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Role of Extracellular Signal - Regulated Kinase and Peroxisome Proliferator - Activated Receptor gamma on Transforming Growth Factor - β1 - Induced Human Endometrial Stromal Cell Decidualization

아 주 대 학 교 대 학 원

의 학 과

장 혜 진

Role of Extracellular Signal - Regulated Kinase and Peroxisome Proliferator - Activated Receptor gamma on Transforming Growth Factor - β1 - Induced Human Endometrial Stromal Cell Decidualization

by

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2006년 12월 22일

- ABSTRACT -

Role of Extracellular Signal-Regulated Kinase (ERK) and Peroxisome Proliferator-Activated Receptor gamma ($PPAR_\gamma$) on Transforming Growth Factor(TGF)-β1-Induced Human Endometrial Stromal Cell Decidualization

Objective : To investigate the role of ERK and $PPAR_\gamma$ on TGF-β1-induced human endometrial stromal cell decidualization *in vitro*.

Materials and Methods : Human endometrial tissues obtained by hysterectomy specimens from patients with conditions other than endometrial diseases such as leiomyoma. Endometrial stromal cells were cultured under the following conditions: DMEM/F-12 (10% FBS, 1 nM E_2 and 100 nM P_4). 5 ng/ml of TGF-β1, 50 nM of rosiglitazone ($PPAR_\gamma$ agonist), and 20 μM of PD98059 (ERK inhibitor) were added according to experimental purposes.

Trypan-blue and a hemocytometer were utilized to count the viable cell number. Enzyme-linked immunosorbent assay (ELISA) and Western blotting was employed to detect proteins.

Results : TGF-β1 inhibited proliferation of cultured human endometrial stromal cells and induced expression of prostaglandin E_2 (PGE_2) and prolactin. This effect was mediated by Smad and ERK activation. Addition of rosiglitazone, a $PPAR_\gamma$ agonist, prevented TGF-β1 effect on endometrial cell proliferation. Furthermore,

rosiglitazone inhibited TGF-β1 induced activation of ERK, consequently reducing PGE_2 and prolactin production.

Conclusion: TGF-β1-induced decidualization of endometrial stromal cells through Smad and ERK phosphorylation. $PPAR_\gamma$ acts as a negative regulator of human endometrial cell decidualization *in vitro*.

Key words: Decidualization, TGF-β1, $PPAR_\gamma$, ERK, PGE_2, Smad

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I. INTRODUCTION

The human uterine endometrium is a dynamic organ that undergoes remarkable periodic growth, remodeling and breakdown under hormonal control. The decidualization of human endometrial stromal cells, which develops in the late secretory stage of the menstrual cycle, is characterized by morphological and functional differentiation. The most obvious features of decidualization are the stromal cell transformation. It is characterized by the transformation of the elongated fibroblast-like phenotype of endometrial stromal cell to the larger, rounder phenotype of the decidual cell (Verma, 1983; Tang et al, 1994; Giudice & Ferenczy, 1996). These differentiation processes are mediated by changes of numerous gene expressions of the endometrial stromal cells *in vivo*. The processes can be induced *in vitro* by progesterone in estradiol-treated cultures (Huang et al., 1987; Classen-Linke et al., 1998), by ligands which are coupled to the cAMP pathway such as PGE_2 (Frank et al., 1994), gonadotropins (Frank & Gurpide, 1993), and by cAMP alone (Tank et al., 1993). It was also recently shown that heparin-binding epidermal growth factors and their receptors (Chobotova et al., 2005) participate in the decidualization process.

Transforming Growth factor-β (TGF-β) was first discovered in the fibroblast of white mice's sarcoma (DeLarco & Todaro, 1978). The TGF-β superfamily, which includes various TGF-βs, activin, bone morphogenetic proteins (BMPs), inhibin and growth/differentiation factors, is known as a multifunctional polypeptide cytokine. They are secreted in a latent form and activated by plasmin (Odekone et al., 1994). In

endometrium, TGF-β is a multifunctional cytokine that regulates many biological functions ranging from growth/differentiation to apoptosis of various cell types (Sporn & Roberts, 1990). Because of their powerful effects on the cellular and molecular processes, they are associated with cellular proliferation (Moses et al., 1987) and differentiation (Moses & Serra, 1996), angiogenesis (Renner et al., 2002), extracellular matrix modification and immunomodulation (Akhurt & Derynck, 2001).

Peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors of the nuclear hormone receptor superfamily. Three subtypes of PPARs, which are activated by polyunsaturated fatty acid and eicosanoid metabolites have been characterized ($PPAR_{\alpha}$, $PPAR_{\beta}$ and $PPAR_{\gamma}$). After binding a ligand (e. g. thizolidinediones, arachidonate, or others), it forms a heterodimer with the *cis*-retinoid acid receptor; retinoic X receptor (RXR), binds to a peroxisome proliferator responsive elements (PPRE), and activates transcription of selected genes. However, their specific endogenous functions are yet to be identified. Of the three PPAR isoforms, $PPAR_{\gamma}$ has been implicated in the control of a broad range of cellular responses, such as differentiation, proliferation, cell death and inflammation (Murphy & Holder, 2000; MacDougald & Mandrup, 2002). It has been reported that PPARs can suppress the growth of different types of human cancer cells isolated from colon, breast and prostatic cancer through distinctive ways (Grommes et al., 2004). Overexpression of $PPAR_{\gamma}$ significantly inhibits TGF-β1-mediated Smad activation in human liver cancer cells (Han et al., 2004), and also $PPAR_{\gamma}$ ligands can repress vascular endothelial cell growth factor (VEGF) gene expression via a $PPAR_{\gamma}$-

responsive element (PPRE) in the VEGF gene promoter in human endometrial cells (Peeters et al., 2006).

It was previously reported that progesterone indirectly induced stromal cell decidualization via enhancing the expression and secretion of TGF-β1 from epithelial cells *in vitro*. The secreted epithelial-derived TGF-β1 acted on adjacent stromal cells, at least in part, to turn on Smad signaling that may lead to stromal decidualization. This was reversed by anti-TGF-β1 antibody (Kim et al., 2005).

Extracellular signal-regulated kinase (ERK) is one of the mitogen-activated protein kinase (MAPK) and its major roles are cell proliferation and differentiation (Torii et al., 2004). Also it is known to induce differntiation/G0 arrest in the differentiation of HL-60 cells (Yen et al., 2006).

In this study, by using cultured human endometrial stromal cells, I investigated the role of ERK and $PPAR_{\gamma}$ on the TGF-β1-induced human endometrial stromal cell decidualization *in vitro*.

II. MATERIALS AND METHODS

A. Materials

1. Clinical subjects and endometrial biopsies

Human endometrial tissues were obtained by curettage of hysterectomy specimens from patients undergoing hysterectomy for conditions other than endometrial diseases (leiomyoma or adenomyosis), according to protocols approved by the Institutional Review Board, Ajou University Hospital. Tissue specimens from patients of ages 30 to 45, who were estimated to be in the mid- or late proliferative phase of the menstrual cycle, were taken for the experiment.

2. Reagents and antibodies

Human recombinant TGF-β1 and reagents for ELISA analysis were purchased from Sigma (St. Louis, MO, USA). The $PPAR_{\gamma}$ agonist, rosiglitazone, was purchased from Alexin (San Diego, CA, USA). The ERK inhibitor PD98059 was purchased from Calbiochem (San Diego, CA, USA). The antibodies against human prolactin (goat), p-Smad2 (goat), p-ERK (mouse), $PPAR_{\gamma}$ (rabbit), and COX-2 (goat) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibody against β-actin was purchased from Sigma. The antibodies against goat horseradish peroxidase (HRP), mouse HRP, rabbit HRP were purchased from Chemicon (Temecula, CA, USA).

B. Methods

1. Endometrial stromal cell isolation and primary cell culture

The protocol for cell isolation was identical to that of a previously reported method (Park et al., 2001). Briefly, the endometrial tissues obtained were placed in a conical tube (Falcon, Becton Dickinson, NJ, USA) containing DMEM/F-12 supplemented with 10 % fetal bovine serum (FBS, Gibco Life Technologies) and transported to the aseptic laboratory, where the tissue was again washed several times with phosphate-buffered saline (PBS) to remove any residual blood clots and FBS. Then the tissues were minced in 2-3 ml of Dulbecco's modified Eagle's medium (DMEM, USA). The minced tissues were centrifuged at 85×g in a conical tube and 10 ml of trypsin-EDTA (Gibco Life Technologies, Grand Island, NY, USA) was added to the pellet and incubated in a shaker at 37 ℃. After 30 min of incubation, 1 ml of 10% heat-inactivated FBS was added to stop the enzymatic reaction. The tissues were centrifuged and the upper portion was removed. The cell pellet was suspended with 5ml of PBS and centrifuged again. After trypsinizing and washing with PBS, the cells were resuspended in 10ml DMEM/F-12 to a final concentration of 5 x 10^6 cells/ml, and 8 ml of the cell suspension was seeded on a 100mm culture dish (Corning, NY, USA). After incubation for 24 hr, cells and red blood cell (RBC) unattached but suspended in the media were washed with PBS, attached cells were cultured for 72 hr in DMEM/F-12 medium (10 % FBS; 1 nM E_2; 100 nM P_4) under the following conditions ; 5 ng/ml of TGF-β1, 50 nM of $PPAR_\gamma$ specific ligand,

rosiglitazone , and 20 μM of phosphorylated-ERK inhibitor, PD98059. Unless stated elsewhere, all reagents were purchased from Sigma.

2. Cell count by using trypan-blue dye

Cell growth was determined by using trypan-blue (Gibco, USA) stain. Viable cells are able to repel the dye and thus can not be stained. After trypsinizing and washing with PBS buffer, the stromal cells were resuspended in 10ml DMEM/F-12 and the same volume of trypan-blue was added. Viable stromal cells that were not stained with trypan-blue dye, were counted by a hemocytometer.

3. Western blot analysis of prolactin, pSmad2/3, PPAR$_\gamma$, COX-2 and pERK

At the end of each treatment, cells were scraped off the plates and centrifuged, resuspended with PBS buffer containing 40 nM Tris-HCl (pH 8.0), 120 mM NaCl, 0.1 % Nonidet P-40 and protease inhibitors. After sonication, the whole cell lysate was collected by centrifugation at 14,000 rpm at 4 ℃ for 20 min. Total proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE; 8, 10, 15 %) and transferred onto nitrocellulose blotting membrane (Satorius AG, Germany). The membranes were blocked with 5 % non-fat dry milk in Tris-buffered saline and incubated with primary antibodies for 1 hour at 4 ℃. Blots were developed by peroxidase-conjugated secondary antibody, and proteins were visualized by enhanced chemiluminescence (ECL) procedure (Santa Cruz Biotechnology, USA), according to the manufacturer's recommendations.

4. Measurement of Prostaglandin E_2 concentrations in the conditioned media

To measure the secretion of prostaglandin E_2 (PGE_2) by cells during culture in various culture media for 72 hr, cells were removed by centrifugation, the cell-free conditioned media was collected and was stored at -75 ℃ until use. After thawing at room temperature, the medium was analyzed for immunoreactive PGE_2 by enzyme-linked immunosorbent assay (ELISA) using anti-PGE_2 antibodies (ELISA kit) according to the manufacturer's specifications with absorbance at 450 nm in a plate reader (SpectraMax 190, Molecular Device, Sunnyvale, CA, USA). Experiments were repeated at least three times with different media under the same condition to minimize intra- and interassay variations.

5. Statistical analysis

Student's t-test was used for the statistical analysis of concentrations of PGE_2 in the culture media. P values of < 0.05 were considered to be statistically significant. Mann-Whitney test was used for the statistical analysis of cell count by trypan blue staining. Unless otherwise indicated, $P < 0.05$ was considered to be statistically significant.

III. RESULTS

A. The effect of TGF-β1 on the human endometrial stromal cell proliferation

Endometrial stromal cells were treated with or without 5 ng/ml of TGF-β1 for 72 hr. After that cells were stained with trypan-blue dye and counted using a hemocytometer. As shown in Fig. 1, TGF-β1 inhibited *in vitro* cell proliferation of endometrial stromal cells significantly compared to the control.

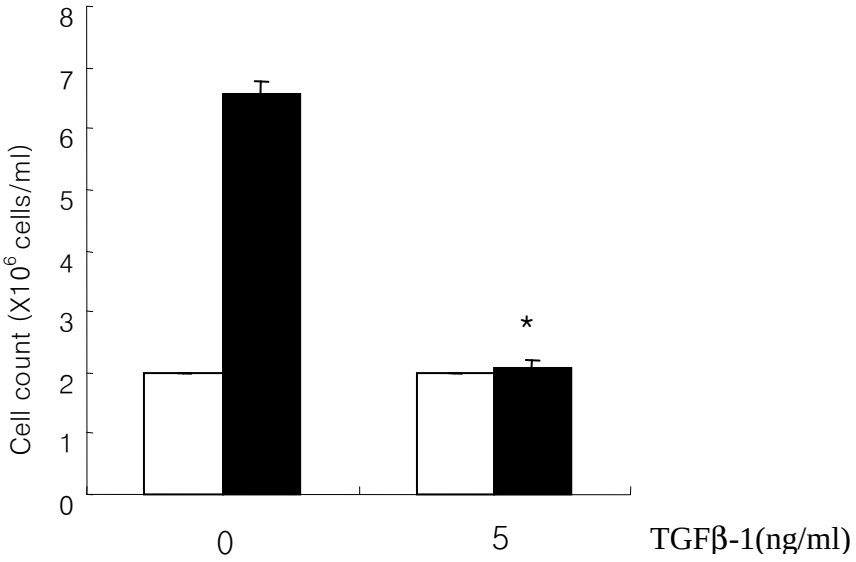

Fig. 1. Effect of TGF-β1 on the endometrial stromal cell proliferation. Endometrial stromal cells were treated with or without 5 ng/ml of TGF-β1 for 72 hr. Cells were stained with trypan-blue and counted using hemocytometer. TGF-β1 inhibited proliferation of cultured endometrial stromal cells. Data are the means ± SE of three independent experiments. Open bar; 0 hr, Black bar; 72 hr. * *p* value < 0.05

B. The effect of TGF-β1 on the expression of prolactin, PPAR$_\gamma$, and pSmad2/3 by endometrial stromal cells during decidualization

To confirm the effects of TGF-β1 on the stromal cell decidualization, stromal cells were maintained under serum-free condition for 24 hr, and then treated with TGF-β1 at concentration of 5 ng/ml for 72 hr. As shown in Fig. 2, 5 ng/ml of TGF-β1 induced expression of prolactin, and Smad 2/3 phosphorylation after 72 hr treatment. On the other hand, at the same time, expression of PPAR$_\gamma$ was significantly decreased by treatment of TGF-β1. We didn't show total Smad 2/3 here, because the value was same indivisually.

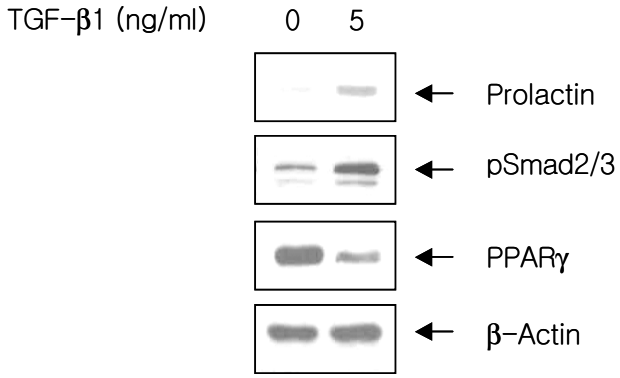

Fig. 2. Expression of prolactin, PPARγ and pSmad 2/3 in cultured endometrial stromal cells. Endometrial stromal cells were treated with or without 5 ng/ml of TGF-β1 for 72hr. Cell lysates were subjected to western blot analysis using anti-prolactin, phosphorylated Smad2/3 and PPARγ antibodies. TGF-β1 induced expression of prolactin and pSMAD2/3 after 72 hr treatment. Expression of PPARγ was inhibited by TGF-β1 treatment for 72 hr.

C. Rosiglitazone restores the mitosis-inhibitory effect of TGF-β1 on endometrial stromal cells

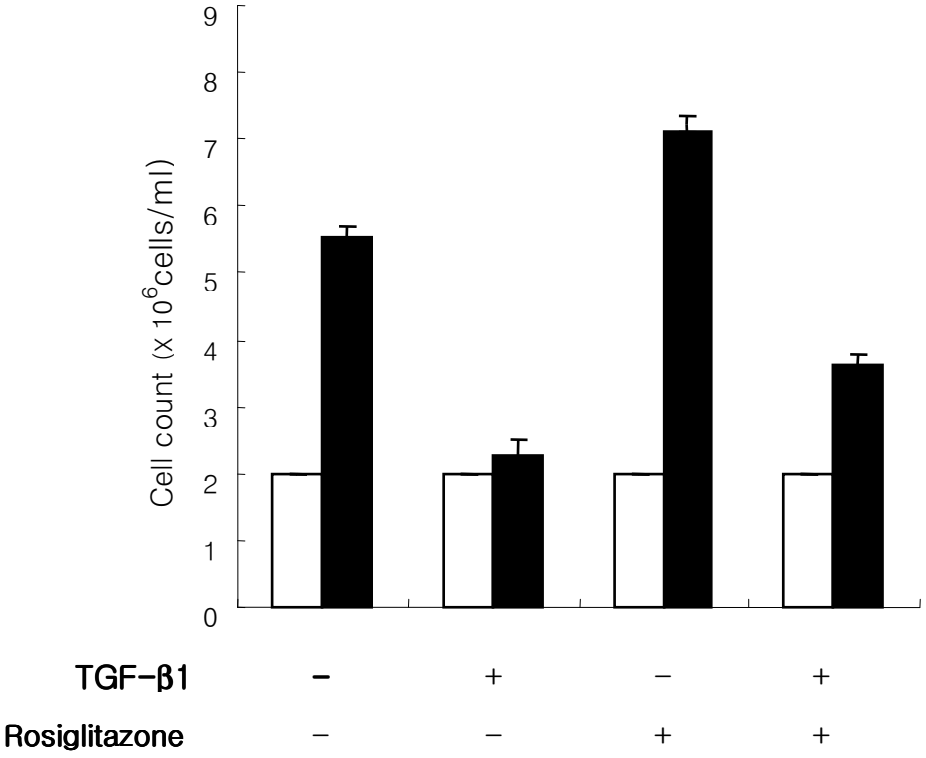

Fig. 3. Effect of TGF-β1 on the cell proliferation was prevented by rosiglitazone (PPAR$_\gamma$ agonist) treatment. Endometrial stromal cells were treated with 5 ng/ml of TGF-β1 and/or 50 nM of rosiglitazone for 72 hr. Cells were stained with trypan-blue and counted using hemocytometer. Cell proliferation was increased by co-treatment of TGF-β1 and rosiglitazone compared with TGF-β1 treatment alone. Data are the means ± SE of three independent experiments. Open bar; 0 hr, Black bar; 72 hr.

It was also examined whether the signaling pathway of TGF-β1 was controlled by $PPAR_{\gamma}$. To do this, endometrial stromal cells were cultured with 5 ng/ml of TGF-β1 and/or 50 nM of rosiglitazone, the specific ligand of $PPAR_{\gamma}$ for 72 hr. As shown in Fig. 3, the mitosis-inhibitory effect of TGF-β1 was reversed by combined treatment with rosiglitazone.

D. The changes of prolactin, pSmad2/3, $PPAR_{\gamma}$, pERK, COX-2 and PGE_2 expression by rosiglitazone

Rosiglitazone treatment alone resulted in the considerable decrease of prolactin expression and Smad2/3 phosphorylation compared with the TGF-β1 treatment alone. Also, COX-2 and pERK expression was significantly inhibited by combined treatment with rosiglitazone (Fig. 4A). COX-2 is known as a key enzyme of prostaglandin production. And thus concentration of PGE_2 in stromal cell cultured medium was analyzed using an ELISA assay. Concentration of PGE_2 in the cell culture conditioned medium was decreased by combined treatment with TGF-β1 and rosiglitazone, whereas rosiglitazone did not affect on PGE_2 concentration in the same medium (Fig. 4B). Total ERK was not shown here, because the vaule was same indivisually.

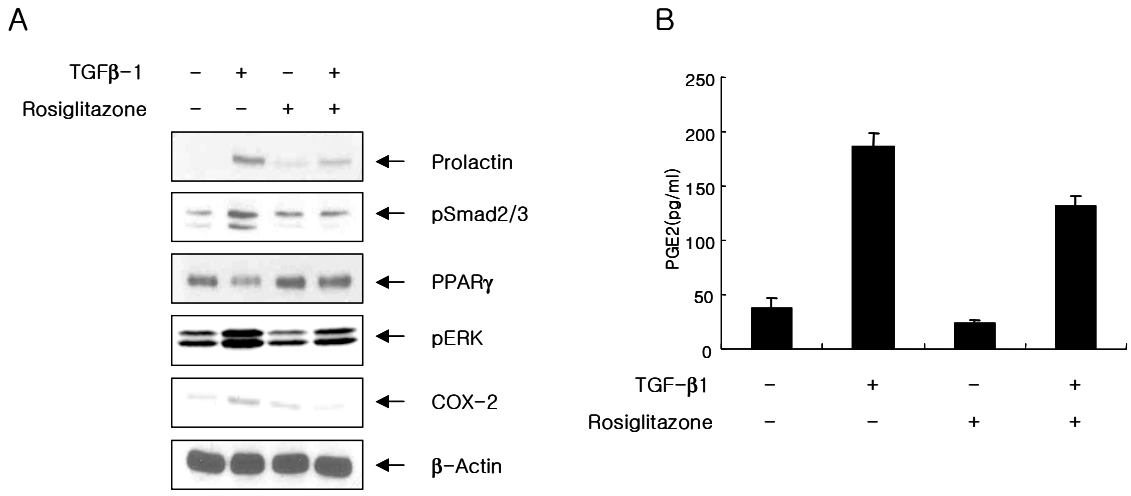

Fig. 4. Rosiglitazone reduced expression of pSmad, pERK, COX-2 and PGE2 in cultured endometrial stromal cells. Endometrial stromal cells were treated with 5 ng/ml of TGF-β1 and/or 50 nM of rosiglitazone for 72 hr. Cell lysates were subjected to Western blot analysis using anti-prolactin, pSmad2/3, PPARγ, pERK, COX-2 antibodies. TGF-β1 induced expression of prolactin, pSmad2/3, pERK, and COX-2 were prevented by combined treatment of rosiglitazone (A). Concentration of PGE_2 in culture medium was measured by ELISA. TGF-β1 induced PGE_2 releasing from cultured endometrial stromal cells was inhibited by combined treatment of rosiglitazone. Data are the mean ± SE of three independent experiments (B).

E. The change of prolactin, pERK, COX-2 and PGE_2 expression by PD98059

To verify the effect of ERK on the COX-2 expression and the prostaglandin production in cultured endometrial stromal cells, endometrial stromal cells were cultured with 5 ng/ml of TGF-β1 and/or ERK inhibitor, 20 μM of PD98059 for 72

hr. As shown in Fig. 5A, PD98059 inhibited the TGF-β1-induced prolactin, ERK phosphorylation and COX-2 expression simultaneously. Concentration of PGE_2 in culture medium was decreased by combined treatment of TGF-β1 and PD98059 (Fig. 5B).

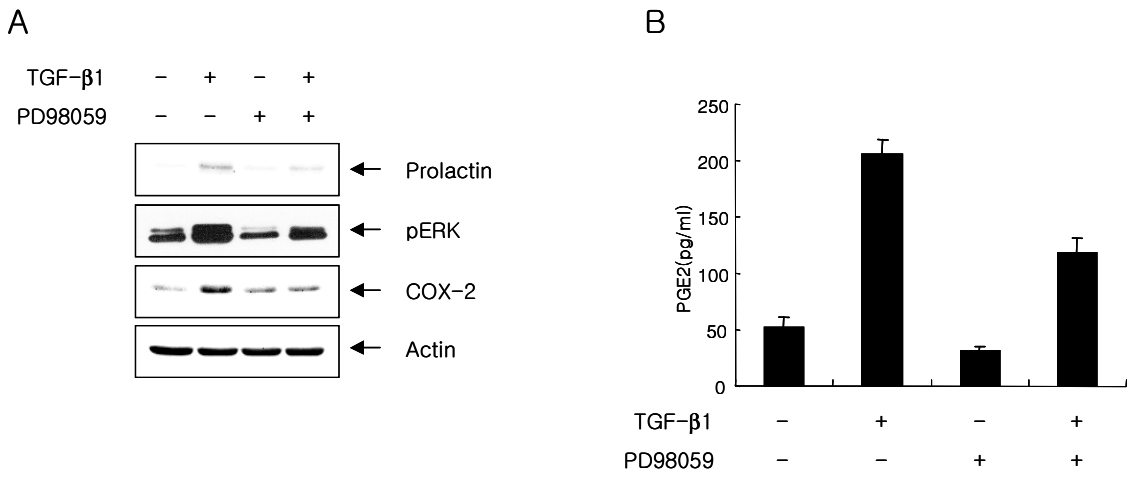

Fig. 5. PD98059 reduced expression of pERK, COX-2 and PGE_2 in cultured endometrial stromal cells. Endometrial stromal cells were treated with 5 ng/ml of TGF-β1 and/or 20 μM of PD98059 for 72 hr. Cell lysates were subjected to western blot analysis using anti-prolactin, pERK, COX-2 antibodies. TGF-β1-induced expression of prolactin, pERK, and COX-2 were inhibited by combined treatment of PD98059 (A). Concentration of PGE_2 in culture medium was measured by ELISA. TGF-β1-induced PGE_2 releasing from cultured endometrial stromal cells was inhibited by combined treatment of PD98059. Data are the mean ± SE of three independent experiments (B).

IV. DISCUSSION

Generally, cell differentiation is known to occur when cell proliferation stops. Previous reports have shown that progesterone induces endometrial stromal cell decidualization via triggering expression and secretion of TGF-β1 by epithelial cells (Kim et al., 2005). In the present study, it was investigated whether TGF-β1 could affect on the endometrial stromal cell proliferation, and that TGF-β1 might inhibit cell proliferation. The results suggest that a possible linkage between the intracellular signaling pathway via TGF-β1-mediated Smad phosphorylation and the molecules associated with cell proliferation and differentiation of endometrial stromal cells might exist.

$PPAR_{\gamma}$ is widely expressed in various tissues including adipose tissue, placenta, amnion and choriodecidual tissue, although the exact role of $PPAR_{\gamma}$ in these tissues remains unclear (Marvin et al., 2000). The present study shows that $PPAR_{\gamma}$ expression is decreased in TGF-β1-induced decidualization of endometrial stromal cells. Consequently it was attempted further to elucidate the role of $PPAR_{\gamma}$ in TGF-β1-mediated stromal cell decidualization process. To ascertain the change in TGF-β1-mediated cellular response by $PPAR_{\gamma}$ activation, cell proliferation of cultured stromal cells was investigated after treatment with TGF-β1 and/or $PPAR_{\gamma}$ ligand, rosiglitazone. The result showed that TGF-β1-mediated mitoinhibition could be restored by adding rosiglitazone. $PPAR_{\gamma}$ activation by rosiglitazone inhibited not only mitoinhibition, but also Smad phosphorylation as well. Based on these

observation, it is suggested as follows ; TGF-β1-induced decidualization takes place via Smad phosphorylation. Subsequently, cell proliferation is suppressed, and endometrial stromal cells differentiate into decidual cells simultaneously. However these mechanisms are regulated by activation of $PPAR_{\gamma}$. Similar findings was made in the previous reports in which the $PPAR_{\gamma}$ inhibited the TGF-β-induced differentiation in pulmonary myofibroblasts (Burgess et al., 2005). To further confirm the above conclusions, whether intracellular signaling transmission of TGF-β1 might influence cell growth factor activity was examined.

ERK is a type of MAPK, which is known to have an important role in cell proliferation and differentiation. Enhanced ERK activation affects differentiation and G0 arrest in specific cell differentiation (Yen et al., 2006). Present study shows that TGF-β1 could induce not only decidualization of endometrial stromal cells, but also inhibition of cell proliferation, and enhancement of ERK phosphorylation. Overall, these results suggests that TGF-β1 could induce ERK phosphorylation, thereby resulting in the differentiation/G0 arrest of endometrial stromal cells, which again could be prevented by $PPAR_{\gamma}$ activation.

Prostaglandins (PGs) are ubiquitous compounds involved in various homeostatic and inflammatory processes throughout the body. They are formed by the combined action of phospholipase A_2, which liberates arachidonic acid from the *sn*-2 position of cellular membrane phospholipids, and cyclooxygenase (COX), which converts arachidonic acid into the endoperoxide intermediate, PGH_2. PGH_2 is subsequently converted to various PGs by the action of cell-specific synthases (Vane et al., 1998). It is well known that PGs have an important role in ovulation, implantation, and

parturition (Poyser, 1995). The suppression of PG biosynthesis is known to inhibit endometrial stromal cell decidualization, which can be induced by artificial manipulation of the mouse uterus (Rankin et al., 1979). Thus, it was investigated whether TGF-β1 might affect PG synthesis, and the process could be prevented by activation of $PPAR_{\gamma}$ in human endometrial stromal cells *in vitro*. The results shows that TGF-β1 could increase the expression of COX-2, in order to possibly induce PG biosynthesis and PGE_2 release from cultured endometrial stromal cells. In addition to this, activation of $PPAR_{\gamma}$ by rosiglitazone prevented this mechanism. From the results, it was found that TGF-β1-induced decidualization occurred through Smad and ERK phosphorylation. In murine fibroblasts, activation of the Ras/Raf-1/ERK signal pathway is required for COX-2 induction (Xie et al., 1994). To further confirm whether the ERK phosphorylation affect PGE_2 biosynthesis, it was examined the COX-2 expression and PGE_2 release from the cultured stromal cells after treatment with ERK phosphorylation inhibitor, PD98059. It was observed that COX-2 expression and PGE_2 concentration in medium significantly decreased by PD98059 treatment, and prolactin expression of stromal cells also decreased. These findings demonstrate the role of ERK-mediated COX-2 activation in the TGF-β1-induced PGE_2 synthesis, and that $PPAR_{\gamma}$ is the counteracting part of TGF-β1-mediated mitoinhibition through the suppression of ERK and Smad phosphorylation.

V. CONCLUSION

In conclusion, TGF-β1 potently induces decidualization of cultured human endometrial stromal cells *in vitro*. The study also demonstrates the expression of pSmad 2/3 and pERK, which are intracellular mediators of TGF-β1 signaling in stromal cells. This proteins would subsequently increase PGE_2 production and finally induce stromal cell mitoinhibition and prolactin expression during decidualization. $PPAR_\gamma$ could reverse these processes. It is evident in the present study that $PPAR_\gamma$ is linked to crosstalk between ERK1/2 and Smad signaling cascades activated by TGF-β1 in decidualization process of cultured human endometrial stromal cells.

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– 국문요약 –

Transforming Growth Factor-β1 에 의해 유도된 인간 자궁내막 기질세포의 탈락막화에서 Extracellular Signal-Regulated Kinase 와 Peroxisome Proliferator-Activated Receptor$_\gamma$ 의 역할

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자궁내막 탈락막화는 수정란의 착상 및 임신의 유지에 중요한 자궁 내막의 분화과정으로 TGF-β1 가 관여한다고 알려져 있다. 본 연구를 통해 TGF-β1 에 의해 유도된 인간자궁내막의 탈락막화 과정에서 ERK 와 PPAR$_\gamma$의 역할을 규명하고자 하였다. 자궁내막 기질세포는 DMEM/F12 (10% FBS, 1nM E2 and 100nM P4) 조건에서 배양하였다. 연구 목적에 따라 TGF-β1 (5 ng/ml), Rosiglitazone (50 nM)과 PD98059 (20 μM)를 배양액에 첨가하였다. Trypan-Blue 와 hemocytometer 를 이용하여 현미경하에서 세포의 개수를 측정하였다. Enzyme-linked immunosorbent assay (ELISA)와 Western blotting 방법을 사용하여 Prostaglandin E_2 (PGE_2) 및 prolactin 단백질의 발현 정도를 관찰하였다. 배양액에 TGF-β1 을 첨가하여 세포의 증식정도를 측정한 결과 TGF-β1 이 세포의 증식을 억제하는 것을 알 수 있었다. 또한 배양된 세포로부터 PGE_2 및 prolactin 의 발현을 유도하는 것을 알 수 있었고,

이러한 TGF-β1 의 작용은 Smad 및 ERK 의 활성화를 통하여 일어남을 알 수 있었다. $PPAR_{\gamma}$의 기질인 rosiglitazone 을 배양액에 첨가한 결과 TGF-β1 에 의한 세포 증식의 억제가 역전되는 것을 알 수 있었다. 뿐만 아니라, 세포 내 ERK 의 활성 역시 억제 시켰으며 이 결과 PGE_2 와 prolactin 의 발현이 감소되는 것을 관찰할 수 있었다. 따라서 본 연구를 통해 TGF-β1 에 의한 자궁내막 기질세포의 탈락막화는 Smad 와 ERK 의 활성화를 통하여 이루어지며 이러한 과정은 PPAR γ 에 의해 억제됨을 확인하였다.

핵심어 : 탈락막화, TGF-β1, $PPAR_{\gamma}$, ERK, Smad