Platelet-activating factor and metastasis: calcium-independent phospholipase A2β deficiency protects against breast cancer metastasis to the lung

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Am J Physiol Cell Physiol 300: C825–C832, 2011. First published January 12, 2011; doi:10.1152/ajpcell.00502.2010.—We determined the contribution of calcium-independent phospholipase A2β (iPLA2β) to lung metastasis development following breast cancer injection into wild-type (WT) and iPLA2β-knockout (iPLA2β-KO) mice. WT and iPLA2β-KO mice were injected in the mammary pad with 200,000 E0771 breast cancer cells. There was no difference in primary tumor size between WT and iPLA2β-KO mice at 27 days postinjection. However, we observed an 11-fold greater number of breast cancer cells in the lungs of WT mice compared with iPLA2β-KO animals (P < 0.05). Isolated WT lung endothelial cells demonstrated a significant increase in platelet-activating factor (PAF) production when stimulated with thrombin [1 IU/ml, 10 min, 4,330 \( \text{dpm} \)] compared with iPLA2β-KO animals. We found that WT lung endothelial cells significantly increased PAF production when stimulated with thrombin [1 IU/ml, 10 min, 4,330 \( \text{dpm} \)], P < 0.01. Adherence of E0771 cells to WT endothelial cells was increased by thrombin (4.8 ± 0.3% vs. 70.9 ± 6.3, P < 0.01). These responses were blocked by pretreatment with the iPLA2β-selective inhibitor (S)-bromoenol lactone and absent in lung endothelial cells from iPLA2β-KO mice. These data indicate that endothelial cell iPLA2β is responsible for PAF production and adherence of E0771 cells and may play a role in cancer cell migration to distal locations.

endothelium; inflammation; bromoenol lactone

Cancer deaths are more often caused by distant metastases than by growth of the primary tumor (23). Organ-specific spreading of tumor cells relies on heterotypic and homotypic adhesive interactions and on chemokines and their receptors (16). Although many studies have characterized the role of adhesion molecules in tumor cell extravasation and endothelial cell attachment (16), the role of platelet-activating factor (PAF) in this process has been less well studied. PAF is one of the most potent inflammatory lipid mediators and partici- pates in various inflammatory conditions, e.g., rheumatoid arthritis and ulcerative colitis. The cPLA2α is expressed constitutively in most cells, prefers substrates with sn-2 arachidonoyl substituents, and provides arachidonic acid for agonist-induced eicosanoid production in many cells and tissues.

We have demonstrated previously that the majority of endothelial cell PLA2α activity is attributable to iPLA2α, which is activated upon agonist stimulation and provides precursor for PAF production (34, 45, 46, 56). Most iPLA2α activity in mammalian cells resides in the group VIA and VIB enzymes designated iPLA2αβ and iPLA2αγ, respectively (8, 14, 31, 57). Homology between iPLA2αβ and iPLA2αγ includes an ATP-binding motif, a consensus GXGXG serine lipase catalytic center, and a region of nine amino acids of currently unknown functional significance (31). These two enzymes exhibit differential sensitivity to inhibition by enantiomers of the suicide substrate bromoenol lactone (BEL) (45). Racemic BEL inhibits iPLA2α activity at concentrations over 1,000-fold lower than those required to inhibit cPLA2α and sPLA2α enzymes (45). In addition, (S)-BEL inhibits iPLA2β preferentially over iPLA2αγ, and the converse is true for (R)-BEL (27). We have used BEl enantiomers to determine that endothelial cell PAF production appears to require iPLA2β activity (49). However, BEL also inhibits phosphatidate phosphohydrolase (1) and serine pro- teases (20). Additionally, hydrolysis of BEL by iPLA2α generates a diffusible bromomethyl keto acid that can alkylate thiol groups of susceptible neighboring enzymes, such as those with active cysteine residues (51). Such “off target” effects complicate interpretation of studies in which BEL is used as a pharmacologic inhibitor of iPLA2α and have motivated studies of genetic manipulations of iPLA2α enzymes to elucidate their roles in biological processes.

Mice that do not express iPLA2αβ have been generated by homologous recombination (5), and these iPLA2αβ-knockout...
(iPLA₂β-KO) mice have been used previously to identify roles for iPLA₂β in insulin secretion and glucose homeostasis, in macrophage functions, and in vascular myocyte biology (3, 4, 6, 44, 42, 49, 58, 59). In this study, we have used iPLA₂β-KO mice to evaluate whether development of cancer cell metastases to distant sites involves a mechanism similar to that described for neutrophil recruitment and transmigration, which requires iPLA₂β activity and PAF biosynthesis.

MATERIALS AND METHODS

iPLA₂β-KO mice. The generation of mice deficient in iPLA₂β has been described previously (5). Mice were housed in a pathogen-free facility, and studies were conducted under protocols approved by Saint Louis University Animal Care and Use Committee.

C57BL/6-derived breast cancer cell line E0771. The C57BL/6-derived breast cancer cell line E0771 was obtained from Dr. Rong Xiang ( Scripps Research Institute, La Jolla, CA) and stably transfected with green fluorescent protein (GFP) cDNA to act as a tumor-specific marker. Cells (2 × 10⁶) were mixed with 50% Matrigel and injected into the mammary pads of syngeneic female wild-type (WT) and iPLA₂β-KO mice. Primary tumor growth was measured at weekly intervals using calipers. Tumor volumes (in mm³) were calculated using the formula: (width)² × length/2, where width is the smaller of the two measurements. Mice were killed at days 14, 19, and 27, and primary tumor, blood, liver, and lungs were harvested. Mice and primary tumors were weighed after death, and final tumor volumes (in mm³) were calculated using the formula: width × length × depth/2. Pieces of tumor tissue and the right apical lobe of the lung from each animal were fixed in formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) for histological examination. Results were analyzed in a blinded fashion.

Endothelial cell isolation and culture. Endothelial cells were isolated from mouse lung by collagenase digestion. Diced lung tissue was incubated in a solution of collag enase (1 mg/ml, 1 h, 37°C), and the digested tissue was passed through a cell strainer. A single-cell suspension was obtained by incubating (10 min) in trypsin-EDTA. Endothelial cells were isolated by incubation with murine immunoglobulins to block Fc receptors and then with rat anti-mouse CD31, rat anti-mouse CD105, and biotinylated isoclin B4. Cells were washed, incubated with rat anti-mouse Ig and streptavidin-conjugated microbeads, and separated using an AutoMACS cell separator. Eluted cells were washed, resuspended in EGM-2MV cell culture medium (Lonza), and plated in 25-cm² culture flasks. Nonadherent cells were removed the next day, and cells were grown to confluency and passaged at 1:3 dilution. Cells from passages 3-4 were used for experiments.

Real-time RT-PCR analysis. The right azygos lobe of the lung and a section of liver were snap frozen, and the tissue was homogenized using a rotor-stator homogenizer (Tissuemiser, Fisher Scientific, St. Louis, MO). Blood was obtained by cardiac puncture. RNA was prepared using the RNeasy Mini Kit (Qiagen, Valencia, CA), and cDNA was prepared using the TaqMan Reverse Transcription Gene Expression Assay Kit (Applied Biosystems, Carlsbad, CA). Real-time PCR analysis of green fluorescent protein (GFP) and 18s RNA was performed using GFP and 18s RNA-specific Taqman primer/probes. PCR analysis of green fluorescent protein (GFP) and 18s RNA was performed using the RNeasy Mini Kit (Qiagen, Valencia, CA), and a section of liver were snap frozen, and the tissue was homogenized by 10.2×0.3 on October 20, 2017 http://ajpcell.physiology.org/ Downloaded from
more GFP was observed in WT compared with iPLA2-WT and KO mice compared with lungs of both WT and KO animals, but the differences were not significant. In both WT and KO mice by quantitative PCR. Therefore, at very low, barely detectable levels of E0771 cells at these sites (Fig. 2). We also evaluated peripheral blood and the liver for metastasis measurements. Figure 2 depicts representative histological analyses of lungs from WT and KO mice and is consistent with the quantitative PCR metastasis measurements. Figure 2 shows the H&E staining evaluation of lungs from WT and KO mice and is consistent with the quantitative PCR analysis of lungs from one set of mice killed at day 19 and another set on day 27. At day 19, GFP was detected in the lungs of both WT and KO animals, but the differences were not significant. By day 27, there was more GFP expression in both WT and KO mice compared with day 19, and significantly more GFP was observed in WT compared with iPLA2-KO mice (Table 1), and was also no significant difference in primary tumor weight or volume in tumors from WT or iPLA2-KO mice (Table 1 and Fig. 1).

Lungs, blood, and liver from WT and iPLA2-KO mice were analyzed for tumor metastasis by quantitative RT-PCR analysis of GFP, which acts as a tumor-specific marker. Table 2 shows the PCR analysis of lungs from one set of mice killed at day 19 and another set on day 27. At day 19, GFP was detected in the lungs of both WT and KO animals, but the differences were not significant. By day 27, there was more GFP expression in both WT and KO mice compared with day 19, and significantly more GFP was observed in WT compared with iPLA2-KO mice (ΔCt 15.8 vs. 19.3, P < 0.05), which reflects an 11-fold difference between the number of metastatic E0771 breast cancer cells in the lungs of WT than of iPLA2-KO mice. Table 3 shows the H&E staining evaluation of lungs from WT and KO mice and is consistent with the quantitative PCR metastasis measurements. Figure 2 depicts representative histological analyses of lungs from KO (Fig. 2A) and WT mice (Fig. 2B). We also evaluated peripheral blood and the liver for evidence of tumor metastasis. In contrast to the lung, we found very low, barely detectable levels of E0771 cells at these sites in both WT and KO mice by quantitative PCR. Therefore, at the times of our analysis, we were only able to detect metastasis to the lung.

To determine whether the observed difference in lung metastases could be, at least in part, a result of decreased endothelial cell PAF production and transendothelial cell migration, we isolated endothelial cells from lungs of WT and iPLA2-KO mice by selecting cells that expressed CD31 and CD105. Confluent monolayers were stained for coagulation factor VIII to determine endothelial cell purity, and preparations consisting of >80% endothelial cells were used for subsequent studies. In previous studies, we have demonstrated increased PAF production in human lung microvascular endothelial cells upon treatment with thrombin or trypsin (47). Increased PAF production is inhibited completely by pretreatment with the iPLA2-selective inhibitor BEL (47). To verify that mouse lung endothelial cells release PAF via iPLA2 activation, we incubated WT and iPLA2-KO lung endothelial cells with thrombin or TNF-α and measured PAF production (Fig. 3). Incubation of WT lung endothelial cells with thrombin (1 IU/ml, 10 min) or TNF-α (10 ng/ml, 2 h) induced a fourfold rise in PAF production, and these responses were completely prevented by pretreatment of the cells with (S)-BEL (5 μM, 10 min, Cayman Chemical), which is consistent with the involvement of iPLA2 in the responses. Stimulation of iPLA2-KO endothelial cells with thrombin or TNF-α failed to induce any increase in PAF production (Fig. 3), which is consistent with a requirement for iPLA2 in thrombin- and TNF-α-stimulated PAF production by pulmonary endothelial cells. PAF expressed by endothelial cells binds to its cognate receptors on circulating inflammatory cells, and results in cell adherence to an activated endothelial cell monolayer. To determine whether E0771 cells may adhere to the endothelium via a PAF-PAF receptor interaction, we performed immunoblot analysis on E0771 cells to verify that they express the PAF receptor (Fig. 4). PAF receptor immunoprotein was detected in E0771 cells (Fig. 4, left lanes), human coronary artery endothelial cells (Fig. 4, middle lanes), and mouse atrial cardiomyocytes (HL-1, Fig. 4, right lanes). We incubated endothelial cells with E0771 breast cancer cells to determine whether increased PAF production was associated with increased cell adherence. As shown in Fig. 5, stimulation of WT lung endothelial cells with thrombin or TNF-α resulted in a sixfold increase in E0771 cell adherence. E0771 cell adherence was inhibited when WT mouse lung endothelial cells were pretreated with (S)-BEL before incubation with thrombin or TNF-α (Fig. 5). Pretreating E0771 cells with the PAF receptor antagonist CV3988 (10 μM, 10 min) before incubating them with WT endothelial cells resulted in complete inhibition of adherence, which demonstrates the requirement of the interaction of PAF with its receptor in order for cell adherence to occur (Fig. 5). In contrast, stimulation of iPLA2-KO lung endothelial cells with thrombin or TNF-α failed to increase their adherence to E0771 cells (Fig. 6). These results were consistent with a requirement for iPLA2 in thrombin and

Table 1. Whole body weight and breast cancer tumor weight and volume for wild-type and iPLA2-β-knockout mice injected with 200,000 E0771 cells into the mammary pad

<table>
<thead>
<tr>
<th></th>
<th>Tumor Volume, mm³</th>
<th>Tumor Weight, g</th>
<th>Mouse Weight, g</th>
</tr>
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<tbody>
<tr>
<td>WT</td>
<td>701 ± 242</td>
<td>0.9 ± 0.4</td>
<td>23.5 ± 0.8</td>
</tr>
<tr>
<td>KO</td>
<td>907 ± 230</td>
<td>1.1 ± 0.5</td>
<td>22.4 ± 0.3</td>
</tr>
</tbody>
</table>

Data represent means ± SE. iPLA2β, calcium-independent phospholipase A2β.

Table 2. Real-time RT-PCR analysis of GFP and 18s RNA expression in the lungs of wild-type and iPLA2-β-knockout female mice at 19 and 27 days following injection of 200,000 GFP-expressing E0771 cells into the mammary pad

<table>
<thead>
<tr>
<th></th>
<th>ΔCt GFP vs. 18s RNA</th>
<th>Fold Change From WT</th>
<th>ΔCt GFP vs. 18s RNA</th>
<th>Fold Change From WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>19.4 ± 1.2</td>
<td>15.8 ± 1.2</td>
<td>(n = 10)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>KO</td>
<td>21.8 ± 1.1</td>
<td>-5.3</td>
<td>19.3 ± 0.8</td>
<td>-11.3</td>
</tr>
</tbody>
</table>

Data represent means ± SE. GFP, green fluorescent protein; C, cycle threshold; WT, wild type; NS, not significant.
TNF-α-stimulated endothelial cell PAF production and inflammatory cell adherence.

**DISCUSSION**

Cancer is the second most prevalent cause of death, and the majority of cancer deaths are caused by distant metastases rather than by growth of the primary tumor (23). Development of new management strategies that target metastasis development are thus required to improve cancer morbidity and mortality outcomes. Here, we have demonstrated that lungs of WT mice accumulate an 11-fold greater number of metastatic breast cancer cells than do those of iPLA2-KO mice in our model, and this identifies iPLA2 as a potentially important target for pharmacologic or other interventions designed to reduce adverse outcomes from metastatic breast cancer, which is the leading cause of cancer death in US women. Our data suggest that the protection against breast cancer metastasis conferred by iPLA2 deficiency is attributable, at least in part, to decreased endothelial cell PAF production and consequently reduced breast cancer cell adherence to, and transmigration across, the endothelium. Several previous studies have investigated the role of adhesion molecules in facilitating tumor cell detachment from primary tumors to gain access to the lymphatic or blood circulatory systems, but few have demonstrated the role of endothelium-derived PAF in promoting tumor cell egress from the circulation and migration into tissue. Tumor cell extravasation employs a process similar to that involved in recruitment of inflammatory cells to the endothelium, which involves a sequence of events that include the interaction of endothelial cell adhesion molecules with corresponding receptors on inflammatory cells and the interaction of PAF with its receptor (35, 36).

In a previous study, we demonstrated an increase in human lung microvascular endothelial cell (HMVEC-L) PAF production, and polymorphonuclear leukocyte adherence in response to thrombin or tryptase (47). Subsequently, we demonstrated that HMVEC-L PAF production required iPLA2β activity, as reflected by inhibition of thrombin- or tryptase-stimulated PAF production by pretreatment with (S)-BEL (49). Endothelial cells isolated from lungs of WT mice exhibited increased PAF production upon stimulation with thrombin or tryptase, but this response was absent with iPLA2-KO lung endothelial cells (49). Murine monocyte/macrophage RAW 264.7 cells exhibited increased adherence to activated WT lung endothelial cells, and this response was blocked by pretreatment of the endothelial cells with racemic BEL (49). Here, we demonstrate that pretreatment of WT lung endothelial cells with (S)-BEL completely inhibited E0771 cell adherence in response to thrombin or TNF-α, while pretreatment with (R)-BEL had little effect (Fig. 4). Moreover, pretreatment of E0771 cells with the PAF receptor antagonist CV3988 completely inhibited their adherence to activated WT lung endothelial cells, and this response was blocked by pretreatment of the endothelial cells with racemic BEL (49). Here, we demonstrate that pretreatment of WT lung endothelial cells with (S)-BEL completely inhibited E0771 cell adherence in response to thrombin or TNF-α, while pretreatment with (R)-BEL had little effect (Fig. 4). Moreover, Pretreatment of E0771 cells with the PAF receptor antagonist CV3988 completely inhibited their adherence to stimulated endothelial cells. These data demonstrate that iPLA2β activity and increased PAF production are involved in adherence of E0771 cells to an activated endothelium. Although these in vitro data support our hypothesis that endothelial cell iPLA2β is involved, we cannot rule out the possibility that the absence of iPLA2β in other cells is playing an instrumental role in metastasis.

E0771 cells are syngeneic to C57BL/6 mice and were originally isolated from a spontaneous medullary breast adenocarcinoma (17, 52). Subcutaneous injection of E0771 cells has been shown to result in secondary metastases in the lung that are morphologically similar to primary E0771 tumors (18, 63). At 27 days postinjection, histological examination dem-

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**Table 3. Histological analysis of lungs removed from WT and iPLA2β-knockout mice at 27 days following injection of 200,000 E0771 cells into the mammary pad**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Diagnosis</th>
<th>Distribution</th>
<th>Severity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT1</td>
<td>Carcinoma, metastatic</td>
<td>Focal</td>
<td>2</td>
<td>3 of 3 sections</td>
</tr>
<tr>
<td>WT2</td>
<td>Carcinoma, metastatic</td>
<td>Multifocal</td>
<td>2</td>
<td>1 of 3 sections</td>
</tr>
<tr>
<td>WT3</td>
<td>Carcinoma, metastatic</td>
<td>Focal</td>
<td>1</td>
<td>1 of 3 sections</td>
</tr>
<tr>
<td>WT4</td>
<td>No evidence of neoplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KO1</td>
<td>No evidence of neoplasia</td>
<td>Focal</td>
<td>1</td>
<td>2 of 3 sections</td>
</tr>
<tr>
<td>KO2</td>
<td>Carcinoma, metastatic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KO3</td>
<td>No evidence of neoplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KO4</td>
<td>No evidence of neoplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Severity graded as 1, minimal; 2, mild; 3, moderate; 4, severe. KO, iPLA2β-knockout.

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**Fig. 2. Hematoxylin and eosin-stained sections of mouse lung from WT and iPLA2β-KO mice. A: representative section of lungs from KO mice, with no evidence of tumor from a section of lung from knockout mouse no. 1. B: section of lung from wild-type mouse no. 2, showing focal metastasis of E0771 cells.**
tumor development in the iPLA2 in the lung (Table 2). This suggests that iPLA2 evaluation of micrometastasis by GFP-expressing E0771 cells means ng/ml, 2 h). Cont, control; dpm, disintegrations per minute. Data represent with thrombin (thr; 1 IU/ml, 10 min), or tumor necrosis factor-

this is due, at least in part, to impaired endothelial cell PAF

decrease in metastasis formation in the lung after injection of

data suggest that the absence of iPLA 2

a significant difference in primary tumor size in WT and

formation of metastases in specific organs involves adhe-

molecules, chemokines, and cancer stem cells (reviewed recently in Ref. 16), but there has been relatively little exam-

ination of the role of PAF in breast cancer metastases. Several breast cancer cell lines produce PAF upon stimulation and express PAF receptors (9). Increased PAF production has been proposed to promote cell proliferation and angiogenesis in several breast cancer cell lines (9). Pretreatment of human breast cancer cells with the PAF receptor antagonist WEB-2086 has been reported to inhibit cell growth and differentia-

tion in vitro. In the studies described here, we did not observe a significant difference in primary tumor size in WT and iPLA2-β-KO mice, although there was a trend toward increased tumor development in the iPLA2-β-KO mice (Fig. 1). These data suggest that the absence of iPLA2 β adjacent to the injected E0771 cells did not affect primary tumor breast cancer cell growth or differentiation in vivo.

Enhanced PAF production in response to stimulation is observed in highly invasive breast cancer cells, which suggests that breast cancer PAF production may play a role in the development of metastases (9). We observed a significant decrease in metastasis formation in the lung after injection of breast cancer cells into iPLA2-β-KO mice, and we propose that this is due, at least in part, to impaired endothelial cell PAF production and transendothelial cell migration from the primary tumor to the circulation, from the circulation to distal sites, or from both of those processes. We attempted to identify circulating tumor cells by analyzing blood for the presence of GFP using quantitative PCR; however, we were not able to detect tumor cells. Our study is the first of which we are aware that demonstrates reduced development of metastases after injection of breast cancer cells into mammary pads of iPLA2-β-KO mice and to provide evidence that endothelial cell iPLA2 β activity and PAF production are important in this process. A role for PAF in development of lung metastasis development after intravenous injection of melanoma cells has been demonstrated previously (36). Im et al. (26) demonstrated that intraperitoneal instillation of PAF resulted in increased development of lung metastases after

![Fig. 3. Effect of pretreatment with (R)-bromoenol lactone (BEL, 5 μM, 10 min) or (S)-BEL (5 μM, 10 min) on platelet-activating factor (PAF) production in lung endothelial cells isolated from WT or iPLA2-β-KO mice stimulated with thrombin (thr; 1 IU/ml, 10 min), or tumor necrosis factor-α (TNF-α, 10 ng/ml, 2 h). Cont, control; dpm, disintegrations per minute. Data represent means ± SE; n = 12. **P < 0.01 compared with unstimulated controls. + + P < 0.01 compared with stimulated samples in the absence of BEL.](http://ajpcell.physiology.org/)

![Fig. 4. Presence of PAF receptor (PAF-R) immunoprotein in E0771 cells (left), human coronary artery endothelial cells (HCAEC, middle), and mouse atrial cardiomyocytes (HL-1, right).](http://ajpcell.physiology.org/)

![Fig. 5. Adherence of E0771 cells to endothelial cells isolated from the lungs of WT mice stimulated with thrombin (1 IU/ml, 10 min) or TNF-α (10 ng/ml, 2 h). Where indicated, endothelial cell monolayers were pretreated with (R)- or (S)-BEL (5 μM, 10 min) before addition of E0771 cells. E0771 cells were incubated with CV3988 (10 μM, 10 min) before addition to the endothelial cell monolayer where indicated. Data represent means ± SE; n = 11–14. ***P < 0.01 compared with unstimulated controls. + + P < 0.01 compared with stimulated samples in the absence of BEL or CV3988.](http://ajpcell.physiology.org/)

![Fig. 6. Adherence of E0771 cells to endothelial cells isolated from the lungs of WT or iPLA2-β-KO mice stimulated with thrombin (1 IU/ml, 10 min) or TNF-α (10 ng/ml, 2 h). Data represent means ± SE; n = 8–11. *P < 0.05, ***P < 0.01 compared with unstimulated controls.](http://ajpcell.physiology.org/)
injection of melanoma cells, and Melnikova et al. (36) have shown that pretreating mice with the PAF receptor antagonist PCA4248 inhibited development of melanoma metastases in the lung. A recent study examining tumorigenesis and ascites formation after intraperitoneal injection of IB8 ovarian cancer cells demonstrated that these processes were much reduced in iPLA₂-β-KO compared with WT mice, which suggests that iPLA₂-β may represent a useful target for the development of antineoplastic drugs or other therapeutic interventions (30).

The importance of an inflammatory microenvironment in cancer initiation and progression offers opportunities for exploration of new approaches to cancer treatment. Inflammatory cells in the tumor microenvironment release cytokines, chemokines, and other factors that can enhance tumor growth, angiogenesis, and metastasis. Thrombin can regulate tumor cell adherence to endothelial cells and is a critical component of the microenvironment that influences tumor cell behavior (reviewed in Ref. 37). We demonstrate here that E0771 cell adherence to isolated endothelial cells is enhanced by stimulation with thrombin, which suggests that thrombin may contribute to transmigration of E0771 cell across the endothelial barrier from the primary tumor. In addition, we observed increased endothelial cell PAF production and E0771 cell adherence in response to TNF-α stimulation. Im et al. (26) have previously demonstrated that PAF mediates metastasis-enhancing activities of TNF-α and IL-1α, and we propose that at least part of this mechanism may involve PAF production by the endothelium in response to TNF-α stimulation.

Phospholipase A₂ enzymes hydrolyze the sn-2 fatty acid on the glycerol backbone of membrane phospholipids to yield products that include a free fatty acid, e.g., arachidonic acid, and a 2-lyso phospholipid, e.g., 1-O-hexadecanoyl-2-lyso-glycerophosphocholine, that can act directly or be converted to myriad phospholipid-derived signaling molecules that affect tumor cell behavior and cancer progression, including PAF and various eicosanoids. Expression of sPLA₂ enzymes is increased in several human cancers, including breast cancer (60–62). Similarly, cPLA₂ is activated by 17β-estradiol in MCF-7 breast cancer cells and inhibition of cPLA₂ expression in MCF-7 cell growth (11). Although the role of iPLA₂ in cancer has been less well characterized, in vitro studies demonstrate that both iPLA₂β and iPLA₂γ are expressed in several human cancers (2, 43, 53). PLAr inhibitors have been proposed as anticancer drugs because inhibition of PLAr could theoretically decrease production of several metabolites implicated in cancer progression. For example, free arachidonic acid can be metabolized to eicosanoids that induce cancer cell growth and proliferation in vitro (for recent reviews, see Refs. 60–62). Nonsteroidal antiinflammatory drugs may reduce breast cancer incidence by up to 20%, and this might be related to their ability to inhibit cyclooxygenase-2 or reduce serum estradiol in postmenopausal women (reviewed recently in Refs. 19, 21, and 24). Our data suggest that inhibition of endothelial cell iPLA₂β could represent a therapeutically advantageous means to reduce PAF production and to prevent or retard development of metastases from the primary tumor. Since the vast majority of breast cancer-related deaths are due to metastatic disease rather than growth of the primary tumor, this represents a novel and potentially important therapeutic approach to the management of breast cancer.

GRANTS
This work was supported by United States Public Health Service Grants R37-DK34388, P41-RR00954, P60-DK20579, and P30-DK56341 (to J. Turk) and by a merit review grant from the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, and Biomedical Laboratory Research and Development (to J. Kornbluth).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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