Expression of Estrogen Receptors α and β, and Progesterone Receptors A and B in Human Mucinous Carcinoma of the Endometrium

NAIM SHABANI[1,2*], IOANNIS MYLONAS[2*], UDO JESCHKE[2], AGIM THAQI[3], CHRISTINA KUHN[2], THOMAS PUCHNER[1] and KLAUS FRIESE[2]

[1]Landes-Krankenhaus Schärding, Abteilung für Frauenheilkunde und Geburtshilfe, A-4780, Schärding; [2]I. Frauenklinik and [3]Institut für Medizinische Informatik, Biometrie und Epidemiologie der Ludwig-Maximilians-Universität München, D-81377, München, Germany

Abstract. *Background and Aim: Endometrial carcinoma is the most common female pelvic genital malignancy. The strong association between the development of endometrial cancer and influence of steroid hormones (especially estrogen) was demonstrated in many studies. Mucinous carcinoma is an uncommon type of endometrial carcinoma. Most cancers are low grade and have a relatively good prognosis. The expression of one type of estrogen (ER) and progesterone receptor (PR) has been well documented. Recently, two new types of receptors (ER-β and PR-B) were demonstrated. The aim of this study was to determine the expression of all four steroid receptors (ER-α, ER-β, PR-A and PR-B) in human mucinous carcinoma of the endometrium. Patients and Methods: An immunohistochemical hormone receptor assay using specific monoclonal antibodies against estrogen receptors (ER-α, ER-β) and progesterone receptors (PR-A and PR-B) was used to study formalin-fixed and paraffin-embedded slides of 12 patients, diagnosed with primary endometrial mucinous carcinoma of different histological grades (G1 n=9; G2 n=3; G3=0). Results: Three types of steroid receptors (ER-α, PR-A and PR-B) were frequently expressed in mucinous adenocarcinoma. ER-β was weakly expressed in only one analyzed case. The immunohistochemical expression of PR-B demonstrated a statistically significant decrease in G1 neoplasms in comparison to G2 ($p \leq 0.001$). Conclusion: We demonstrated the expression of four different steroid receptors in mucinous endometrial carcinoma. No significant differences between different histological grades of tumor with respect to ER-α and ER-β expression were observed. Interestingly, a statistically significant increase in expression of PR-B in G2 neoplasms compared to G1 was demonstrated. The higher expression of PR-B in G2 tumors suggests a substantial function of progesterone, and thus progesterone receptor, in the malignant transformation of mucinous endometrial cancer. Therefore, PR-B expression might be utilized as a tumor marker to distinguish between G1 and G2 mucinous tumors. However, additional studies are necessary to evaluate whether these parameters could be used as tumor markers for endometrial cancer.*

The endometrium is a dynamic tissue in which growth and proliferation during the menstrual cycle is regulated by hormones. It is a target tissue for ovarian steroid hormones, estrogen and progesterone (1). Endometrial carcinoma is the most common female pelvic genital malignancy and the fourth most frequently diagnosed cancer in women. There are several specific histological types of endometrial carcinoma. Mucinous carcinoma is an uncommon type of all endometrial cancer. It represents about 1-9% of all endometrial carcinomas (2). Most cancers are low grade and have a relatively good prognosis. There is a strong association between the development of endometrial cancer and the influence of steroid hormones (especially estrogen). Many studies document that endometrial cancer is associated with estrogen-induced growth stimulation unopposed by the effects of progesterone (3-5). Endometrial hyperplasia with cytological atypia as a precursor lesion of endometrial cancer is induced due to unopposed estrogenic stimulation. In contrast, progesterone inhibits endometrial proliferation and can reverse endometrial hyperplasia (6). Also growth of early, non-

*Both authors contributed equally to this study.

Correspondence to: PD Dr. Udo Jeschke, Ludwig Maximilians University of Munich, Department of Obstetrics and Gynaecology, Maistrasse 11, D-80337 Munich, Germany. Tel: +49 89 5160 4266, Fax: +49 89 5160 4916, e-mail: udo.jeschke@med.uni-muenchen.de

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Table I. *Mucinous carcinomas classified according to grade.*

Classification	Grade 1	Grade 2	Grade 3	Total
WHO (32)	9 (75%)	3 (25%)	0 (0%)	12
FIGO (32)	7 (58%)	5 (42%)	0 (0%)	12

Table II. *Mean IRS for different steroid receptors in relation to the grade of mucinous carcinoma of the endometrium.*

Mean IRS	G1(WHO)	G2 (WHO)	G1 (FIGO)	G2 (FIGO)
ER α	4.6	3	4.6	3.6
ER β	0.1	0	0.1	0
PR A	3	3.3	2.4	4
PR B	3.9	9	4.4	6.2

Table III. *Antibodies used for immunohistochemical characterization of endometrial glandular cells.*

Antibody	Clone	Isotype	Dilution	Source
ERα	1D5	mouse IgG1	1:150	Immunotech, Hamburg, Germany
ERβ	PPG5/10	mouse $IgG2_a$	1:50	Serotec, Oxford, United Kingdom
PR A	10A9	mouse $IgG2_a$	1:50	Immunotech, Hamburg, Germany
PR B		mouse $IgG2_a$	1:50	Immunotech, Hamburg, Germany

ER=estrogen receptor, PR=progesterone receptor.

invasive stages of endometrial cancer can be inhibited by progesterone treatment (7). An increased incidence of endometrial cancer is most common in women with conditions resulting in unopposed estrogens, such as estrogen-only hormone replacement therapy (HRT), obesity, polycystic ovary disease, nulliparity, estrogen-producing tumors and anovulation (3, 4). In contrast, combined estrogen and progesterone therapy prevents the increased risk of endometrial cancer (8).

The effect of the steroid hormones estrogen and progesterone are thought to be mediated through activation of estrogen receptors and progesterone receptors. Estrogen receptor (ER) and progesterone receptor (PR) are closely related to the occurrence of autocrine and paracrine processes that respond to estrogen and progesterone (9). ER and PR status is a well-recognized prognostic indicator in women with breast cancer and also appears to be of clinical importance (as a prognostic factor) in women with endometrial carcinoma (10, 11). The expression of ER and PR are linked because transcription of the PR gene is induced by estrogen and inhibited by progestins. ER and PR belong to the nuclear receptor superfamily. They are ligand-dependent transcriptional factors, which can bind to different DNA sites to initiate the expression of specific genes. In several studies both receptors were measured in paraffin-fixed human endometrium using immunohistochemical assays (4, 9). However, the exact mechanism for the proliferative effects of estrogens on the endometrium and its role in neoplasia remain unknown. The expression of one type of ER and PR has been well documented. The ER and PR expression and distribution pattern might play an important role in normal endometrial function and pathogenesis. The expression and relationship of the two distinct ER and PR could be of essential clinical importance. The ER status is believed to provide prognostic information independent of tumor stage and grade in women with endometrial carcinoma (12). The ER-β/ER-α mRNA ratio was high in advanced invasive carcinoma, suggesting that ER-β is important in the progression of myometrial invasion. The intact synchronized expression of ER-β interacting with ER-α might be disrupted in the neoplastic endometrium (13), playing an important role in endometrial pathogenesis. A significant correlation using regression analysis of ER-α and ER-β was also demonstrated in malignant endometrial tissue, showing dependence in the expression of all these steroid receptors. In a previous article our group showed the presence of steroid receptors in human endometrium, indicating that these cells respond to estrogen and progesterone, playing a significant role in endometrial physiology and tumor genesis (14).

The aim of this study was to determine the expression of all four types of steroid receptor (ER-α, ER-β, PR-A and PR-B) in human mucinous carcinoma of the endometrium and to determine if any type of steroid receptor could be used, in the future, as a tumor marker.

Patients and Methods

Tissue samples. Samples from 245 patients who underwent surgery for endometrial cancer in the First Department of Obstetrics and Gynaecology, Ludwig Maximilians University, Munich, Germany, during 1990-2002 were evaluated.

Only 12 cases (about 4%) were diagnosed as primary endometrial mucinous carcinoma of different histological grade (Table I).

Immunohistochemistry. Immunohistochemistry was performed using a combination of microwave-oven heating and the standard streptavidin-biotin-peroxidase complex using the mouse-IgG-Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA). Mouse monoclonal antibodies used for the experiments are listed in Table III. For positive controls, sections of human breast cancer tissue and normal colon samples were used.

Briefly, paraffin-fixed tissue sections were dewaxed using xylol for 15 min, rehydrated in a series of alcohol and subjected to

antigen retrieval for 10 min on a high setting in a pressure cooker in sodium citrate buffer (pH 6.0) containing citrate acid 0.1 M and sodium citrate 0.1 M in distilled water. After cooling, the slides were washed twice in PBS. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide (Merck, Darmstadt, Germany) in methanol for 20 min. Non-specific binding of the primary antibodies was blocked by incubating the sections with diluted normal serum for 20 min at room temperature. Sections were then incubated at room temperature with the primary antibodies for 60 min. ER-α and PR-A and -B were diluted in dilution-medium (Dako, Glostrup, Denmark), while ER-β was diluted in PBS. After washing with PBS, the slides were incubated in diluted biotinylated serum at room temperature for another 30 min. After incubation with the avidin-biotin peroxidase complex (reagent ABC) for another 30 min and repeated washing steps with PBS, visualization was performed with substrate and the chromagen 3,3'-diaminobenzidine (DAB; Dako, Glostrup, Denmark) for 8-10 min. The slides were counterstained further with Mayer's acidic hematoxylin and washed in a series of alcohol (50-98%). After xylol treatment the slides were covered.

Negative controls were performed by replacing the primary antibody with normal mouse serum. Positive cells showed a brownish color and negative controls, as well as unstained cells, were blue.

Evaluation and statistical analysis. The SPSS/PC software package, version 6.01 (SPSS GmbH, Munich, Germany), was used. *P*-values resulted from two-sided statistical tests and $p \leq 0.05$ was considered to be significant.

The intensity and distribution of the specific immunohistochemical staining reaction was evaluated using a semi-quantitative method (IRS-score) as described elsewhere (15) and used in the evaluation of endometrial steroid receptor expression (16). The IRS score was calculated as follows: IRS=SI x PP, where SI is the optical stain intensity (graded as 0=no, 1=weak, 2=moderate, and 3=strong staining) and PP the percentage of positively stained cells. PP was estimated by counting approximately 200 cells and it was defined as 0=no staining, 1=<10%, 2=11-50%, 3=51-80% and 4=>81%. The samples were evaluated by two different observers and the mean of the results were used. The Mann-Whitney rank-sum test was used to compare the means of the different IRS scores (SPSS GmbH, Munich, Germany). Spearman's-Rho factor and regression analysis were used to assess any correlation between the steroid receptors in endometrial cancer. The ratios of ER-α/ER-β and ER-β/ER-α were calculated and the means were compared using the Mann-Whitney rank-sum test. Significance was assumed at $p \leq 0.05$.

Results

A total of 245 patients were diagnosed with endometrial cancer. Only twelve (12) cases out of the total were primary endometrial mucinous carcinoma. These cases were analyzed immunohistochemically for expression of hormonal steroid receptors.

Three types of steroid receptor (ER-α, PR-A and PR-B) were frequently expressed in mucinous adenocarcinoma. Staining intensity was in most cases high for ER and PR expression. In contrast, ER-β was weakly expressed in only one of the analyzed cases (1/12).

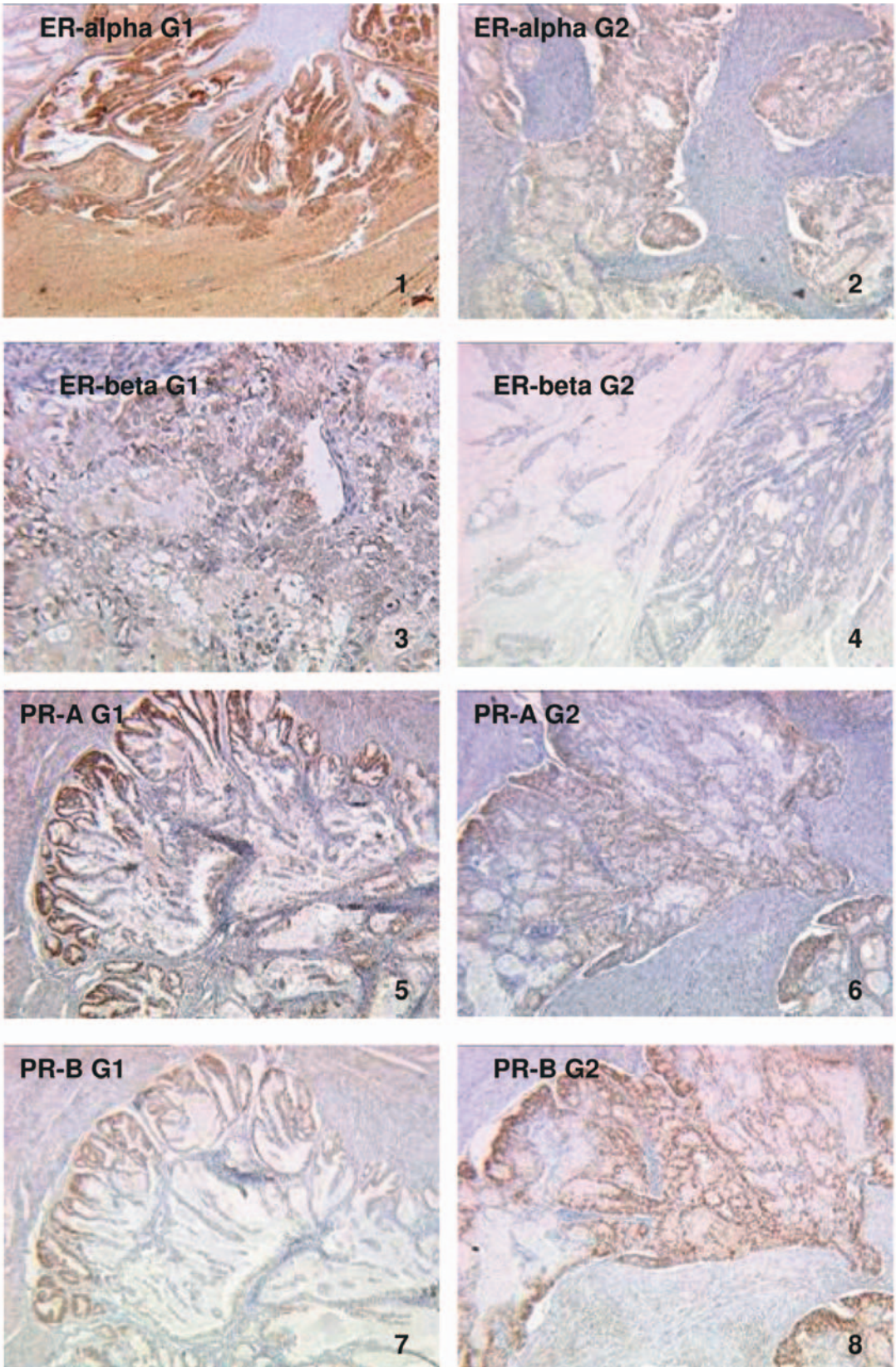
Expression of ER-α. ER-α expression was demonstrated in all tumors and it was localized in the nuclei of epithelial tumor cells. A strong positive expression of ER-α was found in tissue slides of mucinous carcinoma in 10/12 of the cases and weak expression in 2/12 of the cases (Figures 1 and 2). There was a difference in the expression of ER-α in G1 and G2 tumors but these differences were not statistically significant.

Expression of ER-β. Out of 12 cases investigated with mucinous carcinoma of the endometrium, there was only one case identified with positive staining. Weak nuclear expression of ER-β is shown in Figures 3 and 4. Statistical evaluation was inappropriate in this situation.

Expression of PR-A. Positive staining for PR-A was found in all tissue slides of mucinous carcinoma of the endometrium. Strong expression in 5 cases of tumor (5/12) and a weak expression in 7 cases (7/12) of tumor were identified (Figures 6 and 7). PR-A expression was localised in the nucleus of epithelial tumor cells and there was no statistically significant difference between G1 and G2 tumors.

Expression of PR-B. Expression of PR-B as in ER-α and PR-A was demonstrated in all tumor cases. Localisation was found in the nuclei of epithelial tumor cells. The staining intensity was strong in 9/12 of the cases and weak in 3/12 of the cases of mucinous endometrial carcinoma (Figures 7 and 8). In contrast to the other types of ER and PR, there is a statistically significant difference between G1 and G2 tumors (see below).

IRS score. The results of the mean IRS score for the all four receptors are shown in Table II and summarized in Figure 9. Noteworthy is a high mean IRS for PR-B in tumors of moderate differentiation (G2). The analysis of Figure 9 shows that for expression of ER-α, there is no statistically significant difference between well-differentiated (G1) and moderately-differentiated (G2) carcinomas ($p>0.39$). Because ER-β was demonstrated in only one case, a test of statistical significance for this type of receptor was not possible. PR-A expression, like the expression of ER-α, showed no statistically significant difference between well-differentiated (G1) and moderately-differentiated (G2) carcinomas ($p>0.84$). In contrast to the other types of receptors analysed, we identified a statistically significant difference in PR-B expression between well-differentiated (G1) and moderately-differentiated (G2) carcinomas ($p<0.001$).



Figures 1-8. *Immunohistochemical expression of ER-α, ER-β, PR-A and PR-B in the mucinous type of endometrial carcinoma.*

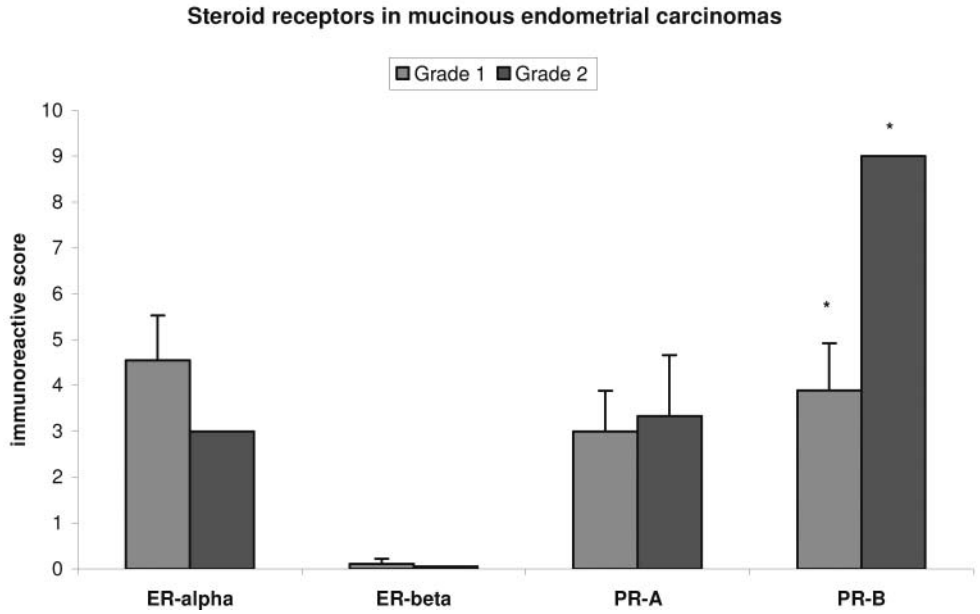


Figure 9. *Steroid receptors in mucinous endometrial carcinomas. The IRS score of PR-B increased significantly (*p<0.001) in mucinous carcinoma.*

Discussion

Normal human endometrium expresses four types of hormone receptors: ER-α, ER-β, PR-A and PR-B (1). Expression of hormone receptors (ER and PR) in both normal and hyperplastic endometrium plays an important role in carcinogenesis of endometrial cancer due to stimulation with estrogen in conditions unopposed by progesterone. The highest expression of ER and PR is demonstrated by the endometrioid subtype of endometrial cancer (17, 18). Our study group demonstrated for the first time expression of four types of different steroid receptors in endometrial tissue (19). Also in this study we demonstrated the expression of four different steroid receptors (ER-α, ER-β, PR-A and PR-B) in mucinous endometrial carcinoma.

For several years it was generally believed that just one single ER existed. In 1996, a second ER (ER-β) with different regulatory functions was cloned (20) However, the discovery of a new nuclear receptor with specificity for estrogens has provided new insights into the estrogen signaling system. The novel receptor, ER-β, has a high (approx. 95%) homology in the DNA-binding domain and 55% homology in the ligand-binding domain with the classic ER (ER-α), which can bind estradiol with a high affinity and bind to a consensus estrogen response element (ERE), stimulating transcription of ER target genes (9). Expression of ER-β was demonstrated in several gynecological tumors including endometrial cancer (21, 22).

It was demonstrated that estradiol may activate transactivation through the classic estrogen receptor (ER-α) but inhibit transcription through ER-β (23). ER-α mRNA showed a stepwise decrease from normal endometrium or grade 1 to grade 3 tumors, suggesting a shift in the ratio of the two ER subtypes during endometrial tumor genesis (21, 22). Similarly, two distinct forms of PR (PR-A and PR-B) exist in the female genital tract and might be differentially regulated in endometrial cancer (23). The down-regulation of PR-B may predict for poorly differentiated endometrial cancers that do not respond to progestin therapy (24).

Progesterone can act on the endometrium through activation of PR-A and PR-B, which can act as transcription factors upon activation by ligand. Transcription of the *hPR* gene is under regulation of two different promoters (10). Transcription initiation from these two promoters results in two distinct mRNAs, which are translated into two distinct proteins: PR-A and PR-B. The PR-A is a truncated form of PR-B, lacking the first 164 amino acid residues at the NH_2 terminus. PR-A and PR-B can be considered as two independent receptors, which display different transcriptional activities. PR-A is not as transcriptionally active as is PR-B and may have a more important function as a cell- and promoter-specific repressor of PR-B. Furthermore, it has been found that a different set of genes is regulated by progesterone in human breast and endometrial cancer cells that express different PR isoforms (25). PR-B appears to have the most substantial growth inhibition effects in human endometrial cancer cells grown *in vitro* (26). PR-B is lost in

poorly differentiated endometrial cancer cell lines such as Hec50 and KLE suggesting that this isoform is important for maintenance of endometrial differentiation (24). Endometrial cancer appears to down-regulate PR-A and PR-B or only PR-A (27, 28). Many studies showed a loss of PR in endometrial cancer (24) (27-31), but reports are conflicting whether this is a consequence of selective down-regulation of PR-A or PR-B, or of both receptors.

The statistical analysis of expression of four steroid receptors in our study showed that only the immunohistochemical expression of PR-B demonstrated a statistically significant decrease in low grade (G1) neoplasms in comparison to intermediate grade (G2) neoplasms ($p \leq 0.001$). In contrast, no significant differences between different histological tumour grades (G1 *vs.* G2) with respect to ER-α and ER-β expression were found.

Interestingly, a statistically significant increase in the expression of PR-B in tumours of intermediate differentiation (G2) compared to well-differentiated tumours (G1) was demonstrated. The higher expression of PR-B in grade 2 tumors suggests a substantial function of progesterone, and thus progesterone receptor, in the malignant transformation of mucinous endometrial cancer. Therefore, PR-B expression might be utilized as a tumor marker to distinguish between well-differentiated (G1) and intermediately-differentiated (G2) mucinous tumors. However, additional studies with more cases of mucinous carcinoma of the endometrium are necessary to evaluate whether these parameters could be used as tumor markers for endometrial cancer.

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