High Immune Expression of Progesterone-Induced Blocking Factor in Epithelial Ovarian Cancer

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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.
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. 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. Progesterone-induced blocking factor has alternatively spliced isoforms. The full-length PIBF may be connected to disturbed cell cycle, and its isoform may be related to local immunosuppression.

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. 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. 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. It is shown in recent studies that PIBF is overexpressed in many malignant tumors such as breast, cervical, lymphoma, and leukemia. 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. 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.

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 and 49.5 (10.3) in the cancer group (P < .05). Distribution of PIBF immunoreactivity according to staining power and specimen number among the groups is shown in Table 1. A
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 approximate 21,000 new patients and 14,000 deaths 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 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 CD8+ T 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 CD8+ T cells can identify and exterminate neoplastic cells, tumors

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

|                | Normal Ovarian Gland, n = 20 | Epithelial Ovarian Carcinoma Gland, n = 20 |
|----------------|-----------------------------|------------------------------------------|
| Positive Staining (+)⁵ | 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.

*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.⁶

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

⁶Values were expressed as median and percentiles (25-75).

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

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).
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.

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References
1. Szekeres-Bartho J, Polgar B. PIBF: the double edged sword. Pregnancy and tumor. Am J Reprod Immunol. 2010;64(2):77-86.
2. Szekeres-Bartho J, Faust Z, Varga P, Szereday L, Kelemen K. The immunological pregnancy protective effect of progesterone is manifested via controlling cytokine production. Am J Reprod Immunol. 1996;35(4):348-351.
3. Lachmann M, Gelbmann D, Kálmán E, et al. PIBF (progesterone-induced blocking factor) is overexpressed in highly proliferating cells and associated with the centosome. Int J Cancer. 2014;112(1):51-60.
4. Bogdan A, Poglar B, Szekeres-Bartho J. Progesterone induced blocking factor isoforms in normal and failed murine pregnancies. Am J Reprod Immunol. 2014;71(2):131-136
5. Srivastava MD, Thomas A, Srivastava BI, Check JH. Expression and modulation of progesterone-induced blocking factor (PIBF) and innate immune factors in human leukemia cell lines by progesterone and mifepristone. Leuk Lymphoma. 2007;48(8):1610-1617.
6. Halasz M, Polgar B, Berta G, Czimbalek L, Szekeres-Bartho J. Progesterone-induced blocking factor differentially regulates trophoblast and tumor invasion by altering matrix metalloproteinase activity. Cell Mol Life Sci. 2013;70(23):4617-4630.
7. Balassa T, Berta G, Jakab L, Bohonyi N, Szekeres-Bartho J. The effect of the progesterone-induced blocking factor (PIBF) on E-cadherin expression, cell motility, and invasion of primary tumour cell lines. J Reprod Immunol. 2018;125:8-15.
8. Anderle C, Hammer A, Polgar B, et al. Human trophoblast cells express the immunomodulator progesterone-induced blocking factor. J Reprod Immunol. 2008;79(1):26-36.
9. Kyurkchiev DS, Ivanova-Todorova E, Kyurkchiev SD. Effect of progesterone on human mesenchymal stem cells. Vitam Horm. 2011;87:217-327.
10. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2015;65(1):5-29.
11. González-Arenas A, Valadez-Cosmes P, Jiménez-Arellano C, López-Sánchez M, Camacho-Arroyo I. Progesterone-induced blocking factor is hormonally regulated in human astrocytoma cells, and increases their growth through the IL-4R/JAK1/STAT6 pathway. J Steroid Biochem Mol Biol. 2014;144(pt b):463-470.
12. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348(3):203-213.
13. Sato E, Olson SH, Ahn J, et al. Intraepithelial CD8 + tumor-infiltrating lymphocytes and a high CD8 +/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci USA. 2005;102(51):18538-18543.
14. Wouters MC, Komdeur FL, Workel HH, et al. Treatment regimens, surgical outcome, and t-cell differentiation influence prognostic benefit of tumor-infiltrating lymphocytes in high-grade serous ovarian cancer. Clin Cancer Res. 2015;22(3):714-724.
15. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004;21(2):137-148.
16. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity’s roles in cancer suppression and promotion. Science. 2011;331(6024):1565-1570.
17. Jensen SM, Twitty CG, Maston LD, et al. Increased frequency of suppressive regulatory T cells and T cell-mediated antigen loss results in murine melanoma recurrence. J Immunol. 2012;189(2):767-776.
18. Garrido F, Ruiz-Cabello F, Cabrera T, et al. Implications for immunosurveillance of altered HLA class I phenotypes in human tumours. Immunol. 1997;18(2):89-95.
19. Dazzi F, D’Andrea E, Biasi G, et al. Failure of B cells of chronic lymphocytic leukemia in presenting soluble and alloantigens. *Clin Immunol Immunopathol*. 1995;75(1):26-32.

20. Koido S, Homma S, Takahara A, et al. Current immunotherapeutic approaches in pancreatic cancer. *Clin Dev Immunol*. 2011;2011:267539

21. Inaba T, Ino K, Kajiyama H, et al. Role of the immunosuppressive enzyme indoleamine 2.3-dioxygenase in the progression of ovarian carcinoma. *Gynecol Oncol*. 2009;115(2):185-192.

22. Aguirre-Ghiso JA. Models, mechanisms, and clinical evidence for cancer dormancy. *Nat Rev Cancer*. 2007;7(11):834-846.

23. Gabrilovich D, Ishida T, Oyama T, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. *Blood*. 1998;92(11):4150-4166.

24. Yoshimura A, Muto G. TGF-beta function in immune suppression. *Curr Top Microbiol Immunol*. 2011;350:127-147.

25. Lob S, Konigsrainer A, Rammensee HG, Opelz G, Terness P. Inhibitors of indoleamine-2.3-dioxygenase for cancer therapy: can we see the wood for the trees? *Nat Rev Cancer*. 2009;9(6):445-452.

26. Quezada SA, Peggs KS, Simpson TR, Allison JP. Shifting the equilibrium in cancer immunoediting: from tumor tolerance to eradication. *Immunol Rev*. 2011;241(1):104-118