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EXPRESSION OF THE ESTROGEN AND PROGESTERONE RECEPTORS AS PROGNOSTIC FACTOR IN SEROUS OVARIAN CANCERS

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Aim: **To study the expression of estrogen receptors (ER) and progesterone receptors (PR) and proliferation marker Ki-67 in ovarian tumors using immunohistochemistry, and evaluate possible prognostic significance of these markers.** ***Methods:*** **Immunohistochemical evaluation of Ki-67, ER and PR expression was performed on serous ovarian cancer (OC) tissue samples from 81 OC patients.** ***Results:*** **Serous OC is characterised by high proliferative activity and increased expression of steroid hormone receptors compared to nontransfomed ovarian surface epithelium. It has been shown that ER and PR expression levels depend on tumor histologic grade and the stage of the disease, and are variable between tumors of the same grade. The ER and PR expression levels correlate with OC patients' survival.** ***Conclusion:*** **Proliferative activity and steroid hormone receptor status along with clinical and morphological characteristics of serous OC possess prognostic significance and may be used for evaluation of the disease course.**
Key Words: **ovarian adenocarcinoma, tumor grade, survival, Ki-67, estrogen and progesterone receptor expression.**

Ovarian cancer (OC) is one of the most aggressive malignancies of female reproductive system, occupying the fourth place in the structure of cancer incidence among Ukrainian women (14.3 per 100 000 women). It has one of the highest levels among genital cancer patients in Ukraine and other countries as well [1]. High mortality in OC patients is caused by the fact that 75% of OC cases are diagnosed at III–IV stage, resulting in poor prognosis of the disease and low efficacy of treatment [2].

OC pathogenesis and ethiology are still poorly understood. Nevertheless, there are several hypothesis of the pathology origin. Consistent with one of them, the ovarian cancer occurrence is caused by a high number of ovulations that leads to enforced proliferation of the ovarian surface epithelium, that fills the wound defect arising from follicule rupture. So, the number of ovulation cycles during lifetime is an indicator of the OC risk [2, 3].

Meanwhile, viral infection (by human papilloma virus, for example, strains 16, 18, 48, 56) of the ovarian epithelium contributes to OC development, especially in case of serous neoplasia [4].

Moreover, genetic factors play an important part in OC occurrence. Accumulation of genetic alterations has been reported to underlie progressive transformation of ovarian benign tumors into malignant ones [5]. Numerous oncogenes and suppressor genes determine ovarian tumors pathogenesis and progression (acquisition of more malignant features during tumor growth). Sporadic OC often carry mutations in *TP53* tumor suppressor gene (in 50% of serous adenocarcinomas, for example). Epithelial ovarian tumors are characterized by changes in expression of a number of cell cycle regulators, such as $p16^{INK4a}$ (in 35% of OC cases), *CDK4*, *cyclin D* and *Rb* (retinoblastoma gene) (in 30% of OC cases), and overexpression of *HER2/neu* oncogene (in 10–50% cases) [6].

A body of experimental, epidemiological and clinical studies allows to characterize OC as a hormone-dependent tumor. By other words, an essential factor in OC pathogenesis is hormonal imbalance determined by an increase of pituitary gonadotrophic function, resulting in ovulation overstimulation and chronic hyperestrogenia along with a decrease in progesterone secretion. Hyperestrogenia can be considered as an additional risk factor of ovarian malignancy [2].

More evidence on OC hormone-dependence was presented when estrogens (ER) and progesterone receptors (PR) were detected. It was shown that ovaries produce sex steroid hormones, and they are a target of their action simultaneously; i. e. realization of hormonal stimuli requires an adequate quantity of the receptors. Ovarian neoplasias are characterized by changes in their receptor status, and, consequently, tumors can be either primary receptor-negative or as a result of their progression they may lost the receptors.

In a number of studies it was shown that both missense and nonsense mutations (resulting in complete loss of expression) in ER genes are common in OC [7].

Steroid hormone receptors are a significant link in hormonal signal transduction. They modulate such important events, as cell differentiation, proliferation and death through interaction with the respective ligands.

ER and PR levels depend on tumor histologic type, patients' age that determines their responsiveness to hormonal therapy with synthetic progestagen and antiestrogen [8].

It was noticed that receptor status and proliferative activity determine tumor malignancy and disease course [9–12]. However, no consensus on prognostic

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Abbreviations used: ER – estrogen receptor; LI – labeling index; OC – ovarian cancer; PR – progesterone receptor.

significance of steroid hormone receptor expression levels in ovarian tumors was reached yet.

Immunohistochemistry enables estimation of biomarkers expression in tumor tissue and determination of morphological structures that express them [9, 13].

In the present paper we report the results of the retrospective immunohistochemical investigation of ER and PR expression and proliferative activity in ovarian neoplasias and evaluation of their possible prognostic significance.

MATERIALS AND METHODS

The current study was carried out on surgically resected tumor samples (including archival ones) from 81 patients with serous OC of I–IV stage (16–79 years old, average age was 46.6 ± 2.4 years), 42 from which were at menstrual period (16–55 years) and 39 — at menopausal period (52–72 years). Morphologically not changed serous epithelium samples of endometrial fibromioma cases (n = 7) were used as the relative negative control.

All patients underwent treatment in the Oncogynecological Department of the National Cancer Institute of Ministry oh Health of Ukraine (headed by prof. Vorobyova) at the period from 1988 to 2005. The stage of tumor process was determined according to FIGO classification [14]. The data about disease clinical course, treatment and patients' outcome were obtained retrospectively from each case history and ambulatory records. According to these data, 20 patients survived for 5–17 years, and for 29 patients survival period was < 5 years.

Immunohistochemistry on ER and PR expression was performed on deparafinized slides, using mouse monoclonal antibodies against ER (clone 1D5), PR (clone PgR636) and Ki-67 (clone MIB-1), and EnVision visualization complex (DakoCytomation, Denmark) according to manufacturer protocol [15]. Marker expression was determined in 700–800 tumor cells.

The results of immunohistochemical reaction were evaluated using semiquantitive method [15], using calculation of positively stained cells or labelling index (LI). ER and PR medians were 29.0% and 37.0%, respectively. Consistent with these data, LI values less and higher than median value Me were considered low and high, respectively. ER or PR expression was considered negative when LI ⩽ 10%.

Proliferation index (PI) was estimated as the number of Ki-67 expressing cells. Proliferation activity was considered low if PI < 10.0%, and high if PI ⩾ 10.0% [15].

Statistical analysis of obtained results was performed using description statistic, the comparison of samples (Mann — Whitney's U-test), using program STATISTICA 6. Survival analysis was provided using Kaplan — Maier method; statistical significance of the differences between survival curves was defined by Cox-test [16–18].

RESULTS AND DISCUSSION

All studied neoplasias were diagnosed as serous adenocarcinomas of different grade: G1 (n = 9), G2 (n = 34) or G3 (n = 38).

We have found that Ki-67 was not expressed in normal ovarian epithelial cells. At the same time, the majority of serous tumors were highly proliferating with PI ranging from 10 to 76.3% (average value 33.6 ± 2.8%). Analysis of steroid hormone receptors in all relative control samples has shown low PR (14.2 ± 3.9%) and negative ER expression.

ER and PR expression in ovarian tumors increased essentially, compared to that in the nontransformed ovarian tissue, and was 29.0 ± 2.6% i 33.0 ± 3.1%, respectively (Fig. 1, 2).

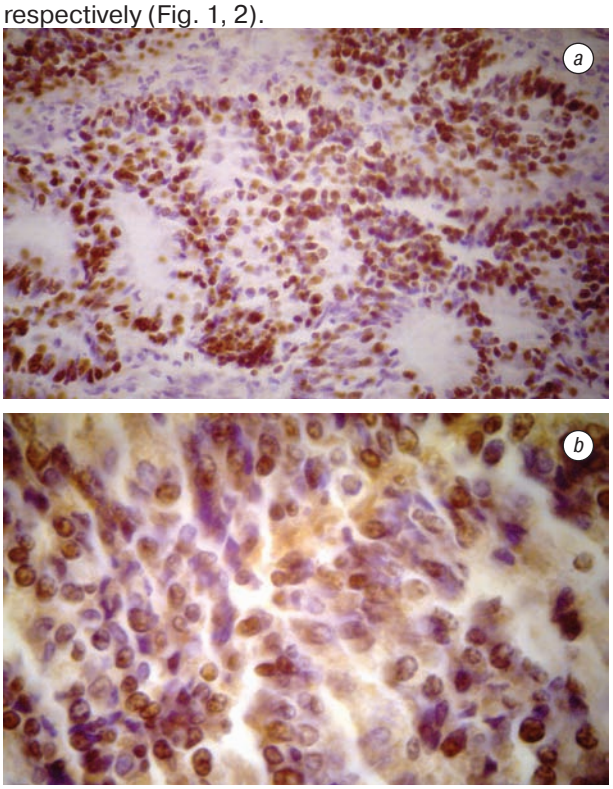

Fig. 1. High expression of estrogen receptors in ovarian cancer cells. *a*, x 400; *b*, x 900

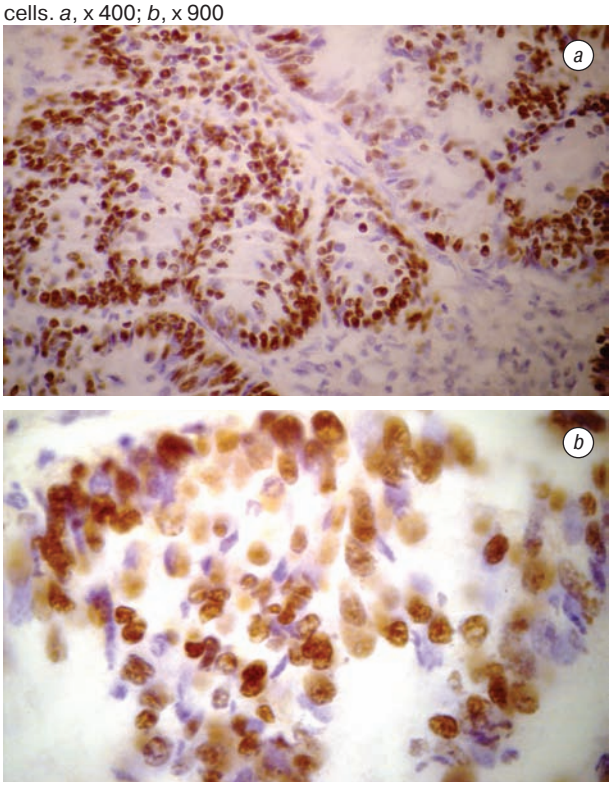

Fig. 2. High expression of progesterone receptors in ovarian cancer cells. *a*, x 400; *b*, x 900

Positive expression was recorded almost in the same number of OC cases: 68.0% of tumors was PR positive, and in 67.0% of tumors ER positive. Meanwhile, tumors with low ER and PR expression comprised 25.5 and 15.0% of total OC cases. High expression of these receptors was found in 42.0 and 53.0% of total OC cases, respectively. Receptor's phenotype of neoplasm is one of the basic criteria of OC hormone sensitivity; together with efficacy of hormonal therapy it predetermines the prognosis of the disease.

The analysis of receptor phenotype of studied ovarian tumors has shown that 54.0% of cases were ER^+PR^+ positive, and in 21.0% of tumors both receptors were not expressed. ER^+PR^- and ER^-PR^+ phenotypes were determined in 14.0% and 11.0% of the patients.

The current study has demonstrated the relationship between the expression of steroid hormone receptors and the state of patients' menstrual function. The number of cells expressing ER in the group of patients with a restored menstrual function was higher compared to this parameter in patients of menopausal age, and were 30.0 ± 2.8% and 26.0 ± 2.4%, respectively. For women at menopausal period PR expression was reliably lower (27.0 ± 2.9%, $p < 0.05$) than for patients with restored menstrual function (34.0 ± 3.2%).

The results of comparison of ER and PR expression in I–II and III–IV stage OC are shown in Table 1. It was shown that a half of I–II stage OC samples expresses receptors versus 43.0% in III–IV stage OC samples. Moreover, in the group of III–IV stage patients' number of receptor-negative tumors was the three folds increased.

Table 1. Steroid hormone receptor expression in ovarian adenocarcinomas of different stages

Stage of disease according to FIGO	Receptor expression profile, % of total case number			
	ER^+PR^+	ER^+PR^-	ER^-PR^+	ER^-PR^-
I–II	50.0	12.5	25.0	12.5
III–IV	43.0	13.0	12.0	32.0

The investigation of ER and PR expression in ovarian neoplasias of different grade has revealed significant heterogeneity of this index, especially in G2 and G3 tumors (Table 2).

Table 2. ER and PR expression in ovarian neoplasias of different grade

Tumor histologic grade	Labelling index of the biomarker, %	
	ER	PR
	min–max	min–max
G1	51.6 ± 4.6	51.8 ± 4.3
	29–70	37–70
G2	31.8 ± 3.6	37.8 ± 4.7
	0–69	0–84
G3	21.0 ± 3.9	24.2 ± 4.4
	0–90	0–84

ER and PR expression was the highest in G1 tumors and decreases along with disease progression reaching its minimal values in G3 ovarian carcinomas ($p < 0.005$).

It should be noted that among patients with G1 ovarian tumors, high ER and PR expression was prevalent, while lower differentiation grade corresponds to higher numbers of cases with low or negative receptors expression. ER and PR expression was absent in 26.5% of G2 tumors, whereas there was a 2-fold increase in the number of such cases among G3 carcinomas (Fig. 3).

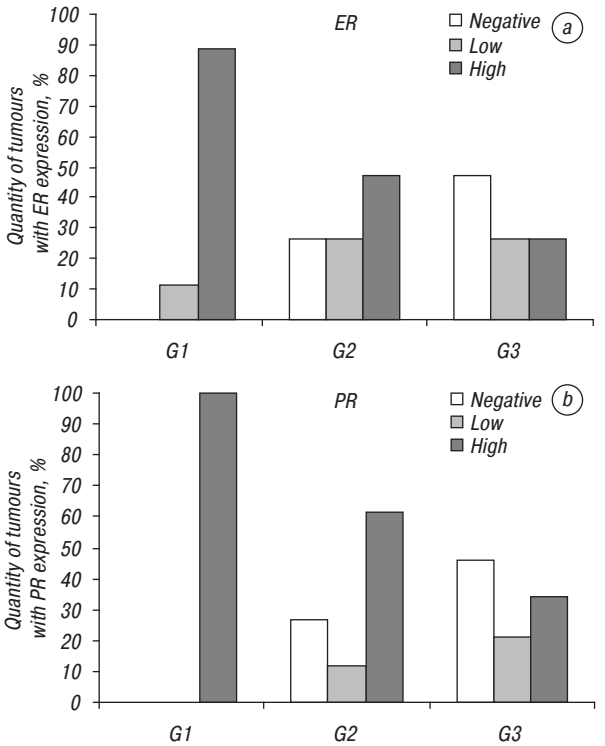

Fig. 3. Distribution of ovarian serous adenocarcinomas of different grade according to the level of steroid hormone receptor expression

The study of receptor expression has revealed that all G1 tumors were positive (ER^+PR^+ phenotype), while ovarian carcinomas of higher grade showed an increase in the number of receptor negative (ER^-PR^-) cases (Table 3).

Table 3. Steroid hormone receptor expression in ovarian adenocarcinomas of different grade

Tumor histologic grade	Receptor phenotype, %			
	ER^+PR^+	ER^+PR^-	ER^-PR^+	ER^-PR^-
G1	100.0	–	–	–
G2	61.7	14.7	8.8	14.7
G3	36.8	15.8	15.8	31.6

In order to estimate the prognostic significance of steroid hormone receptor expression, groups of OC patients were standardized according to type and regimen of applied polychemotherapy.

Based on analysis of survival curves of OC patients, it was possible to determine the ER and PR expression levels (29.0% and 37.0%) that were of critical prognostic significance. We have found that 5-year survival of 75.0% and 65.0% was reliably higher in OC patients with high levels of ER and PR expression (higher than 29.0% and 37.0%, respectively), compared to patients with lower values of expression (Fig. 4).

Obtained data indicate that the expression of steroid hormone receptors could be considered as an independent prognostic factor in ovarian neoplasias. This hypothesis is supported by the number of female reproductive system malignancy studies, showing a great importance of receptor status in hormone-dependent tumors. For example, Ellinidi *et al.* [19] have reported on estrogen-and-progesterone receptor phenotype as an important prognostic parameter, reflecting the presence of two pathogenetic pathways of breast cancer development. Meanwhile, it was shown that endometrial neoplasms that expreesed ER and PR were

characterised by low grade, insignificant depth of miometrium invasion, low number of metastases in regional lymph nodes and better survival, compared to the negative cases. It is noteworthy, that the expression of ER and PR is important for the course and outcome of endometrial cancer [20]. However, for prognosis of OC outcome PR expression is considered to be most valuable [8, 9]. The prognostic significance of ER expression is far from being completely determined, but there is some evidence that the loss of estrogen receptor β promotes OC development [21].

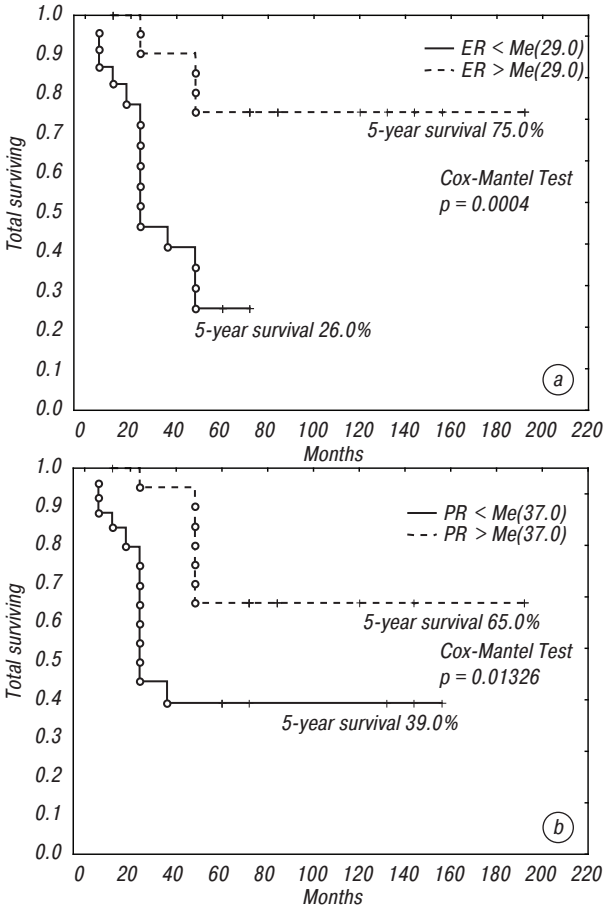

Fig. 4. Survival curves for OC patients dependent on ER (*a*) and PR (*b*) expression

In conclusion, the current study shows that serous OC is characterised by high proliferative activity and increased expression of steroid hormone receptors compared to those in the nonchanged ovarian surface epithelium. It was shown that ER and PR expression depends on tumor histologic grade and varies between the tumors of the same grade. The receptor phenotype of serous ovarian tumors correlates with disease stage, and level of steroid hormone receptor expression is one of the significant factors that determine OC patients' survival. Proliferative activity and steroid hormone receptor status along with clinical and morphological characteristics of the disease have prognostic significance and may be used for evaluation of serous OC course.

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