

Expression of estrogen receptors α and β in term human myometrium

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SUMMARY

We used immunohistochemistry to compare the expression of estrogen receptors (ER α and ER β) in term myometria of 32 pregnant women divided in two groups. Group I comprised of 16 women in labor and group II included 16 non-laboring gravidas. We observed cytoplasmatic localization of both ER isoforms and no differences in the ER expression between the two groups of patients. The abundance and specific localization of ERs in human term myometrium seems to be independent of its contractile activity which may point to the specific role of those receptors in late pregnancy myometrium. *Reproductive Biology* 2004 4(3): 305-311.

Key words: myometrium, pregnancy, ER α , ER β , immunohistochemistry

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INTRODUCTION

There is compelling evidence that steroid hormones, especially estrogens, play a key role in modulating the growth, differentiation and functions of different target tissues including myometrium [2]. Most of the actions of estrogens are mediated by two forms of ER: ER α and ER β . Evidence suggests that ERs might play an important role in the timing of the onset of labor [4, 11, 12, 15]. During pregnancy, ER β expression was increased in human term myometrial tissue compared to non-pregnant tissue, whereas ER α was highly expressed in non-pregnant myometrium, but not in term myometrium [15]. Knowledge of the distribution of ER α and ER β in the human myometrium is therefore useful for studies on the respective functions of these ER subtypes in normal and abnormal labor. In the present study we aimed to compare the pattern of ER α and ER β expression in the myometrium of pregnant women at term with and without labor.

MATERIALS AND METHODS

Two myometrial tissue samples (0.5 x 0.5 x 0.3 cm) were collected from the lower uterine segment during cesarean section from every of 32 pregnant woman who delivered at term. The patients were divided into two groups according to the presence or absence of labor. Labor was defined as a presence of four uterine contractions within 20 minutes with cervical dilation of more than 2 cm. Group I included woman in labor (n=16): cesarean section performed as a result of labor arrest in the first or second stage or abnormal fetal heart rate. Group II (n=16) was selected among non-laboring patients, who had elective cesarean section due to breech presentation, cephalopelvic disproportion, high risk pregnancy or repeated cesarean section. The study was approved by the Ethics Committee of the Medical University of Bialystok.

ER α and ER β were detected by immunohistochemistry using the labeled streptavidin-biotin (LSAB) procedure [6, 7, 8]. Sections were deparaffinized in xylene, and cells were rehydrated in decreasing ethanol solutions. Endogenous peroxidase was neutralized with 2% hydrogen peroxide for 3 minutes. The

slides were subjected to heat-induced antigen retrieval using a microwave. They were then washed and incubated for 30 minutes at 22°C with a monoclonal rabbit anti-human ER α antibody (D-12, Santa Cruz Biotechnology, USA) at dilution 1:100 or a polyclonal rabbit anti-human ER β antibody (H-150, Santa Cruz Biotechnology, USA) at dilution 1:200. The samples were then washed off, and a biotinylated secondary antibody was applied to the slides for 25 minutes in a humidity chamber. The slides were again washed and incubated with streptavidin peroxidase for additional 25 minutes and submerged in DAB bath for five minutes. The tissues were counterstained with hematoxylin. Consecutive sections (5 μ m) from each tissue sample were evaluated by light microscopy (Olympus CX 40) in 10 different myometrial fields. Evaluation was based on the percentage of positive cells assuming 10% as a cutoff and intensity of immunostaining categorized as: weak (+), medium staining (++) and intense staining (+++). Positive control included myometrium taken from the operated leiomyomatosis uterus. Negative control included omission of the primary antibodies. MCF-7 cells, estrogen positive breast cancer cell line, were used to assess the quality of ER α and ER β antibodies. Mann-Whitney test was used for statistical analysis. P-values of less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

All examined myometrial samples from two groups of patients exhibited ER α and ER β expression. Both ER α and ER β showed cytoplasmatic pattern of staining (figs. 1A and B). In a small fraction of examined cells, a nuclear localization of immunostaining for ER β was also observed. Positive control showed nuclear ER α as well as cytoplasmatic and perinuclear ER β staining in the leiomyomatosis uterus (data not shown) as well as in MCF-7 cells (figs. 1C and D). Specific staining was abolished in negative controls (figs. 1E and F).

The percentage of ER α immunopositive cells was similar in two groups and ranged from 63% to 84%. The respective values for ER β were 62% to 86%. Percentage of cells stained positively for ER α (median: 75 vs. 74) and ER β (median: 74 vs. 75) did not differ ($p > 0.05$) between groups I and II.

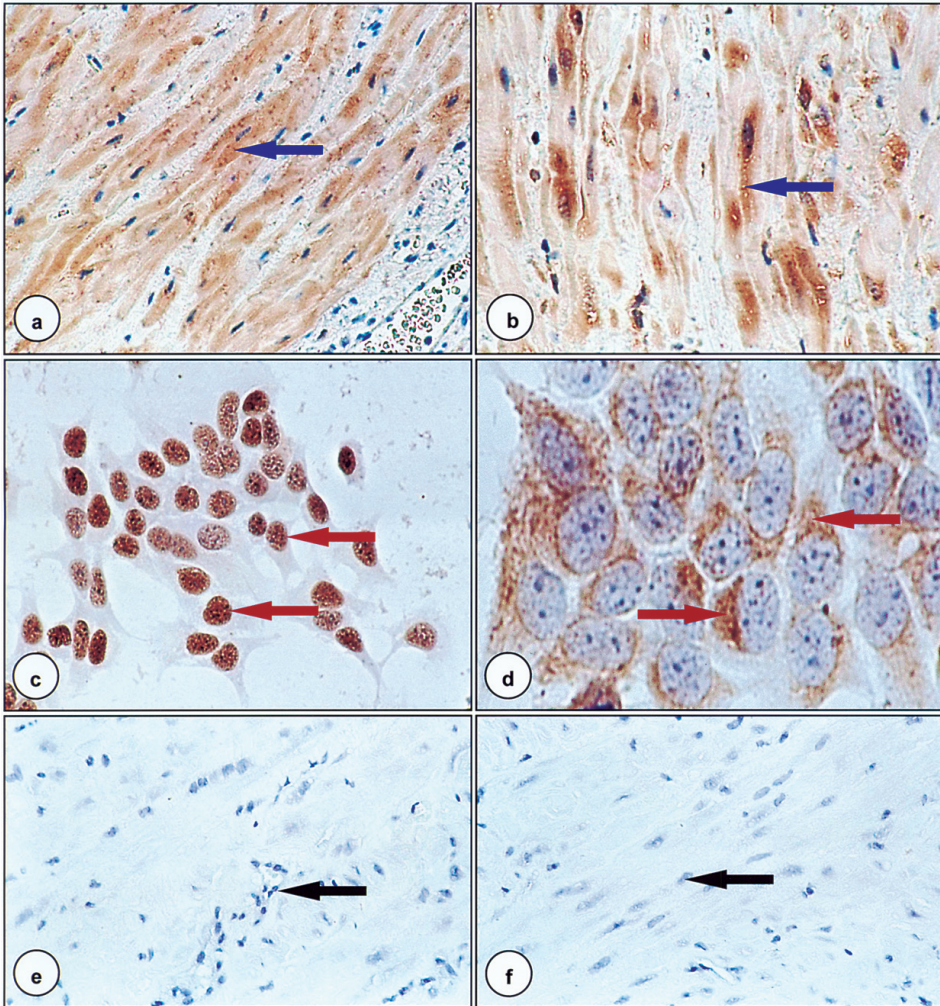


Fig. 1. Immunohistochemical detection of ER α and ER β in myometrium of women in late pregnancy. A, B/ Arrows indicate cytoplasmic localization of ER α (fig. 1A; intensity: ++) and ER β (fig. 1B; intensity: ++/+++) in myometrium. C, D/ Quality of ER α (fig. 1C; arrows indicate nuclear localization of immunostaining) and ER β (fig. 1D; arrows indicate cytoplasmic localization of immunostaining) antibodies was assessed in MCF-7 cells. E, F/ Negative controls included omission of the primary antibodies (E: ER α ; F: ER β), arrows indicate nuclei. The original magnification: 200 \times except fig. 1D: 400 \times .

In the present study, we used an antibody that can recognize all ER β variants. Interestingly, we found that ER β expression in tissue sections was predominantly cytoplasmatic. Although ER β has been found in the nucleus, cytoplasmatic expression of ER β has also been described [5, 14]. To define the specificity of our ER β antibody, we examined ER β expression and localization in MCF-7 cells. In agreement with other reports [10], we previously have shown [6] that ER β cx (one variant the ER β receptor) was cytoplasmatic and did not translocate to the nucleus in response to estradiol (E2) treatment, most probably because it does not bind E2 [9]. It is possible that ER β staining in tissue sections may reflect the expression of ER β cx or other ER β variant characterized by low E2 affinity.

Positive staining of ER α in the cytoplasm is not a novel finding neither. It has recently been reported that cytoplasmatic ER α expression accompanied by a lack of nuclear staining is characteristic for immature cells, whereas nuclear ER α indicates differentiating cells. Also a complete lack of ER α expression accompanies terminal differentiation of estrogen-sensitive cells [1]. It remains to be seen whether the cytoplasmatic staining of myometrial cells should be attributed to a given characteristic of utilized monoclonal antibody recognizing certain epitopes or is a result of immaturity of myometrial cells.

Wu et al. [15] observed switch from ER α to ER β expression in the myometrium during pregnancy. The authors suggested that myometrial ER β may inhibit activator protein-1 activity and thus block induction of the connexin-43 gene and other labor-associated genes which implied that labor may ensue after a loss of myometrial ER β expression [15]. More recently it was noted that a decrease in the expression of ER α as well as progesterone receptor (PR) isoforms A and B characterizes the early phase of first stage of labor [13]. It was not, however, efficiently significant to predict the progress of labor since the concentration of both ER and PR did not correlate with duration of labor [13]. In the present study we also found that in the contractile myometrium, ER α protein expression was similar to that of ER β . This resembles the patterns observed both in the myometrium of premenopausal women [12] and uterine endometrium during the menstrual cycle [3]. It seems plausible that in contractile uterus, estrogen

dependency might be preserved in both myometrium and in endometrium with a characteristic stable expression of ER α with ER β .

In summary, our results provide evidence for the widespread expression of both estrogen receptor isoforms in human term myometrium regardless of its contractile activity. Cytoplasmatic immunohistochemical staining may reflect a specific role of those receptors in late pregnancy myometrium which presumably is a consequence of their functional status.

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