Technology in Cancer Research & Treatment
Volume 17: 1-5

DOI: 10.1177/1533033818783911
journals.sagepub.com/home/tct
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High Immune Expression of Progesterone-Induced Blocking Factor in Epithelial Ovarian Cancer

Yusuf Madendag, MD[1], Erdem Sahin, MD[1], Ilknur Col Madendag, MD[1], Mefkure Eraslan Sahin, MD[2], Gokhan Acmaz, MD[1], and Hatice Karaman, MD[3]

Abstract

Background: Progesterone-induced blocking factor, which is released from maternal lymphocytes during pregnancy mediates the immune effect of progesterone. According to new reports, it is suggested that proliferating cells, such as human trophoblasts, mesenchymal stem cells, and malignant tumors, can excrete progesterone-induced blocking factor at high ratio to escape from maternal immunity. It is shown in recent studies that progesterone-induced blocking factor is overexpressed in many malignant tumors such as breast, cervical, lymphoma, and leukemia. There are no data about progesterone-induced blocking factor expression in ovarian cancer cells. Hence, it is aimed to determine the progesterone-induced blocking factor expression levels in epithelial ovarian cancer. **Methods**: The study which was a retrospective cross-sectional study was conducted in a University Hospital. Twenty tissue specimens of patients with epithelial ovarian cancer and 20 tissue specimens of patients with healthy ovary were included in the study. Primary rabbit polyclonal anti- progesterone-induced blocking factor antibody was used to incubate the sections at a ratio of 1:300. **Results:** When the tissue sections were compared based on immunostaining with progesterone-induced blocking factor, we detected high stromal progesterone-induced blocking factor expression in the epithelial ovarian cancer group as check against to the normal ovarian group ($P = .007$). Similarly, we found high glandular progesterone-induced blocking factor expression in the epithelial ovarian cancer group as check against to the normal ovarian group ($P < .001$). **Conclusion:** Proving the existence of progesterone-induced blocking factor expression in epithelial ovarian cancer cells may lead new visions or new studies for epithelial ovarian cancer immunotherapy. As a result, epithelial ovarian cancer cells have greater levels of expression of progesterone-induced blocking factor protein than normal ovarian tissue according to immunohistochemistry. Further research is needed to understand the clinical importance of this finding, to learn outcomes of high levels of progesterone-induced blocking factor, and to investigate its underlying mechanisms.

Keywords

epithelial ovarian cancer, progesterone-induced blocking factor, PIBF, immunostaining, selective immunological tolerance

Abbreviations

EOC, epithelial ovarian cancer; IL, interleukin; PIBF, progesterone-induced blocking factor; Tregs, T regulatory cells

Received: December 6, 2017; Revised: February 18, 2018; Accepted: May 24, 2018.

Introduction

Progesterone-induced blocking factor (PIBF) that is released from maternal lymphocytes during pregnancy mediates the immune effect of progesterone.[1] According to recent studies, 2 mechanisms are suggested about PIBF action. One of them is the inhibition of activated natural killer cells and the other is the induction of the T-helper (TH) 2-dominant cytokine response after fecundation. Progesterone-induced blocking factor makes

[1] Department of Obstetrics and Gynecology, Health Sciences University Kayseri Education and Research Hospital, Kayseri, Turkey
[2] Department of Obstetrics and Gynecology, Erciyes University Faculty of Medicine, Kayseri, Turkey
[3] Department of Pathology Clinic, Health Sciences University Kayseri Education and Research Hospital, Kayseri, Turkey

Corresponding Author:
Yusuf Madendag, MD, Kayseri Egitim ve Arastirma Hastanesi, MKP Street/ Kocasinan, Kayseri 38010, Turkey.
Email: yusufmadendag@gmail.com

possible to produce interleukin (IL)-10, IL-4, and IL-3 and depresses TH1 cytokines, like IL-12 and interferon-g, both *in vivo* and *in vitro*.[2] It is important to depress the cellular immune system. If selective immunological tolerance is not provided in the maternal–fetal interface, fetus can be rejected by maternal immune system. The full-length PIBF messenger RNA encodes a 90-kD protein with a nuclear localization as well as other 35-, 57-, and 60-kDa proteins with cytoplasmic locations, which symbolize the varied shapes of PIBF.[3] Progesterone-induced blocking factor has alternatively spliced isoforms.[4] The full-length PIBF may be connected to disturbed cell cycle, and its isoform may be related to local immunosuppression.[4]

Immunosuppression is a major phenomenon that helps maintain pregnancy. Several host factors contribute to this phenomenon during pregnancy. There is a plenty of growing evidence implicating the role of these host factors in cancer and chronic viral infections. For example, T regulatory cells (Tregs) are activated during pregnancy to suppress inflammation.[1,2] Now, it is evident that Tregs are also induced by tumor cells to evade inflammatory responses by immune cells. Removal of Tregs improves immune responses and results in the elimination of tumor cells.[5-7] Similarly, PIBF is secreted by lymphocytes after implantation, and it plays an important role in mediating immunosuppression during pregnancy.

According to new reports, it is suggested that proliferating cells, such as human trophoblasts, mesenchymal stem cells, and malignant tumors, can excrete PIBF at high ratio to escape from maternal immunity.[8,9] It is shown in recent studies that PIBF is overexpressed in many malignant tumors such as breast, cervical, lymphoma, and leukemia.[5,6] Balassa *et al* mention that PIBF is strongly upregulated in ovarian tumor cells and that there are no published full articles showing the role of PIBF in ovarian cancer.[7] This makes the current study very important, as it would help us better understand the role of PIBF in cancer, and possibly, PIBF will be used as a target for cancer immunotherapy in future.

There are no sufficient data about PIBF expression in ovarian cancer cells. Hence, we aimed to determine the PIBF expression levels in epithelial ovarian cancer (EOC).

Materials and Methods

The study which was a retrospective cross-sectional study was conducted in the Health Sciences University Kayseri Education and Research Hospital by the Departments of Obstetrics and Gynecology and Pathology. The study was approved by the local ethics committees and was done according to the Declaration of Helsinki.

Twenty patients with epithelial ovarian carcinoma and 20 patients with healthy ovaries were included in the study. The medical archives of the patients were evaluated retrospectively between March 2015 and September 2017. Demographic data such as age, chronic diseases, and drug using history were saved. Patients with polycystic ovarian syndrome, chronic liver and kidney diseases, patients with other cancers, and patients taking chemotherapy or taking oral contraceptive pill or hormone replacement therapy were excluded from this study. The normal ovarian tissue and epithelial ovarian carcinoma tissue specimens of the patients were found in the archives of pathology. Buffered formalin 10% (Sigma-Aldrich, St. Louis, Missouri) was used to fix the tissues, and then the tissues were embedded in paraffin (Sigma-Aldrich). One sample block tissue embedded in paraffin was taken from each case. Each block tissue was cut into 4-micron sections. The tissue sections were purified from the paraffin, rehydrated, and revealed with target-retrieval solution. Endogenous peroxidase activity was inhibited by treatment with 3% H_2O_2 (Sigma-Aldrich), and 10% goat serum (Sigma-Aldrich) was used to block nonspecific immunoglobulin binding in the phosphate-buffered saline (Sigma-Aldrich). Primary rabbit polyclonal anti-PIBF antibody[7] (Sigma-Aldrich, AE030801) was used to incubate the sections at a ratio of 1:300. Following this procedure, the slides were washed with phosphate-buffered saline (Sigma-Aldrich). They were then incubated with secondary antibodies (Sigma-Aldrich) and 3,3′-diaminobenzidine (Sigma-Aldrich). The sections were counterstained with hematoxylin and eosin (Sigma-Aldrich). Each specimen was evaluated by experienced pathologist via polarized light microscopy (Nikon Eclipse Ni-E; Nikon, Japan). For analysis, the section that stained tumor cells at the highest rate was used. 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+: mild dyeing; 2+: moderate dyeing; 3+: severe dyeing). The percentages of positively stained tumor cells were also used in calculations (1+: 1%-20%; 2+: 21%-50%; 3+:≥51%).The preparations were photographed with the camera (Nikon DS-Fi2; Nikon).

Statistical Analysis

To test the normality assumption of the data, Shapiro-Wilk was used. Variance homogeneity assumption was tested with Levene test. Values are expressed as mean (standard deviation). Parametric comparisons were made using a *t* test. Since the measurement level of positive painting variable was ordinal, values were expressed as median (25th percentile-75th percentile). Mann-Whitney *U* test was applied for the collation of distinctions between the groups. Overall calculations were performed with PASW Statistics 18 software (Predictive Analytics SoftWare, Statistics for Windows, Version 18.0., Chicago, Illinois) $P < .05$ probability value was considered as statistically significant.

Results

A total of 40 tissue specimens of patients with normal ovaries ($n = 20$), and epithelial ovarian carcinoma ($n = 20$) were evaluated in the study. The mean age was similar between the groups. The mean age was 50.7 (7.5) in the normal ovary group while 49.5 (10.3) in the cancer group ($P = .556$). Distribution of PIBF immunoreactivity according to staining power and specimen number among the groups is shown in Table 1. A

Table 1. Distribution of PIBF Immunoreactivity According to Staining Power and Specimen Number Among Groups.

Positive Staining (+)[a]	Normal Ovarian Gland, n = 20	Epithelial Ovarian Carcinoma Gland, n = 20	Normal Ovarian Stroma, n = 20	Epithelial Ovarian Carcinoma Stroma, n = 20
0+	20	1	8	2
1+	0	2	8	7
2+	0	15	4	7
3+	0	2	0	4

Abbreviations: PIBF, progesterone-induced blocking factor.
[a]The general staining intensities were used in the calculations (0+: negative; 1+: mild dyeing; 2+: moderate dyeing; 3+: severe dyeing).

Table 2. Distribution of PIBF Immunoreactivity Between Groups.[a]

	Normal Ovarian Gland	Epithelial Ovarian Carcinoma Gland	*P* Value
Positive immunostaining	+0 (0-0)	+2 (2-2)	<.001
	Normal Ovarian Stroma	Epithelial Ovarian carcinoma Stroma	*P* Value
Positive immunostaining	+1 (0-1)	+2 (1-2)	.007

[a]Values were expressed as median and percentiles (25-75).

crosscheck of glandular and stromal PIBF immunostaining is shown in Table 2. All values were expressed as median and percentiles (25-75). When the tissue sections were compared based on immunostaining with PIBF, we detected high stromal PIBF expression in the EOC group as check against to the normal ovary group ($P = .007$). Similarly, we found high glandular PIBF expression in the EOC group as check against to the normal ovary group ($P < .001$). The immunohistochemical dyeing with PIBF is illustrated in Figure 1.

Discussion

Malignant ovarian neoplasm, which is the most common cause of gynecological cancer death in the United States, is the second most common gynecologic cancer. Epithelial ovarian cancer was seen in the United States with an approximative 21 000 new patients and 14 000 decease in 2015.[10]

The aim of the present study was to determine PIBF expression levels in EOC cells. The immunostaining of PIBF in both ovarian gland and stroma was found at high levels in the EOC group. High levels of immunostaining in the EOC specimens can be related to decreased local antitumor immune response.

There are a few studies to evaluate PIBF expression in tumor cells. Recent reports have demonstrated the overexpression of

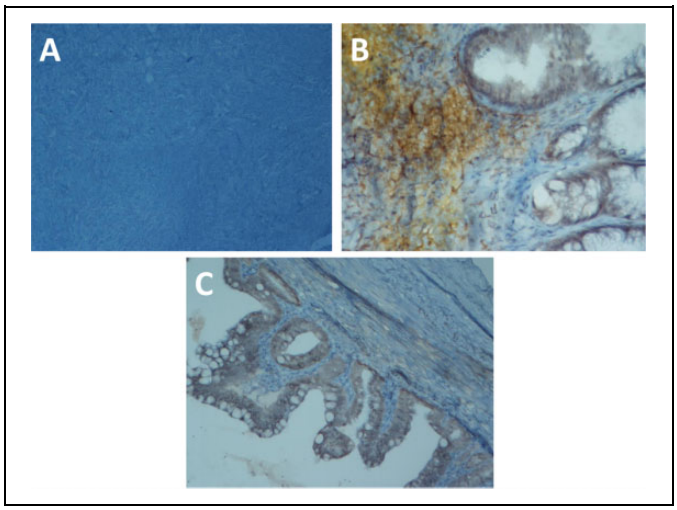

Figure 1. Immunohistochemical staining of progesterone-induced blocking factor (PIBF) in normal ovary and epithelial ovarian cancer. (A) Negative (−) stromal immunostaining with PIBF in normal ovary (×50); (B) diffuse strong (+3) immunostaining with PIBF in ovarian gland and stroma in epithelial ovarian cancer (×400); (C) diffuse strong (+3) immunostaining with PIBF in ovarian gland and stroma in epithelial ovarian cancer (×200). Cells were labeled with polyclonal anti-PIBF antibody (brown). Nuclei were counterstained with hematoxylin and eosin (blue).

PIBF in solid tumors of the cervix and breast as well as in lymphoma, leukemia, and astrocytoma.[8,9,11]

These data demonstrate that tumor cells can secrete PIBF to escape the immune system. The act of the immune reply in ovarian cancer is fine reported like other solid tumors.[12-14] A favorable relationship between the amount of tumor infiltrating lymphocytes (TILs) and overall survival is known,[12] a phenomenon that is supported by various studies.[13,14] Especially, the existence of CD8T cells is connected positively with survival.[13]

In the present study, we found at high levels of expression of PIBF protein in the EOC group relative to the normal ovarian group using immunohistochemistry. The results of this study can be explained as immunoediting, specifically equilibration (immunosurveillance). The immune system aims to inhibit cancer cells via a combination of processes called immunoediting. These processes involve elimination, equilibration, and escape steps.[15] Actually, the immune system frequently preserves equilibrium with tumor cells that can continue for long duration and prohibit any clinical sequela. At this stage, the maximum immunogenic cells are continuously extracted, a course that develops and clarifies the residual tumor population till eventually a group of tumor cells escapes from immunologic control and expands uncontrolled.[16] The getaway from immunological check can happen by some mechanisms such as loss of tumor antigen expression,[17] loss of major histocompatibility complex (MHC -I) expression,[18] or failure of the intracellular antigen promotion way.[19]

Although the immune cells such as natural killer and CD8T cells can identify and exterminate neoplastic cells, tumors

frequently grow in sight uncontrolled in people with normal immune response. This event is owing to some effects such as weak immunogenicity of some neoplasms, suppression of immunity, and editing of immunity.[20-22] Tumors can escape from immunosurveillance by producing a regional or systemic immunosuppressive surrounding. Thus, tumor cells can manufacture several proteins such as vascular endothelial growth factor,[23] transforming growth factor β,[24] and indoleamine 2,3-dioxygenase.[25] Progesterone-induced blocking factor seems to be one of these secreted proteins.[15,26] When the effect of PIBF on the tumor microenvironment is considered, the level of PIBF expression in EOC cells may be a factor for invasion and poor outcome. According to this hypothesis, the level of PIBF expression may be a prognostic marker, but further studies are needed.

The greater parts of patients with EOC still have been diagnosed with advanced disease. While many of them will reply first to chemotherapy, some of them will relapse and die of their illness. Severe therapies like arresting or activating special intracellular signaling ways have disappointed. New investigations have specified possible treatments using the immune system to specify and devastate neoplastic cells which formerly escaped immunosurveillance mechanisms. Proving the existence of PIBF expression in EOC cells may lead new visions or new studies for EOC immunotherapy.

Conclusion

As a result, EOC cells have greater levels of expression of PIBF protein than normal ovarian tissue according to immunohistochemistry. Further research should be needed to understand the clinical importance of this finding to learn outcomes of high levels of PIBF and investigate its underlying mechanisms. This polyclonal antibody used in the study may detect full length form or the isoform of PIBF. It is not clear. However, this may open a horizon for new studies.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Yusuf Madendag http://orcid.org/0000-0002-7622-2991

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