CYP19 gene expression and aromatase activity in endometrial cancer tissue: importance of the type of the disease*

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Aromatase (CYP19) activity in malignant endometrium presents local mechanism with potential ability to support tumor growth. The data on interrelation between activity of this enzyme and its mRNA signal in endometrial cancer (EC) tissue are very scarce and inconclusive. To correct this gap we studied aromatase activity and gene expression totally in 19 samples of EC (17 of them – from postmenopausal women) collected during surgery. Aromatase activity was evaluated by tritium water release assay and CYP19 gene expression – with rt-PCR. Additionally, all studied EC cases were divided on the basis of case history and some characteristics of host and tumor and in accordance with existing classification into cases belonging to type I or II of the disease (correspondingly, 13 and 6 observations). Positive correlation between enzyme activity and CYP19 mRNA signal (R_s=+0.63, $p<0.05$) was revealed in the whole group of studied samples. Aromatase activity varied in evaluated material between 1.45 fM/mg prot./hr and 11.49 fM/mg prot./hr, and in type II cases it was higher (7.27±0.96 fM/mg prot/hr) than in type I observations (4.96±0.66 fM/mg prot./hr, p=0.066). CYP19 mRNA expression was not revealed in 6 cases and all of them belonged to the type I of disease. Thus, although type II of EC is frequently considered as hormone non-dependent, increased ability of this tumor type to estrogen biosynthesis (on CYP19 gene and protein level) may lead to reconsideration of such conclusion and warrants further investigation.

Key words: endometrial cancer, aromatase, activity, CYP19 mRNA

Estrogenic hyperstimulation is considered – together with progesterone deficiency, hyperinsulinemia/insulin resistance, etc. – among leading risk factors of endometrial cancer [9, 11, 12, 13, 25]. In this context, two main estrogen sources can be distinguished in relation to the target of interests. One of them may be called "exogenous" and, besides HRT and other estrogen containing drugs, includes also estrogen-like environmental substances as well as estrogens circulating in blood. Another source of estrogen is "endogenous", or intratissular, and reflects different processes, among which estrogen uptake and local biosynthesis are most essential [2, 17].

The final step in reactions leading to estrogen formation is based on conversion of androgens to estrogens and is catalyzed by enzyme aromatase. The latter belongs to the XIX class of cytochrome P450 (CYP19). Although not completely unanimously [21], the most popular contemporary concept claims that contrary to the situation with breast epithelium, normal endometrium does not contain aromatase mRNA transcripts, and ability to synthesize estrogens is characteristic only for endometrial cancer (EC) tissue itself [8, 18]. It deserves mentioning that aromatase activity in EC tissue was studied significantly much rarely than in breast cancer, and the scope of analysis of correlation between CYP19 activity and clinico-biological features of EC was understandably very far from exhaustive [5, 18, 23]. Additionally, according to our knowledge, expression of CYP19 gene was so far evaluated in less than 25 cases of EC [8, 19, 21], and practically no comparative studies relating levels of aromatase activity and CYP19 mRNA within endometrial cancer tissue have been performed [19].

Important and rather well known feature of EC is its heterogeneity. Several lines of evidence suggest that two different variants of EC can be distinguished [2, 7, 12, 15, 20]. One of them is traditionally called hormone-dependent (type I) or es-

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trogen-dependent and more rarely – as usual type, and the other one – hormone-independent (type II) or estrogen-independent and as a special type [7, 12, 15, 23]. Initial task of the present study was to compare aromatase activity and CYP19 gene expression in EC tissue and to undertake preliminary steps in direction of the evaluation of these parameters separately in cases belonging to type I or II of endometrial cancer.

Material and methods

Subjects. Tumor samples from 19 patients with endometrial cancer were evaluated. Patients' age varied from 43 to 77, 17 patients were postmenopausal. According to existing surgical classification, stage of the disease varied between FIGO IA–IIIC. In 17 cases, or in 89.5 %, it was in a range of IA–IIB (Tab. 1). Morphologically mostly endometrioid adenocarcinomas were presented in this material. To distinguish between patients with type I and type II of EC following criteria were used: absence or presence of infertility/low pregnancy number, late menopause, obesity, hypertension, hyperlipidemia, uterine myoma, endometriosis, ovarian theca hypertrophy, well-, modestly- or low-differentiated carcinomas, superficial (less than 5 mm) or deep invasion into myometrium, involvement of regional lymph nodes. As proposed, patients who are infertile, obese, hypertensive, hyperlipidemic, have menopause timing ≥53 years, myoma or endometriosis and well-differentiated node-negative adenocarcinoma with superficial invasion (or are characterized with more than 50–60 % of these features) belong to type I of disease [7]. As a result, in the studied group of patients 13 women had type I of EC and 6 women had type II (Tab. 1).

Table 1. Data on patients, type of disease, aromatase activity and CYP19 expression in tumor tissue

Initials	Age	MP timing	Stage (FIGO)	Type of disease	AA (fM/mg prot/hr)	CYP19 expression (cond. units)
K.L.A.	43	–	IB	1	3.75	2 (±)
O.K.	74	42	IIB	1	2.09	1 (–)
K.N.A.	65	50	IB	1	2.98	1 (–)
L.Z.	61	53	IC	1	4.05	3 (+)
B.G.	51*	48	IB	2	7.26	2 (±)
K.N.	52	50	IB	1	7.93	2 (±)
V.M.	63	50	IB	1	4.90	2 (±)
F.N.	52	47	IA	1	3.13	1 (–)
B.L.	38	–	IB	1	1.45	1 (–)
J.T.	52	49	IB	2	5.81	2 (±)
T.G.	55	50	IB	1	4.22	1 (–)
T.E.	66	45	IIIC	2	8.06	2 (±)
E.V.P.	52	47	IA	1	6.45	3 (+)
G.L.	70	45	IC	2	6.19	2 (±)
T.P.	77	45	IB	2	11.49	3 (+)
K.M.	63	50	IC	1	8.76	3 (+)
K.L.	62	55	IIIB	1	6.52	1 (–)
Sh.T.	69	52	IB	1	8.25	3 (+)
G.M.	76	50	IIB	2	4.81	3 (+)

Aromatase activity in tumor tissue was estimated by measuring of ^3H_2O release from ^{3}H-1–androstenedione (NEN, Boston, MA; specific activity, 94 GBq/mM) as described [22]. Briefly, the reaction mixture (which contained tumor homogenate, NADPH regeneration system and labeled androgenic precursor) was incubated for 2 hr at 37 °C. Then reaction was stopped by adding 5 vol of cold chloroform, and 5 % suspension of activated charcoal (Norit A) was added to the water phase. The fraction containing ^3H_2O was separated by centrifugation, and counting was performed with dioxane scintillator. Results were presented in fM/mg protein/hr.

CYP19 gene expression was studied in the same 19 samples of malignant endometrium. Total RNA was isolated with guanidinium thiocyanate-phenol-chloroform method and checked by spectrophotometry and electrophoresis. Reverse transcription was performed in standard way and its efficiency was verified with PCR with primers to GAPDH-gene. Expession of the coding site of aromatase mRNA was evaluated examining the region across exons II and III with primers described by KOOS et al [14] – sense: 5'-GAATATTGGAAGGATGCACAGACT-3', antisense: 5'-GGGTAAAGATCATTTCCAGCATGT-3'. The conditions of reaction were the following: 37 cycles, denaturation – 30 sec. at 95 °C, annealing – 30 sec. at 61 °C, synthesis – 1.5 min. at 72 °C. The products were separated in the polyacrylamide gel and visualized by ethidium bromide staining. The expected size of CYP19 product was 293 bp and as a positive control RNA from human placenta was used. Results were presented in a semiquantitative manner using arbitrary or conditional units: 1 ("–" no signal), 2 ("±" weak positive signal) and 3 ("+" distinct positive signal) (Fig. 1).

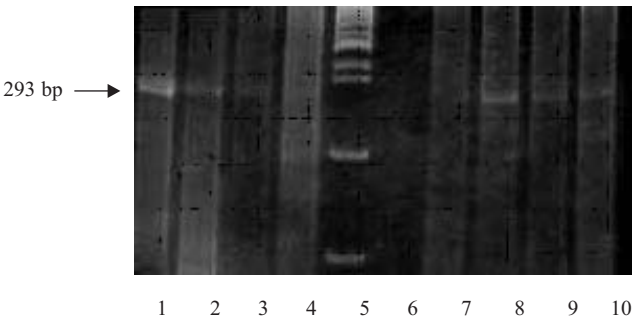

Figure 1. Examples of rt-PCR for aromatase gene in endometrial cancer tissue. Sample 1: (+); sample 2: (±); sample 3: (±); sample 4: (–); sample 5: mol. weight markers; sample 6: internal control, water; sample 7: (–); sample 8: (+); sample 9: (±); sample 10: (±).

Statistical analysis was performed by methods allowing for means, standard errors, χ^2-values and Spearman correlation on the basis of SigmaPlot program. The differences with $p \leq 0.05$ were considered as significant.

Results

Within studied samples of malignant endometrium (n=19) aromatase activity varied between 1.45 fM/mg prot./hr and 11.49 fM/mg prot./hr, respectively (Tab. 1) with average (±SE) on the level of 5.69±0.59 fM/mg prot./hr. Inclination to more higher aromatase activity was revealed in tumors from patients belonging to type II of disease (7.27±0.96 fM/mg prot./hr, n=6) vs. tumors from patients with type I of disease (4.96±0.66 fM/mg prot./hr, n=13; p=0.066). Expression of CYP19 mRNA was not revealed in 6 cases of EC, all of which belonged to type I of disease; 7 cases demonstrated signal on the level "±" and 6 cases – on the level "+". Difference in distribution of aromatase gene signaling between patients/tumors belonging to type I or II of the disease is seen in Tables 1 and 2. In the whole group of studied samples aromatase activity and CYP19 mRNA signal correlated with each other (rank correlation coefficient, or R_s=+0.63, p); this correlation was more pronounced in type I samples (R_s=+0.67) than in type II material (R_s=+0.33).

Table 2. Distribution of the data on CYP19 gene expression in endometrial cancers belonging to the different types of the disease

CYP19 expression	Type I	Type II
Negative (–)	6	0
Weak positive (±)	3	4
Distinct positive (+)	4	2

Data are presented as number of corresponding cases.
Difference in distribution of reactions ("–" vs. "±" and "+") between type I and type II cases is significant (χ^2 = 4.05, p = 0.044).

Discussion

According to epidemiological observations, in the developed countries endometrial cancer ranks fourth among female cancers and first among gynecological cancers [12]. Effects of the known EC risk factors (obesity, anovulation, infertility, late menopause, etc.) are frequently thought to be mediated through a leading hormonal mechanism, that is, relatively high estrogen levels along with absolute or relative progesterone insufficiency affecting endometrium [13, 20, 25]. There are two sources of estrogen production in female organism, namely gonadal and extragonadal, and significance of the latter increases especially in postmenopausal period [2, 17]. As demonstrated, similarly to breast, epithelial tumors EC tissue also possesses ability for estrogen biosynthesis [5, 8, 18, 19]. Although idea on heterogeneity of EC and existence of its two variants (one of which is usually considered as hormone/estrogen dependent and the other one as hormone/estrogen independent) became rather popular during last 40–50 years [1, 6, 7, 12, 15, 20, 23], nobody so far studied aromatase (estrogen synthetase) activity and aromatase gene signal separately in tumor samples belonging to type I or II of the disease. Meanwhile, such approach might help at least partly to solve the problem, whether the hormonal imbalance associated with risk of endometrial cancer and excessive estrogenic stimulation is peculiar only for so called type I of this disease.

In studies described above two principal results were received: aromatase activity as well as CYP19 gene signal in EC tissue were increased in cases belonging to the type II of disease and besides there was a positive correlation between these two parameters reaching significance ($p<0,05$) in the whole group of samples. In one of the previous reports tendency to increased aromatase activity in low-differentiated tumors was revealed [24], and according to our recent observations the ratio of incidence of CYP19 A6A6 genotype (presenting a combination of longer and probably more biologically active allelic polymorphisms) to the frequency of A1A6 and A3A6 genotypes was higher in type II patients (1.0) than in type I patients (0.3) [4].

Thus, notwithstanding that we studied relatively small number of cases, it may be suggested that more aggressive clinically and frequently receptor-negative type II of endometrial cancer [7, 12, 14, 15, 20, 23] is associated with the signs of intratumoral hyperproduction of estrogens both on the level of aromatase protein and gene expression. These data additionally support earlier made assumptions that: 1) EC of type II actually may be also estrogen dependent – with the dependence of neoplastic transformation of endometrium and tumor growth progression only of locally produced estrogens [3], and 2) the existence of two variants of EC is a reflection of two types of hormonal carcinogenesis, i.g. promotional and genotoxic [2, 10, 16]. In case that further studies confirm at least first of these assumptions, such property of endometrial cancer probably might be a sign of increased sensitivity of type II of this disease to the treatment with aromatase inhibitors.

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