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The localization of estrogen receptor α and its function in the ovaries of postmenopausal women

Agnieszka Brodowska[1], Maria Laszczyńska[2], Andrzej Starczewski[1], Beata Karakiewicz[3], Jacek Brodowski[3]

[1]Department of Reproduction and Gynaecology, [2]Laboratory of Embryology, [3]Laboratory of Family Nursing, Pomeranian Medical University, Szczecin, Poland

Abstract: The localization of estrogen receptor α (ERα) in the ovaries of postmenopausal women is a very up-to-date topic in the aspect of using estrogens therapy in the clinical situations of different type. In ovaries of reproductive age women ERα is present in ovary stroma, theca and granulosa cells, ovary surface epithelium (OSE) and in corpus luteum. The ovaries of postmenopausal women are smaller than those of women at the reproductive age, the division into cortex and medulla gets blurred, the ovaries have no follicles any longer, and the stroma is mainly composed of fibrous connective tissue, corpora albicantia, nerves, and blood and lymphatic vessels. The aim of our study was to investigate the immunolocalization and immunoexpression of ERα in the ovaries of postmenopausal women. The study involved 50 postmenopausal women who had their ovaries removed by laparotomy due to non-neoplastic diseases of the uterus. The women were divided into 3 groups (A, B, and C) depending on the time that had passed since the last menstruation. Group A consisted of women who had their last menstruation no more than 5 years earlier, in group B menopause occurred 5 to 10 years earlier, group C was composed of patients who had the last menstruation over 10 years earlier. In all the patients concentrations of follicle stimulating hormone (FSH), luteinizing stimulating hormone (LH), estradiol (E_2), testosterone (T), androstendione (A) and dehydroepiandrosterone sulphate (DHEAS) in blood plasma were measured. Ovarian tissue was obtained during surgery. For morphological studies, ovaries were fixed in Bouin`s solution and 4% formalin and embedded in paraffin. Morphological analysis was carried out after hematoxylin-eosin (HE) staining. Comparing to groups A and B, the ovaries in group C contained a small number of corpora albicantia located in the medullary part as well as thinned blood vessels and few lymphatic vessels and nerves. For immunoohistochemical expression of ERα paraffin-embedded specimens fixed in 4% buffered formalin were used. The sections were next incubated with monoclonal mouse anti-human ERα antibody (N 1575 Dako, Denmark). Immunohistochemical nuclear expression of ERα in OSE, in epithelial inclusion cysts, in stroma, and in group A also cytoplasmic expression of ERα in luteal and paraluteal cells of disappearing corpus luteum were revealed. Immunohistochemical expression of ERα seems to decrease in the ovaries of women after menopause.

Key words: Ovary - Estrogen receptor α - Menopause - Women

Introduction

The use of the estrogen or estroprogestin replacement therapy in female diseases of different type is a very up-to-date subject. Advantages and complications of this therapy are considered with reference to the distribution of estrogen receptors in particular tissues, and the function that estrogens fulfil in a female organism.

Estrogen receptors (ERs) in women are usually found in the female reproductive system (ovaries, Fallopian tube, uterus, vagina), blood vessels, nervous system, bones, breast, intestines, immune system cells, and adrenal glands [1-7]. The following are regarded as the most common estrogen dependent diseases: osteoporosis, neurodegenerative diseases, cardiovascular diseases, insulin resistance, lupus erythematosus, endometriosis, obesity and breast, ovarian, colorectal and endometrial cancer [2,3,8-10].

The biological actions of estrogens are mediated by binding to one of the two specific estrogen receptors ERα or ERβ, which belong to the nuclear receptor superfamily, a family of ligand-regulated transcription factors [8,11-15]. For both types of the receptors, the common ligands are: 17β-estradiol, estron and estriol, but affinity of these ligands for particular receptors

Correspondence: M. Laszczyńska, Laboratory of Embryology, Pomeranian Medical University, Żołnierska 48, 71-210 Szczecin, Poland; tel.: (091-4800910), fax.: (091-4800905), e-mail: laszcz@sci.pam.szczecin.pl

varies [6,15-17]. It is a similar case with synthetic compounds, which can be ligands for ERs, but depending on whether they bind to ERα or ERβ, they become agonists or antagonists. These differences probably result from the different mechanism of receptor phosphorylation affecting the transcription activation [6,11,13,15-17].

Various authors say that ERβ is a dominant receptor in the mammal reproductive system at the reproductive age [18-20]. In the ovary, ERβ was found mainly in granulosa cells, theca cells, stromal cells, corpora luteal cells and epithelial cells, while ERα was present in granulosa cells, theca cells, interstitial gland cells and epithelium as well as in the corpus luteum at the early luteal stage [4-6,8,13,16,20-22]. At the middle luteal stage and in the regressive corpus luteum, ERα is absent. Subsequently, ERα is a dominant ER in the Fallopian tube and uterus of a woman at the reproductive age [17,22]. Therefore, as the main functions of ERα at the reproductive age, we regard: the regulation of fertility through the influence on estrogen concentrations in blood serum, the growth and development of follicles and thus, the modification of ovulation, the regulation of the motor activity of Fallopian tubes, and finally the development and growth of the uterus [1,21,23,24]. According to the most recent data, ERα plays an important part also in the mechanism of menopause [1]. Blocking (estrogen antagonists) or stimulating (estrogen agonists) of these receptors in different tissues of menopausal women, as well as SERM (selective estrogen receptors modulation) are the basic mechanisms of the estrogen therapy. They are applied in the treatment of, among others, menopause symptoms, atrophic changes within the urogenital system, osteoporosis, breast cancer, endometrial cancer, neurodegenerative diseases [15,25].

After the menopause, the structure of the human ovary considerably changes, and the reports on the presence of ER are very scanty. The ovaries of postmenopausal women are smaller, the division into cortex and medulla gets blurred, there are no ovarian follicles any longer, and the stroma is mainly composed of fibrous connective tissue, nerves, blood and lymphatic vessels, as well as corpora albicantia with fibroblasts, macrophages and miofibroblasts [26-28].

Crucial for immunolocalization of ER in the postmenopausal women's ovaries is the presence of epithelial inclusion cysts which are usually located near the surface epithelium, in the stroma and in the surroundings of corpora albicantia. Epithelial inclusion cysts emerge as a result of metaplasia of cuboidal epithelium, or effect of invagination of the surface epithelium, also called ovarian mesothelium. These cysts are lined with columnar epithelium. The epithelial ovarian carcinomas, the group derived from the OSE and in epithelial inclusion cysts represent approximately 90% of all human ovarian malignant neoplasms [9,10,25,29,30].

In this study, we decided to investigate the presence of ERα in the ovaries of postmenopausal women depending on the lapse of time from the last menstruation.

Materials and methods

Patients. The study involved 50 postmenopausal women, who had their ovaries removed by laparotomy due to non-neoplastic diseases of the uterus. Neither of them used substitutive hormone therapy before. The Bioethical Commission gave its consent for this study. All cases were diagnosed at the Department of Reproduction and Gynecology, Pomeranian Medical University, Szczecin.

The women were divided into 3 groups depending on the time from the last menstruation. Group A (25 patients) included women whose last menstruation was no more than 5 years earlier. In group B (15 patients) menopause appeared 5 to 10 years earlier. Group C (10 patients) consisted of patients who had their last menstruation over 10 years earlier.

Hormone measurements. In all the patients concentrations of follicle stimulating hormone (FSH), luteinizing stimulating hormone (LH), estradiol (E_2), testosterone (T), androstendione (A) and dehydroepiandrosterone sulphate (DHEAS) in blood plasma were measured using ELISA method enzyme immunoassay kit (Spi-Bio France).

Morphology. Ovarian tissue (both cortex and medulla) was obtained during surgery. For morphological studies, ovaries were fixed in Bouin`s solution and 4% formalin, and embedded in paraffin. Sections were cut from the respective parts of ovaries. Morphological analysis was done after hematoxylin and eosin (H+E) staining.

Immunohistochemical quantification of estrogen receptor α. For immunohistochemical expression of estrogen receptor α (ERα), the paraffin-embedded specimens fixed in 4% buffered formalin were used. The slides were heated in citrate buffer, pH=9.0 for 30 min. in a water bath at 96°C. The sections were next incubated for 30 min. at the room temperature in humidified chamber with monoclonal mouse anti-human estrogen receptor α antibody (N 1575 Dako, Denmark). The detection of anti-ERα antibody was performed using Dako LSAB 2 KIT/HRP. Aminoethylocarbazole (AEC substrate chromogen) was used to visualize the immunohistochemical reaction. Finally, the sections were counterstained with Mayer's hematoxylin. After each step, the sections were rinsed with Tris-buffered saline (TBS). The control sections were incubated with TBS instead of the primary antibody.

Statistical analysis. The Kruskal-Wallis test was used for statistical analysis. The accepted significance level was $p<0.05$.

Results

Age of the examined patients

In group A average age of the patients was 46.3±1.2 (M±SD), in group B it was 52.4±3.8 (M±SD), and in group C 58.6±2.4 (M±SD). Considerable age differences were found between women from groups: A and B, B and C, and A and C ($p<0.05$).

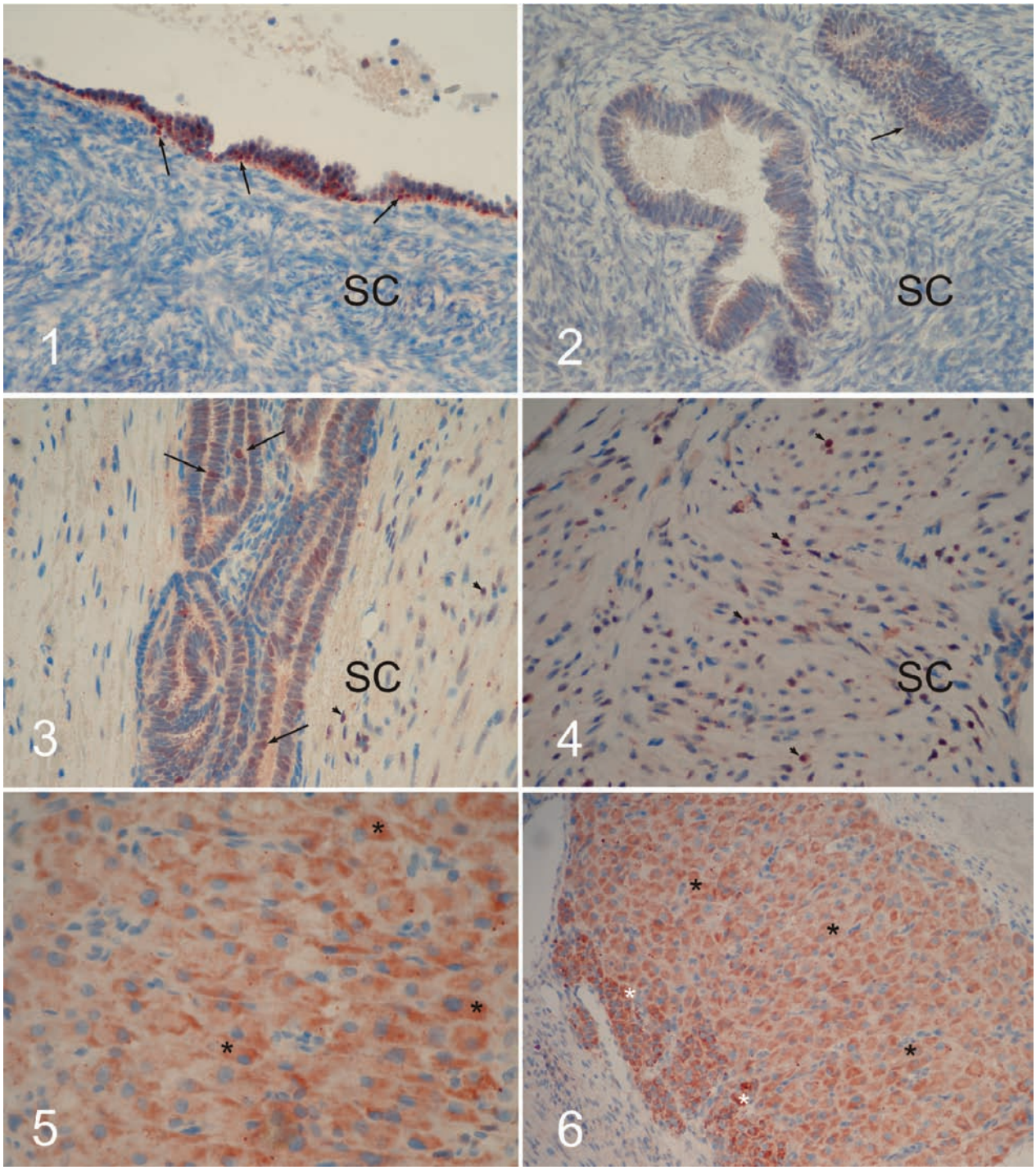

Fig. 1. Ovary of group A postmenopausal women (examined no more than 5 years after menopause). Immunohistochemical localization of ERα. The high level of ERα expression was observed in some nuclei of ovarian surface epithelium (arrows). Stromal cells (SC) (magnification ×400). **Fig. 2**. Ovary of group C postmenopausal women (examined more than 10 years after menopause). Immunohistochemical localization of ERα. Weak nuclear staining is present on some epithelial cells (arrows) (invagination of ovarian surface epithelium metaplastic changes). Stromal cells (SC) (magnification ×400). **Fig. 3**. Ovary of group A postmenopausal women (examined no more than 5 years after menopause). Immunohistochemical localization of ERα. Strong nuclear staining is present in some columnar epithelial cells (invaginations of ovarian surface epithelium metaplastic changes) (arrows). Stromal cells (SC). Nuclear expression of ERα in some stromal cells (arrowheads) (magnification ×400). **Fig. 4.** Ovary of group B postmenopausal women (examined from 5 to 10 years after menopause). Nuclear expression of ERα in some stromal cells (arrowheads) (magnification ×400). **Fig. 5**. Ovary of group A postmenopausal women (examined no more than 5 years after menopause). Cytoplasmic expression of ERα in granulosa lutein cells of disappearing corpus luteum remaining in ovary (black asterisks) (magnification ×400). **Fig. 6.** Ovary of group A postmenopausal women (examined no more than 5 years after menopause). Cytoplasmic expression of ERα in granulosa lutein cells (black asterisks) and in theca lutein cells (white asterisks) of disappearing corpus luteum remaining in ovary (magnification ×200).

Table 1. The levels of E_2, FSH, LH, A, T and DHEAS in serum of the examined patients expressed as means ±SD; * ($p<0.05$)

Group	No	E_2 (pg/ml)	FSH (mIU/ml)	LH (mIU/ml)	A (ng/ml)	T (ng/ml)	DHEAS (μg/ml)
A	25	14.15±9.21	28.60±15.12*	25.15±9.34	2.64±1.18	1.9±0.17	108±32.43
B	15	20.55±6.25	76.45±20.15*	27.25±8.15	2.85±0.65	1.35±0.07*	98±22.4
C	10	23.18±8.85	98.15±14.38*	54.36±10.12	1.30±1.17	0.77±0.08*	50±14.24*

Table 2. Immunohistochemical localization and immunoexpression of ERα in the ovaries of postmenopausal women. Legend: very strong intensity (+++), strong intensity (++), week intensity (+), lack of expression (-).

Patients	Ovarian surface epithelium (OSE)	Epithelial inclusive cysts	Stroma	Luteal cells of disappearing corpus luteum	Paraluteal cells of disappearing corpus luteum
Group A	+++	+++	++/+++	+	+++
Group B	++	++	+/++	-	-
Group C	+/-	+/-	+/-	-	-

The levels of E_2 and FSH, LH, A, T, DHEAS in serum of the examined patients

The mean E_2 , FSH, LH, A, T, DHEAS serum concentrations of the examined patients are shown in Table 1. The mean E_2 serum concentrations did not significantly differ from each other groups, but the mean FSH, LH, A, T and DHEAS serum concentrations significantly differ from each other between the examined groups of women (between groups A and B, B and C, and A and C).

Morphology (H+E staining)

In the ovaries of postmenopausal women, the main structural changes in both the cortex and medulla were observed. In the cortex, they included: 1) the reduction of its thickness; 2) epithelial inclusions forming cysts; 3) a blurring of the line between medulla and cortex; 4) the reduction of follicles number; 5) the tendency to fragmentation of corpora albicantia; 6) surface epithelium invaginations. The changes in the medulla covered: 1) fibrosis and scars in stroma; 2) architectonical changes in blood vessels with hyalinization of the walls and constriction of lumen. Comparing to groups A and B, the ovaries in group C contained small number of corpora albicantia located in the medullary part as well as thinned blood vessels and few lymphatic vessels and nerves. Our morphological results of postmenopausal women ovary (between groups A, B and C) were presented lately.

Immunohistochemical results

Immunohistochemical localization and immunoexpression of ERα in the ovaries of postmenopausal women were present in Table 2.

In our study immunohistochemical nuclear expression of ERα in OSE, in epithelial inclusion cysts, in stroma, and in group A also cytoplasmatic expression of ERα in luteal and paraluteal cells of disappearing corpus luteum were revealed. (Fig. 1-6). Immunohistochemical nuclear expression of ERα seems to decrease in the ovaries of women after 5 and 10 years from menopause. In some ovary of postmenopausal women in group C immunohistochemical nuclear expression of ERα was absent.

Discussion

The distribution of estrogen receptors in the ovaries of postmenopausal women is still poorly known. This the result of the difference between the structures of the human ovary before and after menopause, and also because of the false opinion that the postmenopausal ovary is nothing but the hormonally inactive connective tissue which is not worth investigating.

The results of hormonal tests, which involved 50 patients, proved the significant differences in serum FSH, LH, A, T, DHEAS concentrations between the particular groups (A, B, C), whereas estradiol concentrations in serum did not considerably vary depending

on the length of time from the last menstruation. These data show that estradiol concentration considerably decreases in postmenopausal women comparing to the reproductive period, whereas the time from the last menstruation has no significant impact on serum estradiol concentration. Subsequently, serum FSH, LH, A, T and DHEAS concentration in the examined women depended on the time from the last menstruation. Our results are partially consistent with those reported by some other authors [31-34], but we have not found, however, any reports on the hormonal concentrations in women examined more than 5 years after the last menstruation.

Morphological results of our study show that the structure of female ovaries after menopause depends on the time that passed from the last menstruation, which is consistent with Focci *et al.* and Motta *et al.* results [26-28,35]. At the same time, we provide new information on the ovarian structure in that period. Our results revealed that the main structural changes were observed in both the cortex and medulla. In the cortex, they included: the reduction of its thickness; epithelial inclusions forming cysts; blurring of the line between medulla and cortex; the reduction of follicles number; the tendency to fragmentation of corpora albicantia; surface epithelium invaginations [35].

In immunohistochemical examinations that we carried out, the presence of ERα was clearly dependent on the time that had passed since the last menstruation. In the patients, who had their last menstruation less than 5 years earlier (group A), nuclear expression of ERα was mainly present in the stromal cells, in OSE, in epithelial inclusion cysts and also cytoplasmic expression of ERα in the luteanized cells of the corpus luteum (the early luteal stage). Intensity of the reaction in the mentioned places was assessed as the strong one. Pepe *et al.* [36] described the similar results in amphibian fetuses. In group B patients, an average intensity of the reaction was observed in OSE, the cells of involution cysts, and stromal cells. On the other hand, in group C women, who had their menopause at least 10 years earlier, only week intensity of ERα was observed in OSE and in stroma. The inclusion cysts of the examined patients contained cuboidal epithelium or metaplastically changed columnar epithelium. ERα was found both in cuboidal and columnar epithelium.

Our results show that ERα expression considerably reduces with age of a patient and the lapse of time from the last menstruation. In our research, however, ERα expression does not depend on serum concentrations of FSH, LH, T, A and DHEAS, which is inconsistent with the report of Meza-Munoz *et al.* [37], who concluded that ERα expression in the skin from the external gluteal area in postmenopausal women was associated with estrone and DHEAS concentrations in serum.

To sum up, finding evidence for the existence of epithelial inclusion cysts and the presence of ERα in the epithelium and in OSE is very important for postmenopausal women in the aspect of the use of estrogen replacement therapy and the development of estrogen-dependent neoplasms. 90% of ovarian epithelial neoplasms, and especially serous cancers, are known to develop from epithelial cysts and in OSE. It should be emphasized that the research on steroid receptors in the ovaries of postmenopausal women need to be continued. At present, we are in the middle of the research on the assessment of immunolocalization and immunoexpression of ERβ, as well as androgen and progesterone receptors in the ovaries of postmenopausal women.

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