung Kang, M.D.
Department of Dermatology, Ajou University School of Medicine, 5 Wonchon-dong, Paldal-gu, Suwon 442-721, Korea
Tel: +82.31-219-5190, Fax: +82.31-219-5189
E-mail: wwww@madang.ajou.ac.kr

*S.I., E-S.L., and W.H.K. were supported by grant of the Graduate School of Medicine, 1997, Ajou University, and were partially supported by grant (HMP-98-M-2-0021) of the 1998 Good Health R&D Project, Ministry of Health and Welfare, Republic of Korea.

INTRODUCTION

Evidences suggesting that some dermatoses are related with female sex hormones include the following: 1) dermatoses arising during pregnancy (PUPPP, herpes gestationis, etc.) (1), 2) dermatoses improving or aggravating during pregnancy (psoriasis, systemic lupus erythematosus, etc.) (2), 3) dermatoses showing female predominance (erythema nodosum, melasma, etc.) (3, 4). Major hormones involved are known to be estrogen and progesterone, but only a few studies have examined the effects of estrogen and progesterone on human skin (5, 6) At present, the exact mechanisms and the effects of estrogen or progesterone on cutaneous biology or pathogenesis of certain dermatoses are mostly unknown.

Female steroid hormones regulate growth, differentiation, and function of diverse target tissues, both inside and outside the reproductive system. Most of the actions of steroid hormones appear to be exerted via their specific receptors on target cells that function as ligand-activated transcription factors, regulating the synthesis of specific RNAs and proteins (7, 8). The human ER and PR have been identified in normal target cells and in their neoplastic counterparts, with special emphasis on the female genital tract and tumors of breast (9, 10). In breast cancer, the presence of ER and PR is an indicator for predicting the responsiveness of the tumor to endocrine manipulation and its clinical course (11). In human skin, expression and regulation of ER and PR were poorly known. To clarify the relationship of ER and PR with cellular kinetics of skin diseases, we analyzed the expression of ER and PR in cultured keratinocytes and in samples from inflammatory dermatoses. Our results showed that PR is present in human skin, suggesting that human keratinocytes are direct target of progesterone.

MATERIALS AND METHODS

Keratinocyte cultures

Normal human keratinocytes were prepared from neonatal foreskins obtained by circumcisions. After trimming out the subcutaneous fat, the skin was cut into pieces of 4×4 mm. The specimens were incubated in 0.25% trypsin at 0℃ overnight, and the epidermal sheets were

peeled off from the dermis. 10% fetal calf serum (FCS) was added to inactivate trypsin. The epidermis was agitated gently, centrifuged, and suspended in defined keratinocyte serum-free media containing insulin, epidermal growth factor (EGF) and fibroblast growth factor (FGF; Gibco, Grand Island, NY, U.S.A.). Single cell suspension was plated into 25 cm^2 culture flasks (Corning Glass Works, Corning, NY, U.S.A.) and incubated for 2 days at 37℃ in a humidified 95% air/5% CO_2 atmosphere. Medium was renewed twice weekly. At near confluence, the cells were subcultured into 75 cm^2 flasks. The keratinocytes formed discrete, uniform colonies of undifferentiated, noncornified, cuboidal cells resembling epidermal basal cells. Human breast cancer epithelial cell line MCF-7, the positive control for ER and PR, was grown in RPMI containing 10% FCS. For translocation of PR, keratinocytes were stimulated with estrogen and progesterone (1 and 100 nM each, Sigma, St. Louis, MI, U.S.A.) for 1 hr and for 24 hr.

Reverse transcriptase polymerase chain reaction

Total RNA was extracted from cells by using silica gel-based membrane (RNeasy total RNA kits; QIAGEN, Chatsworth, CA, U.S.A.) and was incubated for 15 min at room temperature with 1 U RNase-free DNase I (Gibco BRL, Grand Island, NY, U.S.A.) to remove the contaminating genomic DNA. Following inactivation of DNAse I with EDTA, the samples were heated and 3 μg of total RNA was reverse-transcribed using oligo-dT and Super-Script II RNase H reverse transcriptase (Gibco BRL, Grand Island, NY, U.S.A.). One tenth of RT product was mixed with 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 165 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dTTP, 22 U recombinant *Taq* DNA polymerase (PCR SuperMix system; Gibco BRL, Grand Island, NY, U.S.A.), and 0.5 μM each of the sense and corresponding anti- sense primer pairs. The following deoxyoligonucleotides were used as specific primers for the PCR: human PR (12); sense 5'-GAGTTGTGTCGAGCTCACAGCG-3', anti-sense 5'-GTTTCACCATCCCTGCCAAT-3', human ER (13); sense 5'-GCACCCTGAAGTCTCTGGAA-3', anti-sense 5'-TGGCTAAAGTGGTGCATGAT-3', GAPDH; sense 5'-GGTCGGAGTCAACGCATTTG-3', anti-sense 5'-ATGAGCCCCAGCCTTCTCCAT-3'. After heating the mixtures at 94℃ for 2 min, the PCR reaction was performed for 35 cycles [30 sec at 94℃, 30 sec at 65℃ (PR and GAPDH) or 55℃ (ER), and 2 min at 72℃]. PCR products were visualized with ethidium bromide staining (Promega, Madison, WI, U.S.A.).

DNA sequencing

Amplified PCR products were electrophoresed on 2% agarose gel. The cDNA fragments were eluted from agarose gel slices by QIAquick gel extraction kit (QIAGEN, Chatsworth, CA, U.S.A.). Single strand DNA template was purified by Big Dye Terminator cycle sequencing kit (Perkin-Elmer, Norwalk, CT, U.S.A.). Sequencing reactions were performed using the dideoxy

Table 1. Expression of ER and PR in various tissue samples

Case No.	Sex	Age (yr)	Site	Diagnosis	Receptor status	
					ER	PR
1	F	31	Abdomen	PUPPP (lesion)	NT	+
2	F	32	Abdomen	PUPPP (lesion)	NT	+
3	M	26	Leg	Psoriasis	−	+
4	M	31	Arm	Psoriasis	−	+
5	M	31	Leg	Psoriasis	−	+
6	F	38	Arm	Psoriasis	−	+
7	M	31	Arm	Psoriasis	NT	+
8	F	38	Leg	Psoriasis	NT	+
9	F	69	Leg	Psoriasis	NT	+
10	M	38	Trunk	Psoriasis	NT	+
11	M	32	Trunk	Psoriasis	NT	+
12	F	1	Knee	Psoriasis	NT	+
13	F	29	Leg	Erythema nodosum	−	−
14	F	29	Leg	Erythema nodosum	−	−
15	F	39	Face	Normal skin	−	−
16	M	17	Axilla	Normal skin	−	−
17	M	56	Arm	Normal skin	−	−
18	M	59	Arm	Normal skin	−	−
19	F	19	Buttock	Normal skin	−	−
20	F	47	Leg	Normal skin	−	−

NT, not tested; −, negative; +, positive immunostaining

chain-termination method and analyzed using an ABI377 sequencer (Perkin-Elmer, Norwalk, CT, U.S.A.).

Examples of PUPPP, psoriasis and erythema nodosum were retrieved from the files of Ajou University Hospital at Suwon, Korea. The location of the lesions are listed on Table 1. Normal skin was obtained from surgical specimens aged from 17 to 59 yr (3 females and 3 males).

Immunostaining of ER and PR

Paraffin blocks from skin samples were cut in 5 μm thickness and mounted on slides. Slides were microwaved in solution containing 10 mM citrate buffer solution (pH 6.0) for 10 min at a frequency of 2.45 MHz and 750 W. Primary keratinocytes grown on Lab-Tek chambers (Nunc, Naperville, IL, U.S.A) were fixed in 4% paraformaldehyde for 30 min at room temperature and were then permeabilized in methanol followed by 0.1% Triton X-100. Slides were placed in 0.3% hydrogen peroxide for 10 min and tissue nonspecific activity was blocked by normal goat serum for 10 min. Anti ER antibody against recombinant protein corresponding to the full-length ER (Novocastra, Claremont Palace, U.K.) and anti-PR antibody against synthetic peptide of highly antigenic PR (Novocastra) were incubated overnight at 0℃ in a 1:40 dilution. Biotinylated antibodies against both mouse and rabbit (DAKO, Carpinteria, CA, U.S.A.) were applied for 20 min. Subsequently, streptavidin peroxidase (DAKO) was added and incubated each for 20 min at room temperature. The substrate chromogen 3-amino-9 ethyl-carbazole (AEC, Biomeda Corp., Foster City, CA, U.S.A.) was applied for approximately 20 min. The sections were counterstained in Mayer's hematoxylin

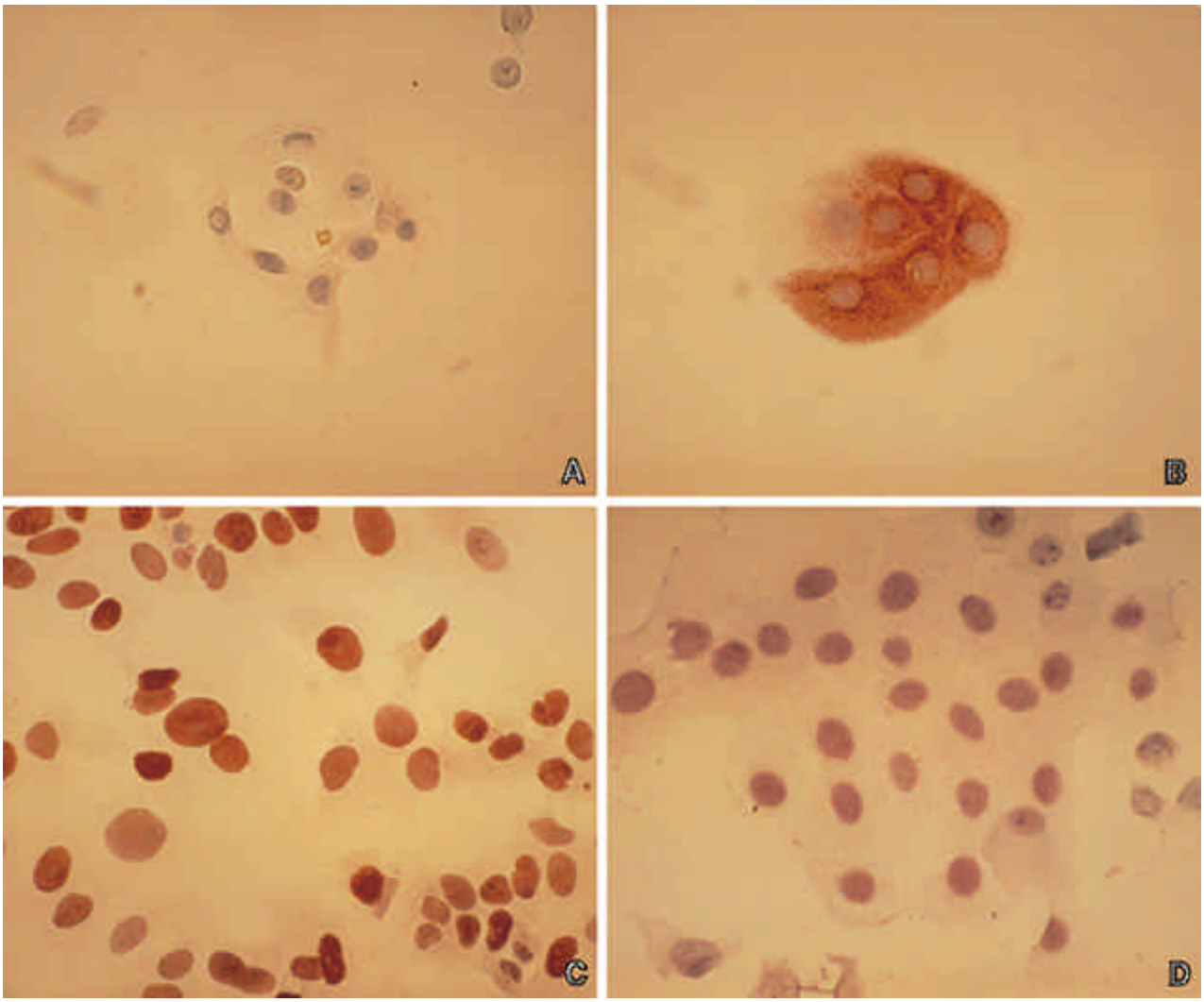

Fig. 1. Immuncytochemistry of ER and PR in cultured keratinocytes (×400). **A:** Keratinocyte-ER staining. No immunoreactivity is observed. **B:** Keratinocyte-PR staining. Cultured keratinocytes show strong cytoplasmic immunoreactivity. **C:** MCF-7-ER staining. Tumor cells show nuclear immunoreactivity. **D:** MCF-7-PR staining. Tumor cells show nuclear immunoreactivity.

(Merck, Darmstadt, Germany) and mounted with universal mount.

Evaluation of the staining result

In tissue ER and PR staining, immunoreactivity on the areas other than upper granular layer and stratum corneum was interpreted as positive. Sections were evaluated independently by two observers and reviewed again by the initial observer.

RESULTS

Immunoreactivity in cultured keratinocyte

For clear assessment of PR and ER expression, keratinocyte monolayer culture was used. ER immunoreactivity was not detected in cultured keratinocytes (Fig. 1A), which were found to express strong cytoplasmic PR (Fig. 1B). When keratinocytes were incubated with progesterone (1 and 100 nM) or estrogen (1 and 100 nM) and both (1 and 100 nM) for 1 hr or 24 hr, no nuclear translocation of PR was observed (data not shown). The human breast cancer cell line MCF-7 showed positive ER and PR immunoreactivity in the nucleus (Fig. 1C, 1D).

RT PCR and DNA sequencing

To confirm that PR immunoreactivity represents the encoded proteins, we next examined the PR and ER transcripts by using PCR. The predicted 195 bp and 470 bp PCR products of PR and ER, respectively, were produced in MCF-7 and human keratinocytes (Fig. 2). Sequence analysis of the RT-PCR products of ER and PR showed 99.9% homology with the reported human ER and PR sequences (PR, GenBank accession number AF016381; ER, GenBank accession number X03635) (data not shown) (14, 15).

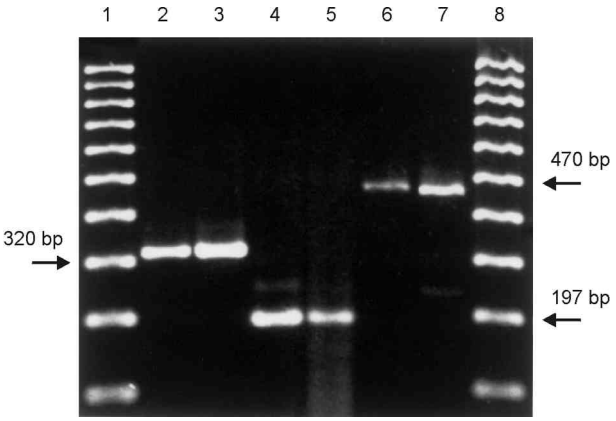

Fig. 2. RT-PCR profiles of cultured human keratinocyte. MCF-7 and human keratinocyte-derived RT-PCR samples show predicted 195 bp PCR product of PR and 470 bp of ER at mRNA level. Lane 1, molecular marker. Lane 2, GAPDH (MCF-7). Lane 3, GAPDH (Keratinocyte). Lane 4, PR (MCF-7). Lane 5, PR (Keratinocyte). Lane 6, ER (MCF-7). Lane 7, ER (Keratinocyte). Lane 8, molecular marker.

Immunoreactivity in inflammatory dermatoses

Six samples of normal skin from different sites showed no definite ER immunoreactivity. PR immunoreactivity was only confined to the upper granular layer. ER and PR were not found in the epidermal keratinocytes and melanocytes, dermal fibroblasts, blood vessels, sebaceous glands, hair follicles, sweat glands and adipocytes.

In contrast to normal skin, all samples from PUPPP (Fig. 3A) and psoriasis (Fig. 3C) showed PR immunoreactivity in the epidermis. Nonlesional skin of the PUPPP patients did not show PR immunoreactivity (Fig. 3B). PR immunoreactivity was confined strictly to cytoplasm of suprabasal keratinocytes. Intensity of PR staining was higher in keratinocytes with more infiltration of inflammatory cells to epidermis and lower dermis. To rule out the possibility of PR crossreacting with cytokeratins, psoriatic tissues were stained with keratin-10 which detects the differentiating cytokeratin. Psoriatic skin showed positive immunoreactivity starting from 2 to 3 layers above the basal cells indicating that PR does not cross-react with cytokeratin (Fig. 3D). Erythema nodosum which is characterized by infiltration of inflammatory cells in the deep dermis and subcutaneous fat was negative for PR staining in the overlying epidermis (Fig. 3E). No definite ER immunoreactivity was observed in all samples stained. As a positive control, breast cancer tissue was used and clear nuclear ER and PR immunoreactivities were observed (Fig. 3F). In contrast to the inflammatory dermatoses, seborrheic keratosis which is characterized by the absence of inflammatory infiltrates showed negative PR staining (data not shown).

PR positivity was not influenced by the age, or anatomical sites (Table 1). PR positivity was expressed not only in female patients but also in male patients at all ages.

DISCUSSION

Various types of tissues and tumors are affected by female sex hormones, for which target cells are known to have specific receptors. In breast cancer, the presence of ER and PR in tumor cells is associated with favorable response to adding or blocking of female sex hormones

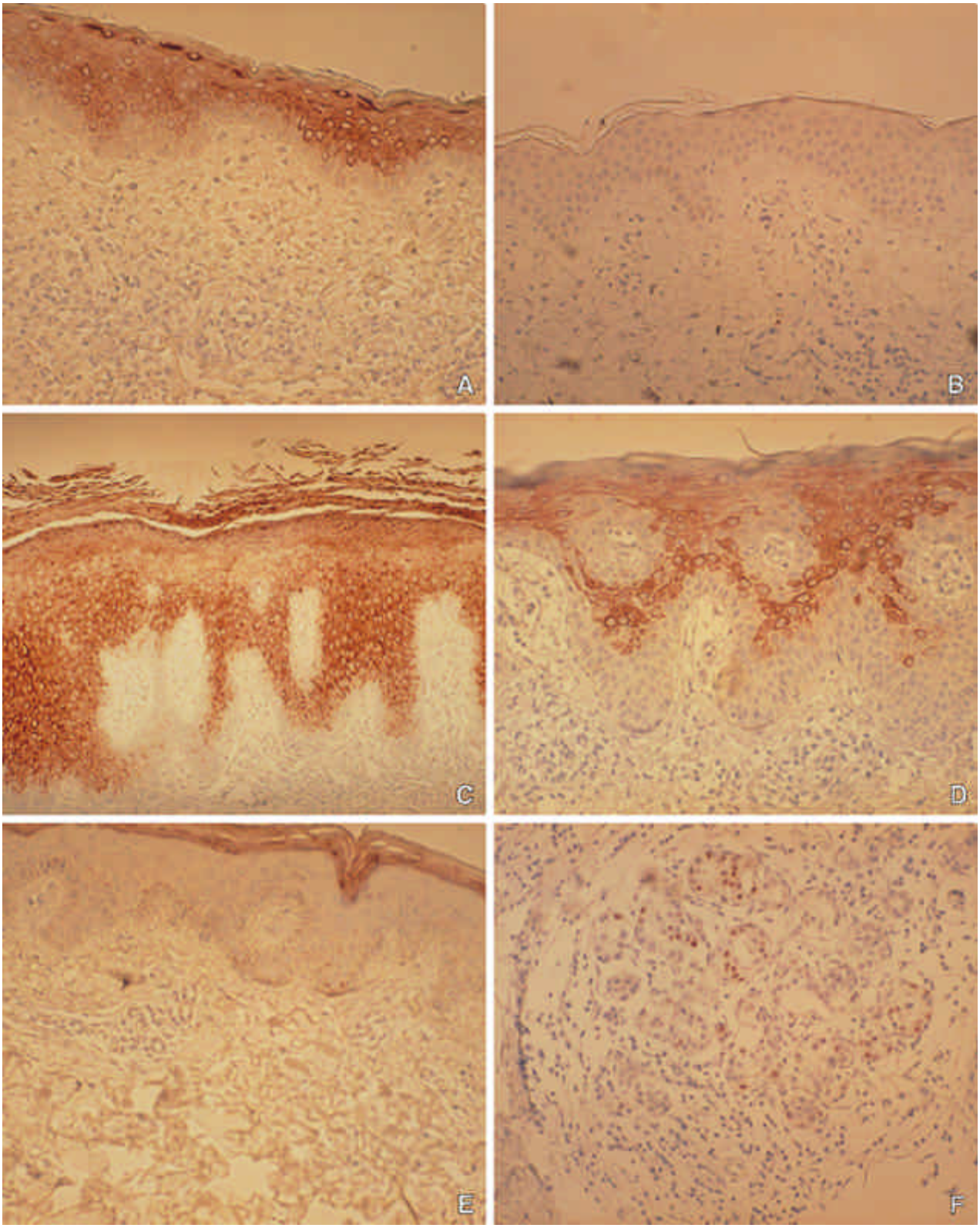

Fig. 3. Immunohistochemistry of PR in tissue (×200). **A:** PUPPP (lesion). Suprabasal keratinocytes show cytoplasmic immunoreactivity. **B:** PUPPP (nonlesion). No immunoreactivity is observed. **C:** Psoriasis. Suprabasal keratinocytes show cytoplasmic immunoreactivity. **D:** Psoriasis. Keratin-10 staining. Psoriatic skin show immunoreactivity starting from the 2 to 3 layers above the basal cells. **E:** Erythema nodosum. No immunoreactivity is observed. **F:** Breast cancer. Tumors cells show nuclear immunoreactivity.

(16). PR in astrocytic tumors correlates with histologic grade, and PR may participate in the growth of these tumors and tumor angiogenesis (17).

Estrogen and progesterone are relevant to skin diseases as seen in pregnancy and menopause. The role of estrogen in skin is known to increase the mitotic rate in the epidermis of man and to stimulate the synthesis, maturation, and turn over of collagen (18). Estrogen appears to increase the vascularization of the skin and the activity of pigment cells while supressing sebaceous gland activity (19). Progesterone has been shown to have anti-inflammatory and immunosuppressive properties. Progesterone increases keratinocyte proliferation (6) and blocks the action of 5α-reductase (20). However, mechanisms by which female sex hormones influence epidermal cell behavior remain to be determined.

In the present study, cultured human keratinocytes were used to detect the PR expression in vitro. Cultured human keratinocytes uniformly express PR in their cytoplasm. The breast cancer cell line MCF-7, which expresses high levels of PR, acted as positive control (21). We employed RT-PCR analysis to detect the expression of PR mRNA. PR mRNA was present in cultured keratinocytes, and by sequencing PCR product was confirmed to code for PR. These results are the first report of PR detection by immunocytochemistry, RT-PCR, and sequencing showing that mRNA for PR is transcribed and translated in human keratinocytes. In our study, ER immunoreactivity was not detected in human keratinocytes, but specific ER mRNA was detected. The absence of immunoreactivity may be due to low concentrations of antigen or to masked antigens in cells (22).

Paucity of data on the expression of ER and PR in human skin rendered us to evaluate expression of these receptor in the skin (23). ER was found in epidermal keratinocytes and dermal fibroblasts of the vulva, perineum, and vagina, but specific ER was not detected in the extragenital skin (24, 25). Recently, the presence of PR in cultured keratinocytes was shown by in situ hybridization. In patients with PUPPP, the lesional skin only showed PR immunoreactivity while perilesional normal skin did not. These findings suggested that PR immunoreactivity was related to the inflammation around the epidermis. The incidence of generalized pustular psoriasis increases in pregnant women and in psoriasis patients taking oral progesterone (26). On the other hand, psoriasis is known to improve during pregnancy (2). The present study revealed the PR immunoreactivity in psoriasis for the first time. PR staining was observed in the cytoplasm of suprabasal keratinocytes. This study will provide important clues in the effect of progesterone on the pathogenesis of psoriasis, which is characterized by inflammation around the epidermis and epidermal proliferation. To further evaluate the role of inflammation in PR immunoreactivity, we examined the samples of erythema nodosum which is characterized by inflammation in the subcutaneous fat. No PR immunoreactivity was observed in the epidermis, dermis and subcutaneous fat in erythema nodosum. These findings suggested that the induction of PR immunoreactivity in the keratinocytes may be due to the inflammation around the epidermis.

After entering the cell, the progesterone binds to the cytoplasmic PR and the resulting complex translocates to the nucleus (27). The monoclonal antibody used in our study detected nuclear PR in breast cancer cells and cytoplasmic PR in keratinocytes. In agreement with our study, the presence of PR in the cell cytoplasm of certain tumors has been demonstrated by other authors (28). Using the same antibody that we used, intense nuclear and cytoplasmic staining for PR was noted in syringoma and cutaneous metastatic breast carcinoma (29, 30).

Understanding the molecular mechanisms that regulate PR is of particular importance in assessing the hormonal dependence of target cells. Biochemically, PR has been reported to be regulated by estrogen or progesterone. Our findings indirectly supported that PR immunoreactivity in human keratinocytes is not affected by female sex hormone and that it is expressed in concert with inflammation. Increased PR expression may produce certain cytokines related to inflammation, or PR may be secondarily induced by this process. Because female sex hormones have recently been shown to regulate the synthesis of interleukin (IL)-6, a key pro-inflammatory cytokine, or because progesterone acts as an immunosuppressant, it is possible to postulate that PR was induced in the keratinocytes by an unknown inflammatory cytokines that initiate the action of progesterone either to increase or to decrease the inflammatory process (31, 32). Previous studies documented the up-regulation of PR by IGF-1, EGF, cAMP, dopamine, and PDGF in MCF-7 breast cancer cells and uterine cells (21, 33-35). Likewise, it is also possible that some mediators as EGF, PDGF, IGF and TGF-beta produced during inflammation induce PR to potentiate the role of progesterone in these processes. It is interesting to note that EGF, related to keratinocyte differentiation, can induce progesterone receptors, and progesterone can potentiate the action of EGF by increasing the number of EGF receptors in MCF-7 cell line (36, 37). Further studies on the possible interaction between PR and candidate molecules in the human keratinocytes are necessary.

The occurrence of PR in keratinocytes of some dermatoses may raise the possibility of a specific endocrine therapy. The antiprogesterone mifepristone (RU486) has been clinically used with some success to treat patients

with unresectable meningioma expressing PR (38). It is conceivable that endocrine therapy using progesterone and anti-progesterone may be an effective approach for the management of these skin diseases.

In this study we provide the first direct evidence that human keratinocytes express PR, indicating that keratinocytes are the targets of progesterone. Further studies are necessary to address the role of progesterone in skin disease and the induction mechanism and functionality of PR in keratinocytes.

ACKNOWLEDGEMENT

We thank Dr. Hee Boong Park and Dr. Jung Il Cha for providing breast skin and foreskin. We also extend our gratitude to Drs. Inhee Mook and Hyunhee Lim for their very helpful contribution, and to Young Bae Kim for his technical expertise.

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