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Original scientific paper

A Correlation between the Expression of Estrogen Receptors and Progesterone Receptors in Cancer Cells and in the Myometrium and Prognostic Factors in Endometrial Cancer

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A B S T R A C T

Endometrial cancer is the most common gynecological malignancy in Croatia. The aim of this study was to determine the immunohistochemical expression of estrogen receptors (ER) and progesterone receptors (PGR) in cancer cells and in the myometrium and to correlate it with prognostic factors of endometrial carcinoma. ER positivity in carcinoma cell nuclei was found in 42 cases (73.7%) and PGR positivity was found in 39 cases (68.4%). Loss of ER in carcinoma cell nuclei correlated with larger tumor size ($p=0.041$), poor carcinoma differentiation ($p=0.012$), a more aggressive histological type ($p<0.001$), lymphovascular space invasion ($p=0.002$) and a higher surgical stage ($p=0.037$). Loss of PGR in carcinoma cell nuclei correlated with an increased age in patients ($p=0.009$), poor tumor differentiation ($p=0.002$), a more aggressive histological type ($p<0.001$), lymphovascular space invasion ($p=0.002$) and a higher surgical stage ($p<0.001$). The lower expression of both receptors did not correlate with the depth of myometrial invasion. Regarding the status of receptors in the myometrium, loss of PGR in the myometrium correlated with a more aggressive histological type ($p=0.005$) and a lack of ER in the myometrium tended to correlate with tumor growth ($p=0.059$). In conclusion, the loss of both hormone receptors in carcinoma cells and loss of PGR in the myometrium was a predictor of a more aggressive type of endometrial cancer and a poor prognosis.

Key words: *endometrial cancer, estrogen receptor, progesterone receptor, myometrium*

Introduction

Endometrial cancer (EC) is the most common gynecological malignancy in the Western world responsible for 1–2% of all deaths from cancer in women[1,2]. According to the Croatian National Institute of Public Health, the incidence of EC in 2009 was 25.3/100,000 which is 6.0% of all new malignancies diagnosed in women every year. The majority of endometrial malignances occur in postmenopausal women. According to the World Health Organization, EC has several histological types. The most common is endometrioid adenocarcinoma (75%). Papillary serous (5–10%) and clear cell (1–5%) carcinomas are less common types[1,2]. Type I endometrioid adenocarcinomas are estrogen dependent, low grade and often diagnosed in early stage with a favorable prognosis. The five-year survival rate is over 80.0%. Type II cancers usually have papillary serous or clear cell histology. They are estrogen non-dependent, high grade, and often with a more aggressive clinical course. Studies regarding EC have shown the presence of multiple prognostic factors: surgical stage, patient age, histological type, tumor grade, depth of myometrial invasion, cervical invasion, lymphovascular space invasion (LVSI), lymph node involvement, tumor size, expression of estrogen receptors (ER) and expression of progesterone receptors (PGR)[3–5]. The endometrium and myo-

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metrium are highly hormone-regulated tissues with the expression of both, estrogen and progesterone receptors. The majority of endometrial cancers are ER and PGR positive (35–90%)[6–8]. Loss of ER correlates with a higher tumor grade, higher clinical stage, deeper myometrial invasion, LVSI and poor overall survival[9]. On the other hand, loss of PGR correlates with a younger age of patients, higher tumor grade, higher clinical stage, LVSI and deeper myometrial invasion[10,11]. Loss of PGR also correlates with a relapse of the disease and contributes to tumor progression but does not correlate with overall survival[9,10,12]. On the contrary, according to Bender, loss of PGR is a predictor of a poor clinical outcome[12,13]. The evaluation of expression levels of ER and PGR in endometrial cancer is a very common procedure in modern practice. However, the significance of such tests remains controversial[11]. Therefore, the aims of this study were to analyze ER and PGR expression in cancer cells and in the surrounding myometrium, and to present the correlation of their expression with other EC prognostic factors.

Patients and Methods

In this study we used paraffin embedded endometrial cancer samples taken from 57 patients surgically treated and diagnosed between 2007 and 2010 at the Department of Pathology, University Hospital for Tumors, Zagreb, Croatia. Data were retrospectively collected from a clinical data base. The patient's age, tumor size (mm), tumor histological grade (I, II, III), depth of myometrial invasion (less or more than 50% of myometrium thickness), LVSI (present or absent) and FIGO surgical stage were collected from pathology reports. For statistical analysis, the FIGO classification was grouped in clinical stage I (disease limited to uterus) and clinical stage II (disease extended beyond uterus). The endometrioid histological type of EC was grouped as low risk (Type 1) while clear cell and papillary serous histological types were grouped as high risk cancers (Type 2).

Immunohistochemical analysis

An immunohistochemical reaction was carried out for the detection of steroid hormone receptors expression in cancer and myometrial cells. Sections of 2–3 μm were cut from FFPE blocks and mounted on silanized slides and dried for one hour at 60 °C. The sections were dewaxed in xylene and rehydrated in a series of graded alcohols. Antigen retrieval was performed in pH 9.0 Tris/EDTA buffer in Dako PT link at 97 °C for 20 minutes. Prior to immunohistochemical staining endogenous peroxidase activity was blocked using 3.0% hydrogen peroxide for five minutes. The primary monoclonal antibodies against estrogen receptor α (ER, clone 1D5, Dako Denmark, diluted 1:40) and progesterone receptor A (PGR, clone PgR 636, Dako Denmark, diluted 1:75) were applied for 30 minutes. Visualization was performed using a universal secondary antibody for 30 minutes (EnVision Flex, K8010, Dako Denmark). Counterstaining was performed using hematoxylin for one minute. The slides were dehydrated, cleared with xylene and covered.

We evaluated the expression of steroid receptors in EC cells and in surrounding myometrium cells on the same slide. Positive immunohistochemical staining has nuclear reaction. We counted 1,000 cells and scored the percentage of immunopositive nuclei, using high-power magnification (×40 objective and ×10 ocular).

Statistical analysis

Differences in the expression of estrogen and progesterone receptors (as a percentage) between binary variables – disease risk type (low or high risk), myometrial invasion (less or more than 50% of depth), LVSI (present or absent) and surgical stage (disease limited to the uterus or extended beyond the uterus) were tested with a Student T-test for independent samples. Correlations between hormone receptor expressions, both in the tumor and myometrium, age, tumor size, histological grade and surgical stage were tested using a nonparametric Spearman correlation analysis. All analyses were performed using StatSoft software Statistica 7.0 (Tulsa, USA). We set the definition of statistical significance to 0.05.

Results

The detailed pathohistological data of 57 patients with EC are presented in Table 1. Endometrial carci-

TABLE 1
PATHOHISTOLOGICAL CHARACTERISTICS OF PATIENTS WITH ENDOMETRIAL CANCER

Pathohistological characteristics of patients	Type 1 EC (n=39) N (%)	Type 2 EC (n=18) N (%)
Histological grade		
Grade 1	18 (46.2)	3 (16.7)
Grade 2	11 (28.2)	5 (27.8)
Grade 3	10 (25.6)	10 (55.5)
Hormone receptors		
ER carcinoma	35 (89.7)	7 (41.2)
ER myometrium	36 (92.3)	17 (94.4)
PGR carcinoma	35 (89.7)	4 (22.2)
PGR myometrium	37 (94.8)	14 (77.7)
Myometrial invasion		
=50%	24 (61.6)	9 (50.0)
>50%	15 (38.5)	9 (50.0)
LVSI		
Positive	13 (33.3)	14 (77.8)
Negative	26 (66.7)	4 (22.2)
Surgical stage (FIGO)		
I	31 (79.5)	9 (50.0)
II	3 (7.7)	1 (5.6)
III/IV	5 (12.8)	8 (44.4)

EC – endometrial cancer, ER – estrogen receptors, PGR – progesterone receptors, LVSI – lymphovascular space invasion, FIGO – International Federation of Gynecology and Obstetrics

noma prevails in older women; the median age was 66 years. Patients with a higher risk type of endometrial cancer were older (median age 70 years) than patients with a lower risk type (median age 65 years). High-risk carcinomas had 55.5% grade III carcinomas compared to 25.6% grade III in low risk carcinomas. Half had myometrial invasion in more than 50% of the myometrium thickness while low-risk carcinomas had an invasion of over 50.0% in 38.5% of cases. Lymphovascular space invasion was also associated with higher risk type, with 77.8% positivity. Larger tumor size and a higher histological grade showed the impact on the depth of myoinvasion (R=0.338, p=0.011 and R=0.437, p<0.001, retrospectively). In addition, larger tumor size and a higher histological grade have an impact on LVSI (R=0.333, p=0.012 and R=0.676, p<0.001).

The immunohistochemical expression of ER was found in 42 cases (73.7%) and PGR was found in 39 cases (68.4%) of cancer nuclei. In addition, hormone receptors were predominantly positive in the myometrium of the uteri (Figures 1 and 2). Negative receptors in the myometrium were found in four cases (7.0%) for ER and six cases (10.5%) for PGR (Table 1) (Figure 3).

Correlations between hormone receptors in tumor cells and the myometrium and EC prognostic factors are presented in Tables 2 and 3. In our results (Table 2), the expression of PGR in cancer inversely correlated with the age of patients (p=0.009). Both receptors, ER and

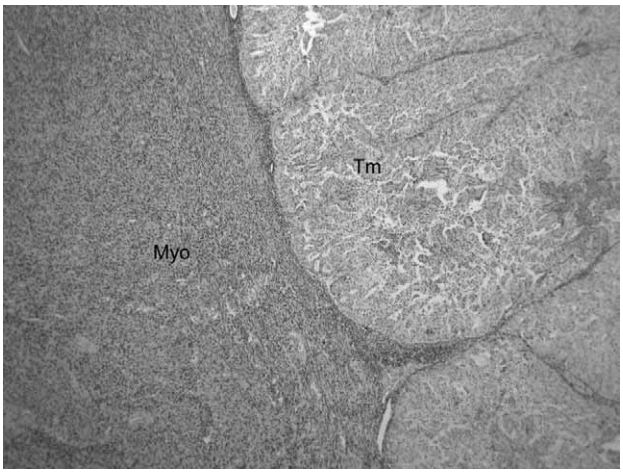

Fig. 2. Nuclear immunohistochemical expression of progesterone receptors; negative expression in carcinoma cells and moderate positive expression in myometrial cells (x50).

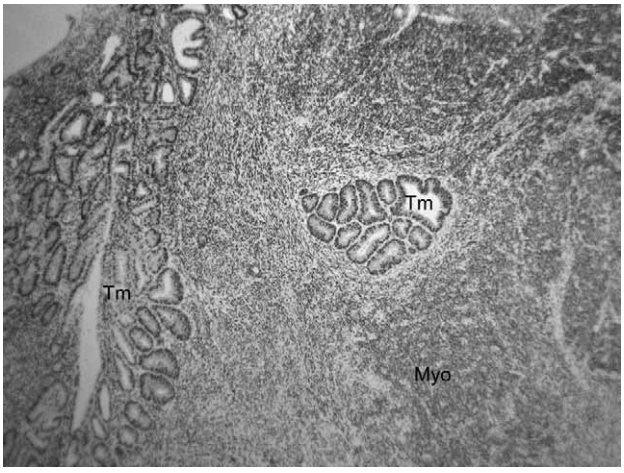

Fig. 1. Nuclear immunohistochemical expression of estrogen receptors; positive expression in carcinoma cells and strong expression in myometrial cells (x50).

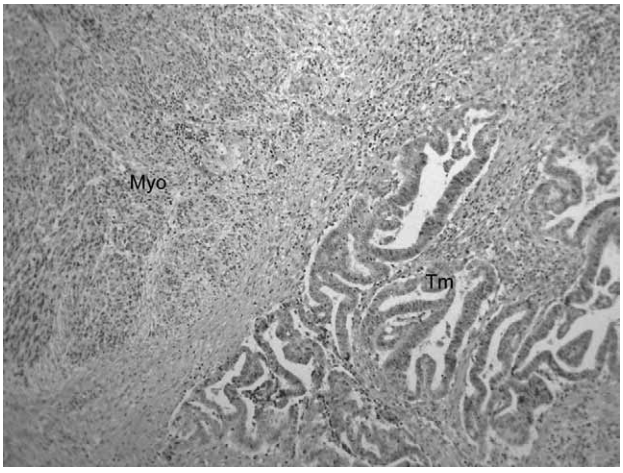

Fig. 3. Nuclear immunohistochemical expression of progesterone receptors; negative expression in carcinoma cells and negative expression in myometrial cells (x50).

TABLE 2
EXPRESSION OF STEROID RECEPTORS IN TUMOR CELLS AND IN THE MYOMETRIUM IN CORRELATION WITH PROGNOSTIC FACTORS IN ENDOMETRIAL CANCER

Prognostic factors		ER tumor	ER myometrium	PGR tumor	PGR myometrium
Age/years	R	–0.17	–0.006	–0.342*	–0.205
Tumor size/mm	R	–0.271*	–0.265	–0.209	–0.162
Histological grade	R	–0.330*	–0.054	–0.388*	–0.072
Surgical stage	R	–0.276*	–0.036	–0.444*	–0.109

ER – estrogen receptors, PGR – progesterone receptors
*p<0.05

TABLE 3
EXPRESSION OF STEROID RECEPTORS IN TUMOR CELLS AND IN THE MYOMETRIUM IN CORRELATION WITH PROGNOSTIC FACTORS IN ENDOMETRIAL CANCER

Steroid receptors		Endometrial cancer		Myometrial invasion		LVSI	
		Type 1 N=39	Type 2 N=18	=50% N=33	>50% N=24	Negative N=30	Positive N=27
ER tumor (%)	Mean value	65.8	18.3*	55.3	44.6	65.3	34.4*
ER myometrium (%)	Mean value	70.6	70.0	73.1	66.8	74.4	67.0
PGR tumor (%)	Mean value	71.9	9.1*	58.6	43.0	67.1	34.2*
pnumPGR myometrium (%)	Mean value	85.0	60.6*	77.2	77.1	80.0	74.4*

LVSI – lymphovascular space invasion, ER – estrogen receptors, PGR – progesterone receptors
*$p<0.05$

PGR, were strongly co-expressed in cancer cells ($R=0.766$, $p<0.001$) as well as in the myometrium ($R=0.441$, $p=0.001$). ER showed a major influence on tumor growth. Tumors were larger in cases with negative ER ($p=0.041$) and, although not statistically significant, lower ER in the myometrium ($p=0.059$). A higher histological grade correlated with the lower expression of ER ($p=0.012$) and PGR ($p=0.002$) in cancer cells, while the status of receptors in the myometrium had no influence on the histological grade. The expression of ER and PGR in cancer influenced the surgical stage of the disease.

Our results showed that ER and PGR in tumor cells were markedly lower in high risk than in low risk endometrial cancer, as presented in Table 3. The lower expression of both receptors in cancer cells also correlated with the presence of LVSI, yet did not correlate with the depth of myometrial invasion. We did not find any correlation between ER expression in the myometrium and disease risk, LVSI or patient outcome but we found that the expression of PGR in the myometrium correlated with disease risk ($p=0.005$).

Discussion

Endometrial cancer is the second most common malignancy among women aged between 50 and 69 years old in Croatia. This cancer is usually diagnosed in early stage and there is good prognosis. The majority of patients with EC in our study were postmenopausal women, with a median age of 66 years. Numerous studies have defined some valuable prognostic factors of this malignancy. The surgical stage and histopathologic tumor grade are the most relevant features for subsequent therapeutic management[14,15]. The depth of myometrial invasion and positive LVSI are parameters in terms of recurrence rate and survival[5,16]. According to the mentioned parameters, the endometrioid histological type of carcinoma belongs to a low-risk malignance while papillary serous and clear cell carcinomas are high-risk malignancies. Our results are similar to those reported in previous studies. The higher risk tumors occurred in older women, with a median age of 70 years. In addition, the high-risk tumors were of a larger size and less differentiated, more often with positive LVSI than low-risk tumors (Table 1). In our study, low-risk carcinomas corresponded to a lesion in clinical stage I/II while high-risk carcinomas corresponded to surgical stage III/IV. Estrogens act as a promoter of growth and proliferation of the endometrium via estrogen receptors, while progesterone acts as an estrogen antagonist in endometrial maturation and inhibition of proliferation[17]. The endometrium is very sensitive to sex hormones, and thus a shift in the balance of estrogens and progesterone can cause the development of endometrial cancer[18]. The expression of steroid hormone receptors is one of the important prognostic factors in endometrial carcinoma[3,4]. Data from the literature reported hormone receptors positivity in 35–90% of endometrial carcinomas[6–8,18]. In our analysis, ER and PGR were positive in 73.7% and 68.4% of endometrial cancer nuclei, with a strong co-expression in cancer cells. In agreement with our results, several studies have reported that a loss of ER expression, correlates with larger tumor size, poor differentiation, positive LVSI and a higher clinical stage[9]. Interestingly, there is disagreement about the impact of ER on myometrial invasion[10,11]. Our results confirm reports by Jorgen et al that ER does not influence myometrial invasion[10]. Jeon et al, however, found a correlation between ER and myometrial invasion[10,11]. Recent studies have proposed progesterone as an ultimate endometrial tumor suppressor[18]. They have reported that progesterone via PGR is an inhibitor of tumorigenesis. Ito et al suggested that the invasiveness and metastatic potential of carcinomas can be influenced by loss of PGR[17]. In our study, loss of PGR expression in carcinoma cells correlated with a higher histological grade and LVSI. We also found a stronger correlation between negative PGR and surgical stage III/IV and a histological high-risk type of disease than with ER. In concordance with others we showed that PGR is a stronger marker of carcinoma aggressiveness[13]. Interestingly, but opposed to reported results, we did not find a correlation between PGR and tumor size or myometrial invasion[9–11]. Some authors suggested that activation of the PGR pathway via estrogen can be interrupted[13,18].

As reported by Yang et al, the relation between cancer cells and surrounding tissue may be involved in endometrial carcinogenesis[18]. Experiments have shown constant

communication between epithelial and stromal cells, and that the critical stages of proliferation are mediated through the stromal expression of hormones[19]. Our results showed that the myometrium is predominantly positive for ER and PGR and they have a strong co-expression (R=0.441, p=0.001). In high-risk carcinomas, we observed lower levels of PGR in the myometrium than ER (77.7% vs. 92.4%). Since, lack of PGR in carcinoma correlated with older age, perhaps this lack of PGR in the myometrium also depends on the age of patients. Lack of ER in the myometrium had a tendency to correlate with tumor size. In our study, loss of PGR in the myometrium influenced the occurrence of high-risk carcinomas. It still remains to be clarified whether PGR has a protective role in endometrial carcinogenesis, as reported in some studies, or whether the loss of PGR is a precondition for carcinoma development[13,20].

In clinical practice, progesterone therapy has been used in recurrent disease but the response rate has not been very high (20–30%)[2,20]. Some results suggest that an improved response to hormone therapy depends on the higher expression of PGR[13]. Although survival analysis was not the subject of this study, we can confirm that PGR is a strong predictor of EC prognosis. Current strategies for the treatment of EC are still not as effective as in other types of hormone dependent carcinomas. Thus, further investigations to improve treatment strategies are necessary.

Conclusion

Our study confirms the prognostic importance of investigating the hormonal status in endometrial carcinomas, considering the direct correlation between the level of the sex steroid hormone receptors in relation to tumor size, tumor differentiation, histological type, LVSI, surgical stage and the age of patients. Loss of ER and PGR in carcinoma cells and loss of PGR in the myometrium was a predictor of a more aggressive type of endometrial cancer and consequently a poor prognosis.

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ODNOS IZMEĐU IZRAŽENOSTI ESTROGENSKIH I PROGESTERONSKIH RECEPTORA U STANICAMA KARCINOMA I U MIOMETRIJU I PROGNOSTIČKIH ČIMBENIKA KARCINOMA ENDOMETRIJA

SAŽETAK

Karcinom endometrija je najčešće maligno oboljenje ženskog spolnog sustava u Republici Hrvatskoj. Cilj je ove studije utvrditi imunohistokemijsku izraženost estrogenskih receptora (ER) i progesteronskih receptora (PGR) u karcinomskim stanicama i u miometriju te utvrditi odnos s prognostičkim čimbenicima karcinoma endometrija. Pozitivni

ER-i u jezgrama karcinomskih stanica pronađeni su u 42 promatrana slučaja (73,7%), a pozitivni PGR-i u 39 promatranih slučajeva (68,4%). Gubitak ER-a u jezgrama karcinomskih stanica povezan je s veličinom tumora (p=0,041), slabom diferencijacijom karcinoma (p=0,012), agresivnijim histološkim tipom (p<0,001), invazijom limfovaskularnog prostora (p=0,002) i višim kirurškim stadijem bolesti (p=0,037). Gubitak PGR-a u jezgrama karcinomskih stanica povezan je sa starijom dobi pacijentica (p=0,009), slabom diferencijacijom karcinoma (p=0,002), agresivnijim histološkim tipom (p<0,001), invazijom limfovaskularnog prostora (p=0,002) i uznapredovalim kirurškim stadijem bolesti (p<0,001). Niži izražaj oba receptora nije pokazao povezanost s dubinom invazije miometrija. Gubitak PGR-a u miometriju povezan je s agresivnijim histološkim tipom (p=0,005), a gubitak ER-a u miometriju imao je tendenciju korelacije s rastom tumora (p=0,059). Zaključak je studije da je gubitak oba hormonska receptora u karcinomskim stanicama i gubitak PGR-a u miometriju naznaka agresivnijeg tipa karcinoma endometrija i loše prognoze bolesti.