Expression of Transcription Factors in Endometrium During Natural Cycles

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Purpose: The sex steroid control of the endometrial cycle is mediated by transcription factors, four of which are the estrogen and progesterone receptors, c-jun and c-fos, all expressed by the endometrium. The aim of this study was to analyze the distribution of the transcription factors in the different endometrial compartments during natural cycles.

Methods: We studied 53 reproductively-normal women, of whom 26 were in the proliferative phase and 27 in the secretory phase. An endometrial biopsy was performed and serum values of LH, FSH, estradiol, and progesterone were determined. We studied the expression of transcription factors using monoclonal antibodies.

Results: A correlation between estrogen receptor and c-jun and c-fos expression was observed in stroma and epithelia, and progesterone receptor expression correlated with c-jun expression in epithelia. C-jun and c-fos presented greater expression in the proliferative phase than in the secretory phase, in the stroma and in both epithelia. No relation was found between estradiol serum levels and any transcription factor, but progesterone serum levels correlated significantly with most such factors.

Conclusion: The two proto-oncogenes could play a decisive role in regulating the endometrial cycle; they could mediate the effects induced by sex steroid, and could be related to other transcription factors.

KEY WORDS: C-fos; c-jun; endometrium; steroid receptor; transcription factor.

INTRODUCTION

The human endometrium is subject to strict hormonal control during the menstrual cycle. Its maximum proliferation, stimulated by 17 β -estradiol, occurs with ovulation. Progesterone inhibits the proliferation

of the endometrial epithelium stimulated by 17 β -estradiol after ovulation and induces secretory differentiation (1,2). The reduction in the presence of ovarian steroids at the end of the luteal phase induces a chain of events, with lysosomal autodigestion and cell involution (2), followed by vascular deterioration, thrombosis, and interstitial bleeding. During menstruation, a variable quantity of the functional layer is lost (3,4), and the remaining mucosa enters an early proliferative phase. Foreseeable cyclic changes, together with the possibility of obtaining biopsies, make it possible to study the human endometrium in relation to the hormonal changes taking place at the end of the menstrual cycle, with regard to proliferation, differentiation, and cell death.

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Growth regulation and sexual differentiation during the menstrual cycle by the sex steroids is achieved by hormones produced locally, by cytokines and proto-oncogenes, whose interactions are as yet incompletely understood. The steroid receptors are intracellular proteins that join in a specific way with steroid hormones, provoking phenomena that in turn lead to cellular change. The steroid receptor/steroid hormone complex regulates the process of transcription of specific genes. One of the possible agents of estrogen-regulated endometrial proliferation is the proto-oncogene *c-fos*, a protein that amplifies the estrogenic message and activates the transcription of other genes that control multiple cellular functions such as division. The transcription of *c-fos* is reinforced by estrogens in the uterus of ovariectomized rats, and its effect is reversed by progesterone (5), which suggests that inhibiting the expression of *c-fos* could be an important mechanism involved in the antiproliferative effect of progestagens (6). The presence of m-RNA *c-fos* in the human endometrium has been established, with the highest levels being found during the proliferative phase of the menstrual cycle (7,8). These findings suggest that the expression of *c-fos* in the human endometrium could also be modulated by sex steroid hormones.

The proto-oncogene *c-jun* is one of the genes whose activation has been related to proliferation, differentiation, and apoptosis, depending on the tissue being studied (9,10). This protein is part of the activating factor (AP-1) that participates in the induction of transcription genes (11,12). This protein AP-1 is constituted with the union of *c-fos* to *c-jun*. The *c-jun* gene is strongly expressed from the proliferative phase to the secretory medium, but is virtually absent in the decidua (8). No cyclical changes have been detected in the endometrial expression of *c-jun* during the normal menstrual cycle, although the significant differences found between gestating and nongestating women support the hypothesis of hormonal regulation (7).

This study proposes an analysis of the distribution of the transcription factors between the different endometrial compartments, both in the proliferative and in the secretory phase, in natural cycles. We also analyze the relation of these factors with the serum values of circulating hormones.

MATERIALS AND METHODS

Subjects

A study was made of 53 reproductively-normal women who attended the Family Planning clinic at

the "Virgen de las Nieves" University Hospital in Granada. All had requested contraception by means of tubaric electrocoagulation, and fulfilled the requisites of fertile age, absence of any history of sterility, or disorders in their menstrual cycle, having become pregnant spontaneously during the previous 3 years and, during the last 6 months, having received no hormonal or IUD contraception.

Of these patients, 26 were in the proliferative phase (between days 9 and 12 of the menstrual cycle) and 27 were in the secretory phase (between days 20 and 22). On the appropriate days, blood samples were taken and an endometrial biopsy performed by aspiration, using a Cournier cannula. The biopsies were dated according to the Noyes method (13).

Estradiol, progesterone, LH, FSH, prolactin, and testosterone serum concentrations were analyzed with commercial kits (VIDAS, Biomerieux, France).

Tissue Processing

The biopsy material was fixed in tamponaded formalin pH 7.2 for 24 h and then embedded in paraffin. Sections were made with a rotary-blade microtome and then stained with hematoxylin–eosin and mucicarmine.

As part of the biopsy, a standard histologic study was made of the three components of the endometrium (epithelium, stroma, and vessels) in order to discount the possibility of any kind of morphologic disorder (irregular maturation, maturative glandular–stromal dissociation, etc.), to determine the presence of specific pathologic signs (endometritis, hyperplasias, etc.), and to attempt to date, as accurately as possible, the functional evolutive moment of the endometrium.

Immunohistochemistry

A streptavidin–biotin peroxidase detection system specific for the TechMate automated immunostainer (Dakocytomation AS, Denmark) was used for the immunohistochemical technique, on 5 μ m paraffin sections. For antigen retrieval, the slides were placed in 200 mL of retrieval solution (pH 6.8) and boiled for 2 min. The slides were cooled at room temperature for 20 min before processing. The following primary monoclonal antibodies were used: Estrogen receptor: monoclonal antibody, clone 1D5 (Dakopatts); Progesterone receptor: monoclonal antibody, clone 1A6 (Dakopatts); *C-fos*: monoclonal antibody, clone *c-fos* 88 (Biogenex); *C-jun*: monoclonal antibody, clone *c-jun* 88 (Biogenex).

The chromogen substrate used was 3'3'-diaminobenzidine tetrahydrochloride. The slides were counterstained with Harris's hematoxylin and mounted with synthetic mounting medium. A semi-quantitative method was performed for evaluation of immunohistochemical assays, as follows: 0: negative; 1: positive at <25%; 2: positive at 25–50%; 3: positive at 50–75%; 4: positive at >75%.

The positivity of the expression of the different transcription factors studied, within the different tissue compartments and cycle phases, was scored between 0 and 4. Interpretation of immunohistochemical techniques was performed blind without knowledge of clinical data.

Statistical Analysis

The Student's *t* test was used to compare hormonal serum levels between phases of the menstrual cycle. The Mann–Whitney *U* test was used for the statistical study of the values of the receptors in the endometrium. Spearman correlation was used to show the degree of association between hormone serum, and transcription factor expressions. Values were considered to be statistically significant for *p* values less than 0.05.

RESULTS

Significant differences ($p < 0.001$) were only found in the serum levels of progesterone (0.97 ± 0.9 ng/mL vs. 12.27 ± 3.52 ng/mL) between the proliferative and the secretory phase (Table I).

In every case, the endometrial morphology and tissue dating were adequate, and considered to be “in phase,” following Noyes' criteria (1950). The specimens in the proliferative phase presented intense proliferative activity, particularly so in the glands, in the surface epithelium, in the stroma, and in the vascular

endothelium of the upper third of the endometrium. The glands appeared to be straight or slightly curled, narrow, unbranched, and uniformly dispersed within an edematous stromal matrix. The tissue samples in the secretory phase presented the characteristic absence of proliferation of the stroma cells. In the glandular epithelium, the distal edge of the cells presented a curled appearance due to the numerous projections of apical cytoplasm filled with secretion material.

The estrogen receptor presented intense expression in the four compartments studied during the proliferative phase of the endometrial cycle but the epithelium in the secretory phase showed minimal levels of expression (Figs. 1 and 8(a)). The progesterone receptor, however, remained unchanged throughout the endometrial cycle in the stromal and endothelial compartments. In the glandular and surface epithelia,

Fig. 1. Expression of the estrogen receptor in the different tissue compartments.

Table I. Hormone Serum Values Obtained for the Two Endometrial Phases

	Follicular phase (n = 26)	Secretory phase (n = 27)
Estradiol (pg/mL)	134.41 ± 98.69 ^a	114.75 ± 76.63
Progesterone (ng/mL)	0.97 ± 0.79	12.27 ± 3.52***
FSH (mU/mL)	6.27 ± 1.85	4.82 ± 3.42
LH (mU/mL)	4.63 ± 2.44	3 ± 2.72
Testosterone (ng/mL)	0.60 ± 0.45	0.46 ± 0.23
Prolactin (ng/mL)	14.74 ± 6.82	12.25 ± 5.13

^a Mean ± SD.

*** $p < 0.0001$.

though, expression was greater during the proliferative phase and decreased in the secretory phase (Figs. 2 and 8(b)). C-jun presented greater expression in the proliferative phase than in the secretory phase, in the stroma, in the glandular epithelium, and in the surface epithelium, but there were no significant cyclical changes in the vascular endothelium (Figs. 3 and 8(c)). C-fos presented a very similar pattern of expression to c-jun (Figs. 4 and 8(d)).

We found a correlation between the expression of the estrogen receptor and that of c-jun in the stroma ($p < 0.005$) and in both epithelia ($p < 0.0001$), but not in the vascular endothelium. With respect to the correlation of the estrogen receptor with that of c-fos, it was found to exist in the stroma and in both epithelia

($p < 0.0001$ in all three cases), but not in the vascular endothelium. A statistical correlation between PR and c-jun was only found in the glandular and in the surface epithelia ($p < 0.001$ and $p < 0.005$, respectively). There was a correlation between PR and c-fos only in the two epithelia ($p < 0.001$) (Table II).

No statistical correlation was found between the serum levels of estradiol and the expression of the receptors in the different tissue compartments. However, the serum levels of progesterone were found to be significantly correlated with the expression of all the receptors studied, in the different compartments, with the exceptions of the progesterone receptor in the stroma and in the endothelium, and the c-fos oncogene receptor in the vascular endothelium (Figs. 5–7).

Fig. 2. Expression of the progesterone receptor in the different tissue compartments.

Fig. 3. Expression of c-jun in the different tissue compartments.

Fig. 4. Expression of c-fos in the different tissue compartments.

DISCUSSION

The expression of estrogen and progesterone receptors in the endometrium depends on the menstrual phase and the cellular compartment (see Fig. 8). We have observed, as did Press *et al.* (14), a higher expression of ER in all cellular compartments in the follicular phase than in the secretory phase. This expression is lower in the secretory phase probably because of the inhibitory effect of progesterone on ER expression (15,16). Progesterone receptor expression is higher in the proliferative phase, probably as a result of estrogen action in the epithelium (14,16). However, we have observed a similar PR expression in the stroma in the two menstrual phases. These results are in agreement with those of other authors (14), but in

Table II. Correlation Between the Expressions of the Different Transcription Factors

	ER	PR	c-jun	c-fos
ER				
Stroma	—	0.17	0.39**	0.55*
Glandular epithelium	—	0.48***	0.78***	0.78***
Surface epithelium	—	0.53***	0.82***	0.87***
Endothelium	—	0.32	0.16	0.25
PR				
Stroma	—	—	0.56	0.003
Glandular epithelium	—	—	0.48***	0.53***
Surface epithelium	—	—	0.49***	0.60***
Endothelium	—	—	-0.05	-0.06
c-jun				
Stroma	—	—	—	0.51***
Glandular epithelium	—	—	—	0.87***
Surface epithelium	—	—	—	0.85***
Endothelium	—	—	—	0.09

* $p < 0.005$; ** $p < 0.001$; *** $p < 0.0001$.

disagreement with Shiozawa *et al.* (17). The latter authors found that almost 75% of stromal cells in the secretory phase express PR, this high value being significantly lower than that observed by the same authors in the proliferative phase (90%). The discrepancies between these results and ours may be due to the fact that in our study the immunohistochemical results are expressed using a semiquantitative scale that does not distinguish such slight differences; on our scale, level 4 of intensity corresponds to expression in more than 75% of the cells.

In general, c-jun is intensely expressed in the human endometrium, but the cyclical pattern varies depending on the cell compartment. This cyclicity of the expression did not appear in studies made to determine

Fig. 5. Correlation between the serum levels of progesterone and the expression of estrogen receptor in the glandular epithelium ($p < 0.0001$).

Fig. 6. Correlation between the serum levels of progesterone and the expression of c-jun receptor in the glandular epithelium ($p < 0.0001$).

overall c-jun, in which the stromal, the glandular, the epithelial, and the surface compartments were included (8) but it was observed when the compartments were differentiated (18), although in the latter study the expression in the stroma varied very little. Taking these data into consideration, and in agreement with the results of Salmi *et al.* (18), the expression of c-jun seems to be associated with proliferation in the stromal and in the epithelial cells, both glandular and surface, but this is not so for the endothelium. In endometria that achieve a complete differentiation, that is, in the gestational decidua, c-jun is completely absent from the stroma (18).

It is interesting to note that the regulation of c-jun expression seems to differ between humans and rats. In the latter, estrogen stimulation produces a diminution in c-jun levels in the epithelial cells (19,20), while

Fig. 7. Correlation between the serum levels of progesterone and the expression of c-fos receptor in the stroma ($p < 0.0001$).

the reduced expression of c-jun and the proliferation in the epithelial cells are correlated (20).

Our results support the view that estrogen is a positive regulator of c-jun in the human endometrium, in the stromal and epithelial compartments; although we did not find a significant correlation between serum levels of estradiol and the expression of the c-jun (Figs. 5 and 6), there were correlations with the presence and the disappearance of estrogen receptors, on the one hand, and c-jun expression, on the other (Figs. 5–8) (8,21,22). In contrast, c-jun expression would be regulated by the progesterone receptor (18), as the receptor of free progesterone stimulates the activity of AP-1, while the addition of progesterone reverses this effect (23). In accordance with this, c-jun expression is stronger in the stroma, the glandular epithelium and the surface epithelium during the follicular phase, when the circulating levels of serum are lower and the expression of the progesterone receptor is greater. Moreover, during the secretory phase, when circulating levels of progesterone rise and the quantity of progesterone receptors in the glandular and surface epithelia falls, the expression of c-jun falls in the same compartments.

Therefore, c-jun expression in the human endometrium is cyclical. On the basis of the temporal association between c-jun expression and the endometrial proliferation of both the stroma and the epithelial cells, c-jun becomes an important regulator of the action of estrogen in the proliferation of endometrial cells.

Various studies (7,8) have shown there is a greater expression of c-fos in the proliferative human endometrium, both in the stromal and in the epithelial cells (6), and also that regulation decreases during the secretory phase (24), to disappear almost completely from the decidua during gestation (7). Our data confirm a cyclical pattern in c-fos expression in the human endometrium, with a greater expression in the proliferative phase, except in the vascular endothelium, a compartment in which no significant variations were found. This reduced regulation by progesterone was found to be significant in the two epithelial compartments and in the stroma, but not in the vascular endothelium. Our data do not support the hypothesis that estrogens stimulate the expression of c-fos (5), although this could be due to the fact that estradiol levels do not vary significantly between the two phases of the endometrial cycle examined in this study. The authors who have studied such an estrogenic control used values for nonphysiological

Fig. 8. Immunohistochemical study of normal cycling endometrium (see text). Evident stromal immunoreactivity of estrogen receptor with negativity in the epithelium (a) and important nuclear positivity of progesterone receptor (b) both in stromal and epithelial cells in secretory phase. Strong expression of c-jun (c) and c-fos (d) in proliferative phase.

estradiol (5,25). We found, thus, not only that the expression of c-fos during the endometrial cycle is controlled by hormonal serum levels, but also that this control varies between different tissue compartments.

In conclusion, both proto-oncogenes could play a decisive role in regulating endometrial growth and in differentiation; they could modulate the effects of estradiol and progesterone and could be closely related to other transcription factors such as the steroid receptors.

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