Role of the Prostaglandin E2 Receptor in Mammary Tumor Metastasis

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ABSTRACT

Both clinical and experimental breast tumors often synthesize high levels of prostaglandins, most notably prostaglandin E2 (PGE2). We have reported previously that metastatic murine mammary tumor cells also express a high-affinity PGE2 receptor. We have now shown that the receptor plays a functional role in the metastasis of two mammary tumor cell subpopulations, lines 66 and 4526. We showed that three agents, LEO101 (LEO Pharmaceuticals), SC19220 (Searle Co.), and AH6809 (Glaxo Co.), antagonize [3H]PGE2 binding to these cells and block PGE2-mediated elevation of intracellular cyclic AMP. Pretreatment of line 66 cells with nontoxic concentrations of any of the three receptor antagonists prior to i.v. injection results in more experimental lung colonies. As shown previously, and confirmed here, pretreatment of these cells with indomethacin (which inhibits endogenous PGE synthesis and therefore increases detectable PGE receptor) inhibits metastasis. Thus, the tumor cell PGE2 receptor contributes to the ability of murine mammary tumor cells to metastasize.

INTRODUCTION

Arachidonate metabolism, leading to the synthesis of eicosanoids, is often elevated in malignant tissue (1). Among the numerous cyclooxygenase products of arachidonate, PGE2 synthesis has been studied most extensively and inhibitor studies indicate that PGE functions to promote the growth and metastasis of many experimental tumors. The mechanisms involved are not completely understood but involve, in some cases, suppression of host immune responses by tumor-PGE2 (2).

Much less is known about the receptor for PGE2 present on some tumor cells and the role that it plays in signal transduction and subsequent tumor behavior. In earlier studies, we showed that murine mammary tumor cells synthesize PGE2 and also possess a high-affinity receptor for this ligand (3, 4). Receptor characteristics differ among related mammary tumor subpopulations but are quite similar for 3 highly metastatic populations examined, suggesting that autocrine effects of tumor-PGE2 may play a role in tumor cell metastasis. Thus, we have proposed that in addition to tumor-PGE host immune cell interactions, autologous effects of PGE2 on tumor cells may also be important.

In the present study, we have asked directly whether the PGE2 receptor plays a role in the metastasis of murine mammary tumors. We have determined the effect of receptor antagonism on tumor cell lines and subsequent tumor behavior. We anticipate that murine mammary tumors may contain a high-affinity receptor for this ligand (3, 4). Receptor characteristics differ among related mammary tumor subpopulations but are quite similar for 3 highly metastatic populations examined, suggesting that autocrine effects of tumor-PGE2 may play a role in tumor cell metastasis. Thus, we have proposed that in addition to tumor-PGE host immune cell interactions, autologous effects of PGE2 on tumor cells may also be important.

In the present study, we have asked directly whether the PGE2 receptor plays a role in the metastasis of murine mammary tumors. We have determined the effect of receptor antagonism on [3H]PGE2 binding, signal transduction, and experimental metastasis of 2 highly metastatic murine mammary tumor cell lines.

MATERIALS AND METHODS

Mice. BALB/c mice 6 to 12 weeks of age were bred in the Animal Care Facility of the University of Maryland School of Medicine using breeding stock produced from cesarean-derived breeding pairs originally obtained from the Cancer Research Laboratory, University of California, Berkeley, CA. Mice were maintained on standard laboratory chow, ad libitum.

Tumor Cell Lines. The derivation and maintenance of the murine mammary tumor cell lines have been described previously (5). Line 66 was derived from a spontaneously arising mammary tumor of a BALB/c C3H mouse. Line 4526 was cloned from cell line 410.4 derived from the cfC3H mouse. Line 4526 was cloned from cell line 410.4 derived from the cfC3H mouse. Line 4526 was cloned from cell line 410.4 derived from the cfC3H mouse. Line 4526 was cloned from cell line 410.4 derived from the cfC3H mouse.

Prostaglandin Receptor Antagonism and Experimental Metastasis. The PGE receptor antagonists LEO101 (polyphloretin phosphate; Pharmacia LEO, Helsingborg, Sweden; Ref. 6) and AH6809 (Glaxo, Middlesex, England; Ref. 7) was dissolved in tissue culture medium, and SC19220 (Searle Co., Skokie, IL; Ref. 8) was dissolved in absolute ethyl alcohol at the appropriate concentrations. Cells were allowed to attach to tissue culture flasks (Corning, Corning, NY) for 24 h. Indomethacin (1 μM) or PGE receptor antagonists, at the concentrations indicated, were added for an additional 24-h period. Cells were trypsinized, washed, and counted, and 2–3 × 105 cells were injected in a volume of 0.1 ml saline into the lateral tail vein of BALB/c mice in random order. Eighteen to 21 days later, mice were sacrificed by cervical dislocation, and lungs were removed, weighed, and examined for the presence of surface colonies.

PGE2 Binding Assay. This assay was performed as described previously (3). Briefly, cells were cultured for 24 h in the presence of indomethacin (1 μM; Sigma Chemical Co., St. Louis, MO) to prevent endogenous PGE2 synthesis. Line 4526 cells, which are tightly adherent, were assayed directly in glass scintillation vials. Loosely adherent line 66 cells were assayed as single cell suspensions. Various concentrations of [3H]PGE2 (160–200 Ci/mM; Du Pont, Boston, MA) or [3H] PGE2 and 400-fold excess unlabeled ligand (Cayman Chemicals, Ann Arbor, MI) were added to assay tubes in assay buffer (NaCl, 0.14 M; Tris-HCl, 0.05 M; CaCl2, 0.01 M; KC1, 0.005 M; MgSO4, 0.001 M; glucose, 0.1%; EDTA, 0.001 M; and bovine serum albumin, 1 mg/ml). Preparations were incubated at 37°C for 30 min. Bound ligand was separated from unbound ligand by repeated rinsing (line 4526 cells) or filtration (line 66 cells). Bound PGE2 was solubilized in ethyl alcohol and radioactivity determined by liquid scintillation spectrometry. Equilibrium binding data were analyzed by Scatchard analysis, plotting the specific [3H]PGE2 bound (total binding minus nonspecific binding in the presence of 400-fold excess unlabeled PGE2) divided by free [3H] PGE2 (Y-axis) versus specific [3H]PGE2 bound on the X-axis.

cAMP Assays. cAMP levels were determined as described previously using a commercially available kit (Du Pont, Boston, MA). Cells growing in 60-mm tissue culture plates were incubated in media containing indomethacin (1 μM) for 24 h prior to assay. On the day of assay, cells were switched to buffer containing isobutylmethylxanthine (100 μM) and PGE2 (10 μM) in the absence or presence of PGE receptor antagonists for 10 min. Samples were rinsed and extracted with 6% trichloroacetic acid for 2 h at 4°C. Recovery was estimated by adding [3H]cAMP to the samples (Du Pont, Boston, MA). Samples were fractionated on a Dowex ion exchange column. Fractions 3–9 were pooled and assayed for cAMP by radioimmunoassay.

PGE2 Assay. PGE was quantitated by radioimmunoassay as described previously (9) using a commercially prepared antibody to PGE2.
(Advanced Magnetics, Inc., Boston, MA). This antibody does not distinguish between PGE1 and PGE2. Conditioned medium from tumor cells was assayed directly and corrected for PGE present in unconditioned medium.

Statistical Analysis. Student's t test was used to compare mean values for binding and cAMP data. Differences were considered significant if P values did not exceed 0.05. Experimental metastasis data were analyzed by Mann-Whitney U test.

RESULTS

Previous studies indicated that pretreatment of tumor cells with nontoxic doses of indomethacin (1 μM) prior to i.v. administration significantly inhibits experimental metastasis of murine mammary tumor lines (10). Table 1 confirms that the experimental metastasis of the related metastatic line 66 is also inhibited by indomethacin pretreatment, by 39% in this representative experiment (P = 0.05). In contrast, pretreatment of tumor cells with the PGE receptor antagonist LEO101 increased experimental metastasis 27% (P = 0.03). We determined the effect of 2 additional receptor antagonists, SC19220 and AH6809, on experimental metastasis. As shown in Fig. 1, pretreatment of tumor cells with any of the 3 receptor antagonists increased experimental metastasis by 158–185%. The effect was statistically significant in the case of LEO101 and AH6809; the P value for SC19220 was 0.06. In 4 experiments, SC19220 increased the mean number of line 66 metastases by an average of 32.5%. In 2 other experiments, when the number of metastases were low in the control animals, SC19220 treatment increased metastasis by 2- and 9-fold. In 3 of 3 additional experiments, both LEO101 and AH6809 increased the experimental metastasis of line 66 cells. Because all 3 agents have the same effect, it seems likely that the ability of these agents to increase metastasis is due to their antagonism of the PGE receptor.

To confirm that these agents are, in fact, PGE2 receptor antagonists for the mammary tumor cells studied here, [3H]-PGE2 equilibrium binding studies were carried out in the presence and absence of LEO101 and SC19220. Fig. 2 shows Scatchard analysis of equilibrium binding data for line 66 cells in the absence or presence of 3 concentrations of LEO101. As shown earlier (11), high-affinity binding of [3H]PGE2 is demonstrable only when mammary tumor cells are pretreated with indomethacin to prevent endogenous PGE2 synthesis. Binding in the presence of indomethacin indicates a high-affinity receptor for PGE2 with a Ka of 6.4 × 10^-9 m/liter and 29,203 binding sites/cell. As indicated by the X-intercept, binding capacity was reduced to 10,451, 5,215, and 1,043 sites/cell in the presence of 5, 10, and 50 μg/ml of LEO101, respectively. The similar slopes of the lines indicate no significant change in the binding affinities, a characteristic of noncompetitive inhibition.

Table 2 illustrates inhibition of [3H]PGE2 binding to another mammary tumor subpopulation (line 4526) by LEO101 and SC19220. We have found LEO101 to be consistently more inhibitory than SC19220.

We have shown previously that stimulation of these cells with PGE2 leads to elevations in intracellular cAMP (3). To determine if receptor antagonism would block signal transduction, line 4526 cells were stimulated with PGE2 in the absence or presence of receptor antagonists. As shown in Table 3, stimulation of line 4526 cells with agonist resulted in a 6.5-fold elevation of cAMP. Treatment with SC19220, LEO101, or AH6809 had minimal effects on basal cAMP levels, but each significantly abrogated the PGE-mediated elevation. Levels of

![Fig. 1](image1.png)

![Fig. 2](image2.png)
Intracellular cAMP was extracted and quantitated by radioimmunoassay. The growth of many experimental tumors is suppressed by the administration of low, nontoxic doses of cyclooxygenase inhibitors such as indomethacin, aspirin, and piroxicam (12). This indicates that, in many cases, tumor eicosanoid synthesis contributes positively to tumor growth. The mechanisms involved are unclear, but considerable evidence implicates tumor-PGE2 as an immunosuppressive molecule that acts to down-regulate host antitumor reactions (2).

In addition to the effects on primary tumor growth, tumor prostaglandins also contribute to successful metastatic dissemination in part through suppression of natural killer cell activity (4, 10). Evidence for eicosanoid effects on platelet function and interactions with vascular endothelium also exist (13). These models have a common premise that tumor-derived prostaglandins are synthesized and released to act exogenously on host components.

We have focused on the question of whether tumor prostaglandins can act in an autocrine fashion to affect the tumor cell directly. In earlier reports, we established that mammary tumor cells that synthesize PGE2 also express a high-affinity receptor for this ligand (3). Finding that receptor characteristics were remarkably similar in a series of metastatic mammary tumor subpopulations derived from a population heterogenous for many other characteristics prompted us to ask if the receptor plays a role in metastasis. Others have suggested that the ability to respond to PGE2 rather than the PGE2 synthetic ability of a tumor cell is critical to metastasis (14).

The present study confirms that 3 agents shown to be PGE receptor antagonists in smooth muscle (15) also antagonize 2 functions of the mammmary tumor PGE receptor, namely, [³H]PGE2 binding and intracellular signal transduction leading to adenylate cyclase activation. Although SC19220 marginally inhibited binding when compared with LEO101, this inhibition was sufficient to significantly block the PGE2-induced elevation in intracellular cAMP.

Studies in vivo show that pretreatment of tumor cells with receptor antagonists prior to i.v. administration enhances experimental metastasis. Conversely, indomethacin pretreatment decreases metastasis. The indomethacin effects, which we have reported previously (4, 10), are, in part, due to the reversal of PGE-mediated suppression of NK function. We cannot, however, eliminate a role for modulation of the PGE receptor on the tumor cell since we have shown that indomethacin effectively increases the expression of detectable receptor presumably by blocking receptor occupancy by endogenously synthesized prostaglandin (11).

From other work in our laboratory, we have some clues as to the mechanisms by which a PGE receptor on the tumor cell could promote metastasis. Since receptor antagonism occurs only prior to in vivo administration, the receptor effects are most likely on very early events in the metastatic cascade. Young et al. (14) have shown that the migration in vitro of metastatic but not nonmetastatic cells is stimulated by exogenous PGE2. Other important early events involve interactions with circulating host cells or with vascular components. We have evidence from studies in vitro that both types of interactions can be affected by the PGE receptor. Thus, antagonism of the receptor on the classic NK target cell YAC-1 prevents effector cell binding and subsequent cytolysis (16). This was somewhat surprising, since, in the past, PGE-mediated suppression of NK cytolysis was believed to be due to inhibitory action on the effector (NK) cell (17). PGE receptor antagonists might promote metastasis by preventing potentially destructive tumor cell-NK cell interactions. Recently, we have shown that receptor

**DISCUSSION**

Since tumor cells derived from many experimental and clinical cancers actively synthesize eicosanoids, there is much interest in defining the role that arachidonate metabolites play in tumor behavior. The growth of many experimental tumors is

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**Table 2** Receptor antagonism and [³H]PGE2 binding to 4526 cells

| Treatment | pmol cAMP/10⁶ cells | -Fold change* | P  
<table>
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<tr>
<td>Control</td>
<td>3.4 ± 0.5</td>
<td></td>
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<tr>
<td>PGE2</td>
<td>22.0 ± 7.0</td>
<td>6.5</td>
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| PGE2 + LEO101 | 4.3 ± 0.4   | NS  
| SC19220   | 2.7 ± 0.9           | 0.8           |
| PGE2 + AH6809 | 2.0 ± 0.1   | NS  
| LEO101    | 4.1 ± 0.1           | 1.7           |
| SC19220   | 2.5 ± 0.0           | 0.7           |
| AH6809    | 2.9 ± 0.1           | 0.9           |

* Fold increase compared with control.

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**Table 3** Effect of receptor antagonists on PGE-stimulated cAMP

| Treatment | pmol cAMP/10⁶ cells | -Fold change* | P  
<table>
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<tbody>
<tr>
<td>LEO101</td>
<td>1,580 ± 5</td>
<td>0.002</td>
</tr>
<tr>
<td>SC19220</td>
<td>7,724 ± 32</td>
<td>0.02</td>
</tr>
<tr>
<td>AH6809</td>
<td>3,138 ± 174</td>
<td>0.01</td>
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**a** Probability by Student’s t test, treatment versus untreated control.

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Control 10,204 ± 722 2.5 nm 14,407 ± 2,544 17,859 ± 1,358 0.007 8.5 nm 7,724 ± 32 0.02 11,563 ± 192 0.13 15,709 ± 745 0.09

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The enhancement of metastasis by receptor antagonists suggests that these agents are not toxic to the cells. Nevertheless, cloning efficiencies in the presence of solvent control, indomethacin (1 ¿¿M), or SCI9220 (1.0 ¿¿M) were 91, 99, and 95%, respectively, in comparison to untreated cells, indicating no significant toxicity.
antagonism on these same mammary tumor cells modulates interactions with laminin and fibronectin. There is extensive evidence that interactions with these and other components of the extracellular matrix are critical to metastatic dissemination. Natural killer cells also appear to use surface laminin to interact with laminin receptors present on tumor cells. Taken together, these data suggest that the PGE receptor either may act as an adhesion molecule or may modify the action of another adhesion molecule or its receptor. Future studies will explore these possibilities.

REFERENCES


S-Z. Zhang and A. M. Fulton, Prostaglandin E receptor interactions with laminin, submitted for publication.
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