Targeting Prostaglandin E EP Receptors to Inhibit Metastasis

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Abstract

It is well established that high cyclooxygenase-2 (COX-2) expression contributes to the aggressive behavior of breast and other malignancies. Due to concerns regarding the safety of long-term use of COX-2 inhibitors as well as a desire to seek more effective alternatives to prevent and treat metastatic disease, we tested the hypothesis that inhibition of downstream signaling by the COX-2 product prostaglandin E2 (PGE2) would be as effective as inhibiting global prostaglandin synthesis. PGE2 acts through four G-protein-coupled receptors designated EP1-4. Here, we summarize data from many laboratories regarding the role of individual E-series of prostaglandin (EP) receptors on cancer behavior and we discuss our own recent findings that antagonists of the PGE receptor subtype 4, EP4, inhibit experimental metastasis in a murine model of hormone-resistant, metastatic breast cancer. These initial results indicate that selective targeting of individual EP receptors should be investigated as an approach to exploit the high COX-2 activity in many epithelial malignancies.

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Cyclooxygenase-2 Is Highly Expressed in Breast Cancer

Many tumors and tumor cell lines, including breast, express elevated cyclooxygenase-2 (COX-2) protein levels that contribute to their malignant behavior (1). Epidemiologic studies indicate that chronic use of nonsteroidal anti-inflammatory drugs that inhibit COX-mediated production of prostaglandins is associated with lower incidence of a number of tumor types, including breast cancers (2). In spite of recent advances in the treatment of hormone receptor-positive breast cancers, effective therapies are lacking for hormone-resistant and metastatic disease. Human breast cancers frequently have high PGE2 levels and breast tumors with high COX-2 protein levels are more likely to metastasize (1).

Protective effects of COX inhibitors both in preventing tumor induction and inhibiting growth of transplanted tumors are well established (3–5). Several mechanisms underlie these therapeutic effects. Direct effects of COX inhibitors on tumor cells have been shown, including inhibition of cancer cell proliferation and induction of apoptosis (6). Other studies support an indirect effect of COX inhibitors on tumor angiogenesis (4). COX inhibitors are also effective at limiting metastatic disease (3, 5). The antimitastatic effect depends on activation of natural killer (NK) cells (3, 7).

The COX-2 Product Prostaglandin E2 Acts through E-Series of Prostaglandin Receptors

In tumors, the principal COX-2 product is prostaglandin E2 (PGE2). Cellular effects of PGE2 are mediated through a family of G-protein-coupled receptors designated EP1,2,3 and EP4 (8). Despite structural and sequence similarities among the four E-series of prostaglandin (EP) receptors, they are coupled to different intracellular signaling pathways. Ligand binding of EP1 is associated with phospholipase C activation and elevations in intracellular calcium, whereas EP2 and EP4 are coupled to protein kinase A/adenyl cyclase and mediate elevations in intracellular cyclic AMP (cAMP; Fig. 1). In addition to protein kinase A-coupled signaling, the EP4 receptor has also been shown to activate extracellular signal-regulated kinase (ERK) 1 and ERK2 by way of phosphatidylinositol 3-kinase (9). EP3 typically mediates decreases in intracellular cAMP, but cAMP-activating properties have also been ascribed to splice variants of this receptor subtype (10).

EP Expression and Function in Cancer Is Complex

EP characterization on tumor cells is only beginning and the precise role of each EP in malignant behavior has yet to be determined. In some cells, EP2 and EP3 may have opposing actions on growth, with EP2 signaling linked to growth stimulation and EP3 linked to either growth inhibition or cellular senescence (11). Much of the data regarding the role of EPs in cancer come from animal models of colon cancer. All four EP receptors have been implicated, but the specific EP subtype differs depending on the model examined. For example, colon carcinogenesis is inhibited in mice lacking EP4 but not EP2 and pharmacologic antagonists of EP4 are protective in the Min model of colon carcinogenesis (12). EP4-mediated ERK activation supports growth of CT26 colon carcinoma cells and also rescues them from growth inhibition mediated by COX inhibitors (13). Conversely, EP2 plays an oncogenic role in polyp formation in the Apc716 model (14), but other studies have identified EP1 as the critical receptor (15). The role of EP3 in cell signaling and cancer biology often opposes the effects of other EP subtypes. For example, azaoxymethane-induced colon carcinogenesis is associated with loss of EP3 expression (16). Late-stage colon carcinogenesis is enhanced in EP3−/− mice. These studies raise the interesting possibility that inhibiting PGE2-mediated cAMP activation by EP3 signaling in normal tissue can initially override the tumor-promoting effect of EP1, EP2, or EP4 signaling.

The role of each EP receptor in skin carcinogenesis seems similarly complex. Considerable evidence exists from several laboratories that EP1 contributes to both UVB-induced and chemically induced skin cancers. UVB-induced squamous cell carcinomas display higher detectable EP1 expression than uninvolved skin (17). Consistent with these conclusions is that topical application of a selective EP1 antagonist protects against UVB-induced tumors (18). EP2 is also important to skin carcinogenesis. EP2−/−
mice are less susceptible to 7,12-dimethylbenz(a)anthracene/12-O-tetradecanoylphorbol-13-acetate–induced malignancies (19). Tumors arising in EP2+/−/− mice contain fewer CD31+ cells and more evidence of apoptosis, suggesting that PGE2-mediated signaling through EP2 drives tumor angiogenesis and apoptosis resistance. In direct contrast, however, loss of EP2 in immortalized keratinocytes was associated with increased invasiveness (20). Also unresolved is the role of EP3. Skin carcinogenesis is associated with loss of EP3 expression in a photocarcinogenesis model (17), whereas no change in EP3 expression was identified in a chemical model of skin cancer (19); evidence for a tumor-promoting role has also been reported (21). In the latter study, skin tumor latency was increased in EP3−/−/− versus wild-type mice, and only benign keratoacanthomas developed in the absence of EP3 expression. A different role for EP receptors in each model is undoubtedly reflective of the different mechanisms of carcinogenesis. The role of EP4 has not been interrogated in skin cancer.


Little clinical information regarding EP receptors is available; however, a recent study reports that coexpression of COX-2 and EP4 is associated with poor prognosis in upper urinary tract tumors (26). No association of EP1-3 with clinical outcome was observed (27). Like urinary tract tumors, EP4 mRNA expression is increased during colon cancer progression (28). EP2 and EP4 mRNA expression are higher in cervical carcinoma compared with normal cervix (29).

Taken together, these studies implicate individual EP subtypes as determinants of carcinogenesis. The majority of studies indicate that EP1, EP2, or EP4 promote early carcinogenesis, whereas EP3 either does not contribute to tumor behavior or actually plays a protective role. No consensus exists regarding why specific receptor subtypes seem to play an organ-specific role. These complexities are undoubtedly a function of the different histologic tumor types, mechanisms of tumor induction, whether inflammation plays a role in carcinogenesis, and other factors. In addition to differences regarding which EP subtype is important, many of these
studies cannot distinguish which of the multiple EP-expressing cell types are mechanistically important. Growth of transplanted MC26 or Lewis lung carcinoma cells was inhibited in EP2 mutant mice, and this was attributed to protection of dendritic cells from PGE2-mediated suppression (30). When inflammation contributes to carcinogenesis, as in some skin cancer models, it is likely that EP receptors on inflammatory cells are critical. In contrast, EP expression on the precursor malignant cell, the vasculature, or other cells may be the more important target in other models. Angiogenesis is regulated directly or indirectly by EP receptor functions. Tumor-induced angiogenesis in the Apc-6T16 model of colon carcinogenesis is mediated by EP2, but not other EP receptors (31), whereas EP3 regulates angiogenesis in a murine sarcoma model (32).

Even less is known about the role of EP receptors in late-stage tumor progression. Several laboratories have shown that EPs mediate migration of tumor cells in vitro (33, 34), suggesting that EP receptors could contribute to metastatic behavior. We sought to determine the functional role of EP receptors in vivo using a murine model of metastatic breast cancer.

**Targeting EP Receptors to Inhibit Metastasis**

We have shown that high COX-2 expression and activity is directly correlated with increased tumorigenic and metastatic capacity (5). Nonselective or selective COX-2 inhibitors limit local mammary tumor growth and spontaneous metastasis. Recent concerns regarding the safety of conventional COX-2 inhibitors (35), as well as the need to identify more effective agents, led us to examine the potential of targeting the EP receptor family. We reasoned that by blocking only PGE2 signaling, rather than global prostaglandin synthesis, we might avoid some of the potential side effects of inhibiting other cardioprotective prostaglandins and might also improve overall therapy.

EP receptors had been described on a range of tumor cells and tumor tissues, often at the mRNA level. We characterized EP receptor expression in several murine breast cancer cells, murine colon carcinoma cells, and on a panel of human breast cancer cell lines (36). All EP isoforms were detected by reverse transcription-PCR and immunoblotting in each murine and human cancer cell line. By flow cytometry, only EP2, EP3, and EP4 were readily detected. Surface expression was apparent on each cell; however, significantly more of each receptor was detected on permeabilized cells, suggesting that intracellular receptor pools exist.

On normal cells, EP2 and EP4 are coupled to adenylyl cyclase and cAMP activation. Treatment of mammary tumor cells with PGE2, an agonist of all EP isoforms, induces elevations in cAMP. Likewise, the selective EP2 agonist, butaprost, or the EP4 agonist, PGE1-OH, induce cAMP. The EP4 antagonists AH23848 or ONO-AE3-208 or the EP1/EP2 antagonist AH6809 block cAMP elevations induced by PGE2 or PGE1-OH. In contrast, antagonists of EP1 or EP3 did not affect the cAMP response. Taken together, these results are consistent with EP2 and EP4 receptors coupling to adenylyl cyclase in mammary carcinoma cells as described on many other cells (8).

**How Does EP4 Antagonism Inhibit Metastasis?**

Intracellular signaling via EP receptors is coupled to many cellular functions. In murine breast cancer cells, COX-2 is constitutively active, producing high levels of PGE2, so an autocrine loop in which tumor-produced PGE2 directs cell behavior by occupying EP receptors is probable (Fig. 1). In some cells, PGE2 directly supports proliferation; however, in our hands, COX inhibitors and EP4 antagonists only modestly affect proliferation of mammary tumor cells at concentrations that effectively block PGE2 synthesis and intracellular signaling. Inhibition of tumor cell migration in vitro by receptor antagonists also indicates that direct effects on cell motility and invasion may be relevant. Host EP expression on immune cells, endothelial cells, and other targets clearly also plays a role in determining tumor behavior (30–32). Our studies examined only the role of the tumor-expressed receptor; however, studies are in progress to determine if antagonism of host EPs can also affect behavior of either the locally growing tumor or metastatic disease. Whether effects of EP4 antagonism on tumor metastasis are directly linked to EP4-mediated signaling or indirectly affect other pathways also remains to be determined. Buchanan et al. (37) have proposed a model in which EP4 occupation causes β-arrestin 1 to translocate to the plasma membrane,
associate with c-Src, and ultimately transactivate epidermal growth factor receptor and AKT. The β-arrestin response was critical to the observed changes in tumor migration, invasion and metastasis.

The ability of COX inhibitors to limit metastasis depends on functioning NK cells (7). We showed that COX inhibitors alter intrinsic properties of tumor cells, rendering them more sensitive to killing by NK cells. Preliminary data suggest that a similar mechanism is relevant to the protective effects of EP4 antagonists.2 That is, antmitotic activity of EP4 antagonists also depends on functioning NK cells.

Although the role of each EP receptor in cancer biology remains complex, evidence is building that EP4 is a critical determinant of cancer cell behavior both in early colon carcinogenesis and in late-stage colon and breast cancer (12, 13, 26, 33, 34, 37). We are currently examining expression of EP receptors in human breast tumors to determine if EP4 or other receptors are relevant therapeutic targets. Using microarrays, we have found that mRNA levels of COX-2 and each EP receptor are positively correlated. Because COX-2 is an indicator of poor prognosis (1), our findings are consistent with a contribution of EP receptors to aggressive disease. Taken together, these studies indicate that antagonists of EP4 and of other EP receptors may be used to exploit the high COX-2 activity common to many epithelial malignancies, and that strategies using more selective targeting of individual receptors may avoid some of the less desirable side effects of COX-2 inhibitors.

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