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Effects of Estrogen and Progesterone on Human Melanogenesis
Effects of Estrogen and Progesterone on Human Melanogenesis

지도교수 강 원 형

이 논문은 의학 박사학위 논문으로 제출함.

2002 년 8 월

 아주대학교 대학원

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이 기변

윤명한

김유찬

 아주대학교 대학원

2002년 6월 21일
ACKNOWLEDGEMENT

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2002 년 8 월

저자서

1973

DAEJOU UNIVERSITY
Effects of Estrogen and and Progesterone on Human Melanogenesis

by

Jeahyun Kim

A Dissertation Submitted to the Graduate School of Ajou University in Partial Fulfillment of the Requirements for the Degree of DOCTOR of PHILOSOPHY

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The Graduate School, Ajou University
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ABSTRACT

Effects of Estrogen and Progesterone on Human Melanogenesis

Purpose: The mechanisms of estrogen and progesterone in human cutaneous pigmentation are largely unknown. The molecular identification of estrogen receptor (ER) and progesterone receptor (PR) in the human melanocytes is of great importance to understand the mechanisms. The present study was done to determine the ER and PR status in cultured melanocytes and the effects of estrogen and progesterone in human pigmentation in vitro in an attempt to elucidate the mechanism of pregnancy-induced pigmentation, melasma.

Materials & Methods: I performed immunocytochemistry analysis to demonstrate the ER and PR expression in cultured human melanocytes. Reverse transcriptase-polymerase chain reaction (RT-PCR) and sequence analysis were done to confirm the expression of ER and PR at the transcriptional level. To determine the melanocyte proliferation and tyrosinase activity after the treatment of estrogen and progesterone, Coulter counter counting, tyrosinase hydroxylase assay and Western blot analysis were done.

Results: I demonstrated the presence of ER and PR in cultured human melanocytes by immunocytochemistry, RT-PCR and DNA sequencing. Despite of the presence of ER and PR, the physiological and pregnant levels of estrogen and progesterone showed different effects on the proliferation and tyrosinase activity of cultured human melanocytes.
Conclusion: These results suggest that human melanocytes express ER and PR, which have individually variable actions in cutaneous pigmentation. Further studies are needed to elucidate the induction mechanism and functionality of these receptors, and the role of estrogen and progesterone in melanocytes.

Key words: progesterone, estrogen, progesterone receptor, estrogen receptor, melanocyte
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I. INTRODUCTION

Melanocyte is a type of epidermal cell located at the basal layer of the skin, where it differentiates and projects dendrites to the upper epidermal layers. It possesses melanosomes which contain active tyrosinase to synthesize melanin, and the resulting pigmented melanosomes protect skin by removing toxic oxygens from UV light. Melanogenesis and melanocyte proliferation appear to be affected by intrinsic and extrinsic factors. For instance, UV-exposed skin produces more melanocytes with an increase in melanin production. Growth factors and cytokines secreted by keratinocytes and fibroblasts may also affect melanocyte proliferation. For example, keratinocytes produce various growth factors and/or cytokines such as transforming growth factor (TGF) \( \alpha \), epidermal growth factor (EGF), tumor necrosis factor (TNF) \( \alpha \), interleukin (IL)-1, IL-6, and IL-8. Fibroblasts are known to secrete basic fibroblast growth factor (bFGF). These growth factors affect their own proliferation as well as melanocyte proliferation by autocrine and paracrine mechanisms.

Melasma is a common acquired symmetric hypermelanosis characterized by irregular light to gray-brown macules and patches on sun-exposed areas of the skin. Although sex, race, or age is not exempt from developing melasma, it is more common in oriental or hispanic women living in sunny locations. Its etiologic factors are genetic backgrounds, exposure to UV radiation, pregnancy, hormonal therapies, cosmetics, phototoxic drugs, and antiseizure medications. The morphologic features of melasma appear to be related to an increase in the number
and activity of melanocytes which appear to be engaged in increased formation, melanization, and transfer of melanosomes to the epidermis.

When hyperpigmentation of the human skin occurs after exposure to ultraviolet light, elongation and increase in the number of dendrites of melanocytes as well as their enlargement are observed. These changes are associated with increased activity of tyrosinase. Tyrosinase is a critical enzyme to produce melanin from amino acid, tyrosine. Other two tyrosinase related gene families, tyrosinase related protein (TRP)-1 and -2 which upregulate the black/brown eumelanin synthesis rather than yellow/red pheomelanin also play an important role in determining the final skin color.

The cause of melasma is unknown, but many factors have been implicated as playing a role in the pathogenesis of melasma. Some of them are estrogen and progesterone, because melasma is frequently associated with pregnancy, contraceptive drugs containing progesterone and estrogen, diethylstilbesterol treatment of prostatic cancer, and the conjugated estrogens in postmenopausal women. I would, therefore, like to think that the ovarian hormones such as estrogen and progesterone may be involved in the pathogenesis of melasma, i.e., their indirect influence of ovarian hormones to melanocytes in vivo remains to be clarified.

The cellular and subcellular targets and the mechanisms of action of estrogen and progesterone in human skin pigmentation are largely unknown. Only a few studies have examined the actions of estrogen and progesterone on the epidermal microenvironment. Most of the actions of female sex hormones appear to be exerted via their specific receptors on target cells and intracellular receptors that function as ligand-activated transcription factors, regulating the synthesis of specific
RNAs and proteins. Human progesterone receptors (PR) have been identified in normal keratinocytes (1).

High estrogen levels in the serum have been reported to be associated with an increase in skin pigmentation (2). However, it is unclear whether the sex hormones play a significant role in the proliferation of cultured human melanocytes and their tyrosinase activity.

The issue of melanocytic expression via estrogen receptors (ER) has been quite controversial (3,4). Although many researchers have shown the ability of melanocyte to bind to the hormones, such as estrogen and progesterone, using a hormone-binding assay, true hormone receptors have not been molecularly identified in human melanocytes (5,6).

The present study used immunocytochemistry and reverse transcriptase polymerase-chain reaction (RT-PCR) to determine the ER and PR status in cultured melanocytes and the effects of estrogen and progesterone in human pigmentation in vitro in an attempt to elucidate the mechanism of melasma.

The occurrence of melanocytes bearing ER and PR may raise the possibility of a specific endocrine therapy for pigmentedary disorders. The antiprogestosterone mifepristone (RU486) has been used clinically with some success to treat patients with unresectable meningioma which express PR. If the progression of some dermatitis is improved or aggravated by progesterone, it is conceivable that endocrine therapy using progesterone and anti-progesterone may be an effective approach for the management of these skin diseases.
II. Materials & Methods

A. Melanocyte culture

Normal human melanocytes were derived from the adult foreskin as previously described (7) and maintained in a growth medium consisting of MCDB-153, 4% heat-inactivated fetal bovine serum (FBS) (Gibco BRL, Grand Island, NY), 10⁻⁷ M α-melanocyte stimulating hormone (α-MSH) (Sigma Chemical Co., St. Louis, MO), 8 nM 12-O-tetradecanoyl phorbol 13-acetate (TPA) (Sigma Chemical Co.), 0.6 ng/ml human recombinant basic fibroblast growth factor (FGF) (Calbiochem, San Diego, CA), 5 µg/ml insulin (Sigma Chemical Co.), 1 µg/ml α-tocopherol (Sigma Chemical Co.), and 1% penicillin-streptomycin (10,000 units/ml and 10,000 µg/ml, respectively) (Sigma Chemical Co.).

All the subjects were Korean with Fitzpatrick skin type between III-VI. In all experiments for the expression of ER and PR, the melanocytes were maintained in culture medium MCDB153 with chelexed FBS (Hyclone, Logan, UT) and in the absence of α-MSH for 3 days prior to beginning of experiment. Conventional FBS were reported to have estrogen.
B. Immunocytochemistry of ER and PR

Melanocytes grown on Lab-Tek® chambers were fixed in 4% paraformaldehyde for 30 min at room temperature, and permeated in methanol followed by 0.1% triton X-100 to achieve a nuclear permeance. Slides were placed in methanol containing 0.3% hydrogen peroxidase for 10 min and the tissue nonspecific activity was blocked by normal goat serum for 10 min. Then they were incubated with mouse monoclonal antibody against recombinant protein corresponding to the full-length estrogen receptors (Novocastra, Claremont Palace, UK) overnight at 0 °C at 1:40 dilution. Biotinylated antibody against mouse and rabbit (DAKO, Carpinteria, CA) was applied for 20 min. Subsequently, the slides were incubated with streptavidin peroxidase (DAKO) for 20 min at room temperature. The substrate chromogen 3-amino-9-ethyl-carbazole (AEC) (Biomedia Corp., Foster City, CA) was applied for 20 min. The sections were counterstained with Mayer’s hematoxylin (Merck, Darmstadt, Germany), and mounted with universal mount. Negative controls were made by applying phosphate-buffered saline (PBS) instead of the primary antibody. They consistently yielded the negative results. MCF-7, human breast cancer cell line grown on the LabTek® chamber were used as a positive control (8).
C. Reverse Transcriptase-Polymerase Chain Reaction

Total RNA was extracted from cells by using a silica gel-based membrane (RNeasy total RNA kits; QIAGEN, Chatsworth, CA), and incubated for 15 min at room temperature with 1U RNase-free DNase I (Gibco BRL, Grand Island, NY) to remove contaminating genomic DNA. Following inactivation of DNase I with ethylenediamine tetraacetic acid (EDTA), the samples were heated and 3 μg of total RNA was reverse-transcribed using Oligo-dT and Superscript II RNase H reverse-transcriptase (Gibco BRL). One tenth of reverse transcription product was mixed with 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 165 mM MgCl₂, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 22 U recombinant Taq DNA polymerase (PCR SuperMix system; Gibco BRL), and 0.5 μM each of the sense and anti-sense primer pairs. The following deoxyoligonucleotides were used as specific primers for the PCR. For human PR (9), 5'-TGGCTAAAGTGGTGATGAT-3' (sense primer) and, 5'-GTTCACCACATCCCTGCCAAT-3' (anti-sense primer), human ER (10), 5'-GCACCCCTGAAGTCTCTGGA-3' (sense primer) and, 5'-TGGCTAAAGTGGTGATGAT-3' (anti-sense primer); for GAPDH, 5'-GGTCGGAGTCATGCAACGC-3' (sense primer) and, 5'-ATGAGCAGCCAGCTTTCCCAT-3' (anti-sense primer) were used respectively. After heating the mixtures at 94°C for 2 min, the PCR reaction was performed for 35 cycles [30 sec at 94°C, 30 sec at 65°C (PR and GAPDH) or 55°C (ER), and 2 min at 72°C]. PCR products were visualized with ethidium bromide staining (Promega, Madison, WI).
D. DNA Sequencing

Amplified PCR products were electrophoresed on a 2% agarose gel. The cDNA fragments were eluted from the agarose gel slices by a QIAquick gel extraction kit (QIAGEN). Single stranded DNA template was purified by a Big Dye Terminator cycle sequencing kit (Perkin-Elmer, Norwalk, CT). Sequencing reactions were performed using the dideoxy chain-termination method and analyzed, using an ABI 733 sequencer (Perkin-Elmer).
E. Skin organ culture

Normal skin of the breast cancer patients during the mastectomy were used for organ culture. All the skin samples were used immediately after the excision. Subcutaneous fat was removed and discs were taken from the skin samples with a 4-mm punch biopsy. Each skin sample was placed on a millicelles (1.2 μm pore size, Millipore, Bedford, MA) and then inserted on a 24-well culture plate in such a way that the medium was only in contact with the dermal side of the sample so that the epidermis remained constantly exposed to the air. Each piece was maintained in a DMEM medium lacking phenol-red (Gibco BRL) and containing 10% Chelexed FBS (Hyclone). Phenol-red and conventional FBS were reported to have an estrogenic stimulatory effect. Treatment with estrogen and progesterone (1 and 100 nM each) and medium change were performed every other day for 6 days. At 6th day, the cultures were fixed in 4% buffered formaldehyde and embedded in paraffin. Routine hematoxylin and eosin stain and Fontana-Masson stain were done on each sample.
F. Determination of melanocyte proliferation and tyrosinase activity after treatment with estrogen and progesterone

To investigate the effects of estrogen and progesterone on the melanocyte proliferation and tyrosinase activity, cells were plated at a density of $1.5 \times 10^6$ cells/60-mm dish. The culture medium was replenished, and a fresh treatment (1,100 nM estrogen and progesterone) was done every other day for a total of 6 days. At the end of each experiment, melanocytes from each individual dish were harvested and counted using a Coulter counter (Coulter Electronics, Hialeah, FL). During the final 24 hr of the experiment, (3,5-$^3$H) L-tyrosine (specific activity, 52 mCi/mmol; Dupont NEN, Boston, MA) at a concentration of 0.7 $\mu$Ci/ml (total of 2.1 $\mu$Ci/3 ml/flask) was added to the culture medium. The conditioned medium from each well was then saved to determine tyrosinase activity. The tyrosine hydroxylase activity was determined by a modification of the Pomerantz’s charcoal absorption method as previously described (11). This assay is based on the measurement of the amount of $^3$H$_2$O released into the culture medium as [$^3$H]tyrosine is converted to L-dihydroxyphenylalanine (L-DOPA) by tyrosinase.
G. Western blot analysis of tyrosinase

For western blot analysis of tyrosinase, cells were plated at a density of 5x10^6 cells/60mm dish and treated 48h later with 1nM and 100nM estrogen and progesterone for a total of 6 days.

Fresh medium and appropriate treatment were added every other day for 6 days. Cell lysates were prepared using RIPA buffer (150mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, and 50mM Tris, pH 8.0) containing the phosphatase inhibitor Na$_3$VO$_4$ (10mM) and the protease inhibitors phenylmethylsulfonyl fluoride (200mM), aprotinin (10μg/ml), and leupeptin (10μg/ml).

Equal amounts of protein (8μg) were electrophoresed on a 7.5% polyacrylamide gel. After transblotting onto nitrocellulose membranes, the membranes were reacted with AhPEP-7 (1:1,500), a polyclonal antibody raised against the COOH terminus of the human tyrosinase.

The membranes were then reacted with horseradish peroxidase conjugated anti-rabbit IgG (Amersham; 1:3,000 dilution after incubation with AhPEP-7) or anti-immunoreactive bands were detected by chemiluminescence, using the Renaissance kit (Dupont NEN, Boston, MA).
III. RESULTS

A. Expression of ER and PR in the cultured melanocytes

Melanocytes grown on slides were analyzed by immunostaining using human ER and PR antibodies. For clear assessment of ER and PR expression, melanocyte monolayer culture was used. The human adult melanocytes expressed ER and PR in the cytoplasm and nucleus, as evidenced by the red colour (Fig. 1A, 1B, and 1C). The human breast cancer cell line MCF-7 showed positive ER and PR immunoreactivity in the nucleus (data not shown) (12,13).

To confirm that the ER and PR immunoreactivity was originated from the encoded proteins, we subsequently examined the ER and PR transcripts by PCR. The predicted 195 bp and 470 bp PCR products of PR and ER, respectively, were found in MCF-7 and human adult melanocytes (Fig. 2). Neonatal melanocytes expressed the same immunoreactivity and mRNA transcripts of ER and PR (data not shown). Sequencing analysis of the RT-PCR products of ER and PR showed 99.9% homology with the reported human ER and PR sequences (Fig. 3). These results confirmed that mRNA coding for ER and PR is transcribed and translated in human melanocytes, providing a strong evidence that human melanocytes are the target for the estrogen and progesterone action.
Fig. 1. Immunocytochemistry of ER and PR in cultured melanocytes (X400)

A: Melanocyte-negative control.
B: Melanocyte-ER positive. Cultured melanocytes show cytoplasmic and nuclear immunoreactivity.
C: Melanocyte-PR positive. Cultured melanocytes show cytoplasmic and nuclear immunoreactivity as evidenced by the red colour.
Fig. 2. RT-PCR profiles of cultured human melanocyte

RT-PCR of MCF-7 and human melanocytes yielded the predicted 195 bp PCR product for PR and 470 bp for ER at mRNA level. Lane 1, molecular size marker; Lane 2, GAPDH (MCF-7); Lane 3, GAPDH (melanocyte); Lane 4, ER (MCF-7); Lane 5, ER (melanocyte); Lane 6, PR (MCF-7); Lane 7, PR (melanocyte); Lane 8, molecular size marker.
Fig. 3. DNA Sequencing

Sequencing analysis of the RT-PCR products of ER and PR showed 99.9% homology with the reported human ER and PR sequences.
B. Effect of estrogen and progesterone on pigmentation in skin samples grown in organ culture

To investigate the influence of estrogen and progesterone on pigmentation, skin organ culture was used as a model system which is more closely resembling in vivo circumstances than those of the melanocyte monolayer culture. Because the steroid hormones are capable of penetrating the skin easily as compared with the peptide hormones, the skin explants were first immersed in the media and estrogen and progesterone were treated for 6 days. During the culture, the overall in vivo cell morphology of keratinocytes was maintained for a period of 6 days. Although a slight dermoeipidermal separation was noted in some experiments, the explants were quite satisfactory for testing. Epidermal cells of the explant proliferated and migrated to form a new epidermis (epibolus) that completely surrounded the explant within 6 days. Estrogen and progesterone either at a physiologic concentration (1 nM) or at a concentration in pregnancy (100 nM) did not show any significant epidermal changes including the thickness and rete ridge pattern. The concentrations did not affect the epibolic outgrowth either. In 2 samples out of 5, estrogen at 1 nM and 100 nM concentration both increased the basal pigmentation as compared to the control (Fig. 4A and 4B). However, progesterone either at 1 nM or at 100 nM concentration did not increase the melanin content in the epidermis in all samples.
Fig. 4. Effect of estrogen and progesterone on pigmentation in skin samples grown in organ culture

Estrogen and progesterone at a physiologic concentration (1 nM) and concentration in pregnancy (100 nM) did not show any significant epidermal changes including the thickness and rete ridge pattern. The concentrations also did not affect the epibolic outgrowth. In 2 samples out of 5, estrogen at 1 nM and 100 nM concentration increased the basal pigmentation as compared to the control. However, progesterone either at 1 nM or at 100 nM concentration did not increase the melanin content in the epidermis in all samples.

A: control, B: estrogen at 100 nM concentration increased the basal pigmentation (Fontana-Masson stain, X400).
C. Melanogenic and proliferative effects of estrogen and progesterone on human melanocytes

Because conventional FBS and phenol red in MCDB media have a mild estrogenic effect, we first maintained the cultured melanocytes in phenol red-free DMEM with chelexed serum that was deprived of estrogen by charcoal. In this condition, melanocytes started to float on day 4 and we could not maintain the melanocytes more than 6 days. We next compared the effect of estrogen and progesterone on melanocytes in MCDB with phenol red and chelexed serum versus regular serum, and observed no difference. Thus the subsequent experiments were done using MCDB with chelexed serum.

Melanocytes from 8 donors treated with 1 and 100 nM estrogen and progesterone for 6 days showed a individual variation (Table 1). Number of melanocytes and tyrosine hydroxylase activity were increased in melanocytes from 3 adult foreskin donors in response to 1 and 100 nM estrogen and progesterone, respectively (Fig. 4A). In one donor, only the tyrosinase activity was increased. In the remaining 4 donors, neither proliferation nor tyrosinase activity of melanocytes showed any significant changes due to estrogen or progesterone (Fig. 4B). The dose response to 1 and 100 nanomolar concentrations of either hormone did not show any significant differences. α-MSH treatment, used for a positive control, was proved to be both mitogenic and melanogenic in melanocytes. However when α-MSH was treated concomitantly with estrogen and progesterone, no additive response was observed, and no difference was noted between neonatal and adult melanocytes (data not shown).
Table. 1 Melanogenic and proliferative effects of estrogen and progesterone on cultured human melanocytes (% of untreated control)

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</table>

Cells were plated at 1.5x10^6 cells/60-mm dish, and triplicate dishes were included in each of the control and experimental groups. Fresh growth medium with 1 nM and 100 nM concentrations of estrogen (E) and progesterone (P) each was added to each dish every other day for a total of 6 days. For proliferation assay, melanocytes from each individual dish were harvested at the end of experiment and counted using a Coulter counter, and % of control was calculated. In the same experiment, tyrosine hydroxylase activity was assayed on day 6. On day 5, each dish was incubated with a medium containing [^3H] tyrosine for 24 hr. The conditioned medium from each dish was then collected and assayed for tyrosine hydroxylase activity.

For THA, tyrosine hydroxylase activity was expressed as DPM/10^7 cells and % of untreated control was calculated. Experiments were repeated three times, that yielded similar results.

The values indicated are mean and standard deviation of % of control in each experiment.

Melanocytes treated for 6 days with 1 and 100 nM estrogen and progesterone in 8 different donors showed an individual variations.
Fig. 5. Effect of estrogen and progesterone on the proliferation and tyrosinase activity of human melanocytes.

A,B,C: Both number of melanocytes and tyrosine hydroxylase activity were increased in melanocytes from adult donor in response to 1 and 100 nanomolar concentrations of estrogen and progesterone.
D,E,F,G: Neither proliferation nor tyrosinase activity showed any changes due to estrogen and progesterone treatment.
H: Only the Tyrosinase activity increased in one donor.
D. Effect of Estrogen and Progesterone on Tyrosinase Expression

Tyrosinase is a critical enzyme for producing melanin from amino acid, tyrosine. The expression level of tyrosinase was further characterized by Western blot analysis. Examination of the autography showed that the levels of tyrosinase were not significantly altered after female sex hormone treatment.

Fig. 6. Western blot analysis of Tyrosinase

The expression level of tyrosinase was further characterized by western blot analysis. Examination of the autography showed that the levels of tyrosinase were not significantly altered after estrogen or progesterone treatment.
IV. DISCUSSION

Since many dermatoses are related to a female predisposition and pregnancy, it is feasible that the female sex hormone may influence the skin directly. Estrogen appears to increase the vascularization of the skin and suppress the sebaceous gland activity. Also estrogen increases the pigment cell activity (14).

Skin pigmentation \textit{in vivo} is determined by genetic, environmental, local, and endocrine factors, which influence both melanin synthesis within each melanocyte and the distribution of melanin throughout the epidermis. Melasma, a common acquired brownish pigmentation occurring exclusively in the sun-exposed areas on the face, is exacerbated by pregnancy and oral contraceptives (15-20).

Sato reported a significantly high level of progesterone in the serum of Japanese patients with melasma (2). On the other hand, Perez et al. reported the increased serum levels of LH alone, and lower levels of serum estradiol in patients with melasma than in normal controls (21).

I, therefore, assumed that the ovarian hormones such as estrogen and progesterone may be involved in the pathogenesis of melasma, i.e., indirect influence of ovarian hormones on the melanocytes in vivo remains to be clarified.

The issue of melanocytic expression via ER has been quite controversial. Investigators found that tyrosinase can mimic the tight binding of 3H-estradiol, giving 3H-labeled products. In this way tyrosinase oxidation of 3H-estradiol led to spurious positive results in early biochemical assays of estrogen binding. Although the true estradiol binding could not be distinguished from tyrosinase
activity with the dextran-coated charcoal assay, the use of immunocytochemistry and RT-PCR obviated this problem.

In this study we provide the first direct evidence that human melanocytes show ER and PR immunoreactivity. ER and PR belong to a superfamily of ligand-induced transactivators that exerts their regulatory activity on discrete genes through DNA binding at individual hormone-responsive elements. Understanding the molecular characterization of ER and PR is of particular importance in assessing the hormonal dependence of target cells. The significance of ER and PR in the melanocytes is an area of considerable interest. To clarify the existence of ER and PR, we analyzed the expression of ER and PR in cultured melanocytes and designed an experiment to evaluate the role of estrogen and progesterone on human pigmentation using an in vitro culture system. Our data provides the evidence for the presence of ER and PR in cultured melanocytes by immunocytochemistry, RT-PCR, and sequencing.

Recent studies have demonstrated the effects of estrogen and progesterone on human melanocytes. However, it is unclear whether the effects were directly exerted by the sex hormones or by tyrosinase activity.

Ranson et al, for the first time, reported that the incubation of neonatal melanocytes with beta-estradiol (10^{-10}-10^{-9} M) for 24 hr resulted in a dose-dependent stimulation of the tyrosinase activity (22). The increased melanogenesis was accompanied by reduced cell number and enhanced melanin extrusion into the medium. They are also aware that, most studies on the melanocytes have extrapolating any of the results to the adult situation.

On the contrary, Jee et al reported that the treatment of 17β-estradiol (10^{-12} to
10^9 M) for 10 days significantly showed the increased neonatal melanocyte number, and for 1 day exhibited the decreased tyrosinase activity and melanin content (6). However, they suggested that the tyrosinase activity and melanin content were expressed on per cell bases. It is suggested that the proliferating activity stimulated by estradiol was greater than that by the tyrosinase activity and the melanin producing activity.

Recent data from the adult melanocytes of Japanese male foreskin showed that estradiol in the concentration range of 0.01 to 1.0 μg/ml and 1μg/ml progesterone significantly increased the amount of TRP-1, and no significant effect on dopa oxidase activity was detected after estradiol and progesterone treatment (23). Estradiol and progesterone also increased the area, the dendrites and the perimeter per cell.

Kippenberger et al. applied the reverse transcriptase-competitive multiplex PCR to normal human melanocytes, and reported that 20 μM treatment of diethylstilbestrol and estradiol for 48hr lead to an increase of about 1.5 to 2.5 fold of tyrosinase and TRP-2 transcripts (24). The authors reported for the first time that the sex steroids cause an increase of melanogenic enzyme transcripts in normal human melanocytes. An increase in the tyrosinase activity could provoke the switch from pheomelanogenesis to eumelanogenesis. Activation of tyrosinase by estradiol might be an alternative to a direct receptor-mediated mechanism for the growth inhibitory effect observed in vivo and in vitro.

In this study, in spite of the presence of receptor for estrogen and progesterone, they had a donor-specific effect on proliferation of melanocytes. There are several plausible explanations for this lack of effect on melanocyte growth in vitro. The
first is that certain prone melanocytes only can respond to estrogen and progesterone with a stimulation of growth. The genetic predisposition and ultraviolet exposure may be prerequisite or costimulating factors. Alternative explanations for the lack of either a stimulatory response to estrogen and progesterone in vitro include the limited amount of receptor present, a requirement for an estrogen- or progesterone-induced autocrine or paracrine growth factor-mediated growth. The dilution of such factors in culture has been proposed to account for the observed absence of estrogen- or progesterone-stimulated cell growth in the breast tumor cells that otherwise respond to estrogen and progesterone in vivo by increased tumor growth.

I provide the first direct evidence that human cultured melanocytes contain ER and PR immunoreactivity. Human melanocytes uniformly expressed ER and PR in their cytoplasm and nucleus. ER and PR mRNA were present in cultured melanocytes. In spite of the presence of receptor for estrogen and progesterone, estrogen and progesterone had a donor-specific effect on proliferation of melanocytes and tyrosine hydroxylase activity.
V. CONCLUSION

1. I demonstrated the presence of ER and PR in cultured melanocytes by immunocytochemistry.

2. Neonatal melanocytes expressed mRNA transcripts of ER and PR.

3. Sequencing analysis of the RT-PCR products of ER and PR showed 99.9% homology with the reported human ER and PR sequences. These results confirmed that mRNA coding for ER and PR is transcribed and translated in human melanocyte.

4. In spite of the presence of receptor for estrogen and progesterone, they had a donor-specific effect on proliferation of melanocytes.
BIBLIOGRAPHY


-국문요약-

에스트로겐과 프로게스테른이 인체 색소 생성에 미치는 영향

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목적: 인체 색소 침착에 대한 에스트로겐과 프로게스테론의 기전은 잘 알려져 있지 않다. 인체 멜라닌세포에서의 에스트로겐 수용체와 프로게스테론의 수용체를 분자생물학적 방법으로 증명하는 것은 이 기전을 이해하는데 중요하다. 저자는 배양한 멜라닌세포에서 에스트로겐 수용체와 프로게스테론 수용체의 발현 여부와 인체 색소 생성에 에스트로겐과 프로게스테론의 역할을 밝혀서 임신시 잘 발생하는 색소 질환인 기미의 생성 기전을 밝히고자 하였다.

재료 및 방법: 저자는 면역 세포 염색 방법을 통하여 배양한 인체 멜라닌세포에서 에스트로겐 수용체와 프로게스테론의 수용체를 증명하고자 하였다. 역전사 중합 효소 반응과 염기 서열 분석을 통하여 전사 단계에서 에스트로겐 수용체와 프로게스테론의 수용체가 표현됨을 증명하고자 하였다. 에스트로겐과 프로게스테론 치료후에 멜라닌세포의 증식과 티로시나제 활성도에 미치는 영향을 측정하기 위하여 클러 카운터와 티로시나제 분석, 웨스턴 블롯을 실시하였다.
결과: 저자는 면역 세포 염색 방법을 통하여 인체 멜라닌 형성 세포의 핵과 세 로질에서 에스트로겐 수용체와 프로게스테론의 수용체를 증명하였다. 역전사 중합 효소 반응과 염기서열분석을 통하여 전사 단계에서 에스트로젠 수용체와 프로게스테론의 수용체가 표현됨을 증명하였다. 에스트로젠 수용체와 프로게 스테론의 수용체가 표현됨에도 불구하고 생리적인 농도와 업신시 농도의 에스 트로젠과 프로게스테론은 인체 배양 멜라닌세포의 증식과 티로시나제 활성도 에 개인에 따라 다른 영향을 보였다.

결론: 이러한 결과들은 인체 멜라닌 형성세포가 에스트로젠 수용체와 프로게스 테론의 수용체를 표현 하지만 인체 색소 침착에는 공여자 개인의 변이를 보인 다는 사실을 시사한다. 이러한 유도기전과 이 수용체들의 기능을 밝히고 에스 트로젠과 프로게스테론이 멜라닌 형성 세포에 미치는 영향을 명확히 하기 위 해 더욱 심층적인 연구가 필요하다.

핵심되는 말: 프로게스테론, 에스트로젠, 프로게스테론의 수용체, 에스트로젠 수 용체, 멜라닌세포