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Estrogen modulates epithelial progenitor cells in rat vagina

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Purpose: The expression of epithelial progenitor cells (EPCs) in rat vagina was recently reported. The aims were to investigate the effects of estrogen on vaginal EPCs in the oophorectomized female rat model.

Materials and Methods: Female Sprague-Dawley rats (230–240 g, n=30) were divided into 3 groups: control (n=10), bilateral oophorectomy (OVX, n=10), and bilateral OVX followed by subcutaneous injections of 17β-estradiol (50 μg/kg/day, n=10). After 4 weeks, the expression of EPC-specific markers (CD44, estrogen receptor alpha [ERα], and progesterone receptor) were evaluated by immunohistochemistry and Western blot.

Results: The CD44/ERα double-labeled cells were mainly expressed in basal cell layers and suprabasal layers as shown by confocal immunofluorescence. Confocal microscopy revealed that the number of CD44+/ERα+ cells decreased in the OVX group compared with the controls but was similar to control levels in rats receiving estrogen replacements. The protein expression of CD44 and ERα decreased after OVX and was restored to control levels after estrogen supplementation.

Conclusions: Markers of EPCs were expressed in the vagina, and the expression of resident EPCs was regulated by estrogen. These findings imply that resident EPCs may have an important role in the regeneration of vaginal mucosa by estrogen replacement.

Keywords: Cells; Epithelium; Estrogens; Rats; Vagina

INTRODUCTION

Female sexual dysfunction is not unusual and can have a significant negative impact on the quality of life of affected female and their partners [1]. Vaginal lubrication is an indicator of female genital sexual arousal and is a complex physiologic process that is not clearly understood [2]. Vaginal mucosal epithelial cells have been reported to have an important role in the mechanism of vaginal lubrication [3]. Also, several studies have reported that vaginal epithelial cells may act as a physical barrier to prevent both external damage and infiltration of pathogens [4,5].

Recently, some studies have investigated epithelial progenitor cells (EPCs) in the female reproductive tract [6,7]. The vagina, similar to the endometrium, undergoes regular cycles in the number of layers of epithelium over the normal menstrual cycle [8]. Vaginal EPCs are likely involved in this change and might be essential for maintaining and restoring normal vaginal function. Recently, we demonstrated the existence of mucosal EPCs in the epithelial layer of the rat vagina [9]. However, there is still insufficient understanding about the role of EPCs in the vagina.

The growth and differentiation of vaginal epithelial cells are regulated by sex steroid hormones. Estrogen is required

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to maintain the proper structural integrity of the vagina and immune response, both of which are necessary to protect the vagina against physical damage and to restore the vaginal environment [10]. Therefore, we hypothesized that mucosal EPCs in the vagina might also be influenced by hormone insufficiency and replacement. On the basis of our previous study of the existence of vaginal EPCs, we investigated the effect of estrogen on vaginal EPCs in the oophorectomized female rat model.

MATERIALS AND METHODS

1. Animals

In this study, we used female Sprague-Dawley rats (230–240 g, n=30) and harvested vaginal tissue for biochemical analyses. Female Sprague-Dawley rats were divided into 3 groups: a control group (CON, n=10), a group that underwent bilateral oophorectomy (OVX, n=10), and a group that underwent bilateral OVX followed by subcutaneous injections of 17β-estradiol (OVX+17β-estradiol treatment [EST], n=10). The CON group underwent a sham operation, and the OVX group was treated with an oil vehicle. The OVX+EST group underwent bilateral OVX followed by treatment with subcutaneous estradiol daily for 7 days after OVX (50 ug/kg/day). After 4 weeks, the expression of EPC-specific markers (CD44, estrogen receptor alpha [ERα], and progesterone receptor [PR]) were evaluated by immunohistochemistry and Western blot. The procedures used and the care of animals were approved by the Institutional Animal Care and Use Committee (IACUC) at Chonnam National University (approval number: CNUIACUC-H-2015-48).

2. Tissue harvesting and immunofluorescence staining

The rat vagina was carefully removed and dissected away from the urethra and surrounding connective tissue under optical magnification. The vaginal tissue specimens were immediately fixed by 4% paraformaldehyde in phosphate-buffered saline (PBS) and cryo-embedded. The embedded tissues were vertically sectioned, and the samples were subjected to immunohistochemical detection of CD44, ERα, and PR. For detection of CD44, ERα, and PR, tissue sections were washed by PBS-T (0.1% tween in PBS). After several washes with PBS, the sections were then incubated with anti-CD44, anti-ERα, and anti-PR primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4℃. After being washed in PBS-T, sections were incubated for 2 hours with fluorescence-conjugated antibodies (Invitrogen, Carlsbad, CA, USA) for confocal microscopy analysis. Immunostained tissue sections were mounted with an aqueous permanent mounting medium with DAPI and were examined by light microscopy and LSM510 confocal microscopy (Carl Zeiss, Jena, Germany).

3. Western blot

The tissue homogenates (50 μg of protein) were separated by 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a PVDF membrane (Amersham Pharmacia Biotech, Little Chalfont, UK). The blots were then washed with Tris-buffered saline Tween-20 (10 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween-20). The membrane was blocked with 5% skim milk for 1 hour and incubated with the appropriate primary antibody. Anti-CD44, anti-ERα (Santa Cruz Biotechnology) (1:4,000), and a monoclonal rabbit antibody against GAPDH (cell signaling) were used. The membrane was then washed and detected with goat anti-rabbit-IgG conjugated to horseradish peroxidase. Antibody incubations were performed in a 4℃ incubator. The bands were visualized by using enhanced chemiluminescence (Amersham Pharmacia Biotech). GAPDH was used as an internal CON. Densitometry analysis was performed with a studio Star Scanner (Agfa-Gevaert, Morstel, Belgium) by using Multigauge 3.0 software (Fuji photo film Co., Ltd., Tokyo, Japan).

4. Assay of serum 17β-estradiol

Blood samples were drawn by direct needle insertion into the heart. Estradiol concentrations (pg/mL) were measured by using an enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions (R&D System, Inc., Minneapolis, MN, USA).

5. Statistics

All results are expressed as mean±standard error of the indicated number of experiments. Statistical significance was estimated by using t-tests, and the differences were compared with regard to statistical significance by one-way ANOVA, followed by Bonferroni's post hoc test. Differences were considered statistically significant at a value of $p<0.05$.

RESULTS

1. Serum estradiol concentrations

All the animals survived for 4 weeks after surgery. After 4 weeks of bilateral OVX, serum estradiol concentrations were significantly lower in the OVX group (11±1.2 pg/mL) than in the CON group (25±1.8 pg/mL). However, in the OVX+EST group, the estradiol concentrations significantly

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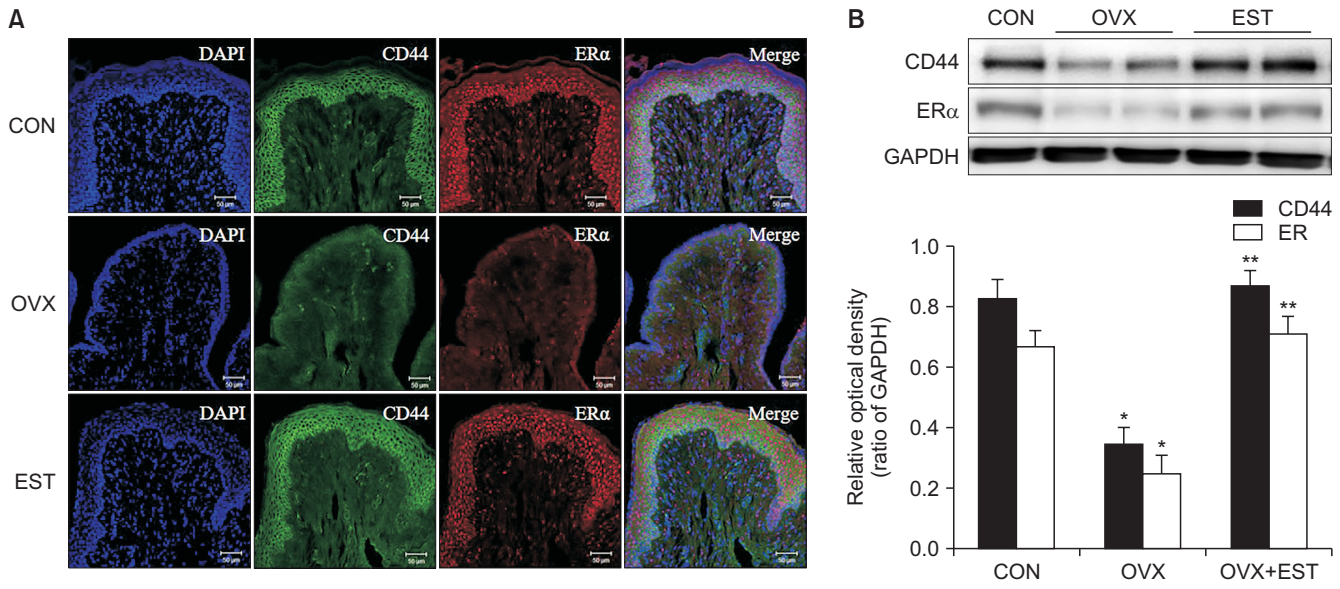

Fig. 1. Effect of 17β-estradiol on epithelial progenitor cells in the rat vagina. (A) Double immunofluorescence detection of CD44 (green) and estrogen receptor alpha (ERα) (red) in animals of the control (CON), oophorectomy (OVX), and OXV followed by 17β-estradiol treatment (EST) groups. (B) Immunoblotting of CD44 and ERα in the rat vagina. *p<0.05 versus CON. **p<0.05 versus OVX.

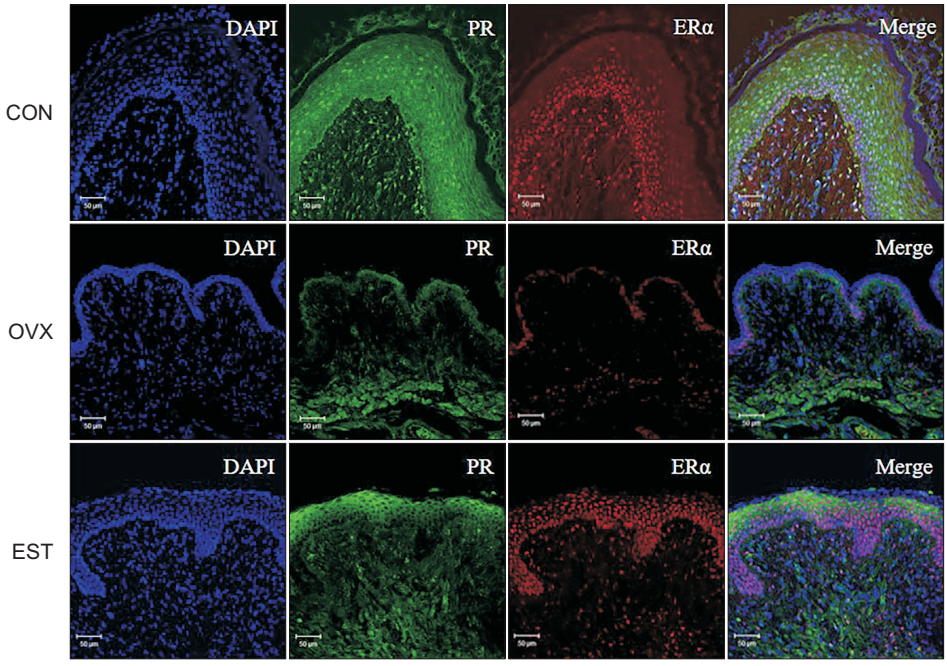

Fig. 2. Double immunofluorescence detection of progesterone receptor (PR; green) and estrogen receptor alpha (ERα; red) in animals of the control (CON), oophorectomy (OVX), and OXV followed by 17β-estradiol treatment (EST) groups.

increased (46±1.97 pg/mL) after EST (p<0.05).

2. Effect of 17β-estradiol on epithelial progenitor cells in the rat vagina

CD44/ERα double-labeled cells were mainly expressed in the basal cell layers and suprabasal layers as shown by confocal immunofluorescence. PR expression was not confined to basal cells but extended to the intermediate layer of epithelial cells. Confocal microscopy revealed that the expression of CD44+/ERα+ and ERα+/PR- cells decreased in the OVX group compared with the CON group but was similar to CON levels in the OVX+EST group receiving 17β-estradiol replacement (Figs. 1A, 2). The protein expression of CD44 and ERα decreased in the OVX group but was similar in the OVX+EST group as in the CON group (p<0.05) (Fig. 1B).

DISCUSSION

In the present study, we identified the altered expression of resident EPCs after OVX and a restoration of expression

following estrogen replacement in the vagina. This implies that mucosal EPCs exist in the epithelial cell layers even after OVX and can be restored after estrogen replacement. Our findings also suggest that resident EPCs may have an important role in the regeneration of vaginal mucosa by estrogen replacement in the postmenopausal state.

Female sexual dysfunction is a common problem in female, and 43% of female experience sexual problems that negatively affect their quality of life [1]. Vaginal lubrication and clitoral engorgement are indicators of genital sexual arousal in female. However, insufficient vaginal lubrication or vaginal dryness in female can cause dyspareunia and other sexual problems. Therefore, lubricants have commonly been used to ameliorate vaginal dryness and increase sexual pleasure [11]. The vaginal mucosa includes a complex network of small capillaries just beneath the vaginal epithelium that may account for vaginal fluid secretion and lubrication [12]. Our previous study showed an important role of mucosal epithelial cells in vaginal lubrication by demonstrating the expression of the aquaporins in rat vagina [3] and also suggested the possible relationship between aquaporin 3 and *all-trans*-retinoic acid in the mechanism of vaginal lubrication [13]. Vaginal epithelial cells may also act as a physical barrier to prevent both external damage and infiltration of pathogens [5,10]. Vaginal epithelial intercellular junctions maintain the integrity and organization of the epithelia by regulating molecular and cellular traffic and by providing a physical barrier to pathogen invasion [4].

Several studies have investigated EPCs and their role at different sites, including the urogenital tract and female reproductive tract [6,7,14]. During menstrual cycles, the basal component of the endometrial glands remains in the basalis layer and epithelial cells re-epithelialize the exposed surface and then proliferate to regenerate the new functional layer under the influence of rising estrogen levels [15]. It was hypothesized that the remaining glands of the basalis contained the EPC population [16]. A recent study reported that the stem-like long-term label-retaining cells from the distal oviduct give rise to progenitor cells (positive for CD44 and ERα, negative for PR, PAEP) for the epithelial lining of the proximal oviduct [7]. In our previous study, we confirmed the existence and localization of EPCs in the female rat vagina [9].

Since the growth and differentiation of vaginal epithelial cells are known to be regulated by sex steroid hormones [10], we further investigated the effects of 17β-estradiol on vaginal EPCs in the oophorectomized female rat model. To our knowledge, the present study is the first report to show the effect of estrogen on vaginal EPCs in the oophorectomized female rat model. According to our study, the expression of CD44+/ERα+ and ERα+/PR- cells decreased after bilateral OVX but was similar to CON levels in the group that received 17β-estradiol replacement. Also, the protein expression of CD44 and ERα decreased after bilateral OVX but was similar to CON levels in the group that received 17β-estradiol replacement. These results strongly suggest that the resident EPCs may have an important role in the regeneration of vaginal mucosa by estrogen replacement.

Similar to the present study, our recent study with the castrated male rat model confirmed that endothelial progenitor cell markers are expressed in the cavernosal sinusoidal endothelial space, and the numbers of resident endothelial progenitor cells are regulated by testosterone [17,18]. These findings indicated that testosterone replacement therapy may improve erectile function by modulating endothelial progenitor cells in patients with hypogonadism. These results have potential implications for future therapeutic options for the treatment of sexual dysfunction.

Vaginal mucosal regeneration is essential to maintain female sexual function, and various mechanisms may contribute to the regeneration of vaginal mucosa. New treatment strategies are needed to repair the damaged mucosal epithelial cells in human vaginal tissue. In this study, we investigated the effect of estrogen on the vaginal EPCs. We found the existence of vaginal EPCs in the vagina even after OVX and that EPCs could be restored after estrogen replacement. Further studies are needed to explore the associated factors and various functional activities of vaginal EPCs without hormonal influences.

CONCLUSIONS

This study showed the existence of mucosal EPCs in the rat vagina. The expression of resident EPCs was regulated by estrogen in the epithelial layer of the rat vagina. These findings imply that resident EPCs may have an important role in the regeneration of vaginal mucosa. Further studies are needed to modulate resident EPCs without hormonal influences.

CONFLICTS OF INTEREST

Kwangsung Park has been the Editor-in-Chief of Investigative and Clinical Urology since 2016. No potential conflict of interest relevant to this article was reported.

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AUTHORS' CONTRIBUTIONS

Research conception and design: Kwangsung Park. Data acquisition: Hyun-Suk Lee. Statistical analysis: Hyun-Suk Lee. Data analysis and interpretation: Hyun-Suk Lee and Kwangsung Park. Drafting of the manuscript: Ho Seok Chung. Critical revision of the manuscript: Kwangsung Park and Ho Seok Chung. Obtaining funding: Kwangsung Park. Supervision: Kwangsung Park. Approval of the final manuscript: Kwangsung Park and Ho Seok Chung.

REFERENCES

1. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. JAMA 1999;281:537-44.
2. Jannini EA, Buisson O, Rubio-Casillas A. Beyond the G-spot: clitourethrovaginal complex anatomy in female orgasm. Nat Rev Urol 2014;11:531-8.
3. Park K, Han HJ, Kim SW, Jung SI, Kim SO, Lee HS, et al. Expression of aquaporin water channels in rat vagina: potential role in vaginal lubrication. J Sex Med 2008;5:77-82.
4. Blaskewicz CD, Pudney J, Anderson DJ. Structure and function of intercellular junctions in human cervical and vaginal mucosal epithelia. Biol Reprod 2011;85:97-104.
5. Wira CR, Grant-Tschudy KS, Crane-Godreau MA. Epithelial cells in the female reproductive tract: a central role as sentinels of immune protection. Am J Reprod Immunol 2005;53:65-76.
6. Gargett CE, Schwab KE, Deane JA. Endometrial stem/progenitor cells: the first 10 years. Hum Reprod Update 2016;22:137-63.
7. Wang Y, Sacchetti A, van Dijk MR, van der Zee M, van der Horst PH, Joosten R, et al. Identification of quiescent, stem-like cells in the distal female reproductive tract. PLoS One 2012;7:e40691.
8. Patton DL, Thwin SS, Meier A, Hooton TM, Stapleton AE, Eschenbach DA. Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle. Am J Obstet Gynecol 2000;183:967-73.
9. Chung HS, Lee HS, Kim ME, Lee JS, Park K. Identification and localization of epithelial progenitor cells in the vagina. Int J Impot Res 2019;31:46-9.
10. Li S, Herrera GG, Tam KK, Lizarraga JS, Beedle MT, Winuthayanon W. Estrogen action in the epithelial cells of the mouse vagina regulates neutrophil infiltration and vaginal tissue integrity. Sci Rep 2018;8:11247.
11. Milford C, Beksinska M, Smit J, Deperthes B. Lubrication and vaginal sex: lubricant use and preferences in general population women and women at risk of HIV. Arch Sex Behav 2020;49:2103-16.
12. Shabsigh A, Buttyan R, Burchardt T, Buchardt M, Hayek OR, D'Agati V, et al. The microvascular architecture of the rat vagina revealed by image analysis of vascular corrosion casts. Int J Impot Res 1999;11 Suppl 1:S23-30.
13. Lee HS, Kim SO, Ahn K, Park K. All-trans retinoic acid increases aquaporin 3 expression in human vaginal epithelial cells. Sex Med 2016;4:e249-54.
14. Goldstein AS, Drake JM, Burnes DL, Finley DS, Zhang H, Reiter RE, et al. Purification and direct transformation of epithelial progenitor cells from primary human prostate. Nat Protoc 2011;6:656-67.
15. Gargett CE, Nguyen HP, Ye L. Endometrial regeneration and endometrial stem/progenitor cells. Rev Endocr Metab Disord 2012;13:235-51.
16. Gargett CE. Uterine stem cells: what is the evidence? Hum Reprod Update 2007;13:87-101.
17. Lee JS, Hwang IS, Lee HS, Kim ME, Seo YW, Park K. Identification of endothelial progenitor cells in the corpus cavernosum in rats. Biomed Res Int 2014;2014:910564.
18. Hwang I, Lee HS, Yu HS, Kim ME, Lee JS, Park K. Testosterone modulates endothelial progenitor cells in rat corpus cavernosum. BJU Int 2016;117:976-81.