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 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.

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 Apc Min 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, aoxymethane-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 tissues 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 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 Apc Min 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, aoxymethane-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 tissues can initially override the tumor-promoting effect of EP1, EP2, or EP4 signaling.

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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 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...
have shown that breast and colon cancer cells migrate in response to PGE2 (33, 34). EP4 function was particularly important to migration of breast tumor cells. Likewise, in colon cells, PGE2-mediated migration occurs via EP4/β-arrestin 1/c-Src signaling that transactivates and phosphorylates the epidermal growth factor receptor (37). We confirmed that the murine mammary tumor cells used for these studies also migrate in response to PGE2 in a dose-dependent manner. EP2 or EP4 antagonists blocked this response.

Taken together, several laboratories provide evidence from studies in vitro that EP receptors, particularly EP4, might contribute to metastatic behavior. We tested this hypothesis directly by determining the effect of selective antagonists of EP4 and EP3 on experimental metastasis. We also considered the possibility that EP3 might play a protective role and that antagonism of this receptor would increase metastatic potential. To distinguish effects of antagonism of tumor EP from effects on host EP function, we pretreated tumor cells in vitro with the EP3 antagonist ONO-AE3-240 or one of two EP4 antagonists, AH23848 or ONO-AE3-208. Cells were washed free of antagonist and immediately injected into the tail vein of syngeneic mice. Three weeks later, pulmonary tumor colonies were quantified. EP antagonist efficacy was compared with pretreatment with indomethacin, which we had previously shown to inhibit metastasis in this model (5). We confirmed the antimitastatic activity of indomethacin and showed that both of the EP4 antagonists significantly inhibited metastasis. The EP4 antagonist, AH23848, was as effective as indomethacin in this regard. In contrast, the EP3 antagonist had no effect on lung tumor colonization. These findings suggest that selective targeting of EP4 may be an effective alternative to inhibiting the entire COX-2 pathway. Although our initial results indicate that EP3 targeting will not affect tumor metastasis, the role of other EP receptors in controlling metastasis may be more complex. We reported in 1991 that pharmacologic antagonism of EP1 and EP2 actually increased lung colonization (38). These findings reinforce the conclusions reached by other laboratories that each receptor may have a unique function in tumor behavior. Thus, it will be critical that receptor-specific antagonists be used based on the precise mechanisms relevant to the effect of each receptor on metastasis.

**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. That is, antimitastatic 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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