New Marker for Blood Vessels in Human Ovarian and Endometrial Cancers

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ABSTRACT

Angiogenesis plays a critical role in tumor biology and may someday be a target for novel therapeutic interventions. To date, however, relatively few markers have been identified that can specifically distinguish between microvessels in benign versus malignant lesions. Here we report that the cationic heme-protein eosinophil peroxidase (EPO) was localized by in situ immunohistochemistry on the vascular endothelial cells and/or connective tissue stroma in 16 of 16 cases of human endometrial carcinoma and in 12 of 15 cases of ovarian carcinoma. Similar deposits of EPO were not detected in normal endometrial tissues or ovaries from five healthy subjects, in adjacent uninvolved tissues from four tumor-bearing subjects, or in any normal organs from five other subjects. These findings imply that eosinophil degranulation is a significant and previously unappreciated component of the interaction between ovarian and endometrial cancers and the host. Moreover, the abundant and highly specific nature of the EPO deposition near and within the microvessels of these cancers suggests that eosinophil degranulation is a new marker for tumor blood vessels that potentially could be exploited to treat these important types of cancers that currently lack highly effective therapies.

INTRODUCTION

A novel approach to the treatment of solid tumors would be to target toxic drugs to the microvasculature of the tumor rather than to the tumor cells themselves (1). As summarized by Thorpe (2), such an approach could have several advantages. For example, tumor vascular endothelial cells are directly accessible to drugs in the blood, whereas tumor cells are mostly inaccessible, especially to large drugs such as monoclonal antibodies. In addition, such an approach might be applicable to a wide variety of solid tumors because all of them require a blood supply for their survival and growth. Furthermore, the endothelial cells within tumors are nonmalignant, thereby making it unlikely that drug-resistant mutants would develop. Finally, this approach has inherent amplification properties because many tumor cells rely on each microvessel for nutrients and oxygenation. Therefore, even partial damage to the blood vessels within tumors might result in relatively massive destruction of tumor cells.

The ideal target molecule for such a therapeutic approach would be expressed at high density on the surface of vascular endothelial cells in solid cancers and be absent on endothelial cells in normal tissues. To date, several promising candidate molecules with these properties have been identified in humans, including tissue factor in breast cancer (3, 4), endoglin (5), endosialin (6), an endoglin-like molecule (7), a fibronectin isoform (8), receptors (9) for vascular endothelial cell growth factor, and vascular endothelial growth factor itself (10).

Using a murine monoclonal antibody called EOS, we have been studying the deposition of EPO,3 a markedly cationic heme protein, in human lymphomas (11, 12) and in breast cancers (13). Normally, EPO is exclusively confined to the intracellular granules within intact eosinophils. In a variety of pathological conditions, however, eosinophils release their granule contents, which then bind avidly to the anionic surfaces of adjacent cells. Here we describe immunohistochemical evidence of a similar degranulation phenomenon occurring in the microvasculature of human ovarian and endometrial cancers.

MATERIALS AND METHODS

Reagents. Immunohistochemical staining of cryostat sections of human ovarian and endometrial cancers was carefully performed, controlled, and evaluated using the same procedures and monoclonal antibodies that we recently used to study human breast cancers (13). In brief, an IgG 2a murine monoclonal antibody called SF25.5 that binds to human EPO was derived as described elsewhere (11). This antibody is highly specific for EPO and does not bind to any other cell types, to myeloperoxidase, or to normal endometrium or ovaries when radiolabeled and administered to human subjects (12). Also included in this study was a mouse monoclonal IgG-negative control antibody (Dako Corp., Carpinteria, CA) diluted to the same working concentration as the anti-EPO antibody (5 μg/ml PBS with 1% FCS). To visualize the architecture of the vascular endothelium, separate serial sections of the tumors were also stained with anti-CD34 (a vascular endothelial marker) monoclonal antibody (Becton Dickinson, San Jose, CA), using the same conditions as were used with the other antibodies.

Tissue Specimens. In this study, we examined all of the cryopreserved, primary ovarian and endometrial carcinomas (listed in Table 1) that were available in the University of California, Irvine, Cancer Center Human Tumor Bank (Orange, CA). All of these cases were well-characterized by routine light

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3 The abbreviation used is: EPO, eosinophil peroxidase.


**Table 1 Immunohistochemical studies of human endometrial and ovarian cancers with EPO antibody**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>Degree of EPO deposition</th>
<th>Location of EPO deposits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Extensive</td>
<td>Present</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial adenocarcinoma</td>
<td>15</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Endometroid carcinoma</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mucinous carcinoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serous carcinoma</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Serous papillary adenocarcinoma</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**RESULTS**

The results of our immunohistochemical staining with the EOS monoclonal antibody are summarized in Table 1. A striking and consistent finding was the highly specific and obvious localization of finely granular EPO deposits in a linear pattern, apparently along and within the microvasculature within the tumors (Fig. 1, a-d). The distribution of staining was luminal and abluminal and was most intense at the edges of the tumors. There was no apparent localization to regions of necrosis. A similar linear distribution of staining was seen in parallel sections of the same tissues that were stained with the CD34 monoclonal antibody (Fig. 1e), but the CD34 staining was homogeneous and not granular or abluminal. Adjacent uninvolved normal tissue was available from four of our tumor-bearing patients, and there was no apparent staining for EPO in these specimens. There was also no tissue staining when a negative control monoclonal antibody was substituted for the EOS monoclonal antibody (Fig. 1f).

At the stromal interface between tumor and peripheral connective tissue, there were extensive fibrillar deposits of EPO (Fig. 2a) that were remarkably similar to those that we have described previously in Hodgkin's disease and in some human breast cancers (11, 13), and faint granular staining frequently extended into the adjacent tumor cells. An identical wavy pattern of staining in stroma at the tumor edges was also seen when the tissues were stained with CD34 antibody (Fig. 2b), but the adjacent tumor cells had no granular staining. Normal endometrial and ovarian tissues obtained from autopsies of five nontumor-bearing subjects were devoid of similar EPO deposits when stained identically with the EOS antibody, as were five specimens of normal heart, liver, spleen, lung, kidney, skin, muscle, and stomach tissues.

In routinely stained tissue sections, intact eosinophils could be identified only in about one-half of our study cases and were easily overlooked unless diligently sought. Eosinophils in our study cases were most evident at the edges of the tumor, in the connective tissue stroma adjacent to blood vessels (Fig. 3a), and extending into the fibrovascular cores between nests of tumor cells (Fig. 3b). This distribution closely paralleled the distribution seen with immunohistochemical staining for EPO. Cytochemical staining of tumor sections for EPO also confirmed the specific presence of EPO and eosinophils within the same locations of the tumors (Fig. 3c).

**DISCUSSION**

Our in situ immunohistochemical and cytochemical study has demonstrated that eosinophil degranulation and deposition of EPO occur within and near the blood vessels and connective tissues of a significant proportion of human ovarian and endometrial cancers. This deposition of EPO occurred in a distribu-

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**Immunohistochemistry and Cytochemistry.** The tumor and control tissues were frozen in Histo-Prep embedding medium (Fisher Chemical, Fair Lawn, NJ), sectioned at 2–6 μm thickness on a microtome, air dried on a glass slide, and fixed in acetone for 1 min. The cryostat sections were then submitted for two separate assays. The first assay was a cytochemical procedure for directly and specifically detecting EPO (11) using a chromogenic substrate for EPO (aminocoumarin carbazole) and 0.01 M potassium cyanide (which inhibits myeloperoxidase but not EPO). For optimal sensitivity, this procedure needed to be performed within 24 h after tissue sectioning. These slides were counterstained with hematoxylin prior to mounting.

The immunohistochemical procedure for detecting EPO and CD34 in tissue sections used an avidin-biotin redox detection procedure (Vector Laboratories, Burlingame, CA) to detect bound primary antibody and was followed by a nuclear fast red counterstain. This detection method was selected to avoid interference by endogenous peroxidase activity that was present within the tissues.

The negative controls for each case consisted of tissue sections that were incubated without the primary antibody or with the irrelevant mouse monoclonal antibody at the same concentration as SF25.5. A positive control was also included and consisted of a cytospin preparation of purified human eosinophils incubated with the SF25.5 antibody.

Each stained slide was reviewed by two observers. For purposes of tabulation, EPO activity in each slide was classified as extensive (present in every ×250 microscopic field), present (detectable in the tissue but not present in every field), or absent (none visible). In addition, the microanatomic location (stromal, peripheral, within tumors, within blood vessels, and others) of the EPO was noted in each case.

**RESULTS**

The results of our immunohistochemical staining with the EOS monoclonal antibody are summarized in Table 1. A striking and consistent finding was the highly specific and obvious localization of finely granular EPO deposits in a linear pattern, apparently along and within the microvasculature within the tumors (Fig. 1, a-d). The distribution of staining was luminal and abluminal and was most intense at the edges of the tumors. There was no apparent localization to regions of necrosis. A similar linear distribution of staining was seen in parallel sections of the same tissues that were stained with the CD34 monoclonal antibody (Fig. 1e), but the CD34 staining was homogeneous and not granular or abluminal. Adjacent uninvolved normal tissue was available from four of our tumor-bearing patients, and there was no apparent staining for EPO in these specimens. There was also no tissue staining when a negative control monoclonal antibody was substituted for the EOS monoclonal antibody (Fig. 1f).

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Fig. 1  Eosinophil peroxidase deposits in endometrial and ovarian cancers. In a-d, small blood vessels (marked by arrows) from four different tumors contained luminal and abluminal blue granules, indicating the immunohistochemical detection of EPO. Similar deposition of EPO was generally absent or decreased in the adjacent adenocarcinoma tumor cells (T) but usually extended into the fibrovascular stroma (S) between tumor cells (d). A similar linear distribution of purple staining was observed when parallel sections were stained with a monoclonal antibody to CD34 (an endothelial cell marker), but the staining appeared to be exclusively luminal and homogeneous rather than granular (e). By contrast, incubation of the tumor sections with a negative control monoclonal antibody produced no staining of tumor, blood vessels, or stroma (f). There was also no staining in normal tissues or in adjacent uninvolved tissues from four tumor-bearing subjects. All immunohistochemical photomicrographs are at an original magnification of ×250. Nuclear fast red counterstain and nitroblue tetrazolium substrate chromogen.

Discussion: the distribution of intact eosinophils seen in routinely stained sections of one-half of the tumors that were diligently examined. Thus, in view of the cytochemical and histological confirmation, we conclude that eosinophils were the source of the EPO rather than ectopic production of EPO by blood vessels or tumor cells. This observation is significant because it implies that occult eosinophil degranulation is an important and previously unsuspected part of the biological interaction between ovarian and endometrial cancers and the host.
Fig. 2  EPO deposition at the edge of an ovarian adenocarcinoma. EPO was detectable in wavy fibrillar deposits (a) at the interface between tumor cells (T) and adjacent connective tissue stroma (S). Fainter granules of EPO staining also extended into the adjacent tumor cells. A similar wavy pattern of staining was observed when parallel sections were stained with anti-CD34, but there was no granular staining within the tumor itself (b).

In specific, the strikingly localized deposition of EPO that we observed in the connective tissues and microvasculature of human gynecological cancers suggests the intriguing possibility that the degranulating eosinophils somehow participated in the neovascularization and extracellular matrix formation that were present within and around these tumors. This possibility is strengthened by the numerous previous reports that eosinophils accumulate, activate, and degranulate in the connective tissues and blood vessels during normal wound healing (14, 15), in utero during the implantation period (16, 17), in hypervascular nasal polyposis (18), and in association with the connective tissue matrix of a variety of other cancers (19–21). The neovascularization and extracellular matrix formation that are observed in all of these conditions have been attributed to transforming growth factors $\alpha$ and $\beta$, both of which are abundantly produced by eosinophils (14, 15, 18–24).

The findings presented in this report also suggest that EPO deposition from degranulating eosinophils is a new marker that can specifically distinguish between microvessels in benign tissues versus ovarian and endometrial cancers, a property that is not shared by other tumor markers that have previously been described in these cancers (25–27). Consequently, EPO deposited within tumor blood vessels could eventually have important practical implications for the management of women with ovarian or endometrial cancer.

For example, we have previously shown (12) that i.v.
administered, radiolabeled EOS antibody localized in a highly specific manner to tumor sites in 18 patients with a variety of lymphomas that contained EPO deposits that were similar to those that we have now also described in ovarian and endometrial cancers. Therefore, intravascular EPO may be an ideal target for a radioimmunoconjugate to be used in women with ovarian or endometrial cancers. In addition, we have recently demonstrated that EPO deposition in murine plasmacytomas sensitized the tumors to killing in vivo by hydrogen peroxide-generating, anionic Stealth liposomes (28). Based on the data in this report, a similar experimental approach might also have value for targeting and chemically killing the EPO-coated blood vessels in human gynecological cancers that are resistant to more conventional treatments.

Finally, it is of interest that eosinophils have sometimes been thought to exert a beneficial antitumor effect in vivo (29). In view of our findings, additional studies are now warranted to determine if eosinophils can modulate the clinical course of ovarian and endometrial cancer in humans.

REFERENCES
New marker for blood vessels in human ovarian and endometrial cancers.

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