Epidermal Growth Factor Receptors in Endometrial Adenomatosis

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The expression of the epidermal growth factor receptors (EGFR) and of progesterone and estrogen receptors (PR and ER, respectively) was studied in 31 women of a reproductive age with endometrial adenomatosis. The receptor phenotype $EGFR^+PR^-ER^-$, indicating mainly local regulation of the proliferative processes, was detected in adenomatous endometrium three times more often than in normal endometrium. The metabolic endocrine disorders were less frequent in patients with endometrial adenomatosis with EGFR, but the disease ran a more severe course in these patients, which was proven by more numerous diagnostic scrapings performed within the same period.

Key Words: *endometrial adenomatosis; epidermal growth factor; steroid hormone receptors*

Extensive investigations of the hyperplastic processes in the endometrium have not facilitated the choice of adequate treatment for this reproductive disorder because of complexity of the mechanisms underlying its development. Precancer diseases of the endometrium, which progress to invasive cancer in 20-30% cases [12,15], deserve special attention.

Hyper- and neoplastic changes in the endometrium cannot be persuasively explained by disorders in the endocrine regulation of proliferative processes in the endometrium. Experimental and clinical studies of the recent decade show that along with sex steroid hormones, locally produced growth factors are involved in the regulation of cell proliferation and differentiation [5,16]. They are the main transmitters of the mitogenic signal stimulating or inhibiting the division and differentiation of various cells. An obligatory condition for realization of the growth factor mitogenic effect is the interactions between these factors and the specific receptors located on cell membrane [7].

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The most active and best studied auto- and paracrine regulators are epidermal (EGF) and α-transforming (α-TGF) growth factors homologous by their primary structure and reacting with a common receptor [7,14]. EGF/α-TGF receptor (EGFR), a large transmembranous protein, is a product of *arb* family oncogene. EGFR has been regarded as one of the main tissue markers of proliferative activity in tumors varying in histogenesis [9,13]. An unfavorable prognostic significance of EGFR expression in osteogenic sarcoma, breast cancer, ovarian cancer, and other tumors has been shown [2-4].

Experimental studies demonstrated the important role of the α-TGF/EGF/EGFR system in the regulation of proliferation and hormone sensitivity of transformed endometrium [10]. The clinical significance of these parameters in proliferative processes in the uterus is still to be investigated.

We studied the expression of EGFR, 17β estradiol, and progesterone in the uterine mucosa of women of a reproductive age with endometrial adenomatosis (EA).

MATERIALS AND METHODS

Thirty-one women of reproductive age (31.6±0.6 years) with relapsing endometrial hyperplasia and symptoms of

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EA were examined. By the moment of the study all of them had anovulations and menstrual cycle disorders: oligomenorrhea (8 patients, 25.8%), oligomenorrhea with menometrorrhagias (22 patients, 71.0%), and secondary amenorrhea (1 patient, 3.2%). Twenty-one patients (67.7%) had cycle disorders starting from the menarche, the rest developed the disorders after they started regular sexual life or after stress situations. The mean duration of the cycle disorders was 13.4±4.1 years, the mean period after the detection of endometrial hyperplasia was 6.3±3.0 years. Despite repeated hormone therapy (progesterone, nonsteroid progestagenes, and 17-oxyprogesterone capronate), 2-8 diagnostic scrapings had to be performed during this period in all women. Cycle disorders were associated with primary sterility in 24 (77.4%) and secondary sterility in the rest 7 (22.6%) patients. Obesity of different degree was observed in 23 (74.2%) patients (body weight index 30.4±1.7 *vs.* the normal no more than 25). Clinical, hormonal, and ultrasonic manifestations of polycystic ovaries were detected in 22 patients (71%).

The reference group consisted of 8 patients of a reproductive age (29.8±0.56 years) complaining of oligomenorrhea, disorders of fatty metabolism (body weight index 31.0±0.8), and infertility. They were subjected to hysteroscopy and diagnostic scraping of the endometrium. Histological findings indicated no endometrial abnormalities; the uterine mucosa corresponded to the proliferation stage.

Hysteroscopy and diagnostic scraping of the endometrium with simultaneous collection of the material for identification of the receptor status of the endometrium were usually carried out during menometrorrhagias and were planned operations in the main group, performed on days 20-24 of the cycle.

The content of EGFR in the membrane fraction of endometrial cells was measured by a modified radioligand method [6] with ^{125}I-EGF as the ligand. Steroid hormone (progesterone and estrogen) receptors (PR and ER, respectively) in the endometrium were detected by the competitive radioligand method with separation of the bound and free steroid on a dextrane-coated activated charcoal. The tissue was considered receptor-positive with EGFR and ER >10 and PR >20 fmole/mg protein.

RESULTS

EGFR were detected in the endometrium of 21 (67.7%) patients with endometrial hyperplasia and EA manifestations. The concentration of EGFR varied from 13 to 169 (mean level 58.0±9.1 fmole/mg membrane protein). In the group without endometrial disease, EGFR were detected only in 3 (37.5%) cases, and their levels were 33, 44, and 55 fmole/mg protein, which is virtually the same as in EA.

PR were detected in 50% patients in the main and reference groups (Table 1). However, their mean level was notably higher in EA than in proliferating endometrium. ER were detected only in 16.1% patients with EA and 25% patients without endometrial disease, and their concentrations varied within a very wide range.

Analysis of the receptor phenotype of the endometrium showed differences in the ratio of the expression of EGFR and steroid hormone receptors in patients with and without endometrial disease (Table 2). In EA, 13 patients (41.9%) with EGFR had no steroid hormone receptors, i.e., the receptor phenotype was $EGFR^{+}PR^{-}ER^{-}$. This phenotype was detected only (41.9%) one patient without endometrial disease. A more favorable ratio of receptors without EGFR with receptors to both steroid hormones ($EGFR^{-}PR^{+}ER^{+}$) was two times more frequent in patients without endometrial disease (2, 25%) than in those with EA (4, 12.9%). The occurrence of all studied receptors simultaneously (EGFR, PR, and ER) was equal in both

TABLE 1. Occurrence and Mean Levels of EGFR, ER, and PR in the Endometrium

Parameter		Hyperplasia with EA (*n*=31)	Proliferating mucosa (*n*=8)
EGFR	number of cases	21 (67.7)	3 (37.5)
	mean level, fmole/mg protein	58.0±9.1	45.0
	range of fluctuations	13.0-169.0	33, 44 and 55
PR	number of cases	16 (51.6)	4 (50.0)
	mean level, fmole/mg protein	97.5±21.4	175.0
	range of fluctuations	23.2-439.2	28.8-594.0
ER	number of cases	5 (16.1)	2 (25.0)
	mean level, fmole/mg protein	85.3	—
	range of fluctuations	12.4-349.0	16.3 and 257.0

Note. Here and in Tables 2 and 3: the percentage is given in parentheses.

TABLE 2. Endometrial Receptor Phenotype of Examined Patients

Parameter	Hyperplasia with EA (n=31)	Proliferating endometrium (n=8)
$EGFR^+PR^-ER^-$	13 (41.9)	1 (12.5)
$EGFR^+PR^+ER^-$	7 (22.6)	2 (25)
$EGFR^+PR^+ER^+$	1 (3.2)	—
$EGFR^-PR^+ER^+$	4 (12.9)	2 (25)
$EGFR^-PR^-ER^-$	2 (6.5)	3 (37.5)
$EGFR^-PR^+ER^-$	4 (12.9)	—
$EGFR^-PR^-ER^+$	—	—
$EGFR^+PR^-ER^+$		

groups of patients in comparison with the normal endometrium, where it was detected in 3 cases (37.5%).

The relationship between the clinical course of the disease in patients with endometrial hyperplasia, EA manifestations and the presence of EGFR in the endometrium has been analyzed (Table 3). No relationship between the incidence of EGFR and patient's age, disease duration, and type of the cycle disorders was detected. On the other hand, patients with EGFR rarely developed metabolic disorders, which was proven by a lower (almost 1.5 times) percentage of obesity. Endometrial hyperplasia ran a relapsing course in all patients who had a history of 2-8 diagnostic scrapings. In patients with EGFR in the endometrium, the disease (of the same duration after similar progestine therapy) seemed to run a more severe course with higher incidence of relapses, which was consistent with an almost twofold greater number of diagnostic scrapings in this group.

Hyperplasia in the endometrium, which can be regarded as a model of proliferative processes at the level of the uterine mucosa, is caused by disorders in the endocrine function of the reproductive system. In the overwhelming majority of the patients (74.2%), endometrial hyperplasia with precancer changes developed in the presence of the metabolic syndrome, which was characterized by metabolic disorders, anovulation, progesterone deficiency, and hyperestrogenism. However, the metabolic syndrome is unlikely to be the only cause of endometrial hyperplasia because no endometrial abnormalities were detected in patients with metabolic endocrine disorders.

The mechanisms regulating the proliferative activity of the endometrium represent hormonal and nonhormonal factors, including locally produced growth factors [3,10,11]. We have demonstrated an almost twofold increase in the occurrence of EGFR in hyperplastic endometrium of EA patients in comparison with the receptor content in patients with similar hormonal disorders without endometrial abnormalities. This finding indirectly points to the involvement of the auto-paracrine system in the regulation of the proliferative activity of the endometrium and development of hyperplastic changes. Higher occurrence of EGFR in precancer endometrium agrees with its higher occurrence in endometrial cancer [2]. However, the mean concentrations of EGFR in patients with EA did not differ from those in patients without endometrial abnormalities and were lower than in endometrial cancer [2]. This suggests that increased proliferative activity of the endometrium and the development of endometrial hyperplasia are associated with increased EGFR expression without any increase in their concentration, as in cancer transformation of the endometrium.

The incidence of ER and PR expression was low in both groups compared with published data [1]. This may be due to a long course of the disease and repeated courses of hormones, sterility, and endometrial hyperplasia therapies. Although there were no appreciable differences in the incidence of sex steroid receptor expression in patients with and without endo-

TABLE 3. Comparative Characteristics of Disease Patterns in Patients with EA with and without EGFR (M±m)

Parameter	$EGFR^+$ (n=21)	$EGFR^-$ (n=10)
Mean age, years	30.8	32.5
Disorders of fatty metabolism	14 (66.7)	9 (90)
Oligomenorrhea	5 (23.8)	3 (30)
Oligomenorrhea with menometrorrhagias	15 (71.4)	7 (70)
Amenorrhea II	1 (4.8)	—
Mean duration of cycle disorders	13.9±4.4	12.4±4.7
Time since the detection of endometrial hyperplasia	6.5±3.1	6.1±2.8
Relapsing course	21 (100)	10 (100)
<3 diagnostic scrapings	6 (28.6)	6 (60)
>3 diagnostic scrapings	15 (71.4)	4 (40)

metrial disease, the concentration of PR was lower in patients with EA than in those without endometrial hyperplasia.

Comprehensive evaluation of the occurrence of EGFR and steroid hormone receptor expression showed a threefold higher occurrence of the receptor phenotype $EGFR^+PR^-ER^-$ in the patients with EA than in those without endometrial involvement; this indicates predominantly local regulation of the proliferative processes [3,8]. The presence of EGFR in the absence of steroid hormone receptors indicates a low hormonal sensitivity of some tumors [2,3] and may serve as an unfavorable prognostic sign in this group of patients.

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