Original Articles/นิพนธ์ต้นฉบับ

Effect of *In vitro* Culture of Human Myometrial and Fibroid Smooth Muscle Cells on Protein Estrogen Receptor

Duangporn Saengwimol[1], Wanwisa Waiyaput[1], Somphoch Pumipichet[2], Areepan Sophonsritsuk[3]

[1] *Research Center Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*

[2] *Department of Gynecology Faculty of Medicine, Srinakharinwirot University*

[3] *Reproductive Endocrinology Unit, Department of Obstetrics & Gynecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*

Abstract

Background: Uterine leiomyomas (fibroids, myomas) are the most common benign smooth muscle cell tumors in the reproductive tract. The sex hormone estrogen has been implicated as a major regulator of fibroid growth. Estrogen exerts its physiological effects on the target cells by binding to specific nuclear receptors, the estrogen receptor α (ERα) and estrogen receptor β (ERβ).

Objective: To assess the expression of ERα and progesterone receptor (PR) on primary culture smooth muscle cells (SMCs) from myometrial and fibroid tissue for the potential use of primary culture SMCs as an experimental model for studying ER activity, PR activity, and drug testing.

Methods: Quantitative real-time PCR (qPCR) and Western blot analysis were used to compare ERα between paired myometrial and fibroid tissues, as well as cells isolated from the same tissues and cultured up to the three passages.

Results: ERα and PR levels in cultured cells were rapidly lost comparing with whole tissues for both myometrium and fibroid. Moreover, ERα protein was degraded after tissue digestion prior to the culturing process.

Conclusions: Primary human myometrial and fibroid smooth muscle cell cultures are not suitable for studying ER *in vitro* model. The stable ER and PR cell lines were the model of choice.

Keywords: Leiomyoma, fibroid, myometrium, estrogen receptor, *in vitro* culture.

Corresponding Author: Areepan Sophonsritsuk
Reproductive Endocrinology Unit, Department of Obstetrics & Gynecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400 Thailand.
Tel. 662-201-1412 Email: areepan.sop@mahidol.ac.th

Introduction

Uterine leiomyoma or fibroid is a benign tumor arising from smooth muscle cells in the uterus. The fibroid is commonly found in the reproductive women age between 20 and 50 years[1], up to 70% of cumulative incidence in menopausal women[2]. Common symptoms of uterine leiomyoma include menorrhagia, miscarriage, difficult pregnancy, frequent urination and constipation. About one-third of women diagnosed with uterine leiomyoma are symptomatic; hence, the fibroid often requires surgical removal. The incidence of uterine leiomyoma in Thai population has never been reported. The exact cause of uterine leiomyoma is still unclear but some studies have reported that the enlargement of fibroid is regulated by estrogen[3-5]. Estrogen activates estrogen receptor (ER) presenting on the nuclear membrane which then translocating into the nucleus to act as a transcription factor. Previous study showed that the expression of ER in uterine leiomyoma is higher than the normal myometrium[6,7]. In addition, clinical evidence also shows a relationship between level of estrogen and fibroid growth. Most fibroids increase in size among women in the reproductive age that associated with a high level of estrogen, and decrease in size after oophorectomy or menopause.

ER is classified into two classes: NR3A1 (ERα) and NR3A2 (ERβ). ERα plays a role in reproductive system such as endometrium and ovarian stromal cells. ERβ acts in the development of brain and cardiovascular system[8]. Previous study demonstrated that mice without *ESR1* gene expression encoding for the expression of ERα failed to naturally reproduce offsprings. On the contrary, *ESR2* gene - knockout mice lacking of ERβ, but not ERα, can successfully reproduce[9]. A study by Gargett et al concluded that the expression of ERβ was not detected in myometrial and fibroid smooth muscle cells (SMCs)[10], whereas the expression of ERβ in fibroid microvascular endothelium was detected[11].

The focus of this study is to assess the expression of ERα on primary culture SMCs for the potential use of primary culture SMCs as an experimental model for studying ER activity or drug testing. To date, human myometrial and fibroid SMC stable cell lines have not been well established; therefore, we tried to develop primary cell culture of myometrial and fibroid SMCs as a model for studying the development of fibroids as well as for ER and PR study. The aim of this experiment is to compare the expression of ERα between myometrial and fibroid tissues collected from patients during culture, before, and after digesting tissue using Western blot analysis and qPCR.

Materials and methods

Tissue collection

Fibroid and myometrial tissues were obtained from five pre-menopausal women age 30 - 40 years who underwent hysterectomy for uterine fibroids (Figure 1) and had not received hormonal or drug therapy. Informed consent was obtained. Ethical approval was obtained from the Human Rights and Ethic Committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University. Tissue was snap frozen in liquid nitrogen immediately after excision, and was also collected in the Hank buffer saline solution (HBSS) (Gibco BRL) during transporting to the laboratory in order to processing and culturing. Snap frozen tissue was stored at -80 °C until protein extraction.

Primary cell cultures

The tissue specimen were minced into small pieces and digested with collagenase type II (Worthing-

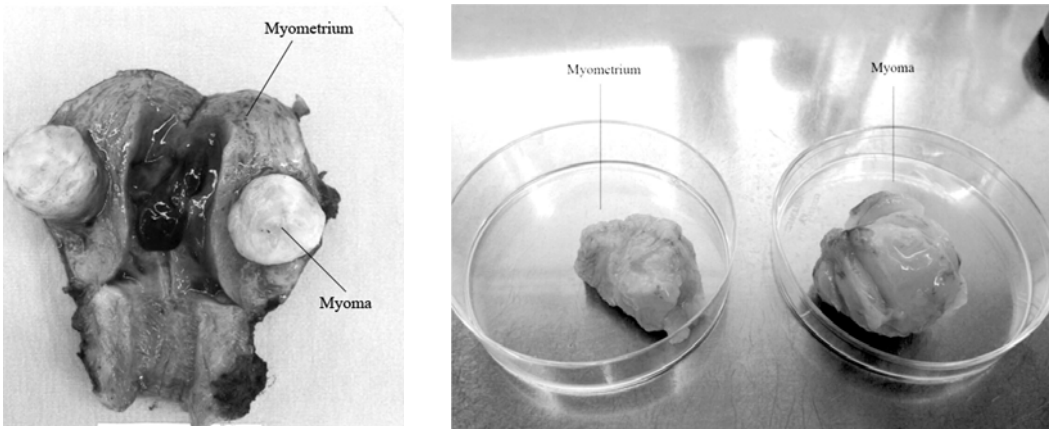

Figure 1 *Fresh operative specimens of a uterus were obtained from woman in the proliferative phase and secretory phase of menstrual underwent elective hysterectomies.*

ton) in the culture medium (phenol red-free DMEM/F12) (Gibco BRL) at 37 °C for 3-4 hours. The digested tissues were passed through the cell strainer. The cells were then collected by centrifugation at 1,500 rpm for 10 minutes and washed several times with phosphate buffer solution (PBS). The resulting cell pellets were resuspended in the culture medium supplemented with 10% fetal bovine serum (FBS) (Gibco BRL) and antibiotic solution ($1x10^5$ U/L penicillin and 50 mg/L streptomycin). The cells were seeded in 75 cm^2 culture flask. The experiments were done on secondary and tertiary cultures. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Growing cells were assessed in the experiments.

Protein extraction and Western immunoblotting

The homogenized tissue and cultured cells were lysed with ice-cold lysis buffer (150 mM NaCl, 50 mM Tris-Cl pH 8.0, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and protease inhibitor cocktail tablets), and incubated at 4 °C for 20 minutes. The extracts were centrifuged at 14,000 xg for 15 minutes and the supernatant were then collected. Protein concentrations were determined by the Bradford assay and equal amount of total protein were loaded into each well. Protein samples (30 μg protein) were resolved by 10% SDS-polyacrylamide gel electrophoresis, and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat dry milk in Tris buffer saline-Tween 20 (TBST) for 1 hour, and incubated overnight with ERα antibody (F10; Santa Cruz Biotechnology, Santa cruz, USA) and GAPDH antibody as control (6C5; Santa Cruz Biotechnology, Santa cruz, USA) at a 1:200 dilution in 5% blocking solution (TBST that contained 5% non-fat dry milk) at 4 °C on a shaker. After being washed three times in TBST, the membranes were incubated with goat anti-mouse IgG-HRP (Santa Cruz Biotechnology, Santa cruz, USA) at 1:2,000 dilution in 5% blocking buffer for 1 hour. The antigen-antibody complexes were detected with the enhanced chemiluminescence detection system (Thermo Scientific, USA) and images were acquired by C-DiGit Blot Scanner (Licor Biotechnology, USA).

Reverse transcription and qPCR

Total RNA from homogenized tissue and culture cells was extracted by RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instruc-

tions. Reverse transcription reactions were carried out using the reverse transcriptase system (Promega, Madison, WI). All qPCR were performed by a real-time PCR machine CFX96 (Bio-Rad Laboratories) with the SoFast EvaGreen (Biorad) and gene-specific primers purchased from Integrated DNA technologies (IDT). Quantification of gene expression was assessed by the comparative cycle threshold ($2^{(-\Delta\Delta ct)}$) method. The following primer pairs were used for amplification: ESR1 forward, 5'-TGATGAAAGGTGGGATACG

A3'; ESR1 reverse, 5'-AAGGTTGGCAGCTCTCA TGT3', GAPDH forward, 5'-TCTCTGCTCCTGTT

C3'; GAPDH reverse, 5'-ACCAAATCCGTTGACT CC3', TFF1 forward, 5' -GCCCTCCCAGTGTGCA

AATA3'; GAPDH reverse, 5' -CTGGAGGGACGTC GATGGTA3' and V-MYC forward, 5' -TGCCGC

ATCCACGAAACT3'; GAPDH reverse, 5' -GTCC TTGCTCGGGTGTTGTAAG3'

Results

ERα protein expression in the primary culture of smooth muscle cells (SMCs)

ERα proteins (66 kilodaltons) from myometrial and fibroid tissues obtained from the same patient before and after digestion with enzyme collagenase type II and the ones from different culture passages were compared by Western blot analysis. With the same concentration of protein used, the expression of ERα was observed in both types of tissues prior to digestion. However, after digestion with collagenase type II the expression of ERα was not observed from any passage (Figure 2). On the contrary, ERα expression of fibroid SMCs was noticed in P0 but not in the later passage (Figure 2). The digestion has no effect on Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression on both myometrial and fibroid SMCs.

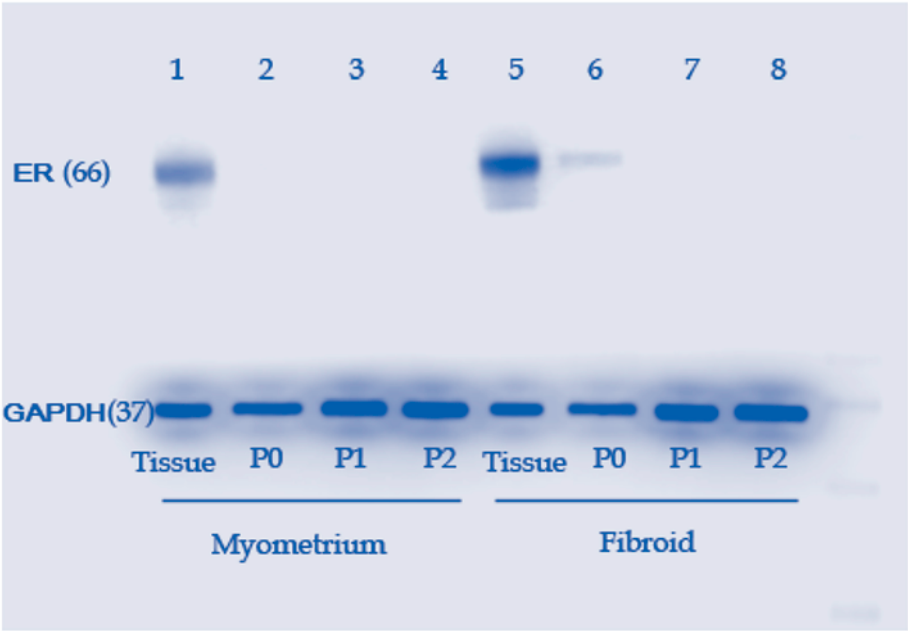

Figure 2 *Effects of sex steroids on the expression of ERα protein (66 kilodaltons) assessed by Western blot analysis in SMCs culture in vitro compared with the whole tissues both myometrial and fibroid. GAPDH (37 kilodaltons) was used as a protein load control.*

The expression of ER was observed in both types of tissues prior to digestion (Lane 1 and 5). After digestion with collagenase type II the expression of ERα was not observed from any passage (Lane 2-4). ERα expression of fibroid SMCs was noticed in P0 (Lane 6) but not in the later passage (Lane 7-8).

ERα and *PR* gene expression in the primary culture of SMCs and fibroid and myometrial tissues

The qPCR analysis of *ESR1* gene between myometrial and fibroid tissues and their respective cells, P0 to P3, showed loss of gene expression in both myometrial cells and fibroid SMCs after the digestion process (P0-P3) (Figure 3). The expression of *ESR1* gene was found to be 2.7 fold higher in fibroid tissue when compared to myometrial tissue using $2^{(-\Delta\Delta ct)}$ method (Figure 4). Loss of *PR* gene expression was demonstrated in both myometrial cells and fibroid SMCs after the digestion (Figure 5). Slightly decreased *avian myelocytomatosis viral oncogene neuroblastoma derived homolog (V-MYC)* without decreased *trefoil factor 1 (TFF)-1* gene expression in both myometrial cells and fibroid SMCs was demonstrated (Figure 6). Both genes were estrogen-regulated target genes.

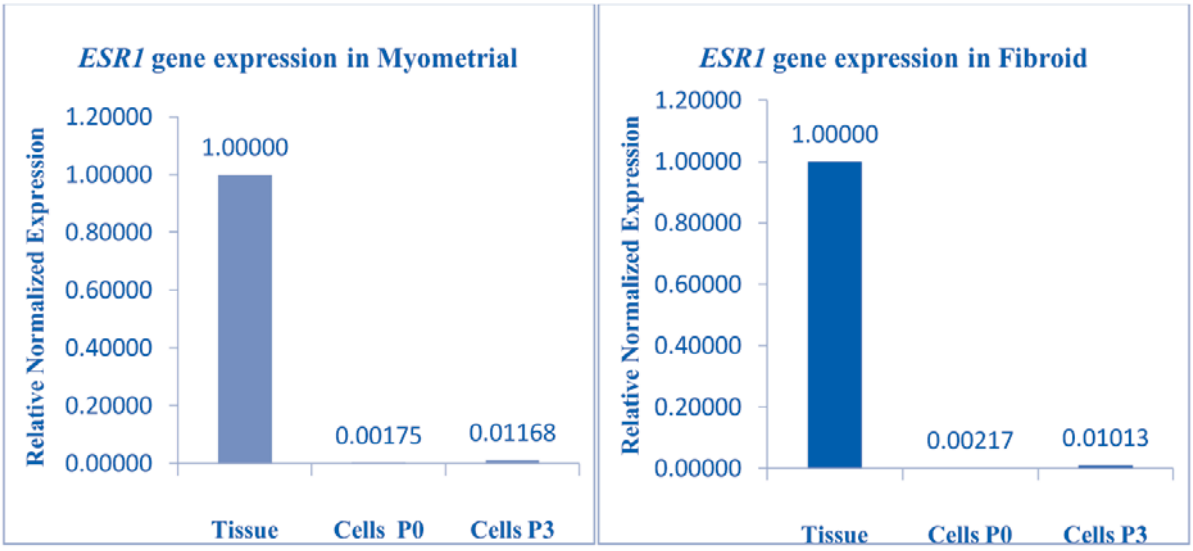

Figure 3 *ESR1 gene expression in myometrial tissue and myometrial cell culture (A) and in fibroid tissue and fibroid SMCs culture (B). After the digestion process (P0-P3), gene expression was lost in both myometrial and fibroid SMCs culture.*

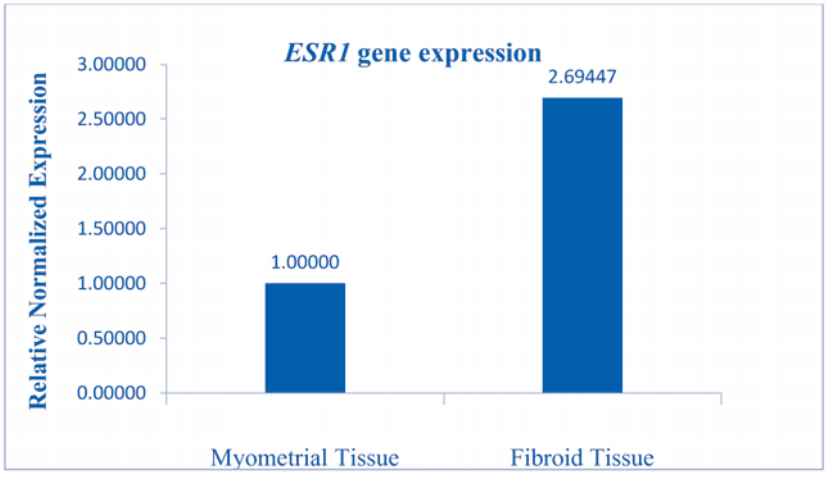

Figure 4 *Comparing of ESR1 expression level between myometrial and fibroid tissues. The ESR1 gene expression in fibroid tissue was 2.7 fold higher than myometrial tissue*

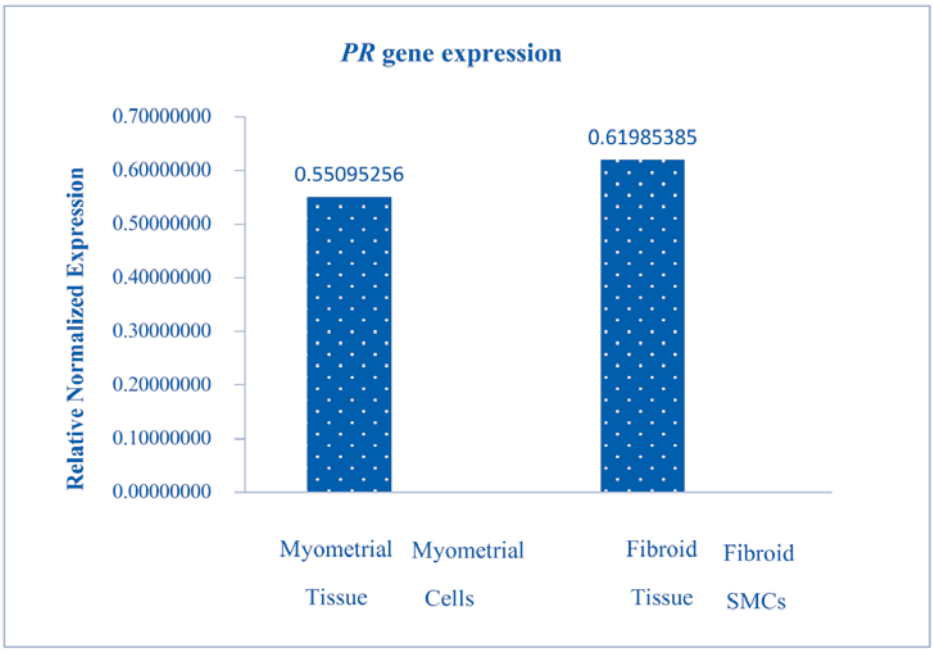

Figure 5 *Comparing of PR expression levels between myometrial and myometrial cells, and fibroid tissues and fibroid SMCs.* PR *gene expression was lost in both myometrial cells and fibroid SMCs after the digestion*

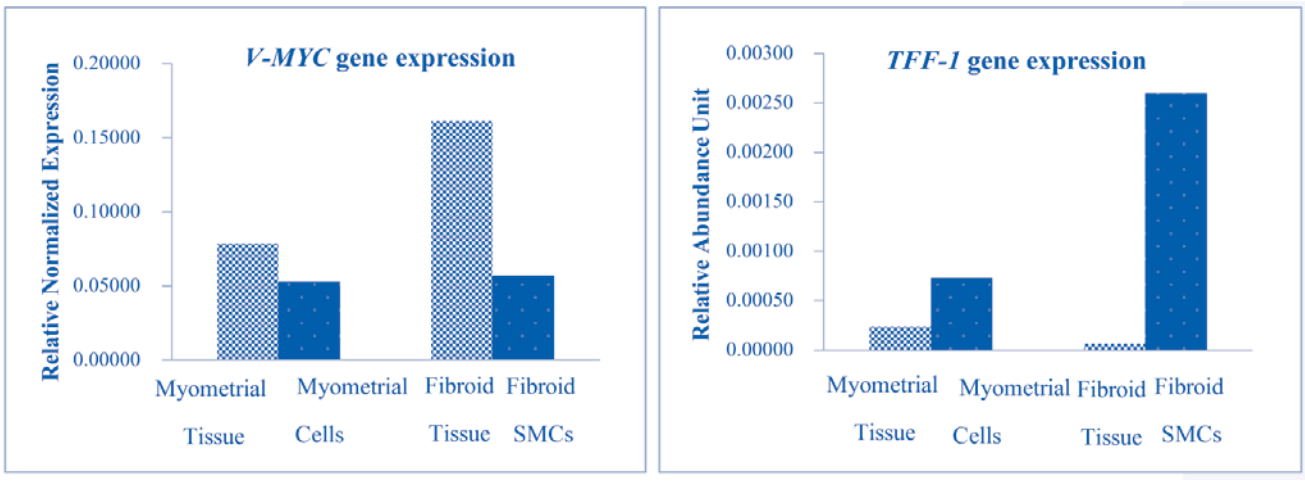

Figure 6 *Comparing of V-MYC (A) and TFF-1 (B) expression levelsbetween myometrial andmyometrialcells, andfibroid tissues and fibroid SMCs.*

Discussion

In the present study we cultured SMCs extracted from the uterine fibroids and adjacent normal myometrial tissues at least 3 passages, and performed gene and protein expression for SMCs and the myometrial tissues. We found that SMCs from uterine fibroids grew slowly in the culture and became senescent earlier than the ones from the normal myometrium. The expression of ER was reduced significantly when the tissues were digested with enzyme collagenase type II. Furthermore, the expression of the receptors was also decreased during the culture

of 3 passages. The expression of estrogen targeted genes, i.e. V-MYC and TFF-1, was slightly decreased. We had also studied different methods, for example, using tissues from younger patients, modifying cell culture conditions, but the results showed no expression of ER and PR at both the mRNA and the protein levels which was consistent to previous studies[12-14]. However, Sadovsky et al[12] studied the expression of ER from SMCs using rabbit model where as Severino et al[13] isolated SMCs from uterine fibroids and myometrium using tissue minced explant method, not enzyme digestion. Zaitseva et al[14] isolated SMCs using relatively similar technique to ours, but the authors digested the uterine fibroids and myometrium with trypsin. They also showed the reduction of ERα and PR in the cell culture of up to 3 passages when incubated with β-estradiol (E_2) and progesterone. Global gene expression with microarray hybridization, and RT-PCR was performed and found that there were 648 genes changed between tissues and cultured cells. ERα was one of 648 genes that found down-regulation. Therefore, the decreased ERα of primary culture of SMCs was not depend on SMC isolation technique, enzyme digestion or tissue explants, and the changes could occur in other species, not just human. We speculate that the characteristics of SMCs would be different when the cells were separated from each other versus when the cells were organized in the tissues. Further studies are needed to identify the cause of these changes, for example, the interaction among the SMCs that enhances the behavior of these cells.

Role of estrogen on the reduction of ERα expression in primary SMCs is questionable. Severino et al[13] reported a loss of ER and PR 72 hours after the incubation with estradiol (E2) in leiomyoma and myometrial explants culture. Their result was in line with our finding. Primary cell culture in our experiment was also treated with hormone but showed no expression of ERα receptors (data not shown). From literature review, only few studies reported the mechanism of the change in ER level by using E2 as a stimulant. Alarid et al[15,16] reported a clear decreased level of ER protein 1 hour after treatment with estrogen, and the basal half-life of ERα was between 3-5 hours depending on cell types. On the other hand, Valley et al demonstrated that the acute E2 administration shortened the ERα half-life, while the chronic E2 treatment prolonged ERα half-life[17]. Therefore, the time taken to transport specimen and digestion of specimen need to be considered when conducting the study regarding these issues.

Both mRNA and protein level analysis showed the expression of ERα in fibroid tissue was higher than the myometrial tissue. Previous study reported that steroid hormones produced from ovary, especially estrogen and progesterone, play important roles in the development of uterine tumor[18]. We did not study ERβ expression because SMCs do not express this receptor[10]. Progesterone has dual effect on growth of tumor, both stimulation and suppression. On the one hand, the stimulation of tumor growth by progesterone mediates through the up regulation of epidermal growth factor and B-cell lymphoma-2 gene involving in the regulation of cells apoptosis especially in malignancy cells, but on the other hand, progesterone also exerts down regulation of cytokines tumor necrotic factor-α and insulin-like growth factor-1 controlling the growth of cells[19]. Therefore, the role of progesterone on tumor growth is still unclear. Comparison of ER expression between normal myometrial and fibroid tissue shows a higher expression of ER in fibroid than the myometrial tissue in the form of α rather than β[7,20,21].

Since the rapid loss of ERα and PR from the primary cell culture of myometrium and uterine

fibroid, in order to study the behavior of SMCs related to estrogen and progesterone, an experimental model which contains stable ERα and PR is needed. Many studies using primary cell cultures from myometrial and fibroid SMCs transfected with adenovirus/ERαη for maintaining the steroid receptors-positive primary cultures as a model for studying the expression level of ER have been conducted[22-24]. Another study could show an increase in ERα in both myometrial and fibroid SMCs after treatment with 17 β-estradiol[25]. In addition, immortalization of fibroid SMC line by using retroviral vector containing human telomerase reverse transcriptase (hTERT) to induce telomerase activity or organotypic culture can be used to maintain *in vivo* environment in the study[26,27].

In conclusion, using primary cell cultures of myometrial and fibroid SMCs as a model for study of effect and mechanism of estrogen analog molecule showed the disappearance of ER and PR during culture and digestion. Therefore, for further study, the immortalized cell line such as estrogen stimulated Ishikawa cells, a human Asian endometrial adenocarcinoma, should be used to maintain the level of ERα. Moreover, clinical study can be conducted to study the effect of estrogen on uterine tumor directly.

Acknowledgement

This work was supported by a research grant from The National Research Council of Thailand (NRC). Part of this paper was presented at the annual conference of the 21th Congress on Innovation in Reproductive Medicine during 14-16 May, 2015, Frankfurt, Germany. The authors would like to thank Faculty of Medicine, Ramathibodi Hospital, Mahidol University for providing financial support to this presentation.

References

1. Verkauf BS. Myomectomy for fertility enhancement and preservation. Fertil Steril 1992;58:1-15.
2. Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. Am J Obstet Gynecol 2003;188:100-7.
3. Bakas P, Liapis A, Vlahopoulos S, Giner M, Logotheti S, Creatsas G, et al. Estrogen receptor alpha and beta in uterine fibroids: a basis for altered estrogen responsiveness. Fertil Steril 2008;90:1878-85.
4. Flake GP, Andersen J, Dixon D. Etiology and pathogenesis of uterine leiomyomas: a review. Environ Health Perspect 2003;111:1037-54.
5. Swartz CD, Afshari CA, Yu L, Hall KE, Dixon D. Estrogen-induced changes in IGF-I, Myb family and MAP kinase pathway genes in human uterine leiomyoma and normal uterine smooth muscle cell lines. Mol Hum Reprod 2005;11:441-50.
6. Sadan O, van Iddekinge B, van Gelderen CJ, Savage N, Becker PJ, van der Walt LA, et al. Oestrogen and progesterone receptor concentrations in leiomyoma and normal myometrium. Ann Clin Biochem 1987;24: 263-7.
7. Tamaya T, Fujimoto J, Okada H. Comparison of cellular levels of steroid receptors in uterine leiomyoma and myometrium. Acta Obstet Gynecol Scand 1985;64:307-9.

8. Raz L, Khan MM, Mahesh VB, Vadlamudi RK, Brann DW. Rapid estrogen signaling in the brain. Neurosignals 2008;16:140-53.

9. Rissman EF, Wersinger SR, Taylor JA, Lubahn DB. Estrogen receptor function as revealed by knockout studies: neuroendocrine and behavioral aspects. Horm Behav 1997;31:232-43.

10. Gargett CE, Zaitseva M, Bucak K, Chu S, Fuller PJ, Rogers PA. 17Beta-estradiol up-regulates vascular endothelial growth factor receptor-2 expression in human myometrial microvascular endothelial cells: role of estrogen receptor-alpha and -beta. J Clin Endocrinol Metab 2002;87:4341-9.

11. Gargett CE, Bucak K, Zaitseva M, Chu S, Taylor N, Fuller PJ, et al. Estrogen receptor-alpha and -beta expression in microvascular endothelial cells and smooth muscle cells of myometrium and leiomyoma. Mol Hum Reprod 2002;8:770-5.

12. Sadovsky Y, Riemer RK, Roberts JM. The concentration of estrogen receptors in rabbit uterine myocytes decreases in culture. Am J Obstet Gynecol 1992;167:1631-5.

13. Severino MF, Murray MJ, Brandon DD, Clinton GM, Burry KA, Novy MJ. Rapid loss of oestrogen and progesterone receptors in human leiomyoma and myometrial explant cultures. Mol Hum Reprod 1996;2: 823-8.

14. Zaitseva M, Vollenhoven BJ, Rogers PA. In vitro culture significantly alters gene expression profiles and reduces differences between myometrial and fibroid smooth muscle cells. Mol Hum Reprod 2006;12: 187-207.

15. Alarid ET, Bakopoulos N, Solodin N. Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation. Mol Endocrinol 1999;13:1522-34.

16. Alarid ET. Lives and times of nuclear receptors. Mol Endocrinol 2006;20:1972-81.

17. Valley CC, Solodin NM, Powers GL, Ellison SJ, Alarid ET. Temporal variation in estrogen receptor-alpha protein turnover in the presence of estrogen. J Mol Endocrinol 2008;40:23-34.

18. Blake RE. Leiomyomata uteri: hormonal and molecular determinants of growth. J Natl Med Assoc 2007;99:1170-84.

19. Maruo T, Ohara N, Yoshida S, Nakabayashi K, Sasaki H, Xu Q, et al. Translational research with progesterone receptor modulator motivated by the use of levonorgestrel-releasing intrauterine system. Contraception 2010;82:435-41.

20. Soules MR, McCarty KS, Jr. Leiomyomas: steroid receptor content. Variation within normal menstrual cycles. Am J Obstet Gynecol 1982;143:6-11.

21. Wilson EA, Yang F, Rees ED. Estradiol and progesterone binding in uterine leiomyomata and in normal uterine tissues. Obstet Gynecol 1980;55:20-4.

22. Sampath D, Zhu Y, Winneker RC, Zhang Z. Aberrant expression of Cyr61, a member of the CCN (CTGF/Cyr61/Cef10/NOVH) family, and dysregulation by 17 beta-estradiol and basic fibroblast growth factor in human uterine leiomyomas. J Clin Endocrinol Metab 2001;86:1707-15.

23. Wijayaratne AL, McDonnell DP. The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. J Biol Chem 2001;276:35684-92.

24. Harris DM, Besselink E, Henning SM, Go VL, Heber D. Phytoestrogens induce differential estrogen receptor alpha- or Beta-mediated responses in transfected breast cancer cells. Exp Biol Med (Maywood) 2005;230:558-68.

25. Mangioni S, Vigano P, Lattuada D, Abbiati A, Vignali M, Di Blasio AM. Overexpression of the Wnt5b gene in leiomyoma cells: implications for a role of the Wnt signaling pathway in the uterine benign tumor. J Clin Endocrinol Metab 2005;90:5349-55.

26. Carney SA, Tahara H, Swartz CD, Risinger JI, He H, Moore AB, et al. Immortalization of human uterine leiomyoma and myometrial cell lines after induction of telomerase activity: molecular and phenotypic characteristics. Lab Invest 2002;82:719-28.

27. Varella-Garcia M, Chen L, Zheng X, Yu L, Dixon D. Karyotypic characteristics of human uterine leiomyoma and myometrial cell lines following telomerase induction. Cancer Genet Cytogenet 2006;170:71-5.

Original Articles/นิพนธ์ต้นฉบับ

ผลการเพาะเลี้ยงเซลล์กล้ามเนื้อเรียบจากมดลูกและเนื้องอกมดลูกจากมนุษย์ต่อโปรตีนตัวรับเอสโตรเจน

ดวงพร แสงวิมล[1], วรรณวิสา ไวยพุฒ[1], สมโภช ภูมิพิเชฐ[2], อารีย์พรรณ โสภณสฤษฏ์สุข[3]

[1] *สำนักงานวิจัย วิชาการ และนวัตกรรม คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล*

[2] *ภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ*

[3] *หน่วยต่อมไร้ท่อนรีเวช ภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล*

บทคัดย่อ

ความเป็นมา: เนื้องอกของกล้ามเนื้อมดลูกชนิดที่ไม่ใช่มะเร็ง เกิดจากเซลล์ของกล้ามเนื้อเรียบของกล้ามเนื้อมดลูกเพียงเซลล์เดียวมีการเพิ่มจำนวนเซลล์ที่ผิดปกติไป การเจริญขยายขนาดของเนื้องอกถูกควบคุมด้วยฮอร์โมนเอสโตรเจน โดยจะออกฤทธิ์ผ่านตัวรับเอสโตรเจนในนิวเคลียสเพื่อควบคุมการทำงานของเซลล์เป้าหมาย คือ ตัวรับเอสโตรเจนชนิดแอลฟาและเบต้า

วัตถุประสงค์: เพื่อศึกษาผลของการแสดงออกของโปรตีนตัวรับเอสโตรเจนชนิดแอลฟา และโปรเจสเตอโรนของเซลล์เพาะเลี้ยงวิธีปฐมภูมิ กล้ามเนื้อเรียบจากมดลูกปกติและเนื้องอกมดลูก เพื่อใช้เป็นโมเดลในการทำการศึกษาทดลองต่อไป

วิธีวิจัย: การศึกษาใช้วิธี Western blot analysis และ Quantitative real-time PCR (qPCR) โดยเปรียบเทียบเนื้อเยื่อระหว่างเซลล์กล้ามเนื้อเรียบจากมดลูกและเนื้องอกมดลูกจากผู้ป่วยรายเดียวกัน ก่อนการย่อยสลายและหลังการเพาะเลี้ยงเซลล์เป็นจำนวน 3 passages

ผลการศึกษา: มีการสูญหายไปของตัวรับเอสโตรเจนและโปรเจสเตอโรนทั้งในเซลล์กล้ามเนื้อเรียบมดลูกและเซลล์จากเนื้องอกมดลูกตั้งแต่การย่อยสลายเนื้อเยื่อและการเพาะเลี้ยงเนื้อเยื่อ โดยเกิดการสูญหายตั้งแต่ภายหลังการย่อยเนื้อเยื่อก่อนการเพาะเลี้ยงเซลล์

สรุปผลการศึกษา: การใช้เซลล์กล้ามเนื้อเรียบจากมดลูกและเนื้องอกมดลูกไม่เหมาะสมที่จะนำมาใช้เป็นโมเดลในการศึกษาฤทธิ์และกลไกการออกฤทธิ์ของสารที่คล้ายฮอร์โมนเอสโตรเจน ดังนั้น การใช้เซลล์ที่มีการแสดงออกของตัวรับเอสโตรเจน และโปรเจสเตอโรนที่มั่นคงจะเหมาะสมกว่า

Corresponding author: ผศ.ดร.พญ.อารีย์พรรณ โสภณสฤษฏ์สุข

หน่วยต่อมไร้ท่อนรีเวชภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

โทร. 662-201-1412 Email: areepan.sop@mahidol.ac.th