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High immunoexpression of progesterone-induced blocking factor (PIBF) in endometrial cancer

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Summary

Purpose of Investigation: This study aims to investigate PIBF expression levels in normal, hyperplastic, and endometrioid adenocarcinoma paraffin blocks. The purpose of this study is to determine the PIBF expression levels in endometrial cancer cells. *Materials and Methods:* A total of 90 patients were investigated. Thirty of those were diagnosed as having normal endometrial tissue, 30 had endometrial hyperplasia, and 30 had endometrioid adenocarcinoma. The expression of PIBF was assessed using immunohistochemically using paraffin-embedded tissue blocks. *Results:* Tissue sections were compared based on immunostaining with PIBF. The authors detected higher stromal PIBF expression in the endometrioid adenocarcinoma group as compared to the endometrial hyperplasia and normal endometrium groups ($p < 0.001$). The glandular PIBF expression did not differ among the groups ($p > 0.05$). *Conclusions:* Endometrial cancer cells have higher levels of expression of PIBF protein than normal endometrial tissue and endometrial hyperplasia tissue based on immunohistochemistry staining. PIBF immunostaining may be helpful in preoperatively differentiating between atypical endometrial hyperplasia (AEH) and endometrioid adenocarcinoma (EC) in suspicious cases.

Key words: Progesterone-induced blocking factor; PIBF, Endometrioid adenocarcinoma; Immunostaining.

Introduction

Progesterone-induced blocking factor (PIBF) mediates the immune effect of progesterone in pregnant women and is released from maternal lymphocytes [1]. The full-length PIBF mRNA encodes a 90 kD protein with a nuclear localization, as well as other 35, 57, and 60 kDa proteins with cytoplasmic locations, which represent the different forms of PIBF [2]. There are two mechanisms of PIBF action. The first is the inhibition of activated natural killer (NK) cells, and the second is the induction of the TH2-dominant cytokine response in pregnancy. PIBF enables IL-3, IL-4, and IL-10 production and suppresses TH1 cytokines, such as IL-12 and IFN-g, *in vitro* and *in vivo* [3]. The suppression of the cellular immune system provides selective immunological tolerance in the maternal-fetal interface.

It has been hypothesized that cancer cells may use the same mechanism to escape immunity. It has been reported that proliferating cells, such as human trophoblasts, mesenchymal stem cells and malignant tumors have higher PIBF excretion [4, 5]. Recent reports have demonstrated that PIBF is overexpressed in solid tumors of the cervical and breast, as well as in lymphoma and leukemia [2, 6, 7]. There are no data about PIBF expression in endometrial cancer (EC) cells. The purpose of this study is to determine the PIBF expression levels in EC.

Materials and Methods

The study was conducted in Kayseri Education and Research Hospital. The Departments of Gynecology and Pathology contributed to the study. The study was approved by the Local Ethics Committees and was in accordance with the Declaration of Helsinki.

A total of 90 patients with normal endometria, endometrial hyperplasia, and endometrial carcinoma were enrolled in the study. The medical records of the patients were collected retrospectively between January 2015 and January 2017. The normal endometria, endometrial hyperplasia, and endometrioid adenocarcinoma biopsy specimens of the patients were found in the archives of pathology. Ten percent buffered formalin was used to fix the tissues, and the tissues were then embedded in paraffin. One sample block tissue that was embedded in paraffin was taken from each case. These block tissues were cut into four-micron sections. The tissue sections were purified from the paraffin, rehydrated, and revealed with target-retrieval solution. Endogenous peroxidase activity was inhibited via treatment with 3% H2O2, and 10% goat serum was used to block non-specific immunoglobulin binding in the phosphate-buffered saline (PBS). Primary rabbit polyclonal anti-PIBF antibody was used to incubate the sections at a ratio of 1:300. Following this procedure, the slides were washed with PBS. They were then incubated with secondary antibodies and 3,3′-diaminobenzidine (DAB). The sections were counter-stained with hematoxylin. Each specimen was evaluated independently by two pathologists via polarized light microscopy. For analysis, the section that stained tumor cells at the highest rate was used.

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Table 1. — *Distribution of glandular PIBF immunoreactivity among groups.*

	Normal Endometria Glandular (n=30)	Endometrial Hyperplasia Glandular (n=30)	Endometrioid Adenocarcinoma Glandular (n=30)	*p*-value
Positive immunostaining	3(2-3)[a]	3(2-3)[a]	3(2-3)[a]	>0.05

Different superscript numbers indicate statistically significant differences. Because the measurement level of the positive staining variable is ordinal, its values are expressed as medians (25th -75th percentile). Kruskal-Wallis H-tests were performed to compare the groups. Mann-Whitney U-tests were used for the double comparisons. All calculations were made with PASW Statistics 18 software. A probability value of $p < 0.05$ was considered to indicate statistical significance.

Table 2. — *Distribution of stromal PIBF immunoreactivity among groups.*

	Normal Endometria Stromal (n=30)	Endometrial Hyperplasia Stromal (n=30)	Endometrioid Adenocarcinoma Stromal (n=30)	*p*-value
Positive immunostaining	0(0-1)[b]	1(0-2)[c]	3(2-3)[a]	<0.001

Different superscript numbers indicate statistically significant differences. Because the measurement level of the positive staining variable is ordinal, values are expressed as medians (25th -75th percentile). Kruskal-Wallis H-tests were performed to compare the groups. Mann-Whitney U-tests were used for the double comparisons. All calculations were made with PASW Statistics 18 software. A probability value of $p < 0.05$ was considered to indicate statistical significance.

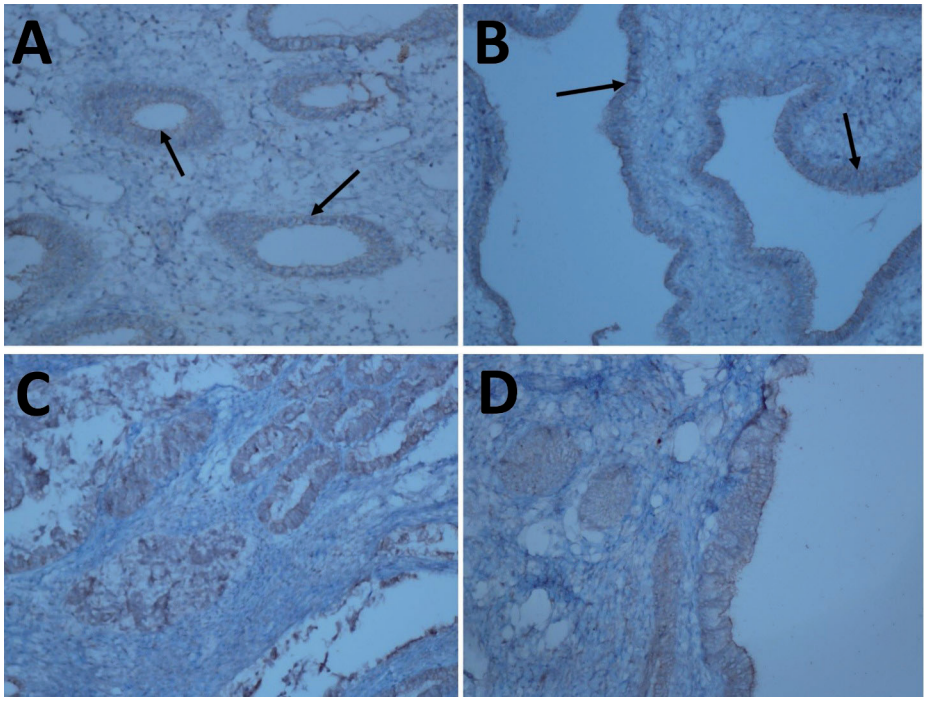

Figure 1. — Immunohistochemical staining of PIBF in normal endometria, endometrial hyperplasia and endometrioid adenocancer.
(A) Intermediate (+2) glandular and negative stromal immunostaining with PIBF in secretory endometrium (×200).
(B) Intermediate (+2) glandular and negative stromal immunostaining with PIBF in simple edometrial hyperplasia (×200).
(C) Diffuse strong (+3) immunostaining with PIBF in endometrial gland and stroma in endometrioid adenocarcinoma (×200).
(D) Diffuse strong (+3) immunostaining with PIBF in endometrial gland and stroma in endometrioid adenocarcinoma (×200).

The quick score for each sample was used to measure PIBF expression levels, and the general staining intensities were used in the calculations (0: negative; 1: weak staining; 2: intermediate staining; 3: strong staining). The percentages of positively stained tumor cells were also made use of in calculations (1: 1-20%; 2: 20-50; 3: ≥ 50%). The preparations were photographed with a Nicon DS-Fi2. Positive (decidua) and negative immunohistochemical controls were routinely used. PIBF staining was observed in the membranes and/or cytoplasm of normal/cancer gland epithelial cells and stromal immune cells.

Because the measurement level of the positive staining variable is ordinal, its values are expressed as medians (25^{th} percentile-75^{th} percentile). Kruskal-Wallis H-tests were performed to determine differences between the groups. Mann-Whitney U tests were used for double comparisons. All calculations were made with PASW Statistics 18. A $p < 0.05$ probability value was considered to indicate statistical significance.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (decision number: 2016/405) and with the 1064 Helsinki Declaration and its later amendments or comparable ethical standards. This article did not contain any studies on animals. Informed consent was obtained from all individual participants included in the study.

Results

A total of 90 patients with normal endometria (n=30), endometrial hyperplasia (n=30), and endometrioid adenocarcinoma (n=30) were enrolled in the study. A comparison of glandular and stromal PIBF immunostaining is provided in Tables 1 and 2.

When the tissue sections were compared based on immunostaining with PIBF, the authors detected high stromal PIBF expression in the EC group as compared to the endometrial hyperplasia and normal endometrium groups ($p < 0.001$). Glandular PIBF expression did not differ among

the groups ($p > 0.05$). The immunohistochemical staining with PIBF is illustrated in Figure 1.

Discussion

EC is the most common gynecologic malignancy worldwide. It was estimated that in 2013, there were 634,437 women living in United States with EC. In 2016, 60,050 new EC cases were identified, and it is estimated that 10,470 women died of this disease [8].

The aim of the present study was to determine PIBF expression levels in EC cells. The immunostaining of PIBF was elevated in the EC group. High levels of immunostaining were detected in the stroma of the EC specimens, which is related with decreased local anti-tumor immune response.

The main effect of progesterone on the endometrium is to antagonize cell proliferation caused by estrogen. Progesterone provides this anti-tumoral effect by binding to nuclear receptors and activating the transcription of various genes [9]. On the other hand, progesterone shows an immune-modulator effect that involves lymphocyte-derived protein PIBF. This immune-modulator effect has been shown by researchers. These researchers have reported that progesterone treatment causes PIBF secretion from the peripheral blood lymphocytes in pregnant women. There is evidence that PIBF may induce a shift from Th1 to Th2 cytokines [10]. The suppression of the cellular immune system provides selective immunological tolerance in the maternal-fetal interface. Recent reports have demonstrated the overexpression of PIBF in solid tumors of the cervix and breast, as well as in lymphoma, leukemia, and astrocytoma [2, 4, 5, 11]. These data demonstrate that tumor cells can secrete PIBF to escape the immune system.

In the current study, the authors showed a higher level of expression of PIBF protein in the EC group relative to the normal endometrial tissue and endometrial hyperplasia groups using immunohistochemistry. The present results can be explained as immunoediting, specifically equilibration (immunosurveillance). The immune system aims to inhibit cancer cells through a combination of processes called immunoediting. These processes involve elimination, equilibration, and escape steps [12]. In the equilibration step, tumor cells secrete certain chemokines and cytokines to suppress the immune response and exchange immune cells in the tumor microenvironment due to the release of the cytokines. In this way, the active Th1, cytotoxic T-cells, and NK cells in the microenvironment can be replaced by increased numbers of inhibitory Th2, myeloid-depressant cells, thus creating a weakened immune response to the tumor. PIBF seems to be one of these secreted proteins [12, 13]. Cytotoxic T-cells and NK cells are the main types of cells that combat tumor cells. There are few studies focusing on NK cells in EC patients. Fergusan *et al.* reported that NK cells were nearly absent from endometrial tumors [14]. Similarly, Witkiewicz *et al.* reported that intratumoral NK cells were immunohistochemically absent from EC patients [15]. These findings support the results of the present study.

The result of the current study can be interpreted in several ways: (1) in some cases, it is difficult to differentiate atypical complex endometrial hyperplasia from well-differential EC histopathology. Atypical complex endometrial hyperplasia is a well-known precursor to EC, and multiple reports indicate that women with a preoperative diagnosis of atypical complex endometrial hyperplasia are frequently found to have EC after hysterectomy [16, 17]. PIBF immunostaining may be helpful in these cases preoperatively. (2) The depth of myometrial invasion is an important prognostic feature. The myometrial invasion ratio has a direct influence on treatment [18, 19]. If the effect of PIBF on the tumor microenvironment is considered, the level of PIBF expression in EC cells may be a factor in myometrial invasion. Based on this assumption, the level of PIBF expression may be a prognostic marker, but further studies are needed. (3) In metastatic and relapsed EC, chemotherapy and hormonal therapy are the only available options for treatment, and survival remains poor [20]. In recent years, cancer immunotherapy has made significant progress, but it has not yet been integrated with other therapies. Currently, immunotherapy may represent an attractive opportunity for EC treatment, especially for advanced or recurrent disease, if no other effective options are available. Demonstrating the presence of PIBF expression in EC cells may open up new horizons for EC immunotherapy.

Conclusion

In conclusion, the data from the present study indicate that EC cells have higher levels of expression of PIBF protein relative to normal endometrial tissue and endometrial hyperplasia tissue based on immunohistochemistry. However, in order to understanding the clinical importance of this finding, the long-term consequences of the elevated expression of PIBF and its underlying mechanisms must be investigated in large-scale studies.

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